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# Biodiversity, Biogeography and Phylogeny of Australian Freshwater Triclad

(Platyhelminthes: Tricladida: Continenticola)



Thesis submitted by  
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B. Sc. (Hons) Monash University

in September, 2016

For the degree of Doctor of Philosophy  
In the College of Marine and Environmental Sciences  
James Cook University



**I would like to  
acknowledge the  
contribution  
made by the  
Freshwater  
Planarians of  
Australia**

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## ACKNOWLEDGMENTS

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First and foremost I would like to thank my primary supervisors David Blair and Ronald Sluys. Their continuous intellectual and moral support, coupled with their extraordinary patience, was well beyond the call of duty. I wish David a very happy retirement in the Wet Tropics, although I suspect the term retirement may not be well applied in this case. Thanks also to Ronald for being such a gracious host on my various trips to Amsterdam, I learnt a lot about worms, and many other things, on my sojourns. In the early stages of my candidature I also received support from Richard Pearson and Leigh Winsor, for which I am exceedingly grateful.

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# STATEMENT OF THE CONTRIBUTION OF OTHERS

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## **Supervision**

- Professor David Blair, College of Marine and Environmental Sciences, James Cook University, Townsville, Queensland, Australia.
- Emeritus Senior Researcher Ronald Sluys, Naturalis Biodiversity Center, Leiden, The Netherlands.

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## STATEMENT ON NEW TAXA

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This thesis introduces several new taxon names that currently should be treated as *nomina nuda* as the provisions of Article 11 of the International Code of Zoological Nomenclature (ICZN 1999) are not met. For this reason these names, as well the accompanying taxonomic descriptions, should not be cited, copied or paraphrased. New taxon descriptions will be formally published in a forthcoming paper.

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## PUBLICATIONS ASSOCIATED WITH THIS THESIS

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### Manuscripts Published

Grant, L. J., R. Sluys and D. Blair. 2006. Biodiversity of Australian freshwater planarians (Platyhelminthes : Tricladida : Paludicola): new species and localities, and a review of paludicolan distribution in Australia. *Systematics And Biodiversity* **4(4)**: 435-471.

Sluys, R., L. Grant and D. Blair. 2007. Freshwater planarians from artesian springs in Queensland, Australia (Platyhelminthes, Tricladida, Paludicola). *Contributions to Zoology* **76(1)**: 9-19.

### Manuscripts in Preparation

Grant, L. J., R. Sluys and D. Blair. *in prep.* A contribution to the current species inventory for Australian Freshwater triclads (Platyhelminthes, Tricladida)(Appendix 1d). To be submitted to Systematics and Biodiversity.

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## ABSTRACT

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Freshwater triclads are a cosmopolitan group. Despite this, our knowledge of their biodiversity, biogeography and phylogeny is surprisingly poor. There has been steady progress worldwide towards remedying this; however, research on the biodiversity of Australian freshwater triclads has been dormant until recently. This project is the first study of the biodiversity, biogeography and phylogeny of Australian freshwater triclads, involving extensive and targeted sampling, contrasting with the more haphazard studies characteristic of the past. The project contributes greatly to our limited understanding of freshwater triclads in general, and the Australian species in particular.

This project began with extensive systematic work performed on a pre-existing, unstudied Australian collection. This work revealed several new species from three separate genera, representing a major contribution to the Australian fauna. New material was then collected from throughout Australia (the Northern Territory being the only notable exclusion), with over 500 sites visited and freshwater triclads discovered at over 400 of these. Molecular and traditional systematic techniques were used to describe new genera and species, draw conclusions concerning their phylogeny, and to examine patterns of geographical distribution and affinities with related taxa elsewhere in the world.

The taxonomic work, which forms the basis for the phylogenetic and biogeographic analyses, revealed a rich and diverse Australian fauna. In total, 16 new species and two new genera (with additional tentative new species and genera) have been described. The phylogenetic analysis, including both new genera and numerous new species, agreed with several previous analyses suggesting that the Dugesiidae shared a closer relationship with their terrestrial counterparts, than they did with other freshwater triclads (the Planarioidea). Where the current analysis disagrees with previous research is around the nature of this relationship. The current research suggests that the entire Geoplanoidea (freshwater and terrestrial) is a simple, monophyletic group within which the Dugesiidae *sensu lato* (*s.l.*) is paraphyletic. The consequence of this result is that the Geoplanidae arose from a freshwater ancestor. This outcome has prompted the development of a tentative new classification for the Geoplanoidea (the Dugesiidae *s.l.* and Geoplanidae) that more accurately reflects the state of our understanding for the group.

In relation to the generic relationships among the Dugesiidae *s.l.*, there are several conflicts with recent morphological and molecular work (Álvarez-Presas et al. 2008; Álvarez-Presas and Riutort 2014; Sluys 2001). This may be related to the markers chosen for the current analysis (i.e. molecular and morphological). However, it is also possible

that these discrepancies are due to the inclusion of new genera, unique to this study. One of the most interesting results is the position of eastern *Romankenkius* as the most ancient of the extant “dugesiid” genera. The position of *Masaharus* and the possible new genus (western *Romankenkius*) are also exciting developments, as they seem to represent links between freshwater and terrestrial genera, and occupy critical position within the paraphyletic Dugesiidae *s.l.*

Australia’s position as the most diverse continent, for both genera and species, has been emphasised through the current research. Endemicity is also a feature of the Australian “dugesiid” fauna. These high levels of diversity and endemicity suggest that Australia was a center of diversity for the Dugesiidae *s.l.*, at least in the Gondwanan era. Within Australia two biodiversity hotspots occur: the Australian Alpine region and the island of Tasmania. These two hotspots are the primary contributors to the status of Australia as the most diverse continent for the Dugesiidae *s.l.* Outside these areas, Australia is dominated by three widespread genera, *Cura*, *Dugesia* and the invasive *Girardia*.

Existing theory, relating to the current distribution of the Dugesiidae *s.l.* throughout the globe, suggests that the taxon must have diversified before the breakup of Pangaea (Ball 1974a; Ball 1975). Regardless of the actual sequence of diversification, by the Jurassic representatives of the Dugesiidae *s.l.* must have been broadly distributed to allow the distribution of its members throughout the Northern Hemisphere. Within Australia the biogeography of the Dugesiidae *s.l.* is defined by what are perceived to be older Gondwanan relics (e.g. *Romankenkius*, *Spathula*) in the southern highlands (Australian Alps and Tasmania) and by the more “modern” genera (*Cura*, *Dugesia*) throughout the remainder of the continent’s waterways.

This study has highlighted the importance of the Australian continent in both freshwater and terrestrial triclad research and provided a substantive contribution to this field. However, there is a great deal more research to be done in relation to the origin of the freshwater and terrestrial Geoplanoidea. I believe that the next major step is a thorough investigation into the relationship between the Geoplanidae, specifically the Australian species, and the Dugesiidae *s.l.* families *Spathulidae* and *Romankenkiidae* and *Masaharidae*. Indeed, such efforts may also lead to the discovery of morphological synapomorphies for the newly described families, allowing the formalisation of the classification.

Practical applications of this research include the development of a key utilising the internal morphology. This would be of use to other taxonomists who may not specialise in this group. The sequencing of the *cox1* region of the mitochondrial genome

and 18S region of the nuclear genome for many new species and several new genera will be useful for future phylogenetic research, and will ideally contribute to an identification system utilising sequence data for the Dugesiidae *s.l.* (i.e. barcoding).

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EU	Europe	dg	diaphragm
NSW	New South Wales	e	eye
NZ	New Zealand	ed	ejaculatory duct
QLD	Queensland	fgd	female genital duct
SA	South Australia	g	gland
TAS	Tasmania	gd	gonoduct
VIC	Victoria	gid	genito-intestinal duct
WA	Western Australia	gl	glands
PA	Palaeartic Region	go	gonoduct
NA	Nearctic Region	gp	gonopore
AT	Afrotropical Region	int	intestine
NT	Neotropical Region	lm	longitudinal muscle
OL	Oriental Region	ma	male atrium
AU	Australasian Region	mc	muscular cavity
ANT	Antarctic Region	mo	mouth
PAC	Pacific Region and Oceanic Islands	od	oviduct
O.	Order	ol	oviducal loop
S.O.	Suborder	ov	ovary
I.O.	Infraorder	pb	Penis bulb
F.	Family	pg	penial glands
ad	adenodactyl	ph	pharynx
ae	atrial expansion	pp	penial papilla
at	atrium	sf	sensory fossae
ao	adhesive organ	sg	shell glands
bc	bursal canal	sp	sperm
br	brain	spe	spermatophore
ca	copulatory apparatus	sph	sphincter
cat	common atrium	sv	seminal vesicle
cb	copulatory bursa	tm	transverse muscle layer
cod	common oviduct	te	testes
cm	circular muscle	vd	vas deferens
cp	ciliated pit	vi	vitellaria
di	diverticulum	vlm	ventral longitudinal muscles
div	diverticulum	vf	valve-like fold

**Please note:** all figures created as part of this thesis unless otherwise referenced.

# Chapter 1: Introduction

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## 1.1 Preamble

Taxonomy, phylogeny and biogeography, will be used to investigate a single family of freshwater triclads, the Dugesiidae Ball, 1974. Familiarisation with the taxonomy and phylogeny of the Platyhelminthes, specifically the Tricladida, is essential to provide context for the exploration of the Dugesiidae, hence this introduction. Some relevant biogeographical patterns are also discussed. Freshwater triclads are introduced as the study taxon, with details of the phylogeny, ecology and life history strategies employed by the group. The introduction concludes with a summary of project aims and the thesis outline.

## 1.2 The Tools

Taxonomy still underpins all biological research, with implications for all basic scientific and applied fields (Tautz et al. 2003). Historically, morphological characters have been used to infer phylogenies and taxonomic systems, an approach that many researchers now view as arcane and unreliable (Hall 2007). Arnold (1990) highlights the difficulties encountered using morphological analyses with issues including: not enough characters developed between branching points, uncertain character polarity, poorly differentiated character-states, homoplasy caused by parallelism or reversal, and extinction. Despite this, a great many of the currently accepted phylogenies were established via morphological analysis.

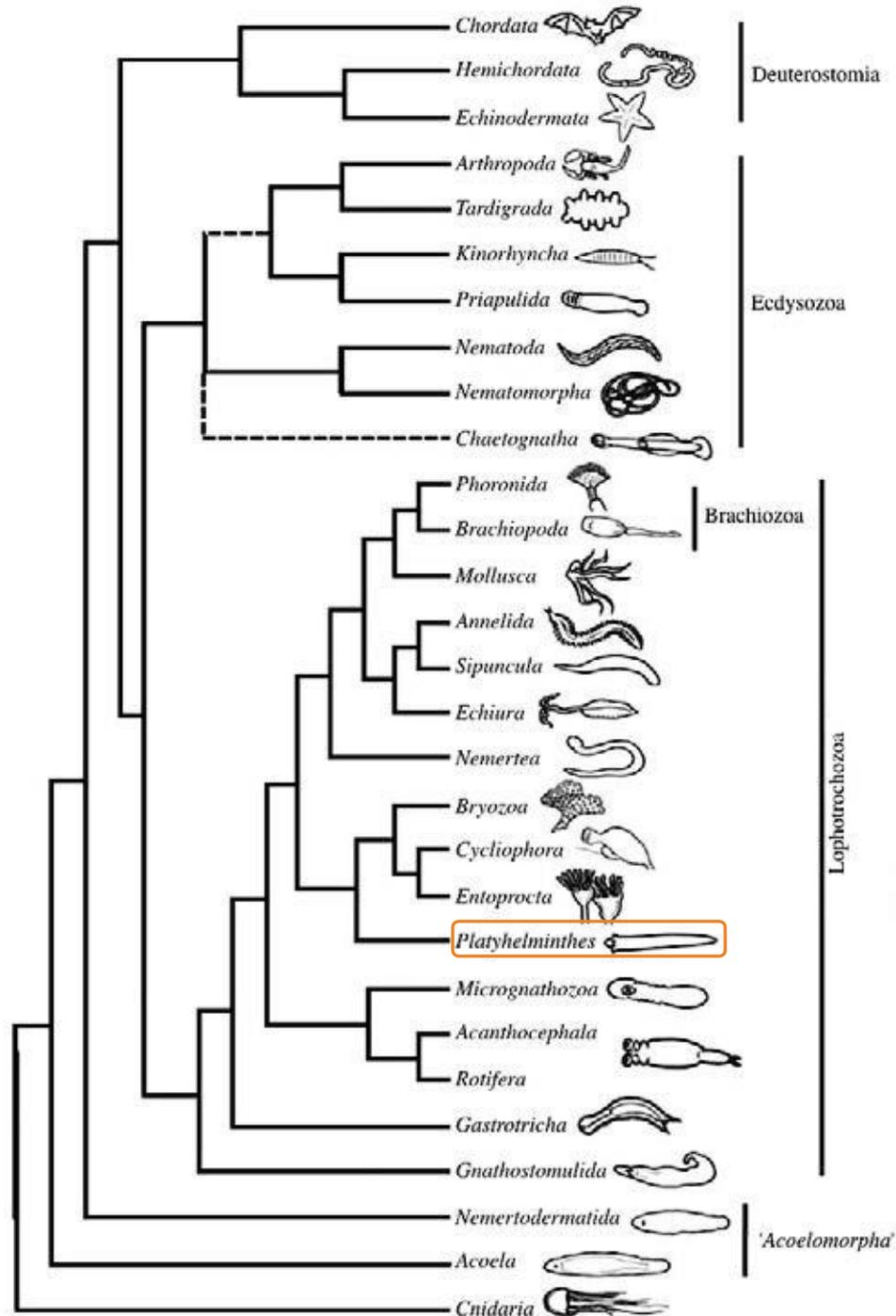
An independent method to test phylogenies, and to overcome some of the difficulties associated with morphological data, is to employ molecular systematics (Carranza et al. 1998a). In the past decade the increased use of molecular techniques has led to traditional views on evolution being challenged and many long-standing hypotheses abandoned (Halanych 2004). Despite the overwhelming support in the scientific community for the use of molecular data to infer phylogenies, there are some reservations regarding its use. Scepticism has focused on uncertainty around the reliability of molecular data, apparent conflict between morphology and molecular data, frequent lack of robust phylogenetic signal, lack of well-defined morphological synapomorphies, and apparent contradictory conclusions from the same data source (Halanych 2004). Many biologists argue that it is counterproductive to abandon morphological phylogenies altogether and are consequently using both morphological and molecular data in their

analysis (Assis 2009). The importance of this “total evidence” approach has been discussed and promoted by many. However, this approach is not without difficulties. Assis (2009), Chippindale and Wiens (1994), Cunningham (1997), Eernisse and Kluge (1993), Kubatko and Degnan (2007), Lee (2001) and Wheeler (1995) are examples of the many authors who have published research specifically addressing the issue of congruence between phylogenetic markers. In addition, Adamowicze et al. (2009), Blair et al. (1998), Daniels et al. (2002), Littlewood et al. (1998), Shull et al. (2005) and Sorensen and Giribet (2006) are examples of researchers presenting “total evidence” approaches for phylogeny of their respective groups and emphasising the importance of any valid phylogeny arising from congruent data. This study aims to develop a “total evidence” phylogeny from congruent data and then to apply this to a preliminary biogeographic analysis of freshwater flatworms of the family Dugesiidae in Australia.

Biogeographical understandings have been significantly enhanced by the recognition of the mobility of landmasses, which led to the identification of vicariance as a method to explain distributional patterns of biota (Brown & Lomolino 1998; Briggs 1987; Cattermole 2000; Cox & Moore 2000; Wegener 1915). This inspired the development of new biogeographical inference techniques including panbiogeography (Croizat 1952, 1958, 1962) and cladistic biogeography (Hennig 1950; Humphries & Parenti 1999), both of which will be considered in an attempt to explain the current distribution of the study taxon. There is still debate regarding the precise movement and connectivity of the continental plates, and constant re-interpretation of dispersal patterns causing regular re-assessment of biogeographical hypotheses. It also needs to be noted that the existence of continental drift does not exclude other biogeographical explanations, such as dispersal and land bridges, to explain biogeographical patterns.

### 1.3 The Platyhelminthes

The main focus for this study is the Dugesiidae, a family of free-living freshwater platyhelminths. The phylum Platyhelminthes, commonly known as flatworms, consists of an anatomically relatively simple group of bilaterian, unsegmented invertebrates. Platyhelminthes are characterised by the lack of a coelom, specialised circulatory system and respiratory organs. This restricts them to a flattened body shape to allow the diffusion of nutrients and oxygen. They have a cephalised nervous system with a cerebral ganglion thought to represent the earliest rudimentary brain to have developed in the Animal Kingdom (Baguña & Riutort 2004a). Platyhelminthes are mostly hermaphrodites having simultaneous male and female reproductive organs (Schockaert et al. 2008).

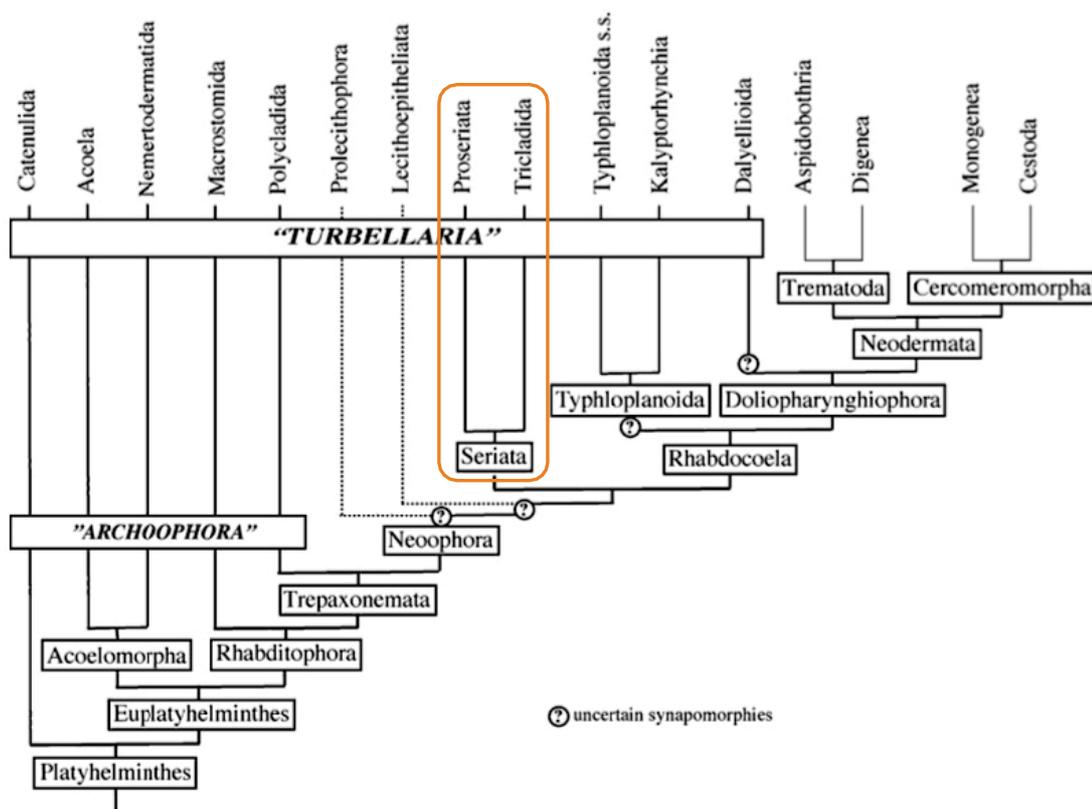


**Figure 1.1** Summary tree adapted from Paps et al. (2009) with the Platyhelminthes highlighted.

The lack of a coelom meant that Platyhelminthes were always placed at the base of the bilaterian tree (Sluys et al. 2009). Platyhelminthes have very few synapomorphies, making their relationships with other groups and the relationships within the phylum difficult to resolve. Whilst traditionally thought to be monophyletic, this lack of synapomorphies lead Smith et al. (1986) to challenge this assumption proposing that the Platyhelminthes was a polyphyletic phylum that formed a sister group to all other

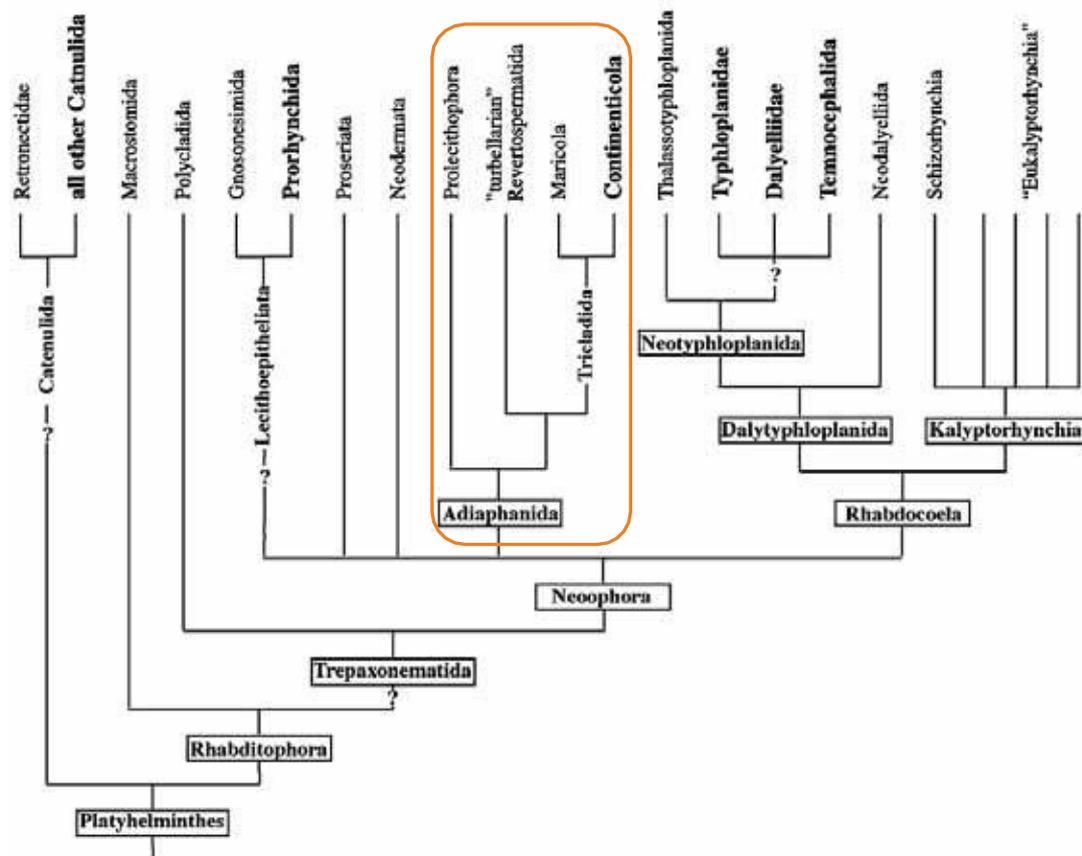
bilaterians (Willems et al. 2006). This polyphyletic state has been confirmed by recent molecular evidence with the erection of a new phylum, the Acoelomorpha, to house the Acoela and Nemertodermatida. Previously classified as platyhelminth “turbellarians”, they are now thought to be the sister to all other bilaterians, including the Platyhelminthes (Baguña & Riutort 2004a; Larsson & Jondelius 2008; Philippe et al. 2007). The remaining Platyhelminthes are now understood to belong amongst the bilaterian superclade Lophotrochozoa, also housing such groups as bryozoans, nemerteans, brachiopods, annelids, echiurians, molluscs, and rotifers (Dunn et al. 2008) (Figure 1.1).

Morphological phylogenies traditionally divided the Platyhelminthes into three “Classes”, the Trematoda and Cercomeromorpha (together forming the Neodermata), which are exclusively parasitic and the Turbellaria, mostly non-parasitic (Ehlers 1985, Sluys et al. 2009). The name “Turbellaria” refers to the whirlpools of microscopic particles created on the epidermis of aquatic species by the movement of their cilia (Ruppert et al. 2004; Schockaert et al. 2008). Ehlers (1985) morphological hypothesis divided the Turbellaria into twelve sub-orders, two of which, the Proseriata and Tricladida, were thought to form a monophyletic order, the Seriata (Carranza et al. 1998a; Álvarez-Presas et al. 2008) (Figure 1.2).



**Figure 1.2** Relationships within the Platyhelminthes based on morphological data according to Ehlers (1985) with the Seriata highlighted; image adapted from Schockaert et al. (2008).

The idea that the Seriata was monophyletic was established on morphological grounds by Bresslau (1928), and was not challenged until relatively recently. The hypothesis was initially contested on the basis of work done on the ultra-structure of the excretory system in Platyhelminthes that suggested a basal location for the Proseriata, and consequently the paraphyly of Seriata (Rohde 1990, 1994). More recently the phylogenetic relationships within the Platyhelminthes have been inferred from molecular data (Baguña et al. 2001a; Joffe & Kornakova 2001; Noren & Jondelius 2002; Willems et al. 2006). The molecular data implied that the ciliated epidermis is a plesiomorphy and the “Turbellaria” a paraphyletic assemblage (Schockaert et al. 2008). The tree inferred from the molecular data confirmed Rohde’s (1990, 1994) hypothesis regarding the Proseriata and triclads with the order Seriata being deemed no longer relevant. The Tricladida are now positioned in a strongly supported clade the Adiaphanida, with the Prolecithophora and the “turbellarian” Revertospermatida (Baguña & Riutort 2004b; Noren & Jondelius 2002; Willems et al. 2006) (Figure 1.3).



**Figure 1.3** Phylogenetic relationships of the Platyhelminthes according to 18S rDNA data with the Adiaphanida highlighted. All named clades are strongly supported, except where indicated by “?” (combined from Baguña et al. 2001b; Joffe & Kornakova 2001; Noren & Jondelius 2002; Willems et al. 2006); image adapted from Schockaert et al. (2008).

In 1774 Müller, who initially grouped triclads with parasitic worms, was the first to place them in their own group, planarians, which at the time also included other turbellarians and nemertean worms (Ball 1981). The order Tricladida (Lang 1884) was erected to encompass all planarians with a triple-branched intestine and anteriorly situated ovaries next to the brain. Note that the name “planarian” is still commonly used interchangeably with triclad, however, triclad or Tricladida, is the more taxonomically correct term. The Tricladida are monophyletic as evidenced by: their unique embryological development, the ventral position of the ovaries, the serial position of the many nephridiopores, the presence of a marginal adhesive zone, and the three-branched intestine (Sluys 1989a; Sluys et al. 2009).

## 1.4 The Tricladida

For over 100 years systematists have recognised three major groups named for the ecological niches they inhabit. The Maricola (marine triclads), Paludicola (freshwater triclads), and Terricolans (terrestrial triclads) (Sluys et al. 2009). More recently a fourth clade was erected, the Cavernicola Sluys, 1990 (triclads inhabiting caves). This latter group includes five enigmatic species that were formerly, albeit tentatively, assigned to the marine triclads (4 species) and the freshwater triclads (1 species). As these groupings suggest, the Tricladida have representatives across a range of ecosystems and are found on every continent, including marine species on the coastline of Antarctica (Sluys 1989a). Marine species, whilst predominantly known from coastal regions, have also been collected from waters as deep as 60m and Sluys (1989a) suggested that there is no reason to believe that their distribution is limited by water temperature, salinity or depth. Freshwater triclads, due to their low vagility and the absence of freezing- or drought-resistant strategies, tend to be restricted to freshwater bodies that do not freeze or dry out (Ball & Reynoldson 1981). Likewise, terrestrial species are restricted to humid environments due to their need for damp soil or moist habitats (Winsor 2003).

Until relatively recently the monophyletic status of the Tricladida was accepted without discussion (Ball 1977c). There are morphological synapomorphies to support the assignment of triclads to infra-orders based on their ecological affinities. Steinbock (1925) divided the Tricladida on the basis of the structure of the nervous system. He coined the taxon name Diploneura for the terrestrial triclads and united the freshwater and marine triclads to form the Haploneura (Sluys et al. 2009). The Diploneura were considered a sister group of the Haploneura. However, Ball (1981) and Sluys (1989b) have demonstrated that the Haploneura is a paraphyletic group; thus this scheme has lost its taxonomic integrity (Ball 1977c; Ball 1981). Meixner (1928) recognised the importance of

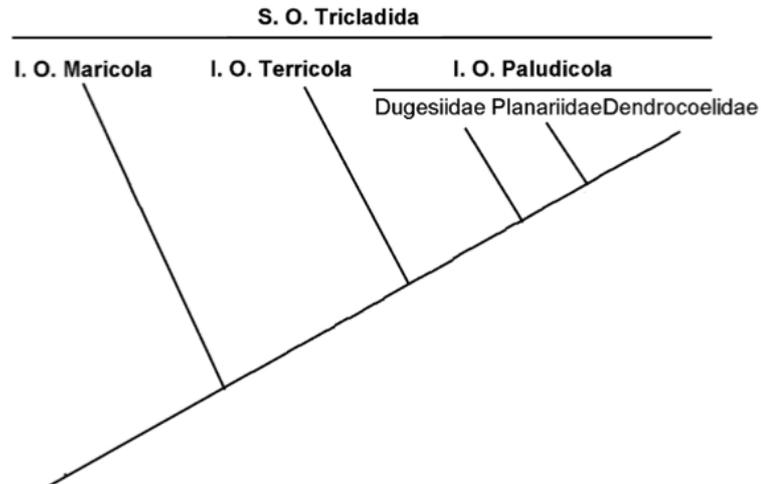
the copulatory apparatus for taxonomic purposes and proposed three distinct reproductive organisations. Meixner's (1928) scheme relied heavily on characters relating to the atrium of the copulatory apparatus; he did not however, erect new phylogenetic classifications based on these groupings. While the integrity of some of these groupings was questionable, the broad premise that the marine and freshwater triclads belong to different evolutionary lines and that the marine representatives were the more primitive of the two groups appeared to be sound (Ball 1974a).

More recently, three infra-orders were established (again aligned ecologically) and, through the efforts of many researchers, two of these infra-orders were assigned a set of characters to define them (Sluys et al. 2009)(Figure 1.4a). The terrestrial triclads are defined by their complex diploneural nervous system, the complex nature of the eyes, distinctive nature of the pharyngeal musculature and the presence of a creeping sole (Ball 1977c, Sluys 1989b). The freshwater triclads are defined by subepidermal musculature consisting of four layers, sperm transfer through spermatophores, the reduced precerebral gut diverticula and the so-called probursal condition of the copulatory bursa (Ball 1981, Sluys 1989b). The third infra-order, marine triclads, are the most difficult group in which to recognise a synapomorphy (Sluys 1989a, b; Sluys et al. 2009).

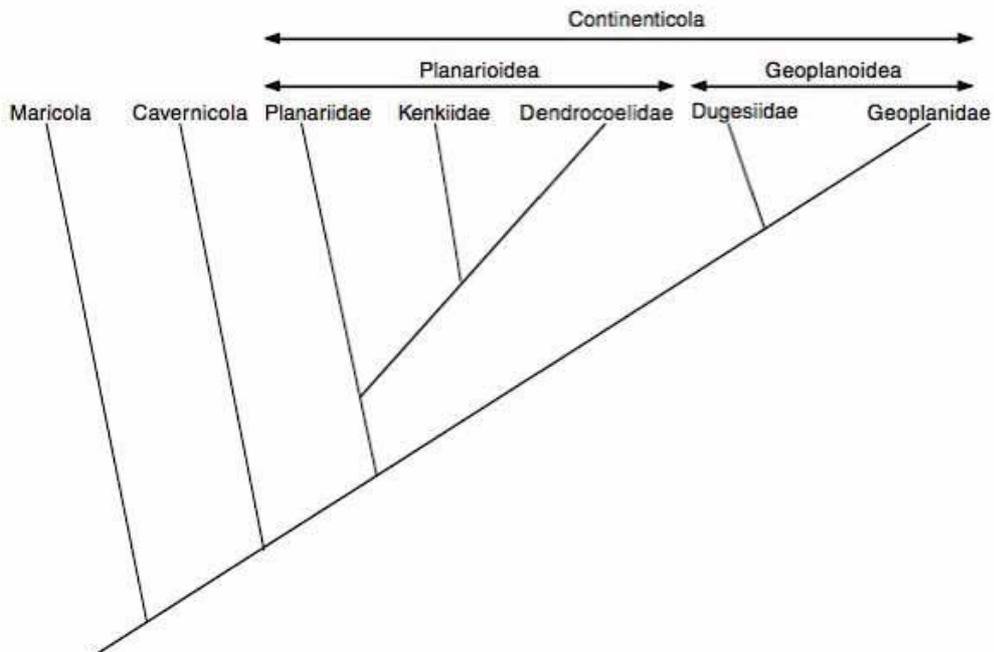
As is evidenced by the above discussion, morphological hypotheses for the Tricladida are difficult to legitimise due to the often-ambiguous nature of their morphology (see section 1.3). When attempting to construct a morphological phylogeny, systematists must rely a great deal on potentially flexible characters. For example, Steinbock (1924) initially suggested that marine triclads were morphologically distinguished from the freshwater triclads based on the position of the bursa. This has proven to be an ineffective division as marine triclads have now been described with both an anterior and posterior bursa (Sluys 1989a).

Sluys (1989b) recognised the inadequacy in previous analyses and subsequently reviewed existing characters and introduced new characters to present a new hypothesis relating to the relationships of the triclads. Sluys (1989b) proposed that the monophyletic status of the Tricladida was supported by several derived features, including unique embryological development and the presence of a marginal adhesive zone (Sluys 1989b). His study was the first to identify marine triclads (Maricolans) as a primitive sister group to the new terrestrial (Terricolan) - freshwater (Paludicolan) clade (Sluys 1989b) (Figure 1.4a). The implication of this new clade was that terrestrial triclads evolved from marine ancestors and that freshwater triclads came from a land or marine ancestor (Sluys 1989b).

a)



b)



**Figure 1.4** a) Phylogenetic relationships of the Tricladida based on morphological characters according to Sluys (1989 a,b) and b) Phylogenetic hypothesis for the Tricladida based on molecular and morphological characters (Sluys et al. 2009).

With the introduction of molecular markers in phylogenetic analysis, it was necessary to completely reassess ideas relating to triclad relationships, particularly of the freshwater and terrestrial representatives. These molecular analyses strongly suggested that freshwater triclad are paraphyletic (Álvarez-Presas et al. 2008, Carranza et al. 1998 a,b)(Fig. 1.4b). Carranza et al. (1998 a,b) suggested the freshwater and terrestrial clade be renamed *Continenticola*, sister to the marine triclad (the *Maricola*).

### 1.4.1 Higher Classification of the Tricladida

The classification of the triclads has undergone substantial change since the adoption of molecular techniques to infer phylogenies. The classification presented below is adapted from Sluys et al. (2009), who have incorporated all modern understandings, most importantly the erection of the suborder Continenticola (Carranza et al. 1998 a,b).

Domain **EUKARYOTA** Whittakey & Margulis, 1978

Kingdom **ANIMALIA** Linnaeus, 1758

**BILATERIA** Hatscheck, 1888

**PROTOSTOMIA** Grobben, 1908

Phylum **PLATYHELMINTHES** Gegenbaur, 1859

Class **RHABDITOPHORA** Ehlers, 1985

Order **TRICLADIDA** Lang, 1884

Suborder **MARICOLA** Hallez, 1892

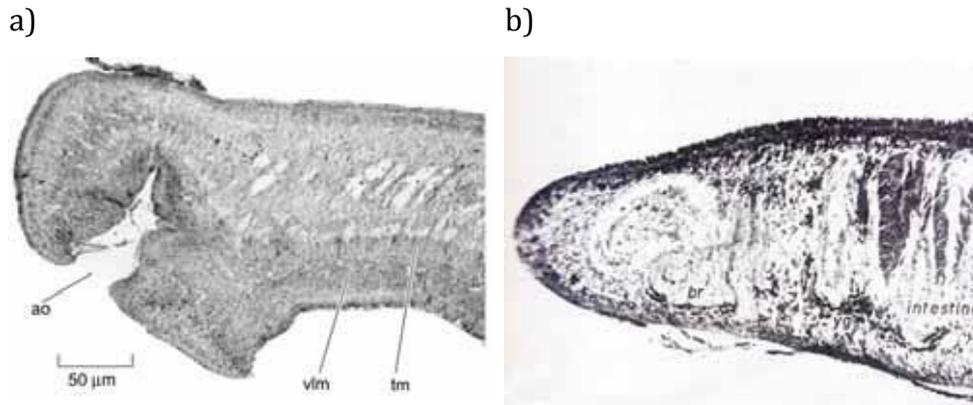
Suborder **CAVERNICOLA** Sluys, 1990

Suborder **CONTINENTICOLA** Carranza, Littlewood, Clough, Ruiz-Trillo, Baguñá and Riutort, 1998

## 1.5 The Continenticola

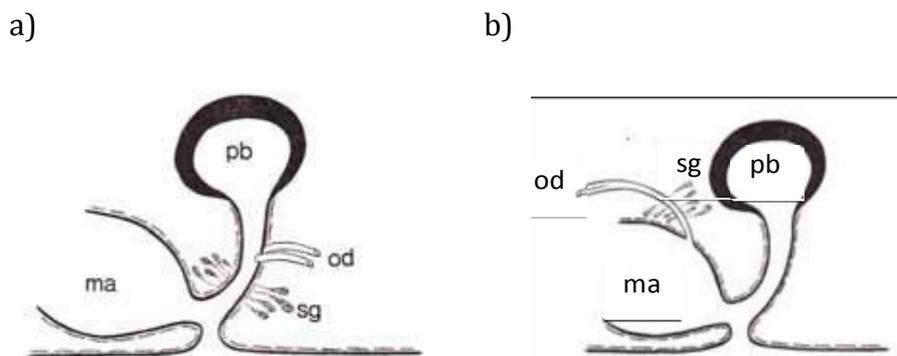
The marine triclads (Maricola) have recently been monographed (Sluys 1989a), as has the cave-dwelling suborder (Cavernicola) (Sluys 1990a). Recent work on the terrestrial triclads (Continenticola) is noticeably lacking, with the most recent monograph being that by von Graff (1899). And, while the freshwater triclads (Continenticola) have not been monographed, this group has received a reasonable amount of attention from researchers (Álvarez-Presas et al. 2008; Ball 1974a; De Vries & Sluys 1991; Sluys 1989b; Sluys 1997; Sluys et al. 1998; Sluys & Kawakatsu 2006). It is the freshwater Continenticola, specifically the family Dugesiidae, which will be the focus of this thesis.

Hallez (1894) recognised nine genera within the freshwater triclads and divided them into two families, the Dendrocoelidae Hallez, 1894 (with an anterior adhesive organ) and the Planariidae Stimpson, 1875 (without an anterior adhesive organ) (Figure 1.5). Von Graff (1912-17) increased the number of families to five, the Curtisiidae Von Graff, 1916, Planariidae Stimpson, 1857, Procotylidae Korotneff, 1908, Podoplanidae Von Graff, 1916 and Dicotylidae Zabusov, 1901. The Procotylidae, Podoplanidae and Dicotylidae contained species from Lake Baikal, which are now considered to be members of the Dendrocoelidae, hence the dissolution of Von Graff's (1912-17) arrangement (Kenk 1974; Sluys et al. 2009).



**Figure 1.5** Example of adhesive organ a) *Bdellocephala cf. angarensis* (Sluys & Kawakatsu 2006) and no adhesive organ b) *Cura patagonica* (Kawakatsu et al. 1984). Abbreviations: ao, adhesive organ, vlm, ventral longitudinal muscles; tm, transverse muscle layer.

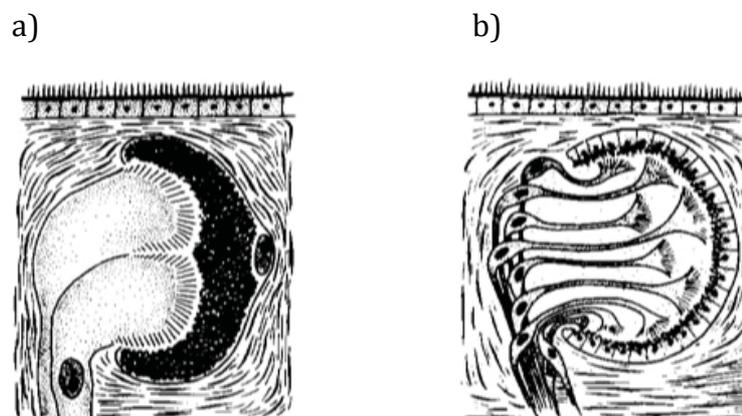
Kenk (1930) deemed the anterior adhesive organ inadequate for family divisions (anterior adhesive organs being found in representatives of both groups), instead defining two families based on the arrangement of the inner muscle layers of the pharynx. Interestingly, this division reflected Hallez's (1894) earlier arrangement, with two families being recognised, the Dendrocoelidae (intermingled musculature) and the Planariidae (separate layers of circular and longitudinal muscle). However, the fact that some dendrocoelids show the planariid-type of musculature complicated matters somewhat (Sluys 2001; Sluys & Kawakatsu 2006). Hyman (1937) found it necessary to erect a third family, the Kenkiidae, to house three genera of cave-dwelling triclads. However, many subsequent taxonomists have questioned the necessity for a new family, instead suggesting that this group represents a subgroup amongst one of the existing families (Ball 1974a; Carpenter 1970; De Beauchamp 1961; Kenk 1976; Mitchell 1968).



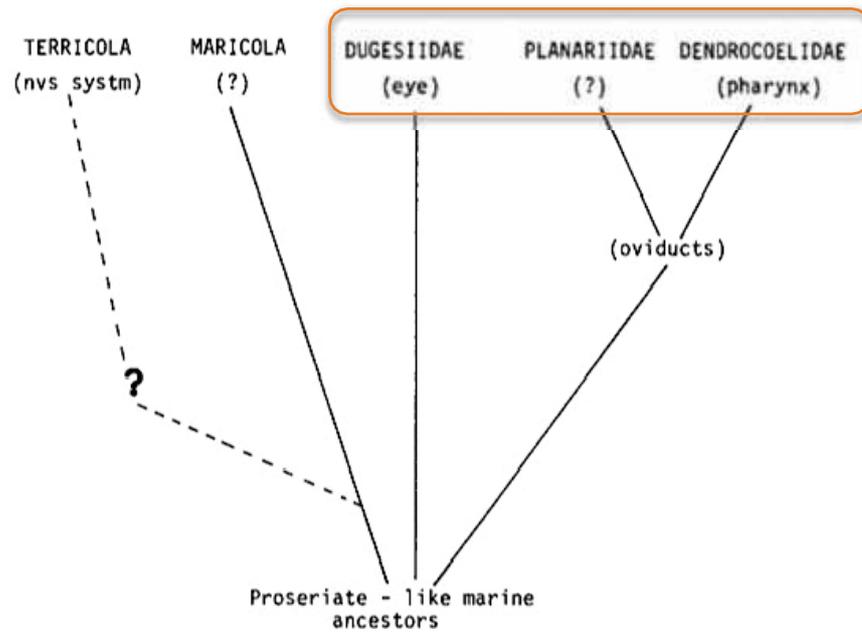
**Figure 1.6** Diagrammatic representation of the atrial organs of aquatic triclads. a) a primitive condition as found in most Maricola and in most Dugesiidae, b) the condition found in the Planariidae and Dendrocoeliidae. Abbreviations: ma, male atrium; od, oviduct; pb, primary bursa; sg, shell glands. Images adapted from Ball (1977c).

Ball (1974a) proposed the family Dugesiidae to house freshwater triclads with oviducts, separate or combined, entering the bursal canal (or very close to its origin in the atrium). The absence of an adhesive organ and the oviducal opening into the bursal canal (also found in marine and terrestrial triclads) prompted Ball (1974a; 1981) to regard the Dugesiidae as the most primitive of the three families.

After phylogenetic analysis, Ball (1974a, 1977c) attempted to describe all families in terms of apomorphic characters. The Dendrocoeliidae were comfortably defined by the pharyngeal musculature and presence of an anterior adhesive organ (Ball 1977c; Sluys & Kawakatsu 2006)(Figure 1.5). The Planariidae formed a sister-group relationship with the Dendrocoelidae, both sharing the same positioning of the oviducts and shell glands (Figure 1.6), however, Ball (1977c) noted a lack of synapomorphies for the Planariidae making it hard to confirm this taxon's monophyly. He suggested that the triangular head of the Dugesiidae may be apomorphic, but immediately pointed out the numerous exceptions to this hypothesis (e.g. southern hemisphere species)(Ball 1974a). To increase uncertainty, the Dugesiidae could not be separated from the Maricola except via a combination of characters which, taken in isolation, could pertain to either group (Ball 1974a). However, within the freshwater triclads, Ball (1974a) settled upon the complex eye structure (resembling that of terrestrial triclad species) of the Dugesiidae as distinguishing this family from the Planariidae and Dendrocoelidae (Figure 1.7). Ball (1974a; 1981) went on to question the monophyly of the freshwater Tricladida, suggesting independent origins of the three families (Figure 1.8). At the conclusion of Ball's (1974a, 1977c, 1981) substantial contribution to the questions surrounding relationships within the freshwater triclads, we were left with three families, the Planariidae Stimpson, 1857, Dendrocoelidae Hallez, 1892 and Dugesiidae Ball, 1974, with the Kenkiidae Hyman, 1937 becoming a subfamily of the Dendrocoelidae.



**Figure 1.7** Sections through the eyes of a) *Planaria* (Planariidae, Tricladida) and b) *Dugesia* (Dugesiidae, Tricladida). Images taken from Ball and Reynoldson (1981).

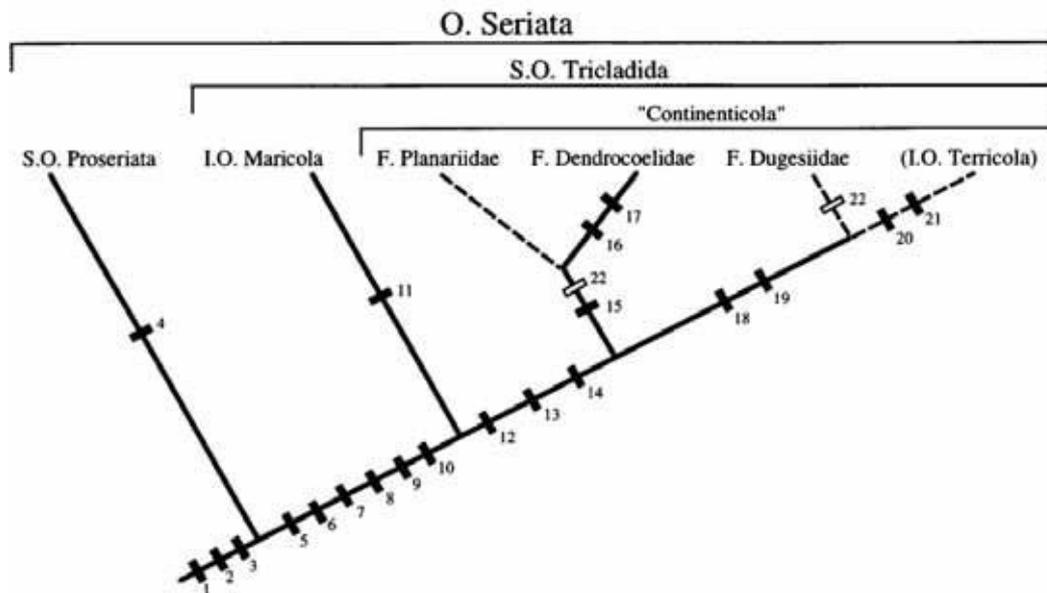


**Figure 1.8** Ball (1974a) proposed this phylogeny of the Tricladida in which the freshwater triclads (box) are diphyletic (? indicates uncertain synapomorphies).

The legitimacy of the Planariidae, Dendrocoelidae and DugesIIDAE has now been supported by molecular data on many occasions (e.g. Álvarez-Presas et al. 2008; Baguña et al. 2001a; Carranza et al. 1998a). Carranza et al. (1998a) cited an 18S gene duplication event shared by both the DugesIIDAE and terrestrial triclads (Terricola) as indicating a sister group relationship between these. The obvious consequence of this discovery was that the freshwater triclads are paraphyletic (Álvarez-Presas et al. 2008; Baguña et al. 2001a; Carranza et al. 1998a) (Figure 1.9). In addition to this, Baguña's work using the 18S and *cox1* markers strongly supports the paraphyly of the freshwater triclads and validity of the Continenticola clade. Furthermore, this same analysis could not validate the monophyly of the terrestrial triclads (Terricola) and the DugesIIDAE (Baguña et al. 2001a). These results support previous statements relating to the weakness of habitat as a phylogenetic character and the dearth of morphological synapomorphies for the group (Ball 1981, Sluys 1989b).

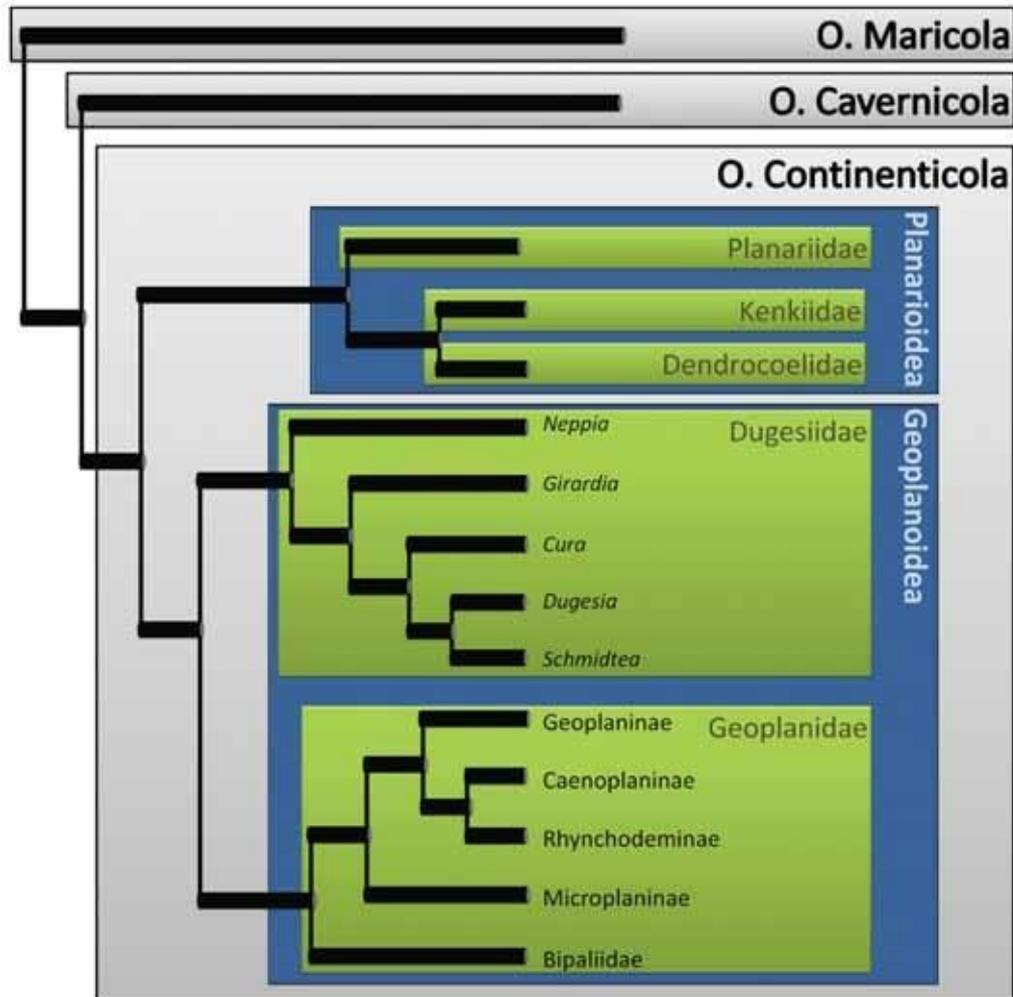
When Ball (1981) questioned the monophyly of freshwater triclads he emphasised the strong relationship between the Planariidae and the Dendrocoelidae invoking the term Planarioidea, originally used by Stimpson (1857) to describe a similar group. The sister-group relationship between these two families has not been questioned in the light of new molecular data and Sluys et al. (2009) used the name Planarioidea Stimpson, 1857 as the superfamily housing the Planariidae, Dendrocoeliidae and the reinstated family Kenkiidae (Table 1.1 and Figure 1.10)(Sluys and Kawakatsu 2006). Finally, the suborder Cavernicola

was erected by Sluys (1990a) in order to house five enigmatic triclads, most of which inhabit caves and formerly belonged to the Maricola (the exception being the freshwater *Rhodax evelinae* Marcus, 1946). He argued that these species more closely resemble freshwater triclads, suggesting a sister group relationship between these two groups (Sluys 1990a)(Figure 1.10).



**Figure 1.9** The first robust molecular hypothesis suggesting the paraphyly of the freshwater triclads, presented by Carranza et al. (1998a). Selected morphological characters from Ball (1981) and Sluys (1989b) have been mapped onto the tree with black rectangles referring to derived characters, and white rectangles to convergences. Dashed lines indicate groups that are not well supported in the molecular phylogenetic analysis (O. - Order, S.O. - Suborder, I.O. - Infraorder, F. - Family).

The most recent contributions to the question of the triclad phylogeny from Álvarez-Presas et al. (2008) and Álvarez-Presas and Riutort (2014) concede that while some parts of the tree remain poorly resolved, there is strong support for monophyly of the terrestrial triclads. However, these analyses were ambiguous when attempting to determine the terrestrial triclad ancestor. According to Álvarez-Presas et al. (2008) a freshwater or marine ancestor are both possibilities. A final interesting point from this research was the idea that some genera are likely to have returned to freshwater (i.e. genera previously thought to belong to the Dugesiidae), a concept with important implications for dugesiid research (Álvarez-Presas et al. 2008; Álvarez-Presas & Riutort 2014). While it is obvious that the terrestrial triclads will, by necessity, play an important role in this thesis, the focus will remain on the freshwater species.



**Figure 1.10** Phylogenetic tree summarising the current understanding of the internal relationships of the Tricladida, from Riutort et al. (2012)(O. - Order).

So, 120 years after Hallez's (1894) initial classification the relationships between the freshwater triclads look very different; the paraphyly of the group and the subsequent erection of the superfamily Geoplanoidea to house both a family of freshwater triclads (Dugesidae) and the terrestrial triclads (Geoplanidae) being the most substantial amendment (Sluys 2009)(Figure 1.10 and Table 1.1). It should be noted that the Planarioidea are under-represented in molecular studies (Sluys 2009)(Figure 1.10 and Table 1.1). Addressing this inadequacy may lead to more substantial resolution of relationships within the Planarioidea (Sluys and Kawakatsu 2006) and possibly within the Continenticola. The current understanding is that while the Planarioidea and Geoplanoidea are sister groups that have arisen from a common ancestor, the ecological preferences of this ancestor are uncertain (Álvarez-Presas et al. 2008; Álvarez-Presas and Riutort 2014; Riutort et al. 2012; Sluys et al. 2009).

**Table 1.1** Summary of taxonomic classification for the *Continenticola* Carranza, Littlewood, Clough, Ruiz-Trillo, Baguña and Ruitort, 1998 according to Sluys et al. (2009).

Superfamilies	Families	Subfamilies
Planarioidea Stimpson, 1857	Planariidae Stimpson, 1857 Dendrocoelidae Hallez, 1892 Kenkiidae Hyman, 1937	
Geoplanoidea Stimpson, 1857	Dugesiidae Ball, 1974 Geoplanidae Stimpson, 1857	Bipaliinae Von Graff, 1896 Microplaninae Pantin, 1953 Rhynchodeminae Von Graff, 1896 Geoplaninae Stimpson, 1856

### 1.5.1 Classification of the *Continenticola*

The classification presented below is adapted from Sluys et al. (2009).

Domain **EUKARYOTA** Whittakey & Margulis, 1978

Kingdom **ANIMALIA** Linnaeus, 1758

**BILATERIA** Hatscheck, 1888

**PROTOSTOMIA** Grobбен, 1908

Phylum **PLATYHELMINTHES** Gegenbaur, 1859

Class **RHABDITOPHORA** Ehlers, 1985

Order **TRICLADIDA** Lang, 1884

Suborder **CONTINENTICOLA** Carranza, Littlewood, Clough, Ruiz-Trillo, Baguña and Ruitort, 1998

Superfamily **PLANARIOIDEA** Stimpson, 1857

Family **PLANARIIDAE** Stimpson, 1857

**9 constituent genera**

Family **DENDROCOELIDAE** Hallez, 1892

**22 constituent genera**

Family **KENKIIDAE** Hyman, 1937

**2 constituent genera**

Superfamily **GEOPLANOIDEA** Stimpson, 1857

Family **DUGESIIDAE** Ball, 1974

**11 constituent genera**

Family **GEOPLANIDAE** Stimpson, 1857

Subfamily **BIPALIINAE** Von Graff, 1896

**4 constituent genera**

Subfamily **MICROPLANINAE** Pantin, 1953

**8 constituent genera**

Subfamily RHYNCHODEMINAE Von Graff, 1896

Tribe *Rhynchodemini* Van Graff, 1896

**6 constituent genera**

Tribe *Caenoplanini* Ogren and Kawakatsu, 1991

**16 constituent genera**

Tribe *Anzoplanini* Winsor, 2009

**2 constituent genera**

Tribe *Eudoxiatopoplanini* Winsor, 2009

**1 constituent genus**

Tribe *Pelmatoplanini* Ogren and Kawakatsu, 1991

**2 constituent genera**

Subfamily GEOPLANINAE

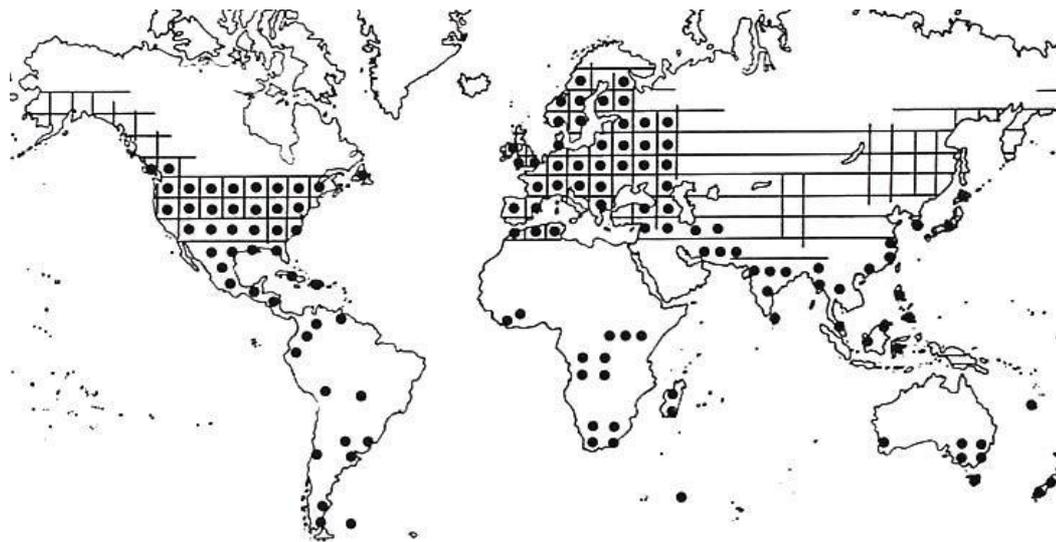
**15 constituent genera**

### 1.5.2 Global Biodiversity and Biogeography of the Continenticola

The Continenticola have never been monographed and their taxonomic status has been in a state of flux for many years. Continenticolans occur on all the major continents excluding the Arctic and Antarctica, but appear to be absent from some oceanic islands (Ball 1981). All continenticolans are sensitive to desiccation. Terrestrial continenticolans live typically in wooded areas or tropical jungles, as they require a humid environment (Kawaguti 1932). Freshwater species are found primarily in aquatic habitats, however some species are capable of surviving in moist soil (Ball & Reynoldson 1981). Freshwater continenticolans can be found in both lentic and lotic environments across a range of ecosystems.

As introduced above, the Continenticola can be divided into two superfamilies: the Planarioidea and the Geoplanoidea. The Planarioidea consists of three families. The largest of these is the Dendrocoelidae, with a total of 22 genera and 256 species. The most species-rich of the Dendrocoelidae genera is *Dendrocoelum*, which accounts for almost half of the species (112) (Sluys et al. 2009). The Planariidae has 12 genera and 156 species. Both families have a Holarctic distribution, meaning that they occur in Asia, Europe, the northern tip of Africa and North America (Ball & Reynoldson 1981)(Figure 1.11). The only known exception is the planariid *Polycelis oculimarginata*, reported from Papua New Guinea (Sluys 1990b). The relatively small cave and ground water dwelling family, the Kenkiidae, contains three genera and seven species with a distribution in central and eastern Asia and North America (Sluys & Kawakatsu 2006).

Within the Geoplanoidea, the Dugesiidae is the only freshwater triclad family represented in the southern hemisphere (Ball 1974a; Ball & Reynoldson 1981) (Figure 1.11). The Dugesiidae, with a relatively cosmopolitan distribution, consists of 11 genera, which will be the focus of this study. The Geoplanidae (terrestrial triclads) have a mainly pan-tropical distribution and consist of four subfamilies, 55 genera and over 800 nominal species (Sluys 1999; Sluys et al. 2009).

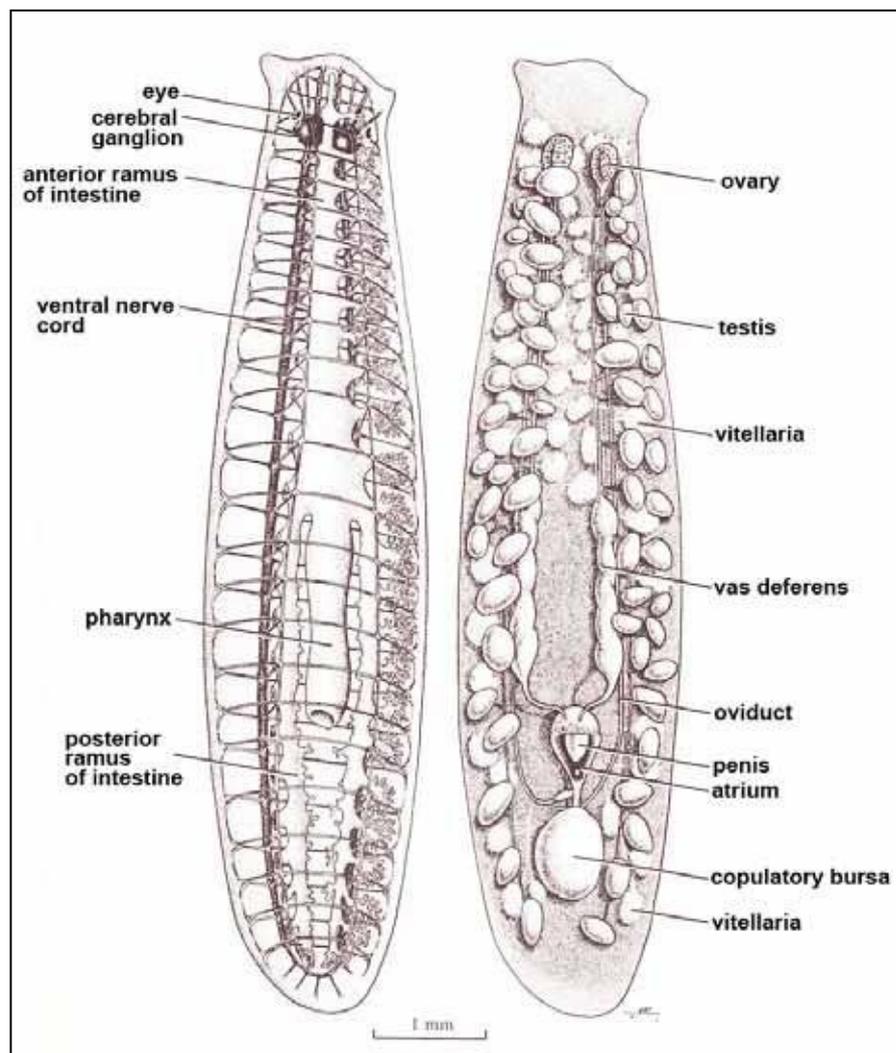


**Figure 1.11** World distribution of the three freshwater Continenticolan families. Dugesiidae, black dots; Planariidae, cross-hatching; Dendrocoelidae, vertical hatching (Ball & Reynoldson 1981).

## 1.6 The study taxon

This project is primarily concerned with freshwater triclads, a cosmopolitan group of obligate freshwater invertebrates with representatives on all continents, excluding Antarctica (Ball & Reynoldson 1981). Platyhelminthes as a whole are characterised by bilateral symmetry and the absence of a coelom: the space between the organ systems is filled with diffuse connective tissue (the mesenchyme); a respiratory system is absent, they use the entire body surface for gas exchange; a circulatory system is also absent, the gut itself functions a gastrovascular system that transports fluids and nutrients; nor are there any skeletal or support structures (Baguña et al. 2001a; Ball and Reynoldson 1981; Ruppert et al. 2004). The alimentary system of the Tricladida consists of a triple-branched intestine that extends throughout the body, and a muscular pharynx (Salo & Baguña 2002)(Figure 1.12). Waste is excreted by protonephridia via nephridiopores opening to both the dorsal and ventral surfaces (Ball and Reynoldson 1981). Sensory structures include eyes, consisting of a pigment cup containing retinal cells, and chemoreceptors,

modified strips of epithelium, often infolded and richly supplied with nerve cells and cilia (sensory pits and fossae) (Ball and Reynoldson 1981). Freshwater triclads possess two prominent ventral nerve cords that thicken at the anterior end of the body to form a bilobed “brain” or cerebral ganglion (Baguñà & Ballester 1978; Nakazawa et al. 2003)(Figure 1.12). Sexual organs of freshwater triclads consist of paired ovaries sitting ventrally behind the brain, and testes, which vary dramatically in size, number and location between species (Figure 1.12). The copulatory apparatus sits posterior to the pharyngeal pocket and consists of both male and female reproductive structures (Figure 1.12). A penial papilla receives the entrance of the vasa deferentia; this papilla sits in an atrium communicating directly or indirectly with the oviducts and a copulatory bursa, responsible for receiving and possibly reabsorbing sperm after copulation (Sluys 1989c)(Figure 1.12). Sperm and cocoons are deposited via a small gonopore opening medially on the ventral surface of the animal (Ball and Reynoldson 1981).



**Figure 1.12** Internal anatomy of *Procerodes littoralis* adapted from Ball and Reynoldson (1981).

Freshwater triclad mobility is based on ciliary and muscular activity, which is expressed as a distinctive gliding movement (Kato et al. 2004). By far the most researched element of freshwater triclad physiology is their remarkable regenerative capacity. When cut into small pieces, each wound surface generates a “blastema” from which a complete new individual can develop (Kobayashi et al. 1999; Salo & Baguña 2002). This type of regeneration has numerous medical implications, assisting in our understanding of basic molecular and cellular processes governing biological function (Sánchez Alvarado 2006). For freshwater triclads this ability has developed to enable flexibility in reproductive strategies, allowing both asexual and sexual reproduction. There are two reproductive modes adopted by freshwater triclads, varying between species within the same genus, and also between populations of the same species. The asexual mode allows reproduction without sexual organs via fission, while the sexual mode requires the production of the hermaphroditic sexual organs with which triclads copulate and produce egg-filled cocoons (Kobayashi & Hoshi 2002).

Australian freshwater triclads usually measure around 10mm in length. However, in some temperate regions, specimens of *Cura pinguis* have been collected measuring well over 20mm (pers. obs.). Very little is known about the life history of Australian freshwater triclads. The only existing ecological study is on effects of temperature on population size and reproductive rate in the Australian Alps (Hay & Ball 1979). This study identified temperature as having a large but varying impact on the survival and reproduction of all species studied (*Spathula tryssa*, *Spathula camara*, *Reynoldsonia reynoldsoni*, *Spathula agelaea*, *Cura pinguis*, *Dugesia sp.*), implying that these species have evolved very different ecological niches (Hay & Ball 1979). The lack of research is surprising as some triclad populations can reach very high densities, thus contributing significantly to the total biomass (pers. obs.). In the northern hemisphere, species are thought to either be perennial, living and reproducing for several years, or annuals, which breed once and then die (Ball and Reynoldson 1981). In the southern hemisphere it is not even clear how species deal with the seasonal drying of many water bodies and common droughts (Hay & Ball 1979).

Freshwater triclads are major predators of freshwater invertebrate organisms, feeding on live or freshly damaged prey items (Adams 1980a, b). They are capable of capturing large active prey, such as Crustacea, but also consume smaller invertebrates, such as nematodes and insect larvae (Weinzierl et al. 1999; Beier et al. 2004). Triclads have fewer predators than their soft-bodied nature suggests. Predators include leeches, dragonfly, damselfly and stonefly nymphs, some small fish species and, interestingly, other

triclad species (Ball and Reynoldson 1981). Freshwater triclads primarily inhabit the undersides of rocks and cobble, or occasionally live within aquatic macrophytes in both lentic and lotic environments. Freshwater triclads are often described as riffle invertebrates, however, they are also very common in shallow runs, off-channel areas and shores (pers. obs.).

Whilst many freshwater invertebrates have efficient active or passive dispersal mechanisms, this is not the case for freshwater triclads (Bilton et al. 2001). Passive dispersal, such as via animal vectors, is considered very rare and in fact all authenticated cases of passive transport can be attributed to modern human actions (De Beauchamp 1940; Reynoldson 1966; Ball 1971). Freshwater triclads have not been recorded in the aerial plankton and they show very little resistance to saline environments or extremes of temperature (Ball 1971). In a study by Brandle et al. (2007), looking at the population structure of the flatworm *Crenobia alpina*, substantial differentiation between populations was found. It was concluded that populations are effectively isolated across rather small spatial scales. This low vagility suggests that the present-day distribution patterns may be reliable guides to historical biogeography.

## 1.7 Project Aims and Thesis Outline

The research outlined in this thesis will provide a continent-level appraisal, for the first time, of the freshwater triclad (Platyhelminthes: Tricladida: Continenticola: Dugesiidae) fauna of Australia, with respect to morphology, taxonomy, molecular systematics and biogeography. This research is deemed important due to the biogeographical and ecological importance, alluded to above, of this poorly known group.

**Chapter 2:** Reports the use of molecular data to produce a phylogeny of the Australian freshwater triclads. Data from the 18S nuclear gene and the *cox1* mitochondrial gene are presented separately and a concatenated data set is also analysed. This chapter also presents a morphological phylogeny of the Australian freshwater triclads. The analyses include all currently known Australian species, including those introduced in this thesis. This chapter will also attempt to provide a “total evidence” (morphological plus molecular markers) hypothesis for the phylogenetic relationships between the genera, investigating the relationships within the newly erected Geoplanoidea. Further, it discusses some taxonomic implications resulting from the phylogenetic analysis.

**Chapter 3:** Summary of taxonomic work performed including a proposed revised classification and a key to the Australian species.

**Chapter 4:** Descriptive biogeography of the Australian freshwater triclads, using the phylogenetic hypothesis developed in the previous chapter to analyse the phylogeography of the worms.

**Chapter 5:** Overview and general conclusions, examining the significance of the work, specifically the biogeographical discussion and also an outline of potential future research arising from this project.

**Appendix 1:** Taxonomic account of all Australian species, including new and revised species, with updated distribution and ecological data (Appendix 1a). This appendix also includes a manuscript that will be co-authored with Dr. R. Sluys to be submitted for publication to a scientific journal (Appendix 1d). In addition, copies of the two taxonomic papers already published from this thesis will be presented (Appendix 1b, Grant et al. 2006; Appendix 1c, Sluys et al. 2007). Finally, detailed taxonomic methods are included (Appendix 1e).

**Appendix 2:** Molecular information to support the phylogenetic conclusions including: additional methods and results (Appendix 2a) and specifics of molecular identification (Appendix 2b).

**Appendix 3:** Morphological information to support the phylogenetic conclusions including specifics of the characters selected for the morphological phylogeny (Appendix 3a), and a detailed character analysis (Appendix 3b).

**Appendix 4:** Summary of Phanerozoic continental movements to support the biogeography discussion.

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## Chapter 2: Dugesiid Phylogeny

### Molecules, Morphology and a Working Hypothesis

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#### 2.1 Introduction

Our current understanding of the phylogeny and taxonomy of the dugesiid genera is discussed in detail to provide a setting for an updated and more inclusive analysis of this group. Phylogenies inferred from portions of the nuclear 18S ribosomal RNA gene and the mitochondrial *cox1* gene are presented. A phylogeny for the Australian freshwater triclad fauna, based on morphology, is also presented. The morphological analyses include all currently known species from Australia, including those introduced in this thesis (Appendix 1d). Finally, this chapter provides a “total evidence” (morphological plus molecular markers) hypothesis, considering data from a character analysis, which will then be discussed with reference to previous research.

##### 2.1.1 The Geoplanoidea Stimpson, 1857

The “new classification of triclad flatworms” proposed by Sluys et al. (2009) summarises the recent molecular and morphological advances in our understanding of this group. As a consequence, Dugesiidae is no longer positioned within the Planarioidea but housed within the Geoplanoidea Stimpson, 1857 with the terrestrial triclads (Section 1.5). The term Geoplanoidea, or variations thereof, dates back to the 1800s (Stimpson 1857) where it was used to describe a group, similar to the one it now represents (including the dugesiids), based on morphological apomorphies (Sluys et al. 2009).

Molecular studies on the Geoplanoidea by Álvarez-Presas et al. (2008) and Álvarez-Presas and Riutort (2014) concluded that Dugesiidae (freshwater triclads) is a paraphyletic taxon, and that the Geoplanidae genera cluster with select dugesiid genera, suggesting possible multiple independent transitions between freshwater and terrestrial habitats (Álvarez-Presas et al. 2008)(Figure 2.1 and 2.2). Álvarez-Presas et al. (2008) indicated that the Dugesiidae and Geoplanidae can be divided into two main monophyletic groups: all dugesiids excluding the genera *Romankenkius* and *Spathula* (Dugesiidae), and all Geoplanidae plus *Romankenkius* and *Spathula* (Geoplanidae - referred to as Terricola by Álvarez-Presas et al. 2008)(Figure 2.1). It follows from this that:

- the terrestrial triclads did not evolve from marine triclads, but from a freshwater triclad, a much simpler (in a physiological sense) transition (Álvarez-Presas et al. 2008; Ball 1981, Sluys 1989b),

- the transition from freshwater to land occurred only once, involving an ancestor common to both,
- the presence of *Spathula* and *Romankenkius* within the Geoplanidae can most parsimoniously be explained by a return to freshwater by these two genera (Álvarez-Presas et al. 2008).

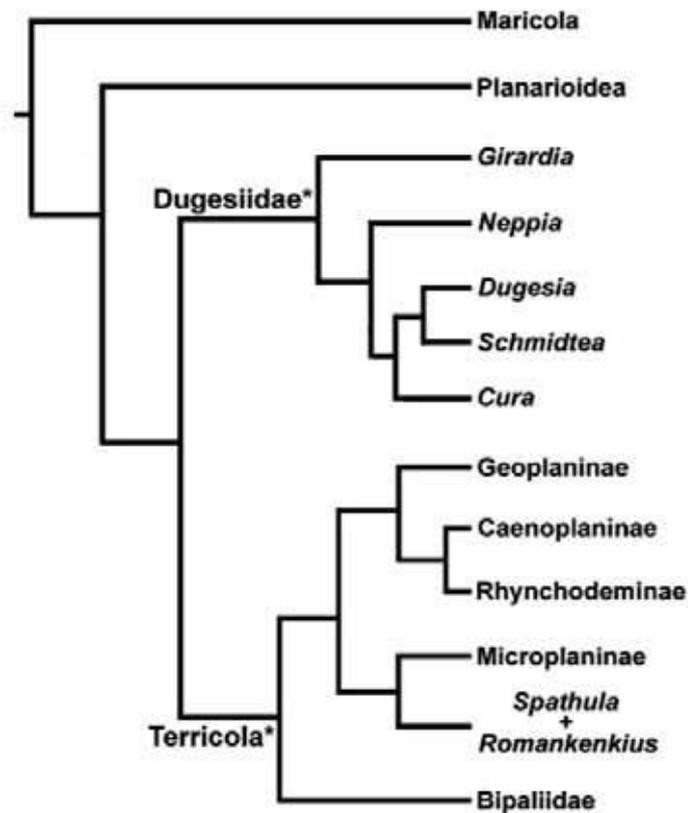
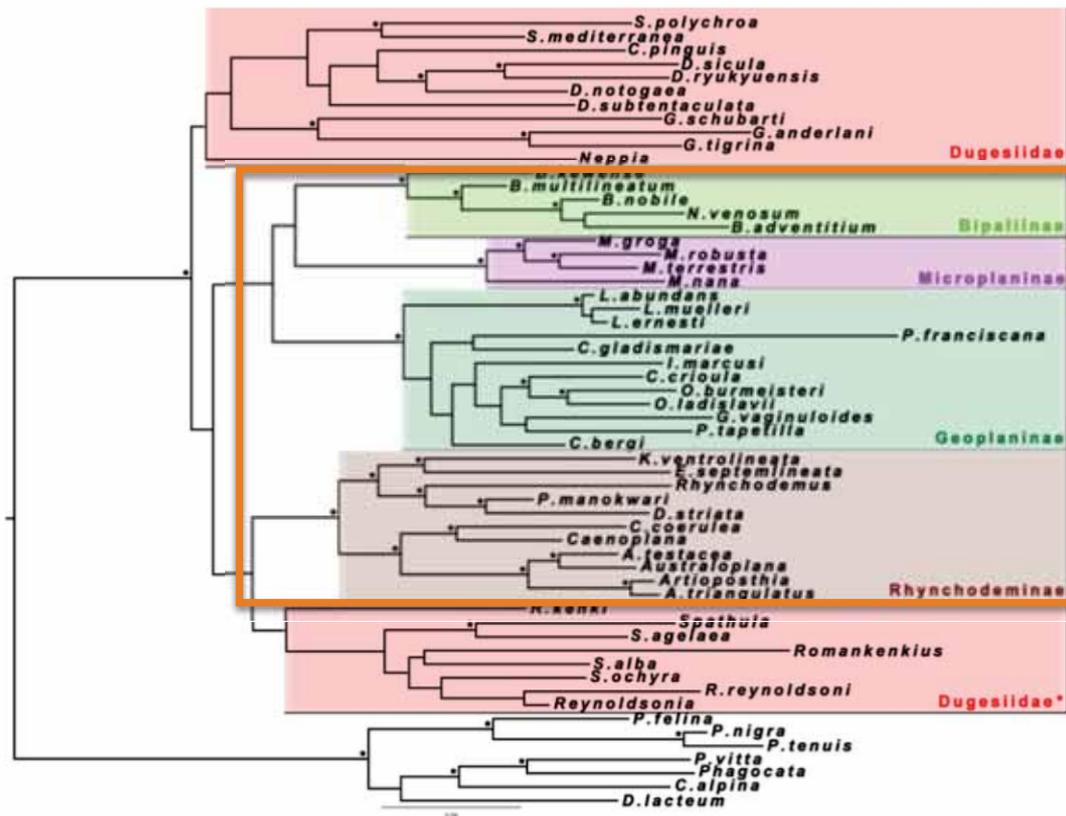


Figure 2.1 A phylogenetic hypothesis for the Geoplanoidea (Álvarez-Presas et al. 2008).

However, there is some cause to question the idea of a return to freshwater. Álvarez-Presas et al. (2008) suggested that *Romankenkius* and *Spathula* shared their most recent common ancestor with the Microplaninae, a relationship that necessitated a return to freshwater (Figure 2.1). In the more recent analysis by Álvarez-Presas and Riutort (2014) the relationships have altered, with *Romankenkius*, *Spathula* and *Reynoldsonia* instead sharing their most recent common ancestor with the Rhynchodeminae (a terrestrial triclad taxon). This phylogeny does not necessitate a return to freshwater; instead both freshwater and terrestrial genera could arise from a freshwater ancestor (Figure 2.2).

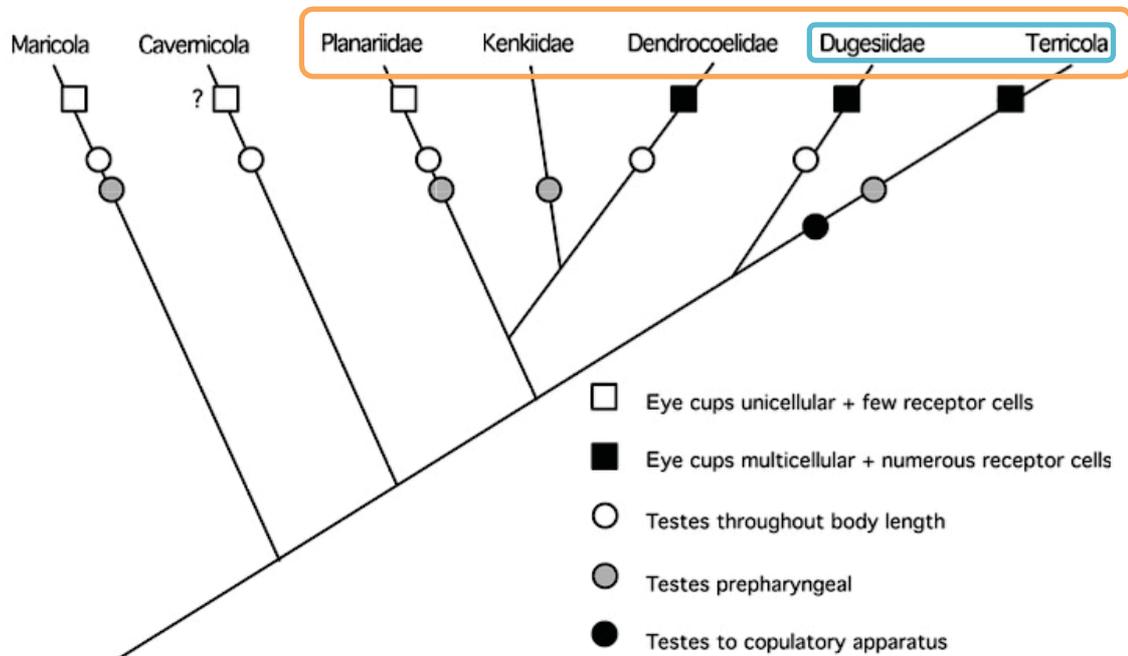


Riutort (2014)(orange box indicates members of the Geoplanidae).

Morphological evidence for the relationships suggested by the molecular phylogenies is difficult to establish. Eye structure, specifically multicellular eyecups with numerous receptor cells, has traditionally been accepted as a synapomorphy for the Geoplanoidea (Baguña et al. 2001a; Ball 1981; Sluys 1989b; Sluys 2001). However, Sluys and Kawatasu (2006) pointed out that this character is also found in many dendrocoelid genera and a few of the Planariidae. While it is not inconceivable that this character arose independently in the Dendrocoelidae, it is more likely that it represents a synapomorphy for the group comprising of the Planariidae, Kenkiidae, Dendrocoeliidae, Dugesiidae and Geoplanidae (Sluys et al. 2009)(Figure 2.3). Falleni et al. (2006) presented a possible synapomorphy for the Geoplanoidea with their investigation of yolk and eggshell globules. They demonstrated that Dugesiidae and Geoplanidae exhibit a type of globule that is not present in other freshwater triclads or marine triclads, however, this needs further investigation (Falleni et al. 2006).

because of the lack of morphological synapomorphies for these groups proposed by Álvarez-Presas et al. (2008) and Álvarez-Presas and Riutort (2014), Sluys et al. (2009) preserved the genera *Romankenkius* and *Spathula* within the Dugesiidae (Sluys et al.

2009). Finally, it is important to note that while the freshwater and terrestrial families are housed within the suborder Continenticola, and more recent developments have not undermined this decision, Sluys et al. (2009) commented that it is even difficult to find unequivocal morphological apomorphies for the Continenticola.



**Figure 2.3** Phylogenetic character state tree of the major taxa in the Tricladida, summarizing the taxonomic distribution of character states concerning the eyes and testes. Continenticola highlighted in orange box and Geoplanoidea highlighted in blue box. Image adapted from Sluys & Kawakatsu (2006).

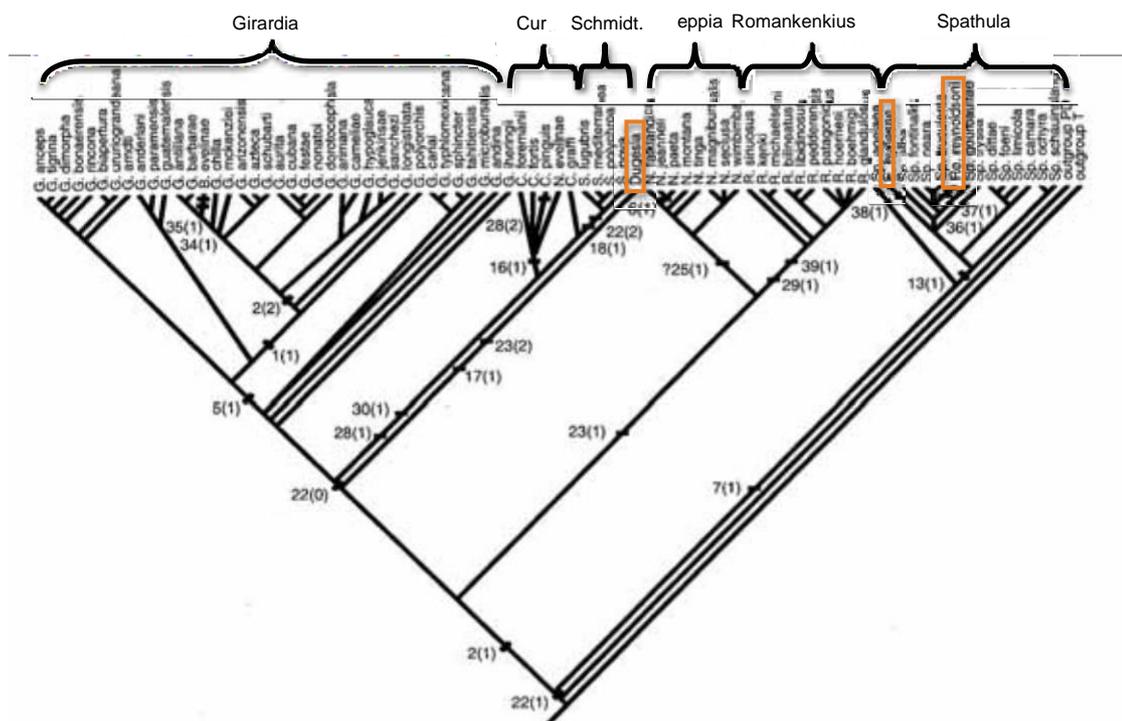
### 2.1.2 The Dugesiidae Ball 1974

Ball (1974a) proposed that ancestral Dugesiidae had been pigmented, with two eyes and with a rounded or spatulate anterior end. He suggested a phylogenetic scheme, which contained only three genera, *Dugesia*, *Rhodax* and *Bopsula*, with six subgenera within *Dugesia* (*Dugesia* Girard, 1851, *Girardia* Ball, 1974, *Cura* Strand, 1942, *Neppia* Ball, 1974, *Spathula* Nurse, 1950 and *Schmidtea* Ball, 1974). These sub-genera were each later elevated to the rank of genus (Ball 1974a; De Vries & Sluys 1991). In Ball's (1974c) review of *Cura* and *Neppia* he described a new genus, *Romankenkius* Ball 1974, to house two Australian species; this genus would later have several existing species reassigned to it (Sluys 1997). A further three monotypic genera have been erected within the Dugesiidae to house problematic species from Australia: *Eviella* Ball, 1974, *Reynoldsonia* Ball, 1977 and *Weissius* Sluys et al., 2007 (Ball 1974b, 1977b; Sluys et al. 2007). Most recently the genus *Recurva* has been described to house two new species from the Mediterranean

(Sluys et al. 2013). It should be noted that despite the monophyly of the Dugesiidae having recently been challenged (e.g. Álvarez-Presas et al. 2008; Álvarez-Presas & Riutort 2014), there have been no formal changes to its status as a family (Sluys et al. 2009).

### 2.1.2.1 Morphological Understandings

Attempts to create a robust phylogeny of the dugesiid genera, based on morphological characters (e.g. De Vries & Sluys 1991; Sluys 1997; Sluys 2001), have been hampered due to the difficulties encountered in developing a useful character set (i.e. very few reliable characters and high levels of plasticity). Regardless, these attempts have been very useful, both in the discussion of the relationships between the genera and also in the resolution of species affinities. By far the most comprehensive morphological phylogeny attempted for the Dugesiidae was by Sluys (2001), which included 39 characters (Figure 2.4). Apomorphic characters for the taxon are often difficult to identify, however a summary of the taxonomic status for each of the dugesiid genera can be found in Table 2.1.



**Figure 2.4.** Relationships of the dugesiid genera based on morphological characters with the monotypic Australian genera and the genus *Dugesia* highlighted (rectangles refer to characters and character states as per Sluys 2001), image adapted from Sluys (2001) (*G.* = *Girardia*, *B.* = *Bopsula*, *C.* = *Cura*, *N.* = *Neppia*, *R.* = *Romankenkius*, *E.* = *Eviella*, *Sp.* = *Spathula*, *Re.* = *Reynoldsonia*).

Finding synapomorphies for proposed closely related genera is possible, but again difficulties arise. For example, a synapomorphy, non-reversed musculature on the bursal canal (character 22, state 0 - Figure 2.4), exists for the cluster *Girardia*, *Cura* and

*Schmidtea*. However, this character is not present in *Dugesia*, hence the close relationship between *Dugesia*, *Cura* and *Schmidtea* is based on a combination of other characters (Sluys 2001). De Vries and Sluys (1991) proposed that ectal reinforcement on the bursal canal (character 23, state 1 or 2) represents a synapomorphy for a group including *Dugesia* and *Neppia*. However, in Sluys' (2001) analysis, *Neppia* was represented as sharing a more recent common ancestor with *Romankenkius* than with *Dugesia*. Interestingly, the synapomorphy proposed for the *Romankenkius* and *Neppia* cluster was also ectal reinforcement of the bursal canal, which is found in some, but not all, representatives of *Romankenkius* (Sluys 2001). This analysis also suggested a very close relationship between *Spathula*, *Reynoldsonia* and *Eviella* based primarily on the synapomorphy "caudally branched oviducts" (character 13, state 1). Sluys (2001) therefore suggested that the monotypic genera, *Reynoldsonia* and *Eviella*, may be atypical representatives of *Spathula*.

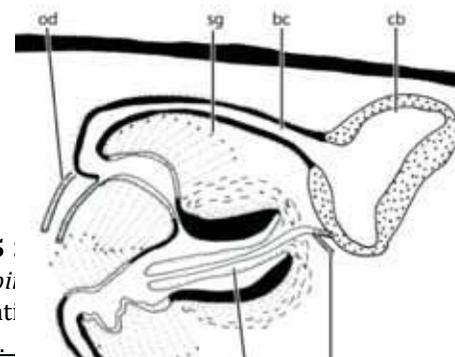
**Table 2.1** Summary of taxonomic status of the Dugesiidae genera, including understandings from Sluys (2009) and any material published as part of this study at the time of submission (i.e. Grant et al. 2006, Sluys et al. 2007)\*

***Cura* Strand, 1942**

**Distribution:** North America, Europe and Australasia.

**Species number:** 3

Many features characterising *Cura* are plesiomorphic, including the arrangement of the testes, bursal canal musculature and rounded head (De Vries & Sluys 1991; Sluys 2001). *Cura* is sometimes considered to be a primitive freshwater triclad and the oldest of the dugesiids due to the unusual, anterior bursal canal communication with the atrium (reminiscent of marine triclads) (Figure 2.5). However, the monophyly of the genus has always been in doubt (Álvarez-Presas et al. 2008; Ball 1977c; Steinbock 1924; Weiss 1910): *Cura pinguis* shares derived features, such as



**Figure 2.5** Saggital section of the anterior region of *Cura pinguis* demonstrating the communication with the atrium.

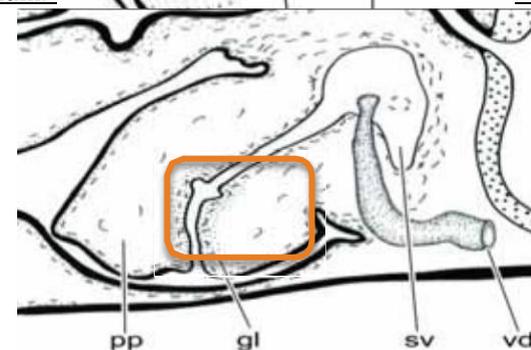
the anterior sensory organs with all other endemic Australian species (Ball 1977c).

***Dugesia* Girard, 1851**

**Distribution:** Distributed throughout Africa, Europe, the Middle East, the Oriental Region, the Far East, and the Australasian region.

**Species number:** 80

Sluys (2001) included *Dugesia* in his analysis as a single operational taxonomic unit sharing a well-defined synapomorphy: a diaphragm in the ejaculatory duct (Figure 2.6). Although this feature occurs in some species that are not assigned to *Dugesia* (e.g. *Romankenkius libidinosus*), in these cases the position of the diaphragm indicates that it is not homologous with that of *Dugesia* species (Sluys 1997).



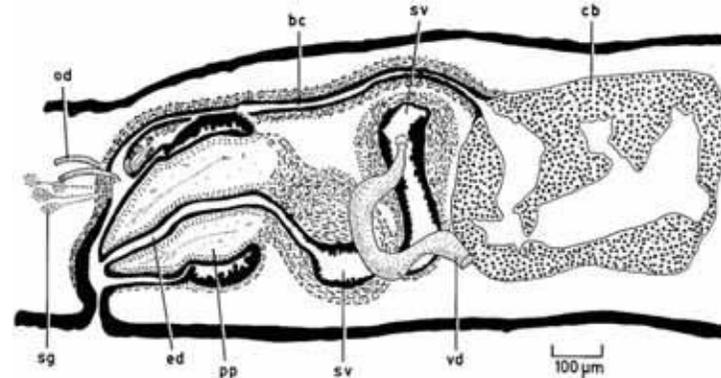
**Figure 2.6** Saggital section of the penial papilla of *Dugesia orientoaustralis* with the autapomorphy for the genus *Dugesia*, the diaphragm, highlighted.

***Schmidtea* Ball, 1974**

**Distribution:** Asia, Europe and North Africa.

**Species number:** 3

*Schmidtea* has two distinctive apomorphies; a double seminal vesicle (Figure 2.7), and intermingled musculature on the bursal canal (Sluys 1997).



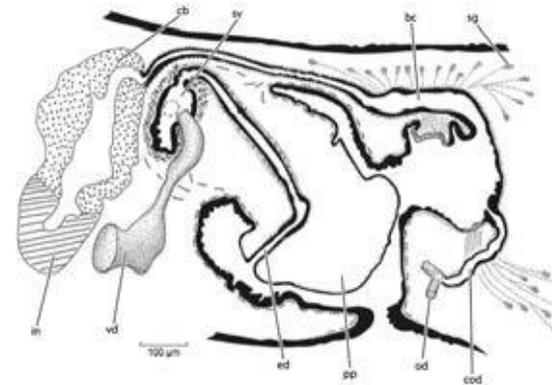
**Figure 2.7** Sagittal reconstruction of the copulatory apparatus of *Schmidtea polychroa* displaying the double seminal vesicle (Harrath et al. 2004).

***Recurva* Sluys, 2013**

**Distribution:** Europe

**Species number:** 2

*Recurva* is recently described and shares several features with *Schmidtea* and *Cura* including a muscular ejaculatory duct and a common oviduct, respectively (Sluys et al. 2013) (Figure 2.8). Unfortunately, there is no apomorphy for this genus and it has been defined on the basis of a unique combination of characters (Sluys et al. 2013).



**Figure 2.8** Sagittal reconstruction of the copulatory apparatus of *Recurva conjuncta* exhibiting a muscular ejaculatory duct and a common oviduct. Image from Sluys et al. (2013).

***Bopsula* Marcus, 1946 and *Rhodax* Marcus, 1946**

***Bopsula***

**Distribution: South America**

**Species number: 1**

While *Bopsula* and *Rhodax* were initially assigned to the Dugesiidae, *Rhodax* is now located within the Cavernicola (Sluys 1990a), while the aberrant characteristics of *Bopsula* should be re-assessed with new material. The genus *Bopsula* is monotypic, with the type species, *Bopsula evelinae*, known only from Sao Paulo, Brazil. Sluys (2001) has re-examined the two known specimens and suggests that they may represent an aberrant *Girardia*. *Bopsula*, which has previously been excluded from phylogenetic analyses, has also been excluded from this study due to the unnecessary complexity (not an Australian genus) this genus adds to any phylogenetic analysis.

***Rhodax***

**Distribution: South America**

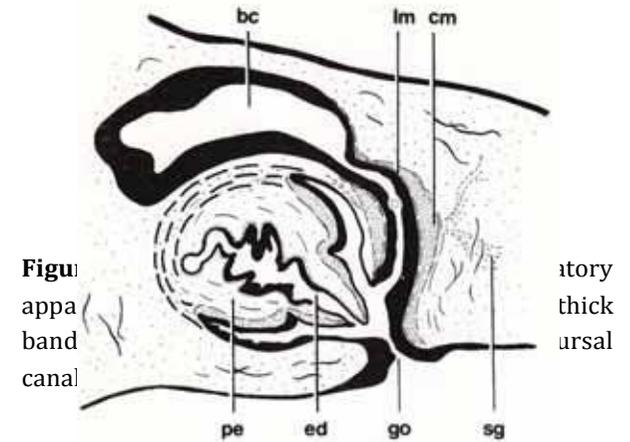
**Species number: 1**

***Neppia* Ball, 1974**

**Distribution: Africa, Australasia and South America.**

**Species number: 8**

De Vries and Sluys (1991) failed to identify any autapomorphies for the genus. The thick circular muscle surrounding the bursal canal and convoluted ejaculatory duct, which were thought to be possible diagnostic characters are absent in *N. schubarti* and *N. evelinae*. However, it is possible that with more information these autapomorphies may hold, as *N. schubarti* may be better aligned to *Girardia* (pigmented pharynx) and *N. evelinae* has been assigned to *Neppia* by a process of elimination, not due to an abundance of shared characters (Sluys 1997, 2001)(Figure 2.9 and 2.8).



**Figure 2.8** Anatomical diagram of a flatworm showing internal structures. Labels include: bc (bursal canal), lm (lateral muscle), cm (convoluted muscle), pe (pharynx), ed (ejaculatory duct), go (gonad), and sg (seminal gland). The diagram shows a cross-section of the body wall and internal organs.

### ***Girardia* Ball, 1974**

**Native distribution:** Carribean, North America and South America - **Invasive distribution:** Asia, Australia and Europe.

**Species number:** 44

*Girardia* is likely to represent a monophyletic taxon. *Girardia* is defined by a unique combination of diagnostic characters; however, an abundance of ill-defined and poorly known species makes its status unclear (Sluys 1997; Sluys 2001).

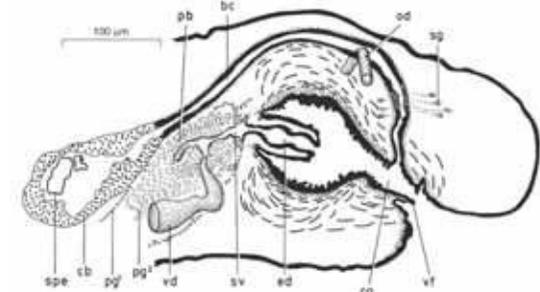
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### ***Weissius* Sluys, 2007**

**Distribution:** Australia

**Species number:** 1

The genus *Weissius* was erected to house a new species, *Weissius capaciductus* (Sluys et al. 2007). It has been placed in the family Dugesiidae, despite the absence of eyes (and hence the diagnostic multicellular pigmented eye-cups). *Weissius* is placed within the Dugesiidae by default as it lacks a common oviduct opening into the atrium (Planariidae) and does not possess a dendrocoelid type of pharynx (Sluys et al. 2007). There are a few characters suggesting a close relationship between *Weissius* and *Cura*, including a finger-shaped penial papilla and a simple circular muscle layer surrounding the bursal canal (Figure 2.5 and 2.10), placing this genus somewhere within the *Schmidtea*, *Dugesia* and *Cura* clade (Sluys et al. 2007). There is currently no molecular data available for this genus.



**Figure 2.10** Sagittal reconstruction of the copulatory apparatus of *Weissius capaciductus*, demonstrating the finger shaped penial papilla. Image taken from Sluys et al. (2007)

***Romankenkius* Ball, 1974 and *Spathula* Nurse, 1950**

***Romankenkius***

***Spathula***

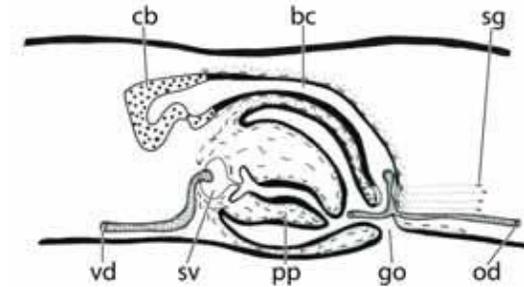
**Distribution: Australasia and South America**

**Distribution: Australasia**

**Species number: 12**

**Species Number: 16**

None of the features used to diagnose *Spathula* or *Romankenkius* are exclusive to the genus (Sluys 2001). The clade housing the genera *Romankenkius*, *Spathula*, *Eviella* and *Reynoldsonia* was defined by caudally branched oviducts, however, many *Romankenkius* lack this feature (e.g. *R. bilineatus* and *R. pedderensis*) (Figure 2.11) (De Vries & Sluys 1991; Sluys 1997).



**Figure 2.11** Sagittal reconstruction of the copulatory apparatus of *Spathula tryssa* demonstrating the caudal branch of the oviducts.

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***Reynoldsonia* Ball, 1974 and *Eviella* Ball, 1977**

***Reynoldsonia***

***Eviella***

**Distribution: Australia**

**Distribution: Australia**

**Species number: 1**

**Species number: 1**

It could be argued that the monotypic genera, *Reynoldsonia* and *Eviella*, are aberrant *Spathula* (Sluys 2001). In the initial description, Ball (1974b) commented on the similarity of *Reynoldsonia reynoldsoni* to members of the genus *Spathula*. However, he concluded that although the species is likely to have evolved from spathuloid ancestors, it had too many apomorphic characters to be placed in this genus (Ball 1974b). Sluys' (2001) morphological phylogenetic analysis places *R. reynoldsoni* and *Eviella hynesae* amongst the *Spathula*, bringing more weight to the theory that these genera are merely abnormal members of the genus *Spathula* (Figure 2.8).

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\*Abbreviations: bc, bursal canal; ca, common atrium; cm, circular muscle; cb, copulatory bursa; di, diaphragm; ed, ejaculatory duct; gl, glands; go, gonopore; lm, longitudinal muscle; od, oviducts; pb, penis bulb; pg, penis glands; pp, penial papilla; sg, shell glands; spe, spermatophore; sv, seminal vesicle; vd, vasa deferentia; vf, valve-like fold.

### 2.1.2.2 Molecular Understandings

#### *Dugesia, Cura, Schmidtea, Neppia and Girardia*

While the molecular data in previous publications has consistently grouped these genera together (Álvarez-Presas et al. 2008; Álvarez-Presas and Riutort 2014; Baguña et al. 2001a; Carranza et al. 1998a; Riutort et al. 1992), the exact nature of their relationships remains to be resolved convincingly. The most recent hypothesis is that of Álvarez-Presas and Riutort (2014)(Figure 2.2).

#### *Romankenkius, Spathula and Reynoldsonia*

Molecular analysis has transformed ideas concerning *Spathula*, *Romankenkius* and *Reynoldsonia*, suggesting that the Dugesiidae is paraphyletic with this cluster grouping with the Geoplanidae (Álvarez-Presas et al. 2008; Álvarez-Presas and Riutort 2014; Baguña et al. 2001a, Carranza et al. 1998a).

Clearly there is some conflict between the morphological and molecular phylogenies. For example, the molecular affinities of *Romankenkius* suggest a close relationship with *Spathula*, whilst the morphological data suggest that *Romankenkius* and *Neppia* share a more recent common ancestor (Figure 2.4). If *Neppia* is indeed a sister group to *Romankenkius* this would place *Neppia* amongst the “Geoplanidae” clade as described by Álvarez-Presas et al. (2008) and Álvarez-Presas and Riutort (2014). Despite these differences, both molecular and morphological analyses have valuable aspects that must be considered when attempting to arrive at a phylogenetic hypothesis for this group. There are currently no molecular data for *Bopsula* and *Eviella*. Additionally, *Recurva* was described after the analysis for this thesis was complete. As a consequence it will not feature heavily in discussions. Likewise, *Bopsula* and *Rhodax*, both monotypic genera from South America, are not considered relevant for many of the following analyses and discussions.

### 2.1.3 The Geoplanidae

At the outset of this project the research focus was intended to be the Australian Dugesiidae (i.e. freshwater triclads). However, it soon became clear that the Geoplanidae (terrestrial triclads) played a pivotal role in the story. Consequently, there was a need to include representatives of the Geoplanidae throughout the analysis in order to present a complete picture of the dugesiid phylogeny. Currently, the Geoplanidae (the terrestrial triclads) consists of four subfamilies (Bipaliinae, Microplaninae, Rhynchodeminae,

Geoplaninae) including over 800 described species, most occurring in the southern hemisphere (Riutort et al. 2012). The relationships between the subfamilies have only been examined in a haphazard fashion, with Winsor et al. (1998) and Sluys (1989b) presenting some preliminary ideas based on morphology. Meanwhile, just two molecular studies including the Geoplanidae have been conducted (Álvarez-Presas et al. 2008; Álvarez-Presas and Riutort 2014)(Figure 2.2).

#### 2.1.4 Aims

The aims of this chapter are to present evidence from both molecular and morphological analyses and subsequently achieve a robust phylogenetic hypothesis for dugesiid genera.

## 2.2 Methods

### 2.2.1 Collection and Preservation

Refer to Chapter 3 for collection methods. Live specimens were placed in a Petri dish, most of the water was removed and the dish flooded with 100% high-grade ethanol. The animals were then transferred into a vial containing 100% high-grade ethanol. Preserved specimens were kept in a cold-room at 4°C prior to the deoxyribonucleic acid (DNA) being extracted.

### 2.2.2 DNA Extraction

DNA was extracted from the tissues of whole specimens using an ammonium acetate protocol with minor alterations (Sambrook & Russell 2001). Details of the protocol are in Appendix 2a.

### 2.2.3 Choice of Markers for Phylogenetic Analysis

There are indisputable advantages of molecular evidence: the genome provides a unifying framework for estimating phylogeny as DNA sequences can be compared among all organisms; DNA provides a record of evolutionary history that is independent of many other sources of historical information, such as the fossil record; DNA sequence data is ideally suited to statistical analysis (Bromham 2008). The assumed antiquity of dugesiid taxa requires that a highly conserved gene be chosen, consequently the 18S region of the ribosomal RNA (rRNA) was selected. This gene is sufficiently conserved so as to give a good picture of the relationships among all genera and families (Carranza et al. 1996; Littlewood et al. 2000). The 18S gene has the additional benefit of being used commonly at

all levels of platyhelminth phylogenetics and will therefore allow for comparisons with other taxa (Álvarez-Presas et al. 2008; Álvarez-Presas and Riutort 2014; Baguña et al., 2001a, Baguña and Riutort 2004a, b; Batistoni et al. 1998, 2001; Blair et al. 1998; Campos et al. 1998; Carranza et al. 1996, 1998a, 1998b, 1999; Littlewood et al. 1998, 1999a, 2000; Riutort et al. 1993, 2012; Rohde et al. 1993, 1995).

Despite its importance in taxonomy, the copulatory apparatus is often only present for the part of the year when individuals are sexually reproducing. However, sequences from asexual specimens can still permit their placement within a known species. For this purpose and to detect finer scale genetic discrimination (between/within species), a portion of the cytochrome c oxidase subunit I (*cox1*) mitochondrial gene was sequenced (Álvarez-Presas et al. 2008; Bessho et al., 1992a, b, 1997; Brandle et al. 2007; Littlewood et al. 1998; Rohde 1990; Ruiz-Trillo et al. 2004; Telford et al. 2000).

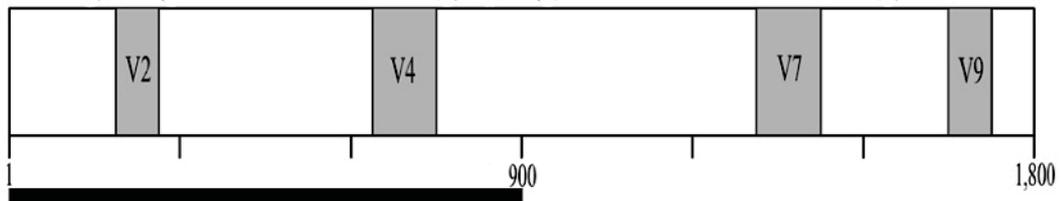
The second class of data used in this analysis was morphological. The value of morphological data has been called into question in recent years (Hall 2007). Any morphological phylogeny for triclads is particularly difficult to justify due a lack of useful characters. Despite these difficulties the relevance of this more traditional data cannot be easily discounted. The rationale and methods behind the use of morphological data will be discussed in detail in Section 2.2.8.

## 2.2.4 Amplification of Target Genes

The 18S region of the ribosomal RNA is, in most phyla including Platyhelminthes, approximately 1,800 bp in length and must be amplified and sequenced in at least two sections (Baguña & Riutort 2004b; Carranza et al. 1996, 1998a; Giribet & Wheeler 2001; Littlewood et al. 1999b). Due to resource restrictions the prospect of sequencing a single shorter section of the gene was examined and found to be a suitable compromise. The first 900 bp will display suitable levels of conservation and variation in order to allow alignment and differentiation (Giribet & Wheeler 2001)(Figure 2.12). This segment was amplified via polymerase chain reaction (PCR) using primers and conditions as described in Littlewood and Smith (1995)(Table 2.2).

Two types (I and II) of 18S rDNA occur in the genome of *Schmitdea mediterranea* Benazzi et al. 1975 (Carranza et al. 1996). While the presence and nature of Type I and Type II rDNA within the Australian dugesiids was unknown, there was a distinct possibility of co-amplifying both types. Both Type I and Type II can be used in phylogenetic analysis as they evolve independently. However, they cannot be used interchangeably (Álvarez-Presas et al. 2008). In order to differentiate between the two

types, cloning, an expensive and time-consuming process, is necessary prior to sequencing (Álvarez-Presas et al. 2008; Carranza et al. 1996). Consequently, differentiation was performed post-sequencing (details in Section 2.2.7). In rare cases both types were sequenced. Fortunately, these cases were easily identifiable due to the double peaks in the chromatograms, making the sequences unusable. Type II evolves 2.3 times faster than Type I, making the latter a much less powerful marker. Fortunately, Type II tends to be more easily amplified and sequenced (Álvarez-Presas et al. 2008; Carranza et al. 1999).



**Figure 2.12** Schematic representation of the 18S rRNA locus adapted from Giribet and Wheeler (2001). The grey squares represent hypervariable regions; the V4 and V7 regions are common in Platyhelminthes. The black bar represents the section of the gene sequenced in this study.

**Table 2.2** 18S and *cox1* primer details

Gene	Primer Name	Bases	Source
<i>cox1</i>	pr-a2 (forward)	AGC TGC AGT TTT GGT TTT TTG GAC ATC CTG AGG T	(Bessho et al. 1992a)
<i>cox1</i>	pr-b2 (reverse)	ATG AGC AAC AAC ATA ATA AGT ATC ATG	(Bessho et al. 1992a)
18S	1F (forward)	TAC CTG GTT GAT CCT GCC AGT AG	(Littlewood and Smith 1995)
18S	5R (reverse)	CTT GGC AAA TGC TTT CGC	(Littlewood and Smith 1995)

A fragment of approximately 300 nucleotides close to the centre of the *cox1* gene was amplified using primers and PCR conditions as described in Bessho et al. (1992a) (Table 2.2). All PCRs were carried out in a DNA Engine TETRAD 2 Peltier Thermal Cycler (Bio-Rad). PCR reactions were catalysed using BIOTAQ™ DNA Polymerase in association with 10x NH<sub>4</sub> Buffer, 50 mM MgCl<sub>2</sub> solution, 100mM dNTP Mix and double-distilled H<sub>2</sub>O, with a total reaction volume of 50 µL (BIOLINE).

## 2.2.5 Quantification, Clean Up and Sequencing

### 2.2.5.1 *Cox1*

PCR products were separated in TBE agarose gels to confirm amplification and ensure adequate PCR products were present. All successful reactions were purified using the

commercially available MinElute 96 UF PCR Purification Kit and protocol (QIAGEN). All PCR products were individually quantified using a ND-1000 spectrophotometer (Nanodrop) to ensure that a volume of 10µl with a concentration of 50ng/µl was available for the sequencing reaction. Sequencing was conducted under BigDye™ Terminator cycling conditions run using Automatic Sequencer 3730xl (Macrogen)(details in Appendix 2a).

### 2.2.5.2 18S

PCR products were run out on a TAE gel from which the DNA band was excised. The DNA was isolated from the gel using UltraClean™ GelSpin™ DNA purification Kits and protocol (MO BIO). Product quantification and sequencing were performed as described for *cox1*.

## 2.2.6 Sequence Alignment

A total of 330 *cox1* sequences were successfully obtained. This included large numbers from a few species (*Cura pinguis*, *Girardia tigrina*, *Dugesia orientoaustralis* Grant and Sluys, sp. nov. and *Dugesia* sp.). Some of the less common genera proved more difficult to amplify, and unfortunately due to time and financial constraints, successful sequencing of all taxa was not possible. Sequence chromatograms were reviewed and edited using Sequencher 4.5 (Genes Codes Corporation, Ann Arbor, Michigan, USA). Sequences were then automatically aligned using ClustalW (Thompson et al. 1994) and the alignment finalised manually using BioEdit (Hall 1999). *Cox1* DNA sequences were aligned using inferred amino acid sequences (flatworm mitochondrial code; Telford 2000) as a guide. The *cox1* minimum evolution tree, inferred using MEGA4 (Tamura et al. 2007), assisted with the identification of ambiguous (i.e. underdeveloped) and asexual specimens (Appendix 2b). This tree was then used to identify 92 candidate specimens for which sequencing of the 18S region was done.

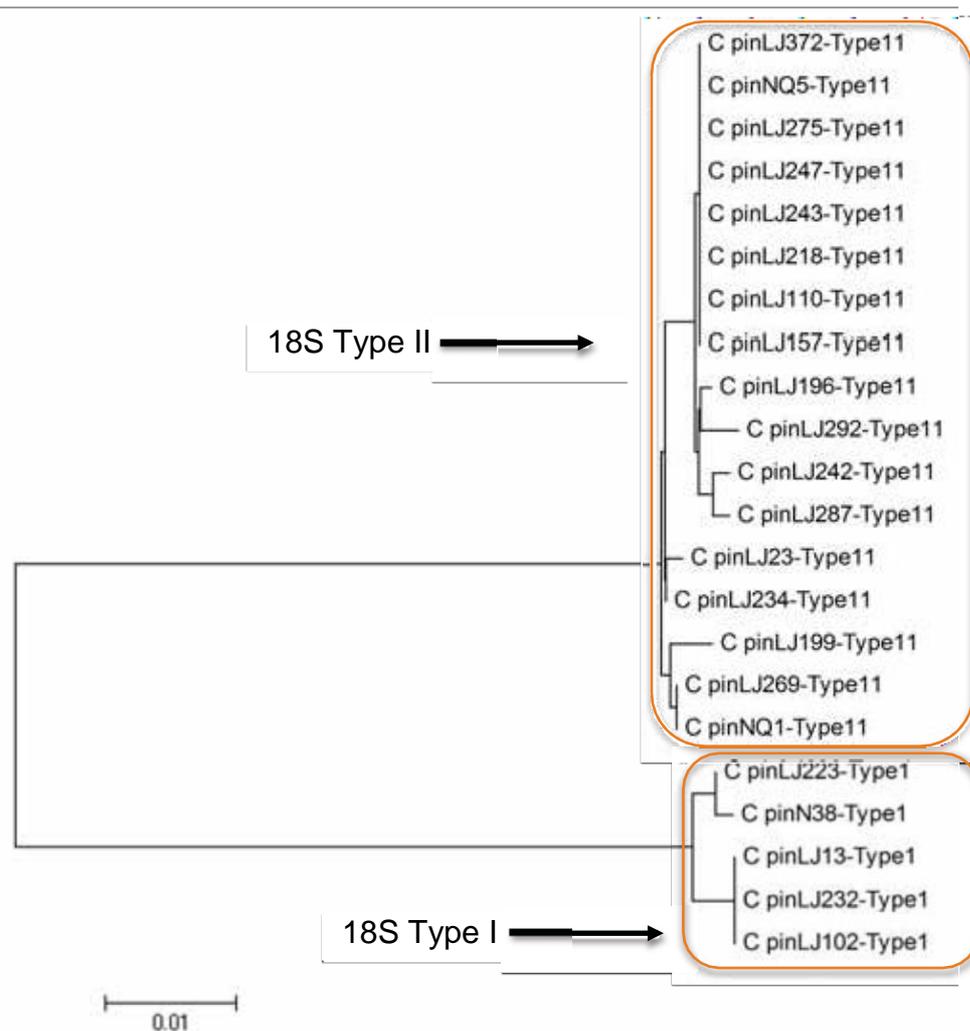
The alignment of the 18S region was done using ClustalW and BioEdit, as above. For this region, secondary structure features were used to aid alignment in hypervariable regions. Selected sequences were manually converted to DCSE format in EditPad Lite (Goyvaerts 2008) and secondary structure viewed in RnaViz (De Rijk & De Wachter 1997). In all cases, those positions that could not be unambiguously aligned were excluded from the analysis. Sequences will be deposited in GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) in due course.

## 2.2.7 Pre-tree Building Analysis

Beyond the quality of the raw sequences, there are several potential issues that need to be addressed when attempting to construct a phylogeny from molecular data. These include: the correct identification of homology in the sequence alignment (see Section 2.2.6); whether substitution rates vary substantially between lineages (relative rate test); long-branch attraction (relative rate test); and the loss of phylogenetic information due to substitution saturation (saturation test) (Xia et al. 2003).

### 2.2.7.1 Sequence Type (I or II for 18S) Test

To test whether the 18S sequences were part of the type I or type II ribosomal cluster, a minimum evolution tree was inferred in MEGA4 (Tamura et al. 2007) and type determined by reference to pre-existing sequences available in GenBank (<http://www.ncbi.nlm.nih.gov/>) (Figure 2.13). Both types were sequenced within five genera (*Cura*, *Dugesia*, *Girardia*, *Romankenkius* and *Spathula*). However, type II is much more easily amplified within dugesiid genera and will therefore be used for the majority of the phylogenetic analyses (Table 2.3 and 2.4).



**Figure 2.13** Differentiation of 18S gene types for *Cura pinguis* via a minimum evolution tree inferred in MEGA4 (C pin - *Cura pinguis*, letters and numbers refer to laboratory codes).

Majority-rule consensus sequences were constructed for each species for which multiple sequences existed. Very little variation occurred within each species (as initially defined by morphology), making it possible to confidently create consensus sequences.

**Table 2.3** Summary list of dugesiid samples used in this study, with sampling locality and GenBank number where appropriate (✓ designates sequences available, <sup>3</sup> designates sequences not available)\*.

Species	Origin	18S Type I	18S Type II	cox1	Morph
<i>Cura pinguis</i>	NSW, QLD, SA, TAS, VIC, WA	✓	✓	✓	✓
<i>Dugesia artesiana</i>	QLD	<sup>3</sup>	<sup>3</sup>	<sup>3</sup>	✓
<i>Dugesia gonocephala</i>	EU (DQ666002, FJ646987)	<sup>3</sup>	✓	✓	✓
<i>Dugesia japonica</i>	Asia (D83382, DQ666034)	<sup>3</sup>	✓	✓	✓
<i>Dugesia notogaea</i>	NSW, QLD, WA	✓	✓	✓	✓
<i>Dugesia ryukyuensis</i>	Asia (AF050433,	<sup>3</sup>	✓	✓	✓

	AF178311)				
<i>Dugesia subtentaculata</i>	EU (AF013155, FJ646996)	3	✓	✓	✓
<i>Dugesia (?) rava</i>	WA	3	3	3	✓
<i>Dugesia orientoaustralia</i>	NSW, QLD, VIC	3	✓	✓	✓
<i>Dugesia sp.</i>	NSW, QLD	3	✓	✓	3
<i>Eviella hynesae</i>	VIC	3	3	3	✓
<i>Girardia tigrina</i>	TAS, QLD, VIC, NSW, WA, SA	✓	✓	✓	✓
<i>Neppia magnibursalis</i>	TAS	3	3	3	✓
<i>Neppia montana</i>	NZ (AF178319, AF050432)	✓	3	✓	✓
<i>Masaharus graffi</i>	WA	3	3	3	3
<i>Masaharus informis</i>	TAS	3	✓	✓	✓
<i>Masaharus sphincter</i>	TAS	3	3	3	✓
<i>Masaharus extensus</i>	TAS	✓	✓	✓	✓
<i>Reynoldsonia reynoldsoni</i>	VIC	3	3	3	✓
<i>Romankenkius cf. bilineatus</i>	TAS	3	3	3	✓
<i>Romankenkius conspectus</i>	VIC	3	3	3	✓
<i>Romankenkius impudicus</i>	VIC	3	3	3	✓
<i>Romankenkius kenki</i>	TAS	3	✓	✓	✓
<i>Romankenkius libidinosus</i>	NSW (Z99951)	3	✓	3	✓
<i>Romankenkius pedderensis</i>	TAS	3	3	3	✓
<i>Romankenkius retrobursalis</i>	TAS	3	3	3	✓
<i>Romankenkius sinuosus</i>	TAS	3	3	3	✓
<i>Romankenkius sp. 2006</i>	VIC	3	3	3	✓
<i>Romankenkius sp. (Appendix 1d)</i>	VIC	3	3	✓	3
<i>Romankenkius boehmigi</i>	WA	3	✓	✓	✓
<i>Romankenkius glandulosus</i>	WA	3	3	✓	✓
<i>Romankenkius hoernesii</i>	WA	3	3	3	✓
<i>Romankenkius musculoglandulosus</i>	WA	3	✓	✓	✓
<i>Romankenkius rutrum</i>	WA	3	✓	✓	✓
<i>Schmidtea mediterranea</i>	EU (U31085, AF178322)	3	✓	✓	✓
<i>Schmidtea polychroa</i>	EU (AF287133, AF013154)	3	✓	✓	✓
<i>Spathula agelaeae</i>	VIC	3	3	✓	✓
<i>Spathula alba</i>	NZ (DQ666006, DQ666054)	3	✓	✓	✓
<i>Spathula camara</i>	TAS, VIC	3	3	3	✓
<i>Spathula dittae</i>	TAS	✓	✓	✓	✓
<i>Spathula cf. foeni</i>	TAS	3	3	3	✓
<i>Spathula foeni</i>	VIC	3	✓	✓	✓
<i>Spathula gourbaultae</i>	VIC	3	3	3	✓
<i>Spathula miserable</i>	VIC	3	3	3	✓
<i>Spathula musculosa</i>	NSW	3	3	3	✓
<i>Spathula simplex</i>	NSW	3	3	3	✓
<i>Spathula tryssa</i>	VIC	3	3	3	✓
<i>Spathula truculenta</i>	VIC, NSW	3	3	3	✓
<i>Spathula oblongata</i>	TAS	3	3	3	✓
<i>Spathula caeca</i>	TAS	3	✓	3	✓
<i>Spathula dupladiaphragma</i>	VIC	3	3	3	✓
<i>Spathula sp. 1</i>	TAS	3	3	✓	3
<i>Spathula sp. 2</i>	VIC	3	✓	✓	3
<i>Spathula sp. 2001</i>	TAS (AF178324)	3	✓	✓	3
<i>Weissius capaciductus</i>	QLD	3	3	3	✓

\*EU - Europe, NSW - New South Wales, NZ - New Zealand, QLD - Queensland, SA - South Australia, TAS - Tasmania, VIC - Victoria, WA - Western Australia.

**Table 2.4** List of non-dugesiid sequences used in this study including the relevant GenBank number (✓ designates sequences available, <sup>3</sup> designates sequences not available).

<b>Species</b>	<b>18S Type I</b>	<b>18S Type II</b>	<b>cox1</b>	<b>Morph</b>
<b>Maricola</b>				
<i>Procerodes littoralis</i>	<sup>3</sup>	Z99950	DQ666050	✓
<b>Geoplanidae</b>				
<b>Bipaliinae</b>				
<i>Bipalium adventitium</i>	<sup>3</sup>	DQ666000	AF178306	✓
<i>Bipalium kewense</i>	AF033039	<sup>3</sup>	<sup>3</sup>	<sup>3</sup>
<b>Geoplaninae</b>				
<i>Arthurdendyus triangulatus</i>	AF033038	AF033044	DQ666027	✓
<i>Geoplana burmeisteri</i>	<sup>3</sup>	DQ666004	DQ666039	<sup>3</sup>
<i>Australoplana sanguinea</i>	AF033041	<sup>3</sup>	<sup>3</sup>	<sup>3</sup>
<b>Rynchodeminae</b>				
<i>Platydemus manokwari</i>	<sup>3</sup>	AF048766	AF178320	<sup>3</sup>
<i>Microplana scharffi</i>	<sup>3</sup>	AF050435	DQ666044	✓
<i>Microplana nana</i>	AF033042	<sup>3</sup>	<sup>3</sup>	<sup>3</sup>
<b>Planarioidea</b>				
<b>Planariidae</b>				
<i>Crenobia alpina</i>	<sup>3</sup>	M58345	EF088231	✓
<i>Phagocata vitta</i>	<sup>3</sup>	DQ665998	DQ666052	✓
<i>Polycelis felina</i>	<sup>3</sup>	DQ665996	DQ666049	✓
<b>Dendrocoelidae</b>				
<i>Dendrocoelum lacteum</i>	<sup>3</sup>	AJ312271	AF178312	✓

### 2.2.7.2 Relative Rate Test

In order to assess the constancy of the rate of evolution over time amongst the lineages, a relative rate test was performed separately on both genes (18S and *cox1*) using all retained sequences. This allowed the exploration of variation of substitution rates and a test of possible long-branch attraction effects. The test was performed in RRTree (Robinson-Rechavi & Huchon 2000), using complete alignments (all codon positions (*cox1*) and bases included), where every taxon was treated as a separate lineage. The outgroup was a maricolan (*Procerodes littoralis*). This test compares all lineages to each other and indicates when there is a significant difference in the rate of evolution between pairs of lineages. The Kimura two-parameter method (Kimura 1980) and the Jukes-Cantor one parameter method (Jukes & Cantor 1969) were used and gave very similar results. Any lineage comparison that gave an exact probability < 0.05 was deemed to be evolving at a significantly different rate.

### 2.2.7.3 Homogeneity Test

A homogeneity test was conducted in Tree-Puzzle v.5.2 (Schmidt et al. 2002; <http://www.tree-puzzle.de>) for all 330 *cox1* sequences and all 92 18S sequences. A Chi-square analysis of base frequency homogeneity among taxa was carried out to determine whether any significant nonstationarity of base frequencies was evident, as sequences may be grouped on this basis rather than phylogenetic history.

### 2.2.7.4 Saturation Test

The saturation test measures whether the alignment has lost too much phylogenetic information due to substitution saturation. These tests were run in DAMBE (Xia & Xie 2001), using the parameters outlined by Xia et al. (2003) (60 replicates). The test requires an estimation of the proportion of invariant sites (pInv), which was obtained in BEAST 1.5 (Drummond & Rambaut 2007) by running a Bayesian analysis on the alignments using a General Time Reversible (GTR) model for both genes and a Gamma and Invariant Sites model (this process also estimated the shape parameter “alpha” of the Gamma distribution, which will be used during analyses when a value for Gamma is required). The log file produced was read in Tracer v 1.4 (Drummond & Rambaut 2007).

### 2.2.7.5 Models for Inferring Tree Topology

Prior to inferring trees, the model of evolution or substitution model was established for each alignment. Model testing for all alignments was performed using PAUP\* (Swofford 2003) (via the cross-platform bioinformatics software Geneious (Version 4)(<http://www.geneious.com>, Kearse et al. 2012)) to infer a maximum likelihood tree, using the Akaike Information Criterion (AIC), heuristic search strategy and rooting the tree to the Planarioidean outgroup.

## 2.2.8 Morphological Data

Despite the decline in the use of morphological data for phylogenetic research, mainly due to the often-ambiguous nature of the morphological characters, morphology is included as an important part of this analysis. This inclusion was deemed essential for two main reasons; firstly due to the valuable information contained within the data, particularly when looking at character evolution (Jenner 2003), and secondly in an attempt to complete the most exhaustive analysis possible (Assis 2009). It is important that DNA is not the sole source of phylogenetic information. This is due to DNA data not being available for all species and phylogenies based on DNA may not always be correct resulting in research groups publishing contradictory phylogenies (Graur and Li 2000;

Wiens 2004). Finally, Jenner (2003) stated that “science derives its greatest strength from its multifaceted nature”. It is the combination of morphological and molecular data that makes this analysis unique.

### 2.2.8.1 Data matrix

A matrix of phylogenetically informative characters for the included taxa was compiled on the basis of data extracted from the literature and newly obtained information. This matrix (Table 2.5) includes seven new species, full descriptions of which are available in Appendix 1; collection, histological processing techniques, reconstruction and identification are described in Chapter 3.

### 2.2.8.2 Character Selection

The literature on the relative merits of different coding approaches was reviewed prior to morphological character selection (Fitzhugh 2006; Poe & Wiens 2000; Sereno 2007; Sluys 1996; Wiens 1998). All coding approaches are intended to provide an explanation of observed shared similarities among individuals distributed among two or more species (Fitzhugh 2006). Therefore, misrepresentative character statements were avoided by the appropriate use of both presence/absence and multi-state character statements. It was important to make a clear distinction between the two types of character statements as presence/absence data were often replicated in multi-state data with the use of “absence” as a variable character state (Sereno 2007). For example, a character statement may be concerned with the presence or absence of an adenodactyl. However, a related multistate character statement deals with the orientation of the adenodactyl. The difficulty is how to code those species without an adenodactyl. Using the variables: anterior, posterior, and absent in this multistate character statement clearly replicates the presence/absence data, distorting the phylogenetic hypothesis. Consequently the use of “absence” in multistate character statements with a partner presence/absence character statement was avoided. “Absence” as a variable was used when the character statement was independent of all other statements and absence represented a true variable. In rare cases when the transformational characters had presence/absence partners and the structure of interest was missing, a “?” (=missing data) was used. In situations where two variables were applicable, both were coded for.

Characters used in this analysis were based on those selected for Sluys’ (2001) analysis. Many characters differ from Sluys’ original definitions due to inappropriateness or lack of information for the Australian representatives of the Dugesiidae. For example, characters including pharynx pigmentation, pharyngeal musculature and interintestinal

testes are only relevant for genera not found in Australia (Sluys 2001). Similarly, there are many characters concerned with whether structures are lined with nucleate or infranucleate epithelium, data that is not available for many Australian species (Sluys 2001). The 14 new characters that were included for this analysis relate to external morphology, shape and nature of the penial papilla, ectal reinforcement of the bursal canal, musculature and position of the copulatory bursa, the presence and glandularisation of the gonoduct, the presence and orientation of an adenodactyl and the nature of the ejaculatory duct. Several proposed autapomorphies were included in the analysis as it was hoped that through the investigation of these characters more reliable diagnostic characters might be discovered for the genera.



### 2.2.8.3 Characters Selected

Table 2.6 briefly describes the characters used for the morphological analysis, for more detailed descriptions, see Appendix 3a.

**Table 2.6** Summary of characters used in morphological analysis (controversial characters indicated by grey shading\*).

Number	Character Description	Character States
1	Head shape (live)	Low triangular (0), High Triangular (1), Bluntly triangular (2), Rounded (3), Spathulate (4), Truncate (5), Pointed (6), Lunate (7), Convex (8)
2	Auricles	Absent (0), Short (1), Long (2)
3	Eyes	Absent (0), Multicelled (1), Single-celled (2)
4	Dorsal pigmentation	Absent (0), Solid (1), Mottled/Patterned (2)
5	Ventral pigmentation	Absent (0), Less than dorsal (1), Same as dorsal (2)
6	Ciliated pits	Absent (0), Present (1)
7	Sensory fossae	Absent (0), Present (1)
8	Position of Mouth	At the hind end of the pharyngeal pocket (0), At 1/5 of the distance between the hind of the pharyngeal pocket and the root of the pharynx (1), At 1/3 (2), Halfway the distance between the hind end and the root of the pharynx (3), At 1/4 (4)
9	Position of testes	Ventral (0), Dorsal (1), Extending from dorsal to ventral body surface (filling the entire space) (2) Situated in the middle of the body (3)
10	Extension of testes	Throughout body length (0), Prepharyngeal (1), To about halfway along the pharyngeal pocket (2), To the mouth at the posterior end of the pharyngeal pocket (3), To the copulatory apparatus (4)
11	Fused Testes	Absent (0), Present (1)
12	Position of ovary	Directly behind the brain (0), At a short distance behind the brain (1), A long distance behind the brain (2)
13	Caudally branched oviducts	Absent (0), Present (1)
14	Oviducal loop	Absent (0), Present (1)
15	Common oviduct/diverticulum	Absent (0), Opening well into the bursal canal (1), Opening into the most postero-ventral section of the bursal canal or even into the common atrium (2)
16	Shell glands	Absent (0), Entering the diverticulum (1), Entering the bursal canal (2)
17	Entrance of the oviducts into the bursal canal	Symmetrical (0), Asymmetrical (1)
18	Musculature of bursal canal	Non-reversed (0), reversed (1), Mixed (2), Circular (3)
19	Ectal reinforcement	Absent (0), Present (1) Extension (2)
20	Sphincter on bursal canal	Absent (0), Present (1)
21	Bursal canal opening into atrium	Posterior (0), Lateral (1), Dorsal (2), Absent (3)
22	Bursal canal connected to	Copulatory bursa (0), Intestine via copulatory bursa (1), Intestine (FID) (2), Expansion (3), Common Oviduct/Oviduct (4)
23	Dactylose projections on bursal canal	Absent (0), Present (1)

24	Musculature around bursa	Absent (0), Present (1)
25	Bursa	Absent (0), Sitting anterior to penis bulb (1), Sitting lateral to penis bulb (2), Sitting posterior to penis bulb (3)
26	Gonoduct	Absent (0), Present (1)
27	Glands entering directly into gonoduct	Absent (0), Present (1)
28	Glandularisation of common atrium	Absent (0), Present (1)
29	Penial papilla	Finger-shaped (0), Conical (1), Rounded (2), Plug (3), Irregular/Flexible (4), Absent (5)
30	Eversible penial papilla	Absent (0), Present (1)
31	Penial folds	Absent (0), Present (1)
32	Separate ejaculatory ducts	Absent (0), Present (1)
33	Diaphragm	Absent (0), Present (1)
34	Seminal vesicle	Absent (0), Present (1), Pleated (2), Double (3)
35	Penis glands opening through the covering epithelium of the penial papilla	Absent (0), Present (1)
36	Penial glands	Absent (0), Opening into the ejaculatory duct (1) Opening into the seminal vesicle (2), Opening into both (3)
37	Adenodactyl	Absent (0), Orientated posteriorly (1) Orientated anteriorly (2), Orientated ventrally (3) Orientated laterally (4)
38	Pleated ejaculatory duct	Absent (0), Present (1)
39	Pigmented pharynx	Absent (0), Present (1)
40	Posterior sucker	Absent (0), Present (1)

### \*A note on Controversial Characters

Sensory organs have traditionally been excluded from phylogenetic analyses of triclads due to the vast array of organ types described within the freshwater Geoplanoidea and it being unknown whether certain structures are homologous with others, i.e. sensory pits and auricular grooves. The presence of marginal sensory organs throughout the freshwater geoplanoid genera indicates that these structures arose early. I have chosen to include sensory structures by simplifying their classification into just sensory fossae (shallow, cup shaped organs) and sensory pits (deep ciliated pits).

The position and extension of the testes was not included in De Vries and Sluys' (1991) phylogeny of the genus *Dugesia* as they considered the large variability of states to be unsuitable for phylogenetic analysis. However, as more species were described some consensus within the genera emerged and Sluys (2001) included testes in his more recent analysis. Sluys (2001) also included the nature of the epithelium surrounding certain structures (nucleate or infranucleate), however, these characters will not be included here due to a lack of information for many of the species included in this analysis.

Triclads are such flexible, soft bodied organisms that there tends to be a great deal of intraspecies variation in shape, colouration and some positional characters. Every effort has been made to only use characters that show a consistent state across and within the species. Characters that might vary due to this intraspecies plasticity include gonoduct & glands entering gonoduct, penis papilla shape, head shape,

auricles long or short, dorsal colouration, ventral colouration, bursal canal communication with atrium (characters 1, 2, 4, 5, 21, 26, 27 & 29). Analyses were run including and excluding these characters in order to judge their impact on the resulting phylogenies.

## 2.2.9 Construction of Phylogenies

### 2.2.9.1 Outgroups

*Procerodes littoralis*, a maricolan, was initially considered as an outgroup taxon for the trees involving representatives from all groups within the Continenticola (Álvarez-Presas et al. 2008). However, it was determined that this species was too divergent as the focus of this study is the internal relationships of the Geoplanoidea. As a consequence, representatives of the Planarioidea (e.g. *Crenobia alpina*) were chosen, as they are a sister group to the Geoplanoidea and therefore represent a much closer root (Álvarez-Presas et al. 2008).

### 2.2.9.2 Methods of Inferring Trees

#### 2.2.9.2.1 Parsimony (Morphological)

Morphological trees were created using a parsimony analysis in PAUP (Swofford 2003). All characters were treated as unordered and multistate taxa were treated as polymorphic. The heuristic search option with random stepwise sequence addition was applied (100 repeats), with seed number 10 and reference taxon *Cura pinguis*. Branch-swapping option tree-bisection-reconnection (TBR) was used, all minimum-length trees were kept, zero-length branches were collapsed, the ingroup was made monophyletic, and multiple furcations were treated as soft polytomies. Dugesiidae was treated as paraphyletic (Álvarez-Presas et al. 2008). Consistency and retention indexes for the data set were obtained in TNT (Goloboff et al. 2008), using a script available from the TNT wiki site (<http://tnt.insectmuseum.org/index.php/Scripts>). As per Sluys (2001), bootstrapping was not an appropriate measure of node support for this data set, due to the low number of characters available, with almost all of the clades having branch support far below 50%. Consequently, group frequencies were used to draw any conclusions about the node support for morphological trees. Group frequencies indicate the number of bootstrap replicates (expressed as a percentage) in which the particular group was found (Swofford 2003). Trees were read and manipulated in Dendroscope (Huson et al. 2007).

#### 2.2.9.2.2 Bayesian (Molecular & Morphological)

MrBayes (Ronquist & Huelsenbeck 2003) was used to infer Bayesian trees. For those alignments specifying a TVM model, the GTR model was used instead. The TVM model is a special case of the GTR

model and is not yet implemented in MrBayes. For all analyses the number of generations ran between 1000000 and 10000000, depending upon how quickly the average standard deviation of split frequencies was reported to be less than 0.01. This number is an indication of the convergence of two parallel runs estimating trees from the same data, and therefore the smaller the standard deviation between the two runs the more reliable the results. The sample frequency was set at 100 and the number of simultaneous chains run was four, for all analyses. It was necessary to manipulate the temperature for many of the analyses in order to achieve the most efficient analysis (i.e. when there were convergence issues it was necessary to chain the temperature parameter in order to increase/decrease the likelihood of chains swapping); as a consequence temperatures ranged between 0.05° and 0.3°. Bayesian posterior probabilities were saved in order to assess the reliability of nodes inferred. The burn-in value was determined by establishing when both parameters (runs) converged upon on a stable value. After discarding the trees sampled during the burn-in the results were summarised in a consensus tree, calculated using the allcompat option (50% majority rule tree); this allows posterior probabilities less than 50% to be displayed. Trees were read and manipulated in Dendroscope (Huson et al. 2007).

The model of evolution specified for morphological data, either on its own, or as part of a concatenated alignment, was Standard Gamma. This model allowed for up to 10 different states and was appropriate for unordered characters (Ronquist & Huelsenbeck 2003). The “variable” coding bias was deemed appropriate for use in situations where the morphological data were analysed independently. When the morphological data were included in a concatenated data set, the “all” coding option was used. This was due to the reduced number of species included in these analyses and the consequential decrease in the number of “constant” sites in the morphological data set (Ronquist & Huelsenbeck 2003).

### 2.2.10 Character Mapping

A morphological tree (including all species), which represented most accurately the hypothesis proposed as a result of all analyses, was manipulated (to exactly resemble the final hypothesis, Figure 2.25) in Mesquite (Maddison & Maddison 2009). The morphological character matrix was linked to this tree using the “trace character history” analysis with a parsimony reconstruction method. This analysis allowed the evolution of all characters to be viewed individually, so that apomorphies and synapomorphies could be identified. The relative merits of parsimony versus “stochastic” methods have been discussed in detail (Bollback 2006; Huelsenbeck et al. 2003; Nielsen 2001, 2002). While this stochastic approach has definite advantages when dealing with large numbers of molecular characters, in this scenario where a low number of morphological characters are being mapped onto a consensus tree it was deemed that the parsimony approach would be more than adequate.

### 2.2.11 Determining Final Hypotheses

Several techniques were used in order to arrive at a final hypothesis for the family-, genus- and species-level trees (Figures 2.25 & 2.22). The reliability of “taxonomic congruence” (consensus from separately derived data sets) has been debated over the last 30 years, particularly following the introduction of technology allowing a “total evidence” (character congruence) approach for phylogenetic inference. It is agreed that total evidence is a more robust technique. However, taxonomic congruence should not be used in competition with total evidence (Crisci 1984). To determine final hypotheses a taxonomic congruence approach will be used when the trees derived from the total evidence approach are unclear or missing data. This approach is supported by Li and Lecointre (2009) who argued that in some scenarios the total evidence approach is not completely reliable as it dismisses interesting information.

## 2.3 Results and Discussion

### 2.3.1 Sequences obtained

Table 2.3 lists the studied species and successfully sequenced genes in this and previous studies. New Type I 18S sequences were obtained for 3 taxa, new Type II 18S sequences were obtained for 17 taxa and new *cox1* sequences were obtained for 19 species.

### 2.3.2 Pre-tree Building Analysis Results

#### 2.3.2.1 Relative Rate Test

Those species that were found to be evolving at a different rate for the 18S region were the representatives of the Dendrocoelidae and Planariidae that have been included in the analysis for comparison (one dendrocoelid, *Dendrocoelum lacteum*, and three planariids, viz. *Phagocata vitta*, *Polycelis felina*, and *Crenobia alpina*). If only the Dugesiiidae and Geoplanidae are tested the pass rate increases substantially, and becomes 100% when the genera *Dugesia* and *Schmidtea* are also excluded (see Álvarez-Presas et al. 2008). The same is true for the *cox1* sequences: many species failed the test, however, the exclusion of groups outside the Geoplanidae/Dugesiiidae clade lead to a 100% pass for all remaining *cox1* sequences. The equivalent rates of substitution demonstrated in the alignments excluding these “outgroups” (the Dendrocoelidae and Planariidae) suggests that biased nucleotide composition and long-branch attraction problems among species are unlikely in these analyses.

#### 2.3.2.2 Homogeneity Test

The *cox1* sequences that had failed the chi-squared test at the first or second codon position were excluded. The third codon position created more of a challenge as many sequences failed at this position. In order to combat this issue the third codon was recoded as either purines (A’s and G’s) or pyrimidines

(C's and T's), allowing several more sequences to pass at the third codon position. Despite this approach, the third base position was still problematic for the majority of species and consequently excluded from all analyses.

The 18S sequences were tested using the same method but the sequences were divided into five regions, two of which were the hypervariable V2 and V4 regions, the remainder being the surrounding, more constant regions. All sequences passed the test for all regions and consequently were kept in the analysis.

### 2.3.2.3 Saturation Test

Saturation tests were run on the standard alignments for both gene regions. There is little saturation if the value for *I<sub>ss</sub>* is significantly lower than *I<sub>ss.c</sub>*. Additional parameters are as discussed by Xia et al. (2003). A summary of the results is found in Table 2.7. Those alignments displaying little saturation (shaded) are the most useful for phylogenetic analysis. Those exhibiting substantial saturation were excluded from further analysis or discussion. Whilst the *cox1* region including all codon positions was shown to be problematic in the homogeneity test (2.2.7.1), it is included here to demonstrate that the inclusion of the 3<sup>rd</sup> base position results in the *cox1* gene displaying substantial saturation. This further justifies the removal of the 3<sup>rd</sup> base position from any future analyses.

**Table 2.7** Results of the substitution saturation test showing the proportion of invariant sites (*P<sub>inv</sub>*),  $\alpha$  parameter ( $\alpha$ ), probability (*P*), the *I<sub>ss</sub>*, *I<sub>ss.c</sub>* and the quality of the data implied by these results.

Alignment*	<i>p<sub>inv</sub></i>	$\alpha$	<i>P</i>	<i>I<sub>ss</sub></i>	<i>I<sub>ss.c</sub></i>	Result
18S	0.311	0.301	0	0.330	0.813	Little Saturation
18S #	0.386	0.313	0	0.4508	0.7383	Little Saturation
<i>cox1</i>	0.211	0.385	0.35	0.628	0.679	Substantial Saturation
<i>cox1</i> # ♦	0.258	0.375	0.0040	0.465	0.666	Little Saturation
<i>cox1</i> 1 <sup>st</sup> codon position	0.256	0.493	0.4235	0.572	0.653	Substantial Saturation
<i>cox1</i> 2 <sup>nd</sup> codon position	0.352	0.437	0.0980	0.468	0.652	Substantial Saturation
<i>cox1</i> ♦	0.268	0.375	0.0124	0.485	0.666	Little Saturation

# Dugesiidae and Geoplanidae only, ♦ 3<sup>rd</sup> codon position removed for *cox1* alignment.

### 2.3.2.4 Models for Inferring Tree Topology

The models identified are summarised in Table 2.8. For concatenated alignments the relevant model will be specified for each partition separately where appropriate. When necessary, the model of evolution specified for morphological data was a likelihood model (Gamma), as suggested by Ronquist and Huelsenbeck (2003) (see section on Bayesian analysis for more details).

**Table 2.8** Model of evolution identified for each alignment. GTR = General Time Reversible (variable base frequencies, symmetrical substitution matrix), TIM = Transition Model (variable base frequencies, variable transitions, transversions equal), G = Gamma (gamma distributed rate variation among sites), I = Invariant sites (static, unchanging sites in a dataset).

Alignment*	Model	Average Pairwise (JC) Distance
18S	GTR + I + G	0.174
18S #	GTR + I + G	0.151
<i>cox1</i> # ♦	TIM + I + G	0.115
<i>cox1</i> ♦	TIM + I + G	0.236
Morphology	G	N/A

\*# Dugesiidae and Geoplanidae only, ♦ 3<sup>rd</sup> codon position removed for *cox1* alignment

### 2.3.3 Analyses Run

For each of the methods discussed above, analyses were run on data sets modified in various ways in order to assess the effect of these variations on the inferred phylogeny. For some analyses certain groups of taxa were excluded to ensure that the inclusion of more divergent but less important species (e.g. non-Dugesiidae species) was not unduly influencing the phylogeny. To this end phylogenies were run including all taxa, including only species of Dugesiidae and Geoplanidae and finally the species of Dugesiidae on their own.

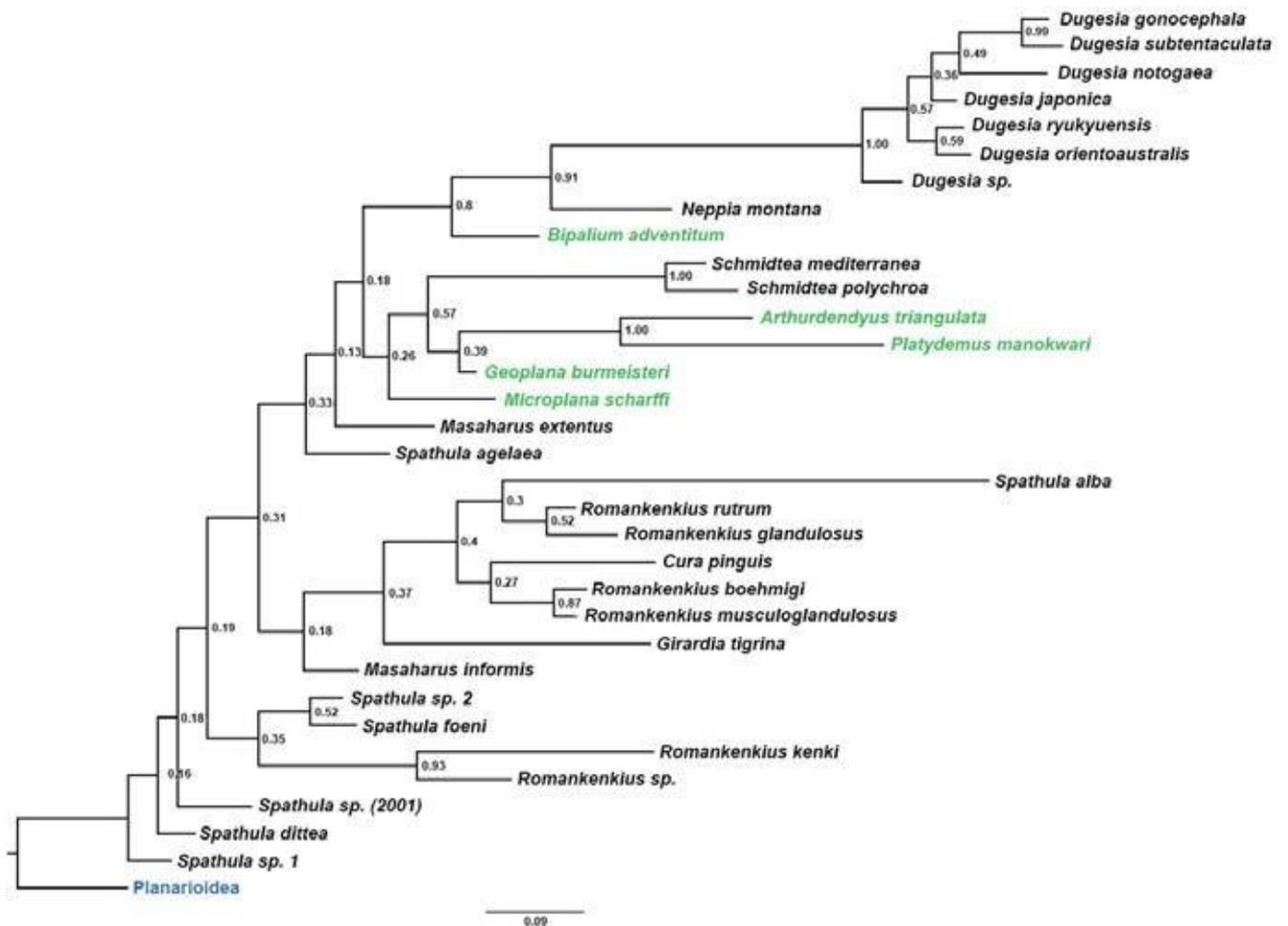
The analyses including the hypervariable regions of the 18S segment passed the homogeneity test (Table 2.6). Therefore, these hypervariable regions were included in all relevant analyses. Issues encountered getting the *cox1* sequences to pass a homogeneity test and substitution saturation test meant that the 3<sup>rd</sup> base position has been removed from all *cox1* sequences for all analyses (Section 2.3.0.2 and 2.3.0.3). Some of the morphological characters are questionable (refer to Table 2.6). Analyses involving morphological data were run both including and excluding these characters.

### 2.3.4 Phylogenies

The inclusion of outgroup species (one dendrocoelid, *Dendrocoelum lacteum*, and three planariids, viz. *Phagocata vitta*, *Polycelis felina*, and *Crenobia alpina*) in the majority of analysis did not change the topology of the tree. However, it did influence the support values negatively. For this reason, all molecular analyses shown here exclude Planarioidea species, excepting the required outgroup *Crenobia alpina* (this also addresses the issue outlined in section 2.3.2.1). Additionally, while analyses were run excluding the Geoplanidae species, due to the importance of this group to the phylogeny of the Dugesiidae, it was deemed uninformative to present these results (there was also very little influence on topology and support values).

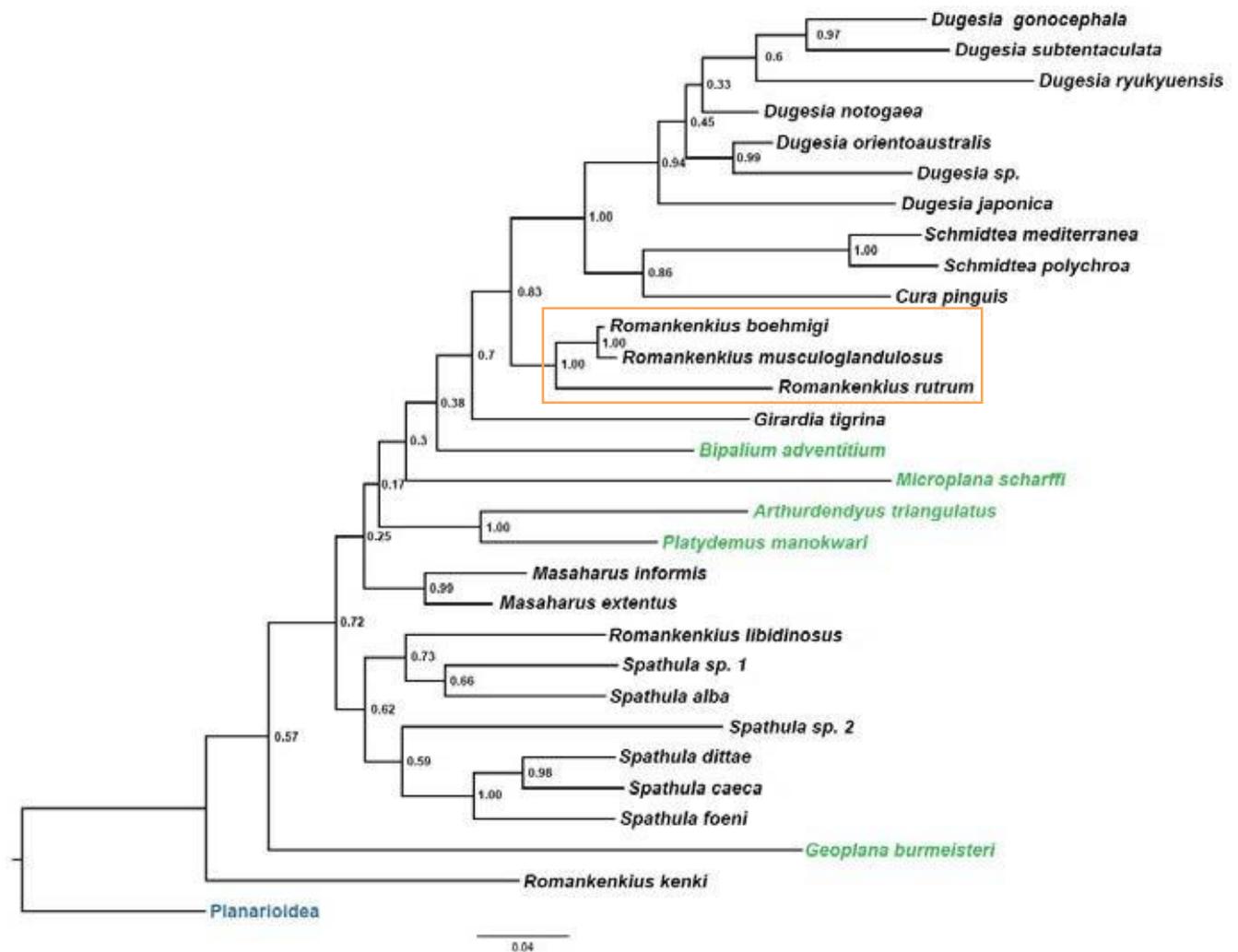
### 2.3.4.1 *cox1* Topology

There is very little phylogenetic information, particularly at deeper levels, in the *cox1* gene region; however some preliminary patterns can be identified. Strong support was found for the genus *Dugesia*, however relationships within it are unclear (Figure 2.14). Expected affinities of several dugesiid species appear with high levels of support (e.g. *Schmidtea mediterranea* and *Schmidtea polychroa*, *Romankenkius boehmigi* and *Romankenkius musculoglandulosus*, *Romankenkius kenki* and *Romankenkius* sp.), yet beyond these, support values are low (Figure 2.14). The Geoplanidae species are distributed amongst the dugesiid species, again with generally low support values (Figure 2.14). Nevertheless, this analysis will be useful in placing some of the species for which 18S data are lacking.



**Figure 2.14.** Tree inferred from *cox1* sequences, 3<sup>rd</sup> base excluded, Bayesian analysis including posterior probabilities. (Geoplanidae representatives highlighted in green, Dendrocoelidae highlighted in blue).

2.3.4.2 18S Topology



**Figure 2.15** Bayesian tree of 18S region, including posterior probabilities and bootstrap supports from other analyses. “Western *Romankenkius*” represented by box (Geoplanidae representatives highlighted in green, Planarioidea highlighted in blue).

The 18S gene region allows much greater resolution of the relationships than the *cox1* analyses, yet some of the deeper level relationships remain unclear. The genus *Dugesia* is still well supported with some further resolution provided for several species relationships. The location of *Schmidtea* as a sister-group to *Dugesia*, which was shown in the *cox1* analysis, is replicated here with strong support (Figure 2.15). *Cura* is sister to *Schmidtea* group, however the support for this placement is only moderate. Surprisingly, the genus *Romankenkius* appears in three, widely separated, places in the tree (eastern and western *Romankenkius*). The western *Romankenkius* species all cluster with high support and this clade is sister to the *Dugesia/Schmidtea/Cura* clade (Figure 2.15 - see box), with *Girardia* basal to these. However, support for the position of *Girardia* is not strong.

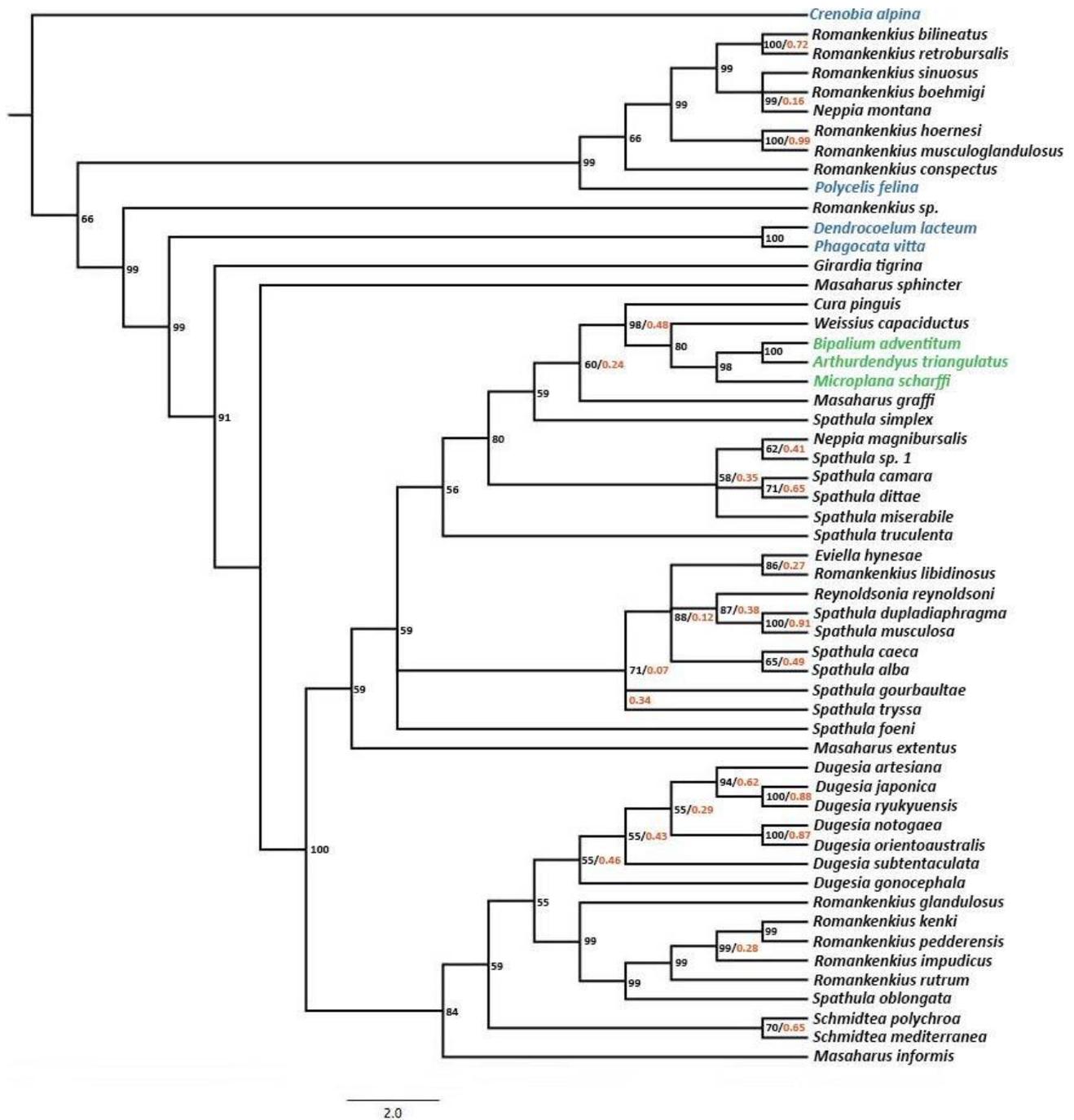
The 18S analysis placed *Romankenkius libidinosus* amongst the *Spathula* species, however support for this positioning is not strong (Figure 2.15). This is the only *Romankenkius* species from which

molecular data were previously available, so the possibility of mis-identification must be considered and investigated. The Geoplanidae are once again distributed amongst the dugesiids, specifically amongst the *Spathula*, *Masaharus* and *Romankenkius* clades, albeit with poor support (Figure 2.15).

### 2.3.4.3 Morphological Phylogeny

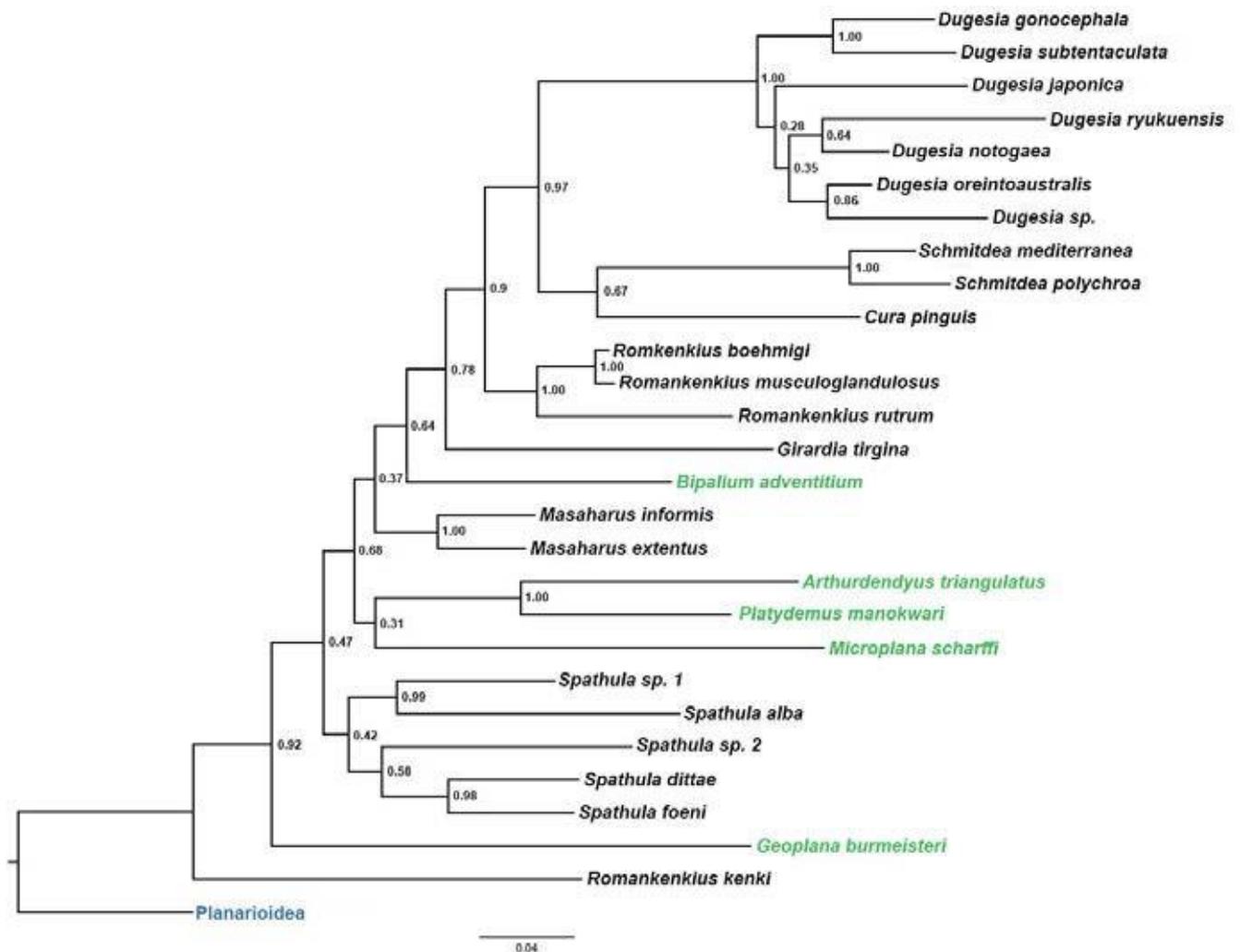
Due to the difficulties encountered in arriving at a reliable morphological hypothesis, two tree inference methods were examined for the morphological phylogeny. Neither analysis provided high levels of support for nodes throughout the trees, particularly at the deeper nodes (likely due to the lack of morphological characters available for comparison). However, the species associations shown in the PAUP and MrBayes analyses concur in many cases, which is especially encouraging). A detailed discussion of these analyses is considered superfluous. Instead Figure 2.16 shows the PAUP analysis with the group frequencies (see Section 2.2.9.2) shown and posterior probabilities (from the Bayesian analysis) shown at shared nodes.

It should be pointed out that several of the species in unexpected locations exhibited even fewer diagnostic characters than the already limited character set for the Dugesidae. This may be due to the fact that a species is asexual (e.g. *Girardia tigrina*) or was described in an immature state (e.g. *Spathula sp. 1*). When species do not have a copulatory apparatus, this removes 30 of the 40 diagnostic characters. Other species were difficult to assign to a genus due to a confused set of diagnostic characters, for example, many of the species assigned to *Masaharus* (formerly *Girardia*) were assigned to this genus by default (i.e. there are not many unifying characters within this genus). Similarly, the morphological similarities between *Reynoldsonia reynoldsoni* and the genus *Spathula* are well documented, including a suggestion that this species may be just an aberrant *Spathula* (Ball 1974b; Sluys 2001). Once these considerations are taken into account the tree presented appears more useful. It is possible that the morphological information will be more useful when scaled back to a few very strong diagnostic characters. This will be done in the character analysis below (Section 2.3.6). The information in this tree will be useful in placing species for which no molecular information is available within their genus group in the final phylogeny; however, no useful comparisons can be made with the molecular trees at the genus level.



**Figure 2.16.** A 50% majority rule consensus tree based on morphological data and inferred using parsimony, all taxa and characters included; steps 304, CI=0.271, RI=0.514, 38651 most parsimonious trees. Group frequencies indicated in black and posterior probabilities from the Bayesian analysis indicated in orange at shared nodes (Geoplanidae represented in green, Planarioidea represented in blue).

2.3.4.4 18S and *cox1*

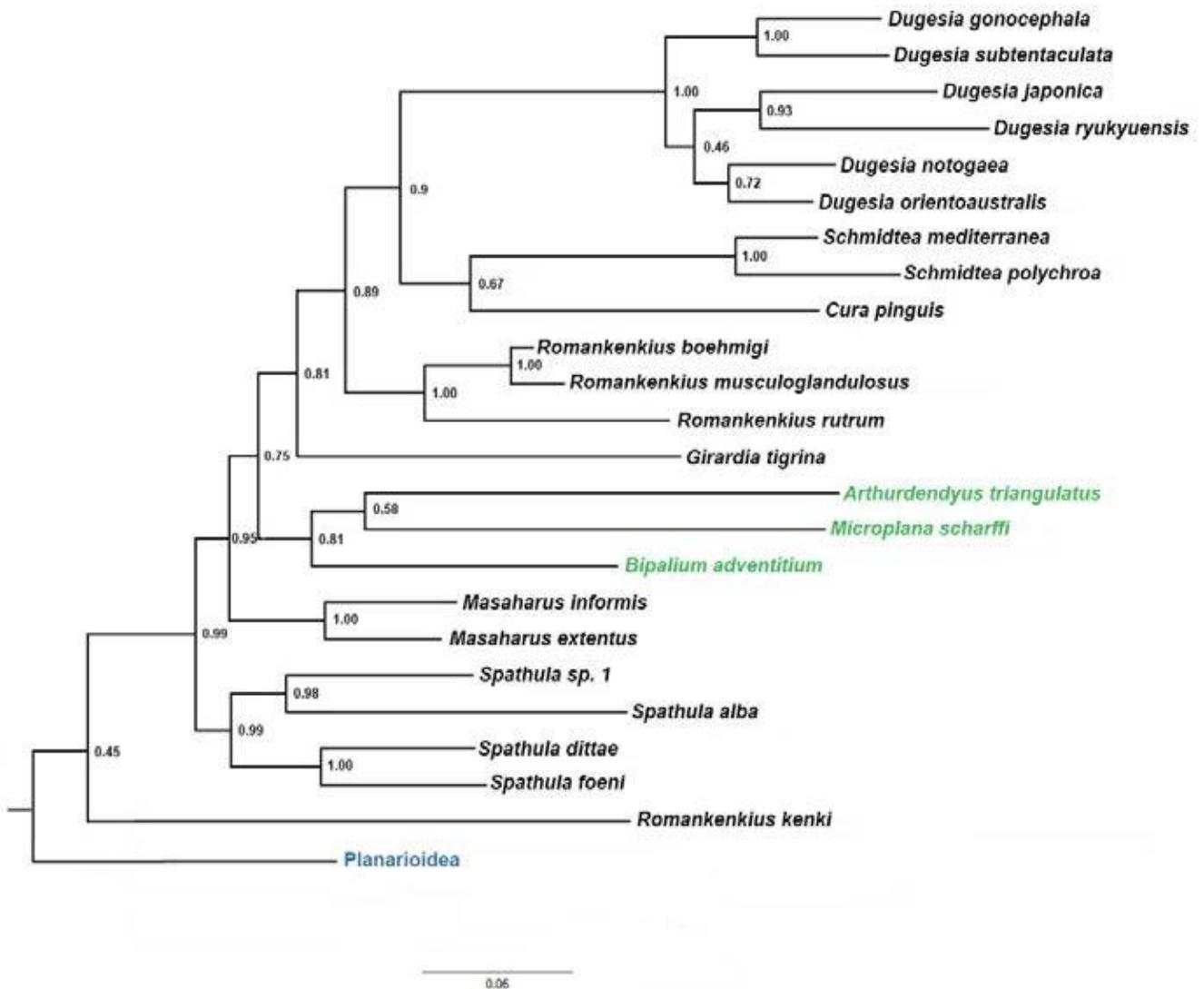


**Figure 2.17.** Cladogram inferred from the Bayesian analysis of an 18S and *cox1* concatenated data set, excluding the 3<sup>rd</sup> base position for *cox1* (Geoplanidae representatives highlighted in green, Planarioidea highlighted in blue).

Whilst the relationship between *Dugesia* and *Schmidtea* remains close, *Cura* is now located as a sister group to *Schmidtea*, introducing a different topology to that found in the individual gene phylogenies (Figure 2.17). *Girardia* is sitting at the base of this group, placing western *Romankenkius* closer to the *Cura*, *Schmidtea* and *Dugesia* cluster, which agrees with the 18S analysis (Figure 2.15).

The concatenated data set does nothing to increase the resolution of the *Spathula*/*Masaharus*/Geoplanidae group. One pattern that is consistent is the presence of two clusters within a monophyletic *Spathula*, one consisting of *Spathula dittae* and *Spathula foeni* and the other *Spathula alba* and *Spathula sp. 1* (Figure 2.17). The Geoplanidae are still showing little resolution.

2.3.4.5 18s, *cox1* and Morphology



**Figure 2.18** Phylogram inferred via Bayesian analysis of all markers excluding the 3<sup>rd</sup> base position of the *cox1* gene and the controversial characters. Posterior probabilities are shown (Geoplanidae represented in green, Planarioidea represented in blue).

The decision was made to include the tree that excluded the controversial morphological characters (Table 2.6) as, while the tree including them showed the same topology, the support values were higher when these characters were excluded from the analysis. The inclusion of the morphological data in the analysis provides a plausible set of sister group relationships (considering the species' geographical origins) with *D. notogaea* and *D. orientoaustralis* (Australia) clustering separately to *D. japonica* and *D. ryukyuensis* (Asia) and *D. gonocephala* and *D. subtentaculata* (Europe and Africa). The Geoplanidae species cluster together in this analysis, however two species for which data was not available are absent (*Geoplana burmeisteri* and *Platydemus manokwari*). Interestingly, *Bipalium adventitium*, which previously

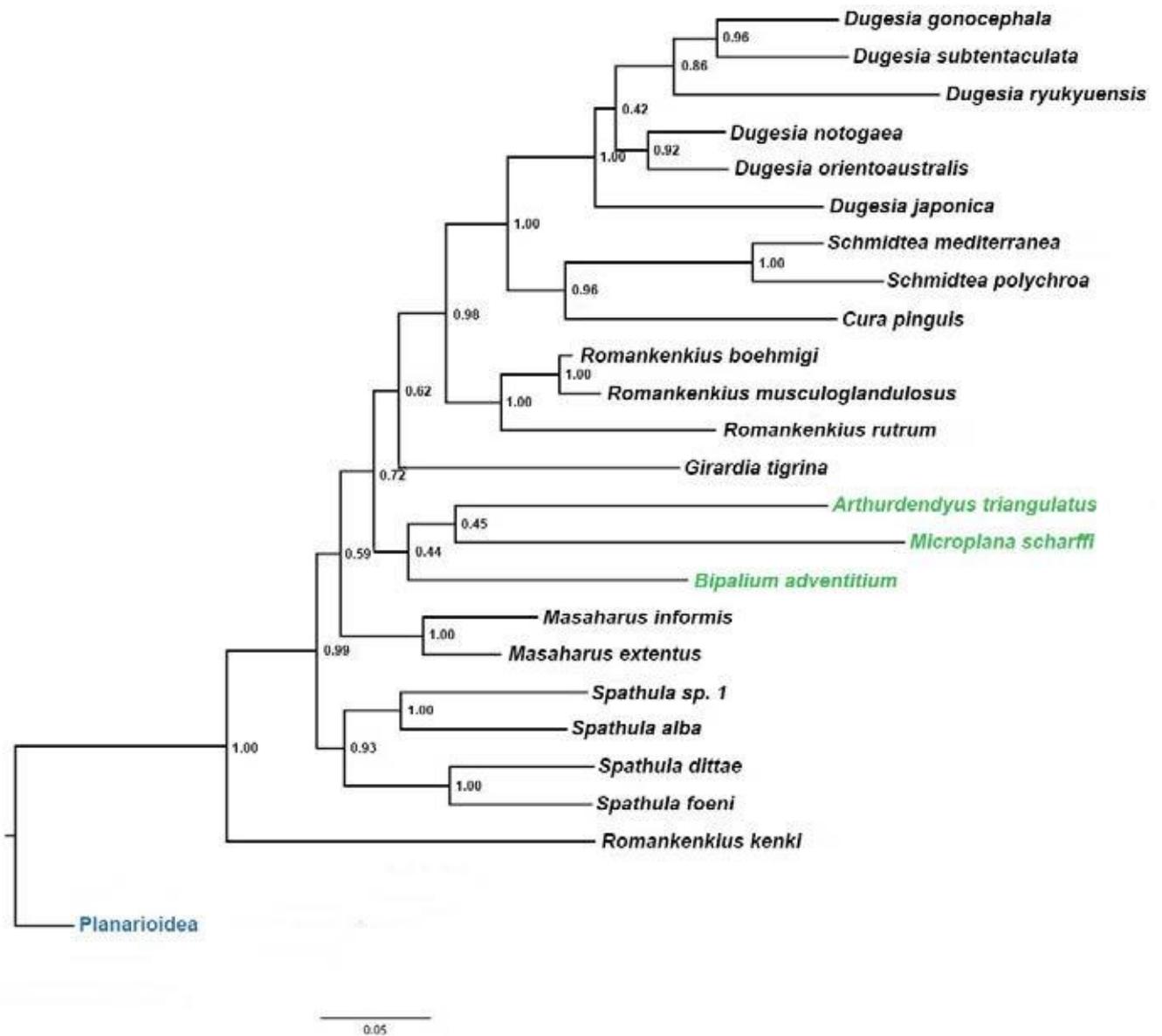
did not cluster with the other Geoplanidae species, is now located at the base of the Geoplanidae cluster. This combination of markers has had a very positive effect on the support levels within *Spathula* (Figure 2.18). We can assume that this is due to the inclusion of the morphological data. Beyond these minor modifications there are no major deviations from the within genus relationships discussed for the previous analyses.

While the genus relationships exhibited in this tree are fairly conventional. It is the structure of the tree that is of interest. The implication is that, aside from the curiously placed eastern *Romankenkius*, the genus *Spathula* is the most basal of the dugesiids followed by *Masaharus* and then the Geoplanidae clade. The *Schmidtea* and *Cura* cluster sits as a sister group to the *Dugesia* clade at the tip of the tree representing the most derived of the dugesiid genera (Figure 2.18). This branching style and generic relationships are reminiscent of earlier morphological work on the Dugesiidae (Sluys 2001).

#### 2.3.4.6 18S and Morphology

For the same reason as outlined for the previous analysis, the tree excluding the controversial characters will form the basis for this discussion. The tree in Figure 2.19 provides strong support for the *Dugesia*, *Schmidtea* and *Spathula* species clusters. *Schmidtea* and *Cura*, for the first time, form a strongly supported clade, which is a sister to the *Dugesia* clade. The position of western *Romankenkius* directly basal to the *Cura/Schmidtea* clade is, here, highly supported. In all analyses (the highest support levels being shown in Figure 2.19) *Spathula* and *Masaharus* are basal to the Geoplanidae.

The arrangement shown in Figure 2.19 is also supported by the 18S, 18S and *cox1* and all markers concatenated analyses (Figure 15, 17 & 18). The morphological data also places Geoplanidae genera near *Masaharus* and *Girardia*. The only competition to this theory is that suggested by the 18S and *cox1* concatenated data set, which places the several Geoplanidae amongst a *Spathula/Masaharus* clade (Figure 2.17). This does not necessarily contradict the relationship shown in Figure 2.19 and it should be noted that regardless of which relationship is most accurate, all reinforce the notion of the paraphyly of the Dugesiidae.

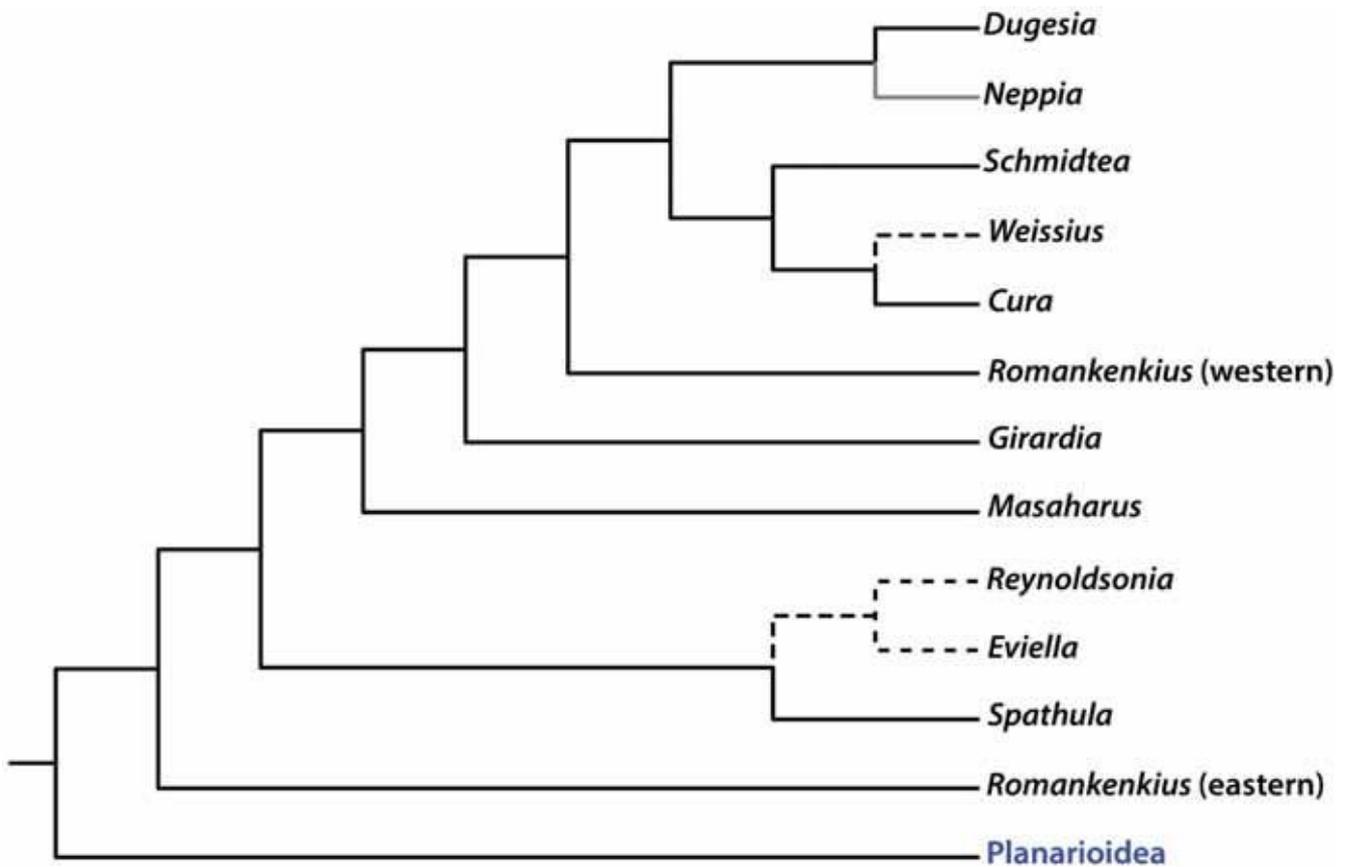


**Figure 2.19** Phylogram for Bayesian analysis of 18S and Morphology concatenated data sets excluding controversial characters. Posterior probabilities indicated (Geoplanidae representatives highlighted in green, Planarioidea highlighted in blue).

## 2.3.5 Phylogenetic Hypotheses

### 2.3.5.1 Genus-level Relationships

As outlined in section 2.2.11, the 18S plus morphology analyses will form the basis of the genus-level hypothesis because these analyses yield the highest support levels, however, it should be noted that this topology is supported by all molecular analyses, excluding the *cox1* marker, which had little resolution at the deeper nodes. The location of any genus not represented in the 18S and morphology analysis (Figure 2.19) will be determined via taxonomic congruence.



**Figure 2.20** Summary tree of genus-level relationships including all dugesiid genera (please note - this tree was developed using a combination of types of evidence, consequently there is no statistical support). Grey line represent a genus positioned due to evidence from *cox1* data only. Dashed lines represent genera placed on the basis of morphological evidence (the Planarioidea outgroup represented in blue).

*Neppia*, *Reynoldsonia* and *Eviella* are the only genera that are not in Figure 2.19. *Neppia* is placed as a sister genus to *Dugesia* in Figure 2.20 on the basis of strong support in the *cox1* analysis. *Reynoldsonia* and *Eviella* are placed in their respective locations based on morphological data. A reasonable amount of confidence can be placed in this topology, as support levels were relatively high and there was a great deal of congruence between the morphological trees for these genera (Figure 2.16). While represented here as separate genera, there is a large body of morphological evidence suggesting that *Eviella hynaesae* and *Reynoldsonia reynoldsoni* represent aberrant *Spathula* species (see Figure 2.21).

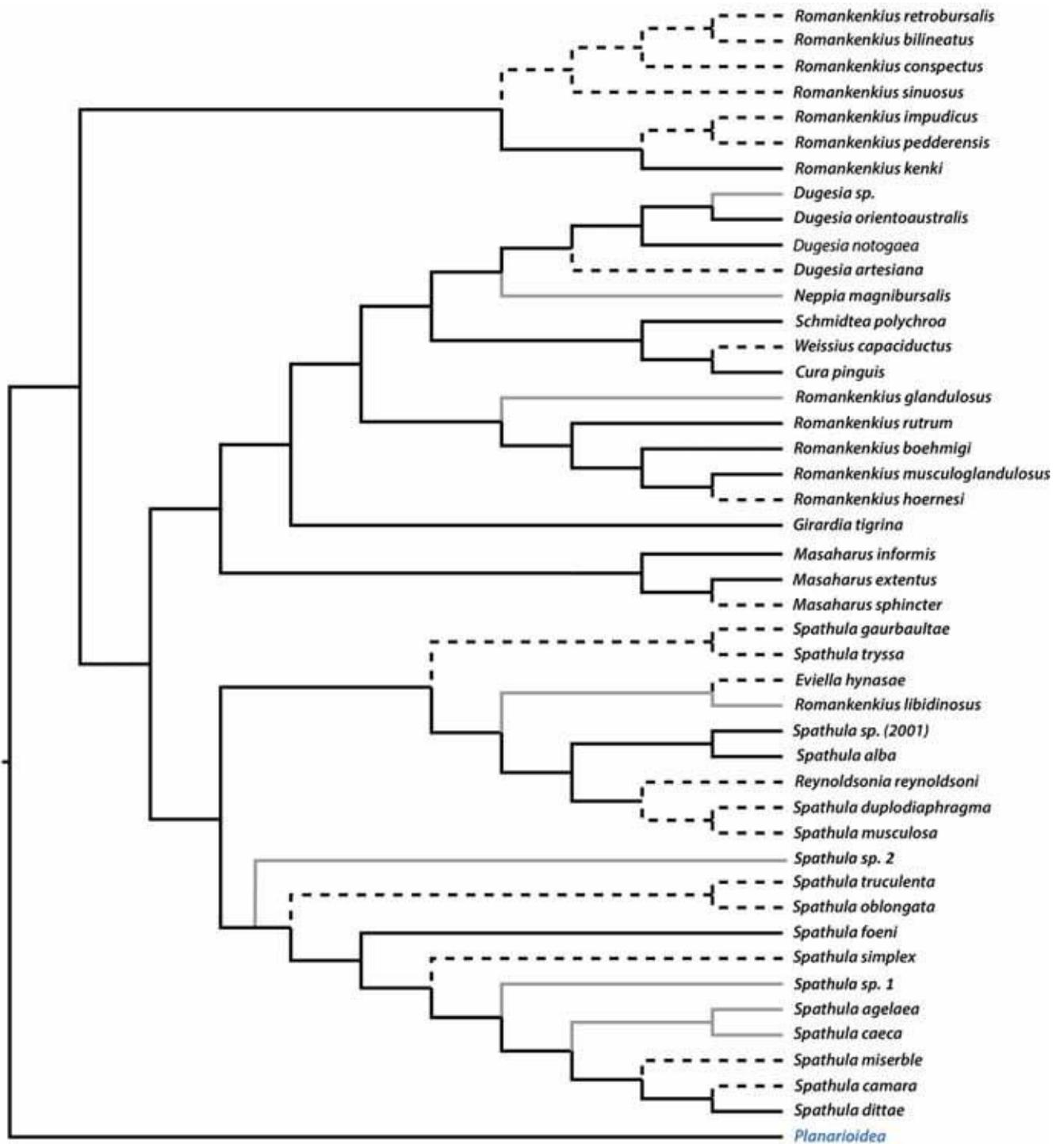
### 2.3.5.2 Species-level Relationships

Using the final genus-level hypothesis (Figure 2.20) as a base, a tree will be presented displaying all Australian triclads using all the evidence available (all markers using support values, total evidence and taxonomic congruence) to arrive at the most plausible tree. It must be emphasised here that no objective

algorithm has been used to arrive at the topology shown in Figure 2.16. This tree has been constructed based on molecular evidence and apparent morphological similarities (based on the morphological analysis where possible) for the species with no molecular data. It should be noted that there were no direct conflicts between the species associations displayed in the morphological tree (Figure 2.16) and those suggested by the molecular data.

*Spathula* was the most difficult group to resolve due to the high proportion of species for which only morphological data were available (Figure 2.21). There was an additional difficulty with this genus resulting from the well-established presence of two sister groups, *Eviella* and *Reynoldsonia* (Section 2.3.3.3). *Eviella hynesae* and *Reynoldsonia reynoldsoni* were located firmly within the *Spathula* cluster, a position determined purely from morphological data (Figure 2.16). The 18S marker placed *Romankenkius libidinosus* within the *Spathula* clade (Figure 2.15), as does the morphological analysis, leaving no option but to place it there (Figure 2.16). This *Spathula* clade houses all a lot of traditionally ‘difficult’ species. It is unlikely that a morphological apomorphy for this group will be discovered; yet this possibility will be explored in the character analysis.

*Romankenkius* species do not form convenient eastern and western groups in the morphological analysis (Figure 2.16), however, they do again form two distinct groups. As a consequence the western *Romankenkius* species, which form a coherent well-resolved group based on molecular data (Figure 2.19), have been positioned between *Girardia* and the *Dugesia/Neppia/Schmidtea/Weissius/Cura* cluster as per Figure 2.20. Molecular data were available for all western *Romankenkius* species, except *R. hoernesi*, a species which was easy to place as it shared a strong affinity with *R. musculoglandulosus* (Figure 2.16) in the morphological tree. The remaining *Romankenkius* species, all eastern *Romankenkius*, remain at the base of the tree, associated with *Romankenkius kenki*, where the morphological and molecular data suggest this group should be (Figure 2.16). The relationships within this cluster are not well resolved as they are based entirely on poorly supported morphological hypotheses. However, the arrangement presented is the most probable given the available data.



**Figure 2.21** Summary tree of species relationships including all known Australian species excluding *Dugesia rava*, *Romankenkius sp.* and *Masaharus graffi* (due to a lack of data). Topology not based on an objective algorithm, but on a combination of molecular evidence and apparent morphological similarities (consequently there is no statistical support). Black lines represent species locations as evidenced by the 18S + Morphology data. Grey lines represent a species positioned due to evidence presented by 18S or *cox1* phylogenies. Dashed lines represent species placed based on morphological data (the Planarioidea outgroup represented in blue).

The *Dugesia* group is very well resolved due to the abundance of molecular data for these species (e.g. Figure 2.19). *Dugesia artesiana* is the only *Dugesia* species lacking molecular data, however, the

morphological data were useful in this case, placing this species at the base of the *Dugesia* cluster (Figure 2.16).

*Masaharus* is also well resolved as reliable molecular data were available for two of the four members of this genus (Figure 2.19). No molecular data were available for *Masaharus graffi* and *M. sphincter* and the morphological data for the *Masaharus* species is confused with very low support values (Figure 2.16). *Masaharus sphincter* demonstrated some association with *M. extentus* and is placed accordingly within the *Masaharus* cluster (Figure 2.16). *Masaharus graffi* is located close to *Cura pinguis* and *Weissius capaciductus* in both morphological analyses (Figure 2.16). However, fewer characters were known for *M. graffi* than for any other species included in the analysis (Table 2.5 - 9 of 40). Owing to the difficulty in placing this species it was concluded that the holes in our knowledge justified the exclusion of *Masaharus graffi* from the final hypothesis.

### 2.3.6 Character Analysis

Questions in evolutionary biology are often best addressed by comparing traits in different species. The mapping of characters on phylogenetic trees allows the nature and number of transformations to be identified (Huelsenbeck 2003). The consensus tree (Figure 2.21) has been explored for features that might define apomorphic characters for particular clades. The tree contains primarily Australian species and, while comprehensive for many genera, does not represent the entirety of species diversity for the Dugesiidae. Consequently, the tree is used to identify potentially useful characters, which will then be investigated fully to ensure that any character states are consistent for this genus across the world. Any exceptions to this will be clearly defined as being for Australian species only. Characters were mapped using Mesquite (Maddison & Maddison 2009).

If character analysis is to be used as a true investigative tool, it follows that characters should be mapped on trees inferred from data other than that used to map the characters. The tree that will be utilised, the final hypothesis (Figure 2.21), bears little resemblance to the morphological tree presented (Figure 2.16) as it uses multiple sources of evidence (preferentially molecular markers and then morphology). However, some species have been placed based purely on morphological evidence, which does lessen the integrity of the character analysis somewhat. However, due to the heavy influence of the molecular data on the deeper nodes (i.e. genera), I believe character mapping on this tree to remain a useful and philosophically justified exercise (Appendix 3b). Please note that the Geoplanidae are not included in this analysis, as this would have necessitated a substantial shift in focus that was deemed unnecessary for the purpose.

#### 2.3.6.1 Diagnosing Species

The Mesquite analysis has shown that the characters in Table 2.9 represent autapomorphies for certain species of Australian Dugesiidae.

**Table 2.9** Autapomorphies for certain species of Australian Dugesiidae.

Character	State	Species
1	<b>Lunate head shape (7)</b> This highly polymorphic character varies a great deal within genera. A lunate head shape is common in Geoplanidae species but currently unknown in any other Australian dugesiids.	<i>Masaharus informis</i>
8	<b>Mouth half way between hind end and root of the pharynx (3)</b> The mouth is positioned at the hind end of the pharyngeal pocket in the Dendrocoeliidae, Planariidae and generally the Maricola. The Dugesiidae also primarily show this arrangement, the only exception being within <i>Girardia</i> , which exhibit a range of states (states 0, 1, 2, 3 & 4). <i>Romankenkius sinuosus</i> is the only Australian species with a different state. The Geoplanidae also exhibit many variations.	<i>Romankenkius sinuosus</i>
9	<b>Testes in middle of the body (3)</b> <i>Neppia jeanneli</i> , <i>Girardia hypoglauca</i> also exhibit this character state, however, within Australia this character state is unique for <i>Romankenkius conspectus</i> . This character state is not found commonly outside of the Dugesiidae; Planariidae (ventral), Dendrocoelidae (dorsal and ventral), Maricola (ventral), Geoplanidae (dorsal, ventral and dorso-ventral).	<i>Romankenkius conspectus</i>
14	<b>Oviducal loop present (1)</b> The fusion of long caudal branches in the posterior end of the body has not been reported for any other <i>Spathula</i> and has been observed in very few triclads in general.	<i>Spathula simplex</i>
16	<b>Shell glands absent (0)</b> Shell glands are present in all Australian dugesiids either entering the diverticulum/common oviduct or the bursal canal. The absence of shell glands could be due to the immature state of this individual.	<i>Romankenkius</i> sp. (2006)
22	<b>Bursal canal connected to expansion (3)</b> The most common character state for the Dugesiidae, Dendrocoelidae, Planariidae and Maricola is the bursal canal communicating with a copulatory bursa. The only exception to this is <i>Eviella hynesae</i> , which has a greatly expanded bursal canal in place of a bursa. The Geoplanidae often lack a copulatory bursa, the oviducts simply communicating with a glandular duct arising from a common atrium.	<i>Eviella hynesae</i>
23	<b>Dactylose projections on bursal canal</b> The presence of these structures seems to be confined to <i>Reynoldsonia reynoldsoni</i> .	<i>Reynoldsonia reynoldsoni</i>
25	<b>Bursa sitting posterior to penis bulb</b> The location of the bursa in most dugesiids is anterior to the penis bulb. There are several dugesiid species exhibiting deviations from this arrangement, i.e. sitting lateral to the penis bulb. <i>Romankenkius retrobursalis</i> is the only dugesiid to have a posteriorly located bursa, a character state it shares with the Maricola.	<i>Romankenkius retrobursalis</i>
30	<b>Eversible penial papilla</b> This character state is rare among freshwater triclads and is otherwise confined to several dendrocoelid species. This character is also uncommon amongst the Geoplanidae and Maricola. In regards to the Dugesiidae it appears that this character is an apomorphy for <i>Reynoldsonia reynoldsoni</i> .	<i>Reynoldsonia reynoldsoni</i>
32	<b>Separate ejaculatory ducts</b> This character state appears to be an apomorphy for <i>Romankenkius impudicus</i> (particularly within the Australian dugesiids), as it is known only in one other dugesiid, <i>Girardia biapertura</i> . This is not a common feature in any of the other triclad groups.	<i>Romankenkius impudicus</i>
37	<b>Adenodactyl ventral</b>	<i>Romankenkius boehmigi</i>

There have been many species described as having an adenodactyl within the Dugesiidae including other *Romankenkius* species and several *Dugesia*. A discussion relating to the likelihood of homology of these structures will follow, however, *Romankenkius boehmigi* is the only species within the Dugesiidae where the adenodactyl sits ventral to the penial papilla.

The remaining species may only be diagnosed via a combination of characters. A complete key to the Australian dugesiids is included below in Chapter 3.

### 2.3.6.2 Diagnosing Genera

The summary tree of species relationships (Figure 2.21) has been explored for characters that can be postulated as defining apomorphies for particular clades. Determining apomorphies for genera has traditionally been a difficult task and this analysis is not different. This thesis includes a detailed character analysis, which is summarised here. For further exploration of these ideas, including character trace trees, please see Appendix 3b. Please note that the characters referred to throughout this discussion are those summarised in Table 2.6 and detailed in Appendix 3a.

**Diagnosis:** *Eviella* is a dugesiid identifiable by non-reversed musculature that extends over a bursal canal that expands in place of a copulatory bursa.

**Diagnosis:** *Reynoldsonia* is a dugesiid identifiable by dactylose projections on the bursal canal and an eversible penial papilla.

**Comments:** There are only two dugesiid genera that exhibit definite apomorphies, *Schmidtea* and *Dugesia*. *Reynoldsonia* and *Eviella* are each defined by an apomorphy but this is a result of their monotypic status (Table 2.9). Figure 2.4 places both of these genera amongst the *Spathula* species. While this location is justified for *Reynoldsonia reynoldsoni*, *Eviella* should maintain its current generic status. For a detailed discussion around this, see the *Spathula* character analysis and Appendix 3.

**Diagnosis:** *Schmidtea* is a dugesiid exhibiting a double seminal vesicle.

**Diagnosis:** *Dugesia* is a dugesiid with a diaphragm that receives the opening of glands in the ejaculatory duct.

**Comments:** The diaphragm found in several *Romankenkius* species, specifically, *R. libidinosus*, *R. beohmigi* and *R. patagonicus*, is probably not homologous with that found in *Dugesia* (Sluys 1997).

The remaining genera can only be defined using a combination of characters. Table 2.10 lists characters that are present in all members of any particular genus. Several characters are not helpful in grouping genera as they relate to species-level apomorphies (8, 11, 14, 20, 23, 30, 32, 39 & 40: Table 2.10). Uninformative characters are those that are found in all representatives of a genus but are also widespread throughout the family and therefore of no diagnostic use.

**Possible Diagnosis:** *Neppia* is a dugesiid with an exceptionally thick layer of circular muscle surrounding the bursal canal and a convoluted ejaculatory duct.

**Comments:** The two characters proposed as defining *Neppia*, convoluted ejaculatory duct and thick circular muscle on the bursal canal, were not included in my analysis. Both are extremely subjective and, in general, do not occur in Australian species. *Neppia* is in clear need of review. The only Australian representative is *Neppia magnibursalis*, which was assigned to this genus via a process of elimination (Sluys & Kawakatsu 2001). In doing so one of the proposed apomorphies, the convoluted ejaculatory duct, had to be ignored as it is not present in the Australian representative.

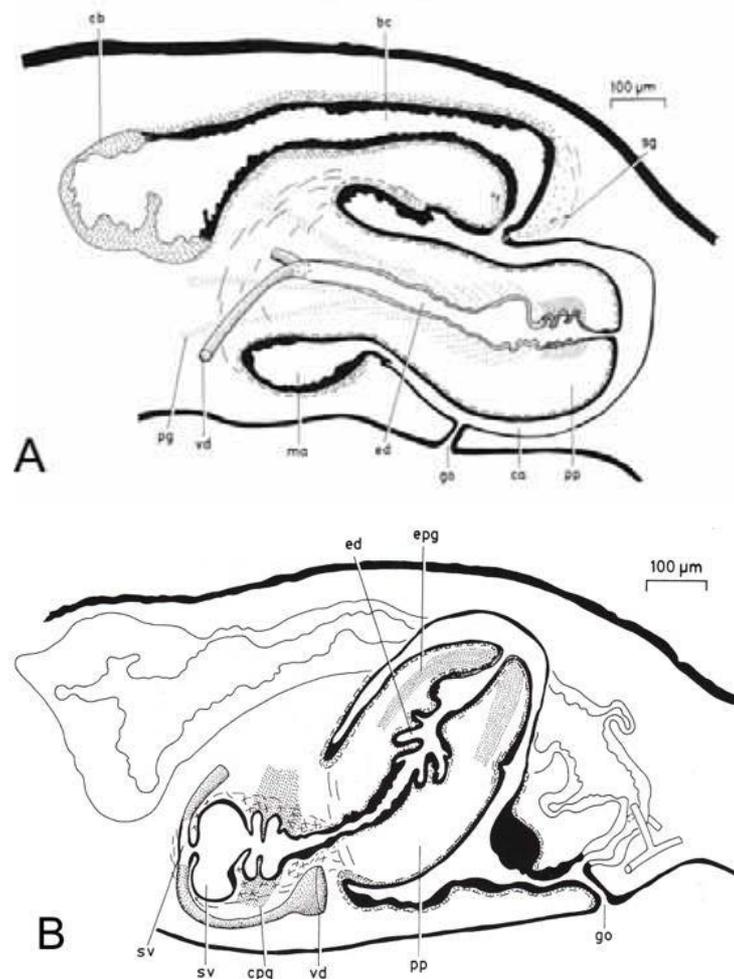
**Diagnosis:** *Cura* is a dugesiid with only circular muscle on the bursal canal and a finger-shaped penial papilla.

**Comments:** *Weissius* is currently represented by a single species, *Weissius capaciductus*. This is an interesting species, as it possesses several characters that are reminiscent of *Cura* and in particular *Cura pinguis*, the only Australian *Cura* species. The absence of a short common oviduct, described as a characteristic feature of *Cura*, led Sluys et al. (2007) to reject *Cura* as a suitable genus for *W. capaciductus*. However, this character is not consistent across the genus and appears to be polymorphic in *C. pinguis*, which is often described as having separate entries of the oviducts into the bursal canal (Grant et al. 2006; Sluys & Kawakatsu 2001)(Appendix 1). It is for this reason that I question the generic placement of this species and suggest that it should sit within *Cura*.

**Table 2.10** Morphological characters shared by all members of each genus (character and character state details as per Table 2.6 and Appendix 3a). Characters highlighted in blue represent proposed apomorphies for the genera from current analysis, Sluys (2001) and Sluys et al. (2007).

Genus	Informative Characters	Uninformative Charaters
Dugesia	6 (0) - ciliated pits absent 7 (0) - sensory fossae absent 9 (1) - dorsal testes 16 (2) - shell glands entering the bursal canal 26 (1) - gonoduct present 29 (3) - plug shaped penial papilla <b>33 (1) - diaphragm</b>	3, 10, 13, 18, 22, 25, 28, 31, 34, 38
Neppia	From Sluys (2001): <b>28 (1) - thick circular muscle on the bursal canal present</b> <b>29 (1) - convoluted ejaculatory duct present</b>	
Schmidtea	From Sluys (2001): <b>34 (3) - double seminal vesicle present</b> 18 (2) - intermingled musculature on bursal canal	
Weissius	From Sluys <i>et al.</i> (2007): 18 (3) - circular muscle around bursal canal 29 (0) - finger shaped penial papilla 21 (0) - posterior point of communication between the atrium and the bursal canal 15 (0) - common oviduct/diverticulum absent	
Cura	From Sluys (2001), Sluys and Kawakatsu (2001) and Kawakatsu and Mitchell (1982): 6 (0) - ciliated pits absent 15 (0) - common oviduct/diverticulum absent 18 (3) - circular muscle around bursal canal 29(0) - finger or thumb shaped penial papilla 21 (1 & 2) - point of communication of the atrium and the bursal canal anterior or middle	
Romankenkius (western)	4 (1) - solid dorsal pigmentation 5 (1) - less than dorsal <b>15 (2) - common oviduct present opening into the most postero-ventral section of the bursal canal or even into the common atrium</b> 16 (1) - shell glands entering the diverticulum (all except <i>R. glandulosus</i> ) 24 (0) - musculature around the bursa absent • common oviduct lacking surrounding musculature	3, 13, 17, 18, 22, 27, 33
Girardia	From Sluys (2001) and Sluys et al. (2005): 2 (2) - long auricles 6 (0) - ciliated pits absent 18 (0 & 2) - non-reversed and intermingled musculature on bursal canal <b>39 (1) - pigmented pharynx present (except several species including three polymorphic for this character)</b>	
Masaharus	2 (2) - long auricles 7 (0) - sensory fossae absent 10 (0) - testes situated throughout the body length 16 (2) - shell glands entering the bursal canal 18 (0 & 2) - non-reversed and intermingled musculature on bursal canal 24 (1) - muscle surrounding bursa (excluding <i>Masaharus</i> sphincter).	15, 17, 19, 22, 25, 26, 27, 28, 31, 33, 36, 37
Eviella	From Ball (1977b): <b>25 (0) - Bursal canal connected to expansion</b> 11 (1) - Fused testes	
Reynoldsonia	From Ball (1974): <b>23 (1) - Dactylose projections on bursal canal</b> <b>30 (1) - Eversible penial papilla</b>	
Spathula	6 (1) - ciliated pits present (except <i>Sp. miserable</i> ) <b>13 (1) - caudally branched oviducts present</b> 16 (2) - shell glands entering the bursal canal (excluding <i>R. libidinosus</i> ) 21 (0) - bursal canal communication with atrium posteriorly. 25 (1) - bursa sitting anterior to penis bulb	17, 22, 28, 31, 33, 35
Romankenkius (eastern)	4 (1) - solid dorsal pigment <b>15 (2) - diverticulum present opening into the most postero-ventral section of the bursal canal or even into the common atrium</b> <b>16 (1) - shell glands entering the diverticulum (all except the underdeveloped <i>R. sp</i> (2006))</b> 18 (1) - reversed musculature on the bursal canal • <b>musculature surrounding the diverticulum</b>	3, 17, 27, 28, 31, 33
Planarioidea		

I suggest the combination of bursal canal musculature (character 18) and penial papilla shape (character 29) should be used to diagnose *Cura*. All *Cura* species, except *Cura fortis*, have a unique (amongst dugesiids) condition of circular musculature surrounding the bursal canal (character 18, state 3). This character is also present in *Weissius capaciductus*. Additionally, Sluys and Kawakatsu (2001) described *Cura fortis* as having a “thumb” shaped penial papilla, presumably to provide a parallel between it and the “finger” shaped penial papilla described for *Cura pinguis* and *Cura foremanii*. I believe the papilla of *C. fortis* could be more accurately described as plug or cylindrical shaped (as described for *Spathula fontinalis*), and has no particular anatomical homology with the finger-shaped penial papilla of the remainder of *Cura* species and *Weissius* (Figure 2.22). I therefore propose that *Cura fortis* is not a true *Cura* and needs to be housed elsewhere within the Dugesiidae. Further, *Weissius* would now fit comfortably within the revised definition of *Cura* and therefore *Weissius* could be regarded as a junior synonym of *Cura* and its only species referred to as *Cura capaciducta*.



**Figure 2.22** A) Sagittal reconstruction of the copulatory apparatus of *Cura fortis*, B) Sagittal reconstruction of the copulatory apparatus of *Spathula fontinalis* (Sluys and Kawakatsu 2001)(Abbreviations: sv - seminal vesicle, vd - vas deferens, pp - penial papilla, go - gonopore, ed - ejaculatory duct, pg - penis gland).

**Diagnosis:** *Girardia* is a dugesiid with or without a pigmented pharynx, possibly with non-reversed musculature on the bursal canal, possibly with pointed auricles and an angled bursal canal.

**Comments:** Until recently the musculature on the bursal canal had provided a useful tool in identifying *Girardia* species (character 18, state 0). Sluys (2001) described all *Girardia* species as having non-reversed musculature, a relatively rare character amongst the Dugesiidae. However, several species have been re-described and many others assigned to this genus, effectively eroding this presumed apomorphy. While there are some shared characters within the genus *Girardia* (e.g. the absence of ciliated pits and the absence of branching oviducts), these are weak diagnostic characters. This genus is invasive in Australia, and so beyond the confirmation that Australia has no native *Girardia*, the current analysis can offer no further resolution of this issue.

**Diagnosis:** *Masaharus* is a dugesiid with long auricles, lacking sensory fossae, testes are situated throughout the body length and either intermingled or non-reversed musculature on the bursal canal.

**Comments:** The description of a new Australian genus and species, *Masaharus extentus* (Appendix 1), prompted a review of the so-called Australian *Girardia* species. As discussed above, *Girardia* has no unequivocal diagnostic characteristics. In fact one of the few features that is consistent across all *Girardia* species is the absence of ciliated pits, a feature that appears in several of the Australian species previously assigned to it. Unfortunately, this new genus, in its current form, does not possess an apomorphy that easily diagnoses its members. The common characters are as listed in Table 2.10. It is true that this is a fairly weak set of characters to define a genus, however, a combination of characters, including the musculature on the bursal canal, precludes assignment to any other currently known genus (character 18, state 0 & 2, Table 2.10).

There is some doubt about the assignment of *Masaharus sphincter* (formerly *Girardia*) to the genus *Masaharus*. *Masaharus sphincter* has a unique combination of characters, making it difficult to define characters for the genus. The exclusion of *Masaharus sphincter* from *Masaharus* allows the recognition of several strong defining characters for *Masaharus* (Section 2.3.6.2). Despite this, a conservative approach has been taken and *Masaharus sphincter* remains in this genus until further species or characters are discovered that would justify the erection of a new genus (Figure 2.25).

**Diagnosis:** *Spathula* is a dugesiid with caudally branching oviducts but lacking a true diverticulum, most likely possessing reversed musculature on the bursal canal.

**Comments:** Bursal canal musculature, and the absence of a true diverticulum (see *Romankenkius* discussion below) combine to provide a reasonably useful diagnosis for this genus ((Sluys 2001), Character 18, state 1 and Character 13, state 1). Unfortunately, there are exceptions to this rule. *Spathula simplex*, possesses only a layer of circular muscle on its bursal canal (Appendix 3b). This species was described from a single specimen and requires further investigation (Grant et al. 2006). When *Reynoldsonia reynoldsoni* was described, Ball (1974b) deemed it necessary to erect a new genus for it. While this is understandable given the dactylose projections on the bursal canal and the eversible penial papilla, the remaining characters fit clearly into our current diagnostic framework for *Spathula* (please note that *R. reynoldsoni* has been described with both non-reversed and intermingled musculature of the bursal canal) (Ball 1974b; Hay & Ball 1979). I therefore concur with Sluys' (2001) musing that this species is in fact an aberrant *Spathula*.

**Diagnosis:** *Romankenkius* is a dugesiid with a muscled diverticulum of the bursal canal that receives the openings of shell glands.

**Comments:** The currently accepted diagnosis for *Romankenkius* includes: the presence of a seminal vesicle (character 34, state 1), reversed musculature of the bursal canal (character 18, state 1) and presence of a diverticulum of the bursal canal (character 15, state 1). The seminal vesicle and the reversed musculature of the bursal canal are not useful diagnostic characters as they are found throughout the Dugesiidae (Table 2.5). There is also confusion surrounding the diagnostic value of the diverticulum (Ball 1974a; Meixner 1928; Sluys 1997). The musculature surrounding the duct/diverticulum does appear to provide a great deal of information. Table 2.11 provides a summary of the musculature and position of the shell glands for all Australian dugesiids possessing a duct/diverticulum.

It is clear from Table 2.11 that there are two different types of duct: muscled diverticula or non-muscled common oviducts. When the details of the musculature are examined a clear pattern emerges. All “eastern” *Romankenkius*, excluding *Romankenkius sinuosus* and *Romankenkius* sp, possess a muscled diverticulum. The description of the latter species was extremely tentative and many features were not mentioned. The lack of musculature on *Romankenkius sinuosus*, on the other hand, warrants further discussion. Sluys described this species as lacking a muscular coat on the diverticulum. Based on our recent supposition, this structure (diverticulum) may therefore be described as a common oviduct. While “common oviducts” are also found in the “western” *Romankenkius*, the presence of a long caudal branch of

the oviducts advocates for the transfer of this species to the genus *Spathula*, placing it amongst species possessing common oviducts (eg. *Spathula libidinosus* and *Spathula truculenta*).

**Table 2.11** Details of the diverticulum/common oviducts of all Australian dugesiids exhibiting this character (+ presence of character state, - absence of character state).

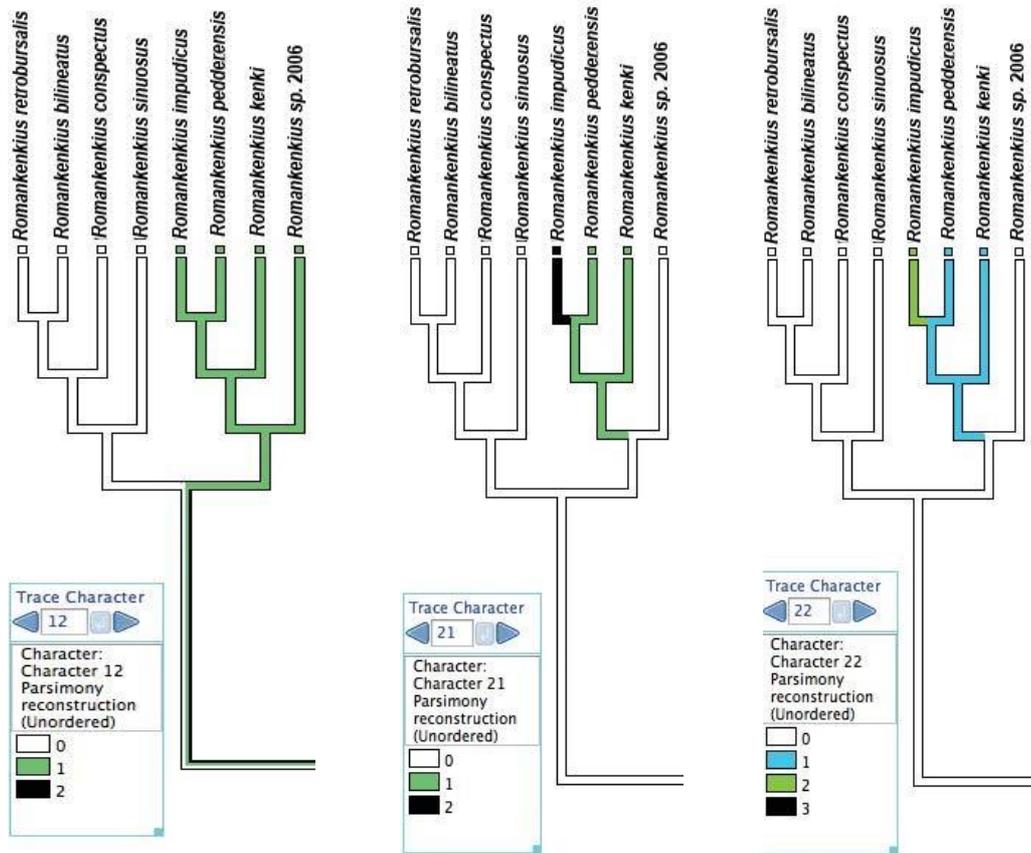
Species	Muscled	Glands Entering	Point of Origin	Location
<i>R. bilineatus</i>	+	Duct	Ventral (Bursal Canal)	Eastern
<i>R. kenki</i>	+	Duct	Ventral (Bursal Canal)	Eastern
<i>R. pedderensis</i>	+	Duct	Ventral (Bursal Canal)	Eastern
<i>R. conspectus</i>	+	Duct	Common Atrium	Eastern
<i>R. impudicus</i>	+	Duct	Ventral (Bursal Canal)	Eastern
<i>R. retrobursalis</i>	+	Duct	Ventral (Bursal Canal)	Eastern
<i>R. sp.</i>	-	?	Common Atrium	Eastern
<i>R. boehmigi</i>	-	Common Atrium	Common Atrium	Western
<i>R. glandulosus</i>	-	Bursal Canal	Common Atrium	Western
<i>R. hoernesi</i>	-	Duct	Common Atrium	Western
<i>R. musculoglandulosus</i>	-	Duct	Ventral (Bursal Canal)	Western
<i>R. rutrum</i>	-	Duct	Ventral (Bursal Canal)	Western
<i>R. libidinosus</i>	-	Duct	Ventral (Bursal Canal)	Eastern
<i>S. truculenta</i>	-	Bursal Canal	Ventral (Bursal Canal)	Eastern
<i>R. sinuosus</i>	-	Duct	Ventral (Bursal Canal)	Eastern
<i>S. oblongata</i>	-	Common Atrium	Common Atrium	Eastern
<i>D. orientoaustralis</i>	-	Bursal Canal	Dorsal (Bursal Canal)	Eastern

This process has introduced an invaluable diagnostic character, the diverticulum (with muscle) or the common oviduct (lacking muscle). This agrees with the molecular data, which shows a clear division between the eastern and western *Romankenkius* species (Figure 2.19). As a result a new genus may be required to house the “western” *Romankenkius*.

**Possible Diagnosis:** A new genus to house western *Romankenkius* would be distinguished by the presence of a non-muscled common oviduct and the absence of a caudal branch of the oviducts.

### 2.3.6.3 Intra-genus relationships

There are three speciose Australian groups, eastern *Romankenkius*, western *Romankenkius* and *Spathula*. Due to the difficulties associated with the morphological tree, the relationships within these genera have been hard to clarify. Consequently, a different approach has been applied in order to place these species on the final tree. This approach utilises a the investigation of a combination of reliable character states to arrive at species relationships.

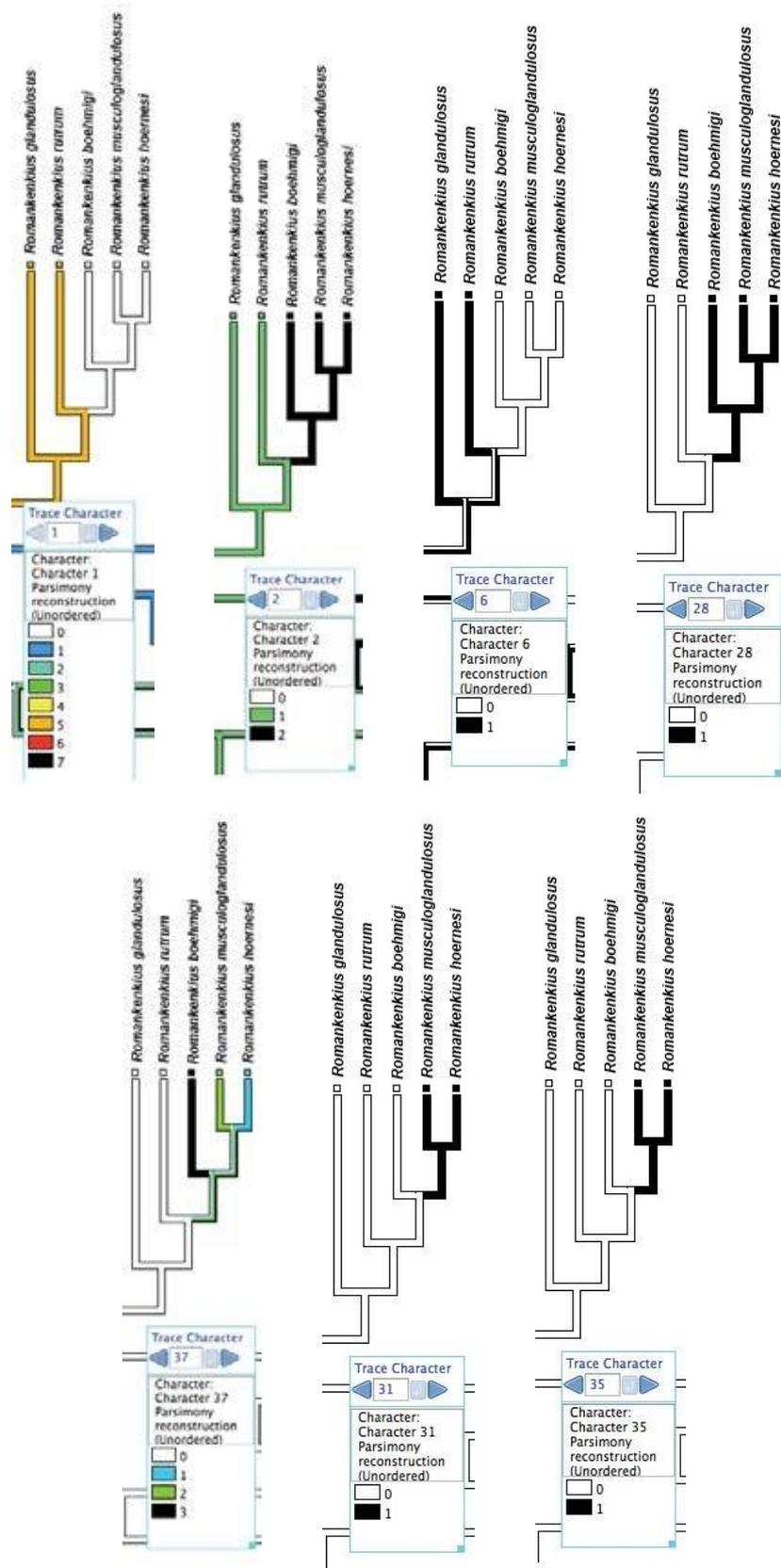


**Figure 1.23** Extracts from character trees for characters 12 (position of ovary), 21 (communication of bursal canal with atrium) and 22 (bursal canal connected to...).

As I am proposing the transfer of *Romankenkius sinuosus* to the *Spathula* group, I will not be including this species as part of the *Romankenkius* analysis. Additionally, a great deal of morphological information is missing for *Romankenkius sp. (2006)* and therefore its location on the tree is extremely unreliable. Eastern *Romankenkius* is divided on the tree into two clusters. Cluster 1, *R. retrobursalis*, *R. bilineatus*, *R. conspectus*; Cluster 2, *R. impudicus*, *R. impudicus*, *R. kenki*. There are several characters that suggest possible clusters within western *Romankenkius* (i.e. *R. glandulosus* and *R. rutrum* share a high proportion of characters as do *R. hoernesii* and *R. musculoglandulosus*). Table 2.12, Figure 2.23 (eastern *Romankenkius*) and Figure 2.24 (western *Romankenkius*) detail the characters that conform to these clusters and support this relationship hypothesis.

**Table 2.12** Summary of characters that link eastern and western *Romankenkius* intra-genus clusters

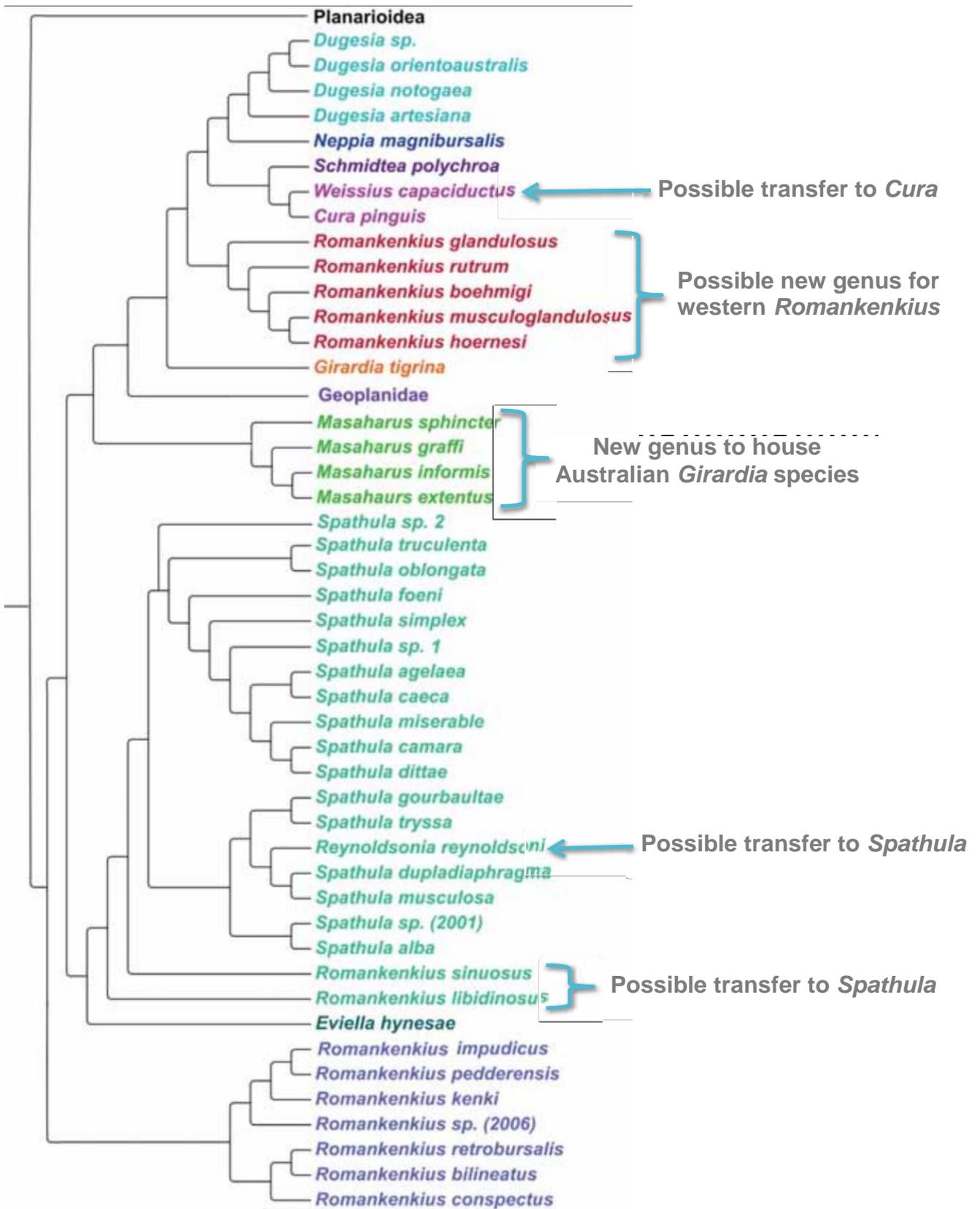
Genus	Character	Character State	Species
<i>Eastern Romankenkius</i>	12 - position of the ovary	0 - directly behind the brain	<i>R. retrobursalis</i> <i>R. bilineatus</i> <i>R. conspectus</i>
		1 - a short distance behind the brain	<i>R. impudicus</i> <i>R. pedderensis</i> <i>R. kenki</i> <i>R. sp (2006)</i>
	21 - communication of bursal canal withatrium 22 - bursal canal connected to...	0 - posterior communication 0 - connected to copulatory bursa	<i>R. retrobursalis</i> <i>R. bilineatus</i> <i>R. conspectus</i> <i>R. sp (2006)</i>
		1 & 2 - lateral or dorsal communication 1 & 2 - connected to intestine	<i>R. impudicus</i> <i>R. pedderensis</i> <i>R. kenki</i>
<i>Western Romankenkius</i>	1 - Head Shape 2 - Auricles 6 - Ciliated pits	0 - low triangular 1 - short auricles 0 - pits absent	<i>R. boehmigi</i> <i>R. musculoglandulosus</i> <i>R. hoernesii</i>
		5 - truncate 2 - long auricles 1 - pits present	<i>R. glandulosus</i> <i>R. rutrum</i>
	28 - Glandularisation of common atrium 37 - Adenodactyl	0 - glandularisation absent 0 - adenodactyl absent	<i>R. glandulosus</i> <i>R. rutrum</i>
		1 - glandularisation present 1 - adenodactyl present	<i>R. boehmigi</i> <i>R. musculoglandulosus</i> <i>R. hoernesii</i>
31 - Penial folds 35 - Penis glands opening through the epithelium of the penial papilla	0 - penial folds absent 0 - glands absent	<i>R. glandulosus</i> <i>R. rutrum</i> <i>R. boehmigi</i>	
	1 - penial folds present 1 - glands present	<i>R. musculoglandulosus</i> <i>R. hoernesii</i>	



**Figure 2.24.** Extracts from character trees for characters 1 (head shape), 2 (auricles), 6 (ciliated pits), 28 (glandularisation of common atrium), 37 (adenodactyl), 31 (penial folds) and 35 (penis glands opening through the epithelium of the penial papilla).

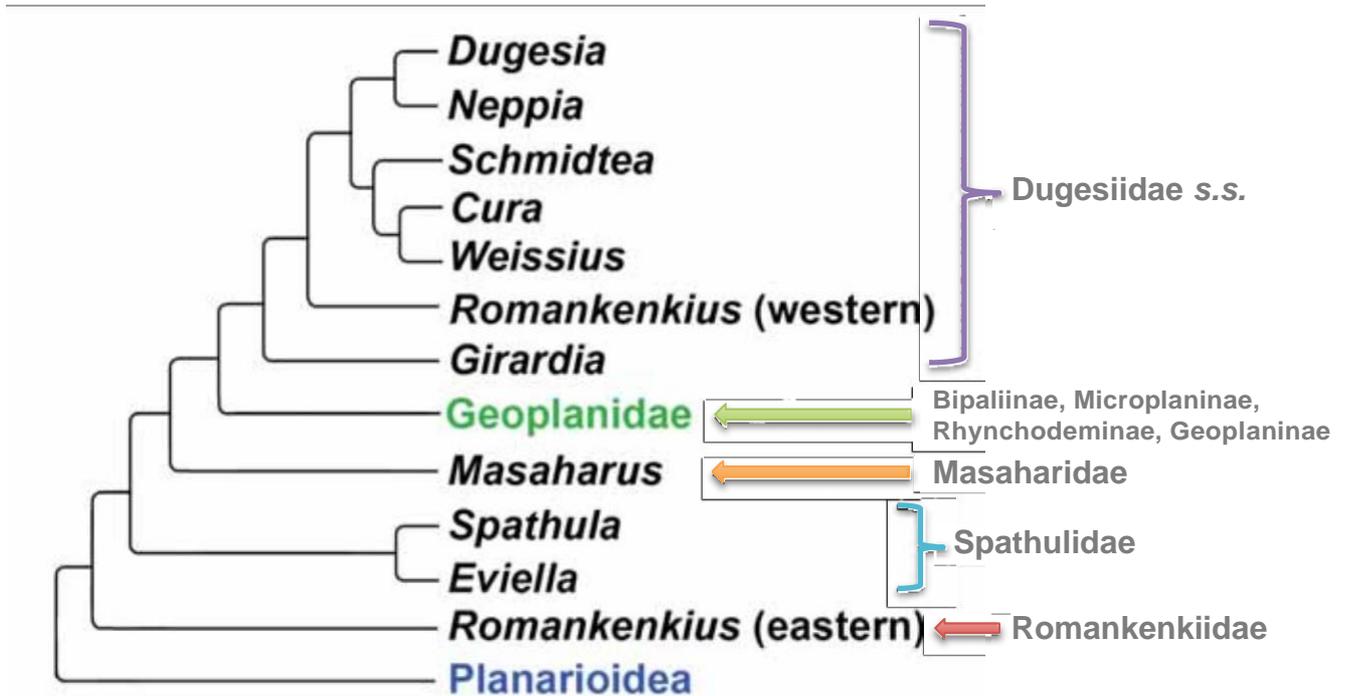
After a lengthy investigation, no characters could be identified that clearly separated the two *Spathula* clusters. *Spathula* was surprisingly consistent for most characters, yet, for each character there would be one or two species displaying an alternative state. The only possible exception to this was the seminal vesicle (Character 34, Appendix 3b - Figure 1). There are three states found in the *Spathula* group for this character, with some congruence with the clusters illustrated. In order to achieve complete congruence many alterations to the original relationship hypothesis would have to be made, but this is not justifiable, as it would mean ignoring much other legitimate evidence for this apparently highly adaptable character. The fact that no “cluster-defining” character could be discovered for *Spathula* could be a comment on the hypothesis or on the nature of the group.

2.3.7 Final Hypothesis



**Figure 2.25** Final hypothesis for relationships among the Australian Dugesiidae species. Proposed taxonomic changes highlighted and the Planarioidea outgroup, represented in black (please note - this tree has been developed using several lines of evidence, consequently statistical support values cannot be assigned to nodes).

Figure 2.25 represents the synthesis of all phylogenetic analyses, molecular and morphological, incorporating any changes that the character analysis advocates. It includes all known Australian species and several representatives from non-Australian genera. As the character analysis suggested several possible changes to the current genus relationships, a summary tree of these changes is also provided (Figure 2.26).



**Figure 2.26** Summary of generic relationships for the Continenticola, focussing on the Geoplanoidea (Geoplanidae highlighted in green, the Planarioidea outgroup represented in blue). Proposed new family relationships for the superfamily Geoplanoidea are highlighted on the right (please note - this tree is developed using a combination of evidence, consequently statistical support values cannot be assigned to nodes).

### 2.3.7.1 Taxonomic Implications

Due to the various conflicts between the molecular and morphological analyses, a conservative approach has been adopted in regards to taxonomic recommendations; regardless there are several points that must be emphasised. The first of these is the fact that the Australian *Girardia* is not *Girardia sensu stricto*. Thus *Masaharus* is coined for all Australian “*Girardia*” species (excepting of course *Girardia tigrina*, which is introduced).

Secondly, it has become increasingly obvious throughout the course of this research that the position of the Geoplanidae renders the Dugesiidae paraphyletic. Figure 2.26 presents a new familial structure, introducing several new families to better represent relationships. The Romankenkiidae (*Romankenkius* (eastern)), Spathulidae (*Eviella* and *Spathula*), Masaharidae (*Masaharus*) and Dugesiidae

*sensu stricto* (s.s.) (*Dugesia*, *Neppia*, *Schmidtea*, *Cura*, *Weissius*, *Romankenkius* (western) and *Girardia*) are suggested to house the freshwater Geoplanoidea genera. Further discussion can be found in Chapter 3. For the purposes of clarity these new family names will be used, where necessary, throughout the remainder of this thesis.

Despite the fact that the taxonomic status of the Dugesiidae has been contentious for some time (e.g. Álvarez-Presas et al. 2008; Álvarez-Presas & Riutort 2014), this family is still the accepted receptacle for all freshwater representatives of the Geoplanoidea (Sluys et al. 2009). While the changes to the family structure will not be formalised at this stage, owing to a lack of clear morphological synapomorphies, throughout the remainder of this thesis this clearly paraphyletic group will be referred to as the Dugesiidae *sensu lato* (or “dugesiid”). This terminology indicates that I am referring to the Dugesiidae *s.l.* in the broadest sense (i.e. all freshwater geoplanoid taxa) as opposed to the newly restricted concept of the family Dugesiidae *s.s.* (Dugesiidae *sensu stricto* in the previous paragraph).

## 2.4 Final Discussion

### 2.4.1 The Romankenkiidae and Spathulidae

Figure 2.26 demonstrates clearly the final hypothesis for the relationships of the Dugesiidae *s.l.* genera resulting from this extended analysis. Molecular evidence suggests that true *Romankenkius* is confined to the eastern states. Morphological evidence has also been presented to suggest a differentiating character between the two *Romankenkius* groups (Section 2.3.6.2, Table 2.11 and 2.16). Eventually this may lead to the erection of a new genus for the “western *Romankenkius*” species, a taxon sharing a more recent common ancestor with *Girardia* than with the eastern *Romankenkius* (Figure 2.26).

Since the description of *Romankenkius* by Ball (1974c) the morphological link between *Spathula* and *Romankenkius* has been the main focus of phylogenetic analyses (De Vries & Sluys 1991; Sluys 1997). Sluys (2001) placed *Spathula* at the base of the Dugesiidae *s. l.* on morphological grounds, suggesting that this is the most ancient of the current genera. While the current analysis disagrees with this assessment, instead placing *Romankenkius* at the base of the tree, new information begins to clarify the relationship between *Spathula* and *Romankenkius* and *Spathula* remains near the base of the tree. While *Spathula* and *Romankenkius* have long been considered closely related it is the position of the aberrant *Eviella* that has presented difficulties (Ball 1977b; Sluys 1997). Because no molecular evidence was available for *Eviella*, it was necessary to rely on character evidence to place this genus. The analysis clearly suggests *Eviella* is more basal than *Spathula* and may in fact represent a link between the *Spathula* and its ancestor.

*Spathula* is interesting as it is the most speciose genus in Australia and achieves its highest diversity in Australia, being only otherwise found in New Zealand. The New Zealand species, *Spathula alba*, slotted in perfectly amongst the Australian *Spathula*, supporting the idea that both the Australian and New Zealand *Spathula* share a common ancestor (Figure 2.15, 2.17-2.19). This is easy to reconcile, as

they represent an ancient genus (further discussion of the links between the Australia and New Zealand fauna is in 2.4.3 and Chapter 4). *Romankenkius libidinosus* may be reassigned to *Spathula* due to many shared characters with this genus. The difficulty in assigning this species to *Spathula* lies in the fact that it also shares several unique characters with *Romankenkius*, hence its position at the base of the *Spathula* clade (Figure 2.25). *Romankenkius sinuosus*, another species that may more comfortably sit within the genus *Spathula* (Figure 2.25), also claims its relatively basal position due to ancient links identified in the character analysis (Section 2.3.6). The other species that may be reassigned to *Spathula*, *Reynoldsonia reynoldsoni*, has some unique features but it is likely that these are derived characters and this species has evolved from the Spathulidae (Section 2.3.6).

Within *Spathula* the molecular data suggests that there may be two clusters (Figure 2.25), but no morphological character can be identified as being typical of one cluster or another. This issue in finding a defining character may lie with the phylogenetic hypothesis, keeping in mind that many species have been positioned using only morphological data, or it could be due to the nature of the evolution of *Spathula* (I suspect a little bit of both). The abundance of shared characters in the genus *Spathula* as a whole suggests a relatively recent radiation (at least for the Australian species).

There is only one *Romankenkius* species reported outside of Australia. The original descriptions of *R. patagonicus* provide no evidence to suggest that this species is not a *Romankenkius* (Böhmig 1902; Borelli 1901, Sluys et al. 2005). This species is most likely more closely related to the eastern *Romankenkius* group due to the fact that the diverticulum is surrounded by musculature (Böhmig 1902). This discovery has obvious biogeographical implications that will be discussed in Chapter 4.

The idea that the DugesIIDae *s.l.* genera share a closer relationship with the Geoplanidae than with their freshwater relatives, the Planarioidea, is not a new one (Ball 1981; Carranza et al. 1998 a,b; Sluys 1989b). Baguña et al. (2001a) were the first to include a *Spathula* (no *Romankenkius* or *Masaharus* samples were available) in their *cox1* analysis, which suggested that *Spathula* might share a closer relationship with the Geoplanidae than it does with the remaining freshwater genera. This was conceivable as morphological analyses routinely placed *Spathula* near the base of the DugesIIDae *s.l.* tree (Sluys 1997; Sluys 2001). With a set of molecular markers Álvarez-Presas et al. (2008) justifiably placed *Spathula* and *Romankenkius* amongst the Geoplanidae. It should be noted that Álvarez-Presas et al. (2008) utilised *Romankenkius libidinosus* in this analysis: I believe that this species is a representative of *Spathula* (Section 2.3.6). Regardless, these results provoked a reassessment of the relationships, with the Terricola\* (Geoplanidae) regarded as housing *Spathula* and *Romankenkius* (Figure 2.1). The current analysis reinforces the relationships between the Geoplanidae and the DugesIIDae *s.l.*, without complete clarification. Although the new data does point to a position between the basal groups including *Spathula*, *Romankenkius*, *Masaharus* and the Geoplanidae, the need for a review of the Geoplanoidea classification is highlighted (Figure 2.26, Section 2.3.3.3). In a more recent analysis, a *Spathula* and *Romankenkius* cluster, with an eastern *Romankenkius* at the base of the cluster, forms a sister group to one of the terrestrial

families (Figure 2.2)(Álvarez-Presas and Riutort 2014). This placement represents a minor change to the original analysis (Álvarez-Presas et al. 2008), namely the lack of a basal terrestrial group for the cluster, and is more concordant with the current hypothesis (Figure 2.26).

The current analysis provides many robust outcomes. The first of these is the placement of eastern *Romankenkius* at the base of the Geoplanoidea (Figure 2.26). Figure 2.26 implies that eastern *Romankenkius* shares an ancient common ancestor with all freshwater and terrestrial Geoplanoidea. The idea that *Romankenkius* has arisen directly from the common ancestor to the entire Geoplanoidea is supported by the high species diversity of *Romankenkius* in southern Australia, which aligns with the Gondwanan centre of origin theory (Ball 1981).

### 2.4.2 The Masaharidae

One of the major points of difference between the current research and all research preceding it is the inclusion of the new genus *Masaharus*. While it is probable that *Masaharus* will be redefined in the future, what is clear is that this taxon is important for an understanding of Dugesiidae *s.l.* evolution. During the molecular analysis this genus provided the greatest difficulty in settling into a location: this difficulty was primarily due to the inclusion or exclusion of the Geoplanidae. When the Geoplanidae are excluded, support values across the board rise, however this could be due to inclusion of fewer taxa in the analysis. While the molecular support values were higher when *Masaharus* was located between *Spathula* and the Geoplanidae there was reasonable support for its position at the base of the *Spathula* clade. Ultimately, the unique combination of characters for this genus left no alternative but to place it between *Spathula* and the Geoplanidae (Figure 2.26).

### 2.4.3 The Dugesiidae *s.s.*

There is much more conformity in the results for the Dugesiidae *s.s.* as all analyses showed very similar relationships between the genera. The genus *Girardia*, in which the majority of the *Masaharus* species were formerly housed, is undoubtedly a representative of the Dugesiidae *s.s.* *Girardia* does, of course, occur in Australia, yet, only in the form of the invasive *Girardia tigrina*. *Girardia* appears to be at, or close to, the root of the Dugesiidae *s.s.*, an idea that is supported by several other researchers (e.g. Álvarez-Presas et al. 2008; Álvarez-Presas and Riutort 2014)

The most interesting taxon within the Dugesiidae *s.s.* is the “western *Romankenkius*” group. While it would have been morphologically most parsimonious to simply include this genus as a sister group to (eastern) *Romankenkius*, this would have conflicted with all of the molecular evidence (Figure 2.19). The morphological resemblance between the two is primarily due to the presence of a diverticulum or common oviduct (Section 2.3.6.2)(Table 2.11). If we accept that all freshwater triclads have evolved from a common ancestor, western *Romankenkius* could simply have retained many of the morphological

characters of this ancestor. This is possibly an unlikely option considering how much more derived western *Romankenkius* is in relation to its eastern counterpart. However, both eastern and western *Romankenkius* live in similar environments (i.e. temperate, mountainous), which could have resulted in the preservation of traits (or alternatively, convergent evolution). Although the weight of evidence is possibly great enough to justify a new genus for the western *Romankenkius* group, we have followed a conservative approach in this thesis and maintained its temporary label (western *Romankenkius*). What is clear is that the origin of the Dugesiidae s.s. will certainly inspire an interesting biogeographical discussion.

*Girardia*, *Cura* and *Schmidtea* were invariably nested together in morphological phylogenies (Sluys 1997; Sluys et al. 1998), and in Sluys' most recent phylogeny of the Dugesiidae s.l., *Dugesia* and *Neppia* join this cluster (Sluys 2001). Early molecular studies supported this arrangement, with some variation regarding the precise relationships (Baguñà et al. 2001a; Carranza et al. 1998a). The analysis by Álvarez-Presas et al. (2008) placed *Dugesia* and *Schmidtea* at the tip of the Dugesiidae s.s. with *Cura* and then *Neppia* located in more basal positions (Álvarez-Presas et al. 2008). The hypothesis proposed here shows an arrangement that sits between the molecular and morphological ideas. At the "tip" of the tree the genera *Cura* and *Schmidtea* sit as a sister group to *Dugesia* and *Neppia* (Figure 2.26). The fact that *Dugesia* has a widespread distribution in Australia is of significant interest. Prior to the discovery of this genus in Western Australia, it was widely assumed that *Dugesia* had migrated from the north, an idea supported by the high diversity of this genus in the northern hemisphere. This new distributional development possibly complicates this hypothesis. A thorough investigation of the biogeographical implications will follow in the biogeography chapter.

Figure 2.19 (Section 2.3.3.3) clearly demonstrates that the Australian *Dugesia* are closely related to *Dugesia* elsewhere, including species from Asia (*Dugesia japonica* and *Dugesia ryukyuensis*) and Europe (*Dugesia gonocephala* and *Dugesia subtentaculata*). Unlike many of the Dugesiidae s.l. genera, *Dugesia* has a clear defining character, the diaphragm, and appears as monophyletic in morphological analyses (Sluys 2001). This means that we can have some confidence that they all have a common ancestor. However, the question remains, does this common ancestor have a northern or southern origin?

Morphological analyses always place *Neppia* and *Dugesia* in close proximity. Until 1974 *Neppia* was considered a sub-genus of *Dugesia* (Sluys 1997; Sluys et al. 1998, Sluys and Kawakatsu 2001). The molecular analysis of Baguñà et al. (2001a), placed *Neppia* in a very similar location to the current analysis, as a sister group to *Dugesia*. The only other molecular analysis including this genus (Álvarez-Presas et al., 2008) placed *Neppia* at the base of this cluster of four genera, and presented *Schmidtea* as the sister to *Dugesia*. Although *Neppia* was not included in the most influential of my analyses (18S and Morphology), this analysis clearly placed *Schmidtea* and *Cura* as sister groups, while the *cox1* and morphological data placed *Neppia* as the sister group to *Dugesia*. The obvious difficulty when dealing with *Neppia* in regards to evolution and biogeography is the poor state of knowledge in regards to this genus. I

suspect that in time this genus will be redefined and the Australian *Neppia magnibursalis* and the New Zealand species, *Neppia montana*, reassigned. However, if these are truly members of *Neppia* (i.e. share a common ancestor with all other *Neppia* species), the fact that they only occur in the southern hemisphere (Australia, New Zealand, Africa and South America) may hold the answer to the question of the precise location of the genus within the Geoplanoidean phylogeny. The fact that we have two highly derived genera, *Dugesia* and *Neppia*, that appear to have a natural distribution in Australia is exciting. The question needs to be asked, how did these highly derived species come to be in New Zealand and Australia?

It is clear that the Dugesiidae s.s. genera share a relatively recent common ancestor. Sluys' (2001) morphological analyses also place *Schmidtea* and *Cura* as sister groups, usually closely associated with *Girardia*. Until recently, molecular analyses, primarily using 18S, had agreed with the nature of the relationship between *Cura* and *Schmidtea* suggested by both the current hypothesis and morphological phylogenies (Baguña et al. 2001a; Carranza et al. 1998a; Sluys 1997; Sluys et al. 1998). Where they differed was in the location of *Girardia*. My morphological analyses place *Girardia* in a more derived location than *Cura/Schmidtea*, while the molecular analyses place *Girardia* at the base of the cluster. The most recent alternative hypothesis, presented by Álvarez-Presas and Riutort (2014)(Figure 2.2), disagrees with some aspects of this arrangement, primarily the placement of *Neppia* at the base of the Dugesiidae s.s. cluster. This discrepancy suggests that even the seemingly stable Dugesiidae s.s. still needs further examination, including all known taxa.

*Schmidtea* consists of only four species distributed in Northern Africa and throughout large parts of Europe. This is the distribution one would expect from a genus so far removed from the proposed Gondwanan groups. Like *Neppia*, the presence of *Cura* in Australia is more difficult to reconcile. There are only four *Cura* species currently known, three exist in Australia/New Zealand and one in North America (although the placement of *Cura fortis*, from New Zealand, has been questioned). This distribution suggests that the Australian species are natives. However, *Cura pinguis* has a very broad distribution in Australia and has the ecological characteristics of an invasive species (relatively broad ecological niche - Appendix 1a); while the presumably closely related *Weissius capaciductus*, has a much more restricted distribution. This issue will be investigated further in the biogeography chapter. The presence of *Cura*, ~~*Neppia*~~ *Dugesia* in Australia requires some extensive biogeographical investigation. All appear to have a relatively recent common ancestor but may have arrived in Australia via different routes.

#### 2.4.4 The Geoplanidae

One of the major characters that has been discussed at length and first led investigators to suggest a closer relationship between the Dugesiidae s.l. and the Geoplanidae is the complex multicellular pigment cup found in the eyes of both land and freshwater triclads (Sluys 2001). This relationship has

since been supported by a range of molecular evidence, with Álvarez-Presas et al. (2008) suggesting that the Geoplanoidea went from freshwater to land and back. However, this idea is questioned in their more recent research (Álvarez-Presas and Riutort 2014). The current analysis (Figure 2.26) also disputes this idea of a return to freshwater, as the relationship shown between the Geoplanidae and Dugesiidae *s.l.* does not necessitate a return to freshwater.

Freshwater and land Geoplanoidea share many characters, which makes the idea of switching between freshwater and land plausible. The main characters in which they differ are: the position of mouth, musculature of bursal canal, and location of bursa. The position of the mouth is very variable in Geoplanidae species, while most Dugesiidae *s.l.* have the mouth sitting at the hind end of the pharyngeal pocket. There are some examples of variation within the Dugesiidae *s.l.*, even within species (eg. *Romankenkius sinuosus* and *Girardia antillana*). Musculature on the bursal canal is commonly non-reversed in the Geoplanidae and reversed in the Dugesiidae *s.l.* Again however, there are a few examples of “dugesiid” species with non-reversed musculature (eg. *Romankenkius libidinosus*, *Eviella hynesae* and *Girardia*). It is interesting that two examples of species with “Geoplanidae” musculature on the bursal canal also happen to be difficult-to-place species from the basal *Romankenkius* and *Spathula* (i.e. do *Romankenkius libidinosus* and *Eviella hynesae* represent transitional evolutionary stages?). One of the most useful diagnostic characters for the Dugesiidae *s.l.* (also for the Planariidae and Dendrocoelidae) is the presence of a bursa sitting anterior to the penis bulb. The situation in land triclads is reversed, with the bursa commonly sitting posterior to the penis bulb. There are of course examples of Dugesiidae *s.l.* exhibiting the same state (eg. *Romankenkius retrobursalis*) and many with the bursa sitting lateral to the penis bulb. Like many positional characters it is not difficult to conceive of the plasticity of these kinds of characters and the ease with which they may evolve and change.

An additional ecological point further supporting the idea of a simple transition between the land and freshwater Geoplanoidea is that freshwater representatives are often described as riffle invertebrates, however, my collection experience suggests that many species are very comfortable living in the slow flowing edges of rivers and streams, and often in areas barely inundated (Appendix 1). This suggests that some freshwater Geoplanoidea are comfortable in an almost-terrestrial environment, albeit a soggy one.

#### 2.4.4 Final Remarks

The Dugesiidae *s.l.* genera share many morphological characters, which have caused issues for taxonomists when attempting to differentiate them. For example, certain *Romankenkius* species have some “*Spathula*”-like characters. While an inconvenience for scientists, this difficulty implies a close relationship between Romankenkiidae, Spathulidae and Masaharidae (not precluded by the current hypothesis), a relationship that will be explored further in the biogeographical discussion (Chapter 4).

The remainder of the freshwater genera (the Dugesiidae *s.s.*) are a much simpler group to resolve, with much higher support values on all trees, but some inconsistencies remain. Some of these inconsistencies were previously known and others noted as a result of the current research.

There are several potential explanations for incongruent results between studies. The issue may lie in the inclusion of new genera, or the differing markers used for the analysis. While Álvarez-Presas et al. (2008) and Álvarez-Presas and Riutort (2014) did not include any morphological data, they used a different set of molecular markers (18S, 28S and elongation factor 1a) in their analysis. One thing that has been agreed upon by all that have attempted to construct a phylogeny of the Geoplanoidea is that the current markers are not sufficient. While each new analysis provides exciting progress, this ancient taxon requires a combination of approaches to piece together its evolutionary history.

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## Chapter 3: Taxonomy of the Australian Dugesiidae s.l.

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### 3.1 Introduction

Provided here is a historical review of prior work on the Dugesiidae s.l. in Australia, a summary of the taxonomic work completed as part of the thesis and a proposal for a new classification for the Dugesiidae s.l. Please note, as discussed in Chapter 2, the family previously known as Dugesiidae is proposed to be paraphyletic and therefore references to it in the broader sense (i.e. referring to all freshwater Geoplanoid genera), will include the qualifier *sensu lato* (s.l.).

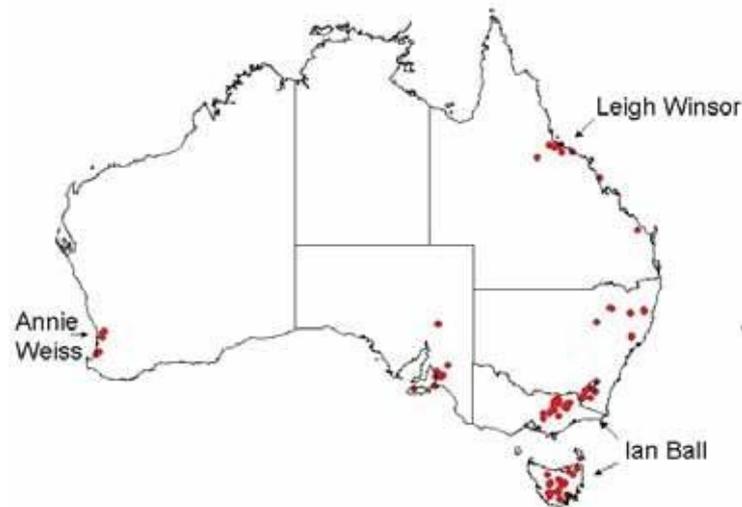
#### 3.1.1 The Dugesiidae s.l. in Australia

Over 30 years after Ball (1974a) wrote his review of Dugesiidae s.l., the family is still considered poorly known globally, nowhere more so than in the Australasian region. “Dugesiids” regularly pose a significant challenge as the genera are often poorly defined and species sometimes assigned purely by a process of elimination. An excellent example of these vague generic diagnoses comes from Nurse (1950).

“Spathula: *Dugesiidae* with rounded, truncate or spathulate head, with 2 eyes, or lacking eyes, pigmented or white. With a single vesicle or none at all, with numerous dorsal or ventral testes.....”

The difficulties involved in the preservation, sectioning and identification of “dugesiids” explain, for the most part, the lack of research performed on this group. They require specific fixatives containing relaxants due to their soft-bodied nature (Schockaert et al. 2008). Most “dugesiids” can only be identified by their sexual apparatus, as the external morphology lacks significant diagnostic characters. Study of the minute detail of the copulatory apparatus requires serial sectioning and graphical reconstruction, an extremely labour-intensive process. Immature or asexual specimens often cannot be identified, further complicating taxonomic study of the Dugesiidae s.l.

Annie Weiss reported the first Australian freshwater “dugesiid” in 1909 when she described six new species from Western Australia, commenting on the diversity and uniqueness of the specimens (Weiss 1909)(Figure 3.1). Despite subsequent collecting efforts by Winsor, Ball, and Sluys, the Australian fauna is still considered poorly known (Sluys & Kawakatsu 2001) (Figure 3.1).



**Figure 3.1** All collecting sites (and main collectors) in Australia prior to 2002.

This lack of knowledge contrasts sharply with other regions of the world, which have received more attention in the past and from which large numbers of species have been reported. The desire to learn more of the Dugesiidae *s.l.* in Australia is amplified by the fact that, in spite of limited previous collecting effort, Australia boasts the highest generic diversity of the Dugesiidae *s.l.* Endemism is also a feature of the Australian fauna, with two endemic genera and all but one of more than 20 known species being endemic (Sluys et al. 1998). The current taxonomic study will considerably increase our knowledge of the Australian fauna, with the associated advances in phylogenetic and biogeographic understandings. This chapter will summarise the taxonomic work done as part of this thesis.

### 3.1.2 The Taxonomic Status of the Australian Dugesiidae *s.l.*

At this stage the Dugesiidae *s.l.* is the only freshwater triclad family known from Australia. There is one report of a Planariidae in Western Australia (*Planaria rava*), however this specimen has been tentatively reassigned to *Dugesia* (Grant et al. 2006). The Australian dugesiid fauna consists of eight genera housing altogether 24 endemic species (Figure 3.5). The only non-endemic species is the invasive *Girardia tigrina*. Chapter 2 (Section 2.1.2) introduced all of the pertinent details relating to our prior understanding of the dugesiid genera. A detailed diagnosis for all Australian genera and species, including comments concerning the assignment process, for all new and revised taxa is provided as part of this thesis. Images are provided displaying examples of the internal and external morphology, as well as updated distribution maps and ecological details. Due to the volume of material, all taxonomic work performed as part of this thesis, both published

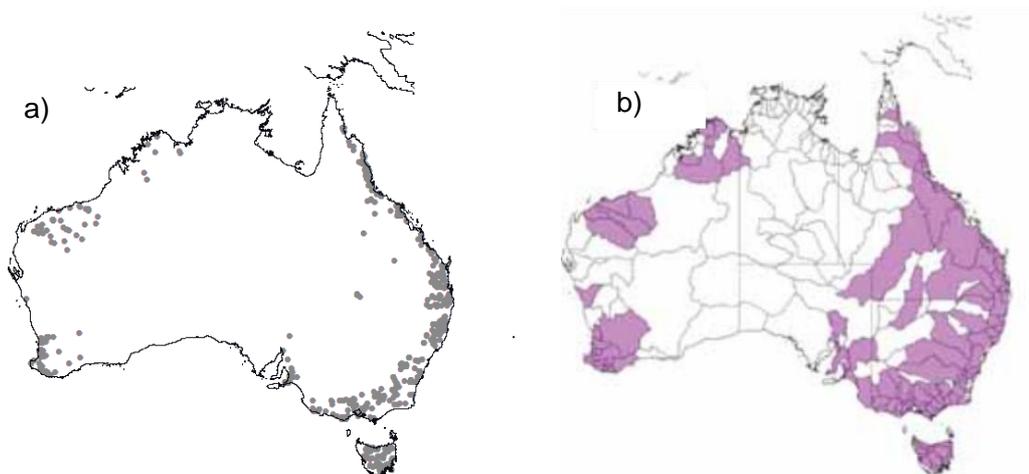
and unpublished, is available in Appendix 1. This chapter will discuss some of the taxonomic implications of this work. In the context of this thesis it was not deemed necessary, or even desirable, to formalise every taxonomic implication by assigning species to other or new genera. Such formalisations will be considered for future publications, notably for the as-yet unpublished information presented in Appendix 1. However, for clarity and consistency, the remaining discussions within this thesis will adopt these “taxonomic suggestions”.

## 3.2 Methods

### 3.2.1 Collection

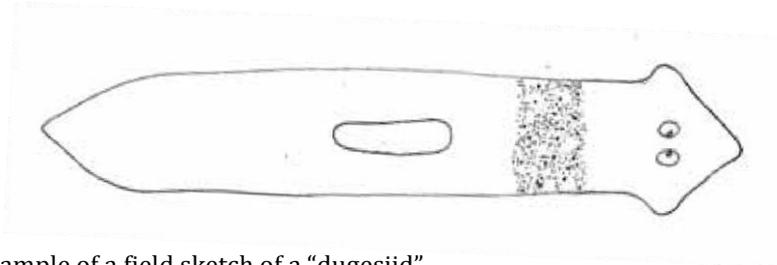
Freshwater triclads were collected from freshwater environments throughout Australia. Rivers, creeks, dams and lakes were sampled with the aim of maximising the number of drainage divisions covered. Two basic collecting strategies were used. The most common collecting method was direct inspection of the underside of rocks or similar objects providing a solid substrate. “Dugesiiids” were removed using a small brush and kept alive in a specimen jar before preservation. The second, less reliable method was the sampling of vegetation. The inhabitants of macrophytes were washed into a sorting tray to enable triclads to be separated from the other invertebrates.

Over 500 sites were visited and “dugesiiids” were found at over 400 of these (Figure 3.2a). Sites in previously unsampled drainage divisions were given priority over sites in drainage divisions from which “dugesiiids” had already been collected. All distribution maps were created in ArcView GIS 3.2 (ESRI 1992). Data were recorded for each site, detailing location and habitat characteristics (for more details see Appendix 1e).



**Figure 3.2** a) Map of Australia with all sampling sites represented b) map of Australia with all river basins sampled during the study highlighted; maps adapted from Geoscience Australia (2015).

Prior to preservation of specimens, details on external morphology were recorded, including: size, form, eyes, other sensory structures, dorsal colouration, ventral colouration, pharynx/intestine, copulatory apparatus and a rough sketch illustrating many of the features described (Figure 3.3).



**Figure 3.3** Example of a field sketch of a “dugesiid”.

After collection, “dugesiids” were preserved separately for molecular analysis or histological processing.

### 3.2.2 Fixation and Preservation

Triclad usually contract and swell and often expel their pharynx during customary fixation. This creates a serious issue as triclads are largely identified by the microanatomy of the copulatory apparatus, a flexible, muscular structure. For this reason a good relaxant must be a component of any fixative. The most successful relaxant for triclads is nitric acid and therefore the fixative chosen, Steinmann’s Fluid, contains this combined with other fixing reagents (300ml concentrated nitric acid (65-70%) + 300ml saturated solution of mercuric chloride in 5% sodium chloride + 300ml distilled water)(Steinmann 1911).

Prior to fixation the triclads were placed in a Petri dish containing a film of water and the triclads were allowed to undertake their normal gliding movements. When the animals were as fully stretched as possible the Steinmann’s fluid was quickly poured over them and left for approximately five minutes. The fixative was removed and the specimens rinsed several times with water (to remove the mercury from the tissues). Specimens were then transferred into a vial containing 70% ethanol for long-term preservation. Individuals preserved for molecular work were simply preserved in 100% ethanol (further details in Chapter 2).

### 3.2.3 Specimen Embedding, Sectioning and Staining

Specimens were placed in embedding cassettes, dehydrated and taken to paraffin wax in a tissue transfer processor. This process involves 12 steps, which are detailed in Appendix 1e.

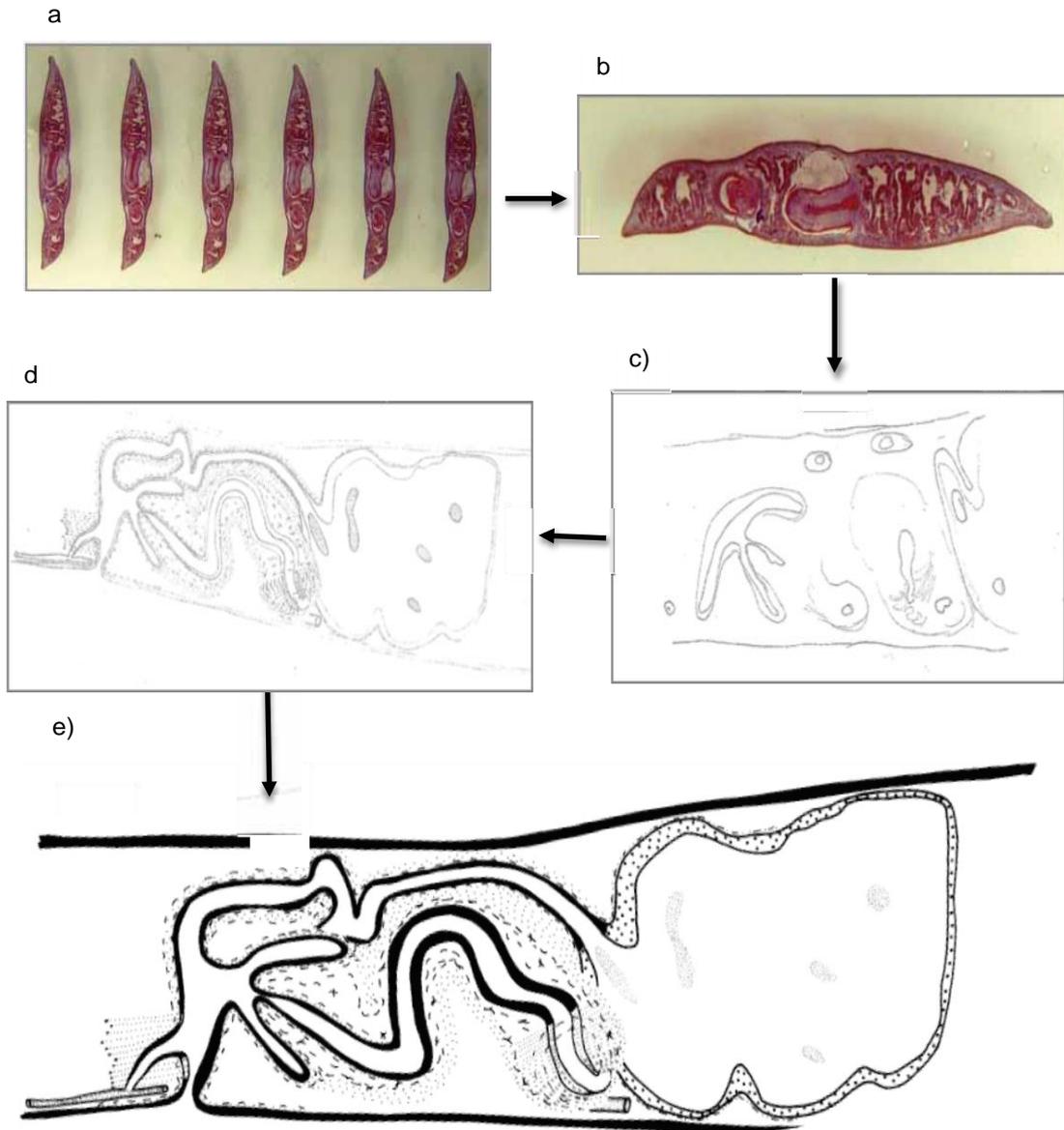
Specimens were examined for the presence of a copulatory apparatus when first cleared. Those specimens that were not sexually mature were placed back in 70% alcohol via a re-hydration process consisting of three 100% alcohol steps. Sexual individuals were embedded in a paraffin block and orientated depending upon whether sagittal, horizontal or transverse sections were desired.

When completely hardened, blocks were trimmed and serially sectioned at 5µm, sections rehydrated and stained using the trichrome stain, Martius-Scarlet-Blue (MSB) (Lendrum et al. 1962). A correctly applied MSB stain will differentially stain nuclei (blue), erythrocytes (yellow), muscle, collagen (blue) and fibrin and is not prone to fading over time (McMichael & Iredale 1959). Bouin's fluid (Bouin 1897) was used as a mordant, the complete staining and mounting process is illustrated in Appendix 1e.

### 3.2.4 Reconstruction and Identification

All species were identified following a reconstruction of their internal anatomy. Specimens studied included those collected as part of this research and those made available from the previously unstudied Ball Collection, collected during the 1980s. These specimens had been processed at the University of Amsterdam, yet never inspected. The slides were fortunately recovered in early 2000 and made available for reconstruction and identification.

Reconstruction and identification were achieved by sketching, with the use of a compound microscope and a drawing tube, approximately every 3<sup>rd</sup> serial section of each specimen (Figure 3.4 a-b). After these sketches were complete it was possible, using tracing paper and overlaying each sketch, to reconstruct the entire internal anatomy of the specimen, thus providing the necessary diagnostic characters for identification (Figure 3.4 c-e).



**Figure 3.4** An overview of the reconstruction process; a) stained serial sections, b) one section from which a sketch will be taken, c) example of such a sketch d) a preliminary reconstruction e) a finished reconstruction.

### 3.2.5 Molecular Identification

Molecular data has the potential to be a useful tool for assisting with the placement of enigmatic species within the correct genus and to suggest the existence of new genera. Furthermore, since the copulatory apparatus is often only present for the part of the year when individuals are sexually reproducing, sequences from asexual specimens can still permit their placement within a known species or genus (see Appendix 2b).

This discussion and Appendix 1a (Systematic Review and Revision of Taxonomy) will reference molecular data when justifying taxonomic decisions. For these purposes the mitochondrial *cytochrome c oxidase subunit I (cox1)* gene was sequenced as well as part of the 18S region of the nuclear ribosomal RNA. A total of 330 *cox1* sequences and 92 18S sequences were successfully obtained. More information regarding the methods used to sequence and analyse these regions can be found in Chapter 2 (Section 2.2). Chapter 2 (Table 2.3) also contains a detailed summary of the molecular data available, either via Genbank or through the author's efforts, for every Australian triclad.

## 3.3 Results

### 3.3.1 Summary of taxonomic work performed as part of this thesis

**Table 3.1.** Summary of taxonomic work presented in this thesis (newly described species appear in bold).

Species	Progress	Reference*
<b><i>Cura</i></b>	<b>Knowledge of distribution of <i>Cura pinguis</i> advanced, description of one new species</b>	
<i>Cura pinguis</i>	Existing species re-description and increased knowledge of distribution and ecology	Grant et al. (2006), Appendix 1d
<b><i>Dugesia</i></b>	<b>Three new species descriptions, one genus re-assignment and one existing species re-description</b>	
<b><i>Dugesia artesiana</i></b>	<b>New species description</b>	<b>Sluys et al. (2007)</b>
<i>Dugesia notogaea</i>	Existing species re-description	Grant et al. (2006), Appendix 1d
<i>Dugesia (?) rava</i>	Re-examination and genus re-assignment from <i>Planaria</i>	Grant et al. (2006)
<b><i>Dugesia orientoaustralis</i></b>	<b>New species description</b>	<b>Appendix 1d</b>
<b><i>Dugesia sp.</i></b>	<b>Tentative species description</b>	<b>Appendix 1d</b>
<b><i>Eviella</i></b>		
<i>Eviella hynesae</i>	Not examined	Ball (1977b)

<b><i>Girardia</i></b>	<b>Four species removed from genus - invasive only</b>	
<i>Girardia tigrina</i>	Re-description and increased knowledge of distribution and ecology	Appendix 1d
<b><i>Neppia</i></b>		
<i>Neppia magnibursalis</i>	Not examined	Ball (1974c)
<b><i>Masaharus</i></b>	<b>New genus erected, two genus re-assignments, one new species description and one tentative species description</b>	
<i>Masaharus (?) graffi</i>	Re-examination and genus re-assignment from <i>Girardia</i>	Grant et al. (2006), Appendix 1d
<b><i>Masaharus informis</i></b>	<b>New species description and genus re-assignment from <i>Girardia</i></b>	<b>Grant et al. (2006), Appendix 1d</b>
<i>Masaharus sphincter</i>	Genus re-assignment from <i>Girardia</i>	Appendix 1d
<b><i>Masaharus extentus</i></b>	<b>New species description</b>	<b>Appendix 1d</b>
<b><i>Reynoldsonia</i></b>	<b>Genus dissolved, species re-assignment</b>	
<i>Reynoldsonia reynoldsoni</i>	Possible identification refer to <i>Spathula sp. 2</i>	Ball (1974b), Appendix 1d
<b><i>Eastern Romankenkius</i></b>	<b>Three new species descriptions, four species re-descriptions and two tentative species descriptions</b>	
<i>Romankenkius bilineatus</i>	Animals closely resembling this species examined	Ball and Tran (1979), Appendix 1d
<i>Romankenkius cf. bilineatus</i>	Species resembling <i>Romankenkius bilineatus</i> described.	Appendix 1d
<b><i>Romankenkius conspectus</i></b>	<b>New species description</b>	<b>Grant et al. (2006)</b>
<i>Romankenkius hoernesii</i>	Not examined	Sluys (1997)
<b><i>Romankenkius impudicus</i></b>	<b>New species description and existing species re-description</b>	<b>Grant et al. (2006), Appendix 1d</b>
<i>Romankenkius kenki</i>	Existing species re-description	Grant et al. (2006), Appendix 1d
<i>Romankenkius libidinosus</i>	Possible new generic assignment discussed, existing species re-description	Grant et al. (2006), Appendix 1d
<i>Romankenkius pedderensis</i>	Existing species re-description	Grant et al. (2006), Appendix 1d
<b><i>Romankenkius retrobursalis</i></b>	<b>New species description</b>	<b>Grant et al. (2006)</b>
<i>Romankenkius sinuosus</i>	Possible new generic assignment	Sluys and Kawakatsu

	discussed, not examined	(2001)
<b><i>Romankenkius sp.</i></b>	<b>Tentative species description</b>	<b>Grant et al. (2006)</b>
<b><i>Romankenkius sp.</i></b>	<b>Tentative species description</b>	<b>Appendix 1d</b>
<b><i>Western Romankenkius</i></b>	<b>Possible new generic assignment discussed, two new species descriptions, two species re-descriptions.</b>	
<i>Romankenkius boehmigi</i>	Existing species re-description	Appendix 1d
<i>Romankenkius glandulosus</i>	Existing species re-description	Grant et al. (2006), Appendix 1d
<i>Romankenkius hoernesii</i>	Not examined	Sluys (1997)
<b><i>Romankenkius musculoglandulosus</i></b>	<b>New species description</b>	<b>Appendix 1d</b>
<b><i>Romankenkius rutrum</i></b>	<b>New species description</b>	<b>Appendix 1d</b>
<b><i>Spathula</i></b>	<b>Seven new species descriptions and six species re-descriptions and one genus re-assignment.</b>	
<i>Spathula agelaea</i>	New specimens collected	Appendix 1d
<i>Spathula camara</i>	Existing species re-description	Grant et al. (2006), Appendix 1d
<i>Spathula dittae</i>	Existing species re-description and species amalgamation with <i>Spathula ochyra</i>	Grant et al. (2006) Appendix 1d
<i>Spathula foeni</i>	Existing species re-description	Appendix 1d
<i>Spathula cf. foeni</i>	Species resembling <i>Spathula foeni</i> described.	Grant et al. (2006)
<i>Spathula gourbaultae</i>	Not examined	Ball (1977a)
<b><i>Spathula miserable</i></b>	<b>New species description</b>	<b>Grant et al. (2006)</b>
<b><i>Spathula muscosa</i></b>	<b>New species description</b>	<b>Grant et al. (2006)</b>
<i>Spathula ochyra</i>	Existing species re-description and species amalgamation with <i>Spathula dittae</i>	Grant et al. (2006) Appendix 1d
<i>Spathula reynoldsoni</i>	Genus reassignment	Appendix 1d
<b><i>Spathula simplex</i></b>	<b>New species description</b>	<b>Grant et al. (2006)</b>
<i>Spathula truculenta</i>	Existing species re-description	Grant et al. (2006)
<i>Spathula tryssa</i>	Not examined	Ball (1977a)
<b><i>Spathula oblongata</i></b>	<b>New species description</b>	<b>Appendix 1d</b>
<b><i>Spathula caeca</i></b>	<b>New species description</b>	<b>Appendix 1d</b>
<b><i>Spathula duplodiaphragma</i></b>	<b>New species description</b>	<b>Appendix 1d</b>
<b><i>Spathula sp. 1</i></b>	<b>Tentative species description</b>	<b>Appendix 1d</b>
<b><i>Spathula sp. 2</i></b>	<b>Tentative species description</b>	<b>Appendix 1d</b>
<i>Spathula sp. (2001)</i>	Not examined	Sluys and Kawakatsu (2001)

<b><i>Weissius</i></b>	<b>New genus erected and new species described, discussion of species re-assignment and subsequent dissolving of genus.</b>	
<b><i>Weissius capaciductus</i></b>	<b>New species description</b>	<b>Sluys et al. (2007)</b>
<b><i>Planaria</i></b>	<b>Re-examination and species re-assignment</b>	
<b><i>Planaria rava</i></b>	Re-examination and genus re-assignment	Grant et al. (2006)

\* Please note that copies of papers published as part of this work can be found in Appendix 1, specifically, Appendix 1b (Grant et al. 2006) and Appendix 1c Sluys et al. (2007).

### 3.3.2 Classification for the Dugesiidae s.l.

**Figure 3.5** Current classification (Sluys et al. 2009) of the Dugesiidae s.l. vs proposed classification with a focus on Australian genera (text highlighted in orange denotes proposed changes).

<b>Current Classification</b>	<b>Proposed Classification</b>
Order <b>TRICLADIDA</b> Lang, 1884	Order <b>TRICLADIDA</b> Lang, 1884
Suborder <b>CONTINENTICOLA</b> Carranza, Littlewood, Clough, Ruiz-Trillo, Baguña & Riutort, 1998	Suborder <b>CONTINENTICOLA</b> Carranza, Littlewood, Clough, Ruiz-Trillo, Baguña & Riutort, 1998
Superfamily <b>GEOPLANOIDEA</b> Stimpson, 1857	Superfamily <b>GEOPLANOIDEA</b> Stimpson, 1857
Family <b>DUGESIIDAE</b> Ball, 1974	Family <b>DUGESIIDAE s.s.</b> Ball, 1974
Genus <b><i>Bopsula</i></b> Marcus, 1946	Genus <b><i>Bopsula</i></b> Marcus, 1946
No Australian representatives	No Australian representatives
Genus <b><i>Cura</i></b> Strand, 1942	Genus <b><i>Cura</i></b> Strand, 1942
<i>C. pinguis</i> (Weiss, 1909)	<i>C. pinguis</i> (Weiss, 1909)
Genus <b><i>Dugesia</i></b> Girard, 1850	Genus <b><i>Dugesia</i></b> Girard, 1850
<i>D. artesianana</i> Sluys, 2007	<i>D. artesianana</i> Sluys, 2007
<i>D. notogaea</i> Sluys & Kawakatsu, 1998	<i>D. notogaea</i> Sluys & Kawakatsu, 1998
<i>D. rava</i> (Weiss, 1909)	<i>D. rava</i> (Weiss, 1909)
<i>D. orientoaustralis</i> Appendix 1d	<i>D. orientoaustralis</i> Appendix 1d
Genus <b><i>Eviella</i></b> Ball, 1977	Genus <b><i>Girardia</i></b> Ball, 1974
<i>E. hynesae</i> Ball, 1977	<i>G. tigrina</i> (Girard, 1850)
Genus <b><i>Girardia</i></b> Ball, 1974	Genus <b><i>Neppia</i></b> Ball, 1974
<i>G. ? graffi</i> (Weiss, 1909)	<i>N. magnibursalis</i> Ball, 1977
<i>G. tigrina</i> (Girard, 1850)	Genus <b><i>Schmidtea</i></b> Ball, 1974
<i>G. informis</i> Sluys and Grant, 2006	No Australian representatives
<i>G. sphincter</i> Sluys and Kawakatsu, 2001	Genus <b><i>Recurva</i></b> Sluys, 2013
Genus <b><i>Neppia</i></b> Ball, 1974	No Australian representatives
<i>N. magnibursalis</i> Ball, 1977	Genus <b><i>western Romankenkius</i></b>
Genus <b><i>Recurva</i></b> Sluys, 2013	<i>W.R. glandulosus</i> (Kenk, 1930)
No Australian representatives	<i>W.R. hoernesi</i> Weiss, 1909
Genus <b><i>Romankenkius</i></b> Ball, 1974	<i>W.R. boehmigi</i> (Weiss, 1909)
<i>R. bilineatus</i> Ball & Tran, 1979	<i>W.R. muscologlandulosus</i> Appendix 1d
<i>R. conspectus</i> Sluys & Grant, 2006	<i>W.R. rutrum</i> Appendix 1d
<i>R. impudicus</i> Sluys and Grant, 2006	Family <b>Spathulidae</b>
<i>R. kenki</i> Ball, 1974	Genus <b><i>Eviella</i></b> Ball, 1977
<i>R. pedderensis</i> Ball, 1974	<i>E. hynesae</i> Ball, 1977
<i>R. sinuosus</i> Sluys & Kawakatsu, 2001	
<i>R. retrobursalis</i> Sluys & Grant, 2006	

- R. boehmigi* (Weiss, 1909)  
*R. glandulosus* (Kenk, 1930)  
*R. hoernesii* Weiss, 1909  
*R. libidinosus* Sluys & Rhode, 1991  
*R. musculoglandulosus* Appendix 1d  
*R. rutrum* Appendix 1d
- Genus **Reynoldsonia** Ball, 1974  
*R. reynoldsoni* Ball, 1974
- Genus **Spathula** Nurse, 1950  
*S. agelaea* Hall & Ball, 1979  
*S. camara* Ball, 1977  
*S. dittae* Ball & Tran, 1979  
*S. foeni* Ball, 1977  
*S. goubaultae* Ball, 1977  
*S. miserable* Sluys & Grant, 2006  
*S. muscosa* Sluys and Grant, 2006  
*S. simplex* Sluys and Grant, 2006  
*S. truculenta* Ball, 1977  
*S. tryssa* Ball, 1977  
*S. oblongata* Appendix 1d  
*S. caeca* Appendix 1d  
*S. duplodiaphragma* Appendix 1d
- Genus **Schmidtea** Ball, 1974  
 No Australian representatives
- Genus **Weissius** Sluys, 2007  
*W. capaciductus* Sluys, 2007
- Family **GEOPLANIDAE** Stimpson, 1857  
 Subfamily BIPALIINAE Von Graff, 1896  
 Subfamily MICROPLANINAE Pantin, 1953  
 Subfamily RHYNCHODEMINAE Von Graff, 1896  
 Subfamily GEOPLANINAE Stimpson, 1857
- Genus **Spathula** Nurse, 1950  
*S. agelaea* Hall & Ball, 1979  
*S. camara* Ball, 1977  
*S. dittae* Ball & Tran, 1979  
*S. foeni* Ball, 1977  
*S. goubaultae* Ball, 1977  
*S. libidinosus* Sluys & Rohde, 1991  
*S. miserable* Sluys & Grant, 2006  
*S. muscosa* Sluys and Grant, 2006  
*S. simplex* Sluys and Grant, 2006  
*S. sinuosus* Sluys & Kawakatsu, 2001  
*S. truculenta* Ball, 1977  
*S. tryssa* Ball, 1977  
*S. reynoldsoni* Ball, 1974  
*S. oblongata* Appendix 1d  
*S. caeca* Appendix 1d  
*S. duplodiaphragma* Appendix 1d
- Family **ROMANKENKIIDAE**  
 Genus **Romankenkius** Ball, 1974  
*R. bilineatus* Ball & Tran, 1979  
*R. conspectus* Sluys & Grant, 2006  
*R. impudicus* Sluys and Grant, 2006  
*R. kenki* Ball, 1974  
*R. pedderensis* Ball, 1974  
*R. retrobursalis* Sluys & Grant, 2006
- Family **MASAHARIDAE**  
 Genus **Masaharus** Appendix 1d  
*M. graffi* (Weiss, 1909)  
*M. informis* Sluys & Grant, 2006  
*M. sphincter* (Sluys & Kawakatsu, 2001)  
*M. extentus* Appendix 1d
- Family GEOPLANIDAE Stimpson, 1857  
 Subfamily BIPALIINAE Von Graff, 1896  
 Subfamily MICROPLANINAE Pantin, 1953  
 Subfamily RHYNCHODEMINAE Von Graff, 1896  
 Subfamily GEOPLANINAE Stimpson, 1857

### 3.3.3 Key to Australian Dugesiidae s.l. incorporating suggested classification changes.

- |   |   |                     |
|---|---|---------------------|
| 1 | Sexual structures present.....  | 2                   |
|   | Sexual structures absent.....   | 40                  |
| 2 | Extended caudal branch of the oviducts present.....                         | (Spathulidae) 9     |
|   | Extended caudal branch of the oviducts absent.....                          | 3                   |
| 3 | Diverticulum of bursal canal receiving oviducts (musculature present) ..... | (Romankenkiidae) 28 |
|   | Muscl'd diverticulum of bursal canal absent.....                            | 4                   |
| 4 | Reversed or circular musculature on the bursal canal.....                   | (Dugesiidae s.s.) 5 |

	Non-reversed or mixed musculature on the bursal canal.....	<b>(Masaharidae) 38</b>
<b>5</b>	Extremely thick layer of circular muscle on diverticulum present.....	<i>Neppia magnibursalis</i>
	Extremely thick layer of circular muscle on diverticulum absent.....	<b>6</b>
<b>6</b>	Finger shaped penial papilla present.....	<b>(Cura) 27</b>
	Non-finger shaped penial papilla.....	<b>7</b>
<b>7</b>	Diaphragm in the ejaculatory duct present.....	<b>(Dugesia) 10</b>
	Diaphragm in the ejaculatory duct absent.....	<b>8</b>
<b>8</b>	Dorsal pigmentation mottled.....	<i>Girardia tigrina</i>
	Dorsal pigmentation not mottled.....	(western <i>Romankenkius</i> ) <b>34</b>
<b>9</b>	Bursal canal connected to an expansion.....	<i>Eviella hynaesae</i>
	Bursal canal not connected to an expansion.....	<b>(Spathula) 12</b>
<b>10</b>	Glands entering ejaculatory duct and seminal vesicle, ectal reinforcement of bursal canal absent .....	<i>Dugesia artesiana</i>
	Glands only entering ejaculatory duct, ectal reinforcement of bursal canal present.....	<b>11</b>
<b>11</b>	Common oviduct opening well into bursal canal.....	<i>Dugesia orientoaustralis</i>
	Common oviduct absent.....	<i>Dugesia notogaea</i>
<b>12</b>	Seminal vesicle absent.....	<b>13</b>
	Seminal vesicle present.....	<b>18</b>
<b>13</b>	Dorsal testes.....	<b>14</b>
	Ventral testes.....	<b>16</b>
<b>14</b>	Common oviduct present.....	<b>15</b>
	Common oviduct absent.....	<i>Spathula goubaultae</i>
<b>15</b>	Musculature around bursa present.....	<i>Spathula oblongata</i>
	Musculature around bursa absent.....	<i>Spathula truculenta</i>
<b>16</b>	Oviducal loop present.....	<i>Spathula simplex</i>
	Oviducal loop absent.....	<b>17</b>
<b>17</b>	Ovary directly behind brain.....	<i>Spathula tryssa</i>
	Ovary a short distance behind brain.....	<i>Spathula foeni</i>

<b>18</b>	Sensory fossae present.....	<b>19</b>
	Sensory fossae absent.....	<b>21</b>
<b>19</b>	Plug shaped penial papilla.....	<b>24</b>
	Non-plug shaped penial papilla.....	<b>20</b>
<b>20</b>	Common oviduct present.....	<b>25</b>
	Common oviduct absent.....	<b>26</b>
<b>21</b>	Dactylose projections on the bursal canal present.....	<i>Spathula</i> <i>(Reynoldsonia)</i> <i>reynoldsoni</i>
	Dactylose projections on the bursal canal absent.....	<b>22</b>
<b>22</b>	Ciliated pits absent.....	<i>Spathula miserabile</i>
	Ciliated pits present.....	<b>23</b>
<b>23</b>	Gonoduct present.....	<i>Spathula sp. (2001)</i>
	Gonoduct absent.....	<i>Spathula caeca</i>
<b>24</b>	Musculature around bursa present.....	<i>Spathula camara</i>
	Musculature around bursa absent.....	<i>Spathula ditae</i>
<b>25</b>	Diaphragm present.....	<i>Spathula</i> <i>(Romankenkius)</i> <i>libidinosus</i>
	Diaphragm absent.....	<i>Spathula</i> <i>(Romankenkius)</i> <i>sinuosus</i>
<b>26</b>	Pleated seminal vesicle.....	<i>Spathula musculosa</i>
	Seminal vesicle not pleated.....	<i>Spathula</i> <i>duplodiaphragma</i>
<b>27</b>	Seminal vesicle present.....	<i>Cura (Weissius)</i> <i>capaciductus</i>
	Seminal vesicle absent.....	<i>Cura pinguis</i>
<b>28</b>	Ovary sitting directly behind brain.....	<b>29</b>
	Ovary sitting a short distance behind brain.....	<b>31</b>
<b>29</b>	Adenodactyl present.....	<i>Romankenkius</i> <i>conspectus</i>
	Adenodactyl absent.....	<b>30</b>
<b>30</b>	Bursa located posterior to penis bulb.....	<i>Romankenkius</i> <i>retrobursalis</i>

	Bursa located anterior to penis bulb.....	<i>Romankenkius bilineatus</i>
<b>31</b>	Musculature around bursa present.....	<b>32</b>
	Musculature around bursa absent.....	<b>33</b>
<b>32</b>	Sensory fossae present.....	<i>Romankenkius kenki</i>
	Sensory fossae absent.....	<i>Romankenkius sp. (2006)</i>
<b>33</b>	Ventral testes.....	<i>Romankenkius pedderensis</i>
	Dorsal testes.....	<i>Romankenkius impudicus</i>
<b>34</b>	Adenodactyl present.....	<b>35</b>
	Adenodactyl absent.....	<b>37</b>
<b>35</b>	Seminal vesicle present.....	<b>36</b>
	Seminal vesicle absent.....	<i>Romankenkius musculoglandulosus</i>
<b>36</b>	Pleated ejaculatory duct.....	<i>Romankenkius boehmigi</i>
	Non-pleated ejaculatory duct.....	<i>Romankenkius hoernesi</i>
<b>37</b>	Dorsal testes.....	<i>Romankenkius glandulosus</i>
	Testes extending from ventral to dorsal.....	<i>Romankenkius rutrum</i>
<b>38</b>	Dorsal testes.....	<i>Masaharus informis</i>
	Ventral testes.....	<b>39</b>
	<b>39</b> Short caudal branch of oviducts present.....	<i>Masaharus extentus</i>
	Short caudal branch of oviducts absent.....	<i>Masaharus sphincter</i>
<b>40</b>	Asexual/Immature: mottled dorsal surface, ventral surface unpigmented, low triangular head with long auricles.....	<i>Girardia</i> or <i>Dugesia</i>
	Other Asexual/Immature: possible identification via site data, distinctive external morphology (see Appendix 1) and molecular.....	All others

## 3.4 Discussion

### 3.4.1 Data Obtained

This research aimed to increase our knowledge of the Australian “dugesiid” fauna. This was achieved by sampling over 500 sites around Australia (Figure 3.2a). Sites were widespread across the continent ranging from Chester River in the northeast of the continent (approximately 13.5° South) and Creekton Creek in the southeast of Tasmania (approximately 43° South). Western Australia was also sampled from Beedelup Brook in the south (approximately 34° South) to King Edward River in the north (approximately 14° South). Within these geographical points most regions sampled contained permanent freshwater bodies (Figure 3.2a). Regions not sampled for logistical reasons include the central north of the continent and remote areas in the arid center of the continent that maintain permanent water. Samples were obtained, however, from the isolated mound springs in western Queensland as well as the isolated Wilpena Pound Creek, in the central south of the continent, which is spring fed.

“Dugesiids” were collected from over 400 of the sites sampled (Figure 3.2a). This intensive sampling effort has led to 120 of 276 Australian river basins being sampled from 11 of 12 drainage divisions (Figure 3.2b). The only drainage division that has not been sampled is the South Western Plateau, a region of over 1,093,000 km<sup>2</sup> that extends into Western Australia, South Australia and the Northern Territory. It consists primarily of sandy or stony desert and is the driest region in Australia (Bureau of Meteorology 2011). Due to the lack of permanent water in this region, it is unlikely that it displays high levels of diversity for any freshwater species, specifically taxa with no resistance to desiccation. In fact in Unmack’s (2001) study on the biogeography of Australian freshwater fish, only one species was recorded from this area. Interestingly, Unmack (2001) did comment that this species has no relationship to any in any other region; therefore a comparison with the triclad fauna could be informative (a detailed discussion of the biogeography and diversity of the Australian fauna can be found in Chapter 4).

### 3.4.2 Taxonomic Work

As part of the taxonomic work performed for this thesis, 16 new species (along with several tentative new species and genera) have been described. This approximately doubles the number of known species from the region (Table 3.1). In addition to this 18 of the previously known species have been re-examined and re-described (Table 3.1).

It has been shown that two monotypic genera may have to be dissolved and two new genera, housing several species, have been erected (Table 3.1). Furthermore, a case

has been made for the erection of another new genus to house the western *Romankenkius*. In the last 40 years only one other new genus has been erected for the Dugesiidae *s.l.* that being *Recurva*, a genus housing two newly described species from the Mediterranean region (Sluys et al. 2013).

This demonstrates the significance of the new Australian genera not only in the Australasian region, but worldwide. The description of these two genera as well as the substantial contribution to the species known from the region also has implications for any analysis of diversity, both Australasian and global (Chapter 4). These new genera and species, as well as the re-assignment of several species, will also influence future phylogenetic studies (see Chapter 2 for more details). This work has a profound impact on the state of our knowledge and understanding of the Australian triclad fauna (for detailed genus and species descriptions see Appendix 1a) and has led to some changes in the classification of the Dugesiidae *s.l.*

### 3.4.3 Classification

Figure 3.5 illustrates the current accepted classification (Sluys et al. 2009), including those alterations resulting from this work that were deemed conclusive enough to be published. Alongside that is a classification that would be introduced if all the proposed changes were accepted (most influentially the paraphyly of the Dugesiidae *s.l.*). In order to accommodate these new concepts, it has been proposed that the superfamily Geoplanoidea house four families of freshwater triclad. This represents a substantial change from the previous classification, which housed all genera under the Dugesiidae *s.l.* (Figure 3.5). The terrestrial triclads will remain within a single family, the Geoplanidae. The addition of the freshwater families best represents the phylogenetic relationships between the freshwater and terrestrial species, as outlined in Chapter 2. The name Dugesiidae *s.s.* has been maintained as the name for the largest of the families, as this family houses the type genus, *Dugesia* (Ball 1974a). Newly proposed families have only one or two constituent genera and therefore naming preferences were much more evident.

### 3.4.4 Identification

A key to the Australian species has been produced. Inevitably, it relies on internal anatomy in order to make a full species identification (Section 3.3.3). External morphological features were not consistent enough to be relied upon for identification (even at the genus level). The key will allow a taxonomist to identify easily to the genus level and, if

necessary, to the species level, any known member of the Australian triclad fauna, providing it has a mature copulatory apparatus. In the absence of a copulatory apparatus the taxonomist is directed to the final step in the key (40), which allows her to discover whether it is a *Dugesia* or *Girardia* (the most common genera lacking the copulatory apparatus) based on external morphology.

The lengthy and expensive process required to prepare specimens for identification means that a histological laboratory, a histologist and a taxonomist are needed. It is for this reason that many generalist biodiversity or ecological studies exclude triclads. However, as a result of the current research a more time-effective and widely accessible identification method could and should be developed. The collection and description of so many new species and new genera, along with the creation of the key, has laid the groundwork for molecular identification. A molecular catalogue for all Australian species could be created for comparison. *Cox1* is potentially a very useful gene for this purpose, particularly as it is utilised commonly as the Bar Code of Life (<http://www.barcodeoflife.org/>). This would enable simple collection (only 100% alcohol needed) and processing (in a molecular lab) of the specimens, making them more attractive as a study group.

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## Chapter 4: Biodiversity and Biogeography of Australian Freshwater Triclad

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### 4.1 Introduction

In this thesis I have proposed the paraphyly of the Dugesiidae *sensu lato* (*s.l.*) and consequential changes in the classification of this group (i.e. a series of families nested within the Geoplanoidea). Therefore, in order to facilitate discussion based around the phylogeny proposed in Chapter 2, the newly proposed classification (Figure 3.5), especially the family structure, will be utilised throughout this chapter.

The implications of the phylogenetic hypotheses developed in the previous chapters are discussed in relation to the biodiversity and biogeography of the Dugesiidae *s.l.* as a whole and within an Australian context.

#### 4.1.1 Global Freshwater Biodiversity and Biogeography

Freshwater environments lend themselves to speciation as they are typically fragmented (Ponder & Colgan 2002). Global freshwater environments, which take up only 0.01% of the total surface area of the globe, provide habitats for approximately 126,000 (9.5%) of all recognised species (UNEP 1972-2002). Although freshwater species that have evolved in one locality should be ecologically capable of surviving in many others, constraints of history and geography (e.g. connectivity) prevent this (e.g. Darlington 1948). For obligate freshwater species, the opportunity for simple range expansion is limited to rare events, such as sea level changes and drainage rearrangement (Unmack 2001).

Despite their almost ubiquitous distribution, freshwater triclad exhibit very low vagility, with no known active dispersal mechanisms. The fact that many species from a range of environments can be found at isolated locations in the landscape, while their dispersal mechanism remains a mystery, has fascinated scientists for years (Cain et al. 2000; Darwin 1859; Gittenberger et al. 2006; Ridley 1930; Van Leeuwen et al. 2012). While “undetected” vicariance events may explain some of these mysteries, dispersal must play a significant role in distribution of all species, including freshwater representatives (Bilton et al. 2001; Queiroz 2014). Some freshwater species, predominantly insects, exhibit large ranges as they have developed effective active dispersal mechanisms (e.g. flight) (Bilton et al. 2001). Others rely on passive dispersal (e.g. by wind), obviously requiring a desiccation-resistant stage, or dispersal via other organisms (e.g. bio-chore).

For organisms lacking an active dispersal phase, such as freshwater triclad, dispersal mediated by animals is one of the most effective forms of passive dispersal

(Vanschoenwinkel et al. 2008). Van Leeuwen et al. (2012) discussed the ability of some invertebrate taxa to disperse via water-bird endozoochory (in the gut). Naturally, there is a requirement that one phase of the animal's life cycle be capable of surviving the extremely acidic environment of the stomach of a vertebrate vector. There is also the possibility of reptiles or land mammals transporting various life stages (e.g. cocoons) via ectozoochory (on their exteriors), protected by mud or fur (Vanschoenwinkel et al. 2008).

Very little is known about the ability of freshwater triclads to utilise passive dispersal mechanisms to move between drainages. In the absence of water, individuals will quickly desiccate. However, there are examples of species for which dispersal is the only plausible explanation for their current distributions in Australia (e.g. *Girardia tigrina*). Pongratz et al. (2003) raised questions about the apparent long distance dispersal of *Schmidtea polychroa* possibly aided by humans. This method of dispersal was also invoked for *Girardia tigrina* and *Planaria torva* (Young and Reynoldson 1999; Ball 1969). Ectozoochory is another possible explanation, as freshwater triclads, or their cocoons, will survive in mud and on macrophytes for short periods of time (pers. obs). Beyond these explanations, for freshwater triclad fauna, we must rely on major vicariance events or temporary connections between adjacent drainage divisions (see section 4.4.2).

When examining possible vicariance explanations for current day distributions, robust phylogenies are extremely valuable. Until recently biogeographical analysis was hampered by the lack of such phylogenies, as phylogenetic schemes were based purely on often-unreliable morphological characters (Cracraft 1994). This is particularly true of freshwater triclads due to the paucity of morphological characters available on which to base a phylogeny (see Chapter 2). Recent developments in this area (e.g. Álvarez-Presas et al. 2008; Álvarez-Presas and Riutort 2014; Sluys and Kawakatsu 2001; this thesis) should allow more confidence when making predictions, whether based on vicariance or dispersal theories.

Current approaches in phylogeny-based biogeography can be roughly categorised as event-based or pattern-based (Sanmartin et al. 2001). Whilst my biogeographical exploration will be primarily descriptive there will be a focus on ideas from the cladistic biogeography school (pattern-based) and some consideration given to event-based biogeography. Our ability to look at events is limited by the fact that no morphological or molecular clock has been imposed in this study and we have no fossil record for triclads. Consequently, we will be looking at the geological history to speculate about the existence and timing of vicariance events (Sanmartin et al. 2001). Similarly, our ability to recognise patterns is also limited due to the differing dispersal mechanisms of other freshwater taxa,

making comparisons difficult (Upchurch 2008). For this reason, the focus of comparisons will be on taxa with similar dispersal restrictions (i.e. obligate freshwater organisms). Additionally, while the concept of a centre of origin or ancestral area has been criticised, the nature of the Dugesiidae *s.l.* and the Australian focus demand some discussion of this idea (Croizat et al. 1974; Humphries & Parenti 1986; Platnick & Nelson 1978).

#### 4.1.2 The Dugesiidae *s.l.* and Geoplanidae Globally

The Dugesiidae *s.l.* consists of 11 genera, which house over 180 species inhabiting both the northern and southern hemispheres. In the south, the “dugesiid” genera appear to have taken advantage of the slow break-up of Gondwana and the dispersal opportunities it provided, particularly between Antarctica and Australia, as the taxon is particularly diverse in Australasia (Ball 1974a, c, 1977a; Sluys et al. 1998). *Dugesia* is the most species-rich (containing more than half of the “dugesiid” species) and widely distributed genus, absent only from the Americas.

There is an assumption that biogeographic patterns in freshwater triclads can be explained by vicariance hypotheses invoking continental drift (Ball 1974a, 1975; Kawakatsu 1968; Sluys 1992). Kawakatsu (1968) placed the centre of dispersal of the Dugesiidae *s.l.* in the Balkan Peninsula. His rationale was based on the fact that this is a well-known evolutionary centre, provides a possible explanation for the distribution of the genus *Dugesia*, and allows for multiple immigration routes into Australasia (e.g. via southern Africa, South America and southeast Asia). Ball (1974a) pointed out the limitations of this scheme, arguing that while there is a great deal of diversification of the Planariidae and Dendrocoelidae in the Balkans, the Dugesiidae *s.l.* is only represented by a few derived species. Ball (1974a, 1975) proposed instead that the Dugesiidae *s.l.* originated in the southern hemisphere, specifically Antarctica.

Sluys et al. (1998) reassessed Ball’s (1975) work and deduced that an origin in either North America or Australia was equally plausible. However, the current distribution of the “dugesiid” genera across the globe (i.e. found on all continents, excluding Antarctica), coupled with the fact that the Dugesiidae *s.l.* achieve their greatest generic diversity in Australia, suggests a Gondwanan origin (Ball 1976; Ball & Reynoldson 1981; Sluys et al. 1998). Further to this, it has also been suggested that present-day distributions of the Dugesiidae *s.l.* are a direct result of the sequential break up of Pangaea and then Gondwana, and that by 220 million years ago (mya) the early diversification of the taxon was complete (Ball 1974a, 1975; Sluys et al. 1998).

The Geoplanidae fauna consists of four subfamilies, which house 55 genera and over 800 species. They have a global, mainly pan-tropical distribution and, as a

consequence of their inability to conserve water, require humid conditions (Kawaguti 1932). Despite clear physiological links to their freshwater counterparts, terrestrial triclads cannot be fully submerged for extended periods of time (Froehlich 1955; Sluys 1999). The close relationship between terrestrial and freshwater triclads is well documented in Chapter 2 and, while a detailed review of the biogeography and biodiversity of the terrestrial fauna is beyond the remit of this study, it is interesting to note that the Geoplanidae is also diverse in Australasia. Sluys (1999) identified New Zealand, southeastern Australia and Tasmania as being hotspots of terrestrial triclad diversity, pointing out that, with some exceptions (e.g. parastacid crayfish, temnocephalan flatworms and some insect groups), these are areas that do not normally feature for other taxa.

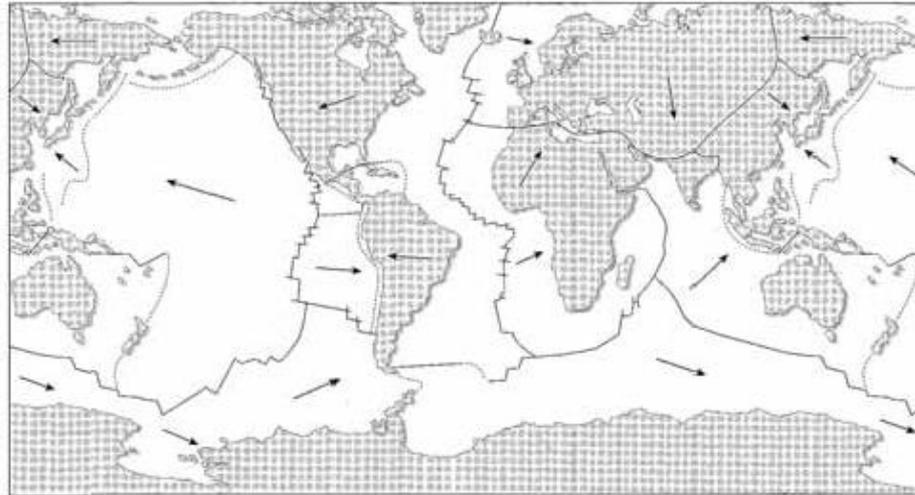
### 4.1.3 Australia within past global settings

The earth's crust was formed approximately 4,600 mya and the Australian continent contains some 4,400 million years of the crust history (Cattermole 2000; Cawood & Korsch 2008). While it is difficult to accurately account for the movement of the plates throughout the earth's history, the most influential continental movements have occurred within the last 570 million years (the Phanerozoic) (Cattermole 2000). A summary of these movements can be found in Appendix 4. The following summarises Australia's movements over the last 65 million years, specifically the late stages of the break-up of Gondwana.

#### 4.1.3.1 Australia's Recent Isolation

### **Cenozoic (65 mya - present)**

The final phase of the break-up of Pangaea (which began in the Jurassic) occurred in the early Cenozoic (65 - 55 mya) when the north Atlantic Ocean opened as rifts appearing on either side of Greenland (Cox and Moore 2000). In the south, Australia's detachment from Antarctica was progressing slowly; 60 million years after the separation began Australia was still not completely isolated (Embleton 1986).



**Figure 4.1** The major tectonic plates today. Arrows show the direction of plate movements and dotted lines show the position of trenches (Cox & Moore 2000).

Australia's separation from Antarctica by about 30 mya (Embleton 1986) released the land mass to be an independent continent for the first time in over 1600 million years (Johnson 2004). Shortly after Australia's separation, South America also separated from west Antarctica (approximately 30 mya) (Cox & Moore 2000). Before its complete separation from Antarctica, the Australian plate (also home to New Guinea, New Zealand and New Caledonia) fused with the Indian Plate, creating the Indo-Australian plate (44 mya)(Embleton 1986; Hall 2001)(Figure 4.1). The Indo-Australian Plate moved rapidly after this separation, drifting 30° northwards in 20 million years. The most recent tectonic event for the Australian Plate was the collision, around 15 mya, between New Guinea (the leading edge of the Australian Plate) and the southwestern part of the Pacific Plate (Hall 2001). Over the last 5 mya Australia has been within 1° or 2° of its present latitude (Veevers 2000).

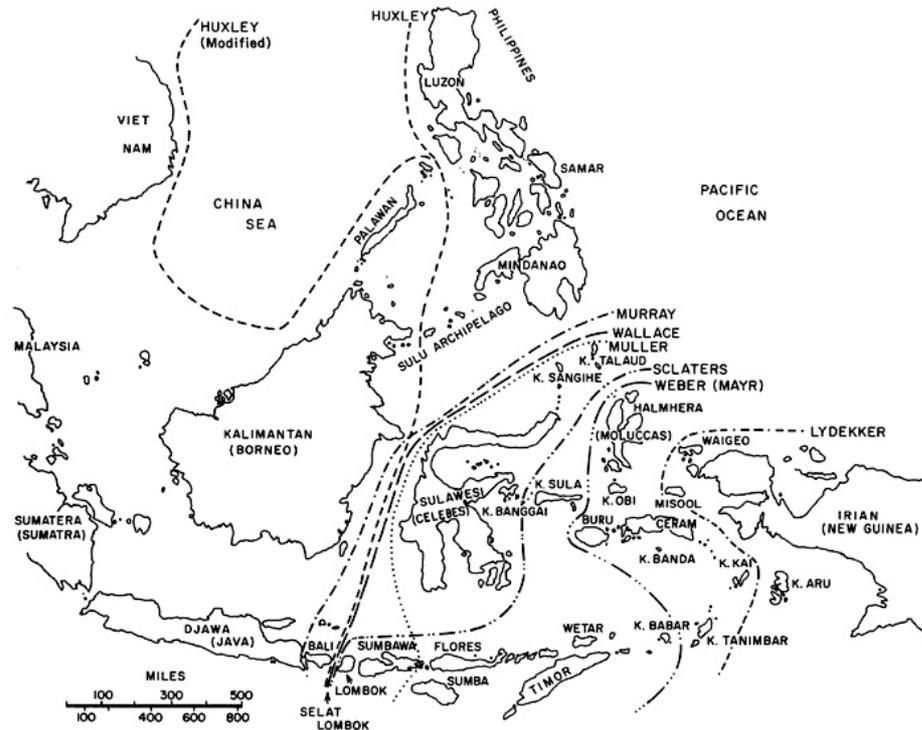
## **The Indo - Australian Plate**

### **New Guinea**

The huge island of New Guinea sits on the northeastern border of Indo-Australian Plate. The southern part of New Guinea is, in fact, the northern part of Australia separated by a shallow sea (maximum depth 70m) and, prior to the last glacial period (10,000 - 15,000 years ago), the two land-masses were connected (Cattermole 2000; Chivas et al. 2001; Smart 1977). Presumably this sea level change also affected the connection with Tasmania, an island sitting 240km south of the eastern side of the continent, which is also thought to have become isolated around 10,000 years ago (Darlington 1969).

As discussed above, New Guinea's position near the Indonesian Archipelago is relatively recent, dating back around 15 mya when New Guinea collided with the southwestern part of the Pacific Plate (Hall 2001). Prior to this collision New Guinea was separated from the Indonesian Archipelago by the deep oceans that existed between the Pacific and Indo-Australian plates (Briggs 1987; Veevers et al. 1978). About 10 mya the gap between New Guinea/Australia and the Indonesian Archipelago is believed to have been at its narrowest; however, the sea level would have needed to fall by at least 200m, an extremely unlikely event, to provide land connectivity (Hall 2001; Haq et al. 1987). It therefore seems likely that there has been no time when land flora and fauna would have been able to avoid crossing ocean water in order to move between the two continents (Hall 2001).

The Indo-Australian Archipelago (modern Indonesia, Malaysia, Brunei and New Guinea) supports one of the highest global levels of species richness and endemism (Myers et al. 2000). Since Wallace's (1876) attempt to identify a clear geographical line separating the faunas of Oriental and Australasian origin, this region has become one of the most discussed biogeographical boundaries in the world and many additional demarcation lines have been proposed (Brown & Guttman 2002; Clode & O'Brien 2001; Simpson 1977)(Figure 4.2). With the inability to settle on a single hypothesis (De Bruyn & Mather 2007), and the value of biogeographical lines being called into question, Simpson (1977) proposed that a 'transitional zone' between the faunas of Oriental and Australasian origin be termed 'Wallacea' (Simpson 1977). While not viewed as a complete resolution to the debate, the recognition of Wallacea is a useful tool to draw attention to the biogeographical importance of this area.



**Figure 4.2** Map of the Indo-Australian Archipelago showing the location of many of the proposed biogeographic dividing lines (Simpson 1977).

### New Zealand

During the initial break up of Gondwana, New Zealand essentially did not exist as a separate geological entity (Laird & Bradshaw 2004). New Zealand and its smaller associated landmasses, including New Caledonia and the Lord Howe Rise (jointly comprising Zealandia), formed part of the Pacific-facing Gondwanan continental margin. The New Zealand that we know today sat at the southern end of the margin connected to the shores of west Antarctica, while in the north, communicating with Australia, was New Caledonia (Laird & Bradshaw 2004; McDowall 2008). This sliver (New Zealand and New Caledonia) became isolated from Gondwana after the onset of seafloor spreading 82 mya (50 million years earlier than Australia), and achieved its present distance from Australia 50 - 60 mya (Cooper & Millener 1993; Ladiges et al. 2003; McDowall 2008; Wallis & Trewick 2009; Worthy & Holdaway 2002). During its isolation, the land area of New Zealand has fluctuated dramatically and most of the now emergent land area (perhaps > 80%) was submerged beneath sea at some time in the Oligocene (Cooper & Millener 1993; Wallis & Trewick 2009). The extent to which any of New Zealand remained above sea level throughout this time is uncertain. Some authors believe none of it was; however, this is

difficult to accept due to apparently archaic elements of the fauna (Cooper & Millener 1993; Daugherty et al. 1993; McDowall 2002; McDowall 2008; Wallis & Trewick 2009).

### **India**

The Indian Plate's fusion to the Australian Plate is relatively recent (44 mya); however, this union did not allow for the exchange of taxa due to the large oceans separating the two landmasses.

## 4.1.3.2 Australian Geography and Climate throughout the Phanerozoic

The Australian biogeographical region is generally considered to consist of Australia and New Guinea. Additionally, due to the historical connections of Australia with New Zealand and New Caledonia, these last two will be included in the discussion where relevant (see section 1.5). Australia is currently situated well within the Indo-Australian plate and is therefore tectonically stable with no active mountain building or major fault systems (Johnson 2004). Australia is one of the lowest and flattest land areas on Earth, with the average elevation being approximately 330m above sea level (Johnson 2004). The Great Divide forms a prominent spine along much of eastern Australia and there are ranges in central Australia (MacDonnell, Musgrave and Petermann Ranges), South Australia (Mount Lofty Ranges) and Western Australia (Hamersley and King Leopold Ranges). These represent the remnants of ancient mountain ranges, but the continent is dominated by a series of broad plains (Johnson 2004).

### **Palaeozoic (542 - 251 mya)**

The emergent parts of Australia were warm and dry for much of the Palaeozoic; however, eastern Australia was covered by a shallow warm ocean (Johnson 2004). By the end of the Devonian (370 mya) the seas had extended and withdrawn several times across the different sections of the Australian continent. Australia was residing in low southern latitudes and the climate remained warm; however, around 330 mya the earth entered a major glaciation event (Johnson 2004; Metcalfe 2001). Australia, covered in an ice sheet, was still attached to northeast Gondwana and was moving from low southern latitudes to high southern latitudes (Metcalfe 2001).

### **Mesozoic 251 - 65 mya**

By the beginning of the Triassic (251 - 199 mya) the ice in Australia had begun to diminish, which resulted in an arid landscape dominated by inland rivers and lakes

(Johnson 2004). Australia's climate has been greatly influenced by the continent's movement into the highest latitude it had reached since the Palaeozoic. The sea level rose and by 140 mya the sea began to spread inland from the developing Indian Ocean to the west and from the north until half of what is now known as Australia was inundated (Johnson 2004). It is thought that by 117 mya the only remaining dry land was in southern Western Australia, the old Yirgarn craton, the Kimberley region, and along the Great Divide in the east (Johnson 2004). The sea withdrew rapidly in the late Cretaceous and the continent was mostly cleared by 99 mya (Johnson 2004).

### **Cenozoic 65 mya - present**

The Indo-Australian Plate drifted 30° northwards in 20 million years; thus Australia was not subject to any further major glaciation events and became progressively dryer over this time (Byrne et al. 2008; Cox & Moore 2000; Frakes 1999). Its broad latitudinal spread (approximately 40°) shielded the continent from the loss of any major climate type during this movement (Unmack 2001). Australia's separation from Antarctica allowed a complete circumpolar current to form, causing global water and air temperatures to cool rapidly (Frakes 1999). The polar ice caps formed in Antarctica; however, Australia's climate warmed slightly, reflecting a balance between the opposing trends (global cooling and latitudinal shift) (Bowman & Yeates 2006). It is important to note that the polar ice caps are relatively young (around 15 million years old) and that for most of the last 250 million years, Antarctica was warm with prolific flora and fauna (Johnson 2004).

The effect of the global drying was compounded in Australia 15 mya by the collision between New Guinea (on the leading edge of the Australian Plate) and the southwestern part of the Pacific Plate (Hall 2001). This pushed up the New Guinea highlands, causing a rain-shadow effect and the Australian climate became progressively drier (Frakes 1999). The aquatic fauna dwindled and the rainforest biotas retreated to the wetter periphery of the continent (Schodde 1989). About 10 mya the sea level dropped significantly, exposing the Australian landmass to a similar extent as today (Johnson 2004). The dry conditions have continued in Australia to the present, except for a brief wet interlude between 5 and 2 mya (Johnson 2004). Australia's dry environment and relative isolation have been the key drivers in the evolution of the unique Australian biota (Bowman & Yeates 2006).

Currently, the Great Divide forms a watershed running down eastern Australia from Cape York to Victoria, separating the rivers that flow eastwards from the longer westward draining rivers (Johnson 2004). While there is debate surrounding the origin of the Great Divide, evidence suggests that it was formed at least 90 mya and could possibly

be the remnants of an old mountain chain dating from the Carboniferous (300 mya) (Johnson 2004).

#### 4.1.4 Australian Freshwater Biodiversity and Biogeography

Freshwater diversity is relatively impoverished in Australasia for both vertebrate and invertebrate taxa (Balian et al. 2008a). This has been attributed to the harshness and uncertainty of the climate and the absence of a yearly energy input in the form of litter fall (Bunn & Davies 1990; Williams & Wan 1972; Winterbourn 1980). It is believed that species have had to develop flexible life history patterns and broad niches in order to survive in these difficult conditions (Bunn & Davies 1990a; Williams & Wan 1972; Winterbourn 1980).

This assessment of the Australian fauna has been called into question by Lake et al. (1985) who cautioned about the practice of making judgements about species richness from region to region, commenting that there is a lack of uniformity in collecting effort and methods, differing degrees of habitat heterogeneity, and variable precision of the taxonomy. Despite the issues surrounding the analysis of global biodiversity, there are certain freshwater groups that show higher than expected levels of diversity on the Australasian plate. Among them are the Platyhelminthes, which display similar levels of diversity to those seen in the Neotropical and Afrotropical regions. It should be noted that most of Australia's flatworm diversity comes from the Tricladida (*Dugesiiidae s.l.*) and the unrelated Temnocephalidae (Balian et al. 2008a; Schockaert et al. 2008).

Due to the relatively scarce and fragmented nature of Australia's inland freshwaters, its fauna has traditionally been regarded as having high endemism but low diversity (Bunn & Davies 1990; Groombridge 1992; Outridge 1987; Unmack 2001; Williams 1981). While there has not been a great deal of research aimed at identifying areas of endemism for freshwater species, several recent studies looking at obligate freshwater species have utilised this concept as a measure of diversity (Crandall & Buhay 2008; Munasinghe et al. 2004; Unmack 2001; Whiting et al. 2000). Indeed, the concept that the Australian freshwater fauna exhibits low diversity has been challenged with some success for southeastern Australia, Tasmania and some tropical regions (Lake 2000; Outridge 1987; Whiting et al. 2000; Williams 1981). However, there remain large areas of the country still considered lacking in diversity (Bunn & Davies 1990; Outridge 1987).

Australia, because of its age, stability and aridity also presents a unique situation for investigating freshwater biogeography (Unmack 2001). Aquatic insects with close Gondwanan affinities, such as Diptera, Blephariceridae, Ephemeroptera, Plecoptera and Trichoptera, are restricted to cool lotic waters, suggesting that such systems have existed on the Australian plate since at least the Jurassic (200 Ma ago). All river basins currently

present in the biogeographical region were established by the Palaeocene (65 - 55 mya); however, the last 15 million years has seen increased drying, resulting in decreases in surface water (Unmack 2001; Veevers 1991). As a consequence, Australia displays a paucity of permanent freshwater lakes and rivers in comparison with other landmasses of a similar size (Groombridge 1992; Williams 1981).

#### 4.1.5 The Dugesiidae *s.l.* within Australia

The biodiversity and biogeography of Australian “dugesiids” is of considerable interest for a number of reasons. One of these is the diversification of the taxa into many genera and species in Australia, which provides a stark contrast to North America, where the “dugesiid” fauna consists of only two genera (Ball 1975, Sluys et al. 2005). Despite this, there has been very little biogeographical study of Dugesiidae *s.l.* within the Australian biogeographical region. Species’ distributions, with cursory comments, have been published in several works, however the lack of collecting effort has made any analysis relatively pointless (Grant et al. 2006; Sluys & Kawakatsu 2001) (Figure 11). This is frustrating considering that many of the regions that have been neglected are of substantial biogeographical interest. For example, southwestern Australia, which, prior to this study could claim a total of only five collecting sites, has been effectively isolated from other Australian temperate regions by Australia’s expanding arid zone. This region has been identified as an area of high endemism for both freshwater and terrestrial species, most notably birds, flora and freshwater crayfish (Burbidge 1960; Cracraft 1991; Crisp et al. 2001; Unmack 2001; Whiting et al. 2000). It is hoped that as a result of the current study, and the consequent increase in sites sampled, it will be possible to examine Australian “dugesiid” biogeography and diversity in a more meaningful way.

#### 4.1.6 Aims

The focus of this chapter is on the descriptive biogeography of Australian freshwater triclads by (1) presenting up-to-date distribution maps for Australia and discussing the implications of these on a global and regional scale, and (2) identifying hotspots for “dugesiid” genera on a global and regional scale.

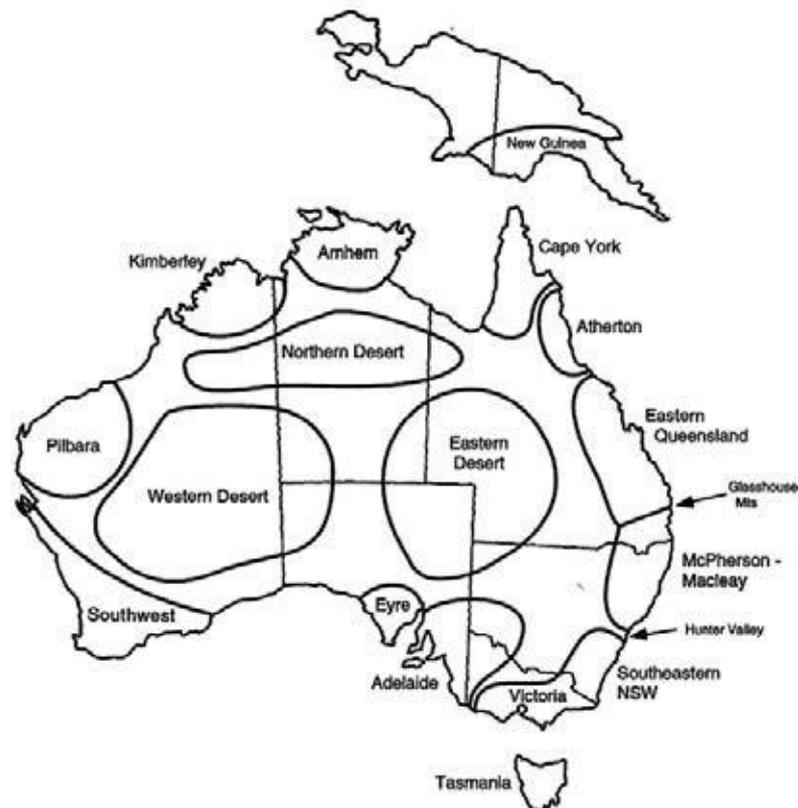
## 4.2 Methods

### 4.2.1 Data Accumulation

Data for this analysis were gathered from the taxonomic and distributional literature available for species worldwide, as well as data collected as part of this thesis. For the global analysis, the country in which the species sighting has been made was recorded, while for the Australian analysis the specific site was recorded. In all cases only the proposed centre of origin for invasive species was included: if there was no firm hypothesis surrounding this, the species was excluded from the analysis.

### 4.2.2 Delimitation of Areas

#### 4.2.2.1 Areas of Endemism



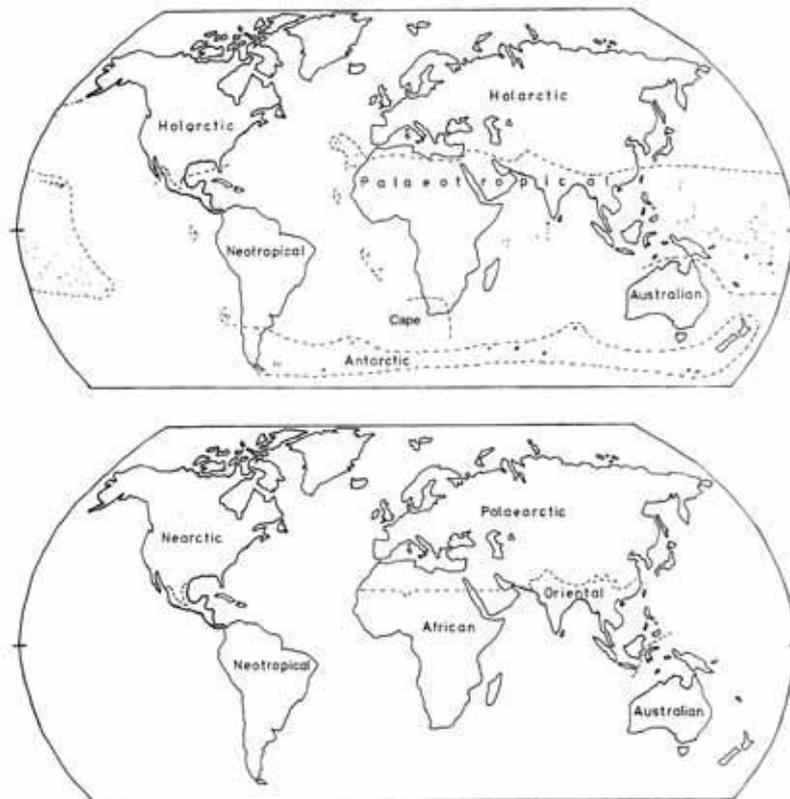
**Figure 4.3** Map of Australia and New Guinea, showing areas of endemism modified from Cracraft (1991)(Crisp et al. 1995).

An accepted approach used for analysing biodiversity is the study of areas of endemism. Areas of highly concentrated endemics within the Australasian region across a range of flora and fauna have been identified (Burbidge 1960; Cracraft 1991; Wallace 1855). Cracraft (1991) identified 14 such areas, accounting for a large percentage of Australia's

landmass, based on avian distribution patterns. The validity of this was emphasised when Crisp et al. (1995) was able to use Cracraft's (1991) areas of endemism, with a few modifications, in their cladistic biogeographical study of multiple plant taxa in Australia and New Guinea (Figure 4.3). Further interpretations of these areas of endemism can be found in accounts by, amongst others, Andrews (1916), Barlow (1981), Burbidge (1960) and Crisp et al. (1999).

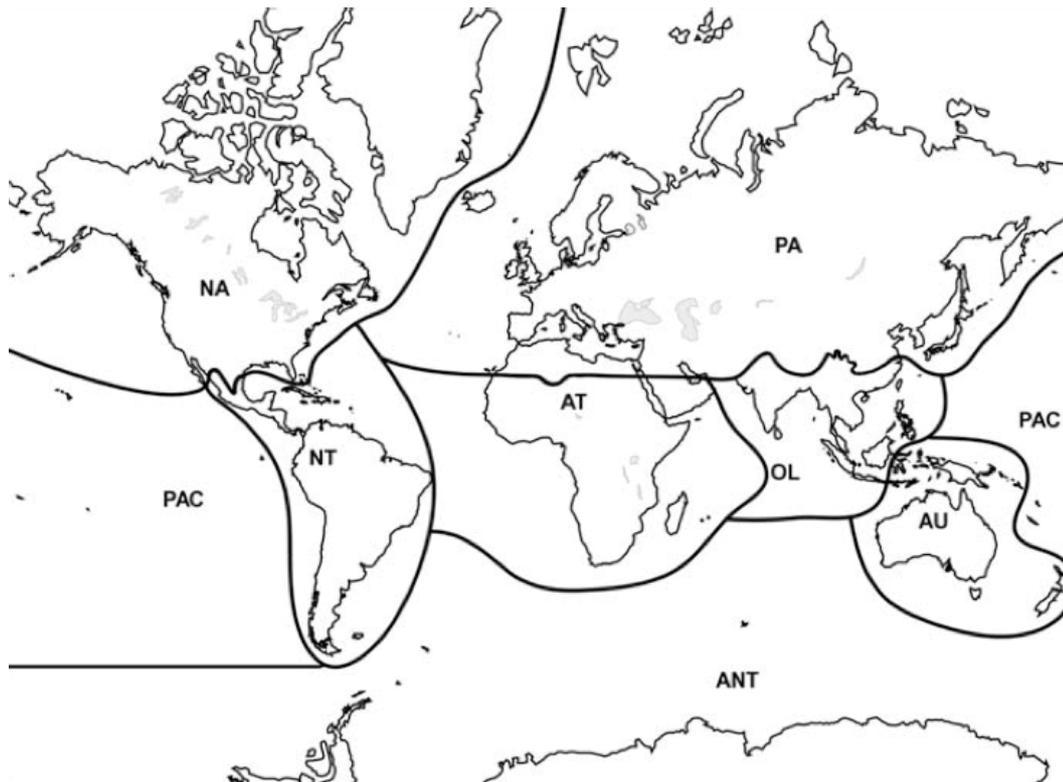
#### 4.2.2.2 Global Biogeographical Areas

There have been many attempts to divide the world into biogeographical regions. The most useful interpretation is that of Cox (2001) (Figure 4.4). It is possible that these biogeographical regions, relating primarily to terrestrial groups, may not be relevant for their freshwater counterparts. An alternative system of global ecoregions, based primarily on freshwater fish distributions (Abell et al. 2008), was considered; however, global information on freshwater flatworms is too limited to permit analysis within this framework.



**Figure 4.4** Floral Kingdoms (a) and mammal zoogeographical regions (b) as currently recognised (Cox 2001).

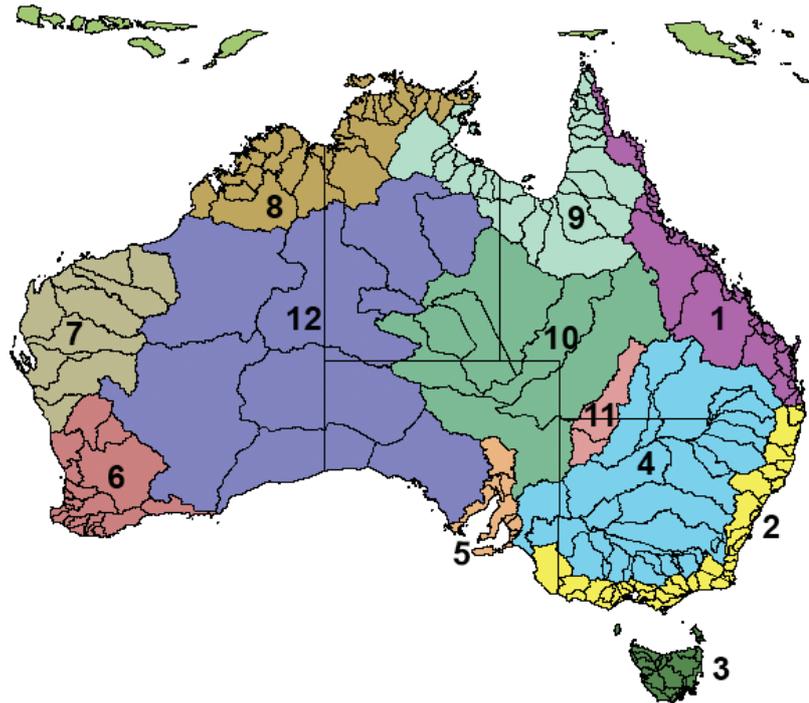
It seems that the regions defined by Wallace (1876), later updated by Cox (2001)(Figure 4.4) and others, are still the most relevant zoogeographical regionalisations in use today. For example, in the recent Freshwater Animal Diversity Assessment (Balian et al. 2008b), eight regions were delineated based on “classic” zoogeographic regions to allow for the standardisation of data. While the issues with these regions cannot be ignored (transitional zones between regions etc.), this is currently the most useful regionalisation that exists for the examination of global patterns (Figure 4.5).



**Figure 4.5** Map of zoogeographical regions. PA: Palearctic Region, NA: Nearctic Region, AT: Afrotropical Region, NT: Neotropical Region, OL: Oriental Region, AU: Australasian Region, ANT: Antarctic Region, PAC: Pacific Region and Oceanic Islands (Balian et al. 2008b).

### 4.2.2.3 Australian Biogeographical Units

The atlas of the Australian hydrologic system currently recognises 12 drainage divisions, encompassing Australia’s 276 river basins (NLWRA (National Land and Water Resources Audit (2001))(Figure 4.6). This scheme is also accepted by the Australian Water Resources Council and is the most appropriate for any analysis within Australia (Bureau of Meteorology 2015b)(Figure 4.6). It should be noted that these drainage divisions are the regions used in the new map of biogeographic units for freshwater species as proposed by Abell et al. (2008) (discussed above).



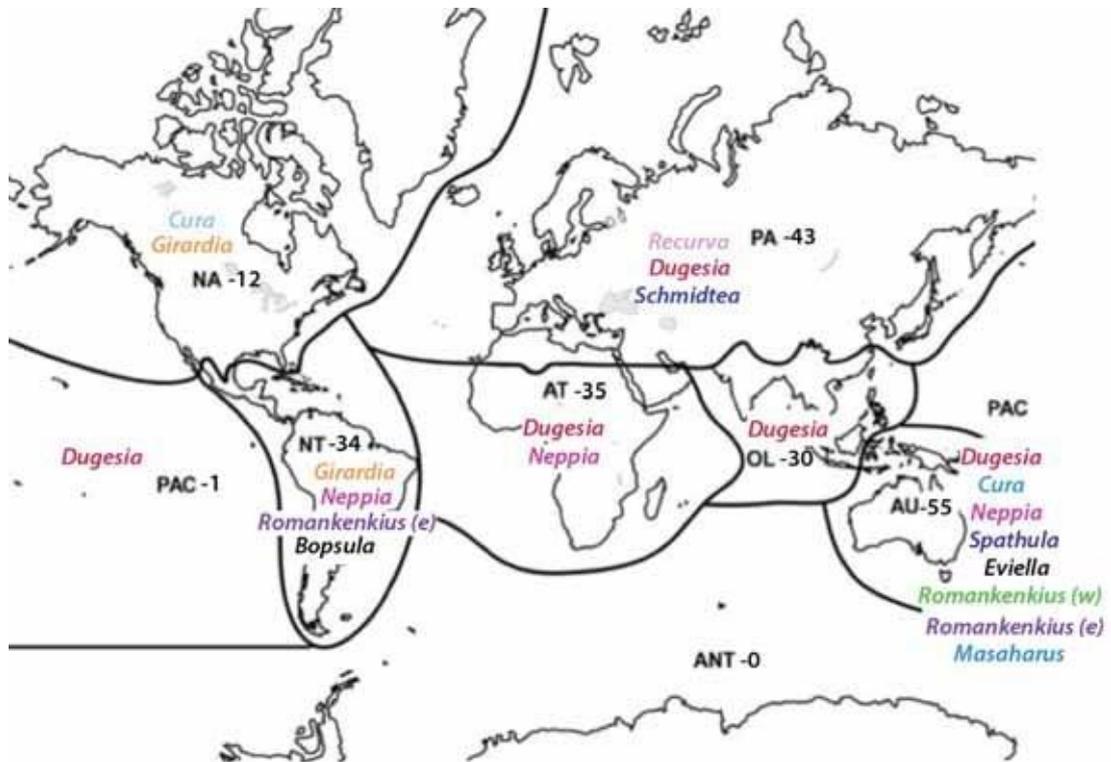
**Figure 4.6** Simplified map of Australian Drainage Divisions (adapted from Bureau of Meteorology, 2015b). 1) Northeast Coast, 2) Southeast Coast, 3) Tasmania, 4) Murray-Darling, 5) South Australian Gulf, 6) Southwest Coast, 7) Indian Ocean, 8) Timor Sea, 9) Gulf of Carpentaria, 10) Lake Eyre, 11) Bulloo-Bancannia, 12) Western Plateau.

### 4.2.3 Analysis

The biogeographical analysis will be primarily descriptive due to the constraints of the data available for the *DugesIIDae s.l.*, making more stringent analysis impractical (Crisci 2001). All distribution maps were created in ArcView GIS 3.2 (ESRI 1992) and edited in Adobe Illustrator (Adobe Illustrator 2012).

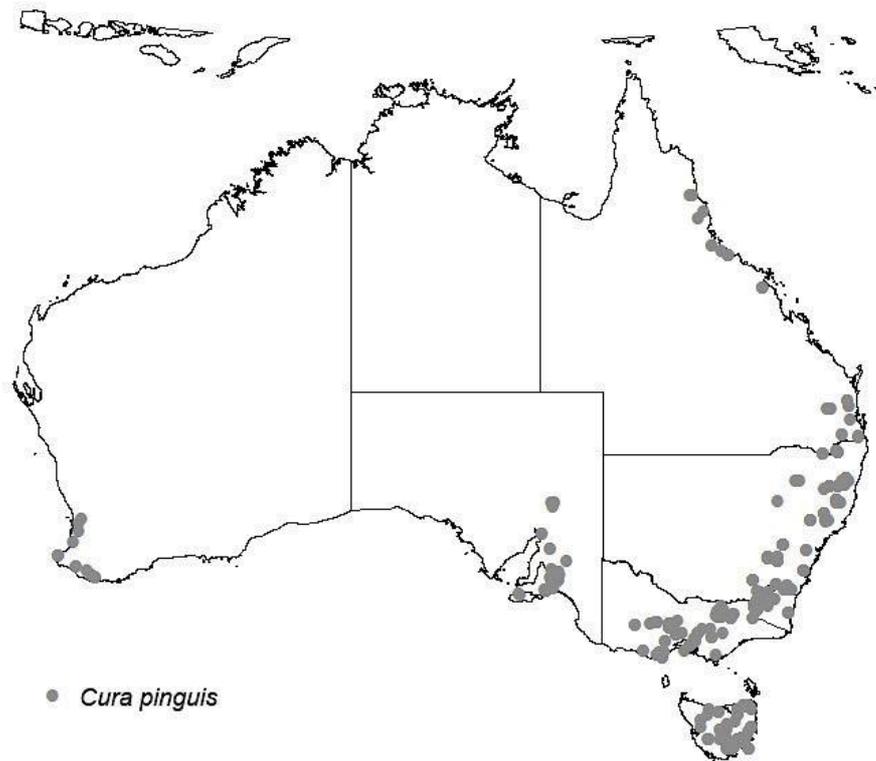
## 4.3 Results

### 4.3.1 Global Distribution

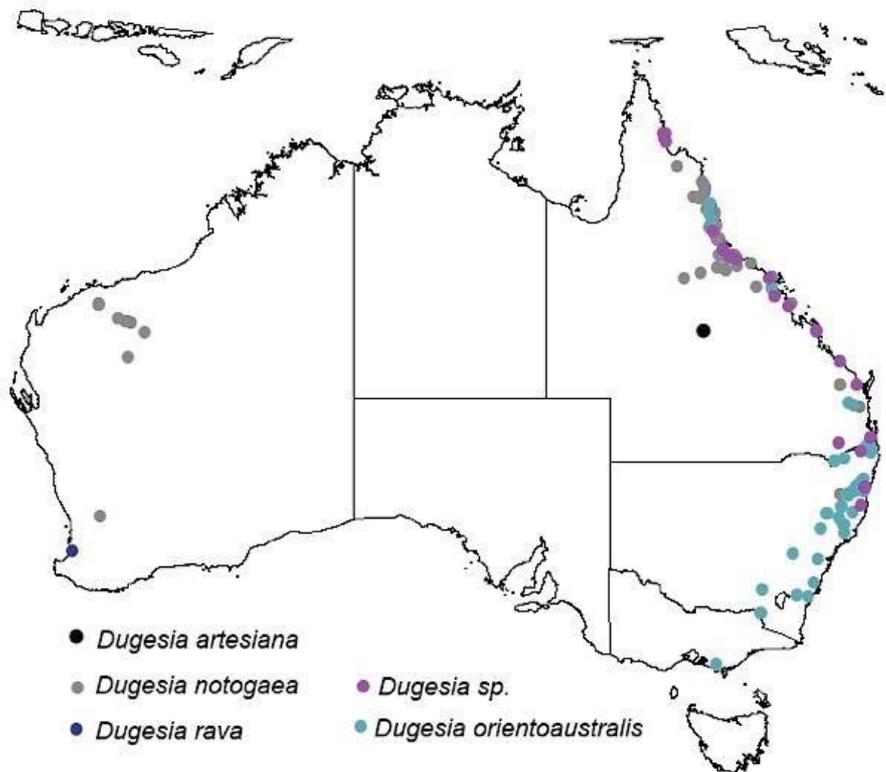


**Figure 4.7** Map of distribution of “dugesiid” genera in relation to biogeographical areas as in Balian et al. (2008b) (autochthonous species only). Numbers in black describe total number of species in the region. PA: Palaeartic Region, NA: Nearctic Region, AT: Afrotropical Region, NT: Neotropical Region, OL: Oriental Region, AU: Australasian Region, ANT: Antarctic Region, PAC: Pacific Region and Oceanic Islands.

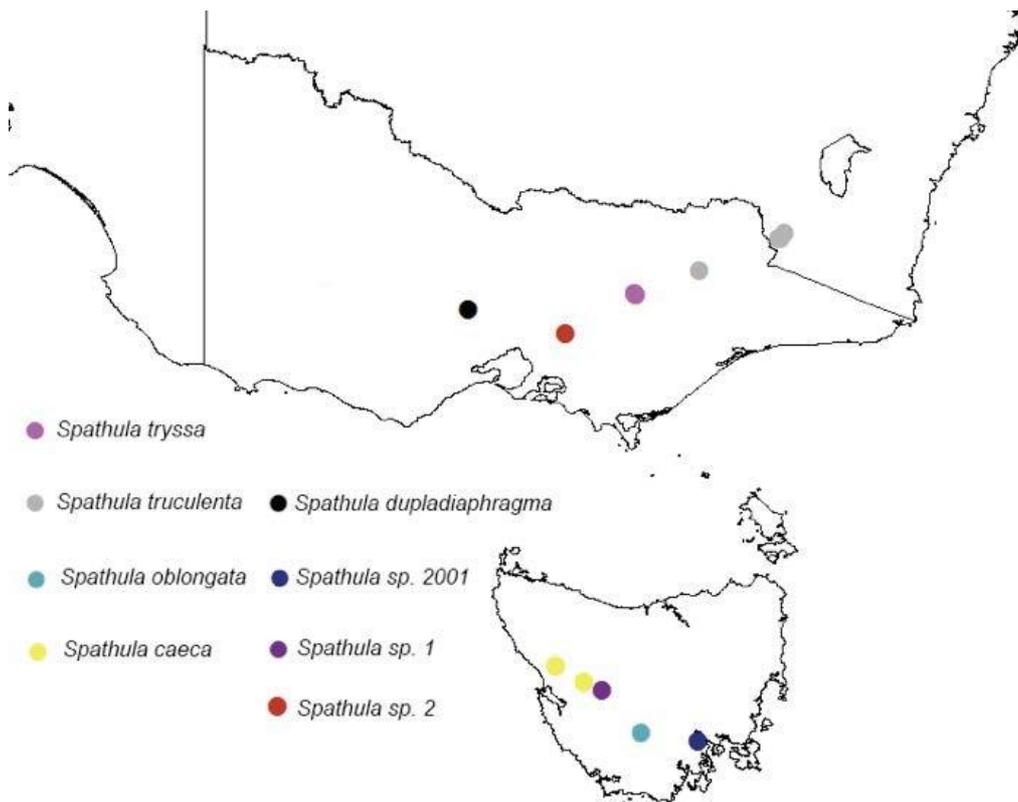
### 4.3.2 Australian Distribution



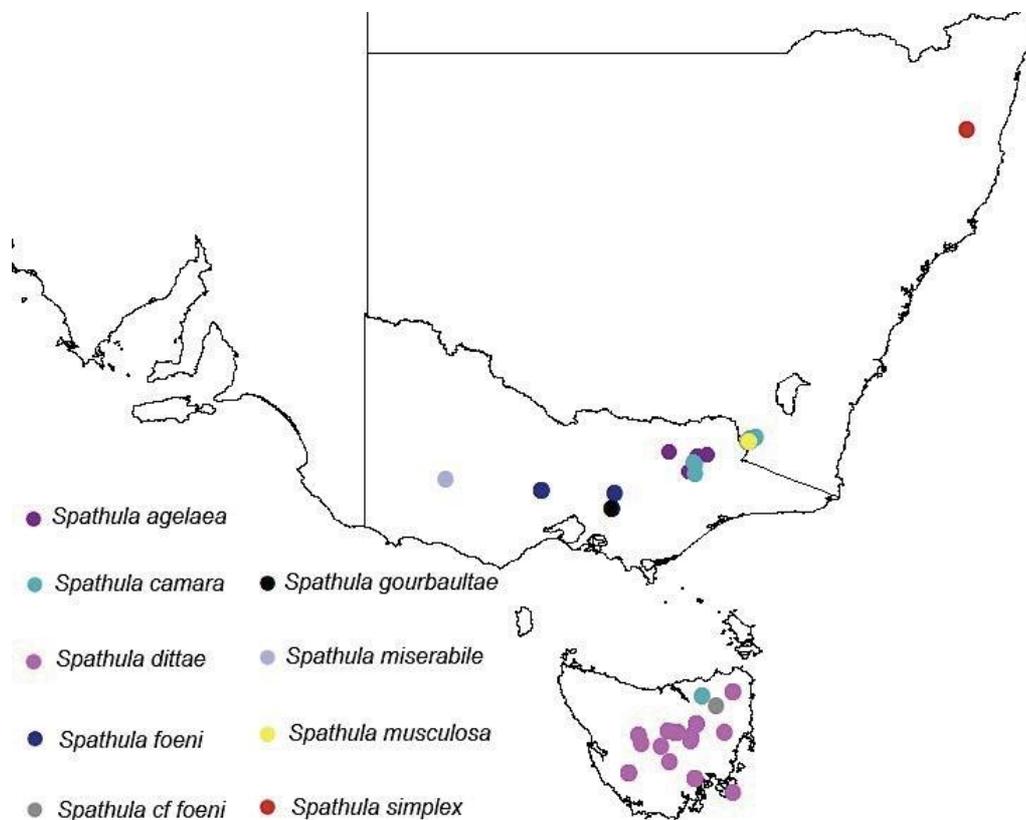
**Figure 4.8** Distribution of the genus *Cura* in Australia.



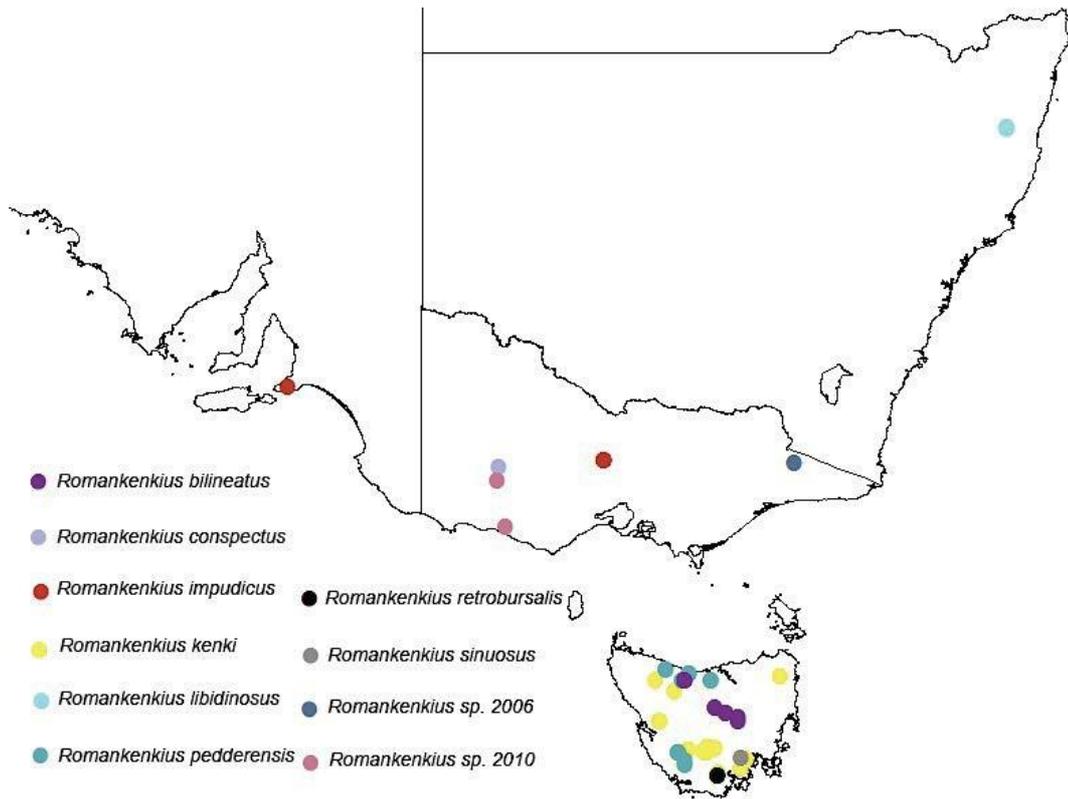
**Figure 4.9** Distribution of the genus *Dugesia* in Australia.



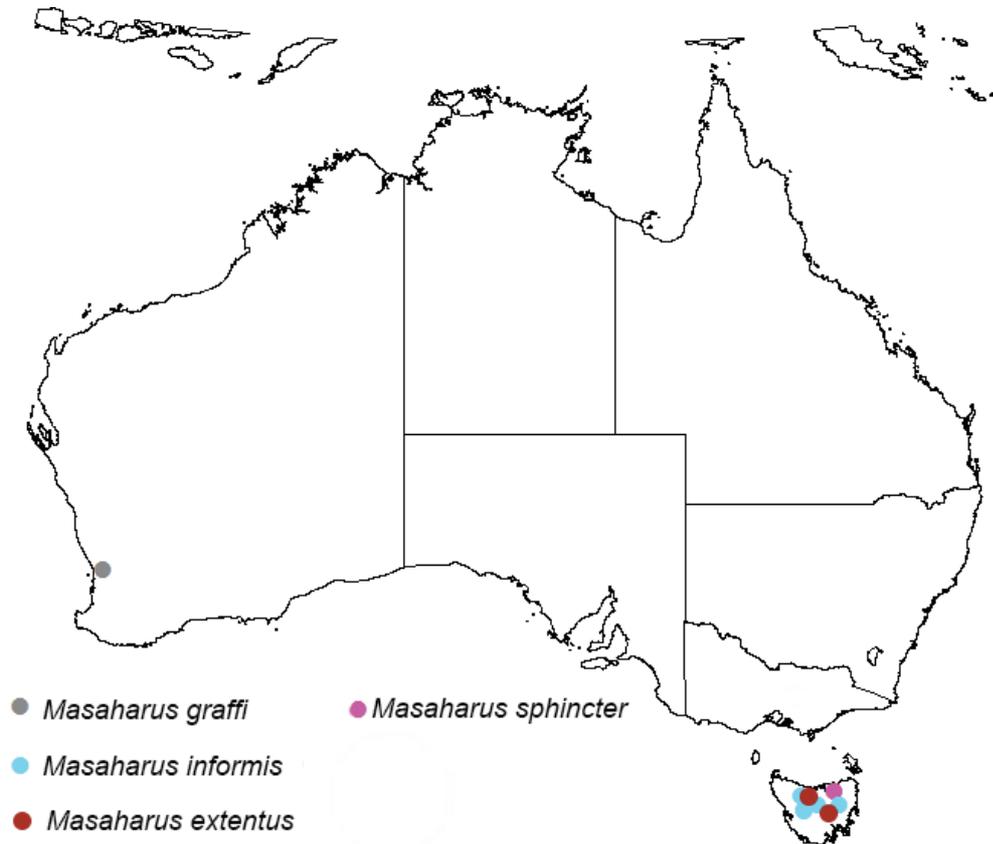
**Figure 4.10** Distribution of some representatives of the genus *Spathula* in Australia (separate maps used due to overlap of many species).



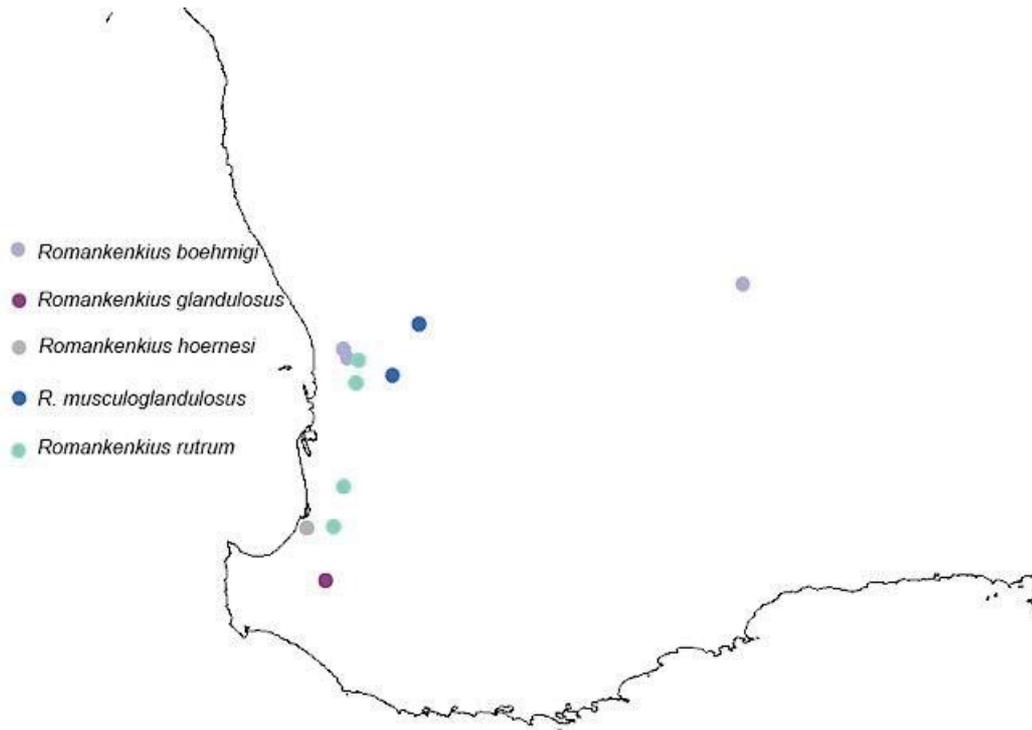
**Figure 4.11** Distribution of the remaining representatives of the genus *Spathula* in Australia (separated due to overlap of many species).



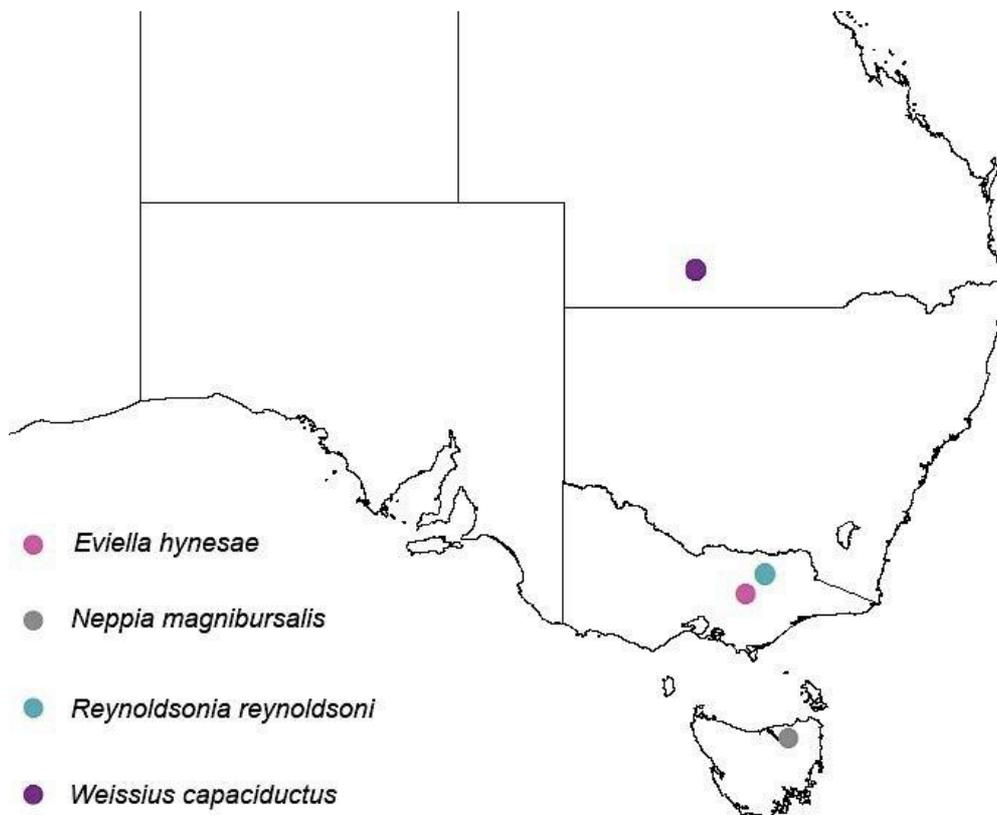
**Figure 4.12** Distribution of the taxon “eastern *Romankenkius*” in Australia.



**Figure 4.13** Distribution of the genus *Masaharus* in Australia.



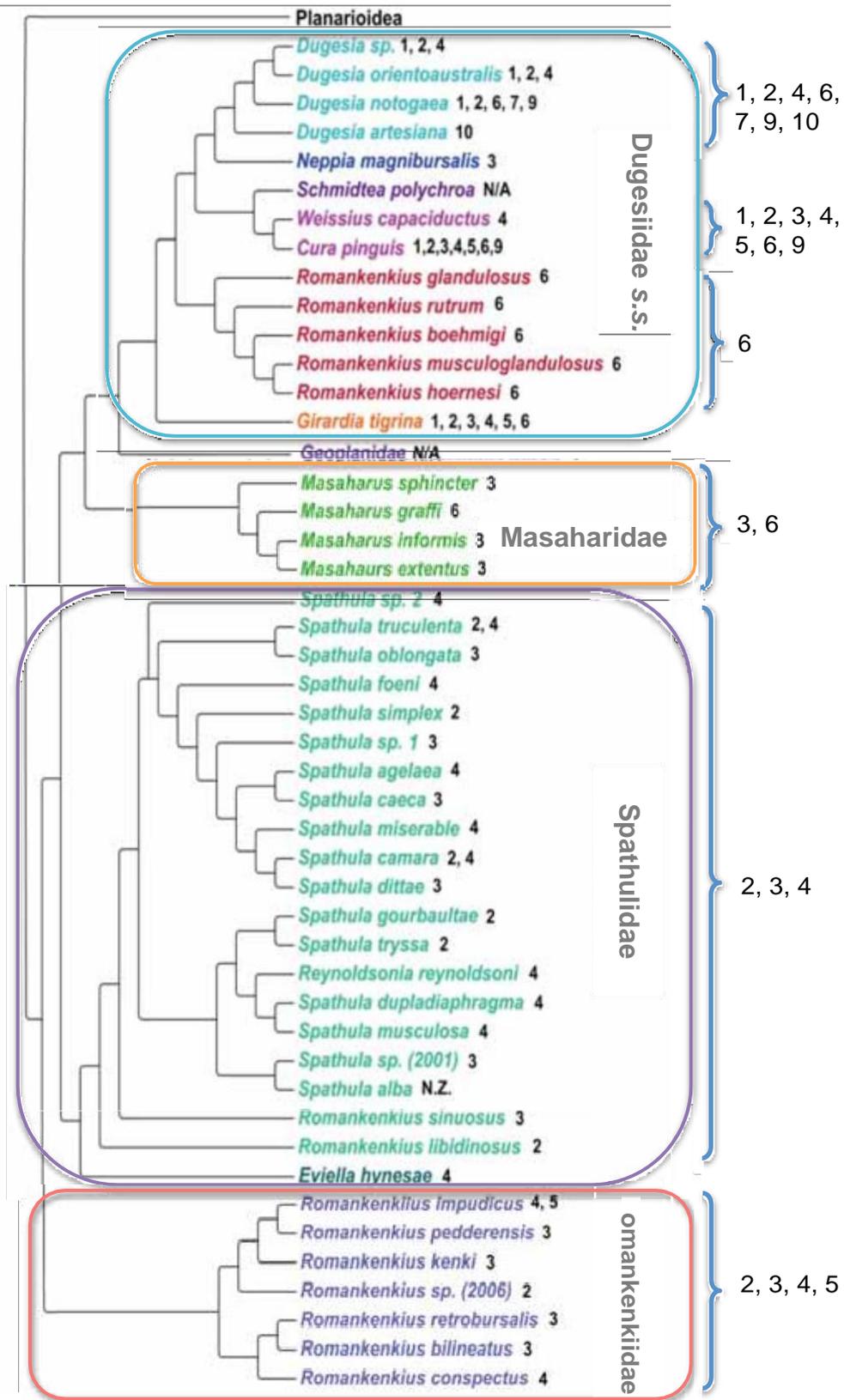
**Figure 4.14** Distribution of the taxon “western *Romankenkius*” in Australia.



**Figure 4.15** Distribution of monotypic genera or those with only one representative within Australia.

**Table 4.1** Species used for Australian analysis (areas referenced are drainage divisions as in Figure 4.6).

<b>Species</b>	<b>Areas</b>
<i>Cura pinguis</i>	1, 2, 3, 4, 5, 6, 9
<i>Dugesia artesiana</i>	10
<i>Dugesia notogaea</i>	6, 7, 2, 1, 9
<i>Dugesia (?) rava</i>	6
<i>Dugesia orientoaustralis</i>	1, 2, 4
<i>Dugesia sp.</i>	1, 2, 4
<i>Eviella hynesae</i>	4
<i>Neppia magnibursalis</i>	3
<i>Girardia tigrina</i>	1, 2, 3, 4, 5, 6
<i>Masaharus graffi</i>	6
<i>Masaharus informis</i>	3
<i>Masaharus sphincter</i>	3
<i>Masaharus extentus</i>	3
<i>Reynoldsonia reynoldsoni</i>	4
<i>Romankenkius cf. bilineatus</i>	3
<i>Romankenkius conspectus</i>	4
<i>Romankenkius impudicus</i>	4, 5
<i>Romankenkius kenki</i>	3
<i>Romankenkius libidinosus</i>	2
<i>Romankenkius pedderensis</i>	3
<i>Romankenkius retrobursalis</i>	3
<i>Romankenkius sinuosus</i>	3
<i>Romankenkius sp. 2006</i>	2
<i>Romankenkius sp. 2010</i>	2
<i>Romankenkius bilineatus</i>	3
<i>Romankenkius boehmigi</i>	6
<i>Romankenkius glandulosus</i>	6
<i>Romankenkius hoernesi</i>	6
<i>Romankenkius musculoglandulosus</i>	6
<i>Romankenkius rutrum</i>	6
<i>Spathula agelaea</i>	4
<i>Spathula camara</i>	2, 4
<i>Spathula dittae</i>	3
<i>Spathula cf. foeni</i>	3
<i>Spathula foeni</i>	4
<i>Spathula goubaultae</i>	2
<i>Spathula miserable</i>	4
<i>Spathula musculosa</i>	4
<i>Spathula tryssa</i>	4
<i>Spathula simplex</i>	2
<i>Spathula truculenta</i>	2, 4
<i>Spathula oblongata</i>	3
<i>Spathula caeca</i>	3
<i>Spathula dupladiaphragma</i>	4
<i>Spathula sp. 2001</i>	3
<i>Spathula sp. 1</i>	3
<i>Spathula sp. 2</i>	4
<i>Weissius capaciductus</i>	4



**Figure 4.16** Final phylogenetic hypothesis including all Australian species from Chapter 2. Numbers in black represent catchments as designated in Figure 4.7. Boxes designate families as proposed in Chapter 3.



## 4.4 Discussion

### 4.4.1 Global Diversity

The Australasian region contains the greatest diversity of DugesIIDae *s.l.* at the level of genera and of species, accounting for around 27% of the globe's species and hosting nine out of the 12 known genera (Grant et al. 2006; Sluys 1997; Sluys et al. 1998; Sluys & Kawakatsu 2001)(Figure 4.7). All species are endemic, the only exception being the invasive *Girardia tigrina*. The Australasian region also exhibits a high level of endemism at the genus level with five endemic genera (*Spathula*, *Masaharus*, *Weissius*, *Eviella* and western *Romankenkius*). Only two other regions have endemic genera. The Nearctic with *Bopusla* (Marcus 1946), and the Palaeartic, with the newly described *Recurva* (Sluys et al. 2013).

The Palaeartic is the next most species-rich region, boasting 20% of the globe's species, with four of the 12 known genera (Figure 4.7). The data for the Palaeartic must be treated with some reservations due to the possible bias discussed in the introduction (Section 4.1.1)(Balian et al. 2008a; Lake et al. 1985). However, this potential sampling bias also means that the low generic diversity reported from this region may, in fact, be correct. The Neotropics and Afrotropics also have relatively high diversity, both having less species diversity than the Palaeartic (16% and 17% respectively), but relatively high generic diversity (three and four genera respectively)(Figure 4.7). This pattern of diversity is expected, to certain extent, as Balian (2008a) found the Palaeartic and Neotropics to be the most species-rich for freshwater taxa generally; the high level of diversity found in the Australasian region seems anomalous in this context.

The high generic diversity found in the DugesIIDae *s.l.* in Australasia, has been discovered in some other freshwater groups; for example, Temnocephalida, Ephemeroptera, Oribatida and the rare crustaceans Spelaeogriphacea and Tanaidacea (Barber-James 2008; Cannon and Joffe 2001; Jaume 2008; Jaume & Boxshall 2008; Schatz et al. 2008; Schockaert 2008; Segers 2008; Wilson 2008). However, when compared to groups with similar life history strategies (i.e. no dispersal or drought resistant phases) such as freshwater nematodes, oligochaetes and nemerteans, no over-representation in Australasia is found (Abebe 2008; Glasby & Timm 2008; Poinar 2014; Sundberg & Gibson 2008). In fact, Balian's (2008a) Freshwater Animal Diversity Assessment places the Australasian region well below all other biogeographical regions (excepting Antarctica and Pacific Region and Oceanic Islands) for both freshwater species and genus diversity.

The presence of such high DugesIIDae *s.l.* generic and species diversity on a continent that has been isolated for the last 30 million years, suggests that Australia was a

centre of diversity for freshwater triclads during the Gondwanan era. While we will probably never have any indication of what the freshwater triclad fauna in Antarctica was like (it supported a rich warm-temperate biota in the core of Gondwana throughout the Triassic), it is likely that this also would have been highly diverse (Pugh & Convey 2008).

#### 4.4.2 Global Biogeography

Existing theory, relating to the current global distribution of the freshwater triclads, suggests that the Dugesiidae *s.l.* must have diversified before the breakup of Pangea (Ball 1974a; Ball 1975). This theory relies on the monophyly of the Geoplanoidea and there is currently no evidence to dispute this assumption (see Chapter 2). New research supports this idea, as regardless of the sequence of diversification, representatives of the Dugesiidae *s.l.* must have been broadly distributed by the Jurassic; specifically, the Romankenkiidae and Spathulidae in Gondwana, and the Dugesiidae *s.s.* throughout Pangaea (as in Figure 3.5, the proposed classification and as illustrated in Figure 4.16). As established above, Gondwana, specifically Australia, was a diversification centre. However, it was the Dugesiidae *s.s.* that dispersed successfully throughout the northern hemisphere (Laurasia), while the Romankenkiidae and Spathulidae are predominantly still only found in Australia (*Romankenkius patagonicus* is the exception). It is clear from the restricted distributions of the current Romankenkiidae and Spathulidae species that the Dugesiidae *s.s.* are currently able to disperse more effectively (further discussion below). It is likely that Australia's isolation has led to the preservation of these relict taxa, as is the case for so much of Australia's native flora and fauna (Byrne et al. 2011; Unmack 2001).

#### **Romankenkiidae**

##### *Eastern Romankenkius*

The Romankenkiidae appears at the base of the geoplanoid phylogeny. The distribution of the Romankenkiidae is concordant with the idea that it is a remnant of an ancient, widespread group (Figure 4.12). Romankenkiidae is represented in South America by *R. patagonicus*, endemic to Chile and Argentina (Sluys et al. 2005). Trans-Pacific links are apparent in a range of flora and fauna, including the Geoplanidae and Maricola (Sluys 1995, 1998). While the vicariance history of the southern continents has been questioned by Sanmartin and Ronquist (2004), they do agree that, for fauna, the distribution of taxa agrees with the breakup sequence of Gondwana and can best be explained by the Antarctic "land bridge" that existed between Australia and South America (Briggs 2004; Sanmartin & Ronquist 2004).

If we assume that the distributions of these Romankenkiidae species are due to vicariance, the genus must have been in existence in the mid-Jurassic (170 - 160 mya), as according to current theory eastern Gondwana (including Australia) and western Gondwana (including South America) severed their connection at this time (Figure 4.12 and 4.14). While the triclad fauna that inhabited Antarctica will forever remain a mystery, the question could be asked as to why no Romankenkiidae have been discovered in Africa. However, this is not an uncommon pattern for Gondwanan biota. Crisci et al. (1991) concluded that southern South America, Australia, Tasmania, New Guinea, New Caledonia and New Zealand constitute a monophyletic group in regards to their biota, reflecting the existence of an ancient Austral biota. While Africa was part of Gondwana, it appears to show a history distinct from both Gondwana and Laurasia as early as the Late Jurassic (160 mya) (Gheerbrant & Rage 2006). Since this time, interchanges with the rest of Gondwana were rare and were mainly dispersals out of Africa. Additionally, despite some contact with Laurasia, Africa exhibits significant absences, low diversity and many endemic taxa (Gheerbrant & Rage 2006).

### **Spathulidae**

#### *Spathula and Eviella*

*Spathula* is found only in the Australasian region, where it is quite diverse (Figure 4.10 and 4.11). The presence of this genus in New Zealand suggests that the genus was well established in this region from at least 82 mya when New Zealand became isolated from Antarctica (Boyer & Giribet 2009). Breaking away from Gondwana first, New Zealand missed out on many groups. However, there was clearly ample opportunity for most freshwater invertebrate taxa (such as triclads) to be present (McDowall 2010; Schodde 1989). There is some speculation that the freshwater environments in New Zealand were drowned by sea level rises at one point in the Miocene (Toon et al. 2010). However, the distribution of several other freshwater groups, including crayfish, provide evidence against this hypothesis (Toon et al. 2010). Prior to recent analyses, *Spathula* was thought to have evolved in isolation in Australasia from *Cura*-like ancestors (Ball 1977a, c). However, the current analysis suggests that the common ancestor to these genera gave rise to most other freshwater geoplanoid genera and that *Spathula* and *Cura* are not sister taxa (Figure 2.24 and 4.17).

### **Masaharidae**

#### *Masaharus*

*Masaharus* appears to have been the last freshwater taxon to diverge from the ancestral lineage before the appearance of the Geoplanidae (Chapter 2). Concordantly, its current

distribution suggests that it is primarily aligned with the Romankenkiidae and Spathulidae. Its distribution, which is restricted to Tasmania (the existence of the Western Australian representative is very speculative), and the fact that it shares a common ancestor with the Geoplanidae, suggests that it is another example of an older genus that has been conserved in the Australian refugia (Figure 4.13).

### **The Dugesiidae s.s.**

#### *Dugesia*

The old-world genus *Dugesia* was reported only recently in Australia when Sluys et al. (1998) described *D. notogaea*. Its presence in northern Australia has global implications (Sluys et al. 2007). It is tempting to rule out dispersal in the case of the Dugesiidae s.s.; however, many classic Gondwanan groups combine recent dispersal events with ancient vicariant patterns (Cooper et al. 2001; Waters et al. 2000). *Dugesia* is the only genus for which a dispersal hypothesis is preferred to explain its presence on the Australasian plate.

*Dugesia* is species-rich, with over 90 described species distributed primarily across Europe, Asia and Africa and is proposed to have had an origin in Laurasia (De Vries 1985). The genus *Dugesia* can be divided, based on morphological phylogenies, into two groups; one with mainly a western distribution (Africa & Europe) and one with a primarily eastern distribution (Asia and Australia) (Sluys et al. 1998). The presence of both *Dugesia* groups only in Africa argues for an origin there (Álvarez-Presas et al. 2008; Ball & Fernando 1969; Ball 1975; c.f. Croizat et al. 1974; Sluys et al. 1998). The absence of *Dugesia* from South America suggests that the genus might have started to diversify between 120-100 mya; however, this is problematic due to the presence of the genus in India/Madagascar (separated from Africa approximately 150 mya) (Jokat et al. 2003; Scotese 2004; Sluys et al. 1998; Upchurch 2008). Perhaps this problem will be resolved if we look beyond vicariance as an explanation for distribution patterns.

The current phylogeny (Figures 2.24 and 4.17) is concordant with the idea that *Dugesia* is a relatively recently derived genus that has dispersed from Africa (after this plate collided with the European plate) through Europe and Asia to the Australian mainland (Appendix 4). New Guinea (the northern extent of the Australasian plate) connected with Asia 15 mya. However, there are no known occasions when there was a land bridge between the two continental plates (Hall 2001; Haq et al. 1987; Voris 2000). So might *Dugesia* have been present in Australia since the break-up of Gondwana?

There are several problems with this hypothesis. For example, if *Dugesia* had an ancient Gondwanan distribution, its presence in the Americas is to be expected (Figure 4.7). Secondly, if this genus had been on the Australian continent since the breakup of

Gondwana, higher levels of speciation would be expected, as in Europe (De Vries 1985; De Vries 1988). Instead, the distribution pattern of the genus strongly suggests that it dispersed from Asia to Australia during the Pleistocene (1.8 mya to 10,000 years ago) when sea levels were much lower (Figure 1.16) (Sluys et al. 2007; Sluys et al. 1998). A precedent for this has been set by many other, albeit usually more vagile, flora and fauna that have dispersed from Southeast Asia to Australia (Bowman et al. 2010).

Whilst de Queiroz (2005) argued that oceanic dispersal is not as rare for freshwater and terrestrial species as often believed, freshwater triclads must require some kind of freshwater pathway. It is possible that this has occurred without the presence of a land connection. For example, during the last glacial period the sea level was at least 120m lower than it is today (22,000 years ago). While this may have not created a land bridge, much more of the present-day Indonesian and New Guinea continental shelves were exposed (Geoscience Australia 2014). Since the collision of the Australasian and Asian plates (15mya) there have been many sea level fluctuations of a similar magnitude (Geological Timescale Foundation 2015). Events like this could have permitted riverine flood plumes to provide a mode of dispersal between otherwise unconnected drainage divisions (Chenoweth & Hughes 1997; Grimes & Kingsford 1996; Jerry 2008; Unmack 2001; Wolanski & Jones 1981). Finally, whilst biochore, a mode of dispersal for adult freshwater triclads, is an unlikely scenario, it must be considered possible that their cocoons have been transported between adjacent islands on birds or even on vegetation mats (Campbell et al. 2000). This dispersal theory is suggested as the most likely mechanism behind the presence of *Dugesia* in Australia by Sluys et al. (1998, 2007).

### *Girardia*

Until recently, *Girardia* was thought to have a natural distribution on the Australasian plate (Sluys 1995, 1998; Sluys & Kawakatsu 2001). However, the erection of *Masaharus*, which now houses all Australian species formerly in *Girardia*, has removed this difficulty from the biogeographical history of the group (see Chapter 3). The only species found in Australia is the invasive *Girardia tigrina*. This species has also recently colonised Japan and Europe (Kawakatsu et al. 1993; Riutort et al. 2012; Sluys et al. 2010), presumably via the international trade in aquarium plants (Ball 1971).

### *Cura*

*Cura* is a widely distributed genus, consisting of four species found throughout North America, Australia, New Zealand and New Caledonia (Ball 1974c, 1977; Grant et al. 2006). This disjunct distribution makes speculation on the origins of the genus particularly

difficult. If *Cura* was not present in Australia, one could simply hypothesise that the common ancestor of *Cura* and *Schmidtea* existed in the northern hemisphere and after the separation from Gondwana the two genera diversified and dispersed from there (Figure 4.7). However, the apparent natural presence of *Cura* in Australia makes this hypothesis untenable.

The current distribution of *Cura* (Australasia and North America) might indicate that the genus was present throughout Gondwana before its breakup, and has since colonised North America and been lost from South America (or simply not yet discovered)(Figure 4.7) This hypothesis is also more concordant with the fact that *Cura* has long been considered the most ancient of the Dugesiidae *s.l.* genera by virtue of its simple anatomy (Ball 1974c). However, recent molecular phylogenies (e.g. Álvarez-Presas and Riutort 2014 and Chapter 2) suggest that *Cura* is a derived species, leaving two alternatives. Either the Dugesiidae *s.l.* had diversified a great deal before the break up of Gondwana, or *Cura* has exceptional dispersal capabilities. While Ball (1974c) and Ball and Fernando (1969) agreed with Brink's (1960) comment that *Cura* has an ability to disperse that warrants further investigation, vicariance was their preferred theory for its current global distribution.

This general pattern of distribution is also demonstrated in Romankenkiidae, a family that is proposed to have diversified from a common ancestor in Australia and to have inhabited much of the supercontinent Gondawana prior to its breakup. There is nothing in the current phylogeny that precludes this being a possibility for *Cura*, as *Girardia* and western *Romankenkius* (genera that appear slightly more basal than *Cura*) are also found in the southern hemisphere. Additionally, *Neppia*, a similarly derived genus, is also found across all the southern continents (excluding Antarctica). The alternate possibility is, of course, that *Cura formanii* (the North American representative) is not a true *Cura* and that the two taxa are not closely related. However, we currently have no molecular data for *Cura formanii*: all theories are based on morphological similarities.

### *Neppia*

The genus *Neppia* consists of only ten species but has one of the broadest distributions of any of the Dugesiidae *s.l.* *Neppia* shows a similar, but broader, distribution to Romankenkiidae (Australasia, Africa and South America), suggesting that this genus was also in existence during the mid-Jurassic (170 mya). The presence of both *Neppia* and Romankenkiidae in southern Australia makes dispersal an unlikely explanation for the distribution of the genus (vicariance being the alternative). The only conceivable dispersal route would be via Asia after the plates collided (15mya). This would necessitate

migration of *Neppia* and Romankenkiidae from Africa through Europe and Asia, a dispersal that should produce a more ubiquitous distribution (it also does not account for the presence of both groups in South America). It is likely that widespread populations of *Neppia* and of Romankenkiidae co-existed in Gondwana before east and west Gondwana had begun to separate 180 mya. The existence of *Neppia* in Africa is interesting as this genus likely represents a Gondwanan relict.

### Western *Romankenkius*

Western *Romankenkius* appears near the base of the phylogeny for the Dugesiidae *s.s.* and is clearly very distinct from the eastern *Romankenkius* (Romankenkiidae) (Chapter 2). It is currently known only from Australia (Figure 4.14), so it either represents another relict, or it has more recently colonised the west from the east of the continent (there have been many opportunities since Australia's isolation). Several researchers have determined that the formation of the Nullarbor Plain, due to the increasing aridity during the Miocene (14-16 Ma), would have made east-west migration, at least in the south of the continent, of freshwater species after this time unlikely (Barendse 1984; Roberts & Maxon 1985; Unmack 2001). This suggests that this species is most likely an isolated remnant of a taxon that previously had a much broader distribution. As part of the Gondwanan supercontinent, western Australia was closely associated with northern Antarctica, which in turn had connections with the Cimmerian and Indian plates (Scotese 2004). Samples from India and Tibet could further elucidate the history of western *Romankenkius*.

### Broad Themes

With our current knowledge of the distribution of the Dugesiidae *s.l.*, the most likely scenario explaining the current day distributions is that most of the extant genera diversified before the breakup of Gondwana. The only exceptions to this are *Dugesia* and *Schmidtea*, which appear to have diversified after this separation. The current global distribution of freshwater geoplanoid genera is primarily a result of vicariance (especially the breakup of Gondwana), with dispersal explaining more modest movement between recently connected landmasses.

Carranza et al. (1999) used the duplication of the 18S gene to suggest that a common ancestor of *Dugesia*, *Schmidtea* and *Girardia* dated back to 120 - 80 mya. Table 3 (Chapter 2) details several other species for which the duplication is present, most notably *Spathula ditae*, *Masaharus extentus* and *Cura pinguis*. The presence of this duplication in these Australian endemic genera, coupled with its existence amongst Geoplanidae (Carranza et al. 1998a), leads to the conclusion that the duplication event must have

occurred even earlier. The common ancestor of *Girardia* and the Geoplanidae, which exhibited the duplication, must have been distributed across Gondwana prior to separation of eastern and western Gondwana (170 - 160 mya).

#### 4.4.3 Australian Biodiversity

It is clear from figures 4.17 and 4.18 that there are two biodiversity hotspots for Australian freshwater triclads. These are Victoria (predominantly the Australian alpine region) and Tasmania. Of these two, Tasmania is the most species rich with 19 species being recorded (14 local endemics), whilst Victoria houses 17 species (11 endemics). These regions share a geological history and have only recently become geographically separated (10,000 years ago). As a consequence, affinities between species in these two areas are common (Brinkhurst 1971; Crisp et al. 1995; Hynes 1974; Jamieson 1973)(Figure 3.23). Southwestern Australia presents the next highest species diversity, with nine Dugesidae *s.l.* species (seven endemics)(Figures 4.17 and 4.18). This is of substantial interest, noting the sampling effort in southwestern Australia has been less than in Tasmania and Victoria (see Chapter 3). The northern/tropical regions of Australia exhibit relatively low species diversity. This pattern is consistent with comments by Blackburn and Gaston (1996) and Balian (2008a, b) who pointed out that, while marine and terrestrial taxa are most diverse in tropical regions, this is generally not the case for freshwater taxa. While the central and northwest regions have not been sampled because of the lack of permanent water sources there, it is unlikely that large numbers of species would be found. The generic diversity shows similar patterns to the species diversity, with Victoria and Tasmania exhibiting the highest levels of diversity (Figure 3.12).

It was proposed at the beginning of this thesis that Australia is likely to be very species-rich for freshwater triclads. Although this is true both at the level of genus and species (9 genera, 56 species), this diversity is concentrated in two biodiversity hotspots. Otherwise Australia is dominated by three widespread genera: *Cura*, *Dugesia* and the invasive *Girardia*. This pattern is not uncommon in Australian freshwater invertebrates. For example, the greatest diversity of freshwater crayfish is found on the northwest coast of Tasmania and the southeast of mainland Australia (Crandall & Buhay 2008; Whiting et al. 2000).

The reasons for these areas exhibiting high levels of diversity are not difficult to infer. Australia and New Zealand inherited from Gondwana a prevailing cool temperate to subtropical rainforest biota (Schodde 1989). The primary contributors to the high freshwater triclad species diversity in these regions (Spathulidae and Romankenkiidae) are believed to be ancient Gondwanan triclads that are ecologically suited to the cooler

mountainous regions of southeastern Australia (Ponder & Colgan 2002). Not only are Romankenkiidae and Spathulidae species isolated by temperature tolerances, but the mountainous terrain and associated drainage systems create physical barriers, leading to more opportunities for speciation (Byrne et al. 2008). It is also likely that these regions have provided refugia for ancient freshwater triclad genera from various events such as sea level rises that created inland seas on the continent, ice sheets and, more recently, aridification (Byrne et al. 2008; Cox & Herbert 2001; Metcalfe 2001).

Most Australian freshwater triclad species are restricted to single drainage divisions. The main exceptions to this are representatives of the Dugesiidae *s.s.*; *Cura*, *Dugesia* and *Girardia* (Table 4.1). *Girardia tigrina* has managed to achieve a very broad distribution considering that it must have been introduced within the last 100 - 200 years (discussed above). In contrast, *Dugesia* and *Cura* have both had millions of years to achieve their current distributions. Within the Romankenkiidae and Spathulidae there are only three examples of species with distributions spanning two drainage divisions and in these cases the divisions are adjacent. It would appear that these ancient taxa currently have a highly restricted niche, confined not only by a lack of interconnecting drainage divisions, but also by temperature (Ponder & Colgan 2002). The ecological data collected as part of this study demonstrates that widespread genera all exhibit broad temperature tolerances, which presumably contributes to their dispersal ability (Ponder & Colgan 2002; Stewart et al. 2013). Another factor affecting species' ability to disperse is their micro-habitat; for example, members of the Romankenkiidae and Spathulidae are primarily found in riffles, whereas *Cura*, *Dugesia* and *Girardia* are just as comfortable in the slow moving and often barely wet regions of a creek (pers. obs.). This is a definite advantage in such a dry continent.

#### 4.4.4 Australian Biogeography

##### **Romankenkiidae, Spathulidae and Masaharidae**

The discussion above relating to the Tasmanian and Victorian diversity hotspots is primarily a discussion around the distribution of eastern *Romankenkius*, *Spathula* and *Masaharus*. As a consequence there is no need to repeat these points. However, there are two additional elements of interest that should be discussed.

Firstly, the presence of *Masaharus* in Western Australia is tentative (Figure 4.13). *Masaharus graffi* has been placed in this genus only because sufficient diagnostic characters are not available to assign it to any other genus. It is likely that if more examples of this species are examined it will be assigned elsewhere. Secondly, why is

there such an affinity (i.e. high diversity and closely related species) between Victoria and Tasmania? While significantly eroded, the Great Dividing Range (approximately 300 million years old) houses a high level of diversity and endemism for triclad taxa (Johnson 2004)(Figure 4.18). This suggests that these ranges, specifically the taller, cooler southern extents, have been a centre of diversity for freshwater triclads for an extended period of time. While historically these taxa inhabited the reasonably continuous, but mountainous area, the two regions of diversity (Tasmanian and Victoria) have been separated for least 12,000 years by Bass Strait, meaning that any recent gene flow between the two areas would have been unlikely. Molecular data are available for many species of *Spathula*, but the phylogeny does not offer a great deal of congruence in regards to the locations of these species (Figure 4.16). Indeed, many *Spathula* species are more closely related to a congener from across Bass Strait than to the *Spathula* on the next mountain. This suggests that, not surprisingly, the last 12,000 years of separation have not been very influential in *Spathula* evolution.

### **The Dugesiidae s.s.: Widespread species**

#### *Dugesia*

The discovery of *Dugesia* virtually throughout Australia is one of the most surprising findings of this thesis. The implication is that it must have migrated from Asia in the northeast and then dispersed across the continent (Figure 4.9). Further support for this hypothesis is provided by the strong links shown between a range of proposed recent arrivals and the Oriental-Papuan fauna (Austin et al. 2004). These include freshwater species, as phylogenetic relationships between Australia and southern New Guinea are consistent with episodic connection via the freshwater Lake Carpentaria (between about 35,000 and 12,000 BP) during periods of low sea level (McGuigan et al. 2000). New Guinea has only a single known freshwater triclad species, *Dugesia novaguineana*; however, there is a high likelihood that more *Dugesia* species will be found there.

If we are proposing that *Dugesia* arrived in Australia in the last 15 million years, we need to look at relatively recent history to explain its presence in Western Australia, which has long been isolated by the arid centre. While the collision with the Pacific plate compounded the drying of the continent (Byrne et al. 2008; Cox & Moore 2000; Frakes 1999), there was a wet interlude between five and two mya, which could have provided enhanced opportunity for drainages to connect and new drainages to appear (Johnson 2004). In addition to this, lowered sea levels would have provided an opportunity for movement around the north of the continent as both adjacent and non-adjacent drainages could have become continuous (Unmack 2001).

The presence of *Dugesia* in the Pilbara, a large semi-arid region of northwestern Australia, suggests that this genus is capable of adapting to challenging environments (Figure 4.9). The river and aquifer systems date back to the Mesozoic and house a large variety of endemic groundwater fauna (Beard 1998). The region, which has been relatively unchanged geologically for 100 mya, has experienced episodes of wet and dry climates but has been arid since the mid-late Pliocene, and likely experienced periods when no permanent sources of water remained (Frakes 1999; MacPhail & Stone 2004). During these periods of extreme aridity, freshwater species presumably sought refuge in subterranean water (a possible strategy employed by Australian freshwater triclads)(Bradbury & Williams 1997; Humphreys 2001). Movement between otherwise unconnected catchments in these arid environments may not be as difficult as the environment might suggest due to the lack of topographical relief. During climate fluctuations and rare flood events, isolated catchments have the potential to connect with others and their constituent fauna to disperse (Carini & Hughes 2004; Hughes et al. 2004). These conditions and restrictions go a long way to explaining why this region is populated by a single species, as less tolerant genera (e.g. *Spathula*) would have perished probably long before *Dugesia* arrived in the region.

In addition to its ecological tolerance, the genus *Dugesia* has one more feature which assists it in colonising new territory: asexual reproduction. This mode of reproduction is likely prevalent in Australian *Dugesia* (*Dugesia* sp. and *Dugesia orientoaustralis*)(Riutort et al. 2012). It has been observed in *Dugesia* in the western Mediterranean that the fissiparous forms have superior colonising abilities over their sexual counterparts in certain habitats (Beveridge 1982; Roca et al. 1992). This is due to the ability of a single individual to establish a population and the higher rates of reproduction in asexual individuals as energy is not being invested in reproductive structures, mating and cocoon production (Baguñà et al. 1999). It has been pointed out by many authors that the advantages of asexual reproduction are magnified in unpredictably poor habitats such as hot dry areas, a habitat type found in abundance in Australia (Baguñà et al. 1999; Beveridge 1982; Calow et al. 1979; Reynoldson 1961; Roca et al. 1992).

### *Cura* and *Weissius*

*Cura pinguis*, with its broad distribution and ecological tolerance, has the characteristics of an invasive species. However, this species must have been on the continent for at least the last 30 million years (since separation from Antarctica) (Figure 4.8). Sluys and Kawakatsu (2001) also reached this conclusion, suggesting that the global distribution of *Cura*

represents an extensive biogeographic track. The radiation of *Cura* must therefore have been well before the Pleistocene. While this is true of other more localised genera (e.g. *Romankenkius*, *Neppia*), the ecological and location data collected as part of this study suggests that *Cura pinguis* has the widest temperature tolerance (Figure 4.7)(Appendix 1d), allowing it to colonise readily and possibly survive in regions where others have perished. *Cura* does appear to be absent from the northwest; however, this could be due to limited sampling effort in this area. The occurrence of *Cura* does decrease considerably in the northeast and the temperatures in these regions are not as harsh as those in the northwest. For example, the average temperature in the Pilbara region (north Western Australia) is 35°C but temperatures in excess of 45° are not uncommon, while in the Wet Tropics (northeastern Australia) temperatures average around 25°C with maximums well below 40°C (Bureau of Meteorology 2015a). It could have also been outcompeted by *Dugesia* for the very limited permanent water in the western region.

One of the interesting features of the closely related genus *Weissius* in Australia is its presence in isolated springs in western Queensland (Figure 4.15). These oasis-like freshwater springs are fed by the Great Artesian Basin, the largest artesian system in the world (Perez et al. 2005). It is likely that increasing aridity around Lake Eyre in the early Pleistocene (1-2 mya) led to the isolation of springs in this region (Byrne et al. 2008). Species unable to cope with the changing environment adapted to ground water habitats or went extinct (Byrne et al. 2008). The species that still exist in these springs, such as *Weissius capaciductus*, represent potential relicts of the previously widespread mesic fauna (Murphy et al. 2012; Perez et al. 2005; Ponder 1986).

### *Girardia*

The American *Girardia tigrina* was almost certainly introduced to the UK and other landmasses, such as Australia, via the international trade in ornamental fish and aquatic plant species (Reynoldson 1956; Young & Reynoldson 1999). *Girardia tigrina* likely flourishes in Australia, as it is a warm-water species capable of various modes of reproduction (Young & Reynoldson 1999). The transport of cocoons is possible; however, in this case the dispersal phase is most likely the adult animal, as all populations appear to be asexual. *Girardia tigrina* adheres to surfaces better than other triclad species (at least those found in British fresh waters), which may contribute to its ability to expand its range (Gee 1990). *Girardia tigrina* can reproduce asexually, hence a population can be founded from a single individual (Kenk 1940; Young & Reynoldson 1999). It has also been shown to be an opportunistic feeder and an aggressive competitor and has even been observed

preying on other triclad species (under laboratory conditions) (Gee & Young 1993; Pickavance 1968; Reynoldson & Sefton 1976).

### **The Dugesiidae s.s.: Geographically restricted species**

#### *Western Romankenkius*

In the discussion above it is proposed that the western *Romankenkius* has been isolated in southwestern Australia for at least 15 million years. However, the duration of their residence there could have been much longer. The high diversity of the genus in this region suggests that an extended presence in the somewhat mountainous terrain has resulted in relatively high levels of speciation. In addition, southwestern Australia appears to have been free from significant drainage re-arrangements from the Late Pliocene (approx. 3 mya) to the present (Gouws et al. 2006), limiting dispersal opportunities.

The similar environments they inhabit could explain the morphological similarities between *Masaharus* and the western *Romankenkius*. These environmental parallels could mean that after diversifying from a common ancestor these genera have not changed functionally, or that these genera exhibit convergent evolution (the first option is obviously the most parsimonious). These modes of evolution are well known and there are many examples where morphologically similar species share a distant common ancestor (e.g. Australian freshwater crayfish; (Munasinghe et al. 2004)).

#### *Neppia*

When Nurse (1950) first discovered a *Neppia* in New Zealand she entertained the possibility that the genus could have been introduced following European colonisation. However, she eventually concluded that this was unlikely as *Neppia montana* is known only from New Zealand, which would mean a rapid speciation event or an extinction event from the original location. In addition, *Neppia montana* lives in mountainous areas across New Zealand, locations that are difficult to disperse into over a short period (Nurse 1950). This logic holds for the existence of a single *Neppia* species currently inhabiting the southern highlands of Australia (Figure 4.15). It is likely that this genus, while less successful than the Romankenkiidae or Spathulidae, has managed to survive in these regions for at least 85 million years (since the separation of New Zealand from Antarctica).

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## Chapter 5: General Discussion

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### 5.1 Introduction

Chapter 5 presents an overview and general conclusions, examining the significance of the thesis. Potential future research arising from this study will also be discussed.

### 5.2 What, Why and How

Freshwater triclads are a cosmopolitan group. Despite this, our knowledge of their biodiversity, biogeography and phylogeny is surprisingly poor. There has been steady progress worldwide towards remedying this; however, research on the biodiversity of Australian freshwater triclads has been dormant until recently. This project is the first study of the biodiversity, biogeography and phylogeny of Australian freshwater triclads, involving extensive and targeted sampling, contrasting with the more haphazard studies characteristic of the past. The project contributes to our limited understanding of freshwater triclads in general, and the Australian species in particular.

This project began with extensive systematic work performed on a pre-existing, unstudied Australian collection (Appendix 1b). This work revealed several new species from three separate genera, representing a major contribution to the Australian fauna. New material was then collected throughout Australia (the Northern Territory being the only notable exclusion), with over 500 sites visited and freshwater triclads were present at over 400 of these. Molecular and traditional systematic techniques were used to describe new genera and species, draw conclusions concerning their phylogeny, and to examine patterns of geographical distribution and affinities with related taxa elsewhere in the world.

### 5.3 What I Found

The taxonomic work, which forms the basis for the phylogenetic and biogeographic analyses, revealed a rich and diverse Australian fauna. In total, 16 new species and two new genera (with additional tentative new species and genera) have been described. This approximately doubled the number of species that were previously known to inhabit the region - a result that was not unexpected, as previous researchers had predicted that the Australian continent harboured very high freshwater triclad diversity (Sluys et al. 1998). These discoveries will have an impact on Australian triclad research, and will also influence global understanding of the taxon. This is demonstrated by the enhanced

understanding that these discoveries have already yielded concerning the phylogeny of the Geoplanoidea.

While the intention was initially to exclude terrestrial representatives of the Geoplanoidea from the phylogenetic analysis, over the course of the research it became clear that the group formally known as the “Dugesiidae” (Dugesiidae *s.l.*) had a complex and significant relationship with the terrestrial triclads (Geoplanidae) (Álvarez-Presas et al. 2008; Álvarez-Presas and Riutort 2014; Ball 1981, Sluys 1989b). The phylogenetic analysis agreed with several previous analyses (e.g. Álvarez-Presas et al. 2008; Álvarez-Presas and Riutort 2014) suggesting that the Dugesiidae *s.l.* shared a closer relationship with their terrestrial counterparts, than they did with other freshwater triclads (the Planarioidea). While this relationship was not well resolved, the current research suggests that the terrestrial triclads may have derived directly from a freshwater “dugesiid” ancestor. Álvarez-Presas and Riutort (2014), suggested that the Dugesiidae *s.l.* can be divided into two sister groups, one of which (*Spathula*, *Romankenkius* and *Reynoldsonia*) includes the terrestrial triclads. My results are slightly different and do not support the sister group relationship, instead suggesting that the entire Geoplanoidea (freshwater and terrestrial) is a simple, monophyletic group, rendering the Dugesiidae *s.l.* a paraphyletic group.

In relation to the generic relationships among the Dugesiidae *s.l.*, there are several conflicts with recent morphological and molecular work (Álvarez-Presas et al. 2008; Álvarez-Presas and Riutort 2014; Sluys 2001). One of the most robust results from the current research was the finding of a close relationship between *Spathula* and *Romankenkius*. While only partly supported by previous morphological research (Sluys 2001), it is supported by more recent molecular research (Álvarez-Presas et al. 2008; Álvarez-Presas and Riutort 2014). In general, generic relationships at the crown of the tree (Figure 2.26) were reminiscent of those shown in recent molecular research. However, there are clear conflicts (i.e. all the same genera but slightly different relationships shown). This may be related to the markers chosen for the current analysis (i.e. 18S plus morphology), as the current hypothesis presents many relationships reminiscent of those presented in morphological work (Sluys 2001). It is also possible that these discrepancies are due to the inclusion of new genera, unique to this study. One of the most interesting results, in relation to the “dugesiid” genera, is the position of eastern *Romankenkius* as the most ancient of the extant “dugesiid” genera. Morphological research has generally pointed to *Cura* as occupying this role, however, molecular data (including the current study) strongly suggests that *Cura* is one of the more derived genera (Álvarez-Presas et al. 2008; Álvarez-Presas and Riutort 2014). Finally, the position of *Masaharus*

and the possible new genus (western *Romankenkius*) are an exciting development as they seem to represent links between freshwater and terrestrial genera, and occupy critical position within the paraphyletic Dugesiidae *s.l.*

These results have prompted the development of a tentative new classification for the Geoplanoidea (the Dugesiidae *s.l.* and Geoplanidae). The existing scheme (Figure 3.5) still sees the Dugesiidae *s.l.* and Geoplanidae as separate families (Sluys et al. 2009); however, the evidence, from both this study and other research, would suggest that this arrangement is now inadequate and that a scheme must be developed to more accurately represent the relationship between these two groups. As with the classification outlined by Sluys et al. (2009), the suggested scheme places all “dugesiids” and geoplanids under a single superfamily - the Geoplanoidea. The family Geoplanidae is maintained to house the terrestrial representatives, however the family Dugesiidae *s.s.* is much reduced and a series of additional families is erected to house those genera that do not align to this cluster and to avoid recognition of paraphyletic groupings. This more accurately reflects the state of our understanding as the families of the terrestrial representatives align well with those created for the freshwater representatives.

The curiously high diversity of the Dugesiidae *s.l.* in Australia (curious due to the lack of diversity in so many other Australian freshwater taxa) was well documented even prior to this study (Ball 1974c, 1977a; Sluys et al. 1998). Australia’s position as the most diverse continent, for both genera and species, has been emphasised through the current research. Endemicity is also a feature of the Australian “dugesiid” fauna. These high levels of diversity and endemicity suggest that Australia was a centre of diversity for the Dugesiidae *s.l.*, at least in the Gondwanan era.

Within Australia two biodiversity hotspots occur: the Australian Alpine region and the island of Tasmania. It has been suggested, and this study certainly supports the idea, that the Dugesiidae *s.l.* of the Australian Alps and Tasmania represent relics of a previously more widespread fauna (Ball 1974a, 1975). These two hotspots are the primary contributors to the status of Australia as the most diverse continent for the Dugesiidae *s.l.* Outside these areas, Australia is dominated by three widespread genera, *Cura*, *Dugesia* and the invasive *Girardia*.

Existing theory, relating to the current distribution of the Dugesiidae *s.l.* throughout the globe, suggests that the taxon must have diversified before the breakup of Pangaea (Ball 1974a; Ball 1975). This theory relies on the monophyly of the Geoplanoidea and there is currently no evidence to dispute this assumption. Regardless of the actual sequence of diversification, by the Jurassic representatives of the Dugesiidae *s.l.* must have

been broadly distributed to allow the distribution of its members throughout the Northern Hemisphere.

As the discussion around “dugesiid” diversity within Australia implies, the biogeography of the Dugesiidae *s.l.* is defined by what are perceived to be older Gondwanan relics (e.g. *Romankenkius*, *Spathula*) in the southern highlands (Australian Alps and Tasmania) and by the more “modern” genera (*Cura*, *Dugesia*) throughout the remainder of the continent’s waterways. Points of interest include the presence of the genetically very dissimilar, but morphologically aligned eastern *Romankenkius* and western *Romankenkius*. It is unclear whether these groups have diversified due to isolation, or converged on a particular morphological blue-print.

The discussion around *Dugesia* is always a fascinating one. Sluys et al (1988) proposed that this genus migrated from Asia after the Indo-Australian plate collided with the Pacific plate. While this is certainly still the most parsimonious explanation (phylogenetically and from a global biogeographical perspective), the presence of the genus in remote Western Australia and the apparent lack of a land bridge between Asia and Australia, does provoke some reflection. The extensive distribution of the genus *Cura* also provided some cause for consideration. However, the presence of another *Cura* species in New Zealand and its apparent broad ecological tolerance suggest that this genus has been on the continent for at least 30 million years (as opposed to being a recent introduction).

#### 5.4 Implications for Practice

It was hoped that, as a result of this research, a simple key could be developed for identifying the Dugesiidae *s.l.* genera using only their external morphology. This would have allowed non-taxonomists to identify the genera. Unfortunately, this task proved impossible; the external morphology was far too homogeneous. Instead, a key utilising the internal morphology has been developed. This would be of use to other taxonomists who may not specialise in this group. However, the internal morphology would initially need to be reconstructed, most likely using the lengthy histological process, which may be a limiting factor. The sequencing of the *cox1* region of the mitochondrial genome and 18S region of the nuclear genome for many new species and several new genera will be useful for future phylogenetic research, and will ideally contribute to an identification system utilising sequence data for the Dugesiidae *s.l.* (i.e. barcoding).

## 5.5 Implications for Research

This study has highlighted the importance of the Australian continent in both freshwater and terrestrial triclad research. There is a great deal more research to be done in relation to the origin of the freshwater and terrestrial Geoplanoidea. For example where do the Planarioidea fit in? Which branch of the Maricola gave rise to the Continenticola? While the analysis within this thesis has occasionally ruminated upon such questions, these are well beyond the remit of this research and any further discussion would constitute pure conjecture. I believe that the next major step is a thorough investigation into the relationship between the Geoplanidae, specifically the Australian species, and the Dugesiidae *s.l.* families *Spathulidae* and *Romanenkiidae* and *Masaharidae*. I believe a targeted sampling effort combined with morphological and molecular analysis could resolve this issue. Indeed, such efforts may also lead to the discovery of morphological synapomorphies for the newly described families, allowing the formalisation of the classification.

It has also become clear throughout the course of this research that more work needs to be done on the ecology of the Dugesiidae *s.l.*, specifically on the dispersal and drought resistance capabilities of some of the taxa (especially *Cura pinguis*). The fact that "dugesiids" are so diverse and widespread on a continent with relatively little permanent water is of significant interest. Finally, it would be fascinating to find and collect samples from permanent water sources in the centre and Northern Territory of Australia to present a more complete picture of the Australian fauna.

## 5.6 Limitations of the Study

This study was limited, as with most research, by the available resources (finances and time). It would have been ideal to sequence one or two more gene regions to strengthen the molecular phylogenies; however, this was not financially viable. Additionally, it would have been ideal to collect samples from some of the more remote regions of Australia; again, not logistically possible with the resources available. Resources would have been less of a limitation if freshwater triclads were easier to collect, as more external assistance could have been utilised. However, complexities around fixation techniques often created a barrier to this solution. Finally, the collection techniques used at most sites were chosen for their simplicity and speed, allowing for a greater number of sites to be visited. Most samples were collected by turning over rocks, or sifting through macrophytes and leaf litter. Ideally, baiting would also be used in order to try and attract those species that inhabit different ecological niches (but this was generally too time consuming).

## 5.7 A final reflection

While freshwater triclads are difficult to work on, primarily due to the labour-intensive identification process, I believe they have a lot to offer the scientific community as a whole. While research on their amazing regenerative capabilities is well known, it concerns me that so little is known about the ecology of a taxon that must constitute an important part of Australia's and the world's precious freshwater ecosystems. Additionally, I believe that the age (i.e. Pangaeian radiation) of the DugesIIDae *s.l.* makes it a fascinating study due to the biogeographical secrets it may hold in its genome.

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## APPENDIX 1a: Systematic Review and Revision of DugesIIDae s.l. Taxonomy

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The content below is a taxonomic account of all Australian species, including new and revised species, with updated distribution and ecological data. Those items highlighted in orange make reference to the proposed classification as outlined in Chapter 3. Additionally, as discussed in Chapter 2, the family previously known as DugesIIDae is proposed to be paraphyletic and therefore references to it in the broader sense (i.e. referring to all freshwater Geoplanoid genera), will include the qualifier *sensu lato* (s.l.).

**Abbreviations used in figures:** ad=adenodactyl, bc=bursal canal, br=brain, ca=copulatory apparatus, cat=common atrium, cb=copulatory bursa, cod=common oviduct, cp=ciliated pit, di= diverticulum, div=diverticulum, dg=diaphragm, e=eye, ed=ejaculatory duct, fgd=female genital duct, g=gland, gd=gonoduct, gid=genito-intestinal duct, gl=glands, go=gonoduct, gp=gonopore, int=intestine, ma=male atrium, mc=muscular cavity, mo=mouth, od=oviduct, ol=oviducal loop, ov=ovary, pg=penial glands, ph=pharynx, pp=penial papilla, sf=sensory fossae, sg=shell glands, sp=sperm, spe=spermatophore, sph=sphincter, sv=seminal vesicle, te=testes, vd=vas deferens, vi=vitellaria.

### Order TRICLADIDA Lang, 1884

#### Suborder CONTINENTICOLA Carranza, Littlewood, Clough, Ruiz-Trillo, Baguña and Riutort, 1998

#### Superfamily GEOPLANOIDEA Stimpson, 1857

#### Family DugesIIDae s.s. Ball, 1974

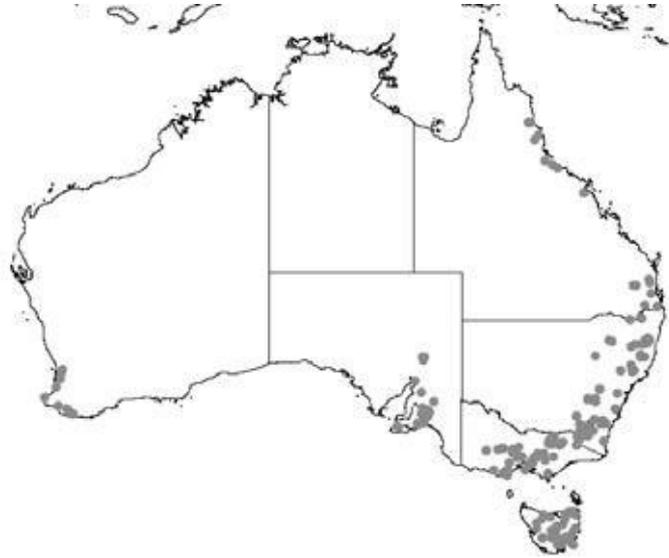
#### Genus *Cura* Strand, 1942

The genus *Cura* is distributed as illustrated in Figure 1a.1 and is defined as a “dugesiid” with: head of truncate or low triangular form; auricular slits; testes small, discrete and very few in number, pre-oral, dorsal or ventral; bursal canal musculature inner circular

## APPENDIX 1a

### SYSTEMATIC REVIEW AND REVISION OF DUGESIIDAE S.L. TAXONOMY

muscles surrounded by a fine discontinuous layer of longitudinal fibres; entrance to the bursal canal high; atrium usually divided in that the bursal canal expands above the level of the shell glands to form a female atrium; finger or thumb shaped penial papilla (Ball 1974b).



**Figure 1a.1** Distribution of the genus *Cura* in Australia.

### ***Cura pinguis* (Weiss 1909)**

*Planaria pinguis* Weiss, 1909

*Curtisia stagnalis* - Nurse 1950

*Cura pinguis* - Marcus 1955

*Dugesia pinguis* - Beauchamp 1968

*Dugesia (Cura) pinguis* - Ball 1974

*Cura pinguis* - Kenk 1974

### **Contribution**

Throughout the course of this research *Cura pinguis* has been redescribed twice, however, neither description involved any major revelations in regards to this species' character set (Grant et al. 2006, Appendix 1d). The considerable increase in our knowledge of *Cura pinguis*' distribution represents a substantial contribution to our understanding of Australian Dugesiids and throws up some interesting considerations (Appendix 1d).

### **Diagnosis**

*Cura pinguis* is easily identifiable owing to the "finger-shaped" penial papilla, the dorsal communication of the bursal canal and the ventral testes (Figure 1a.2-b,c).

### **Comments**

*Cura pinguis* has been described from Australia on many occasions. The only feature within the copulatory apparatus that is still questionable is the presence/absence of a common oviduct (Grant et al. 2006). All of the specimens inspected as part of this thesis exhibit separate communication of the oviducts with the bursal canal (Appendix 1d). As these specimens have been collected from all over Australia I believe that this feature is consistent for all Australian *Cura pinguis*. Attempts to categorise the polymorphic external morphology according to specimen origin (e.g. Australia or New Zealand) have been made (Figure 1a.2-a) (Ball 1974b). It has been my observation that it is impossible to assign morphological types based on locality. This species expresses polymorphisms within drainage divides and often within populations (Appendix 1d).

### **Ecology and Distribution**

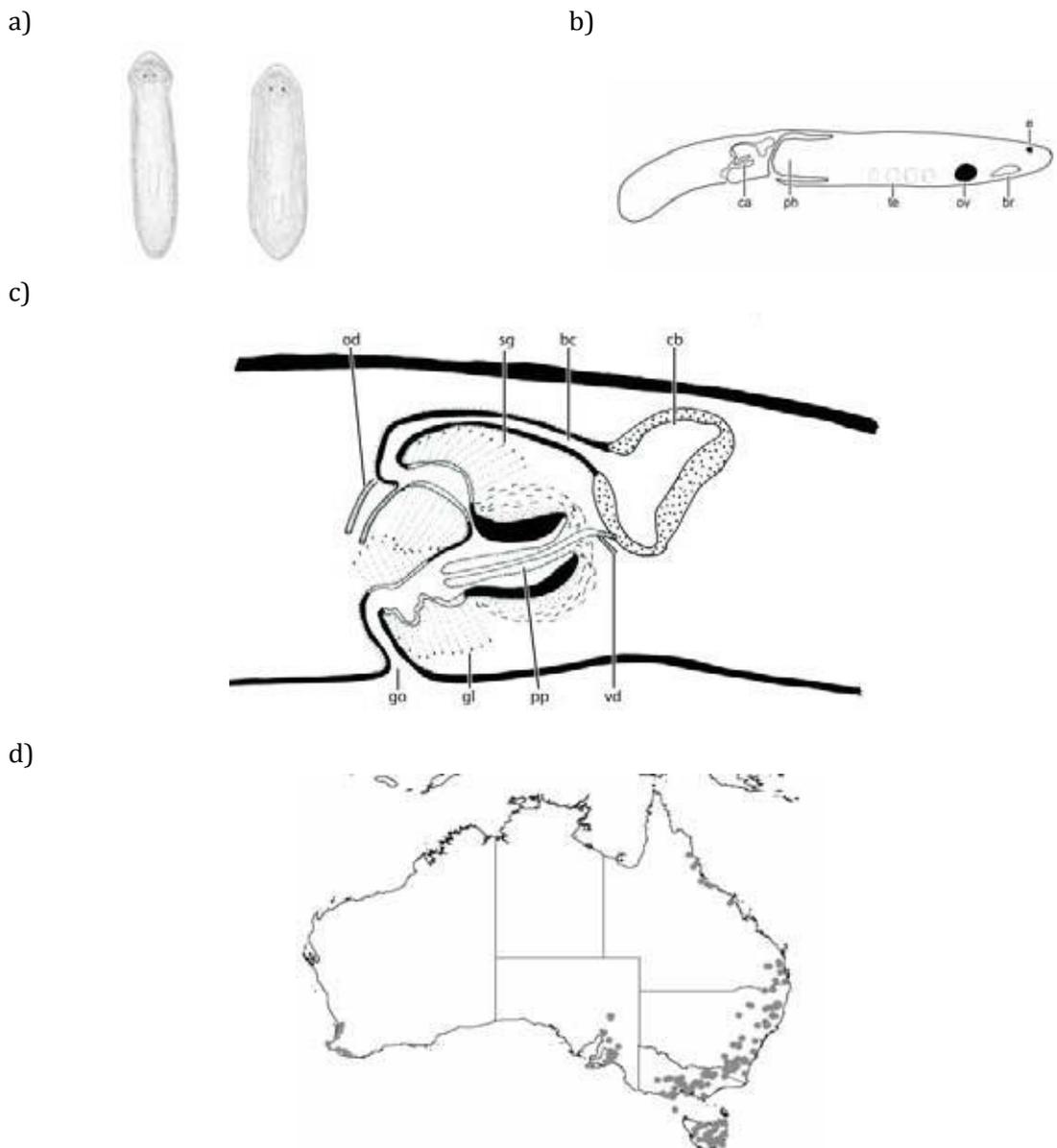
*Cura pinguis* is the most widespread of all Australian species having been collected from all Australian states and territories, excluding the Northern Territory, and is therefore found across a diverse range of both lentic and lotic habitats (Figure 1a.2-d) (Ball 1974b, Grant et al. 2006, Kawakatsu 1969, Sluys and Kawakatsu 2001, Weiss 1909, Appendix 1d). *Cura pinguis* exists commonly at low altitudes and produces stalked cocoons (Hay and Ball 1979). This species is found most commonly on the off-channel area of streams and rivers on the underside of rocks. Worms are often associated with leaf litter and in the absence of rocks can be found on macrophytes. The substrates of the habitats are variable, including mud, sand, gravel, cobble and bedrock (Appendix 1d). *Cura pinguis* appears to be adept at taking advantage of residual pools left in drying streambeds, which are a common feature of Australian freshwater systems (Appendix 1d). *Cura pinguis* was found in the company of many other species, including representatives from the genera *Spathula*, *Romankenkius*, *Dugesia*, *Masaharus* and the invasive *Girardia tigrina*.

Several of the over 100 specimens collected from sites around Australia were identified via molecular data. On the majority of occasions this was done due to an absence of histological specimens from the relevant site (Appendix 2c). However, there were a very few that were found to be lacking a copulatory apparatus (Appendix 2c). The fact that there were so few *Cura pinguis* lacking sexual organs suggests that this species is not often in this state. Of course the lack of sexual organs could be due to any of the following three possibilities; these individuals may be from an asexual population: they may be immature representatives of a sexual population: or they may be members of a physiological population that switches seasonally between asexual and sexual reproduction (Baguña et al. 1999, Brandle et al. 2007, Grasso and Benazzi 1973, Harrath et

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al. 2004, Hoshi et al. 2003, Kobayashi et al. 2002, 1999, Krichinskaya 1986, Roca et al. 1992). There has been very little work done on the ecology of Australian triclads. Hay and Ball (1979) published a paper on the biology of freshwater triclads from the Victorian Alps, in which they discussed some of the ecological features of *Cura pinguis*. While they discussed the seasonal variation in the production of cocoons they make no mention of any variation in reproductive strategies between populations or seasons. Owing to the lack of research in this area it would be unwise to speculate as to which of the three reproductive categories those *Cura pinguis* lacking reproductive apparatus, fall in to.

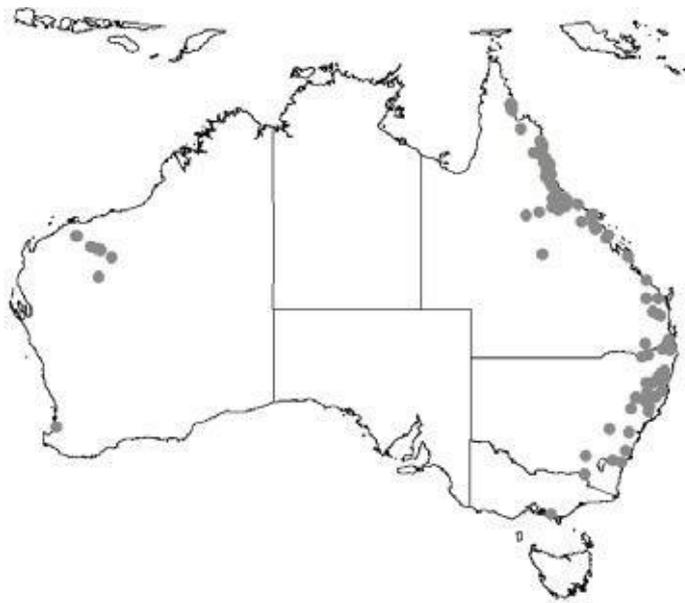


**Figure 1a.2** *Cura pinguis* a) external features of living specimens; b) sagittal reconstruction of the reproductive system; c) sagittal reconstruction of the copulatory apparatus; d) distribution of *Cura pinguis* in Australia.

**Family Dugesiidae s.s. Ball, 1974**

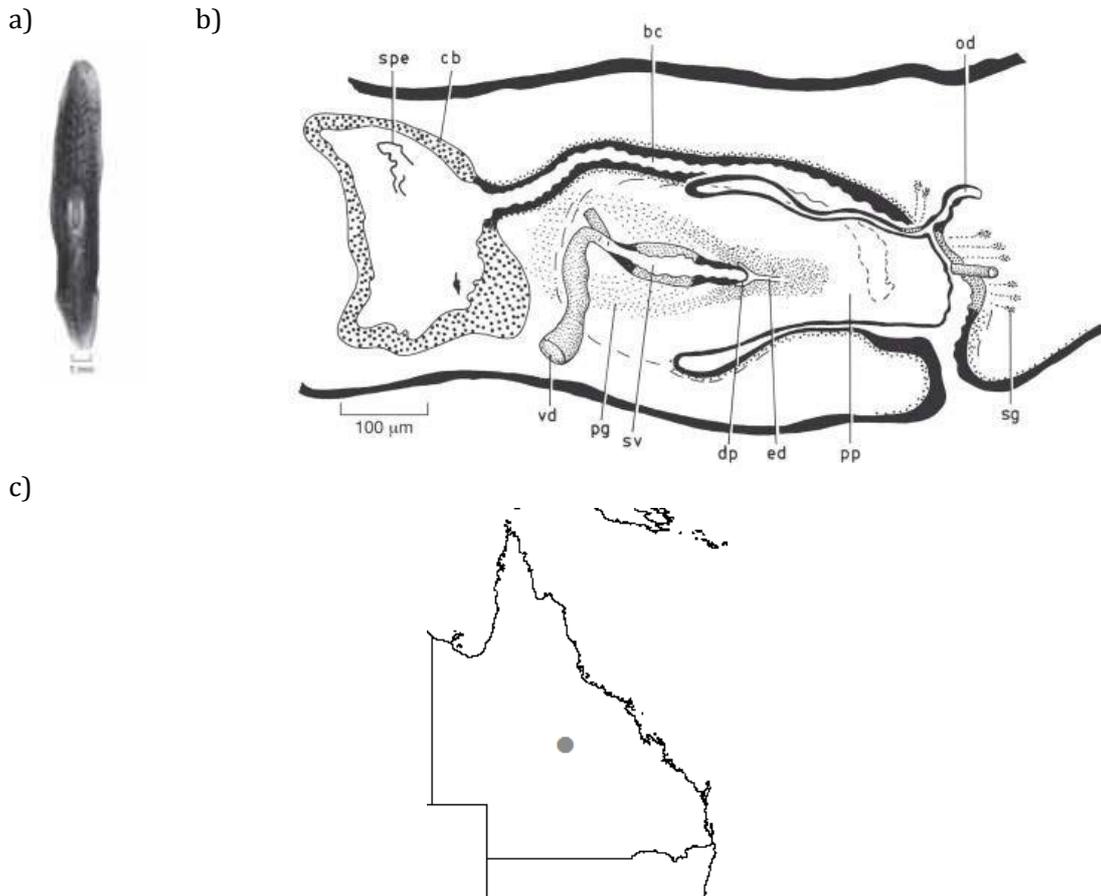
***Dugesia* Girard, 1850**

The genus *Dugesia* is distributed as illustrated in Figure 1a.3 and is defined as a “dugesiid” with: more or less triangulate heads; numerous testes distributed throughout the body length; without cadual branching oviducts; diaphragm in the ejaculatory duct; reversed bursal canal musculature; and ectal reinforcement of the of the bursal canal (Girard 1850). While the presence of a strong apomorphic character (diaphragm within the ejaculatory duct) already provided strong evidence for Australian *Dugesia*'s, the molecular data confirms the taxonomic assignment. The molecular phylogeny established in Chapter 2, demonstrates how the Australian species identified as *Dugesia*'s sit comfortably within a *Dugesia* clade including *Dugesia gonocephala* (Europe) and *Dugesia subtentaculata* (southern Europe and North Africa).



**Figure 1a.3** Distribution of the genus *Dugesia* in Australia.

***Dugesia artesiana* Sluys and Grant, 2007**



**Figure 1a.4** *Dugesia artesiana* a) external features of living specimen (Sluys et al. 2007); b) sagittal reconstruction of the reproductive system (Sluys et al. 2007); c) distribution of *Dugesia artesiana* in Australia.

**Contribution**

*Dugesia artesiana* was newly described during the course of this research (Sluys et al. 2007). The material from this rather unique location was provided by Dr. W.F. Ponder (Australian Museum, Sydney).

**Diagnosis**

*Dugesia artesiana* is characterized by a presumably central ejaculatory duct, asymmetrical openings of the oviducts into the bursal canal, infra-nucleated bursal canal, absence of ectal reinforcement, small diaphragm, and absence of a duct between intrabulbar seminal vesicle and diaphragm (Sluys et al. 2007).

### **Comments**

At the time of description, apart from the problematic *Dugesia* (?) *rava* (Grant et al. 2006, Weiss 1909), only one other species of *Dugesia* has been described for Australia, *D. notogaea* (Sluys et al. 1998) from northern Queensland (Sluys et al. 2007). The latter shows several features in which it differs from *D. artesiana*: hyperplastic ovaries; testes that begin at a short distance behind the brain; large, thin-walled seminal vesicle; relatively long duct between seminal vesicle and diaphragm; distinctly acentral ejaculatory duct (Figure 1a.4-b). In our opinion these differences indicate that the worms from the Edgbaston locality are essentially different from *D. notogaea*, which occurs also in northern Queensland (Figure 1a.4-c).

Considerable consideration was given to the many non-Australian *Dugesia* species; however, none had the same unique combination of characters as *Dugesia artesiana* and were consequently discounted (Sluys et al. 2007). Subsequently, two new Australian *Dugesia* species have been described, one complete with internal morphology and one based on molecular sequences (Appendix 1d). Neither of these species invited comparison with *Dugesia artesiana* and so its taxonomic status remains unchanged.

### **Ecology and Distribution**

The species is known only from two springs in the Great Artesian Basin at Edgbaston, which are separated by a distance of about 8 km. The holotype was collected from shallow water at the spring's edge, and the other specimen was found in a flowing seepage at the head of another spring (Sluys et al. 2007).

## ***Dugesia notogaea* Sluys and Kawakatsu, 1998**

### **Contribution**

*Dugesia notogaea* was described twice as part of this research. While neither description differs a great deal from existing descriptions, they are given to demonstrate the variability within the species (Grant et al. 2006, Appendix 1d).

### **Diagnosis**

*Dugesia notogaea* is characterised by an acentral ejaculatory duct with a terminal opening at the tip of the penial papilla, a well developed duct between the intrabulbar seminal vesicle and the diaphragm, asymmetrical openings of the oviducts into the bursal canal, and hyperplastic ovaries (Sluys et al. 1998).

### ***Comments***

Intraspecific variations include the presence or absence of glands entering the gonoduct. Sluys et al. (1998) make no mention of glands entering the gonoduct in their original description, while this investigation suggests that they are visible in many but not all individuals (Appendix 1d). A similar situation is found with the ectal reinforcement of the bursal canal (Figure 1a.5-c). It appears to be described irregularly in the literature and I have found its presence common but not constant (Appendix 1d).

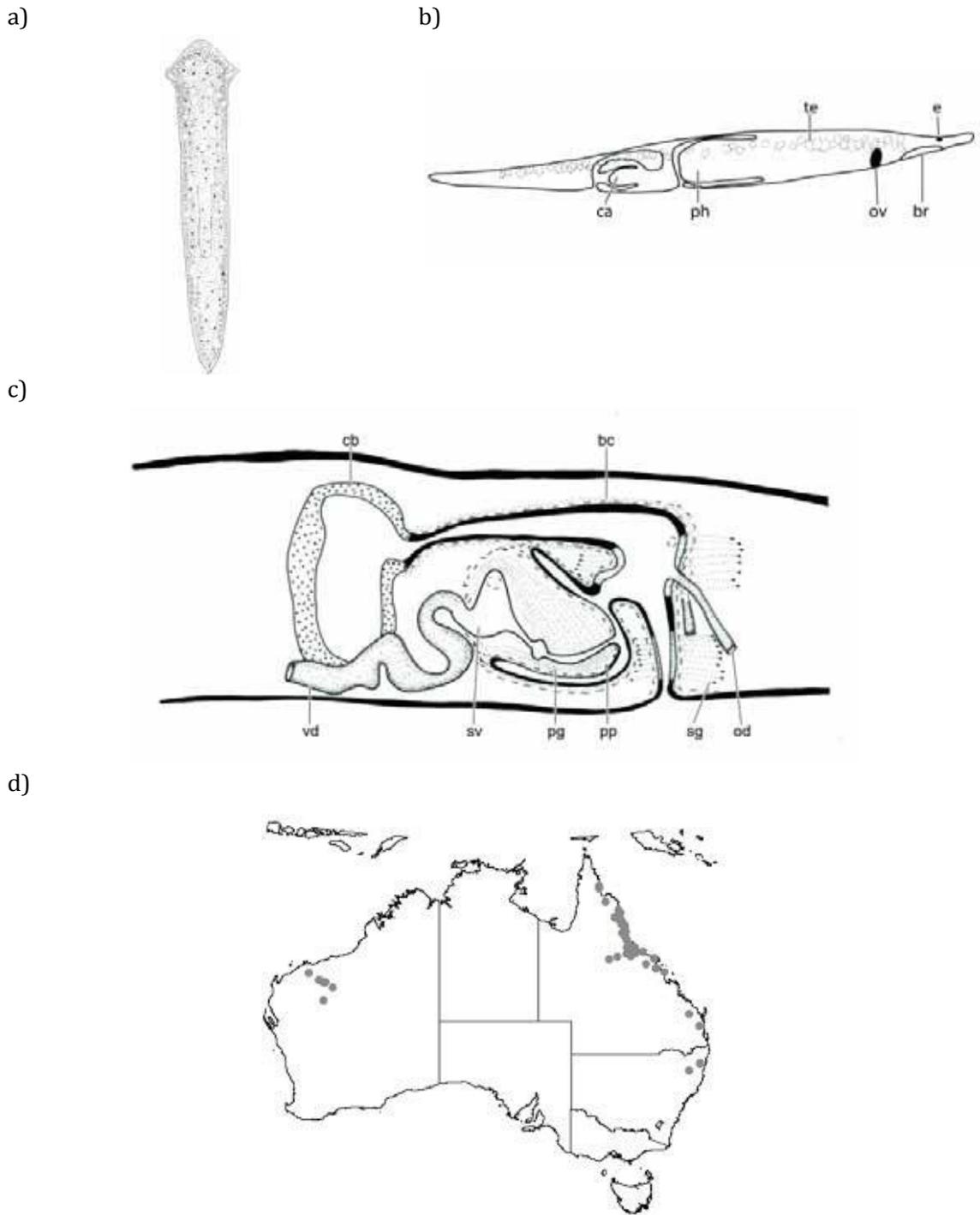
So far the species has been described with hyperplastic ovaries (Grant et al. 2006, Sluys et al. 1998,) yet this was not the case for the more recently collected individuals (Appendix 1d, Figure 1a.5-b). I therefore suggest that this state is not diagnostic for the species. The lack of hyperplastic ovaries allowed for the identification of the dorsal communication between the ovaries and the oviducts, supporting Sluys' proposal that this feature is an apomorphy for the genus *Dugesia* (Sluys et al. 2001, Appendix 1d).

### ***Ecology and Distribution***

*Dugesia notogaea*, whilst generally inhabiting rivers and creeks, has been found thriving in isolated springs and billabongs. Specimens are commonly found on the underside of rocks or logs within riffles or the off-channel area. In the absence of any firmer substrate worms could be detected living within the macrophytes and leaf litter in the slower or stagnant parts of the stream (Appendix 1d). This species is found throughout coastal northern Australia, an area that is typified by non-permanent freshwater (Figure 1a.5-d). During the summer months many creeks and rivers are reduced to residual pools, which often support populations of *Dugesia notogaea*. While *Dugesia notogaea* was primarily found with no other species, it was on occasion found in the presence of *Cura pinguis*.

Several specimens of *Dugesia notogaea* were discovered to be lacking any sexual structures and were identified via molecular data (e.g. N35 and LJ16 - Appendix 2c). There appears to be no obvious relationship between the immature/asexual condition of some specimens and the environmental conditions, since all immature/asexual specimens were found in close proximity to sexual specimens and at the same time of year. In the above ecological discussion of *Cura pinguis*, possible explanations for a lack of reproductive structures are suggested. Those same suggestions apply here, as well as the inability to arrive at a supported answer. This species is found in northern Western Australia and northern Queensland, extending to the north of New South Wales (Grant et al. 2006, Sluys et al. 1998, Sluys and Kawakatsu 2001, Appendix 1d).

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**Figure 1a.5** *Dugesia notogaea* a) external features of living specimen; b) sagittal reconstruction of the reproductive system; c) sagittal reconstruction of the copulatory apparatus; d) distribution of *Dugesia notogaea* in Australia.

***Dugesia orientoaustralis* Grant & Sluys, sp. nov.**

***Contribution***

*Dugesia orientoaustralis* is a new species described as part of this thesis (Appendix 1d).

### ***Diagnosis***

*Dugesia orientoaustralis* can be distinguished from its congeners by the presence of a common oviduct, ectal re-enforcement of the entire length of the bursal canal and the long gonoduct communicating to the common atrium (Appendix 1d).

### ***Comments***

The external features of this species are very similar to that of *D. notogaea* and I suspect that this species has been accidentally assigned to *D. notogaea* in the past (Figure 1a.6-a). However, there are several very important differences between *D. notogaea* and this species. The most useful diagnostic character is the common oviduct, which is not present in *D. notogaea* (Grant et al. 2006, Sluys et al. 1998, Sluys and Kawakatsu 2001, Appendix 1d). The extension of the ectal re-enforcement to the termination of the duct was found without exception amongst *Dugesia orientoaustralis* specimens (Appendix 1d)(Figure 1a.6-c). This feature has been described on rare occasions from *D. notogaea*. It remains to be examined in detail whether this presumed variability is based on a misidentification of a few animals. The long gonoduct and the ventral point of termination of the ejaculatory duct are additional features characteristic for *Dugesia orientoaustralis* (Appendix 1d). This species can also be separated from *D. notogaea* by the absence of ventral pigmentation; this pigmentation is consistently described as light brown in *D. notogaea* (Grant et al. 2006, Sluys et al. 1998, Appendix 1d).

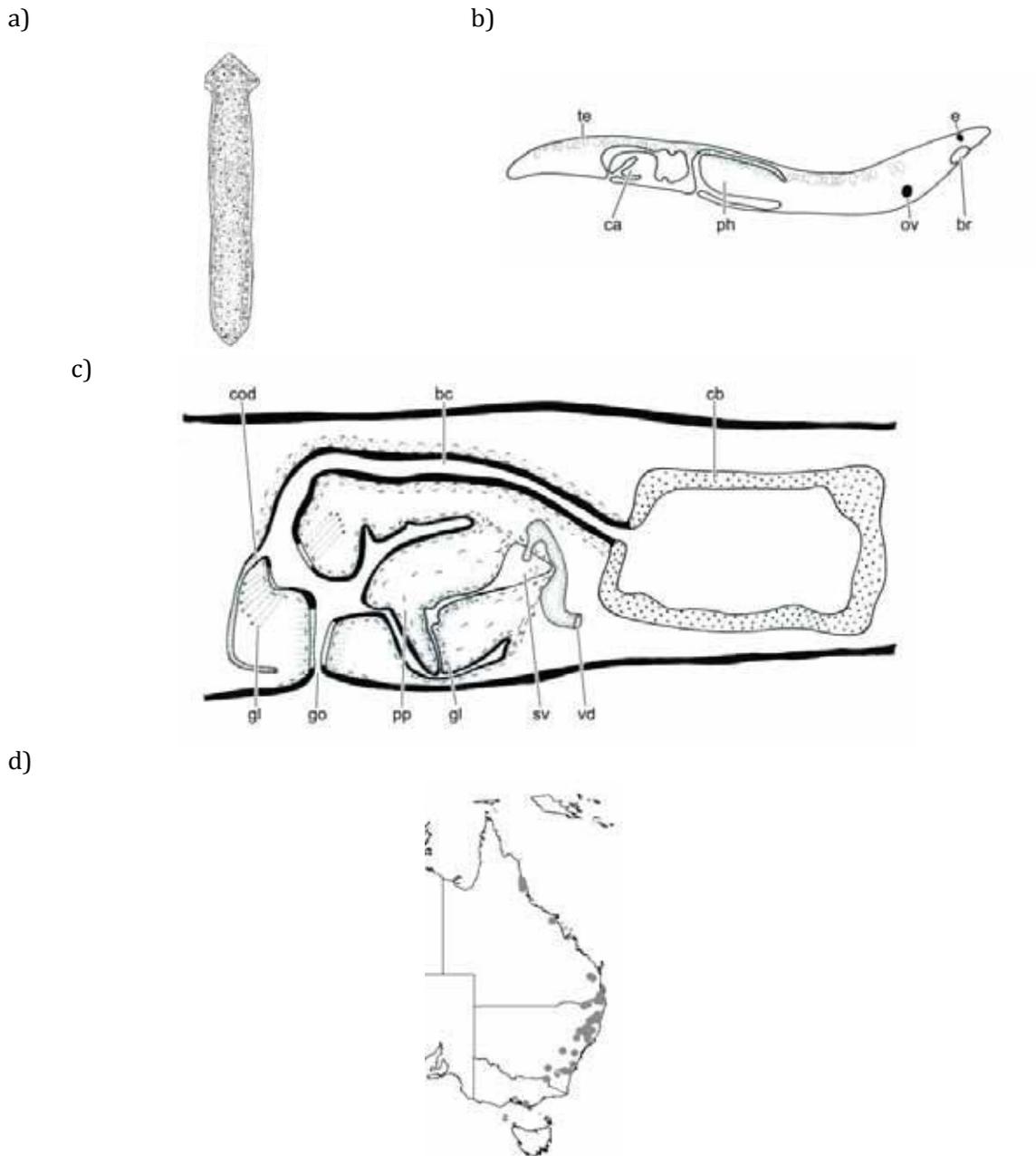
While comparison with *D. notogaea* is important owing its distributional similarities with *Dugesia orientoaustralis* there are other non-Australian *Dugesia* species that have a common oviduct. The discussion detailed in Appendix 1d demonstrates that while there are many similar species to *Dugesia orientoaustralis*, we believe that this new species exhibits a unique combination of characters and can therefore be considered distinct from all other *Dugesia*'s (De Vries 1988, Kawakatsu and Mitchell 1989, Steinmann 1914, Appendix 1d). The molecular data adds a considerable amount of support for this decision as a clear demarcation between specimens of *Dugesia notogaea* and *Dugesia orientoaustralis* is evident in phylogenetic trees (Chapter 2 and Appendix 2c).

### ***Ecology and Distribution***

Currently *Dugesia orientoaustralis* is known only from lotic environments most commonly in the off-channel area, but occasionally amongst the riffles. Found in creeks and rivers with variable substrates but always on the underside of rocks. This species is restricted to eastern Australia, extending from northern Queensland to northern Victoria (Appendix 1d)(Figure 1a.6-d). *Dugesia orientoaustralis* was found in one occasion in the

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presence of *Dugesia sp.*, however, more commonly this species was found with *Girardia tigrina* or *Cura pinguis*.



**Figure 1a.6** *Dugesia orientoaustralis* a) external features of living specimens; b) sagittal reconstruction of the reproductive system; c) sagittal reconstruction of the copulatory apparatus; d) distribution of *Dugesia orientoaustralis* in Australia.

Over 50% of the specimens collected for this species were discovered to be lacking a copulatory apparatus (e.g. KR and LJ101 - Appendix 2c) and identified via molecular data, however, there were no obvious differences in location or environmental conditions

between the sexual and asexual/immature specimens. In the above ecological discussion of *Cura pinguis*, possible explanations for a lack of reproductive structures are suggested. Those same suggestions apply here, as well as the inability to arrive at a supported answer.

***Dugesia ? rava* (Weiss, 1909)**



**Figure 1a.7** Distribution of *Dugesia ? rava* in Australia (Appendix 1d).

*Planaria rava* Weiss, 1909

*Dugesia ? rava* - Grant and Sluys 2006

***Contribution***

Weiss' (1909) holotype was re-examined and the genus re-assigned (Grant et al. 2006). No new material has been assigned to *Dugesia (?) rava* since Weiss' (1909) original description.

***Diagnosis***

Weiss (1909) describes an asexual worm with a brown dorsal surface interrupted by a dark medial stripe and a grey ventral surface.

***Comments***

In her description Weiss (1910) indicated that the type specimen is completely asexual, without reproductive organs, and we concur (Grant et al. 2006). It is highly unlikely that the species belongs to the genus *Planaria* as defined by Kenk (1930) and Ball and Gourbault (1978) since this monotypic genus is restricted to western Europe. More importantly, the sections revealed eyes with multiple retinal cells, indicating that the

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### SYSTEMATIC REVIEW AND REVISION OF DUGESIIDAE S.L. TAXONOMY

species does not belong to the Planariidae but to the Dugesiidae *s.l.* On this basis it was deemed appropriate to tentatively transfer this species to the genus *Dugesia* (Grant et al. 2006). The tentative element of this assignment is completely justified as all *Dugesia*'s known from Australia are described as having a mottled appearance (excluding *Dugesia artesiana*, which was only examined in a fixed state). This is not a feature described for *Dugesia rava*. However, in the absence of more information, *Dugesia* is an appropriate genus.

Grant et al. (2006) discovered several asexual species as part of their research, however, the likelihood of any of these species being *Dugesia rava* is extremely unlikely (see Chapter 2, Appendix 1d).

### ***Ecology and distribution***

*Dugesia rava* is known only from the type location, Brunswick, a dairy-farming region, in the southwest of Western Australia (Figure 1a.7). These specimens would have been collected from the main channel or tributary of the Brunswick River (Weiss 1909).

## ***Dugesia sp.***

### ***Contribution***

Tentative species description based on molecular evidence (Appendix 1d).

### ***Diagnosis***

Due to the lack of any diagnostic characters, this taxon has been identified as a separate species only via molecular data and, consequently, a complete species description cannot be provided.

### ***Comments***

It is unknown whether this species is lacking sexual organs because it is asexual or simply immature at the time of collection (Appendix 1d). Both explanations are plausible as Australian species are often subjected to drying episodes, causing periods of starvation, known to be capable of initiating the re-absorption of sexual organs (Woollhead 1983). It has been well documented that Australian species are regularly found with immature or non-existent sexual apparatus (Grant et al. 2006, Sluys 1997, Sluys and Kawakatsu 2001, Appendix 1d). Upon inspection of the field data pertaining to these specimens it should be noted that while the majority of collections were made from rivers and creeks with low or no flow, there are several examples of sites where the water level was normal or high

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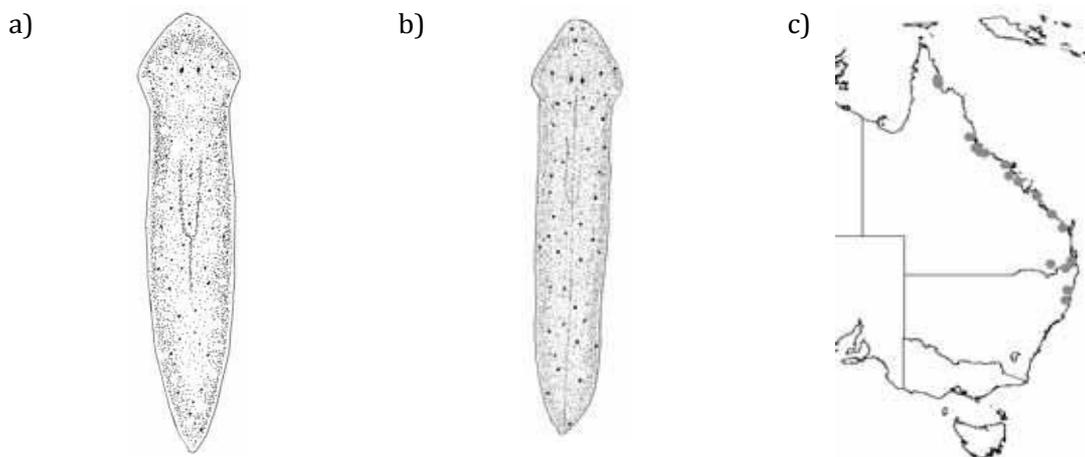
### SYSTEMATIC REVIEW AND REVISION OF DUGESIIDAE S.L. TAXONOMY

(Appendix 1d). These populations were collected in the same asexual state as their presumably stressed counterparts, making it hard to discount the possibility that this is an asexual species reproducing solely via fission (similar to the many invasive populations of *Girardia tigrina*) (Ball and Reynoldson 1981).

This species falls within the genus *Dugesia* when both the mitochondrial and the nucleic data are examined (Appendix 2c). The external morphology also supports this placement with *Dugesia sp.* displaying the characteristic mottled dorsal pigmentation. While this branch does sit close to the *Dugesia orientoaustralis*, the molecular evidence suggesting that this is a separate *Dugesia* species is compelling to the point of certainty (Appendix 1d and 2c).

### **Ecology and Distribution**

This species has been found predominantly on the underside of rocks in the off-channel areas and residual pools of creeks and rivers. The distribution extends from far northern Queensland to northern New South Wales (Appendix 1d)(Figure 1a.8-c). *Dugesia sp.* was found in the presence of *Dugesia orientoaustralis* and *Girardia tigrina*, however, more commonly this species was found in the company of *Cura pinguis*.



**Figure 1a.8** *Dugesia sp.* a) external features of living specimens; b) external features of living specimens; c) distribution of *Dugesia sp.* in Australia.

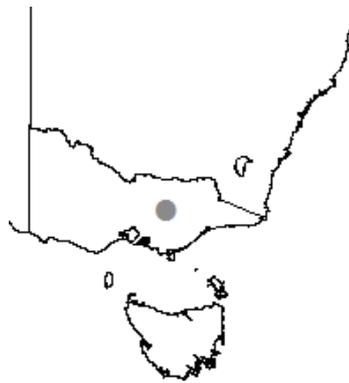
In the ecological discussion of *Cura pinguis*, possible explanations for a lack of reproductive structures are suggested. Those same suggestions apply here. The possibility that these specimens are simply immature is unlikely. The fact that there were no sexual individuals collected for *Dugesia sp.* makes it possible that all of these populations reproduce solely via fission, similar to *Girardia tigrina* (Baguñà et al. 1999, Sluys et al. 2005). However, it is also possible that these populations are physiological, changing

between sexual and asexual throughout the year. Without further observation in a laboratory environment it is impossible to determine which of these scenarios is the true situation.

### Family Spathulidae nov. fam.

#### *Eviella* Ball, 1977

*Eviella* is a monotypical genus distributed as illustrated in Figure 1a.9 and is defined as a dugesiid: without eyes, with one pair of anterior sensory pits, female copulatory organs posterior to the male copulatory organs. Oviducts enter the female genital duct (bursal canal) separately from the sides, and each has a caudal branch. Vasa deferentia enter the penis bulb separately from the sides. Testes fused, predominantly ventral, and throughout the body length (Ball 1977b).



**Figure 1a.9** Distribution of the genus *Eviella* in Australia

#### *Eviella hynesae* Ball, 1977

##### **Contribution**

This species was not examined as part of this thesis.

##### **Diagnosis**

As this species is monotypic, see genus description above.

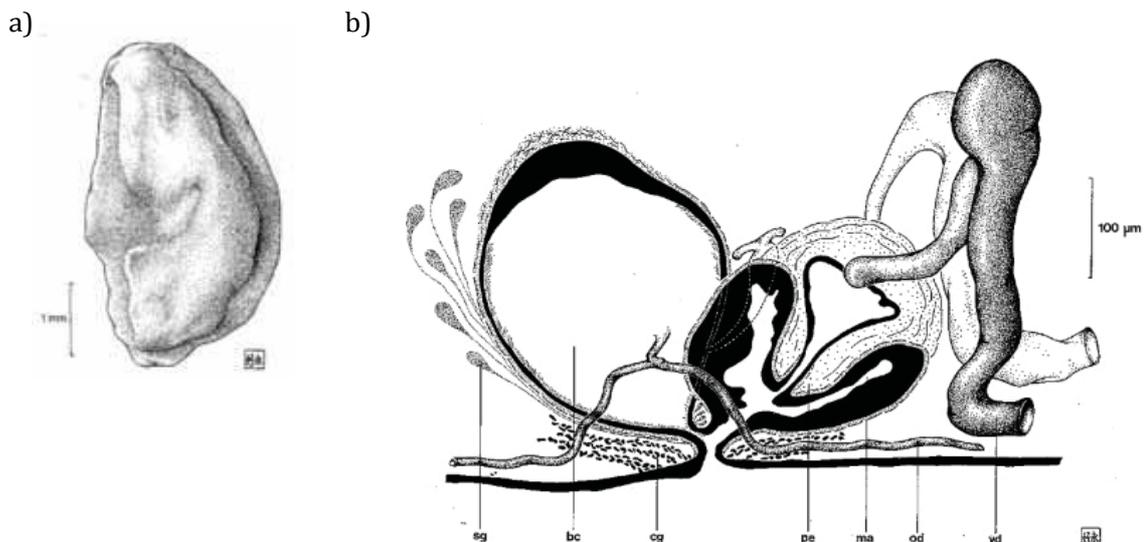
##### **Comments**

In Ball's (1977b) discussion of *Eviella hynesae* he suggests that this *species* represents a primitive direct descendant of marine ancestors. This is suggested due to the retrobursal condition of the species, a state generally found only in marine triclads and a small number of freshwater triclads, also proposed to be marine relicts (e.g. *Rhodax*

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*evelinae*)(Figure 1a.10-b). Since its original description no further comment has been made on the status of *E. hynesae*, however, over the last 30 years several more retrobursal species have been described from the Dugesiidae s.l. (e.g. *Romankenkius retrobursalis*) (Grant et al. 2006). It is therefore possible that *E. hynesae* is simply an aberrant *Spathula*, as suggested by Sluys (2001), however I believe more information is needed before a genus re-assignment can be attempted.



**Figure 1a.10** *Eviella hynesae* a) external features of living specimens (Ball 1977b); b) sagittal reconstruction of the copulatory apparatus (Ball 1977b).

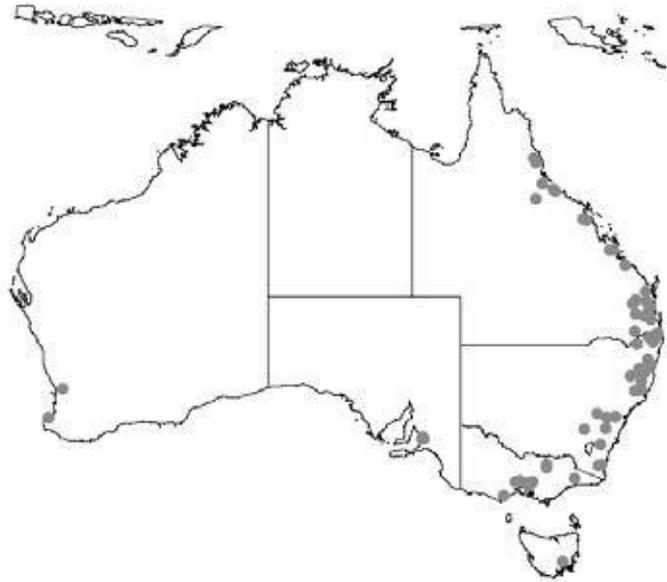
### ***Ecology and Distribution***

*Eviella hynesae* is known only from the Howqua River, approximately 50km southeast of Mansfield, Victoria (Figure 1a.9). Ball (1977b) described the habitat as a swiftly flowing river, 3 - 4 m wide with fixed boulders, rocks and some gravel.

## **Family Dugesiidae s.s. Ball, 1974**

### ***Girardia* Ball, 1974**

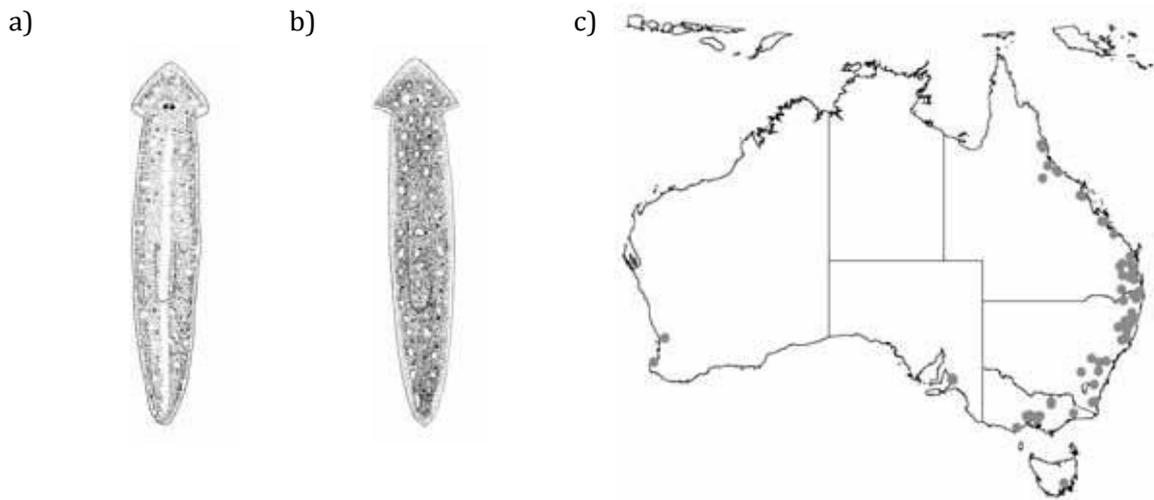
*Girardia* has only invasive members within Australia and is distributed as shown in Figure 1a.11. *Girardia* is described as having a head typically high triangular, but may be truncate. Seminal vesicle absent or of the bifid nonmuscular type. Diaphragm absent. Bursal canal musculature of inner circular muscles surrounded by longitudinal fibres. A pigmented pharynx is present, and numerous testes, distributed throughout the body length and usually ventral (Ball 1974c).



**Figure 1a.11** Distribution of invasive *Girardia* in Australia.

***Girardia tigrina* (Girard 1850)**

- Planaria tigrina* Girard, 1850
- Dugesia maculata* - Girard 1851
- Phagocata tigrina* - Diesing 1862
- Planaria lata* - Sivickis 1923
- Euplanaria maculata* - Kenk 1930
- Euplanaria lata* - Kenk 1930
- Euplanaria novangliae* - Hyman 1931
- Euplanaria tigrina* - Kenk 1935
- Euplanaria tigrina novangliae* - Miller 1938
- Dugesia tigrina* - Hyman 1939
- Dugesia tigrinum* - Stunkard 1950
- Planaria complanata* - Stella & Margaritora 1966
- Dugesia tegrina* - Mihaita 1970
- Dugesia (Girardia) tigrina* - Ball 1974



**Figure 1a.12** *Girardia tigrina* a) external features of living specimen; b) external features of living specimen; c) distribution of *Girardia tigrina* in Australia.

### ***Contribution***

*Girardia tigrina* has been re-described as part of this thesis and while there have been no great revelations regarding this species' morphology, the data gained relating to its distribution, as well as the molecular data, is incredibly informative (Appendix 1d).

### ***Diagnosis***

This species is easily identifiable due to the presence of a pigmented pharynx and distinctive external morphology (Girard 1850).

### ***Comments***

Individuals resembling *Girardia tigrina* were collected from all over Australia (Figure 1a.12-a,b) and the similarity of these molecular sequences, with published *G. tigrina* sequences, allow no other conclusion than that these animals concern the invasive *G. tigrina* (Appendix 2c)(Appendix 1d). The absence of sexual organs does not affect this reasoning as Ball and Reynoldson (1981) note that in this species it is normally the asexual or fissiparous forms that have become established outside of North America. The first confirmed record of this species within Australia was from Brisbane in 1995 when *G. tigrina* was identified from external morphology alone (Sluys et al. 1995). Prior to this there had been tentative identifications from the Victorian Alps and Western Australia (Ball 1974a, Hay and Ball 1979, Sluys et al. 1995). The work completed in Appendix 1d confirms and also expands this distribution, and it appears that *G. tigrina* has been able to undertake another successful invasion.

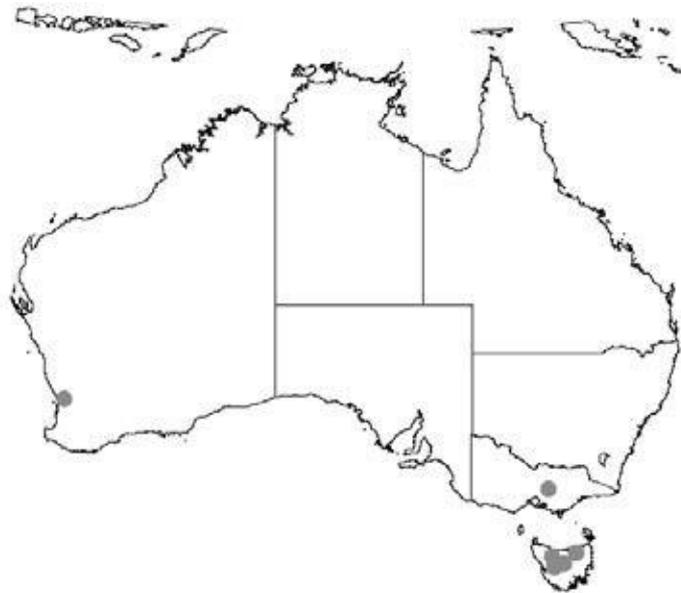
### ***Ecology and Distribution***

As would be expected from a successful invader, *G. tigrina* is found in a wide variety of lentic and lotic environments. Living on the underside of rocks, leaf litter, and macrophytes in environments ranging from fast flowing riffles to residual pools. This species exhibits a remarkable thermal tolerance, with successful populations existing in temperatures ranging from 9°C to 33°C. *G. tigrina*'s Australian distribution can now be expanded to include all states and territories, excepting the Northern Territory (not sampled) (Appendix 1d)(Figure 1a.12-c). *Girardia tigrina* has been found in the company of *Dugesia* sp., *Dugesia orientoaustralis*, *Romankenkius impudicus* and commonly *Cura pinguis*.

## **Family Masaharidae nov. fam.**

### ***Masaharus (formerly Australian Girardia) gen. nov.***

Seminal vesicle absent or of the non-muscular type. Separate oviducal entries to the bursal canal with one species exhibiting a caudal branch. Bursal stalk musculature of inner circular muscles surrounded by longitudinal fibres or intermingled. Copulatory bursa often surrounded by musculature. Penial glands, entering the ejaculatory duct, are present in all species. Testes numerous, distributed throughout the body length and usually ventral. No pharyngeal pigmentation (Appendix 1d). Species within this new genus were formerly assigned, rather tentatively (owing to a lack of synapomorphies), to the genus *Girardia*. However, molecular data demonstrates that these species are not closely associated with the genus *Girardia* and strongly suggests the erection of a new genus to accommodate them (Appendix 2c and Chapter 2). Molecular data were available for two species of the proposed new genus and therefore relationship to each other will be examined further in Chapter 3. Members of the genus *Masaharus* are distributed as shown in Figure 1a.13.



**Figure 1a.13** Distribution of the Australian *Masaharus*.

### ***Masaharus extentus* Grant and Sluys, sp. nov.**

#### ***Contribution***

*Masaharus extentus* was described as part of this research and immediately assigned to the newly erected genus *Masaharus* (Appendix 1d).

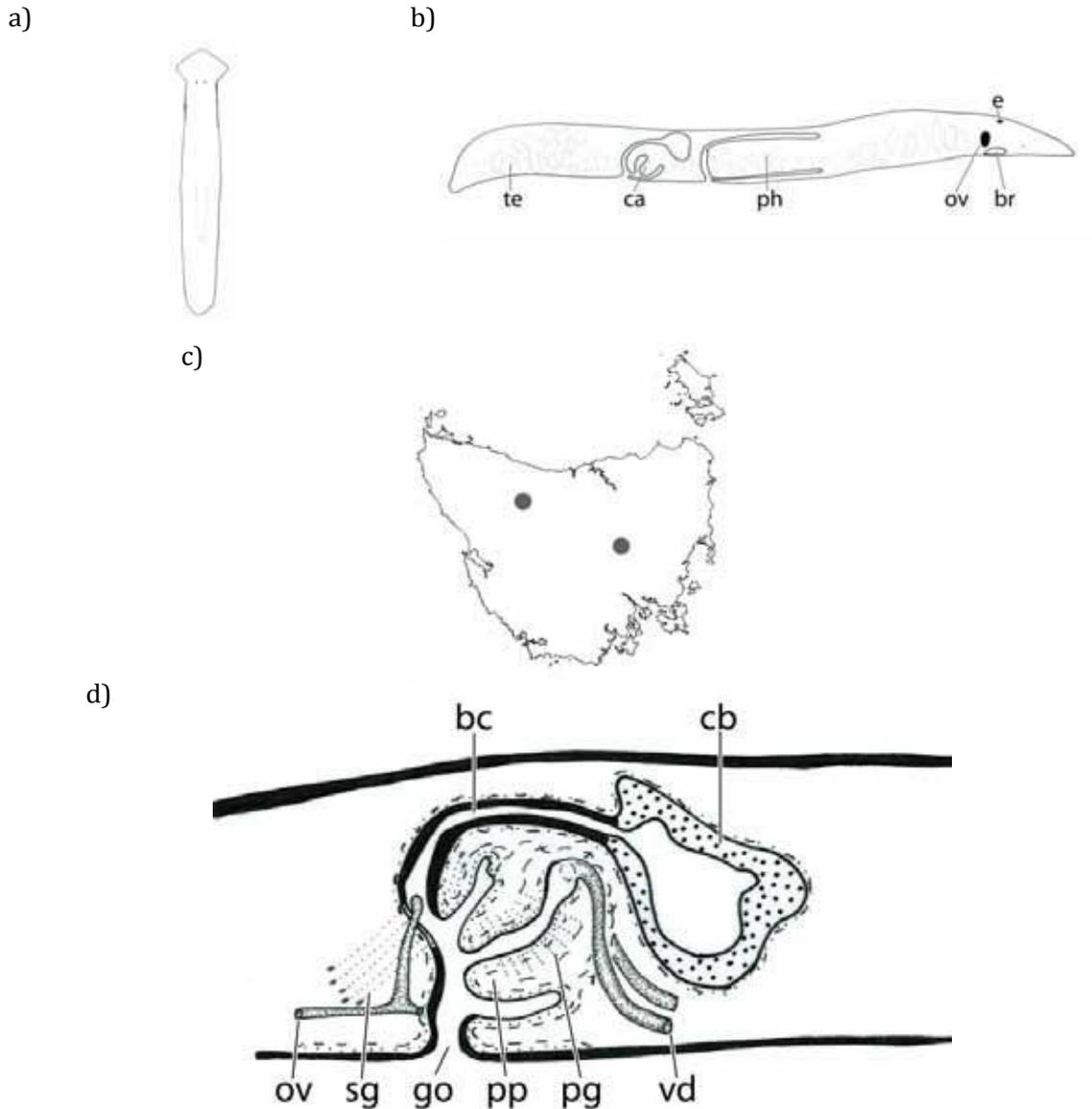
#### ***Diagnosis***

The caudal branch of the oviducts and lack of dorsal pigment differentiate this species from its congeners (Appendix 1d).

#### ***Comments***

The separate entries of the oviducts to the bursal canal and the caudal branch should easily place this species within the genus *Spathula*. However, there are several features that complicate this assignment, including the presence of intermingled musculature on the bursal canal, and musculature surrounding the copulatory bursa (Appendix 1d)(Figure 1a.14-d).

The lack of external pigmentation has been recorded for many *Spathula* species (e.g. *Sp. foeni*, *Sp. tryssa*, *Sp. gorbaultae*, and *Sp. agelaea*). However, none of these species exhibit intermingled musculature on the bursal canal and muscularisation around the copulatory bursa (Appendix 1d)( Figure 1a.14-d).



**Figure 1a.14** *Masaharus extentus*. a) External features of live specimen; b) sagittal reconstruction of the reproductive system; c) distribution of *Masaharus extentus* in Australia; d) sagittal reconstruction of the copulatory apparatus.

*Spathula triculenta*, *Girardia tigrina* and *Masaharus informis* are the only Australian species for which intermingled musculature on the bursal canal has been reported. *Sp. triculenta* has dorsal testes, a common oviduct and no musculature surrounding the copulatory bursa (Grant et al. 2006). *Girardia tigrina* is invasive and possesses a pigmented pharynx, a feature lacking in this species. *Masaharus informis* has the intermingled musculature, the musculature surrounding the copulatory bursa and separate oviducal entries. *Masaharus informis* differs due to its lunate head, dorsal testes

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### SYSTEMATIC REVIEW AND REVISION OF DUGESIIDAE S.L. TAXONOMY

and lack of a caudal branch of the oviducts (Figure 1a.14-a,d). The author was therefore unable to assign this specimen to any known species.

*Masaharus* has the most characters in common with this species and it is therefore proposed that this species belongs to this genus (Appendix 1d). The molecular evidence also supports this assignment with a clear association between this species and *Masaharus informis* (see Chapter 2). It is presumed that the presence of caudally branching oviducts in this species represents a link between this genus and the *Spathula*, *Reynoldsonia* and *Eviella* group (similar to that found in *Romankenkius*). The relevance of the caudally branching oviducts is discussed by Sluys and Rohde (1991) in relation to *Romankenkius* and the *Spathula* group where they propose that the lack of a caudal branch is the primitive state. While this is a possibility I believe that the relationship between these genera and the importance of the oviducts will be clarified with further phylogenetic analysis (see Chapter 2).

### ***Ecology and Distribution***

Collected from both lotic and lentic environments, this species was found on the underside of cobble sitting on the bank/shore of both environments (respectively). *Masaharus extentus* is known from two pristine sites in Tasmania as shown in Figure 1a.14-c. *Masaharus extentus* has been found in the presence of *Masaharus informis* and *Romankenkius kenki* and *Spathula ditatae*.

### ***Masaharus ? graffi* (Weiss, 1909)**

*Planarian graffi* Weiss, 1909

*Euplanaria graffi* - Kenk 1930

*Dugesia graffi* - Ball 1974

*Cura graffi* - Sluys 1997

*Girardia graffi* - Grant & Sluys 2006

### ***Contribution***

The original material examined by Weiss (1909, 1910) was re-examined by Grant et al. (2006) with a subsequent genus reassignment.

### **Diagnosis**

*Masaharus (?) graffi* lacks clear diagnostic characters that would allow the clear assignment of this species to a specific genus (Figure 1a.15-a). *Masaharus (?) graffi* is currently assigned by default to *Masaharus* (Appendix 1d).

### **Comments**

As already described by Weiss (1910) one specimen is completely devoid of a reproductive system (the transversally sectioned animal), while in the second specimen the reproductive organs appear to be partly degenerated in that testes and ovaries are completely lacking and the copulatory apparatus is poorly developed.

Weiss (1910) provided a detailed description of the specimens, which Grant et al. (2006) could only partially corroborate. Sluys (1997) wondered whether the pharynx is pigmented or not. Examination of the specimens suggests that the pharynx is unpigmented; the mouth opening is located at the posterior end of the pharyngeal pocket.

According to Weiss (1910), the oviducts open separately into the female atrium. In view of her reconstruction of the copulatory apparatus one might also reformulate this by stating that the oviducts open separately into the proximal section of the bursal canal. However, Grant et al. (2006) were unable to discern the openings of the oviducts into the female system (Figure 1a.15-a). Furthermore, we were unable unequivocally to discern the junction between the two vasa deferentia. According to Weiss' description the two ducts fuse well within the penial bulb to give rise to the ejaculatory duct.

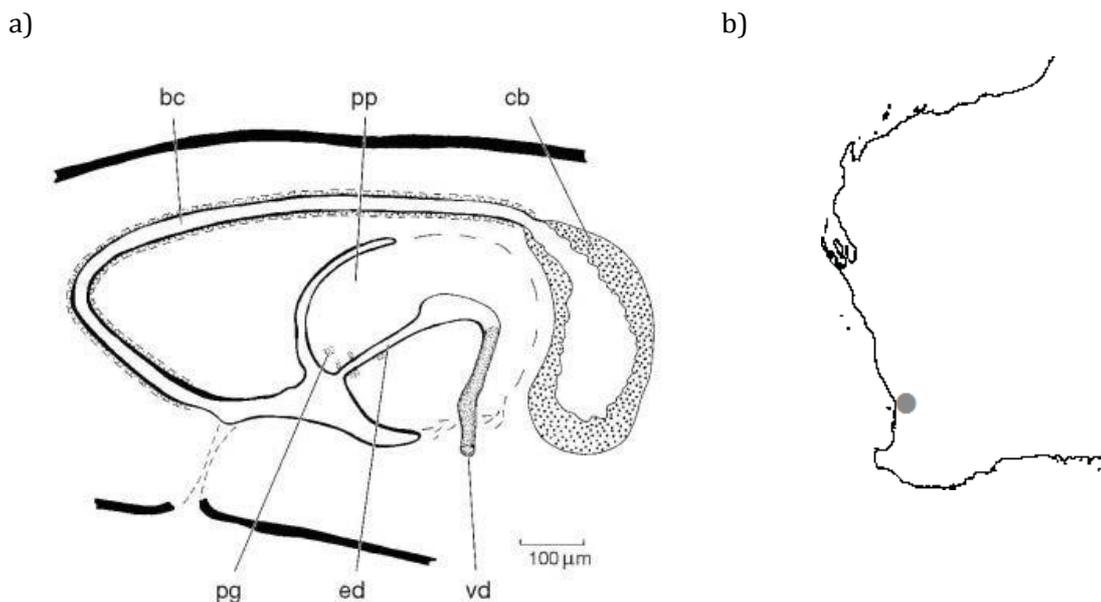
Grant et al. (2006) agree with Weiss' (1910) account of the angled bursal canal, i.e. the canal showing a sharp ventro-antieriad bend before opening into the atrium, being surrounded by a sub-epithelial layer of circular muscle followed by a layer of longitudinal muscle (Figure 1a.15-a). Although the nucleated epithelium and the musculature of the bursal canal are somewhat degenerated, the suggestion is indeed that the musculature around the canal is of the non-reversed type.

Unfortunately, the partly degenerated holotype specimen does not provide enough information to resolve satisfactorily the taxonomic status of this enigmatic species. For example, it could not be determined whether or not the oviducts branch in the posterior end of the body, albeit that generally one would not expect caudally branched oviducts in the presence of a non-reversed bursal canal musculature (Grant et al. 2006). Sluys (1997) provided a detailed argumentation for assigning *G. graffi* to the clade of *Cura*, *Schmidtea*, and *Girardia* in general and to the genus *Cura* in particular. His tentative choice for the genus *Cura* was based on the fact that at the time this was the only genus of that clade with autochthonous representatives in the Australian region. This situation changed with the

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description of non-introduced species of *Girardia* from Australia by Sluys & Kawakatsu (2001) and Grant et al (2006). Furthermore, in a recent analysis Sluys (2001) suggested the following characters as diagnostic features for the monophylum comprising the genera *Cura* and *Schmidtea*: (1) a bursal canal communicating with the middle section of the male atrium, (2) presence of an atrial fold, tall atrial epithelium, and penis bulb musculature extending over the male atrium. A finger- or thumb-shaped penial papilla was suggested as the diagnostic feature for the genus *Cura* (Sluys 2001). The two presumed defining features of the *Cura-Schmidtea* clade do not seem to be present in *Masaharus ? graffi* and neither does the species exhibit a finger- or thumb-shaped penis papilla. Thus, Sluys' (1997) tentative assignment to the genus *Cura* seems no longer warranted. Following this conclusion this species was tentatively assigned to the Australian *Girardia* (Grant et al. 2006). As a result of the current research, the natural presence of *Girardia* in Australia has been discredited and as a consequence all native species assigned to this genus re-assigned to *Masaharus* (Appendix 1d).



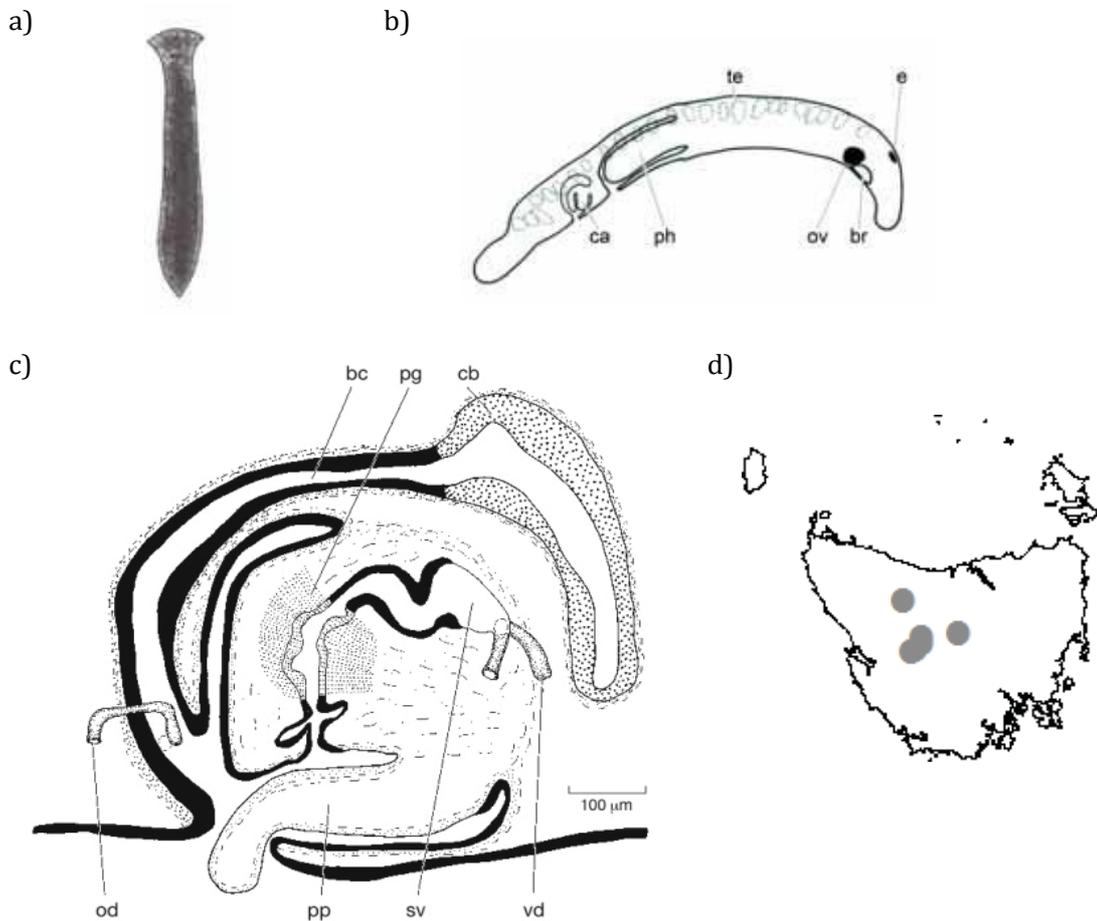
**Figure 1a.15** *Masaharus ? graffi* a) sagittal reconstruction of the copulatory apparatus (Grant et al. 2006); b) distribution of *Masaharus ? graffi* in Australia.

### ***Ecology and Distribution***

This species is known only from Mount Helena (known as Lion Mill until 1924), which is now a rural suburb on the outskirts of Perth (Figure 1a.15-b). This area is hilly and largely covered by bushland.

***Masaharus informis* (Sluys and Grant, 2006)**

*Girardia informis* Sluys and Grant, 2006



**Figure 1a.16** *Masaharus informis* a) external features of living specimens; b) sagittal reconstruction of the reproductive system; c) sagittal reconstruction of the copulatory apparatus; d) distribution of *Masaharus informis* in Australia.

***Contribution***

*Masaharus informis* is a new species described twice throughout the course of this research (Grant et al. 2006, Appendix 1d). The initial description by Grant et al. (2006) placed this species in the genus *Girardia*. This species was then redescribed and reassigned, due to the new material examined by in Appendix 1d.

### ***Diagnosis***

*Masaharus informis* can be distinguished from its congeners by its flexible, asymmetrical penis papilla, intermingled bursal canal musculature, and abundant penis glands opening into the mid-section of the ejaculatory duct (Grant et al. 2006) (Figure 1a.16-c).

### ***Comments***

The only discrepancy between the description presented in Appendix 1d and that of the holotype is the lack of the flexible penial papilla described in Grant et al. (2006). The external morphology was previously unknown, and has proven to be interesting, as the lunate head shape described is quite unique amongst the Australian Dugesiids (Appendix 1d)(Figure 1a.16-a). This species is one of those previously assigned to *Girardia*. Molecular data were obtained for this species and the branch containing this *Masaharus informis* does not sit near the *Girardia tigrina* branch, allowing a high level of confidence when re-assigning this species to the newly erect genus (Chapter 3).

### ***Ecology and Distribution***

*Masaharus informis* has been found on the underside of rocks in the off-channel area of creeks, rivers and lakes. This species has been collected from cool water at high latitudes in the northwest of Tasmania in and around Cradle Mountain National Park (Grant et al. 2006, Appendix 1d)(Figure 1a.16-d). *Masaharus informis* has been found in the presence of *Romankenkius kenki* and *Masaharus extentus*.

## ***Masaharus sphincter* (Sluys and Kawakatsu, 2001)**

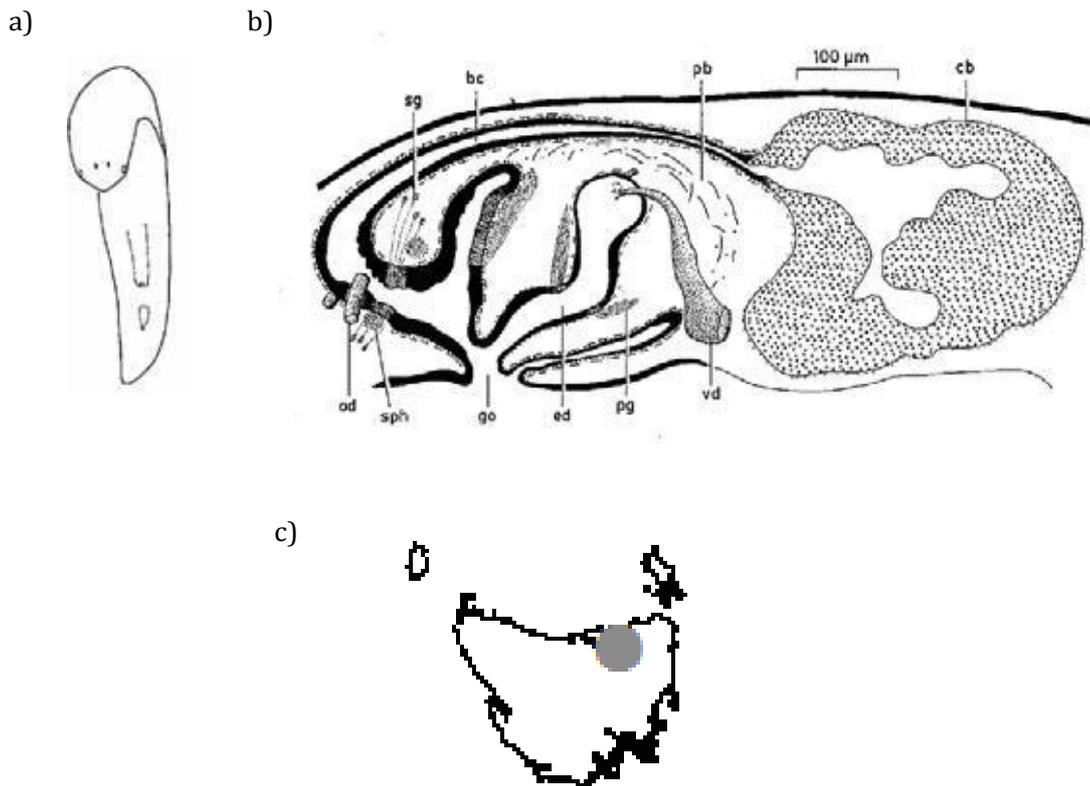
*Girardia sphincter* Sluys and Kawakatsu, 2001

### ***Contribution***

*Masaharus sphincter* was not examined as part of this thesis; however, this species was reassigned to *Masaharus* when this new genus was erected.

### ***Diagnosis***

*Masaharus sphincter* is differentiated from its congeners by the presence of a sphincter at the base of the bursal canal.



**Figure 1a.17** *Masaharus sphincter* a) free hand sketch of preserved specimen (Sluys and Kawakatsu 2001); b) sagittal reconstruction of the copulatory apparatus (Sluys and Kawakatsu 2001); c) distribution of *Masaharus sphincter* in Australia.

### **Comments**

This species was placed in the genus *Girardia* by default as it had non-reversed bursal canal musculature placing it within the *Cura*, *Schmidtea* and *Girardia* clade (Sluys 2001)(Figure 1a.17-b). However, the gross morphology of the copulatory apparatus precluded its placement anywhere but within the *Girardia*. Removing this species from within the *Girardia* is not a controversial step as, like all the Australian *Girardia*'s, it lacks the pigmented pharynx found in true *Girardia*'s worldwide. For similar reasons to those discussed by Sluys and Kawakatsu (2001) (i.e. the non-reversed musculature of the bursal canal), I believe that this species can be comfortably placed within *Masaharus* (Appendix 1d).

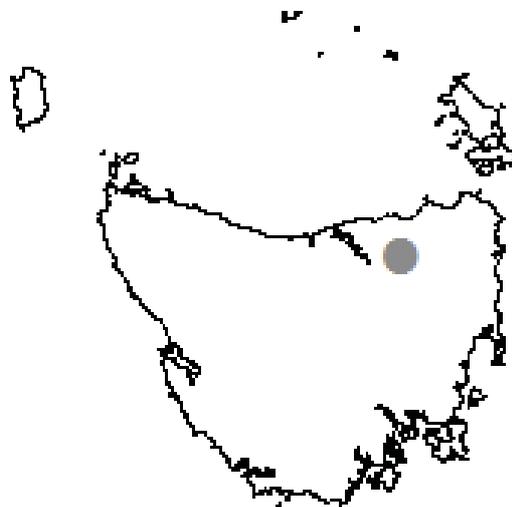
### **Ecology and Distribution**

*Masaharus sphincter* was collected from a pool on Mt. Barrow, a 3 km long plateau in northeast Tasmania (Sluys and Kawakatsu 2001)(Figure 1a.17-c). It has a maximum altitude of 1413 m and receives regular snowfalls in winter.

**Family DugesIIDae s.s. Ball, 1974**

***Neppia* Ball, 1974**

Head typically of low triangular form. Seminal vesicle a single muscular cavity, diaphragm absent. Bursal canal musculature of inner longitudinal fibres surrounded by an exceptionally thin layer of circular fibres. Testes numerous, dorsal, not extending beyond the copulatory apparatus. Ejaculatory duct is typically convoluted (Ball 1974c). This genus only has one Australian representative, with a distribution currently restricted to northern Tasmania (Figure 1a.18).



**Figure 1a.18** Distribution of *Neppia* in Australia.

***Neppia magnibursalis* Sluys and Kawakatsu, 2001**

***Contribution***

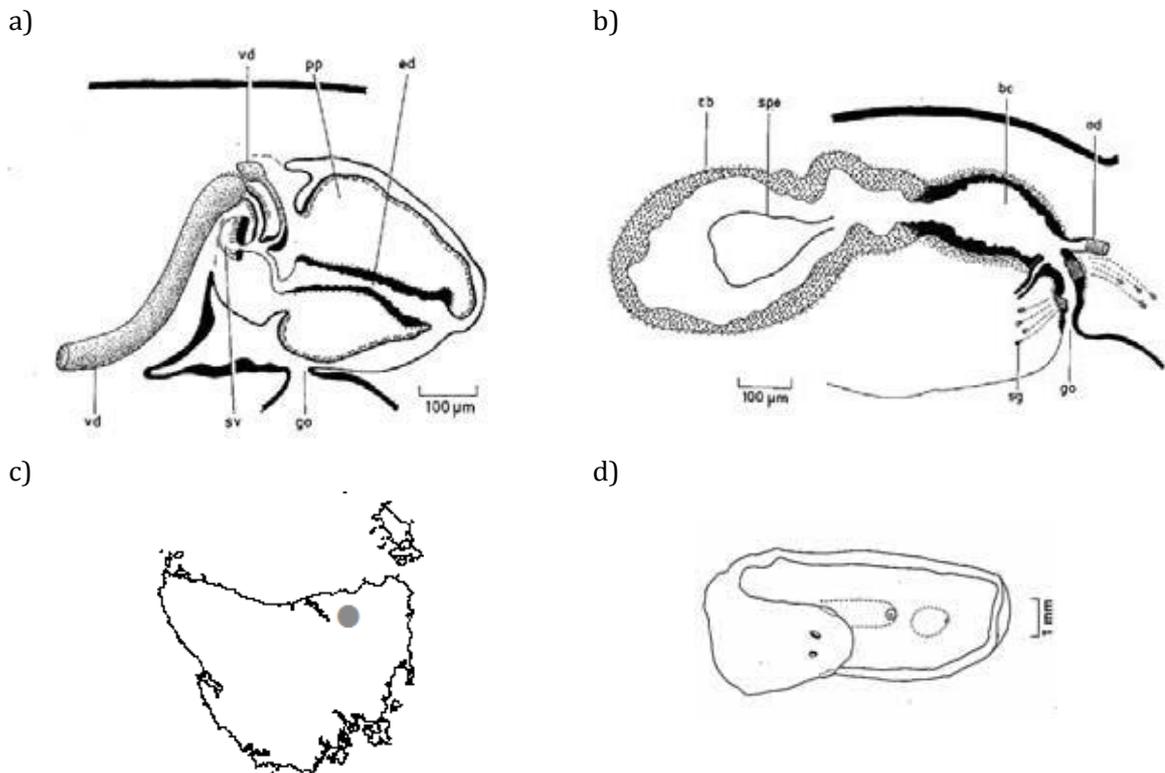
This species has only recently been described and has not been addressed during the course of the current research.

***Diagnosis***

*Neppia magnibursalis* can be distinguished from its congeners by having ventral testes extending throughout the body length, and oviducts that open asymmetrically into the bursal canal (Figure 1a.19-a,b).

### ***Ecology and Distribution***

This species is known only from Mt. Barrow, a 3 km long plateau in northeastern Tasmania (Figure 1a.19-c). It has a maximum altitude of 1413 m and receives regular snowfalls in winter (Sluys and Kawakatsu 2001).



**Figure 1a.19** *Neppia magnibursalis* a) Sagittal reconstruction of the male copulatory apparatus (Sluys and Kawakatsu 2001); b) sagittal reconstruction of the female copulatory apparatus (Sluys and Kawakatsu 2001); c) distribution of *Neppia magnibursalis* in Australia; d) external morphology of *Neppia magnibursalis* (Sluys and Kawakatsu 2001).

### **Family Spathulidae nov. fam.**

#### **Reynoldsonia Ball, 1974**

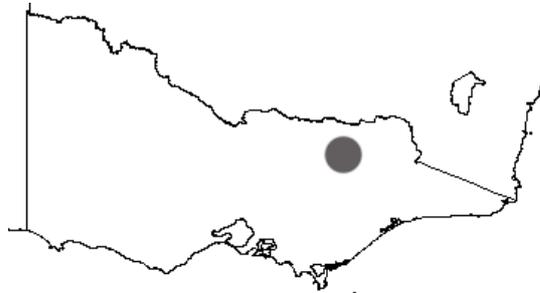
**Genus potentially dissolved and constituent species moved to *Spathula*.**

Slender with two eyes, rounded head with two sensory pits, lacking pigment. Testes numerous, discrete, distributed throughout the body-length, principally ventral in position. Penis large, muscular, with an eversible tip, with a bifid seminal vesicle, without a diaphragm in the ejaculatory duct. Bursal stalk with very thick ectal musculature

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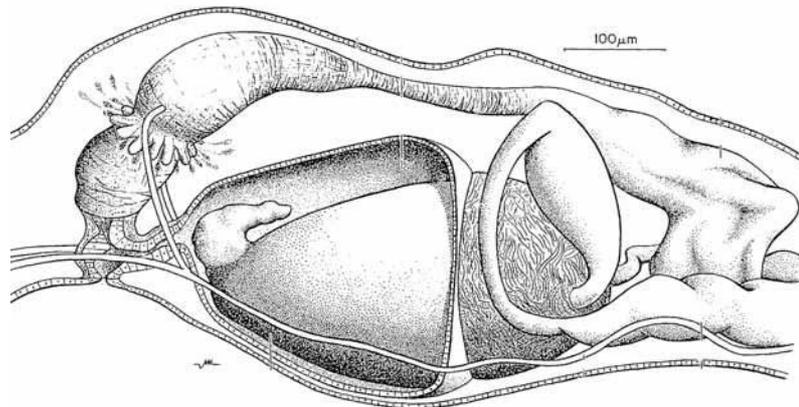
consisting of intermingled circular and longitudinal fibres. Oviducts with caudal branch, enter bursal canal separately above zone of dactylose projections of the bursal canal, and above zone of shell glands (Ball 1974a). *Reynoldsonia* is a monotypical genus that is distributed as shown in Figure 1a.20.



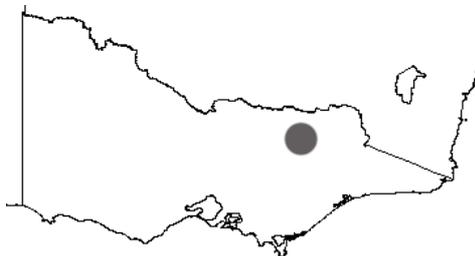
**Figure 1a.20** Distribution of *Reynoldsonia* in Australia.

***Reynoldsonia (Spathula) reynoldsoni* Ball, 1974**

a)



b)



c)



**Figure 1a.21** *Reynoldsonia reynoldsoni* a) Sagittal reconstruction of the copulatory apparatus (Ball 1974a); b) distribution of *Reynoldsonia reynoldsoni* in Australia; c) external morphology of *Reynoldsonia reynoldsoni* (Hay and Ball 1979).

### ***Contribution***

While the collection of this species cannot be confirmed throughout the course of this research there is a possible identification discussed in the comments relating to *Spathula* sp. 2 (Appendix 1d).

### ***Diagnosis***

*Reynoldsonia reynoldsoni* is the type species for this genus and the only *Reynoldsonia* described to date.

### ***Comments***

This species is the sole representative of its genus, and while it certainly displays a unique combination of characters including, the intermingled musculature on the bursal canal, the eversible tip of the penial papilla and the dactylose projections of the bursal canal, it is possible that *Reynoldsonia reynoldsonii* may simply be an aberrant species of *Spathula* (Sluys et al. 2001)(Figure 1a.21-a). While the collection of new specimens of this species during this research cannot be unequivocally confirmed, a possible identification is discussed in the discussion of *Spathula* sp. 2 (Appendix 1d).

### ***Ecology and Distribution***

This species was obtained from beneath loose stones in a small runnel on the rock, draining a wet patch beneath The Thumb, a rock formation near the summit of Mt. Buffalo (Ball 1974a)(Figure 1a.21-b). Mt Buffalo is part of the Victorian Alpine Region in the northeast of the state. *R. reynoldsoni* is characteristic of temporary waters, caused either by rain in summer or by melting snow in winter. This species displays a rare behavioural characteristic; it can be found gliding across the surface of rocks, not exhibiting any aversion to sunlight. Most Australian species are photophobic excepting *Spathula trunculenta* and *Romankenkius musculoglandulosus* (Hay and Ball 1979).

## **Family Romankenkiidae nov. fam.**

### ***Romankenkius* Ball, 1974**

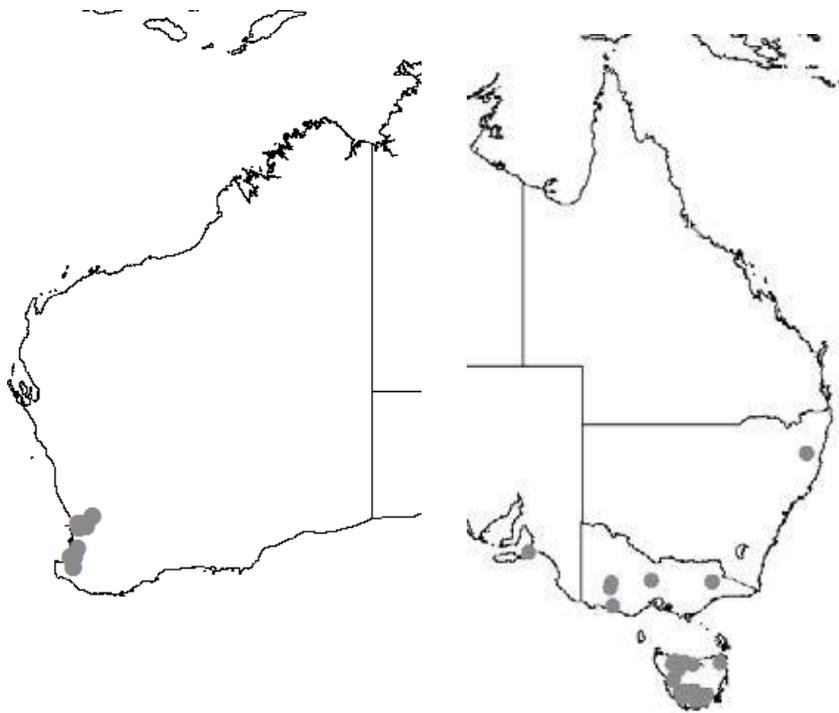
Genus potentially split and a new genus erected to house western Australian representatives.

## APPENDIX 1a

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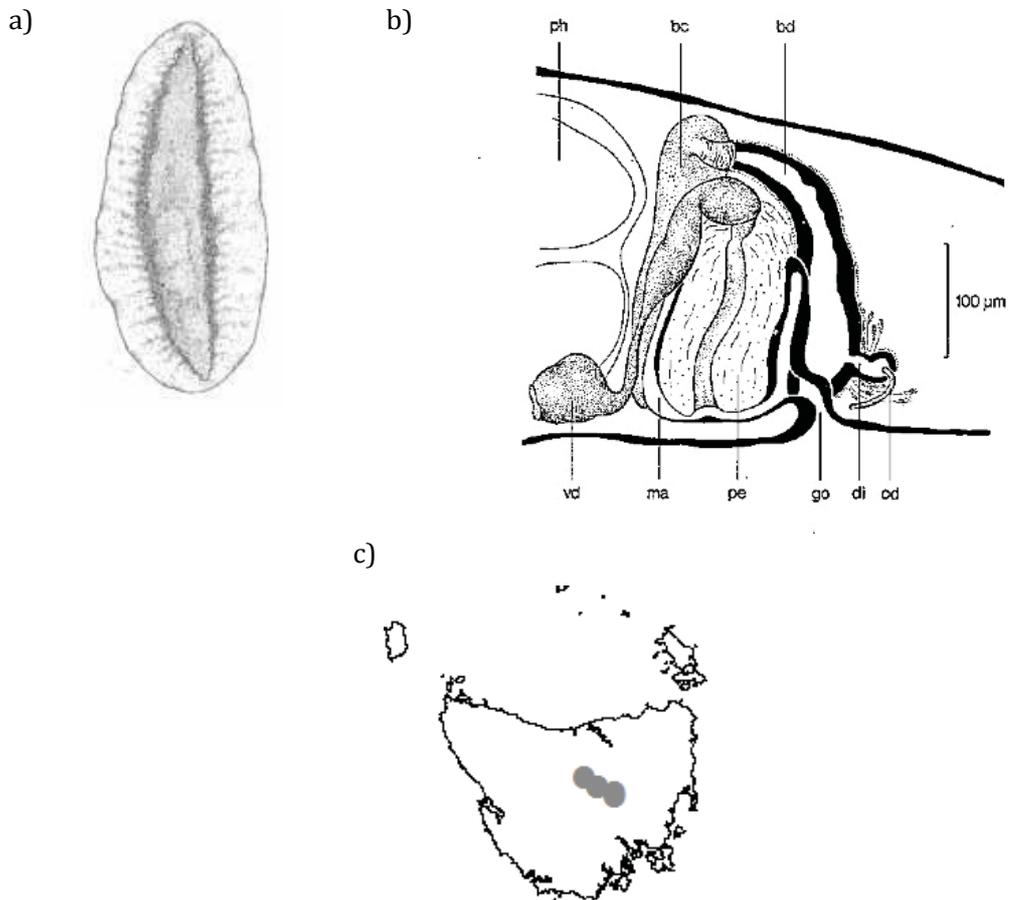
Pigmented, with two eyes each composed of several pigment cells and numerous retinal cells. Head rounded, with auricular slits, without auricular lobes. Pharynx not pigmented. Testes large, numerous but discrete, dorsal or ventral, and prepharyngeal. Penis large, with seminal vesicle, without diaphragm in ejaculatory duct, and asymmetrically placed in atrium. Bursa copulatrix situated laterally or anteriorly to penis. Bursal canal with posterior diverticulum, which receives the shell glands, and the oviducts separately or combined. Bursal canal musculature of inner longitudinal and outer circular fibres (Ball 1974b). Genus is distributed as shown in Figure 1a.21.

The molecular data analysed in Chapter 2 presents some concerns relating to the assignment of the Western Australian *Romankenkius* to this genus. While the morphological data suggests that the Western Australian species should sit within the genus *Romankenkius*, the molecular data presents a relationship inconsistent with this affiliation (Appendix 2c and Chapter 2). The implications of the molecular relationship between the two groups is discussed further in Chapter 2, however, ultimately it was decided that due to the lack of a clearly discernable morphological apomorphy for the Western Australian group, they must remain, for the time being, members of the genus *Romankenkius* (Figure 1a.22).



**Figure 1a.22** Distribution of *Romankenkius* in Australia.

***Romankenkius bilineatus* Ball and Tran, 1979**



**Figure 1a. 24** *Romankenkius bilineatus* a) External features of preserved specimen (Ball and Tran 1979); b) sagittal reconstruction of the copulatory apparatus (Ball and Tran 1979); c) distribution of *Romankenkius bilineatus*.

***Contribution***

No individuals exactly matching the description of *R. bilineatus* were examined during the course of this thesis; however, an extremely similar worm was described (*Romankenkius cf. bilineatus* - see below)(Appendix 1d)(Figure 1a.23-b).

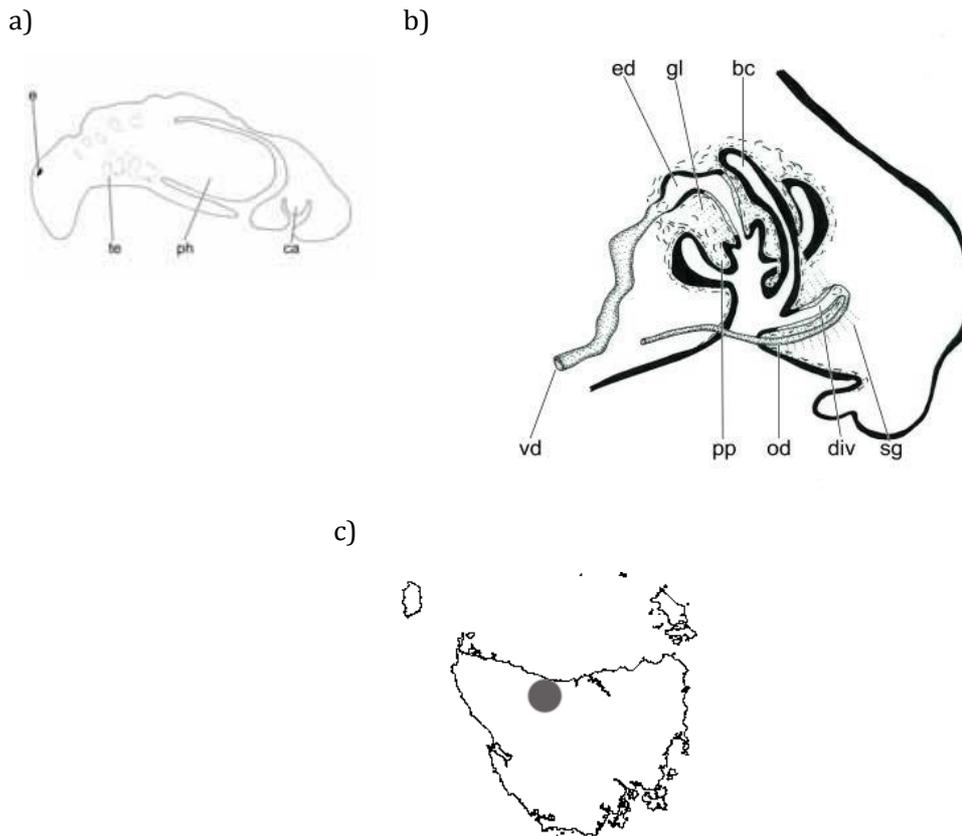
***Diagnosis***

*Romankenkius bilineatus* can be distinguished from its congeners by its distinctive striped colour pattern, the presence of both dorsal and ventral testes and the lack of a true seminal vesicle (Ball and Tran 1979).

### ***Ecology and Distribution***

This species has been confirmed from Arthurs Lake in the sparsely inhabited central highlands of Tasmania (Figure 1a.23-c). There are also several other unconfirmed sightings from several other lakes in Tasmania (Ball and Tran 1979).

### ***Romankenkius cf. bilineatus* Ball and Tran, 1979**



**Figure 1a.24** *Romankenkius cf. bilineatus* a) sagittal reconstruction of the reproductive system; b) sagittal reconstruction of the copulatory apparatus; c) distribution of *Romankenkius cf. bilineatus* in Australia.

### ***Contribution***

Description of a species extremely similar to *Romankenkius bilineatus* (Appendix 1d).

### ***Diagnosis***

This specimen shares the same diagnostic characters to those described above for *Romankenkius bilineatus*, however, due to an inability to identify the copulatory bursa or ovaries, this individual is only tentatively assigned to this species (Appendix 1d) (Figure 1a.24-a,b).

### **Comments**

The assignment of new specimens to *R. bilineatus* represents the first since its original description by Ball and Tran (1979). It is true that these specimens are only tentatively assigned to *R. bilineatus* but this is only due to the inability to identify copulatory bursa or ovaries, which is certainly a fixation artefact (Appendix 1d) (Figure 1a.24-a,b).

Beyond these inadequacies, this description does not contradict the original description (Ball and Tran 1979) and many notable similarities can be identified. These similarities include: the presence of fusing ventral and dorsal testes, a feature lacking in all other species of *Romankenkius*; the lack of a true seminal vesicle, a rare state for this genus; the reduced musculature on the diverticulum (Appendix 1d)(Figure 1a.24-a,b).

Ball and Tran (1979) describe the copulatory apparatus as being remarkably variable, owing to a difference displayed in the orientation of the penial papilla, and suggest that their description may actually be dealing with two very closely related species. While the material presented in Appendix 1d does not resolve the question of this variability, the orientation of the penial papilla in these new specimens is dorso-ventral, confirming at least that this state was not a result of fixation artefacts and may be a common feature of this species.

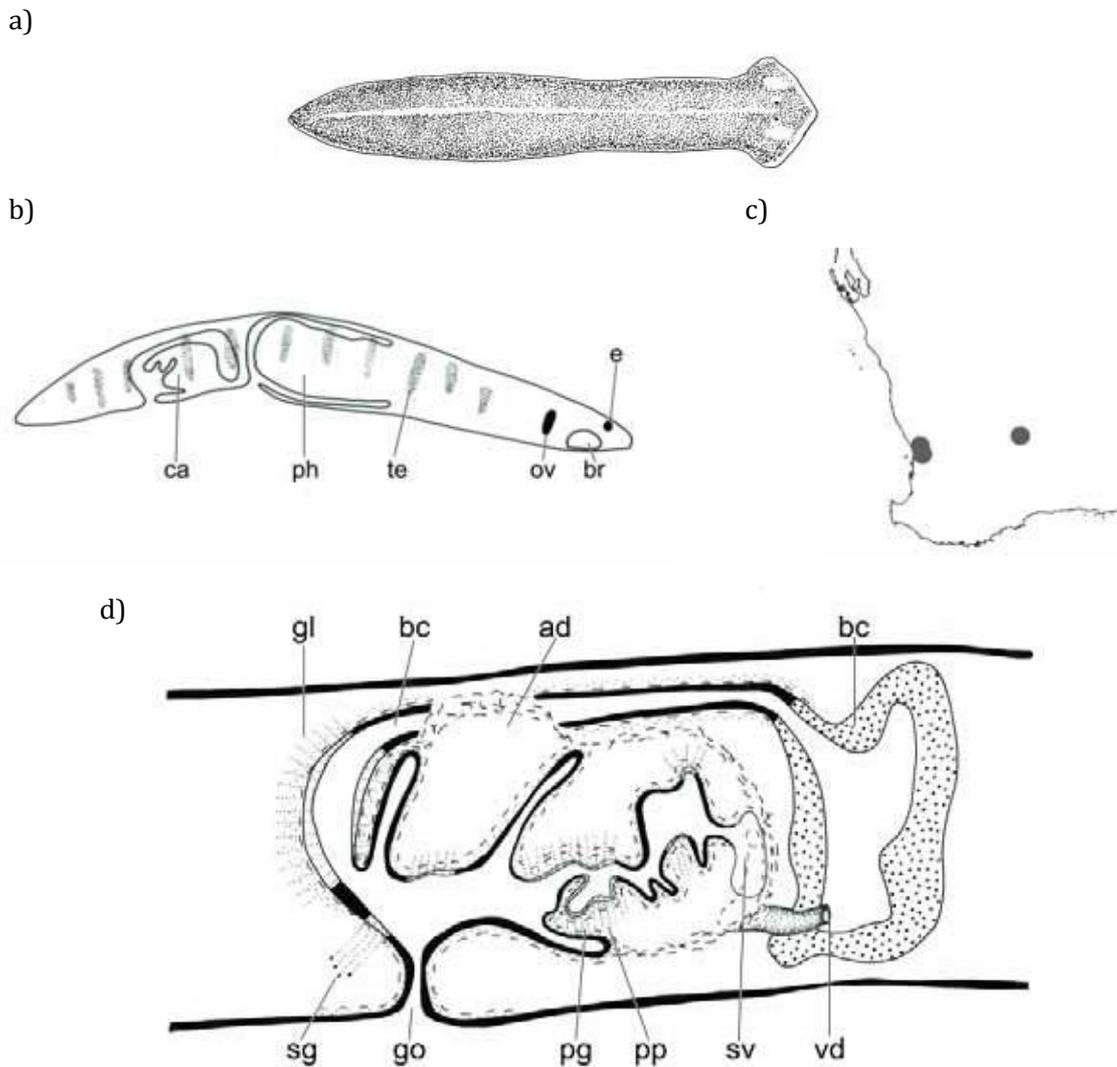
### **Ecology and Distribution**

*Romankenkius bilineatus* has been described from central northern Tasmania in the Great Lake area. *R. cf. bilineatus* was collected from slightly north of this site but still within the Tamar River basin (Appendix 1d) (Figure 1a.24-c). This supports Ball and Tran's (1979) speculation that this species is more widespread in Tasmania than the current distribution data suggests.

### ***Romankenkius (western Romankenkius) boehmigi* (Weiss, 1909)**

*Planaria boehmigi* Weiss, 1909

*Romankenkius boehmigi* - Sluys 1997



**Figure 1a.25** *Romankenkius boehmigi* a) External features of living animal; b) sagittal reconstruction of reproductive system; c) distribution of *Romankenkius boehmigi* in Australia; d) sagittal reconstruction of copulatory apparatus.

### ***Contribution***

New specimens of *Romankenkius boehmigi* were collected as part of this thesis. This species was subsequently redescribed with a few minor amendments.

### ***Diagnosis***

*Romankenkius boehmigi* can be distinguished from its congeners by the dorso-ventral orientation of the adenodactyl, large seminal vesicle, broad, irregular ejaculatory duct and non-folded penial papilla.

### ***Comments***

The specimens presented in Appendix 1d match very closely the re-description of *Romankenkius boehmigi* by Sluys (1997). The new material has allowed for the accurate description of the external morphology that, before now, had only been described from preserved specimens (Figure 1a.25-a). Despite this, the similarities are remarkable, with the extreme paleness on the anterior margin and large pigment free areas close to the eyes already having been suggested in previous descriptions (Sluys 1997).

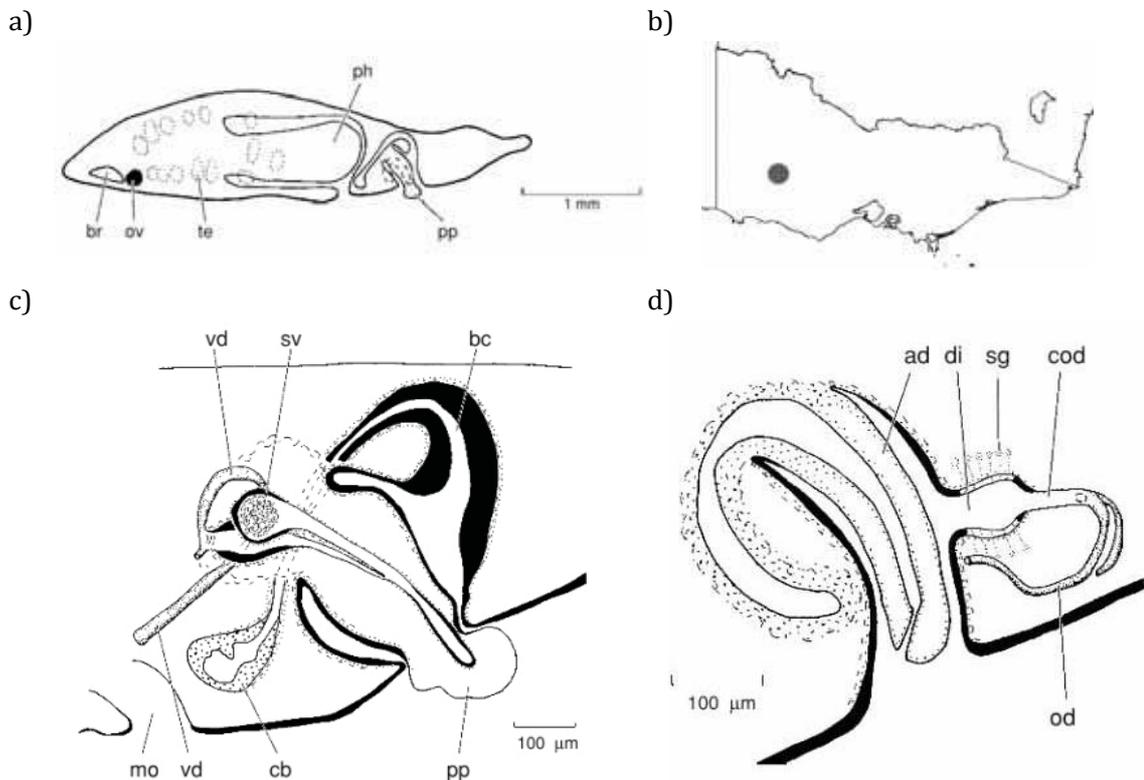
This species is easily located within the genus *Romankenkius* due (1) to the long diverticulum of the bursal canal receiving the oviducts and (2) the large seminal vesicle. Within the genus *Romankenkius* there are five species with an adenodactyl, viz. *R. libidinosus*, *R. hoernesii*, *R. boehmigi*, *R. conspectus* and *R. impudicus* (Grant et al. 2006, Sluys 1997, Sluys and Rohde 1991, Appendix 1d). *R. hoernesii* exhibits many similar features to *R. boehmigi*. However, the posterior orientation and lateral position of the adenodactyl differentiate these two species. The adenodactyl of *R. boehmigi* is not orientated in line with the penial papilla (eliminating any possibility that *R. boehmigi* specimens should be assigned to *R. conspectus* or *R. libidinosus*); only *R. impudicus* shares a similar orientation (Appendix 1d). Yet, this is where the similarity between these species ends. The orientation, which is directly opposing the penial papilla, of *R. impudicus*' adenodactyl and the organisation of the afore mentioned structure, are markedly different from that of *R. boehmigi* (Appendix 1d)(Figure 1a.25-d).

The unique dorso-ventral orientation of the adenodactyl, large seminal vesicle, broad, irregular ejaculatory duct and non-folded penial papilla constitute a diagnostic set of characters for this species. The adenodactyl is extremely interesting as it exhibits a high degree of flexibility not reported in the original description (Sluys 1997, Appendix 1d). Finally, Sluys' (1997) description of *R. boehmigi* reports the presence of a diaphragm or fold in the ejaculatory duct. This new material suggests that this structure is simply a fold and subject to intraspecific variation.

### ***Ecology and Distribution***

*Romankenkius boehmigi* has been collected from the underside of rocks in the off-channel area of small streams. The location of the newly collected specimen is 28km east of Perth and only 5km north of the type locality at Gooseberry Hill (Weiss 1909, Sluys 1997). Another specimen identified via molecular data was collected approximately 50km north of Perth (LJ253 - Appendix 2c and 1d)(Figure 1a.25-c).

***Romankenkius conspectus* Sluys and Grant, 2006**



**Figure 1a.26** *Romankenkius conspectus* a) Sagittal reconstruction of the reproductive system; b) distribution of *Romankenkius conspectus* in Australia; c) sagittal reconstruction of copulatory apparatus; d) sagittal reconstruction of the adenodactyl.

**Contribution**

*Romankenkius conspectus* is a new species described as part of this thesis (Grant et al. 2006).

**Diagnosis**

*Romankenkius conspectus* can be distinguished from its congeners by the presence of an adenodactyl papilla with the same orientation as the penis papilla, and an accessory intrabulbar seminal vesicle communicating with the ejaculatory duct (Grant et al. 2006).

**Comments**

The presence of a diverticulum at the posterior wall of the atrium, directly ventrally to the point where the bursal canal communicates with the atrium, immediately suggests that this animal is a member of the genus *Romankenkius* (Grant et al. 2006). Within this genus, *R. conspectus* is uniquely characterized by the presence of an adenodactyl with the same

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orientation as the penis papilla, and by an accessory intrabulbar seminal vesicle communicating with the ejaculatory duct (Figure 1a.26-c,d). Whilst *R. libidinosus* also has an adenodactyl displaying the same orientation as the penis papilla, in this species the basal parts of the two structures are actually merged into one unit, which is not the case in *R. conspectus*. Furthermore, the small copulatory bursa of *R. conspectus* is situated in an unusual ventral position. Although it could be argued that the extremely ventral location of the bursa is the result of considerable contraction during fixation, Grant et al. (2006) are of the opinion that such an explanation cannot be reasonably invoked for the other unique characteristics of this species.

### ***Ecology and Distribution***

*Romankenkius conspectus* was collected from Turret Falls, just outside of Halls Gap in the Grampians National Park, Victoria (Grant et al. 2006)(Figure 1a.26-b). The Grampians are a series of five spectacular sandstone ridges running north to south with steep and craggy slopes on the eastern side and gentler slopes to the west.

### ***Romankenkius (western Romankenkius) glandulosus (Kenk, 1930)***

*Dugesia glandulosa* Kenk, 1930

*Romankenkius glandulosus* - Sluys 1997

### ***Contribution***

This species was re-described twice throughout the course of this research with some minor amendments to Weiss' (1909, 1910) original description. Grant et al.'s (2006) description was based on a re-examination of the type material, whilst the description presented in Appendix 1d was completed with the benefit of new material.

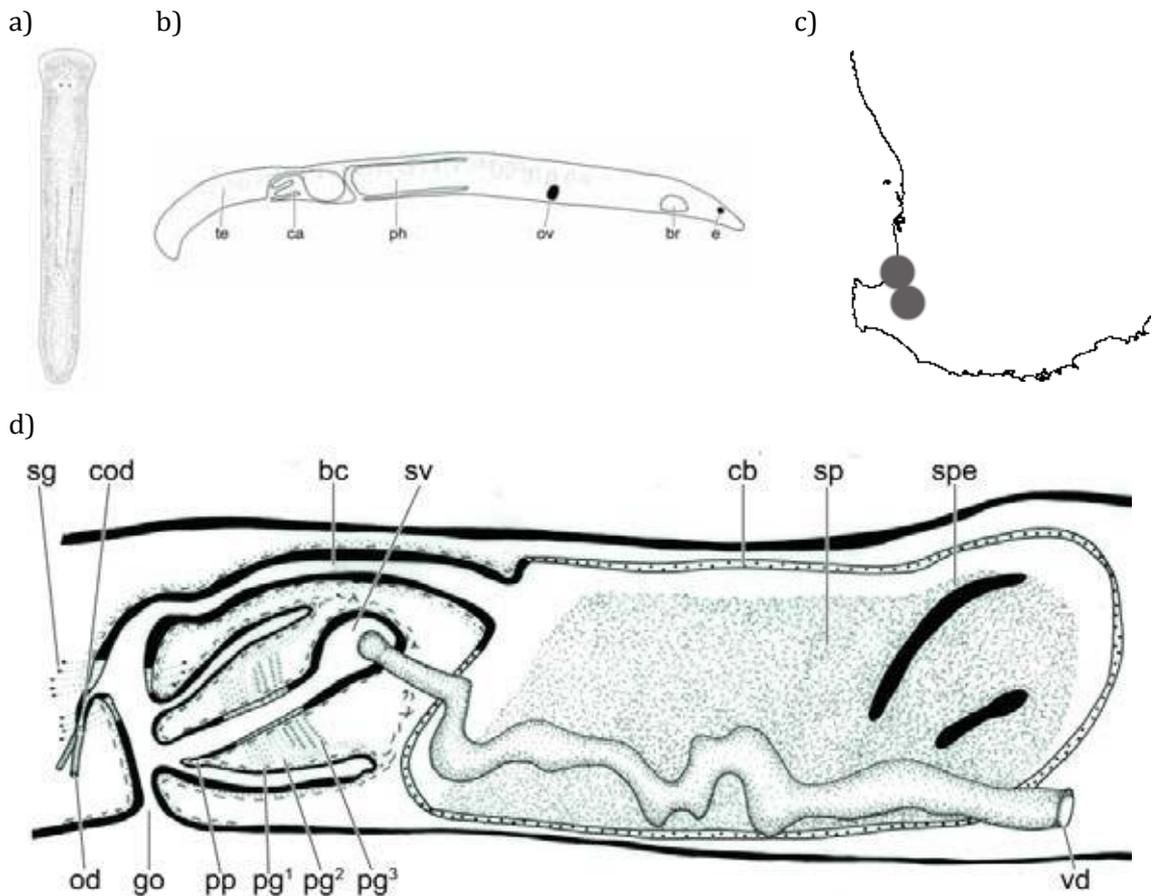
### ***Diagnosis***

*Romankenkius glandulosus* is differentiated from other *Romankenkius* by the presence of three types of glands in the ejaculatory duct and the lack of shell glands entering the diverticulum (Grant et al. 2006).

### ***Comments***

Grant et al.'s (2006, Appendix 1d) descriptions do not differ substantially from the original description of *Romankenkius glandulosus* (Weiss 1910). Subsequent descriptions have

noted that Weiss' original description of non-reversed bursal canal musculature was incorrect and these specimens supports this correction (Ball 1977a)( Figure 1a.27-d). The new material illustrates a minor deviation from previous description's, the presence of a clearly defined seminal vesicle receiving the vasa deferentia. This level of variation is not uncommon in other species and is likely to be due to intraspecific variation.



**Figure 1a.27** *Romankenkius glandulosus* a) External features of live specimen; b) sagittal reconstruction of the reproductive system; c) distribution of *Romankenkius glandulosus* in Australia; d) sagittal reconstruction of the copulatory apparatus.

### ***Ecology and Distribution***

Specimens of *Romankenkius glandulosus* can be found in low numbers across a broad range of habitats including riffles, runs and off-channel areas in a large, predominantly sandy, river (Appendix 1d). Individuals were collected from the underside of rocks not associated with any macrophytes or leaf litter. The new site is located within kilometres of the type locality, Boyanup, in the southern corner of Western Australia and yielded the second series of specimens that have become available after Weiss' (1910) first detailed

**APPENDIX 1a**

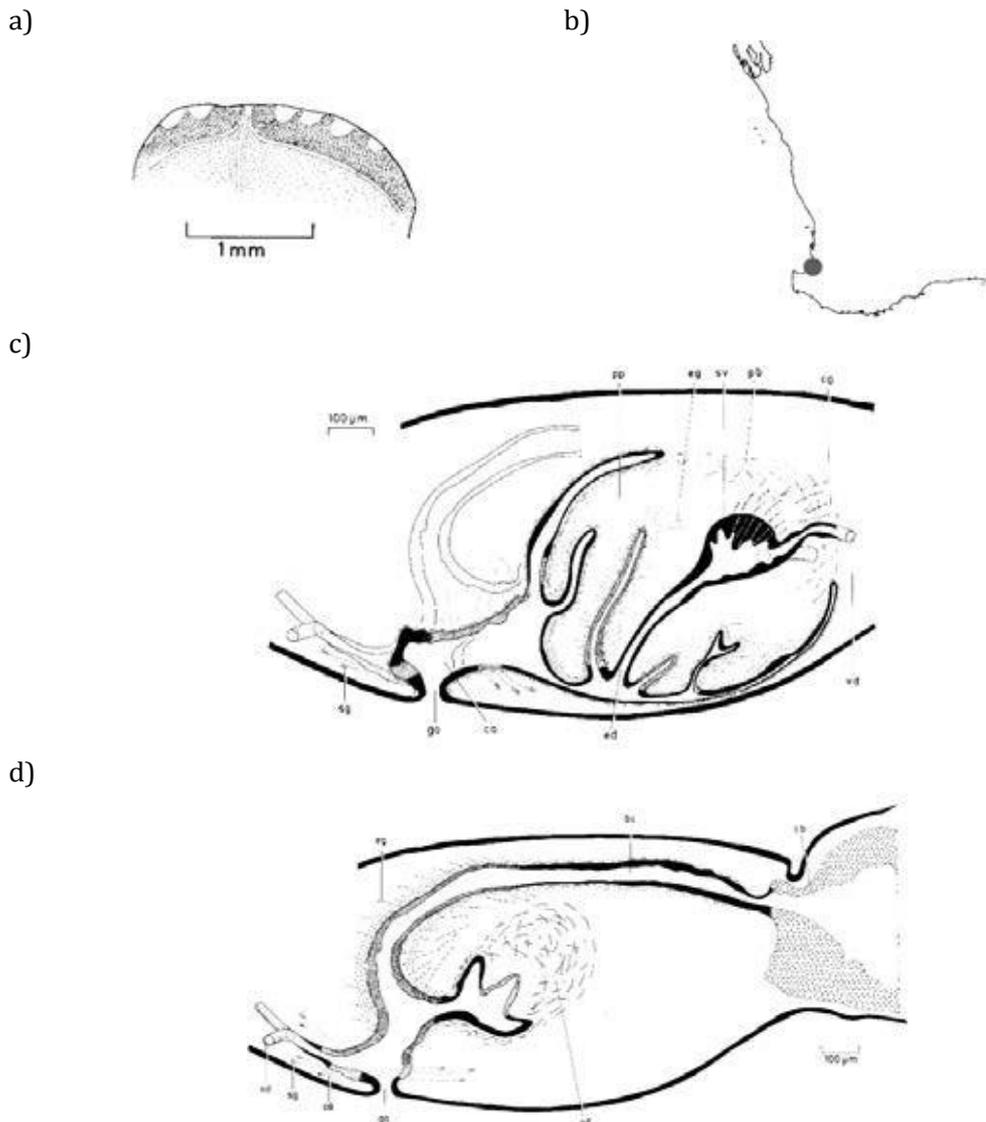
SYSTEMATIC REVIEW AND REVISION OF DUGESIIDAE S.L. TAXONOMY

description of the species (Appendix 1d)( Figure 1a.27-c). Boyanup is located in a predominantly agricultural region and sits on the shores of the Preston River.

***Romankenkius* (*western Romankenkius*) *hoernesi* (Weiss, 1909)**

*Planaria hoernesi* Weiss, 1909

*Romankenkius hoernesi* - Sluys 1997



**Figure 1a.28** *Romankenkius hoernesi* a) Frontal margin with sensory patches, marginal stripes, and median groove (Sluys 1997) b) distribution of *Romankenkius hoernesi* in Australia; c) sagittal reconstruction of the male copulatory apparatus (Sluys 1997); d) sagittal reconstruction of the female copulatory apparatus (Sluys 1997).

***Contribution***

No new specimens of this species were examined as part of this thesis.

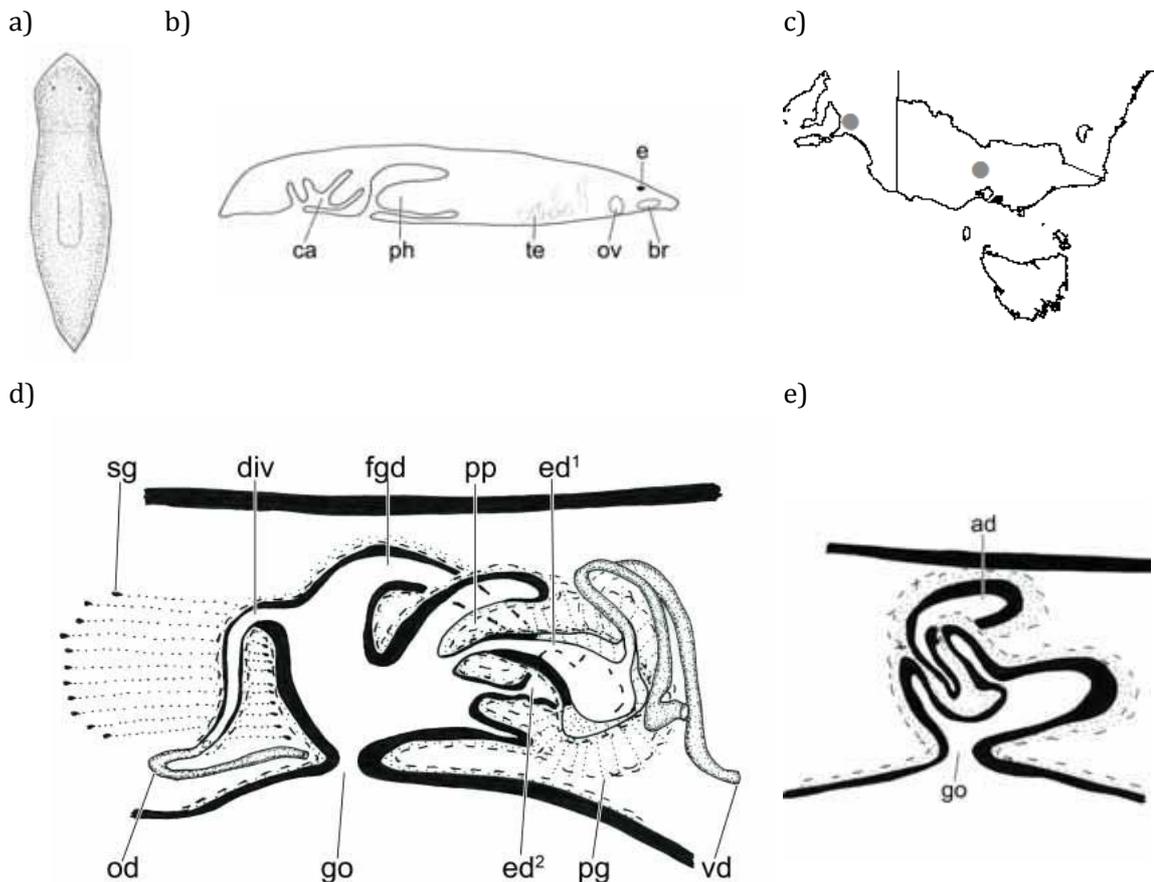
**Diagnosis**

*Romankenkius hoernesii* can be distinguished from other *Romankenkius* by the presence of an adenodactyl orientated posteriorly and the large glandularised folds in the penial papilla (Sluys 1997)( Figure 1a.28-c,d).

**Ecology and Distribution**

*Romankenkius hoernesii* is known only from the type locality Boyanup, Western Australia (Weiss 1909). Boyanup is located in a predominantly agricultural region and sits on the shores of the Preston River (Figure 1a.28-b).

***Romankenkius impudicus* Sluys and Grant, 2006**



**Figure 1a.29** *Romankenkius impudicus* a) External features of a live specimen; b) sagittal reconstruction of the reproductive system; c) distribution of *Romankenkius impudicus* in Australia; d) sagittal reconstruction of copulatory apparatus; e) sagittal reconstruction of the adenodactyl.

### ***Contribution***

This species was newly described and then re-described as part of the research done for this thesis (Grant et al. 2006, Appendix 1d).

### ***Diagnosis***

*Romankenkius impudicus* is differentiated from other *Romankenkius* by the unique adenodactyl and by the presence of two ejaculatory ducts (Grant et al. 2006).

### ***Comments***

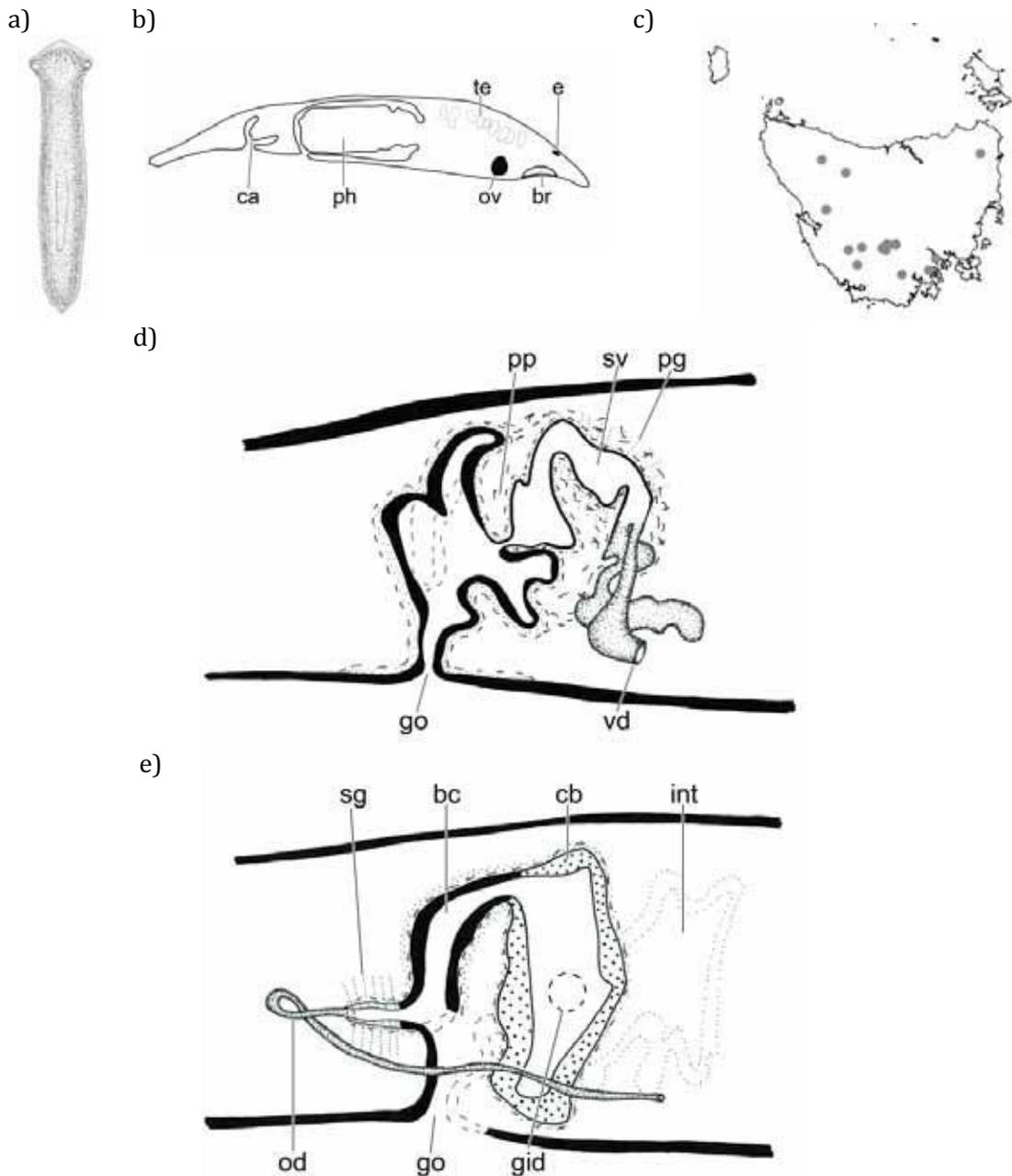
This species possesses a long diverticulum and large seminal vesicles, indicating that these specimens are members of *Romankenkius*. The adenodactyl with its anterior orientation and the unique free papilla are distinct characters for *Romankenkius impudicus* (Grant et al. 2006, Appendix 1d)(Figure 1a.29-d,e). The new material examined as part of this thesis (Appendix 1d) allowed a re-examination and re-interpretation of the poorly stained type material (Grant et al. 2006).

Prior to this re-description, *R. impudicus* was already well-defined by its unique adenodactyl. Now the animal appears to be even more aberrant than initially thought (Appendix 1d). The presence of two ejaculatory ducts opening independently at the tip of the penial papilla, only the second case in the triclads (first case being *Girardia biapertura* (cf. Sluys et al. 1997)), presents an excellent diagnostic character (Appendix 1d). Another notable feature is the absence of a copulatory bursa; instead a female genital duct communicates directly with a branch of the intestine, the latter incorrectly considered to be a copulatory bursa by Grant et al. (2006)(Figure 1a.29-d,e).

### ***Ecology and Distribution***

The newly collected specimens of *Romankenkius impudicus* were collected from the underside of cobble in the warm, off-channel area of a sandy river. Grazing activity had heavily disturbed the river, riparian zone and surrounding region, making suitable habitat sparse. *R. impudicus* is now known from two sites in southern Australia, the type specimen was collected from south of Adelaide while this new material was collected from northwest Melbourne (Grant et al. 2006, Appendix 1d)(Figure 1a.29-c). *Romankenkius impudicus* was discovered in the presence of *Girardia tigrina* and *Cura pinguis*.

***Romankenkius kenki* Ball, 1974**



**Figure 1a.30** *Romankenkius kenki* a) External features of live specimen; b) sagittal reconstruction of reproductive system; c) distribution of *Romankenkius kenki* in Australia; d) sagittal reconstruction of the male copulatory apparatus; e) sagittal reconstruction of the female copulatory apparatus.

### ***Contribution***

This species has been re-described twice throughout the process of this research, with a substantial increase in our knowledge of *Romankenkius kenki*'s distribution (Grant et al. 2006, Appendix 1d).

### ***Diagnosis***

*Romankenkius kenki* can be distinguished from other *Romankenkius* by the postero-lateral position of the copulatory bursa and the direct connection of this organ to the intestine (Grant et al. 2006).

### ***Comments***

Ball's (1974b) original description of *Romankenkius kenki* mentions a double seminal vesicle and dorsal testes as being diagnostic characters for this species. Due to the increase in species assigned to this genus, these features are no longer very helpful in distinguishing *R. kenki* from the five other *Romankenkius*' with dorsal testes (Grant et al. 2006, Sluys 2001, Appendix 1d). The double seminal vesicle has also become somewhat redundant, as this character has proven to be influenced heavily by the highly flexible penial papilla. The most striking feature of the seminal vesicle is the extreme variability between representatives. However variability is not a very useful diagnostic character.

As has been noted in more recent studies, the most characteristic feature of *R. kenki* is the postero-lateral position of the copulatory bursa (Grant et al. 2006). Indeed this new material supports this feature as a defining character for the species (Appendix 1d)(Figure 1a.30-d,e).

The other interesting feature of *R. kenki* is the presence and occasional absence of the direct connection between the copulatory bursa and the intestine. This feature was described by Ball (1974b) in the initial description, however, this feature was only recorded in one of Grant et al.'s (2006) specimens. All specimens examined as part of Appendix 1d have a clear connection between the lateral side of the copulatory bursa and a branch of the intestine. This discrepancy may be due to the poor staining of previous specimens, preventing us from seeing this level of detail. Nevertheless, there is a possibility that the connection is absent in some specimens. This could be an independent state or may be related to the observed variability in the development of the copulatory bursa (Grant et al. 2006, Appendix 1d).

### ***Ecology and Distribution***

*Romankenkius kenki* can be collected from the underside of cobble on the shores of lakes or the off-channel area of rivers predominantly with muddy substrates. All specimens were collected from pristine, forested environments throughout the entire Tasmanian drainage division (Ball 1974b, Grant et al. 2006, Sluys and Kawakatsu 2001, Appendix 1d)(Figure 1a.30-c). *Romankenkius kenki* was discovered in the presence of *Romankenkius pedderensis*, *Cura pinguis*, *Masaharus informis* and *Masaharus extentus*.

### ***Romankenkius (Spathula) libidinosus* Sluys and Rohde, 1991**

#### ***Contribution***

*Romankenkius libidinosus* has been redescribed as part of this thesis (Grant et al. 2006) and in addition some notes relating to a potential genus reassignment are included in the taxonomic work presented in Appendix 1d.

#### ***Diagnosis***

*Romankenkius libidinosus* can be distinguished from its congeners by its large adenodactyl and genito-intestinal connections through small openings in the bursal canal (Sluys and Rohde 1991).

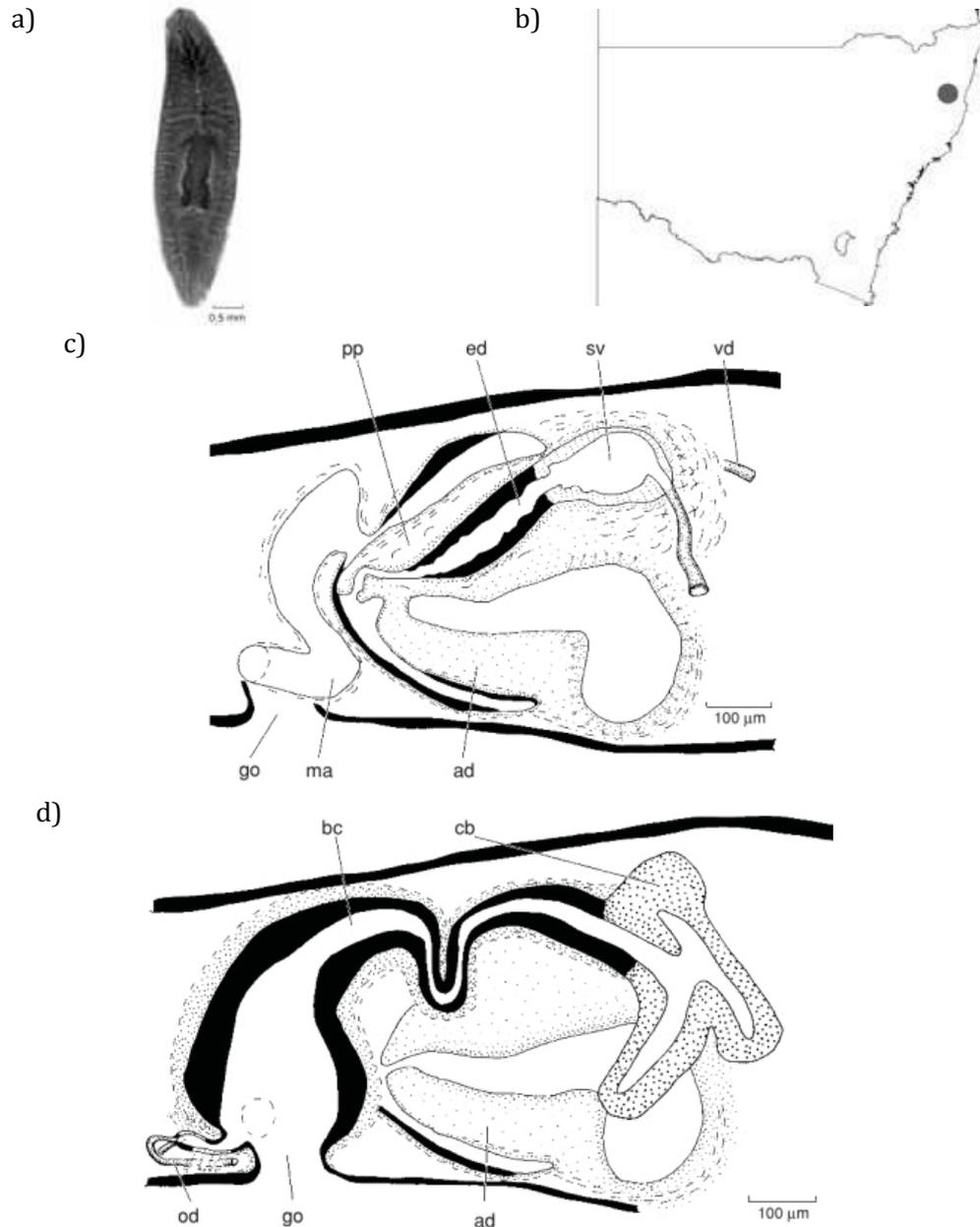
#### ***Comments***

In Sluys and Rohde's (1991) description, one diagnostic feature mentioned was the presence of genito-intestinal connections along the bursal canal. These connections were not obvious in Grant et al.'s (2006) specimens; however, other features present can leave very little doubt as to their assignment. In the specimens examined by Grant et al. (2006) there was also less well defined glandularisation on the adenodactyl and seminal vesicle; however, this is likely to be an artefact of the staining process.

*Romankenkius libidinosus* is considered something of an anomaly due to the non-reversed musculature of the bursal canal (Figure 1a.31-c,d). At the time of description this character state was known only from *Schmidtea*, *Eviella* and a few species of *Girardia* (Sluys and Rohde 1991). This character has also been described for *Masaharus* (Appendix 1d). The presence of a short diverticulum of the bursal canal prompted Sluys and Rohde (1991) to assign this species to *Romankenkius* despite the presence of the long caudal branch of the oviducts, a synapomorphy of the genus *Spathula*. The presence of an

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adenodactyl was at the time of description thought rare in DugesIIDae s.l.; however, several species have subsequently been described with this character from the genera *Romankenkius* and *Spathula*.



**Figure 1a.31** *Romankenkius libidinosus* a) Photograph of preserved, cleared specimen; b) distribution of *Romankenkius libidinosus* in Australia; c) sagittal reconstruction of the male copulatory apparatus; d) sagittal reconstruction of the female copulatory apparatus.

The presence of two “non-*Romankenkius*” like characters in the description of *R. libidinosus* throws some doubt on its assignment to *Romankenkius*. The molecular

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evidence presented suggests that this doubt is justified (see Chapter 2). The newly presented molecular analyses place *R. libidinosus* within the *Spathula clade*, considerably removed from other *Romankenkius* species. While this new evidence is convincing and suggests that a reassignment to *Spathula* may be warranted, Sluys and Rhode's (1991) arguments for placing it within the *Romankenkius* are equally convincing. Due to the fact that there is no new taxonomic data that would justify the reassignment of this species, or construction of a new genus, it will remain amongst the *Romankenkius* for now (see Chapter 2 for further discussion) (Appendix 1d). However, I predict that this will change when more information is available regarding the DugesIIDae s.l. from this region.

### ***Ecology and Distribution***

Grant et al.'s (2006) specimens were found in cool rainforest area, with water percolating through the rock of an inland cliff to form runnels and trickles. In one such *R. libidinosus* (white) was found exclusively. Higher up it mixed with *Spathula simplex* (black), and 100m down the path only *S. simplex* could be found (Appendix 1d). All known specimens have been collected from New England National Park in northeastern New South Wales (Sluys and Rohde 1991, Appendix 1d)( Figure 1a.31-b).

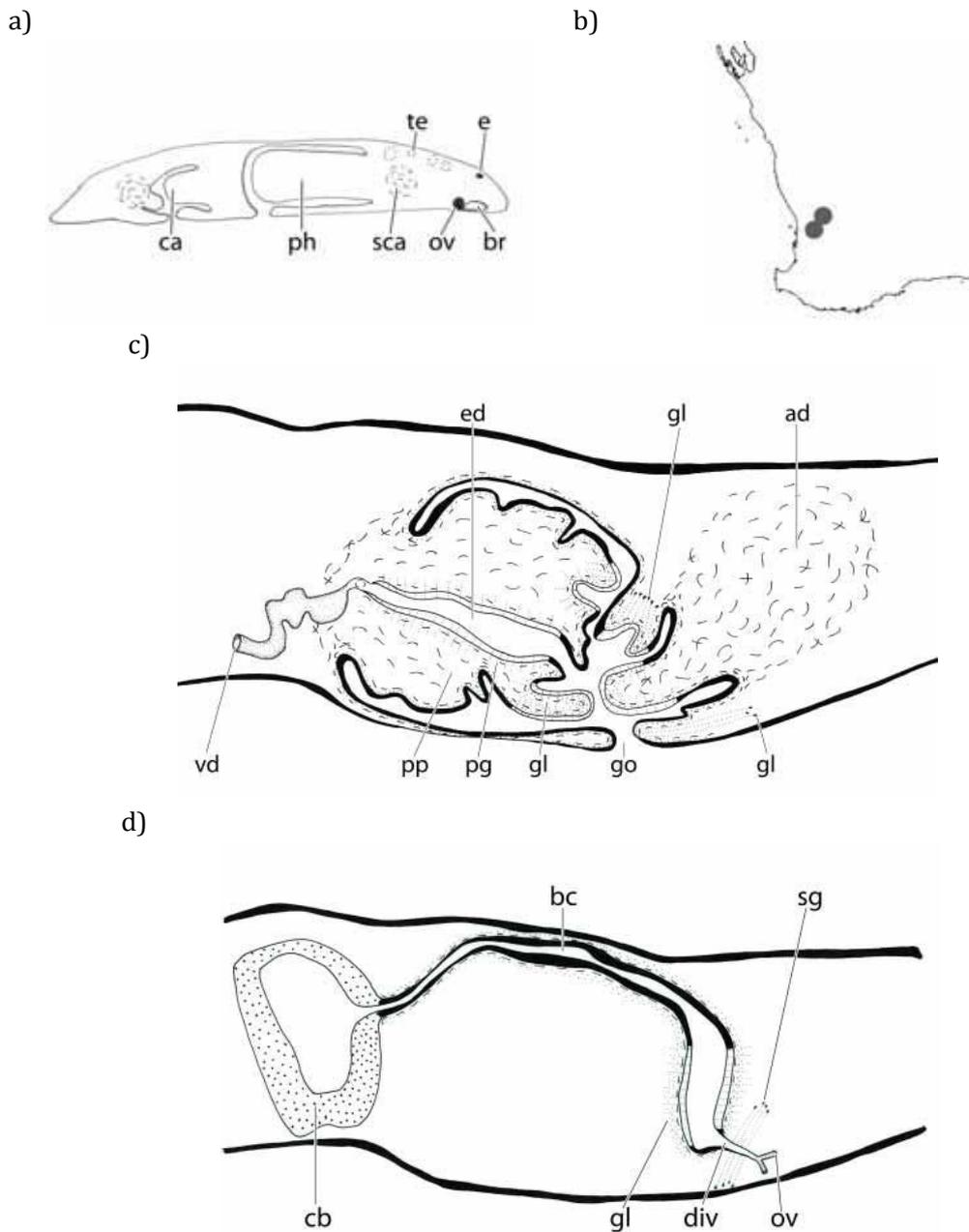
### ***Romankenkius (western Romankenkius) musculoglandulosus* Grant & Sluys, sp. nov.**

### ***Contribution***

This species was described as part of the taxonomic work done for this thesis (Appendix 1d).

### ***Diagnosis***

*Romankenkius musculoglandulosus* is distinguishable from its congeners due to the presence of a muscular adenodactyl opposing the penial papilla, pre-pharyngeal dorsal testes, antero-lateral copulatory bursa and glands exiting through the epithelium of the penial papilla (Appendix 1d).



**Figure 1a.32** *Romankenkius musculoglandulosus* a) Sagittal reconstruction of the reproductive system; b) distribution of *Romankenkius musculoglandulosus* in Australia; c) sagittal reconstruction of the male copulatory apparatus; d) sagittal reconstruction of the female copulatory apparatus.

**Comments**

Despite the lack of a seminal vesicle, the lengthy diverticulum suggests that this species is a member of *Romankenkius*. The presence of an adenodactyl and the nature of the glandularisation in the penial papilla invites comparison with *R. hoernesii* (Sluys 1997).

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Despite some remarkable similarities between the two species there are several significant points of difference (Appendix 1d). *R. hoernesii* has a clear seminal vesicle; testes that extend up to the gonopore; and ovaries located some distance behind the brain, none of which are found in *R. musculoglandulosus*. In addition to all of these conflicts, the adenodactyl has an opposing orientation and a lateral position in *R. hoernesii*. It would be unlikely that the latter fact is a result of intraspecific variability or individual polymorphism, particularly as such variability has not been encountered in other species of *Romankenkius* with adenodactyls (Appendix 1d).

All other *Romankenkius*' with adenodactyls have conflicting orientation to that of *R. musculoglandulosus*, excluding *R. impudicus* and *R. boehmigi*. However, *R. impudicus* can be immediately discounted owing to the double ejaculatory duct and absence of a copulatory bursa (Appendix 1d). The external morphology of *R. boehmigi* and *R. musculoglandulosus* are almost identical. Other similarities include the nature of the glandularisation on the adenodactyl and the extended glandularisation on the bursal canal. Despite these similarities, *R. boehmigi* exhibits a seminal vesicle, non-folded papilla, an absence of glands exiting through the epithelium of the penial papilla and the bursal canal does not run laterally to the penial papilla, conflicting with observations for *R. musculoglandulosus*. Additionally, the testes in *R. boehmigi* extend into the posterior of the animal (Appendix 1d)(Figure 1a.32-c,d).

### ***Ecology and Distribution***

This species was collected from *Acacia* forest inland from Perth, Western Australia (Figure 1a.32-b). Individuals of *R. musculoglandulosus* were found in large numbers gliding across granite bedrock and swimming freely in the water channel of a small stream (Appendix 1d). This is only the third Australian "dugesiid" known to not display photophobic behaviour (cf. Hay and Ball 1979).

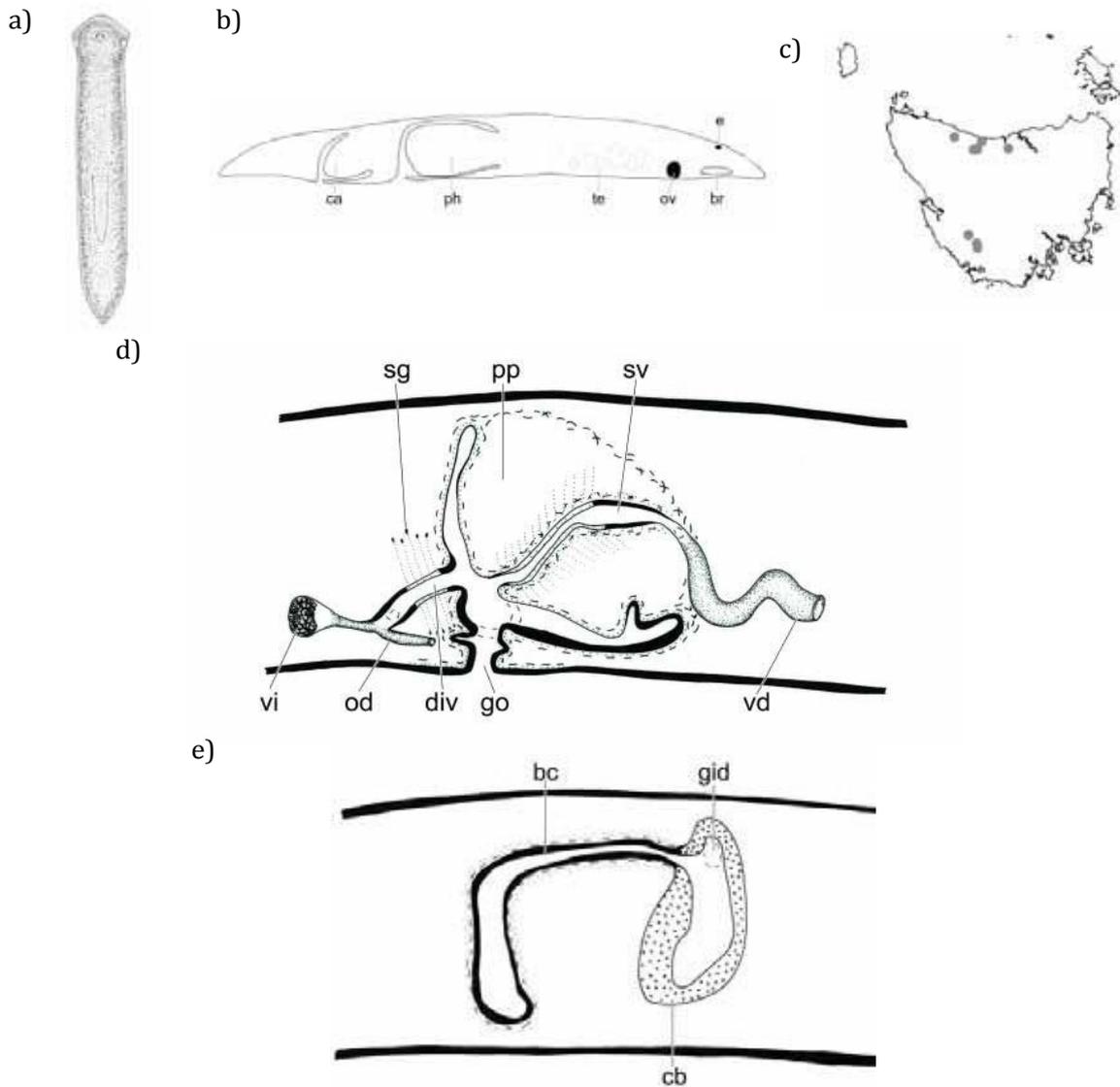
### ***Romankenkius pedderensis* Ball, 1974**

### ***Contribution***

This species was redescribed twice throughout the course of this thesis with some adjustment to Ball's (1974a) original description, supporting Sluys' (1997) earlier amendments (Grant et al. 2006, Appendix 1d).

**Diagnosis**

*Romankenkius pedderensis* can be distinguished from its congeners by its ventrally located testes and by the point of communication of the diverticulum, which sits clearly within the common atrium, not at the base of the bursal canal (the condition found in other *Romankenkius* species) (Ball 1974b, Sluys 1997).



**Figure 1a.33** *Romankenkius pedderensis* a) External features of live specimen; b) sagittal reconstruction of reproductive system; c) distribution of *Romankenkius pedderensis* in Australia; d) sagittal reconstruction of the male copulatory apparatus; e) sagittal reconstruction of the female copulatory apparatus.

### ***Comments***

The long diverticulum receiving the oviducts and reduced seminal vesicle allow us to restrict our generic allocation to the genus *Romankenkius*. The lateral position of the bursa and the presence of a gastro-intestinal duct are both useful diagnostic characters for *R. pedderensis* as the only other species exhibiting these characters is *R. kenki* (Grant et al. 2006, Appendix 1d). While the bursa is lateral in both species, the bursa in *R. kenki* is clearly positioned towards the posterior of the animal, unlike *R. pedderensis* where the bursa sits slightly antero-lateral to the penis bulb (Grant et al. 2006, Appendix 1d)(Figure 1a.33-d,e). Beyond these characters it is the arrangement of the diverticulum that provides the most conclusive diagnostic characters for *R. pedderensis* (Appendix 1d). The diverticulum arises from a point clearly separate from the root of the bursal canal in the common atrium, a detachment not found in any of *R. pedderensis*' congeners (Sluys 1997)(Figure 1a.33-d,e). This feature has been discussed in the literature at some length and, while not included in the original description of the type specimen, is considered to be the most useful diagnostic character for this species (Ball 1974b, Grant et al. 2006, Sluys 1997, Appendix 1d).

### ***Ecology and Distribution***

This species has been collected from the underside of rocks and logs in the off-channel area of cool lakes and streams (Appendix 1d). Prior to the authors newly collected specimens, *R. pedderensis* was known only from Lake Pedder, however it appears that this species is quite widespread throughout Tasmania (Ball 1974b, Grant et al. 2006)(Figure 1a.33-c). *Romankenkius pedderensis* was found in company with *Romankenkius kenki*.

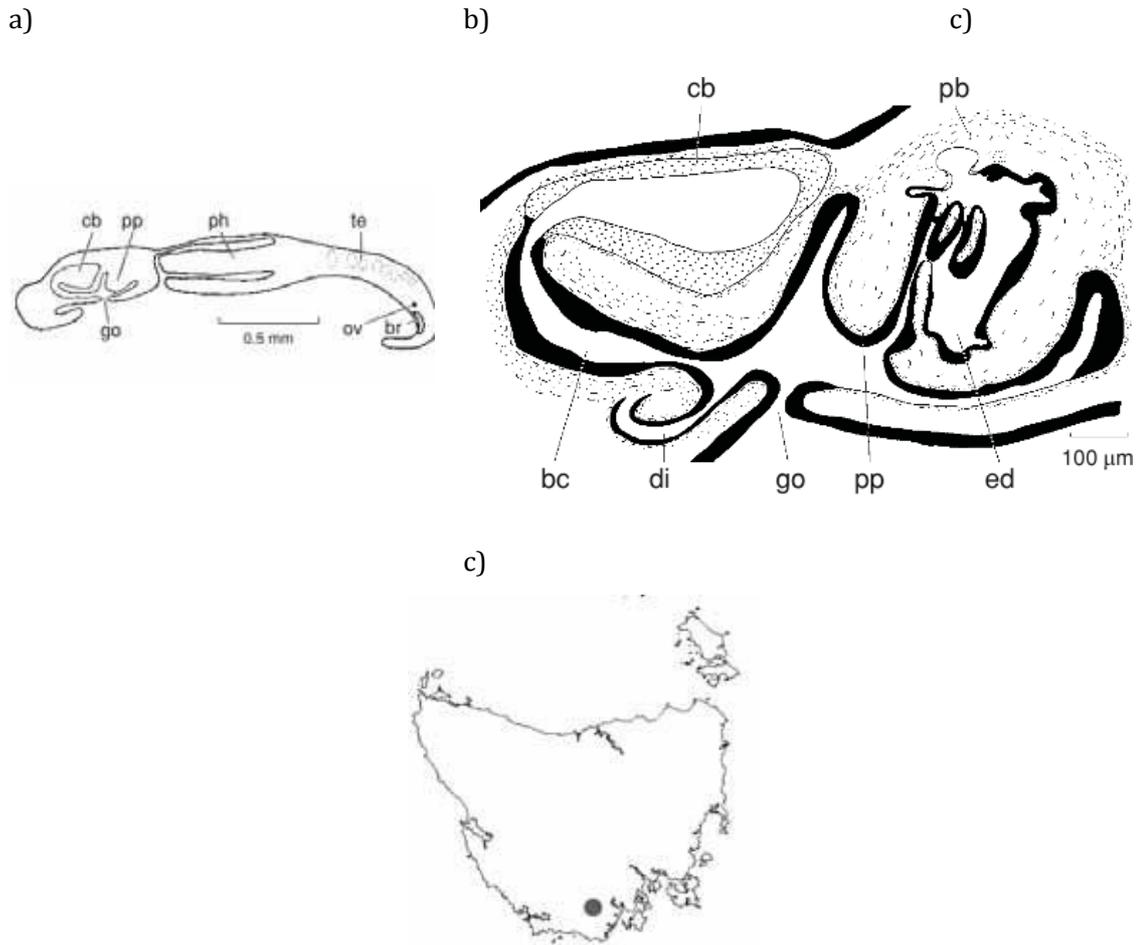
## ***Romankenkius retrobursalis* Sluys and Grant, 2006**

### ***Contribution***

This species was newly described throughout the course of this thesis (Grant et al. 2006).

### ***Diagnosis***

*Romankenkius retrobursalis* can be distinguished from its congeners by its long and laterally displaced penis bulb, housing a large seminal vesicle, and a copulatory bursa situated laterally to the male copulatory apparatus (Grant et al. 2006).



**Figure 1a.34** *Romankenkius retrobursalis* a) Sagittal reconstruction of the reproductive system; b) sagittal reconstruction of copulatory apparatus; c) distribution of *Romankenkius retrobursalis* in Australia.

**Comments**

Although the anatomy of this species is rather different from any other species of *Romankenkius*, it shows some resemblance to *R. sinuosus*. In *R. sinuosus* species the sperm ducts also open into a long, muscular seminal vesicle that eventually communicates with the ejaculatory duct. However, this long and winding seminal vesicle is situated anterior to the penis papilla, in contrast to *R. retrobursalis*, in which the vesicle is displaced laterally to the rest of the male complex (Grant et al. 2006)(Figure 1a.34-b). Both species have a highly irregularly shaped penis papilla. The two species also agree in the thick coat of muscle around the bursal canal, but the course of the canal is quite different in each species (Grant et al. 2006).

With respect to its posteriorly displaced bursa, *R. retrobursalis* resembles *R. kenki*. However, in the latter the posteriorly directed shift is somewhat less pronounced since a fully developed bursa sits laterally to the male complex and not posterior to it, as is the case in *R. retrobursalis* (Grant et al. 2006).

### ***Ecology and Distribution***

Found only in very small streams in the Hartz Mountains. The Hartz Mountains are an area of outstanding natural beauty in the southwest of Tasmania containing regions of wet eucalypt forest, rainforest and alpine heath (Grant et al. 2006)(Figure 1a.34-c).

### ***Romankenkius (western Romankenkius) rutrum* Grant & Sluys, sp. nov.**

### ***Contribution***

This species was newly described throughout the course of this thesis (Appendix 1d).

### ***Diagnosis***

*Romankenkius rutrum* can be distinguished from its congeners by the combination of primarily dorsal testes extending throughout the body, small seminal vesicle, only one type of penial gland, and a short diverticulum entering the bursal canal receiving the openings of shell glands (Appendix 1d).

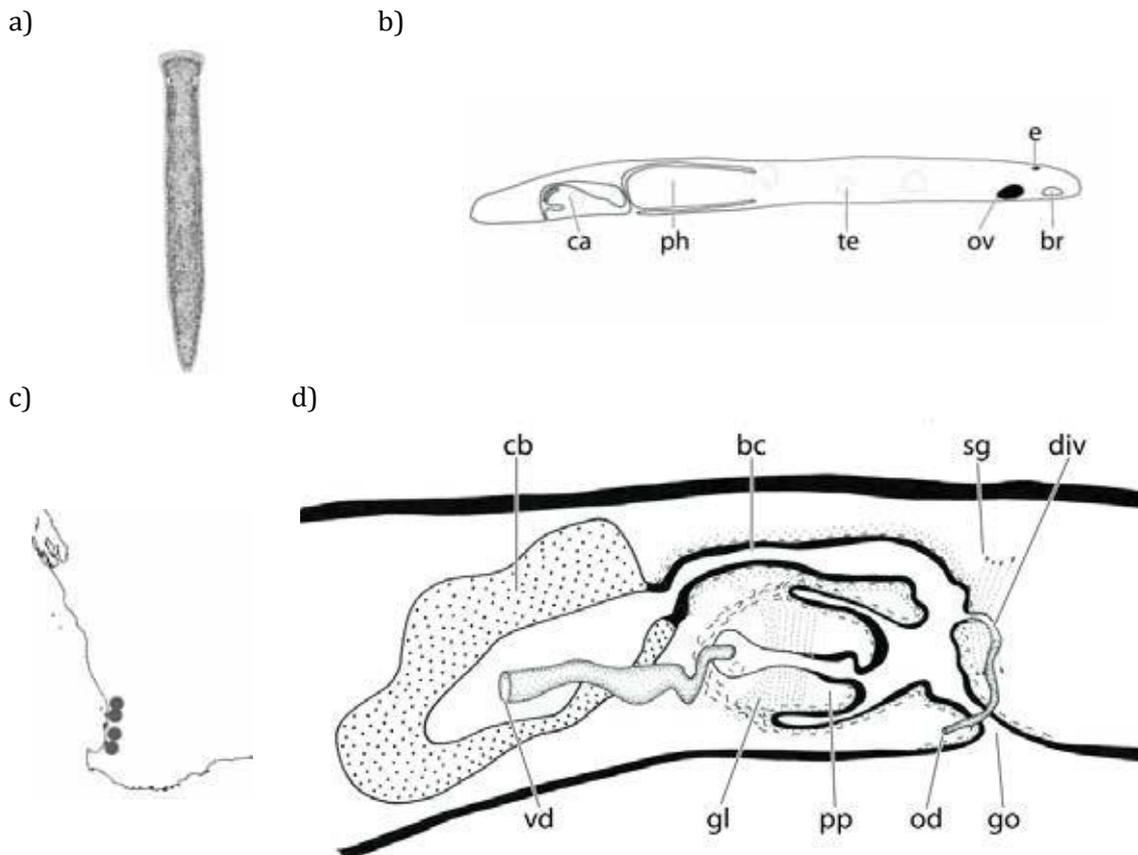
### ***Comments***

The reversed bursal canal musculature, seminal vesicle, diverticulum and lack of a caudal branch of the oviducts eliminate all other genera excepting *Romankenkius* for this species. *Romankenkius* has several species that appear superficially similar to *R. rutrum*, including, *R. glandulosus*, *R. kenki*, and *R. pedderensis*. *Romankenkius rutrum* shares many features with *R. glandulosus* including the head shape, dorsal testes, short diverticulum, small seminal vesicle, and a large copulatory bursa (Figure 1a.35-b,d). However, *Romankenkius rutrum* lacks the distinctive penial glands, conical papilla and shell glands entering the bursal canal characteristic of *R. glandulosus* (Appendix 1d). The polymorphic *R. kenki* also shares some characters with *R. rutrum*, however, the presence of pre-pharyngeal testes, postero-lateral copulatory bursa, and genito-intestinal duct differentiate this species from *R. rutrum*. A similar case applies to *R. pedderensis*, as this species shows ventral testes and presence of genito-intestinal duct (Ball 1974b, Grant et al. 2006, Appendix 1d). The only other possibility worth reviewing is the similarity of *Romankenkius rutrum* to the

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*Romankenkius sp.* described by Grant et al. (2006). The dorsal testes, short diverticulum, simple penial papilla, and large copulatory bursa are all in accord between the two species. However, the lack of diagnostic characters, particularly the glands and the curious pharyngeal arrangement in *Romankenkius sp.* (Grant et al. 2006) suspend any further comparison between the two taxa. By a process of progressive elimination the authors came to the conclusion that their specimen represented a new *Romankenkius* species.

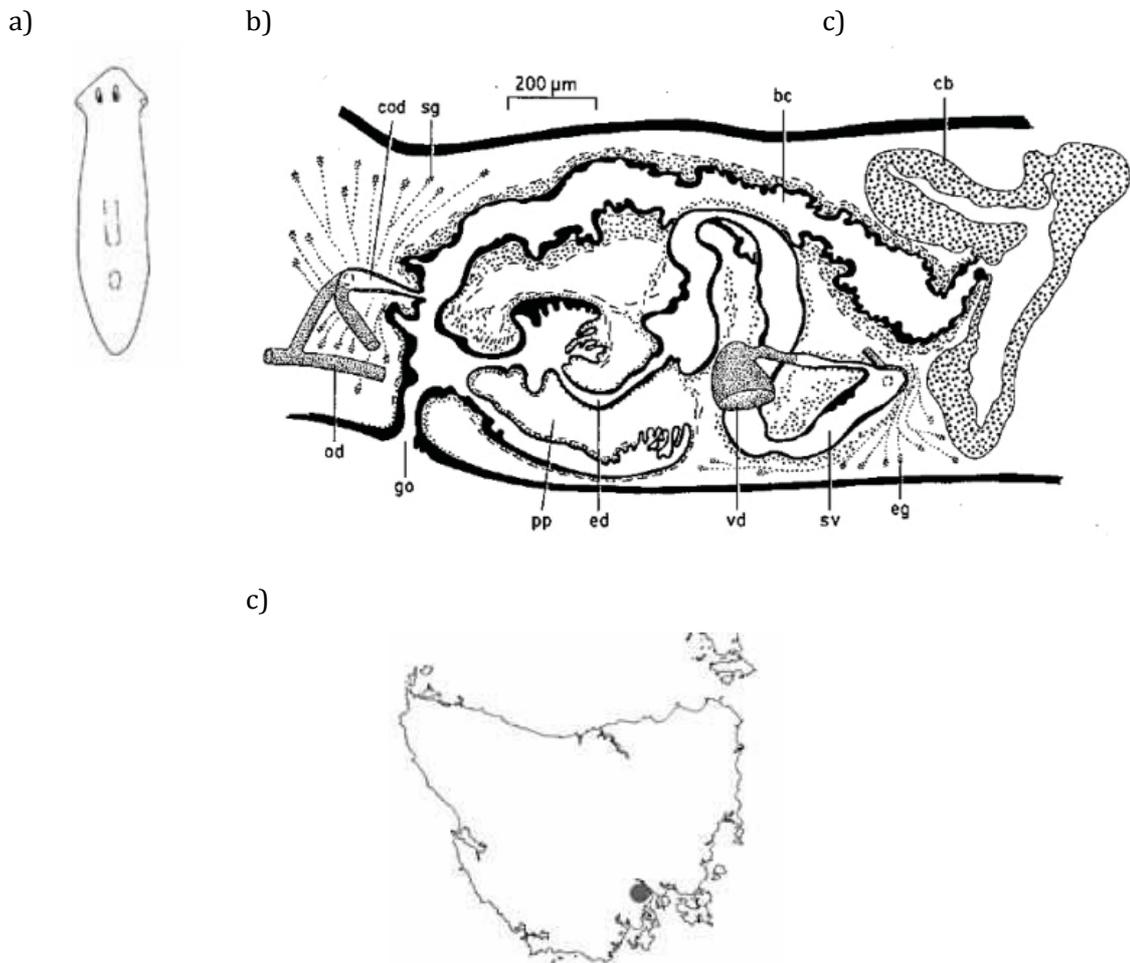


**Figure 1a.35** *Romankenkius rutrum* a) External features of a live specimen; b) sagittal reconstruction of the reproductive system; c) distribution of *Romankenkius rutrum* in Australia; d) sagittal reconstruction of the copulatory apparatus.

### ***Ecology and Distribution***

This specimen was collected from the underside of rocks in the off-channel area of small, sandy bottom creeks. All collecting sites were south of Perth, Australia, within 50 km (Appendix 1d)(Figure 1a.35-c). *Romankenkius rutrum* was discovered in the presence of *Cura pinguis*.

*Romankenkius (Spathula) sinuosus* Sluys and Kawakatsu, 2001



**Figure 1a.36** *Romankenkius sinuosus* a) Free-hand sketch of live specimen (Sluys and Kawakatsu 2001); b) sagittal reconstruction of copulatory apparatus (Sluys and Kawakatsu 2001); c) distribution of *Romankenkius sinuosus* in Australia.

**Contribution**

*Romankenkius sinuosus* has not been addressed during the course of the current research.

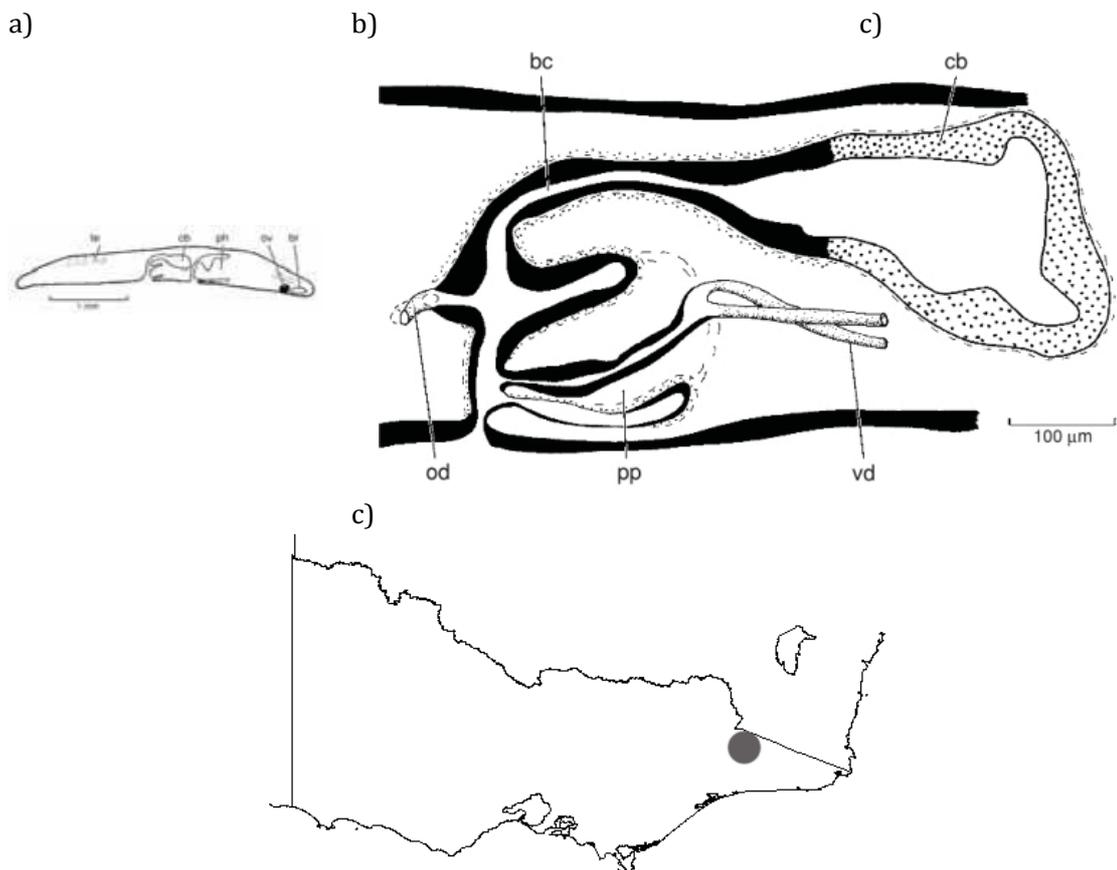
**Diagnosis**

*Romankenkius sinuosus* differs from its congeners by having an asymmetrical penial papilla, an elongated and winding seminal vesicle, a sinuous bursal canal, and a mouth opening located at a more anterior position than usual (Sluys and Kawakatsu 2001)(Figure 1a.36-b).

### ***Ecology and Distribution***

This species is known only from the type locality, the foot of Mt. Wellington (Grant et al. 2006)(Figure 1a.36-c). Mount Wellington is a mountain on whose foothills is built much of the city of Hobart, Tasmania, Australia.

### ***Romankenkius sp. - Sluys and Grant 2006***



**Figure 1a.37** *Romankenkius sp.* a) Sagittal reconstruction of the reproductive system; b) sagittal reconstruction of copulatory apparatus; c) distribution of *Romankenkius sp.* in Australia.

### ***Contribution***

This species was tentatively described as part of this thesis (Grant et al. 2006).

### ***Diagnosis***

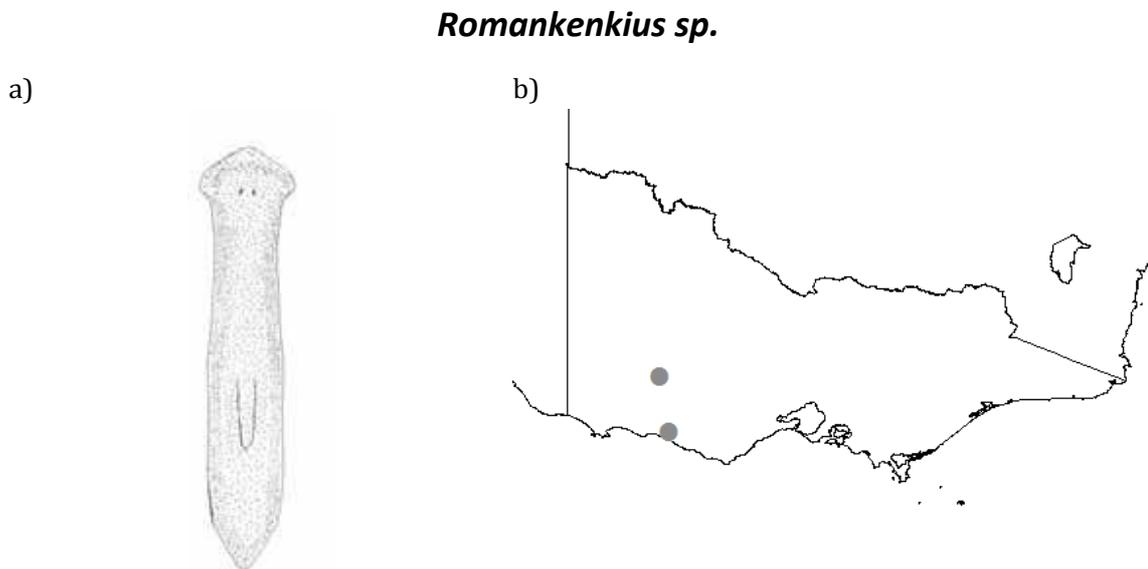
Characteristic for *Romankenkius sp.* is the short pharynx situated in an unusual anterior position. In view of the fact that not all anatomical details could be adequately observed in this specimen, Grant et al. (2006) refrain from describing a new species on the basis of their material.

### **Comments**

By default and successive elimination this animal has been assigned to the genus *Romankenkius*. In some respects, the animal resembles *Spathula truculenta*, albeit that in this species the pharynx is located in the middle of the body (Figure 1a.37-a). However, *S. truculenta* also exhibits a short common oviduct and an asymmetrical penis papilla with a larger dorsal lip (Grant et al. 2006). Unfortunately, it is not known whether the oviducts branch in *Romankenkius sp.*, as is generally the case in species of *Spathula*. A common oviducal section is a rare feature among species of *Spathula* but is characteristic for the genus *Romankenkius*, in which the oviducts may either branch or not (Grant et al. 2006)(Figure 1a.37-b).

### **Ecology and Distribution**

*Romankenkius sp.* was collected from Boundary Creek, near the township of Wulgulmerang (Grant et al. 2006)(Figure 1a.37-c). Wulgulmerang is located on the outskirts of the Snowy River National Park, a mountainous alpine region in the northeast of Victoria.



**Figure 1a.38** *Romankenkius sp.* a) External features of live specimen; b) distribution of *Romankenkius sp.* in Australia.

### **Contribution**

This species was tentatively described as part of this thesis. All specimens were lacking reproductive apparatus and therefore a complete description cannot be provided (Appendix 1d).

### ***Diagnosis***

These individuals have been identified as belonging to a separate *Romankenkius* (eastern) species via the molecular data (Appendix 2c and Chapter 2).

### ***Comments***

The location data does suggest that this species is quite possibly *Romankenkius conspectus* (Grant et al. 2006)(Figure 1a.38-b). The presence of large sensory pits is consistent with the original description of *R. conspectus*. Unfortunately the external morphology of *R. conspectus* is unknown, making a complete comparison impossible (Appendix 1d)(Figure 1a.38-a).

### ***Ecology and Distribution***

*Romankenkius sp.* was initially identified from the Grampians, Victoria and all three specimens tentatively assigned to this species were found in the same river system (Appendix 1d)(Figure 1a.38-b). The Grampians are a series of five spectacular sandstone ridges running north to south with steep and craggy slopes on the eastern side and gentler slopes to the west. *Romankenkius sp.* was discovered in company with *Cura pinguis*.

## **Family Spathulidae nov. fam.**

### **Spathula Nurse, 1950**

It is proposed that three existing species be transferred to this genus: *Romankenkius libidinosus*, *Romankenkius sinuousus* and *Reynoldsonia renoldsoni*.

Head rounded or spathulate, or of the low triangular form. Seminal vesicle a single cavity. Diaphragm absent. Bursal canal musculature of inner longitudinal muscles surrounded by circular fibres. Testes numerous, dorsal or ventral and extending throughout the body length. Oviducts branched caudally (Nurse 1950). The genus *Spathula* is distributed as shown in Figure 1a.39.

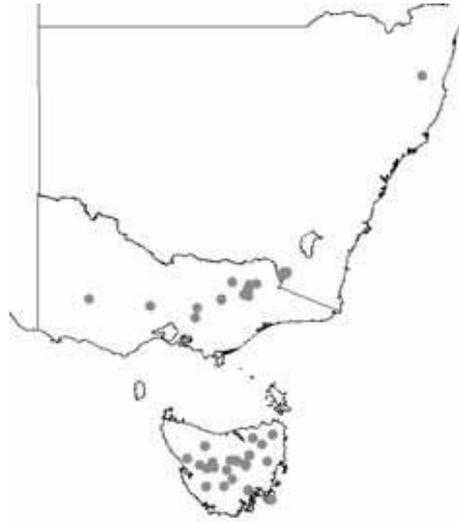


Figure 1a.39 Distribution of the Genus *Spathula* in Australia.

### ***Spathula agelaea* Hay and Ball, 1979**

#### ***Contribution***

New specimens of *Spathula agelaea* have been collected.

#### ***Diagnosis***

*Spathula agelaea* can be identified by its light colour, rounded head, without eyes and one pair of deep ciliated pits in the frontal-lateral margin. The copulatory apparatus is currently unknown, however, discrete ventral testicular follicles have often been observed (Hay and Ball 1979).

a)



b)

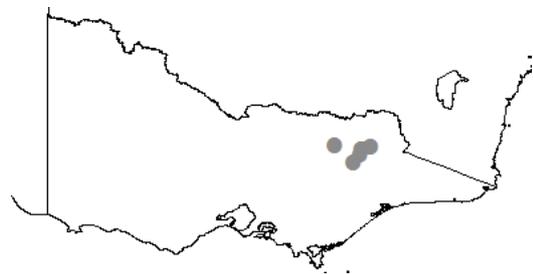


Figure 1a.40 *Spathula agelaea* a) external features of a live specimen (Hay and Ball 1979);  
b) distribution of *Spathula agelaea* in Australia.

### **Comments**

An individual collected from the type locality of *Sp. agelaea*, matching perfectly the description of external morphology for this species was collected as part of the recent work presented in Appendix 1d (Figure 1a.40-a,b). These undeniable coincidences have prompted the assignment of the individual collected to *Spathula agelaea*.

It is possible that this species reproduces purely via fission as many hundreds of specimens have been examined, none displaying any traces of a copulatory apparatus (Hay and Ball 1979, St. Clair et al. 1999). However, Hay and Ball (1979) did mention the presence of ventral testes in some specimens, suggesting that *Spathula agelaea* is capable of sexual reproduction. It may be that the harsh environments this species inhabits make resources scarce, forcing the reabsorption of the copulatory apparatus in most individuals (Baird et al. 2005, Bowen et al. 1976, Connella and Stern 1969, Romero and Bagnuà 1991). Additional to the question of the internal morphology of this species, the author has been unable to confirm its discovery during the course of her research. However, discussion regarding a potential description can be found in the musings surrounding *Spathula dupladiaphragma* (see below).

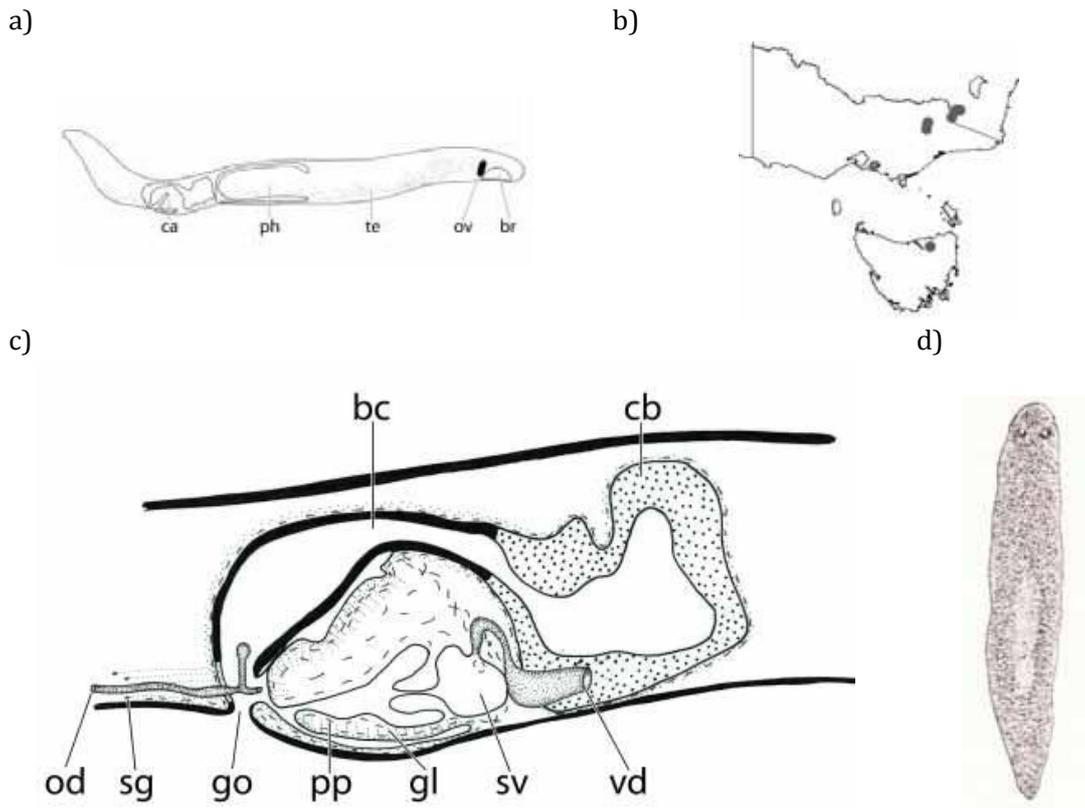
### **Ecology and Distribution**

*Spathula agelaea* is the dominant species on Mt. Buffalo, although it is also abundant in the Falls Creek - Mt. Hotham area (Hay and Ball 1979, Appendix 1d)(Figure 1a.40-b). The creeks in which *Sp. agelaea* are commonly found are frequently covered by several feet of snow. Whilst, *Sp. agelaea* inhabits the same mountains as *Reynoldsonia reynoldsoni*, research suggests that the two species never occur together (Hay and Ball 1979). *Sp. agelaea* shows two very distinctive behaviours; the first being that they are most commonly found in large clumps of individuals, the second is the unusually high frequency of cannibalism found in this species (Hay and Ball 1979). Hay and Ball (1979) ponder the possibility that the two behaviours are linked, suggesting that the clumping behaviour may facilitate cannibalism during times when resources are limited.

## ***Spathula camara* Ball, 1977**

### **Contribution**

*Spathula camara* has been redescribed twice throughout the course of this research (Grant et al. 2006, Appendix 1d). These descriptions add several new details to Ball's (1977a) original description.



**Figure 1a.41** *Spathula camara* a) Sagittal reconstruction of the reproductive system; b) distribution of *Spathula camara* in Australia; c) sagittal reconstruction of the copulatory apparatus; d) external morphology of *Spathula camara* (Hay and Ball 1979).

**Diagnosis**

*Spathula camara* is the only pigmented freshwater triclad with two pairs of ciliated pits. The huge folded copulatory bursa, and the vaulted seminal vesicle are other unusual features of this species (Ball 1977a).

**Comments**

The separate entries of the oviducts into the bursal canal and the caudal branch eliminate the possibility of *Sp. camara* being assigned to any other genus excepting *Spathula* (Figure 1a.41-c). The most useful characters to use for the differentiation of this species are the absence of true eyes, the pleated seminal vesicle, the musculature extending over the copulatory bursa, and the glands exiting through the epithelium of the penial papilla. Whilst Ball (1977a) did not describe eyes in his original description, Hay and Ball (1979) later describe the presence of eyes of ‘unusual appearance and structure’. Grant et al. (2006) also detected an unusual structure located directly posterior to the ciliated pits,

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### SYSTEMATIC REVIEW AND REVISION OF DUGESIIDAE S.L. TAXONOMY

however, do not believe that these structure constitute eyes, a conclusion reinforced in by the work done in Appendix 1d (Figure 1a.41-d).

Other *Spathula*'s in which the eyes are more or less absent are: *Sp. agelaea*, *Sp. camara*, *Sp. miserabile*, *Sp. goubaultae*, and *Sp. tryssa*. Within this group *Sp. goubaultae* can be easily differentiated from *Sp. camara* as it has large dorsal testes and lacks a seminal vesicle (Ball 1977a). It is difficult to assign any species to *Sp. agelaea* as very little is known of the reproductive system beyond ventral testes (Hay and Ball 1979). The presence of a small vesicle, somewhat similar to the statocyst of lower Turbellarians, above the brain is the most effective diagnostic character for *Sp. agelaea*, a feature not found in *Sp. camara* (Hay and Ball 1979). Of the three remaining *Spathula* species all have a seminal vesicle. However, the vesicle in *Sp. miserabile* is singular and its bursa is lacking any surrounding musculature (Grant et al. 2006). Although *Sp. tryssa* does have a double seminal vesicle, the musculature surrounding the bursa is also lacking in this species (Ball 1977a). This leaves *Sp. camara* as the only *Spathula* with no eyes, often lacking pigmentation, with a huge folded copulatory bursa, and vaulted seminal vesicle.

#### ***Ecology and Distribution***

*Spathula camara* is the most widespread species of the genus, having been collected from the underside of rocks in cool creeks at high altitudes in southern New South Wales, Victoria and now also Tasmania (Ball 1977a, Grant et al. 2006, Hay and Ball 1979, Appendix 1d)(Figure 1a.41-c).

### ***Spathula caeca* Grant & Sluys, sp. nov.**

#### ***Contribution***

*Spathula caeca* was described throughout the course of this research (Appendix 1d).

#### ***Diagnosis***

*Spathula caeca* can be distinguished from its congeners by its lack of eyes and pigment, lack of penial glands entering the ejaculatory duct, and presence of a double seminal vesicle (Appendix 1d).

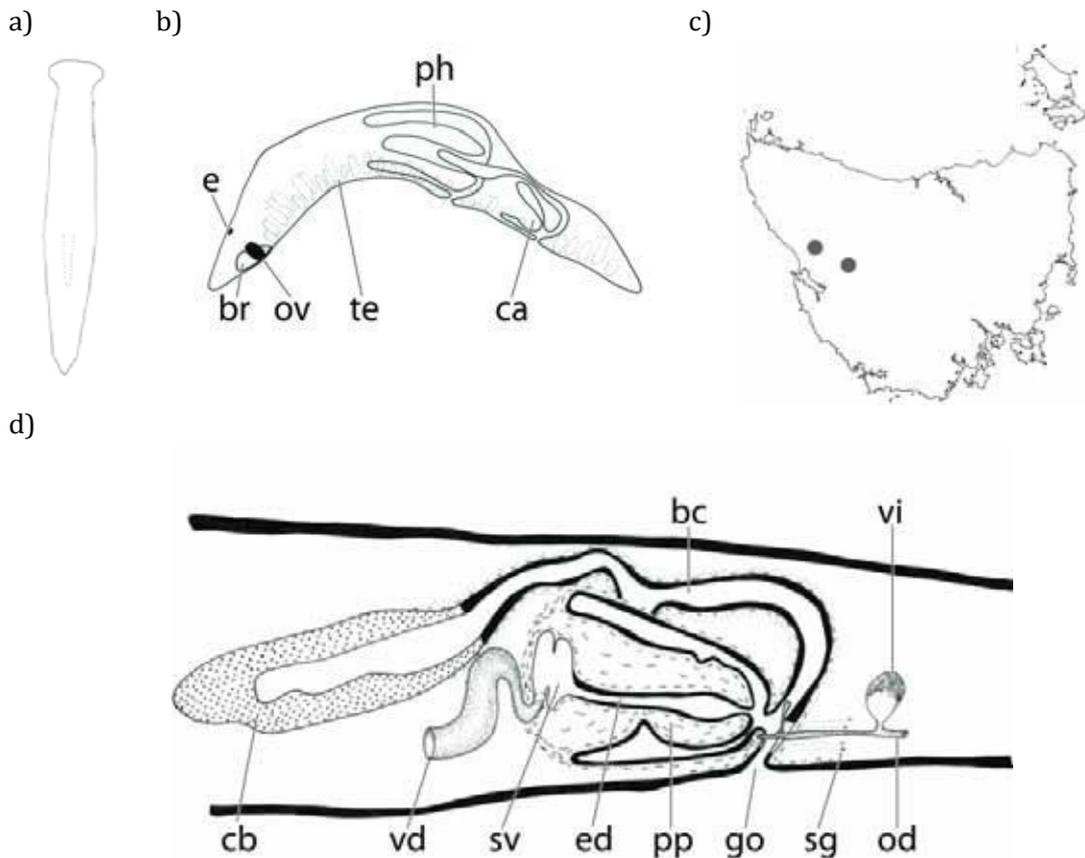
#### ***Comments***

The species examined as part of Appendix 1d is clearly a *Spathula* and has many characters in common with *Spathula ditta*, albeit there are a few important differences that exclude it from this assignment. The external morphology, lacking pigment and eyes,

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cannot be ignored (Figure 1a.42-a). Although *Sp. dittae* displays considerable variability in external morphology, to suggest that some populations may lack eyes and pigment is difficult to justify. The penial papilla of the new material is a completely different shape to that found commonly in *Sp. dittae* and displays none of the characteristic irregularity or folding found in the latter (Figure 1a.42-d). The ejaculatory duct is lacking penial glands altogether and the copulatory bursa is not voluminous, both of which are features consistently described for *Sp. dittae* (Appendix 1d).



**Figure 1a.42** *Spathula caeca* a) External characters of a live specimen; b) sagittal reconstruction of the reproductive system; c) distribution of *Spathula caeca* in Australia; d) sagittal reconstruction of the copulatory apparatus.

Another potential species assignment for the material is the blind *Sp. tryssa*, which has a similar external morphology to the specimens examined as part of Appendix 1d. However, *Sp. tryssa* can be discounted due to the lack of a seminal vesicle, the presence of penial glands and two distinct pits on the frontal margin. Furthermore, the head shape of *Sp. tryssa* is rounded and not shovel-shaped (cf. Appendix 1d). *Sp. miserabile* is the only possible species assignment remaining for these specimens. The virtual absence of eyes described for *Sp. miserabile* could be interpreted as consistent with the state found in

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these specimens. However, the presumed absence of ciliated pits in *Sp. miserabile* is in direct contradiction to the situation described in Appendix 1d. The double seminal vesicle and narrow, muscularised ejaculatory duct support the notion that these specimens cannot be assigned to *Sp. miserabile* (Figure 1a.42-d). The only remaining conclusion is that these specimens represent a new *Spathula* species (Appendix 1d).

### ***Ecology and Distribution***

Both populations of *Spathula caeca* were collected from the underside of rocks in the off-channel area of cool creeks in central western Tasmania (Appendix 1d) (Figure 1a.42-c).

## ***Spathula dittae* Ball and Tran, 1979**

*Spathula ochyra* Ball and Tran, 1979

### ***Contribution***

*Spathula dittae* has been redescribed on several occasions throughout the course of this research. Twice as *Sp. dittae* and once as *Sp. ochyra* (Grant et al. 2006, Appendix 1d). These two species were collapsed into one in Appendix 1d, when the taxonomic evidence became too compelling to ignore.

### ***Diagnosis***

*Spathula dittae* can be differentiated from other *Spathula* by the ventral testes, plug like penial papilla and irregular seminal vesicle (Ball and Tran 1979).

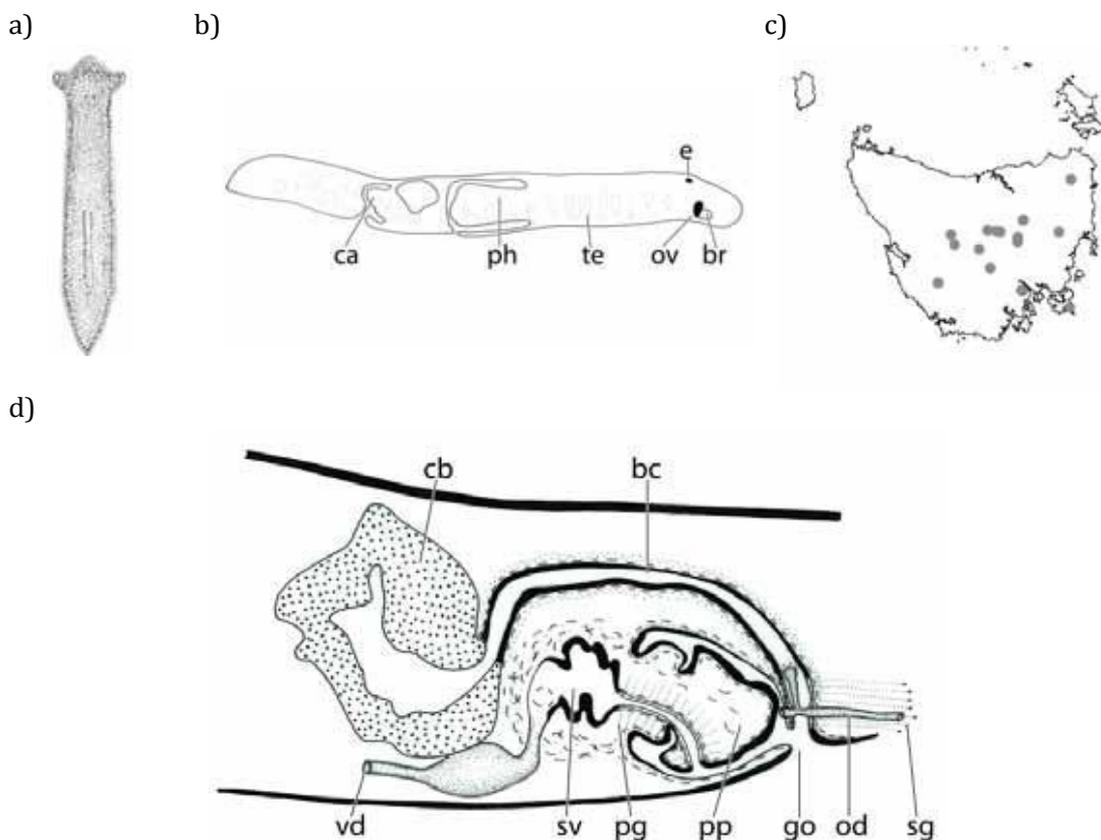
### ***Comments***

The specimens examined as part of Appendix 1d align comfortably with the descriptions of both *Sp. ochyra* and *Sp. dittae* (Ball and Tran 1979, Grant et al. 2006) (Figure 1a.43-a,b,d). That these two nominal species may be one and the same has been suggested previously (Grant et al. 2006). It is unfortunate that there are no new *Spathula* specimens from the type locality of *Sp. dittae*, Lake St. Clair, as this would enable a definitive answer to this question. Despite this, the author believes there is a strong case to be made for amalgamation of *Sp. dittae* and *Sp. ochyra*. The type material of *Sp. dittae* is very poor and *Sp. ochyra* was described from just one specimen (Ball and Tran 1979). These initial restrictions have meant that as more material has become available, the morphological differences have been systematically discounted. So we are left with a situation where the

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only dividing character between the two species is the presence or absence of folds in the papilla (i.e. those with folds are *Sp. ochyra*) (Grant et al. 2006). This is not a very strong diagnostic character and would not be considered an acceptable differentiation for any new species. The use of this feature seems even more tenuous when the variability that *Sp. ochyra* displays throughout a range of other characters is considered. The examination conducted by the author has the added advantage of molecular data, which does not discriminate between those individuals with a folded papilla and those without (see Chapter 3). This chain of reasoning led the author to amalgamate the two species, adding that any specimens displaying these characters should be considered as *Sp. dittae*, in conformity with the previous suggestion by Grant et al. (2006).



**Figure 1a.43** *Spathula dittae* a) External features of live specimen b) sagittal reconstruction of the reproductive system; c) distribution of *Spathula dittae* in Australia; d) sagittal reconstruction of the copulatory apparatus.

### ***Ecology and Distribution***

*Sp. dittae* has been collected from the underside of rocks in the off channel area of creeks and rivers and on the shores of lakes. This species is widespread throughout Tasmania and is found in a range of environments from pristine to highly disturbed areas, with water temperatures ranging from 9°C - 22°C (Ball and Tran 1979, Grant et al. 2006, Sluys and Kawakatsu 2001, Appendix 1d)(Figure 1a.43-c). *Spathula dittae* was found commonly in the presence of *Cura pinguis* and on one occasion *Masaharus extentus*.

### ***Spathula dupladiaphragma Grant & Sluys, sp. nov.***

#### ***Contribution***

*Spathula dupladiaphragma* was described throughout the course of this research (Appendix 1d).

#### ***Diagnosis***

The presence of the secondary chamber within the penial papilla eliminates all known *Spathula*'s for which the copulatory apparatus has been described (Appendix 1d).

#### ***Comments***

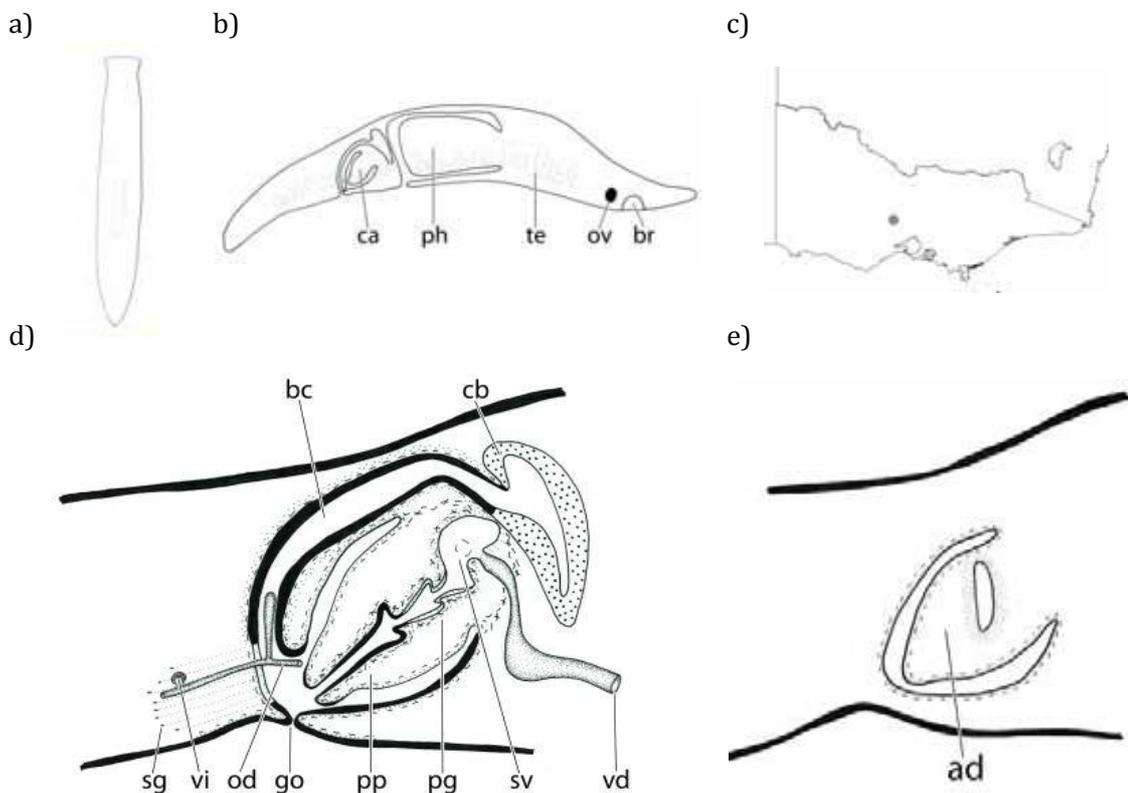
The “folds or diaphragms” in the ejaculatory duct could indicate that the new Victorian material is a possible *Dugesia*, however, there are currently no reported cases of a *Dugesia* with a caudal branch of the oviducts (Appendix 1d). The seminal vesicle and muscularised cavity are reminiscent of *Romankenkius*. *Romankenkius libidinosus* and *R. sinuosus* are the only Australian *Romankenkius*' species with a substantial caudal branch of the oviducts, yet the newly collected material is neither of these (Appendix 1d). The caudal branch of the oviducts, ciliated pits and ventral testes extending throughout the body length suggest that this specimen belongs to *Spathula* (Sluys 2001; Sluys and Rohde 1991)(Figure 1a.44-d).

The presence of the secondary chamber within the ejaculatory duct eliminates all known *Spathula*'s for which the copulatory apparatus has been described (Figure 1a.44-d,e). *Sp. agelaea* has been described from Victoria, without eyes, with ventral testes throughout the body length and with light brown or dirty white with light sepia external pigmentation. All of these features are consistent with the description presented in Appendix 1d. Unfortunately, the internal morphology has never been fully described (Appendix 1d), making a definitive identification difficult. While it would appear possible that Grant et al.'s (Appendix 1d) specimen is *Sp. agelaea*, the situation is complicated by

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the fact that the external features are not rare in this part of Australia. Victoria is home to many other species with very little pigment and small to no eyes (i.e. *Sp. foeni*, *Sp. tryssa*, *Sp. goubaultae*, *Reynoldsonia reynoldsoni*) (Appendix 1d). So it is not unreasonable to suggest that this species is another example of this morphological type. Also, the body shape of the specimen is somewhat different from that reported for *Sp. agelaea* (Appendix 1d). Hay and Ball (1979) describe live specimens of *Sp. agelaea* with a distinct, bluntly pointed anterior end. Further, we have access to an unpublished drawing of a live specimen of presumed *Sp. agelaea* collected from Mt. Buffalo National Park (just above Tekal at 1554m). The body shape and other external features of this animal fully agree with the account of Hay and Ball (1979). In contrast, the anterior end of Grant et al.'s (Appendix 1d) material is truncated and not bluntly pointed.



**Figure 1a.44** *Spathula dupladiaphragma* a) External features of live specimen; b) sagittal reconstruction of the reproductive system; c) distribution of *Spathula dupladiaphragma* in Australia; d) sagittal reconstruction of the copulatory apparatus; e) sagittal reconstruction of adenodactyl.

There are two other diagnostic features known for *Sp. agelaea*. The first concerns sensory structures, which are described similarly to the description presented in Appendix 1d. The second character, which has not been discovered in the specimen described in Appendix 1d, is the presence of a small vesicle, somewhat similar to the statocyst of lower Turbellarians, above the brain.

In view of the fact that (1) several of the known features of *Sp. agelaea* do not fully agree with those of the Appendix 1d specimen and (2) the fact that the specimen was not collected from the precise type locality (Mt. Buffalo National Park), the author has refrained from assigning this specimen to the former and instead has chosen to erect a new species.

### ***Ecology and Distribution***

*Spathula dupladiaphragma* was collected from the base of Trentham Falls on the Upper Coliban River, near Trentham, Victoria (Appendix 1d) (Figure 1a.44-c). Trentham Falls is the longest single drop waterfall in Victoria, plunging some 32 meters over basalt columns. This species was discovered in company with *Cura pinguis*.

## ***Spathula foeni* Ball, 1977**

### ***Contribution***

*Spathula foeni* was redescribed throughout the course of this thesis research (Appendix 1d).

### ***Diagnosis***

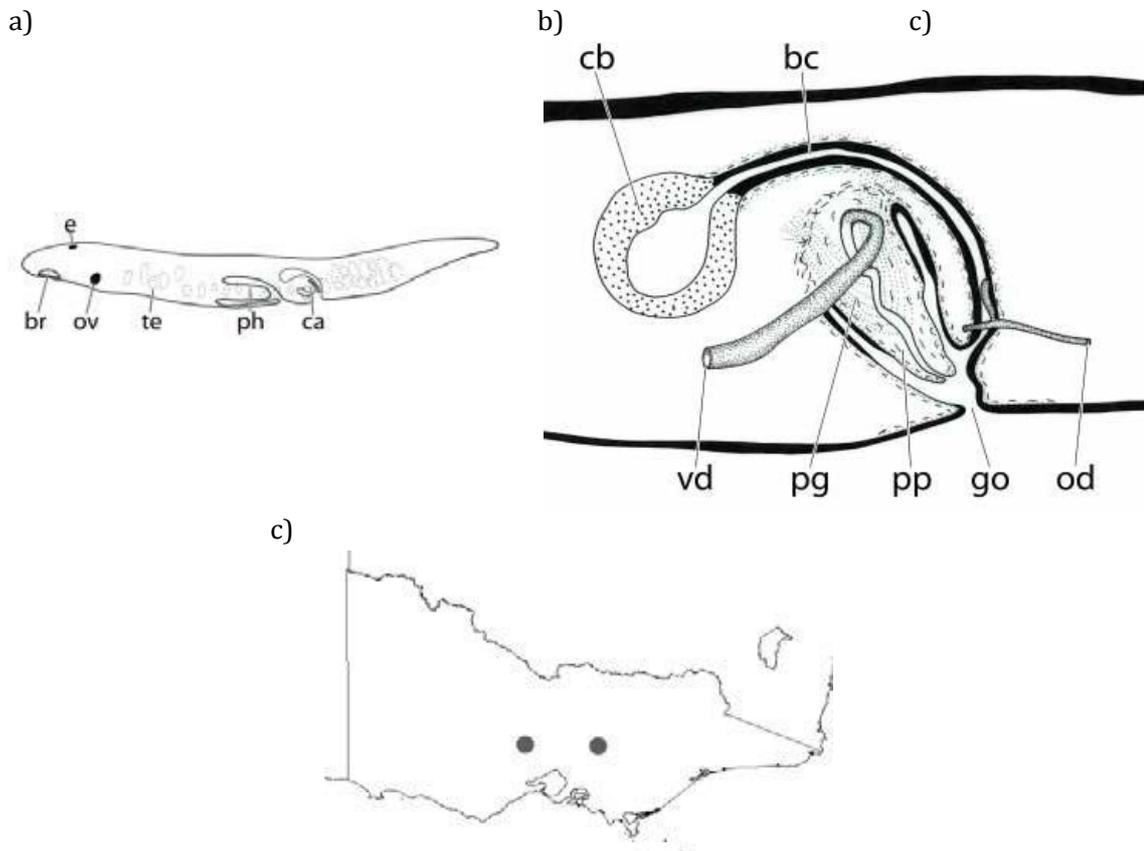
*Spathula foeni* can be distinguished from its congeners, not by a single apomorphy, but by the combination of ventral testes, conical penial papilla, lack of a seminal vesicle and presence of eyes (Ball 1977a).

### ***Comments***

Undoubtedly a *Spathula*, these animals have a conical penial papilla, which is the most useful character in the species assignment (Figure 1a.45-b). When the author encountered a species resembling *Sp. foeni*, it was necessary to eliminate several other similar species before a final decision could be made. Australian *Spathula* species with comparable, in regards to shape or orientation, penial papillae are *Sp. simplex*, *Sp. truculenta*, *Sp. tryssa*, *Sp. gourgaultae* and *Sp. foeni*. *Sp. truculenta* has dorsal testes, which instantly eliminated it as a potential assignment. *Sp. simplex* has an oviducal loop and heavy external pigmentation,

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diagnostic characters that have not been detected in these specimens. In *Sp. tryssa* and *Sp. gorbaultae*, penial glands are present in the ejaculatory duct. However, both of these species are discounted due the absence of eyes.



**Figure 1a.45** *Spathula foeni* a) Sagittal reconstruction of the reproductive system; b) sagittal reconstruction of the copulatory apparatus; c) distribution of *Spathula foeni* in Australia.

The specimen examined in Appendix 1d was most suitably assigned to *Sp. foeni*. The only discrepancy between their specimen and the original description was the absence of penial glands in Ball's (1977a) description, which is most likely due to the poor staining of the preparations described by Ball (1977a). Additionally, the large bursa and the tendency of the testes to fuse in *Sp. foeni* are likely to vary depending upon maturity of the animals. Despite this, these characters are not profound enough to warrant the description of a new species. Grant et al.'s (2006) specimen shares the majority of its characters with *Spathula foeni* and was therefore tentatively assigned to this species.

### ***Ecology and Distribution***

This species has been collected from the underside of cobble in the off-channel area of rivers in the relatively untouched central Victorian wilderness (Ball 1977a, Appendix 1d)(Figure 1a.45-c). Grant et al. (2006) also reported a *Sp. cf. foeni* from northeastern Tasmania (see below).

### ***Spathula cf. foeni* Ball, 1977**

#### ***Contribution***

This individual, that is very similar to *Spathula foeni*, was described during the course of this thesis (Grant et al. 2006).

#### ***Diagnosis***

The specimen, described by Grant et al. (2006), is only tentatively assigned to *Sp. foeni*, due to the poorly developed state of the testes and some anatomical differences between the animal examined and the original species description.

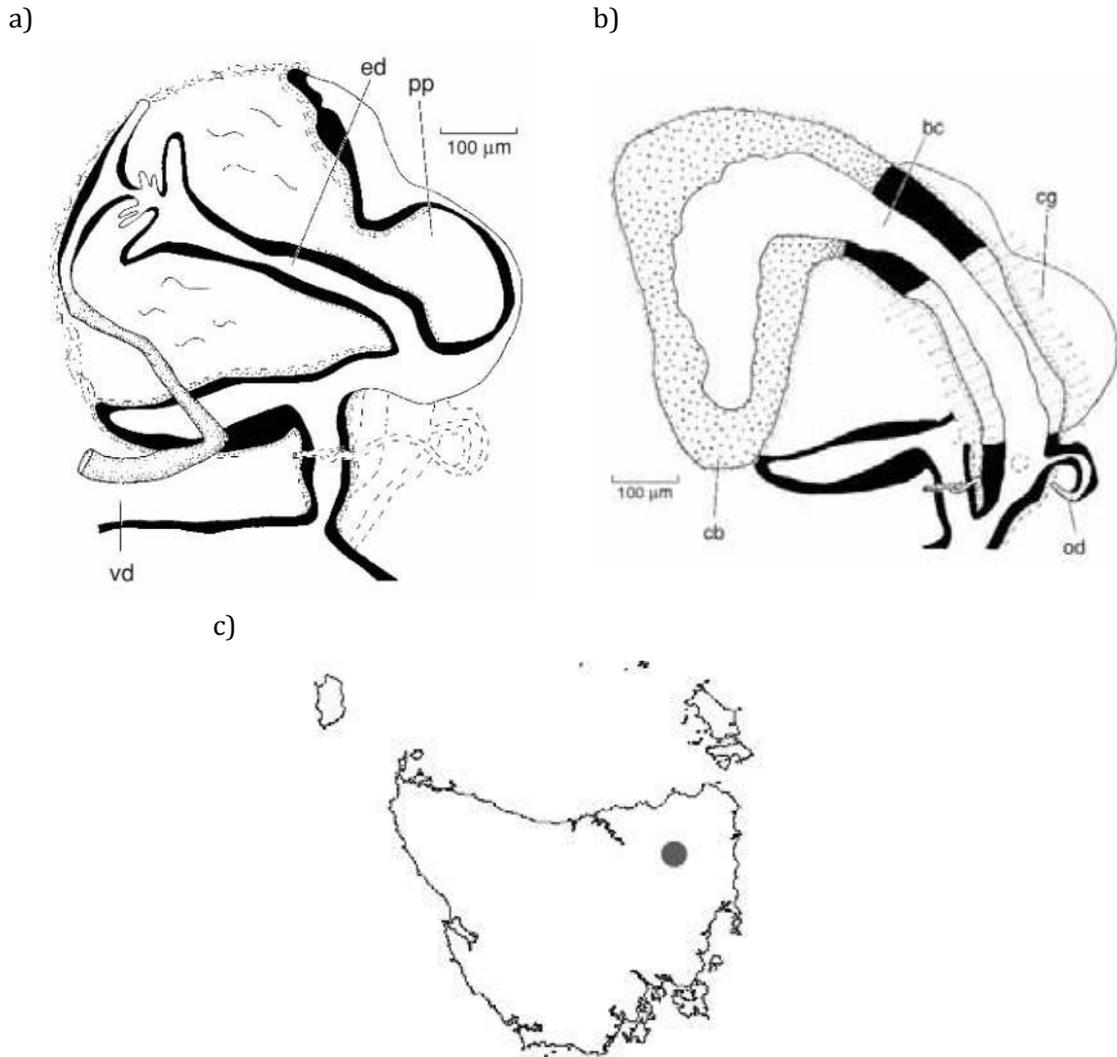
#### ***Comments***

The issues encountered in assigning Grant et al.'s (2006) specimen were primarily due to the presence of a pleated ejaculatory duct (Figure 1a.46-a). Pleated sections of the ejaculatory duct and/or seminal vesicle have been described also for *Sp. camara* and *Sp. fontinalis*. However, Grant et al.'s (2006) specimen cannot be assigned to either one of these two species; the first-mentioned being a blind species, while *Sp. fontinalis*, from New Zealand, is characterized by the presence of a highly developed muscular sphincter on its bursal canal (cf. Sluys and Kawakatsu 2001), neither character being present in Grant et al.'s (2006) specimen (Figure 1a.46-a).

A pleated seminal vesicle or ejaculatory duct was originally not reported for *S. foeni*, the vasa deferentia opening into the wide proximal section of the ejaculatory duct. Grant et al.'s (2006) specimen differs also from the original account due to the presence of cyanophilic glands opening into the bursal canal.

### ***Ecology and Distribution***

*Spathula cf. foeni* was collected from Ben Lomond National Park, an alpine plateau over 1500 meters high in northeastern Tasmania (Grant et al. 2006)(Figure 1a.46-c). This species was found in the presence of *Cura pinguis*.



**Figure 1a.46** *Spathula cf. foeni* a) Sagittal reconstruction of the male copulatory apparatus; b) sagittal reconstruction of the female copulatory apparatus; c) distribution of *Spathula cf. foeni* in Australia.

### ***Spathula gorbaultae* Ball, 1977**

#### ***Contribution***

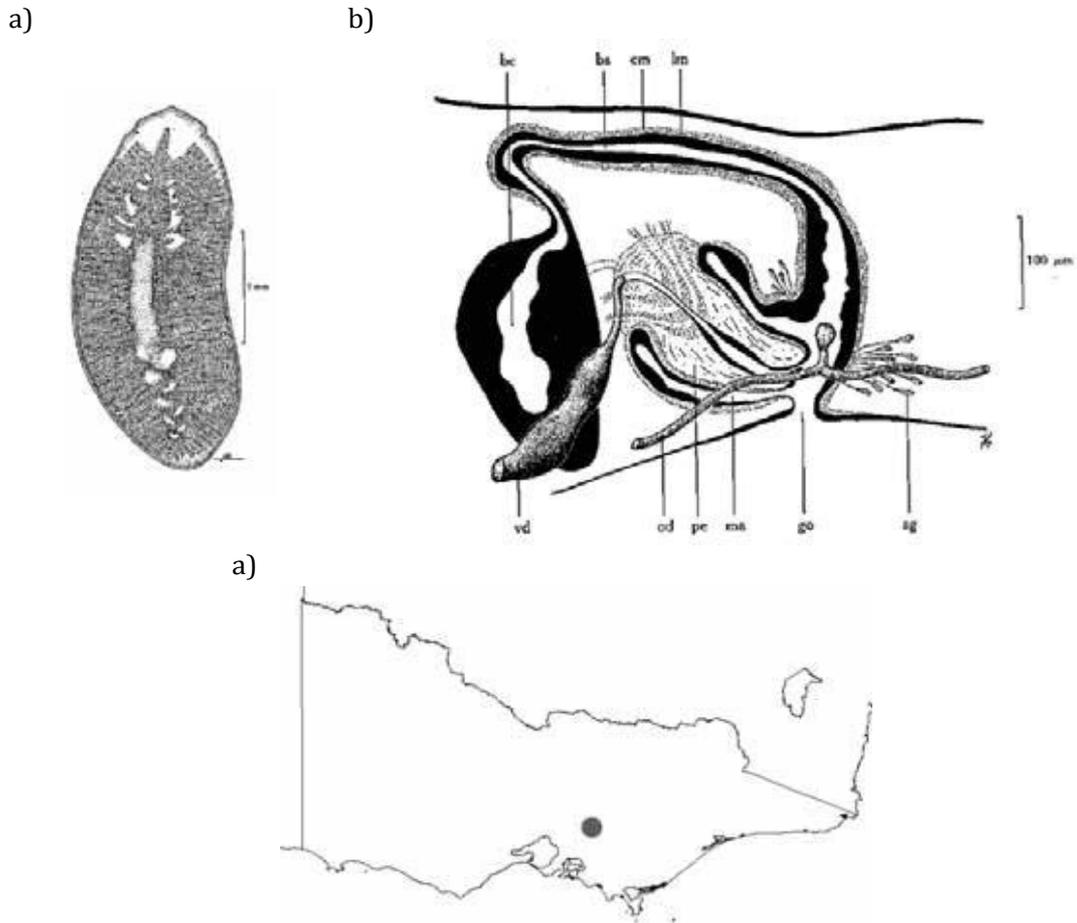
This species was not collected during the course of this research and therefore I have no advancement on the original description to offer.

#### ***Diagnosis***

*Spathula gorbaultae* can be distinguished from other *Spathula*'s as it is unpigmented, blind, has dorsal testes, has only one pair of sensory pits and a short caudal branch of the oviducts (Ball 1977a)(Figure 1a.47-b).

***Ecology and Distribution***

This species is known only from the type locality at the summit of Mt Donna Buang, a cool, well oxygenated, montane water (Ball 1977a)(Figure 1a.47-c). Mt. Donna Buang is part of the Yarra Ranges and receives some snowfall in winter. It lies approximately 80km northeast of Melbourne.



**Figure 1a.47** *Spathula gorbaultae* a) External features of living specimen (Ball 1977a); b) sagittal reconstruction of the copulatory apparatus (Ball 1977a); c) distribution of *Spathula gorbaultae* in Australia.

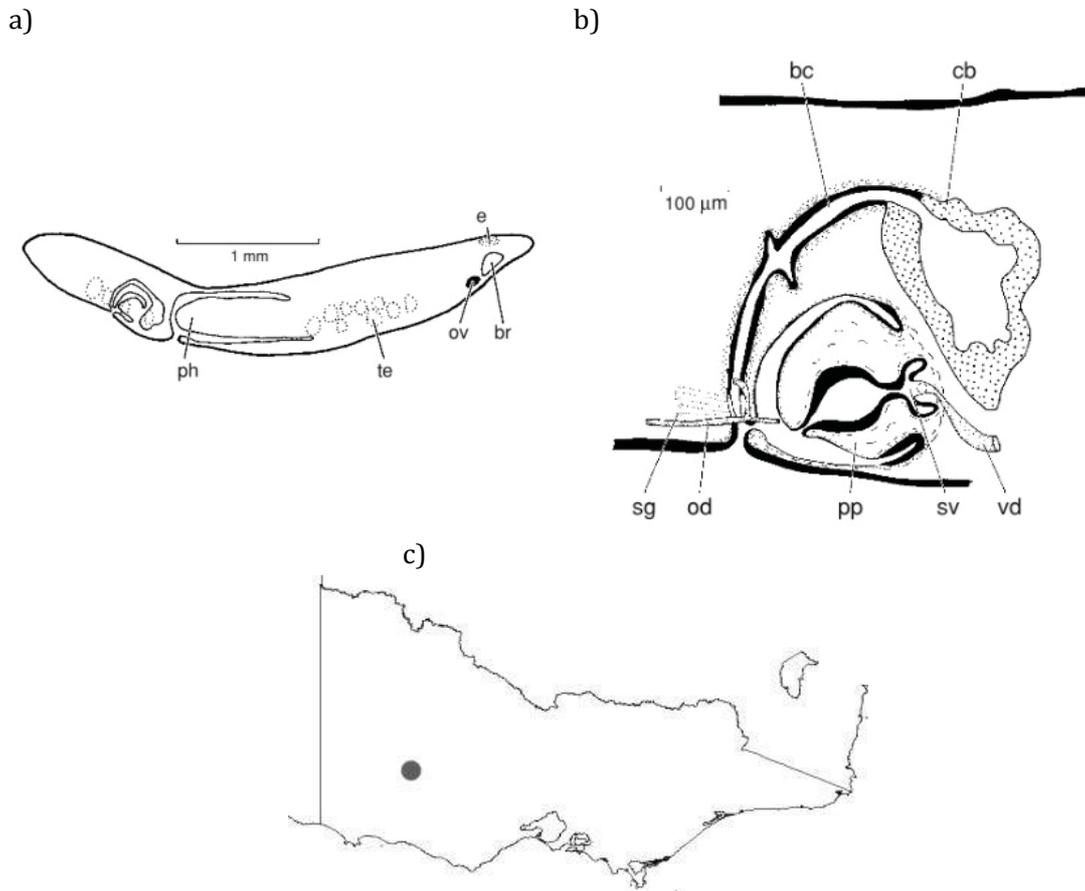
***Spathula miserabile* Sluys and Grant, 2006**

***Contribution***

*Spathula miserabile* was described throughout the course of this research (Grant et al. 2006).

### Diagnosis

*Spathula miserabile* can be distinguished from its congeners by the presence of (1) a plump, asymmetrical penis papilla of which the dorsal lip is larger than the ventral one, (2) ventral testes, and (3) the virtual absence of eyes (Grant et al. 2006a).



**Figure 1a.48** *Spathula miserabile* a) Sagittal reconstruction of the reproductive system; b) sagittal reconstruction of the copulatory apparatus; c) distribution of *Spathula miserabile* in Australia.

### Comments

With respect to the virtual absence of eyes, *S. miserabile* resembles *S. gorbaultae*, *S. tryssa*, *S. camara*, and *S. agelaea*, which were reported from Mt. Donna Buang, Mount Buller, Falls Creek, and Mt. Buffalo in Victoria, respectively (Grant et al. 2006). Unfortunately, the reproductive apparatus of *S. agelaea* is unknown. *S. gorbaultae*, *S. tryssa*, *S. camara*, and *S. agelaea* possess ciliated pits, which appear to be absent in *S. miserabile*. Generally, species of *Spathula* do possess such pits, with the exception of *S. schauinslandi* (possesses eyes and very distinctive penial glands differentiating it from the

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current species) and *S. miserabile*. In *S. goubaultae* the testes are dorsal, in contrast to the ventral testes in *S. tryssa*, *S. camara*, and *S. miserabile*. The penis papilla of *S. tryssa* is also provided with a wide lumen but in contrast to *S. miserabile* the papilla in the former is not asymmetrical, a condition that also applies to *S. goubaultae* (cf. Ball 1977a). *Spathula camara* is characterized by a large, pleated seminal vesicle located in the penis bulb, a feature not present in *Sp. miserabile* (Grant et al. 2006)(Figure 1a.48-b).

The presence of a plump, asymmetrical penis papilla, ventral testes, and the absence of eyes, set this animal apart from all currently known species of *Spathula* (Figure 1a.48-b). In combination with the fact that blind species of *Spathula* have previously only been described from Victoria, Grant et al. (2006) argued that the description of a new species was warranted, in spite of the fact that the available material is limited and no information exists on the external features. New material may also enable us to determine whether the expansion of the bursal canal is an artefact of the holotype or represents a true feature of the species (Grant et al. 2006).

### ***Ecology and Distribution***

*Spathula miserabile* was collected from Ben Lomond National Park, an alpine plateau over 1500 meters high in northeastern Tasmania (Grant et al. 2006)(Figure 1a.48-c).

## ***Spathula musculosa* Sluys and Grant, 2006**

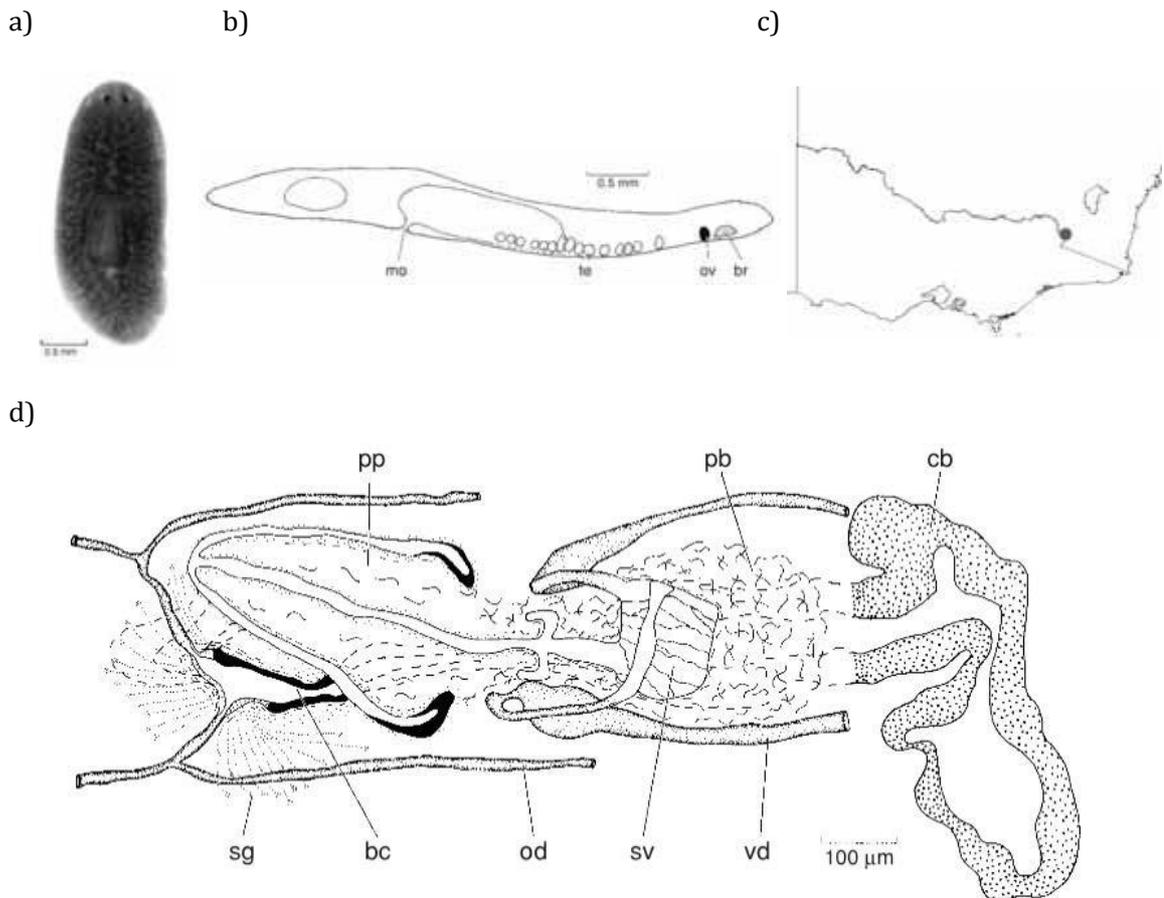
### ***Contribution***

*Spathula musculosa* was described as part of the research done for this thesis (Grant et al. 2006).

### ***Diagnosis***

*Spathula musculosa* can be distinguished from its congeners by the presence of (1) a large, elongated and highly muscular penis bulb, and (2) a large adenodactyl of similar size and orientation as the penis papilla; this adenodactyl lacks a distinct lumen and its musculature is of the reversed type (Grant et al. 2006).

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**Figure 1a.49** *Spathula musculosa* a) Photograph of whole mount; b) sagittal reconstruction of the reproductive system; c) distribution of *Spathula musculosa* in Australia; d) sagittal reconstruction of the copulatory apparatus.

**Comments**

The presence of a large adenodactyl precludes confusion with any known species of *Spathula*, since *S. musculosa* represents the first species in the genus for which such a structure has been reported (Grant et al. 2006)(Figure 1a.49-d). It is striking that the musculature of the adenodactyl is of the reversed type. In other Australian species for which adenodactyls have been reported, such as *Romankenkius libidinosus*, *R. impudicus*, and *R. boehmigi*, the musculature on the adenodactyls is non-reversed (Grant et al. 2006).

Other characteristic features of *S. musculosa* are the large and highly muscular penis bulb and the odd communication between the sperm ducts and the seminal vesicle (Grant et al. 2006)( Figure 1a.49-d).

**Ecology and Distribution**

This species was found in two connected streams on Mt. Kosciuszko in southeast New South Wales (Grant et al. 2006)( Figure 1a.49-c). Mt. Kosciuszko is the highest mountain

on the Australian mainland with a height of 2,228 metres, meaning that the summit is covered with snow for much of the year.

### ***Spathula oblongata* Grant & Sluys, sp. nov.**

#### ***Contribution***

*Spathula oblongata* was described throughout the course of this research (Appendix 1d).

#### ***Diagnosis***

This species can be differentiated from its congeners by the presence of an elongated penis bulb (Appendix 1d).

#### ***Comments***

The specimens examined by as part of Appendix 1d represent a new species not only because of the nature of the penis bulb but also due to the fact that the unique combination of features eliminates all other Australian dugesiids (Figure 1a.50-b). A combination of characters not often seen in Australian dugesiids is the presence of a common oviduct and a caudal branch of the oviducts. All other species with both of these features have other characters precluding the assignment of these specimens to any of these species: *Romankenkius libidinosus* (has a seminal vesicle, adenodactyl and gastrointestinal communications), *R. pedderensis* (the testes are ventral and pre-pharyngeal), *Sp. triculenta* (lacks the large muscular penis bulb, the three types of glands entering the broad ejaculatory duct and the massive copulatory bursa) (Appendix 1d).

Perhaps the most important comparison is with *R. sinuosus*. This species exhibits a common oviduct and short caudal branch of the oviducts and, unlike other species with these features, also has an elongated penis bulb. *R. sinuosus* is easily differentiated from the material examined by Grant et al. (Appendix 1d), however, as it has a large seminal vesicle, no ciliated pits, no glandular differentiation in the penis bulb, a common oviduct receiving the openings of shell glands, and the entire copulatory apparatus is sinuous (Figure 1a.50-a,b).

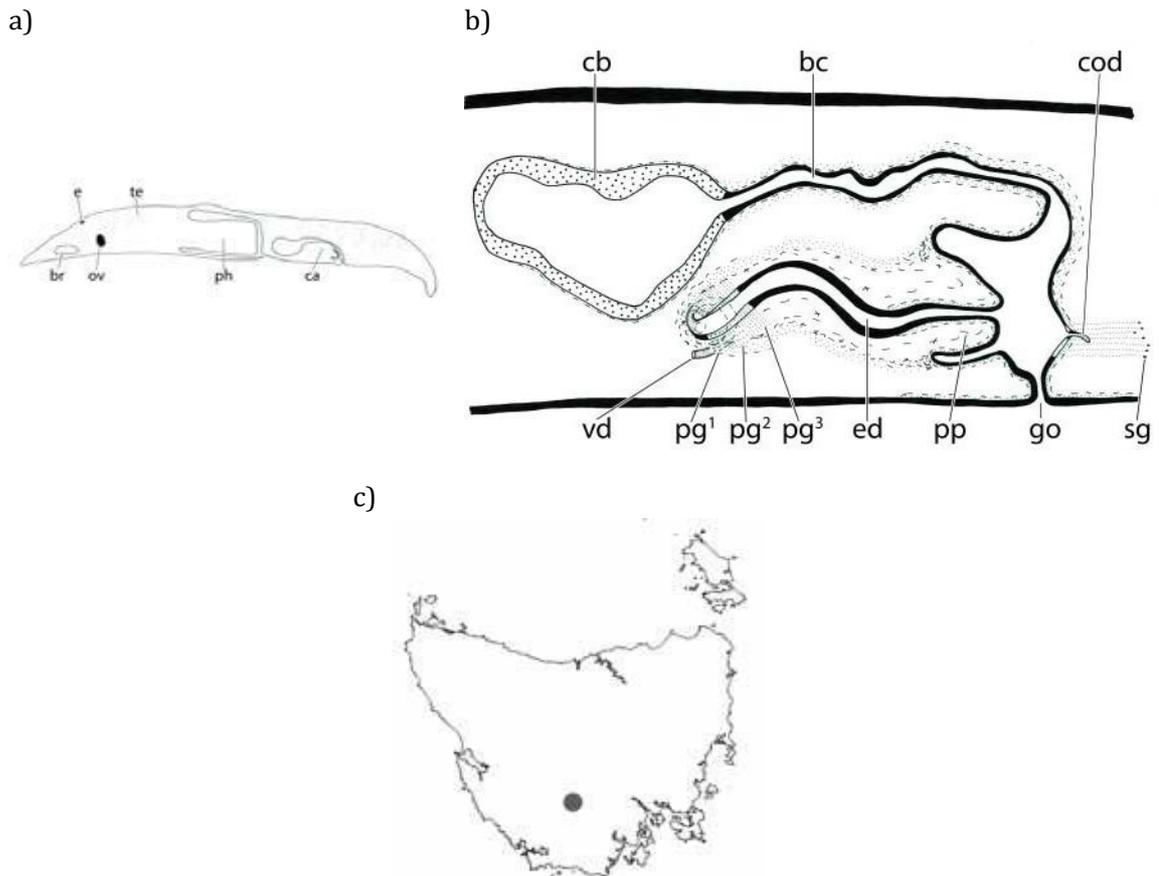
As the specimens inspected as part of Appendix 1d cannot be easily assigned to any known species, the genus needs to be identified. This species is most likely a *Spathula* due to the presence of large sensory pits, shell glands penetrating the bursal canal and caudally branching oviducts (Figure 1a.50-a,b). All of these characters are uncommon outside of *Spathula* and the combination of all three is quite conclusive (cf. Sluys and

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Kawakatsu 2001). This genus assignment allows us to be confident in proclaiming a new species, *Spathula oblongata* (cf. Sluys and Kawakatsu 2001).

Within *Spathula* an elongated penis bulb excludes many known species, excepting *Sp. alba*, *Sp. fontinalis*, and *Sp. musculosa*. However, the former is a blind species with a bursal canal provided with a sphincter (cf. Appendix 1d). *Sp. fontinalis* also possess a sphincter on the bursal canal, in contrast to *Sp. oblongata*. Finally, *Sp. musculosa* has a large adenodactyl, which is absent in *Sp. oblongata* (Allison 1997).

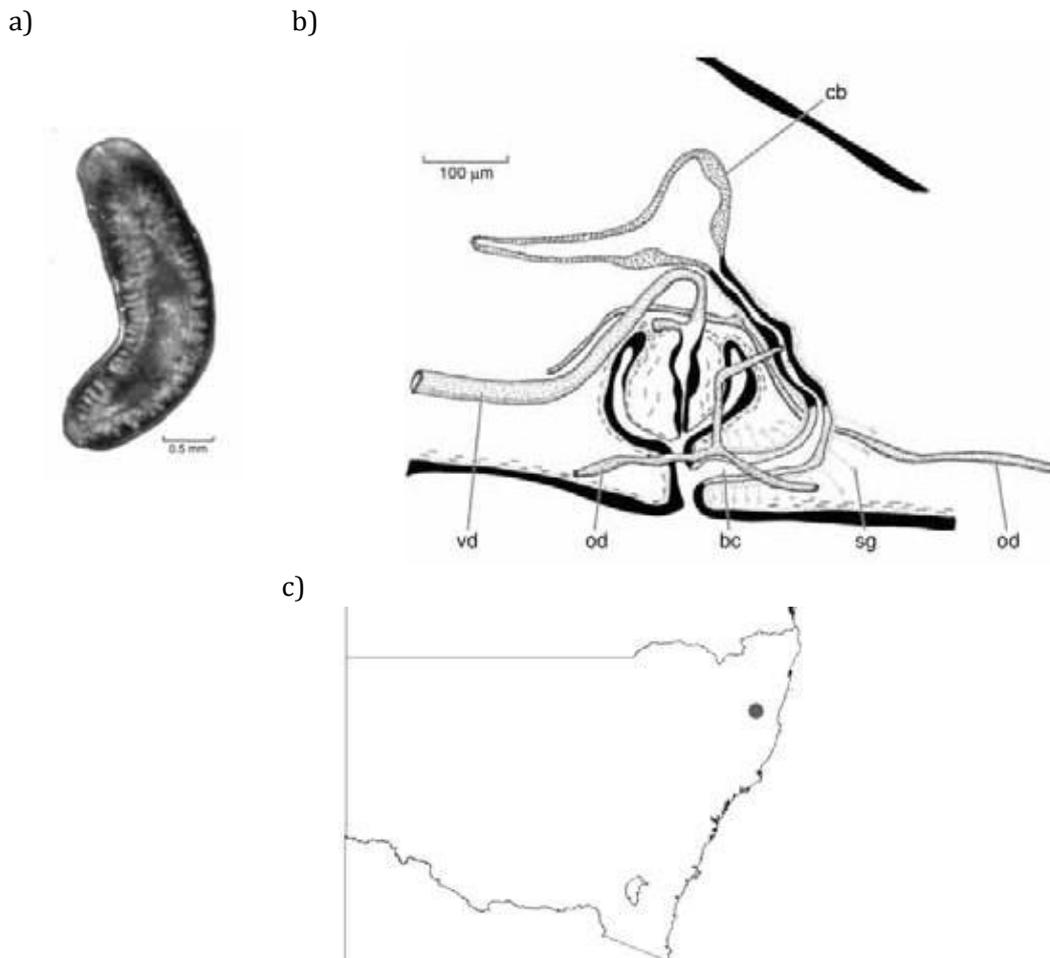


**Figure 1a.50** *Spathula oblongata* a) Sagittal reconstruction of the reproductive system; b) sagittal reconstruction of the copulatory apparatus; c) distribution of *Spathula oblongata* in Australia.

### ***Ecology and Distribution***

*Spathula oblongata* was collected from the Gordon River system, approximately 37km east of Strathgordon (Appendix 1d)(Figure 1a.50-c). The Gordon River is one of the major rivers of Tasmania and runs entirely through uninhabited wilderness.

*Spathula simplex* Sluys and Grant, 2006



**Figure 1a.51** *Spathula simplex* a) Photograph of whole mount; b) sagittal reconstruction of the copulatory apparatus; c) distribution of *Spathula simplex* in Australia.

**Contribution**

*Spathula simplex* was described as during the course of this research (Grant et al. 2006).

**Diagnosis**

*Spathula simplex* can be distinguished from its congeners by oviducts that communicate with the bursal canal in a very dorsal position and caudal oviducal branches forming a closed loop in the posterior end of the body. Furthermore, one oviduct runs almost dorsally to the penis bulb before communicating with the bursal canal (Grant et al. 2006).

**Comments**

The gross morphology of the copulatory apparatus of *S. simplex* offers little that differentiates this species from its congeners, apart from its simplicity. However, in particular details regarding the course of the oviducts, *S. simplex* is rather different from

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other species of *Spathula*. In most species the oviducts open into the most proximal, ventral section of the bursal canal, whereas in *S. simplex* the medial branches of the oviduct communicate with the bursal canal in a very dorsal position (Figure 1a.51-b). Such a dorsal communication between oviducts and bursal canal has been described for only two other species, viz. *S. limicola*, and *S. tryssa*. However, in other features these two last-mentioned species are rather different from *S. simplex* (cf. Ball 1977a; Sluys & Kawakatsu, 2001).

Another characteristic feature of *S. simplex* is the fact that one of its oviducts runs almost dorsally to the penis bulb before giving off a medial branch that travels ventrally to communicate with the bursal canal (Figure 1a.51-b).

The third characteristic detail regarding the course of the oviducts concerns the fusion of the long caudal branches in the posterior end of the body, resulting in an oviducal loop, which has not been reported for any other species of *Spathula* and has been observed in very few triclads in general.

### ***Ecology and Distribution***

*Spathula simplex* is known only from the type locality, New England National Park, in the northeast of New South Wales (Grant et al. 2006)(Figure 1a.51-c). New England National Park is situated on the precipitous escarpment on the east of the undulating northern tablelands plateau, the wilderness consists of impressive cliffs, rugged ridges, spurs and streams.

## ***Spathula truculenta* Ball, 1977**

### ***Contribution***

This species has been redescribed once during the course of this thesis with only minor revisions to the original description (Grant et al. 2006).

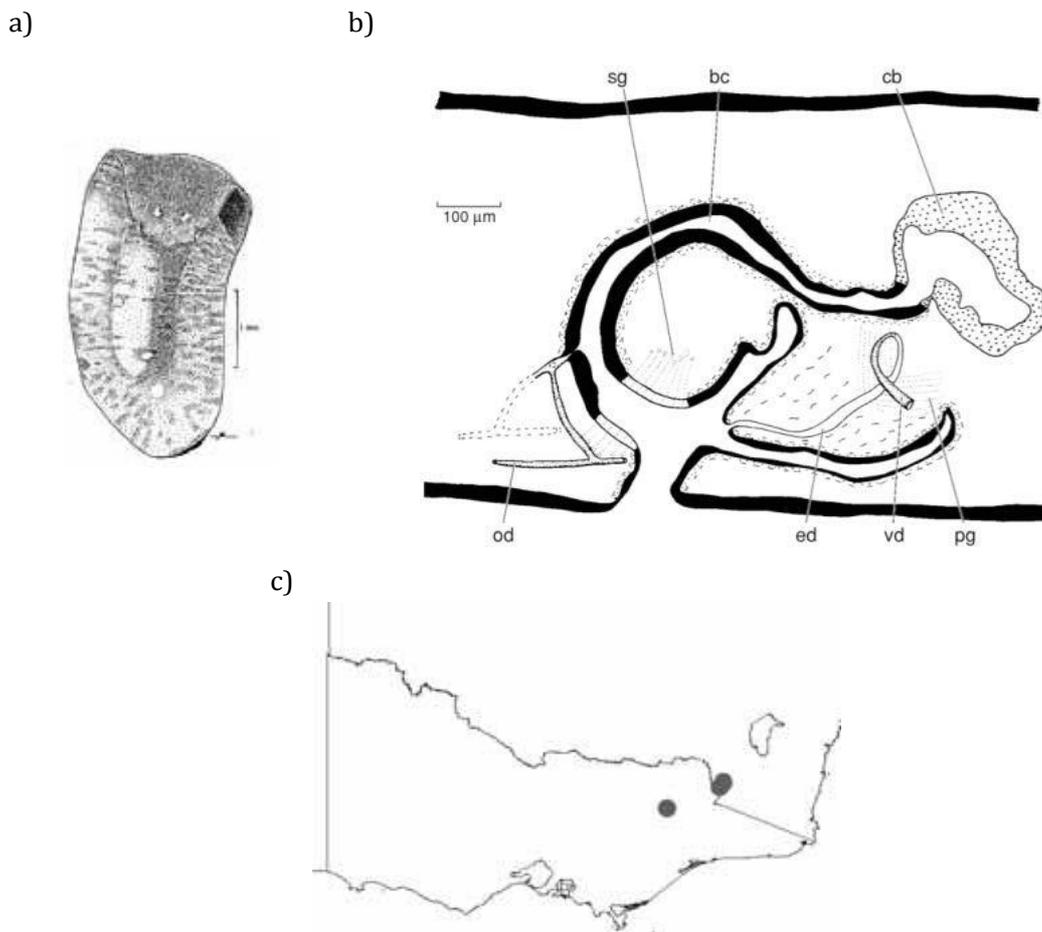
### ***Diagnosis***

The large size, deep pigmentation, and tendency to triangular head are features that distinguish this species from other pigmented *Spathula* species. However, the most useful diagnostic character for *Spathula truculenta* is the presence of a short common oviduct, a character unique for this genus (Ball 1977a).

### ***Comments***

The material examined by Grant et al. (2006) represents the first series of specimens that has become available after the original description of the species by Ball (1977a). The new

specimens conform closely to the original account. Noteworthy features concern the deviant bursal canal musculature, with its reversed, mixed, and non-reversed sections, and the location of the testes (Figure 1.53-a,b). With respect to the latter, Ball (1977a) correctly observed that the pharyngeal region is free of testes but did not notice that in the posterior end of the body testis follicles occur only between the gut branches (Grant et al. 2006).



**Figure 1a.52** *Spathula truculenta* a) External features of preserved specimen (Ball 1977a); b) sagittal reconstruction of the copulatory apparatus; c) distribution of *Spathula truculenta* in Australia.

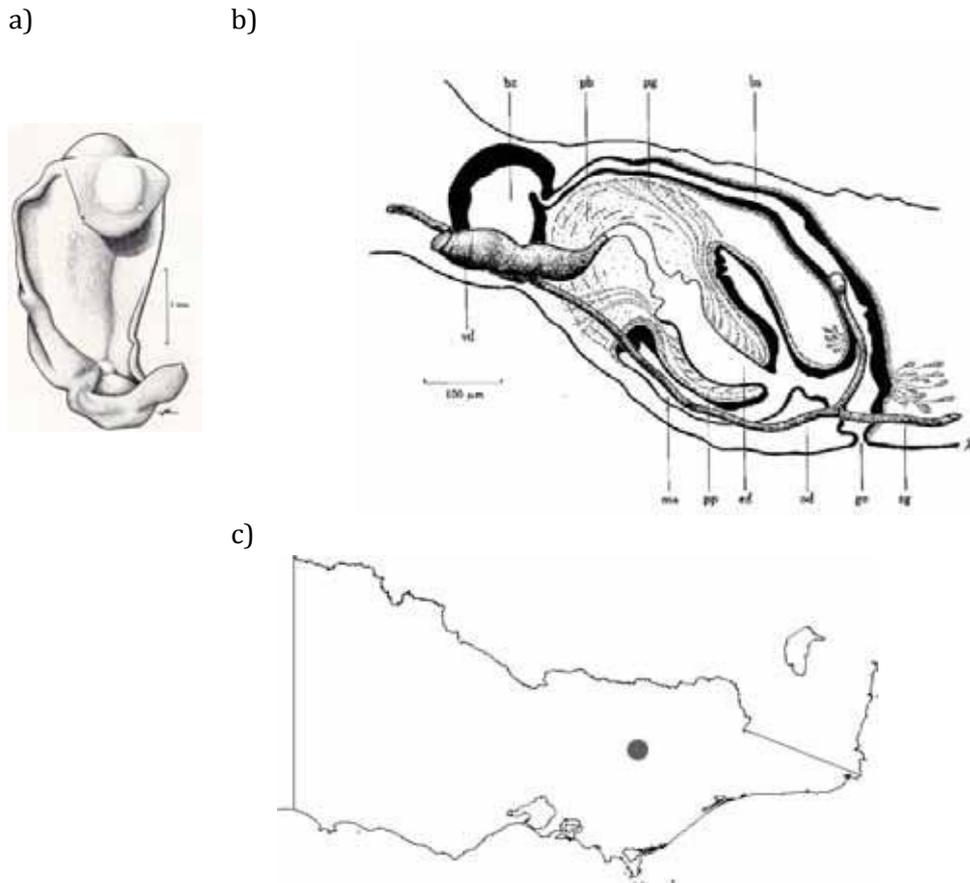
### ***Ecology and Distribution***

*Spathula truculenta* is known from sites surrounding Mt. Kosciuszko in southeast New South Wales, and a single collection from Mt. McKay, Victoria (Ball 1977a, Grant et al. 2006)(Figure 1a.52-c). While these sites are in different states, they are in close proximity to each other, in the Australian Alpine Region. *Spathula truculenta* is one of the three Australian species that is known to not exhibit photophobic behaviour (Hay and Ball 1979).

***Spathula tryssa* Ball, 1977**

***Contribution***

*Spathula tryssa* was not collected during the course of this research and therefore I have no advancement on the original description to offer.



**Figure 1a.53** *Spathula tryssa* a) External features of live specimen (Ball 1977a); b) sagittal reconstruction of the copulatory apparatus (Ball 1977a); c) distribution of *Spathula tryssa* in Australia.

***Diagnosis***

*Spathula tryssa* is unusual in relation to other members the genus for its capacious and glandular ejaculatory duct and for its very long dorsad oviducal branch (Ball 1977a).

### ***Comments***

*Spathula tryssa* is similar to *Spathula camara* in that they both possess two pairs of ciliated pits, however *S. tryssa* is easily distinguishable from *S. camara* by its lack of pigment and penial morphology (Ball 1977a)(Figure 1.54-a,b).

### ***Ecology and Distribution***

*Spathula tryssa* is known only from sites on Mt. Buller, a mountain in the Victorian Alpine Region, where it was collected from a cool, small channel, in open moorland country (Ball 1977a, Hay and Ball 1979)(Figure 1a.53-c). This species is stenothermal (i.e. it never experiences temperatures above 9°C) because its habitat is supplied by underground springs and at least some individuals retreat underground when the surface water dries up (Hay and Ball 1979).

## ***Spathula sp.* - Sluys and Kawakatsu 2001**

### ***Contribution***

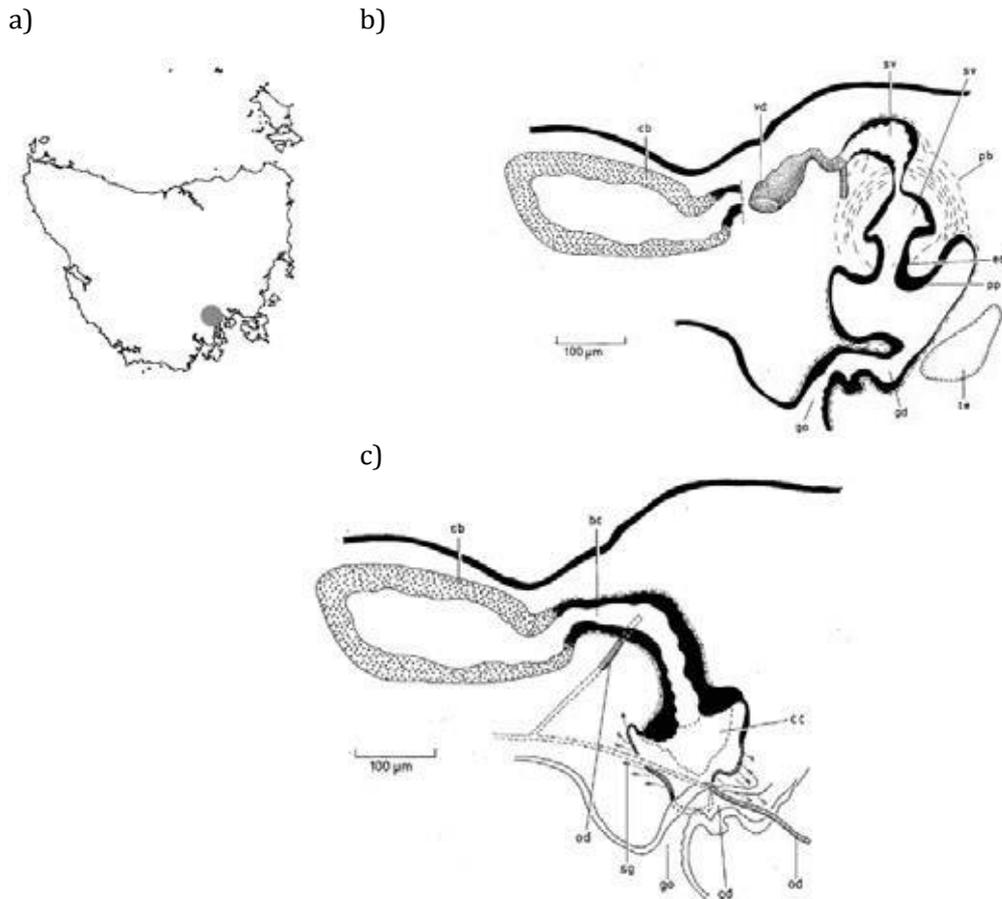
Unfortunately no species resembling this description was collected during the course of this research and therefore our knowledge of this species cannot be advanced.

### ***Diagnosis***

*Spathula sp.* was only tentatively described by Sluys and Kawakatsu (2001). Due to a lack of detail in the copulatory apparatus Sluys and Kawakatsu (2001) were unable to complete a diagnosis (Figure 1a.54-b,c).

### ***Ecology and Distribution***

*Spathula sp.* was collected from a stream trickling down Mt. Wellington, Tasmania (1a.54-a). Mount Wellington is a mountain on whose foothills is built much of the city of Hobart, Tasmania, Australia.



**Figure 1a.54** *Spathula sp.* a) Distribution of *Spathula sp.* in Australia; b) sagittal reconstruction of the male copulatory apparatus (Sluys and Kawakatsu 2001); c) sagittal reconstruction of the female copulatory apparatus (Sluys and Kawakatsu 2001).

### ***Spathula sp. 1***

#### ***Contribution***

*Spathula sp. 1* was only tentatively described as part of the research conducted for this thesis.

#### ***Diagnosis***

*Spathula sp. 1* was only tentatively described as part of this thesis (Appendix 1d). Due to a lack of detail in the morphological sections it was impossible to complete a diagnosis.

#### ***Comments***

Most likely, *Spathula sp. 1* represents a new species, which unfortunately cannot be described fully from the available material. Molecular data unmistakably identifies this species as a *Spathula* (LJ141, Appendix 2c), and the morphological data allows all other

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*Spathula*'s to be eliminated (Appendix 1d). The only other *Spathula* recorded with little pigment and with eyes is *Spathula foeni* (Appendix 1d)(Figure 1a.55-a). While this specimen could represent the first confirmed Tasmanian record (a heavily pigmented *Spathula* cf. *foeni* was recorded from Tasmania (cf. Grant et al. 2006)); the molecular data suggests that *Spathula* sp. 1 is separate from *Sp. foeni*. Unfortunately, in the absence of more information it was difficult to conclusively assign this material to a known species or conclusively describe a new one, leaving it tentatively assigned to *Spathula* sp. 1.

a)



b)



**Figure 1a.55** *Spathula* sp. 1 a) External features of live specimen; b) distribution of *Spathula* sp. in Australia.

### ***Ecology and Distribution***

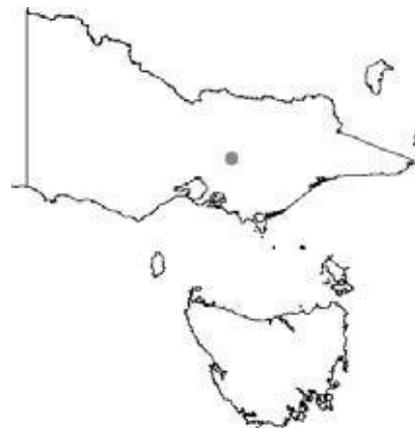
*Spathula* sp. 1 specimens were collected from the Franklin River in the Franklin - Gordon Rivers National Park, in the mid northern part of the Tasmanian Wilderness World Heritage Area (Appendix 1d)(Figure 1a.55-b).

### ***Spathula* sp. 2**

a)



b)



**Figure 1a.56** *Spathula* sp. 2 a) External features of living specimen; b) distribution of *Spathula* sp. 2 in Australia.

### **Contribution**

This species has been tentatively described and assigned to *Spathula* as part of this thesis (Appendix 1d).

### **Diagnosis**

While the internal anatomy of this species is unknown, the most distinctive character is the lack of pigment throughout the specimen (Appendix 1d).

### **Comments**

Although the internal anatomy of this specimen remains unknown, its discovery presents a potential opportunity and is worth examining (Appendix 1d). Hay and Ball (1979) describe *Reynoldsoni reynoldsonia* as a “Slender species up to 7.5 mm long. Head spathulate with two close-set eyes. Usually white or yellowish”. The description of the external morphology of *Spathula sp. 2* presented in Appendix 1d is nearly identical with the description given above (Figure 1a.56-a). This specimen was found at the base of the Victorian Alpine Region, close to the type locality of *R. reynoldsoni* (Figure 1a.56-b).

Recent morphological phylogenies have suggested that *R. reynoldsonia* is just an aberrant *Spathula* (Sluys et al. 2001). With this in mind one would expect *R. reynoldsonia* to sit close to the *Spathula*'s in any molecular analysis. The molecular analysis of the current study places this *Spathula sp. 2* amongst the other *Spathula* species, thus providing further support for the idea that this specimen may be *R. reynoldsoni* (Chapter 2). The only other species resembling *Spathula sp. 2* are *Spathula foeni* and *Romankenkius libidinosus*, both of which are pigment-free with eyes. The possibility that *Spathula sp. 2* is in fact *Sp. foeni* was examined thoroughly due to the proximity of the collection points. However, the size and head shape discrepancies eliminate the possibility of this specimen being assigned to *Sp. foeni* or *R. libidinosus* (Chapter 2).

So there are two possible scenarios. If we accept that *Spathula sp. 2* is *R. reynoldsoni*, then this assignment would be significant since it would imply that we have the molecular data for *R. reynoldsoni* with all the resulting implications. Alternatively, if this specimen is not *R. reynoldsoni* we are left with a new species, superficially very similar to *R. reynoldsoni* that can be comfortably assigned to *Spathula*. It is considered judgement of the author that without more information the latter option is the more conscientious choice (Appendix 1d). Therefore, this is the assignment that will be adhered to throughout the remainder of this thesis, as well as any publications arising from it.

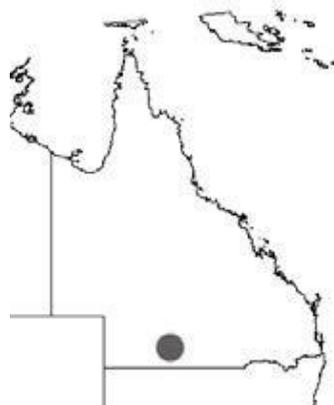
***Ecology and Distribution***

This species was collected from a narrow section of Steavensons River at the small township of Buxton, northeastern Victoria (Figure 1a.56-b). The river is surrounded by eucalypt forest and some urban development. Worms were collected from the underside of cobble in the off channel area (Appendix 1d). *Spathula* sp. 2 has been found in the presence of *Cura pinguis*.

**Family DugesIIDae s.s. Ball, 1974****Weissius Sluys, 2007**

Genus dissolved and constituent species moved to *Cura*.

DugesIIDae s.l. with a ciliated pit on either side of the head and with the copulatory apparatus located in the most posterior part of the body, close to the posterior body margin. Testes ventral and prepharyngeal. Small ovaries directly behind the brain. Anterior sections of the oviducts gradually expanding to form a spacious ampulla communicating with the ovary. Finger-shaped, short penis papilla. Penis bulb elongated, consisting of circular muscle, housing an elongate seminal vesicle, which receives the separate openings of the sperm ducts and the secretion of two types of erythrophilic penis glands. Male and common atrium covered by a well-developed layer of circular muscle and a broad, ball-shaped zone of longitudinal muscle, with some of these fibres attaching around the gonopore. Common atrium with a valve like constriction before communicating with the gonopore. Oviducts separately enter the bursal canal well above the zone of shell glands. Bursal canal covered with layer of circular muscle and communicating with copulatory bursa. Sperm transfer through exchange of spermatophore (Appendix 1d). The genus *Weissius* is distributed as shown in Figure 1a.57.



**Figure 1a.57** Distribution of the genus *Weissius* in Australia.

### *Weissius* (*Cura*) *capaciductus* Sluys, 2007

#### **Contribution**

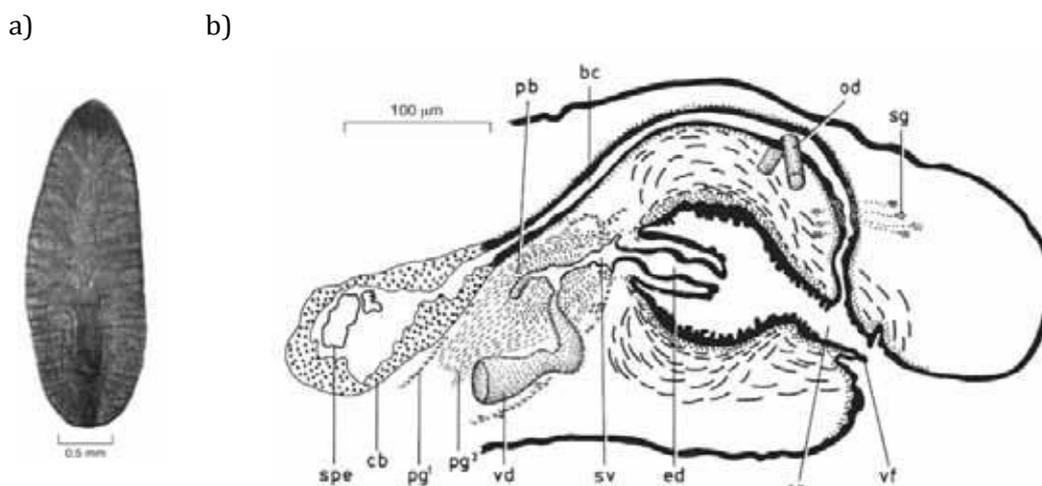
*Weissius capaciductus* was described throughout the course of this thesis (Sluys et al. 2007). The material from this rather unique location was provided by Dr. W.F. Ponder (Australian Museum, Sydney).

#### **Diagnosis**

This species is currently the only member of this newly described genus (Sluys et al. 2007).

#### **Comments**

Although the presumed apomorphic characters for the Dugesiidae s.l., the presence of multicellular pigment cups with numerous retinal cells (Baguña et al. 2001, Sluys 1989, Sluys and Kawakatsu 2001, Sluys and Kawakatsu 2006) cannot be assessed on *W. capaciductus*, the species cannot be placed in the Planariidae or the Dendrocoelidae since it clearly does neither possess a common oviduct opening into the atrium, nor does it show the dendrocoelid type of pharynx. Therefore, Sluys et al. (2007) assigned *W. capaciductus* to the Dugesiidae s.l.



**Figure 1a.58** *Weissius capaciductus* a) Photograph of a whole mount (Sluys et al. 2007); b) sagittal reconstruction of the copulatory apparatus (Sluys et al. 2007).

Considering the taxonomy of “dugesiid” species and genera as expressed in Sluys’ (2001) “guestimated” tree, Sluys et al. (2007) found it impossible (1) to synonymize *W.*

*capaciductus* with one of the currently known species, and (2) to fit the new species comfortably within one of the current genera (Sluys et al. 2007). Absence of a branched oviduct in *W. capaciductus* precludes assignment to *Spathula*, *Eviella*, and *Reynoldsonia*, while absence of a diverticulum makes it impossible to assign the species to *Romankenkius* (Sluys et al. 2007). It does certainly not belong to *Dugesia* because it lacks the characteristic diaphragm of that genus. And neither does *W. capaciductus* show the double seminal vesicle that is characteristic for *Schmidtea*. The genera *Neppia* and *Girardia* are poorly defined from a phylogenetic point of view. However, in most species of *Neppia* the bursal canal is surrounded by a thick zone of circular muscle, which is absent in *W. capaciductus* (Sluys et al. 2007). In contrast to *W. capaciductus*, many species of *Girardia* have a pigmented pharynx, while in *Girardia* species and also in aberrant *Bopsula*, the testes generally occur throughout the body.

An important feature in the taxonomy of “dugesiid” genera is the arrangement of the muscle layers around the bursal canal. In the non-reversed condition the bursal canal is surrounded by a subepithelial layer of circular muscle, followed by a layer of longitudinal muscle. In the reversed condition there is a subepithelial layer of longitudinal muscle, followed by a layer of circular fibres (Sluys et al. 2007)(Figure 1a.58-b). For example, species of *Spathula*, *Romankenkius*, and *Dugesia* are generally characterized by a reversed musculature, whereas *Girardia* exhibits the non-reversed condition. However, the state of this particular phylogenetic and taxonomic character cannot be assessed in *W. capaciductus* since it merely possesses one layer of muscle around the bursal canal, viz. a circular muscle layer (Sluys et al. 2007). A single zone of circular muscle around the bursal canal is known also from *Cura pinguis* (cf. Sluys et al. 2007)(Figure 1a.58-b).

In addition, there are a few other features that suggest a close relationship between *Weissius* and *Cura*. Sluys (1997, 2001) restricted the genus *Cura* to the species *C. pinguis*, *C. foremanii*, *C. fortis*, *C. evelinae*, and *C. graffi*. In another paper written by Grant et al. (2006) it is argued that *C. graffi* does not belong to *Cura* and the species is tentatively transferred to the genus *Girardia*. *Weissius* resembles the four remaining species of *Cura* in that all five have a finger- or thumb-shaped penis papilla as well as a male atrium that is set off from the common atrium and at the same time is surrounded by broad zones of circular and longitudinal muscle fibres (Grant et al. 2006)(Figure 1a.58-b). The last-mentioned, compound feature suggests that *Weissius* belongs to the clade that already comprised *Cura* and *Schmidtea*, since Sluys et al.’s (2007) postulated this compound character regarding the male atrium as a synapomorphy for the two last mentioned genera. *Weissius* may be more closely related to *Schmidtea* since it lacks the short common oviduct and particularly the anteriorly displaced point of communication between atrium

and bursal canal that are characteristic features for species of *Cura* (Sluys 2001). Although *Weissius* may be taxonomically close to *Schmidtea*, it cannot be assigned to this genus because it lacks the double seminal vesicle and the mixed bursal canal musculature of the last-mentioned genus (Sluys et al. 2007). Furthermore, in *Schmidtea* the testes are situated dorsally and occur throughout the body, in contrast to the conditions in *Weissius*. As a consequence, the combination of features present in *W. capaciductus* requires the erection of a new genus, albeit that it is difficult to describe apomorphic features that uniquely identify the genus (Sluys et al. 2007).

### ***Ecology and Distribution***

*W. capaciductus* is known only from springs and pools connected to the Great Artesian Basin at Bundoona Station, in southwestern Queensland (Sluys et al. 2007)(Figure 1a.57). The basin is the largest and deepest artesian basin in the world, stretching over a total of 1,711,000 square kilometres (661,000 sq mi), with temperatures measured ranging from 30°C to 100°C. These specimens were collected from cooler surface waters.

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**APPENDIX 1a**

## SYSTEMATIC REVIEW AND REVISION OF DUGESIIDAE S.L. TAXONOMY

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**APPENDIX 1a**

SYSTEMATIC REVIEW AND REVISION OF DUGESIIDAE S.L. TAXONOMY

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# Biodiversity of Australian freshwater planarians (Platyhelminthes: Tricladida: Paludicola): new species and localities, and a review of paludicolan distribution in Australia

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**Abstract** On the basis of one extensive collection, the study provides new information on the diversity, taxonomy, anatomy and geographic distribution of 21 species of Australian freshwater planarians, including 7 species that are described as new. The material includes old type specimens of three species that have remained enigmatic since their collection and description almost 100 years ago, viz. *Planaria rava* Weiss, 1909, *Romankenkius glandulosus* (Kenk, 1930), and *Planaria graffi* Weiss, 1909. A biogeography of Australian freshwater planarians is documented by plotting all published distributional records for every known species. Thus, the present study forms a baseline for future studies on the diversity and biogeography of Australian freshwater planarians. Attention is drawn to the fact that the biotic relationships of several Australian taxa extend across the Pacific Ocean.

**Key words** Platyhelminthes, Tricladida, *Cura*, *Dugesia*, *Girardia*, *Romankenkius*, *Spathula*, Australia, biodiversity, taxonomy, biogeography, morphology, homoplasy

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# Freshwater planarians from artesian springs in Queensland, Australia (Platyhelminthes, Tricladida, Paludicola)

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Key words: artesian springs, Australia, Dugesiidae, Platyhelminthes, Tricladida, *Dugesia*, *Weissius*

## Abstract

Two new species of triclad flatworm are described from artesian springs in Queensland, Australia, viz. *Dugesia artesiana* Sluys and Grant, sp. nov. and *Weissius capaciductus* Sluys, gen. et sp. nov. Some historical biogeographic scenarios are discussed that may explain the occurrence of the new species and their close relatives in Australia.

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## Introduction

Although the commencement of studies on the freshwater planarians of Australia dates back as far as the beginning of the 20<sup>th</sup> century (cf. Weiss, 1909, 1910), our knowledge on the biodiversity of these animals on the Australian continent is still in its infancy. Only very recently major studies were undertaken that aimed to document the diversity and biogeography of Australian paludicolans (Sluys and Kawakatsu, 2001; Grant *et al.*, 2006), some decades after the publications of another student of Australian triclads (Ball, 1974, 1977; Ball and Tran, 1979).

The present paper describes for the first time the planarian fauna of artesian springs associated with the Great Artesian Basin, the major part being located in Queensland. These springs, located in otherwise arid

regions, provide a unique habitat for a variety of endemic invertebrates. Notably hydrobiid snails have radiated in these artesian springs (Ponder *et al.*, 1989; Ponder and Clark, 1990). In this paper we describe two new species of freshwater planarian, the first ones to be reported from an artesian habitat in Australia.

## Material and methods

Animals were initially fixed in formalin but were postfixed in Steinmann's fluid. Serial sections were made at intervals of 5 or 8  $\mu$ m. The sections, prior to staining, were treated by an acidic dichrome mordant solution and were subsequently stained in Martius Scarlet Blue (cf. Bradbury and Gordon, 1977). The material is deposited in the Australian Museum, Sidney (AM), the Queensland Museum, Brisbane (QM) and the Zoological Museum Amsterdam (ZMA).

Abbreviations used in the figures: amp, ampulla; at, atrium; bc, bursal canal; ca, common atrium; cb, copulatory bursa; cp, ciliated pit; dp, diaphragm; ed, ejaculatory duct; go, gonopore; ma, male atrium; od, oviduct; ov, ovary; pb, penis bulb; pg, penis glands; ph, pharynx; pp, penis papilla; sg, shell glands; spe, spermatophore; sv, seminal vesicle; te, testis; vd, vas deferens; vf, valve-like fold, vnc, ventral nerve cord.

## Systematic section

Suborder Tricladida Lang, 1884  
 Infraorder Paludicola Hallez, 1892  
 Family Dugesiidae Ball, 1974

Genus *Dugesia* Girard, 1850  
*Dugesia artesiana* Sluys and Grant, sp. nov.

Material examined.—Holotype: AM W.29441, Edgbaston Station, Blue eye spring (22°43'13''S - 145°26'20''E), Queensland, Australia, 6 October 2002, coll. W.F. Ponder and A. Davis, sagittal sections on 12 slides.

Additional material: ZMA V.Pl. 3054.1, Edgbaston Station, spring 12, at spring head (22°45'20''S - 145°25'31''E), Queensland, Australia, 6 October 2002, coll. W.F. Ponder, J.H. Waterhouse, and A.C. Miller, sagittal sections on 21 slides.

**Etymology.**– The specific epithet is derived from the English adjective “artesian” and alludes to the fact that the animals have been collected from the artesian system in the Great Artesian Basin.

**Diagnosis.**– *Dugesia artesianiana* is characterized by a presumably central ejaculatory duct, asymmetrical openings of the oviducts into the bursal canal, infra-nucleated bursal canal, absence of ectal reinforcement, small diaphragm, and absence of a duct between intrabulbar seminal vesicle and diaphragm.

**Ecology and distribution.**– The holotype was collected from shallow water at the spring’s edge, and the other specimen was found in a flowing seepage at the head of another spring. The species is known only from these two springs in the Great Artesian Basin at Edgbaston, which are separated by a distance of about 8 km.

**Description.**– Preserved specimens up to 14 mm long and 2 mm wide. Dorsally a light yellow-brown base is consistent throughout all specimens, yet the density of



Fig. 1. *Dugesia artesianiana*. Dorsal view of preserved specimen.

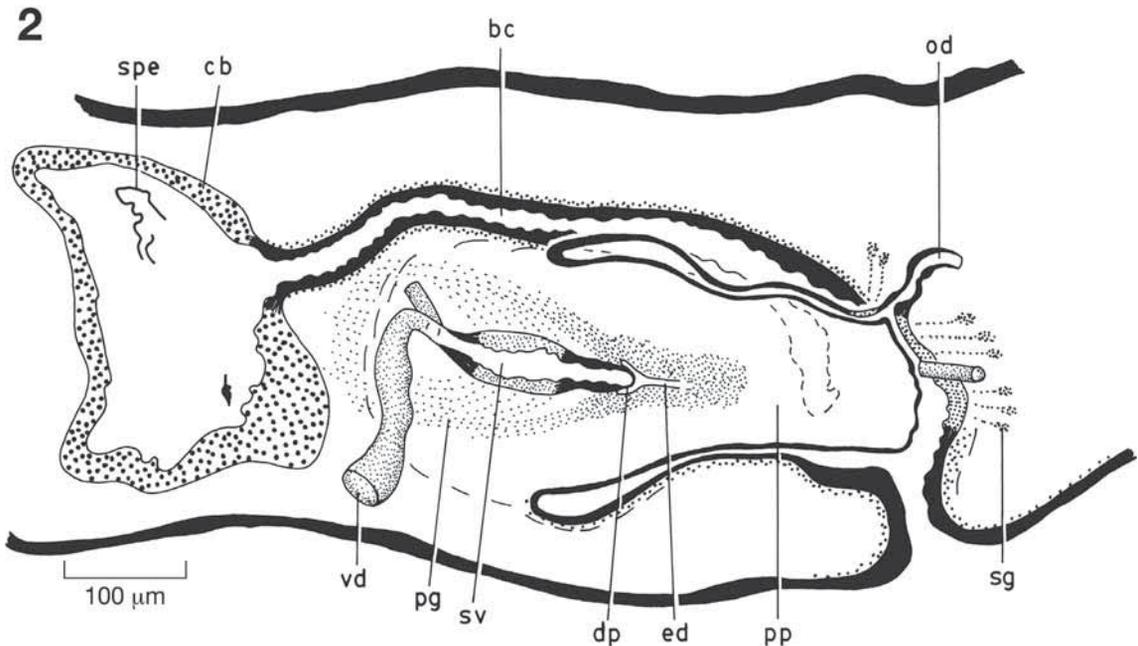


Fig. 2. *Dugesia artesianiana*. Holotype. Sagittal reconstruction of the copulatory apparatus. Anterior to the left.

the fine dark specks varies. Consequently, dorsal pigmentation ranges from dark brown to light yellow-brown. However, regardless of the level of pigmentation it always lightens considerably at the body margins and over the pharyngeal region. Likewise the ventral surface is always paler than dorsal, as pigment is less intense. Examination of the histological sections revealed that the dorsal surface of the holotype is densely pigmented, whereas pigment granules appeared to be absent or very sparse underneath the dorsal epidermis of the other specimen. In the preserved state the front end is rounded (Fig. 1), with no evidence of auricles, owing to preservation effects. A pair of small, pigmented eye cups sit at the point where head tapers in, and positioned closer to each other than to lateral margins. No other sensory structures are evident. The pharynx is positioned posteriorly, in most cases, occupying between one-fourth and one-fifth of the total body length. In the highly contracted holotype and in specimen V.Pl. 3054.1 the pharynx is located in the middle of the body and measures between 1/5-1/6<sup>th</sup> and 1/8-1/9<sup>th</sup> of the body length, respectively. The mouth opening is located at the posterior end of the pharyngeal pocket.

The small testes are situated dorsally and extend from the level of the ovaries, or slightly anterior to the female gonads, to well beyond the copulatory apparatus. The ovaries are located at about 1/3<sup>rd</sup> of the distance between the brain and the root of the pharynx. The oviducts arise from the dorsal surface of the ovarial wall, with these proximal ends of the ducts being

much wider than the major part of the oviducts traversing the body; the oviducts do not branch, i.e. they do not extend backwards beyond the level of the copulatory apparatus.

The sperm ducts communicate separately with the very proximal, anterior section of an elongated, intrabulbar seminal vesicle. The latter is lined with a relatively tall epithelium, receiving the secretion of erythrophilic penis glands. The distal, posterior end of the vesicle tapers to form a small diaphragm conus, which receives the abundant secretion of erythrophilic penis glands. The diaphragm conus projects into the funnel-shaped, proximal section of the ejaculatory duct, the latter receiving the secretion of the erythrophilic penis glands. It is difficult to ascertain whether the ejaculatory duct runs a central or an acentral, ventrally displaced course through the penis papilla. In the holotype the ejaculatory duct could be traced for only part of its length, while its opening at the tip of the penis papilla is not apparent (Fig. 2). In specimen V.Pl. 3054.1 the ejaculatory duct runs a central course through the penis, with a clear opening at the tip. However, in this animal the penis papilla is highly contracted, which may have resulted in an artefactual condition with regard to the course of the ejaculatory duct (Fig. 3). The penis papilla is covered with a nucleated epithelium.

The bursal canal arises from the lateral wall of the common atrium and runs antieriad latero-dorsally to the male atrium and the penis bulb. Immediately anterior to the bulb, the bursal canal communicates with the sac-shaped copulatory bursa. In the holotype the

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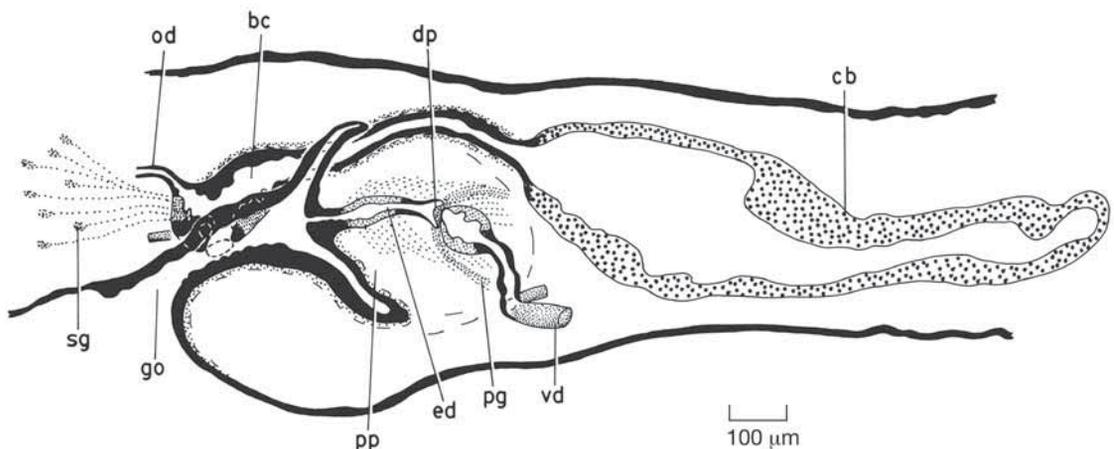


Fig. 3. *Dugesia artesiensis*. ZMA V.Pl. 3054.1. Sagittal reconstruction of the copulatory apparatus. Anterior to the right.

bursa contains remnants of a spermatophore. The bursal canal receives the asymmetrical openings of the infranucleated oviducts very close to the junction with the common atrium. One oviduct opens into the postero-ventral section of the bursal canal, or even into the atrium itself, whereas the other oviduct approaches the canal from a more ventro-lateral direction. Shell glands open into the ventral section of the bursal canal, i.e. in the region where the canal receives the openings of the oviducts. The bursal canal is lined with an infranucleated epithelium for most of its length; in specimen V.Pl. 3054.1 only a short section of the bursal canal immediately adjacent to the copulatory bursa is provided with nucleated cells. The most prominent muscle layer around the bursal canal is one consisting of circular muscle. However, in specimen V.Pl. 3054.1 a thin sub-epithelial layer of longitudinal muscle is also present; this layer of longitudinal muscle could not be traced in the holotype.

Discussion.— Apart from the problematic *Dugesia(?) rava* (Weiss, 1909) (cf. Grant et al., 2006), only one other species of *Dugesia* has been described for Australia, viz. *D. notogaea* Sluys and Kawakatsu, 1998 (Sluys et al., 1998) from northern Queensland. The latter shows several features in which it differs from *D. artesiana*: hyperplastic ovaries; testes that begin at a short distance behind the brain; large, thin-walled seminal vesicle; relatively long duct between seminal vesicle and diaphragm; distinctly acentral ejaculatory duct. In our opinion these differences indicate that the worms from the Edgbaston locality are essentially different from *D. notogaea*, which occurs also in northern Queensland.

With respect to other, non-Australian species of *Dugesia*, the following species share with *D. artesiana* the presence of both a small diaphragm and the absence of a duct between seminal vesicle and diaphragm: *D. aethiopica*, *D. bactriana*, *D. biblica*, *D. colapha*, *D. debeauchampi*, *D. didiaphragma*, *D. indica*, *D. lamottei*, *D. lanzai*, *D. leclerci*, *D. nannophallus*, *D. nanshae*, *D. neumanni*, *D. sicula*. Distinct anatomical features differentiate most of these species from *D. artesiana*, excepting five species for which anatomical resemblance to *D. artesiana* at first sight is much greater, viz. *D. biblica*, *D. colapha*, *D. indica*, *D. leclerci*, and *D. neumanni*. However, in *D. biblica* and *D. neumanni* the bursal canal is provided with distinct ectal reinforcement muscles that extend from the vaginal area to the copulatory bursa; ectal reinforcement is absent in *D. artesiana*. In *D. colapha* and

*D. indica* the bursal canal is nucleate, in contrast to the infranucleated canal in *D. artesiana*. For the rest, the simple copulatory apparatuses of *D. colapha* and *D. indica* resemble much that of *D. artesiana*. However, in *D. indica* the penis papilla is clearly asymmetrical, in contrast to the other two species. Furthermore, in the African species, *D. colapha*, the outer pharynx musculature consists of three layers, a condition that is absent in *D. artesiana* and *D. indica*, which show the more common two-layered condition.

#### Genus *Weissius* Sluys, gen. nov.

Diagnosis.— Dugesiidae with a ciliated pit on either side of the head and with the copulatory apparatus located in the most posterior part of the body, close to the posterior body margin. Testes ventral and prepharyngeal. Small ovaries directly behind the brain. Anterior sections of the oviducts gradually expanding to form a spacious ampulla communicating with the ovary. Finger-shaped, short penis papilla. Penis bulb elongated, consisting of circular muscle, housing an elongate seminal vesicle, which receives the separate openings of the sperm ducts and the secretion of two types of erythrophilic penis glands. Male and common atrium covered by a well-developed layer of circular muscle and a broad, ball-shaped zone of longitudinal muscle, with some of these fibres attaching around the gonopore. Common atrium with a valve-like constriction before communicating with the gonopore. Oviducts separately enter the bursal canal well above the zone of shell glands. Bursal canal covered with layer of circular muscle and communicating with copulatory bursa. Sperm transfer through exchange of spermatophore.

Etymology.— In conformity with the fact that most of the current generic names in the Dugesiidae are derived from names of planarian workers, the genus is named for Annie Weiss in recognition of her early contribution to our knowledge of Australian freshwater planarians. Gender: masculine.

#### *Weissius capaciductus* Sluys, sp. nov.

Material examined.— Holotype: AM W.29442, Bundoona Station (Eulo station 15B), main spring, main flow area (27°57'120"S - 144°46'150"E), Queensland, Australia, 4 April 2002, sagittal sections on 6 slides. Paratypes: QM: G 225677, *ibid.*, sagittal sections on 8 slides; ZMA V.Pl. 3055.1, *ibid.*, sagittal sections

on 7 slides; AM W.29443, *ibid.*, horizontal sections on 3 slides; ZMA V.Pl. 3055.2, *ibid.*, horizontal sections on 3 slides; QM G 225678, *ibid.*, horizontal sections on 3 slides.

Additional material: AM W.29444, Bundoona Station (station no.: Eulo 15A1) (27°57'07''S - 144°46'09''E), Queensland, Australia, 4 April 2002, sagittal sections on 6 slides; ZMA V.Pl. 3056.1, *ibid.*, sagittal sections on 7 slides; ZMA V.Pl. 3056.2, *ibid.*, horizontal sections on 3 slides; AM W.29445, *ibid.*, sagittal sections on 5 slides; AM W.29446, *ibid.*, sagittal sections on 4 slides. ZMA V.Pl. 3057, Bundoona Station (station no.: Eulo 29A), west of road to Quilpie, pool nearest to creek (27°56'29''S - 144°46'42''E), Queensland, Australia, 6 April 2002, preserved specimens; ZMA V.Pl. 3057.1, *ibid.*, sagittal sections on 5 slides; AM W.29447, *ibid.*, sagittal sections on 8 slides.

QM: G 225679, Bundoona Station (station no.: Eulo 15A) (27°57'07''S - 144°46'09''E), Queensland, Australia, 4 April 2002, sagittal sections on 5 slides; G 225680, *ibid.*, sagittal sections on 3 slides; G 225681, *ibid.*, sagittal sections on 4 slides; G 225682, *ibid.*, sagittal sections on 6 slides; G 225683, *ibid.*, sagittal sections on 5 slides. ZMA V.Pl. 3058.1, Bundoona Station (station no.: Eulo 29D) (27°56'29''S - 144°46'42''E), Queensland, Australia, 6 April 2002, sagittal sections on 5 slides; AM W.29448, *ibid.*, horizontal sections on 3 slides. ZMA V.Pl. 3059.1, Bundoona Station (station no.: Eulo 29B) (27°56'29''S - 144°46'42''E), Queensland, Australia, 6 April 2002, 1 whole mount on 1 slide; AM W.29449, *ibid.*, 1 whole mount on 1 slide. AM W.29023, Bundoona Station (station no.: Eulo 16A) (27°56'56''S - 144°46'23''E), 4 April 2002, preserved specimens.

QM: G225685, Bundoona Station (station no.: Eulo 15/1A) (27°57'07''S - 144°46'09''E), 4 April 2002, preserved specimens.

All samples were collected by W.F. Ponder, J.H. Waterhouse, and A.C. Miller.

**Etymology.**— The specific epithet is derived from the Latin adjective “capax”, spacious, and the noun “ductus”; it alludes to the situation that particularly the anterior section of each oviduct is expanded to form a well-developed ampulla.

**Diagnosis.**— With the characteristics of the genus.

**Ecology and distribution.**— All samples were taken in a small group of coalescing springs on Bundoona Station, near the homestead, with the specimens from samples W.29444-6, V.Pl. 3056, W.29442-3, G225677-8, and V.Pl. 3055 having been collected on the same day from various locations at the largest spring. Samples V.Pl. 3057, W.29447, V.Pl. 3059, W.29449, G225684, V.Pl. 3058.1, W.29448, W.29023, and G225685 were taken at several different pools within 100 m from the largest spring. At most of these sites animals were collected from wet sand and mud and on

vegetation out of the water flow or from seepage areas of the springs, with short sedges and duck weed.

**Description.**— Preserved specimens up to 5 mm long and 1 mm wide. Light base with darker pigment speckled throughout, concentrating particularly around the pharynx, giving an overall dark brown appearance. This dark pigment is much sparser on the ventral surface, leaving the light base exposed with a resulting paler appearance. In its preserved state the animal exhibits a broad round head and tail. Eyes absent. An unpigmented ciliated pit is located on either side of the head, slightly anterior to the brain and rather close to the margin of the body (Figs 4, 5). Each relatively deep pit is made up of invaginated, nucleated epidermal cells, which are devoid of rhabdites and are provided with long cilia (Fig. 6).

The unpigmented pharynx is always positioned entirely in the posterior half of the animal (Fig. 4), occupying between 1/4-1/6<sup>th</sup> of total body length in preserved specimens. The mouth opens at the most posterior extent of the pharyngeal pocket.

The testes are situated ventrally, extending from some distance behind the brain and the ovaries up to the root of the pharynx (Fig. 7). The small ovaries are located directly behind the brain. For such a small animal, the oviducts are rather thick tubes, lined with cuboidal or rectangular, nucleated cells. In addition, the anterior sections of the oviducts are developed to even greater extent since these parts gradually widen to give rise to a spacious ampulla, frequently containing sperm (Fig. 8). Surprisingly, the ampulla appears to communicate with the dorsal section of the ovary, with this part of the female gonad containing the germ centre.

The vasa deferentia are swollen to form spermiducal vesicles. In the proximity of the penis bulb, the sperm ducts narrow considerably and are surrounded by a distinct layer of circular muscle. The sperm ducts open separately into the anteriormost part of the elongated intrabulbar seminal vesicle, the latter communicating with the relatively broad ejaculatory duct. There is a distinct constriction at the point where the seminal vesicle opens into the ejaculatory duct, i.e. at the level of the root of the penis papilla. The section of the seminal vesicle adjacent to this point of communication receives the openings of penis glands producing a coarse-grained, orange-brown secretion. Another type of penis gland, producing a red and slightly more fine-grained secretion, discharges into the anterior part of the seminal vesicle. This vesicle is surrounded by a thick coat of circular muscle, thus constituting the penis bulb.

The short penis papilla is covered with a nucleate epithelium and projects into a spacious male atrium. The latter shows a distinct constriction before communicating with the common atrium, a female atrium virtually being absent. In turn, the common atrium is provided with a valve or fold just before it connects with the gonopore. Male and common atrium are lined with a simple columnar, nucleated epithelium and are surrounded by a relatively thick coat of subepithelial circular muscle. In addition, male and common atrium are surrounded by a broad zone of thick and loosely arranged longitudinal muscle fibres (Fig. 9). Notably around the male atrium these strong longitudinal muscle fibres are arranged in a ball-shaped zone. However, some of these muscles extend also well over the common atrium, attaching around the gonopore (Fig. 10).

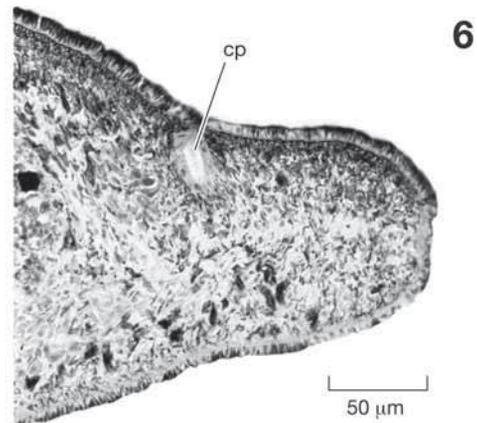
A nucleated bursal canal arises from the common atrium, curves over the ball-shaped mass of muscle around the male atrium and subsequently communicates with a sac-shaped copulatory bursa that lies directly in front of the penis bulb. The bursa contains remnants of a spermatophore; a ruptured spermatophore projects also out of the gonopore of specimen V.Pl. 3055.1 (Fig. 11). The bursal canal is covered by a layer of circular muscle and receives the openings of shell glands well below the point where the oviducts open separately into the canal.

Discussion.— Although the presumed apomorphic characters for the Dugesiidae, the presence of multicellular pigment cups with numerous retinal cells (cf. Sluys, 1989, but see Baguna *et al.*, 2001, Sluys, 2001 and Sluys and Kawakatsu, 2006 for a possible differ-



Fig. 4. *Weissius capaciductus*. ZMA V.Pl. 3059.1. Dorsal view of whole mount.

ent perspective on these characters) cannot be assessed on *W. capaciductus*, the species cannot be placed in the Planariidae or the Dendrocoelidae since it clearly does neither possess a common oviduct opening into the atrium, nor does it show the dendrocoelid type of pharynx. Therefore, the species is here assigned to the Dugesiidae.



Figs. 5, 6. *Weissius capaciductus*. 5, ZMA V.Pl. 3059.1. Dorsal view of head, showing the ciliated pits (indicated by arrows). 6, ZMA V.Pl. 3058.1. Microphotograph of ciliated pit.

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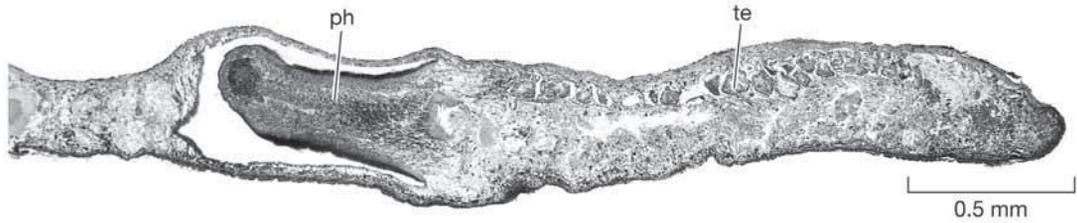


Fig. 7. *Weissius capaciductus*. Holotype. Microphotograph of sagittal section through testes. Anterior to the right.

Sluys (2001) pointed out and researched the problem that several of the current genera in the Dugesidae lack adequate, phylogenetic diagnoses, thus complicating the taxonomic placement of new species. Subsequent to his cladistic analysis of a morphological dataset, incorporating all dugesiid species, Sluys (2001) explored possible diagnostic features of the current genera through manipulation of his preferred 50% majority rule consensus tree. Although the study did not formally publish new diagnoses for the various genera, because of its exploratory nature, it forms nevertheless the most recent and up-to-date reference point for the taxonomy of dugesiid genera.

Considering the taxonomy of dugesiid species and genera as expressed in Sluys' (2001, fig. 7.15) "guestimated" tree, we find it impossible (1) to synonymize *W. capaciductus* with one of the currently known species, and (2) to fit the new species comfortably within

one of the current genera. Absence of a branched oviduct in *W. capaciductus* precludes assignment to *Spathula*, *Eviella*, and *Reynoldsonia*, while absence of a diverticulum makes it impossible to assign the species to *Romankenkius*. It does certainly not belong to *Dugesia* because it lacks the characteristic diaphragm of that genus. And neither does *W. capaciductus* show the double seminal vesicle that is characteristic for *Schmidtea*. The genera *Neppia* and *Girardia* are poorly defined from a phylogenetic point of view. However, in most species of *Neppia* the bursal canal is surrounded by a thick zone of circular muscle, which is absent in *W. capaciductus*. In contrast to *W. capaciductus*, many species of *Girardia* have a pigmented pharynx, while in *Girardia* species and also in aberrant *Bopsula*, the testes generally occur throughout the body.

An important feature in the taxonomy of dugesiid genera is the arrangement of the muscle layers around

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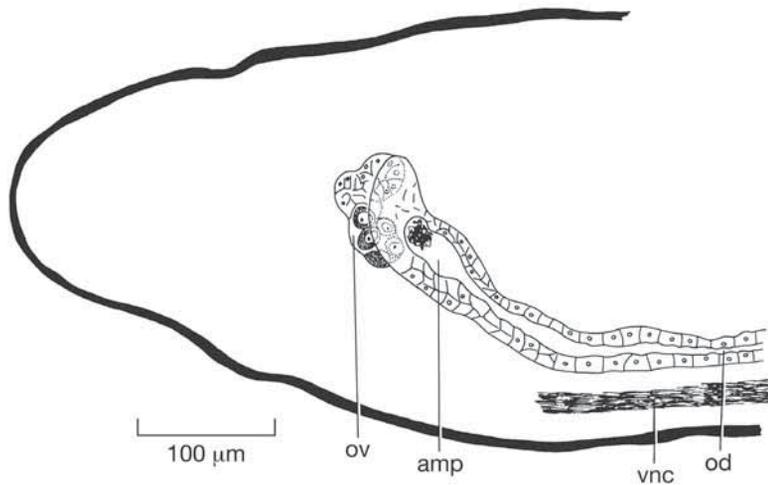


Fig. 8. *Weissius capaciductus*. Holotype. Sagittal reconstruction of ovary and oviducal ampulla. Anterior to the left.

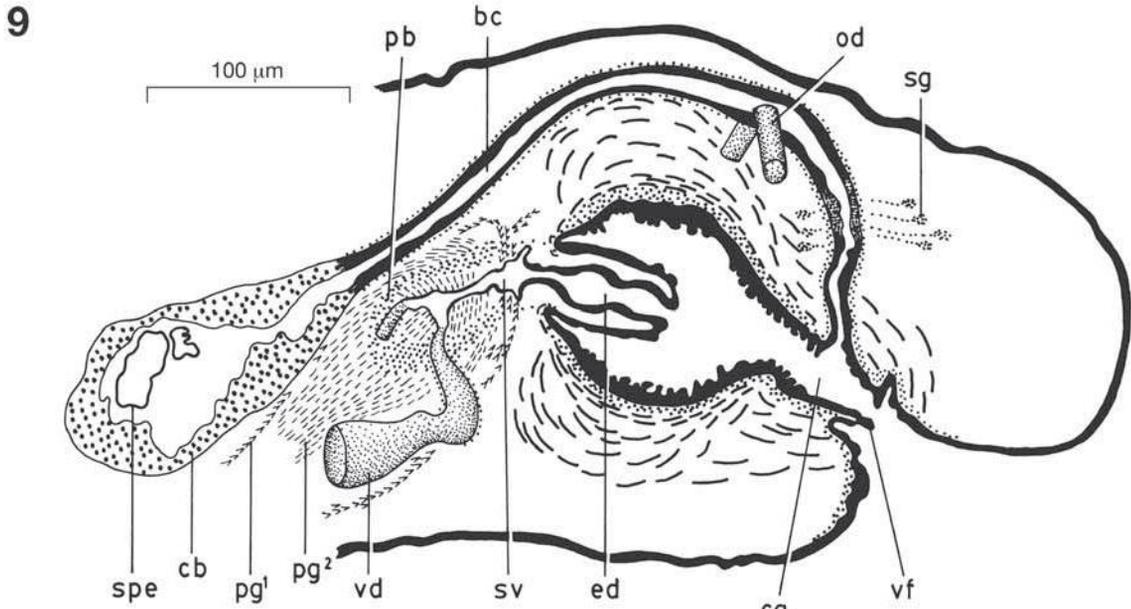
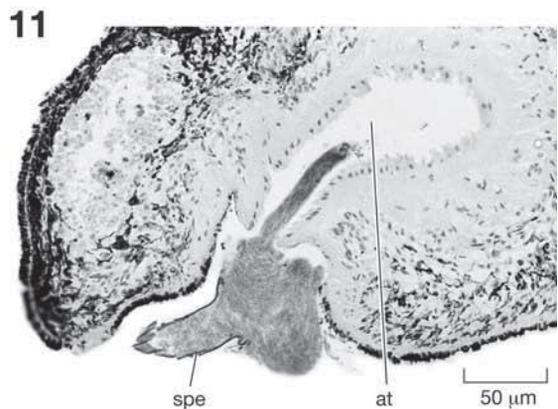
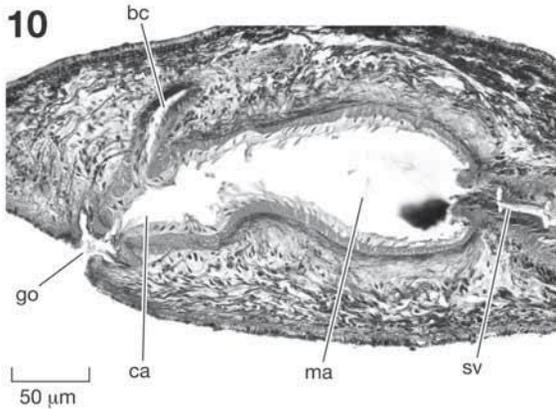


Fig. 9. *Weissius capaciductus*. Holotype. Sagittal reconstruction of the copulatory apparatus. Anterior to the left.

the bursal canal. In the non-reversed condition the bursal canal is surrounded by a subepithelial layer of circular muscle, followed by a layer of longitudinal muscle. In the reversed condition there is a subepithelial layer of longitudinal muscle, followed by a layer of circular fibres. For example, species of *Spathula*, *Romankenkius*, and *Dugesia* are generally characterized by a reversed musculature, whereas *Girardia* exhibits the non-reversed condition. However, the state of this particular phylogenetic and taxonomic character can-

not be assessed in *W. capaciductus* since it merely possesses one layer of muscle around the bursal canal, viz. a circular muscle layer. A single zone of circular muscle around the bursal canal is known also from *Cura pinguis* (cf. Sluys and Kawakatsu, 2001). In addition, there are a few other features that suggest a close relationship between *Weissius* and *Cura*.

Sluys (1997, 2001) restricted the genus *Cura* to the species *C. pinguis*, *C. foremanii*, *C. fortis*, *C. evelinae*, and *C. graffi*. In another paper (Grant et al., 2006) it is



Figs 10, 11. *Weissius capaciductus*. 10, ZMA V.Pl. 3057.1. Microphotograph showing musculature around atrium and gonopore. 11, AM W.29447. Microphotograph of spermatophore projecting out of gonopore. Anterior to the right.

argued that *C. graffi* does not belong to *Cura* and the species is tentatively transferred to the genus *Girardia*. *Weissius* resembles the four remaining species of *Cura* in that all five have a finger- or thumb-shaped penis papilla as well as a male atrium that is set off from the common atrium and at the same time is surrounded by broad zones of circular and longitudinal muscle fibres. The last-mentioned, compound feature suggests that *Weissius* belongs to the clade that already comprised *Cura* and *Schmidtea*, since Sluys (2001) postulated this compound character regarding the male atrium as a synapomorphy for the two last-mentioned genera. *Weissius* may be more closely related to *Schmidtea* since it lacks the short common oviduct and particularly the anteriorly displaced point of communication between atrium and bursal canal that are characteristic features for species of *Cura*. Although *Weissius* may be taxonomically close to *Schmidtea*, it cannot be assigned to this genus because it lacks the double seminal vesicle and the mixed bursal canal musculature of the last-mentioned genus. Furthermore, in *Schmidtea* the testes are situated dorsally and occur throughout the body, in contrast to the conditions in *Weissius*. As a consequence, the combination of features present in *W. capaciductus* requires the erection of a new genus, albeit that it is difficult to describe apomorphic features that uniquely identify the genus.

## Biogeography

This study is the first one dealing with the flatworm fauna of the artesian springs in Queensland since Sluys (1986) reported the first flatworm - from a completely different Order - from an artesian habitat in South Australia. The latter, *Promacrostomum palum* Sluys, 1986, turned out to be a unique species from a genus for which only two other species have been described, one living in Spain and Italy and the other in Lake Ohrid, former Yugoslavia. Presence of the Old World genus *Dugesia* in Australia was reported only relatively recently when Sluys *et al.* (1998) described *D. notogaea* Sluys and Kawakatsu, 1998 from localities in northern Queensland. Further records in the same region are detailed in Sluys and Kawakatsu (2001) and Grant *et al.* (2006), with the last-mentioned publication providing a distribution map of all published records. The Edgbaston locality from which the present paper describes the second species of *Dugesia* for Australia, is approximately

245 km removed (almost directly south) from the nearest site that yielded *Dugesia notogaea*, viz. Porcupine Creek, Porcupine Gorge National Park, Hughenden, Queensland.

It is interesting to note that from the Edgbaston pools and springs also two new endemic fish species were described, viz. the goby *Chlamydogobius squamigenus* Larson, 1995 and the blue-eye *Scaturiginichthys vermeilipinnis* Ivantsoff, Unmack, Saeed and Crowley, 1991 (Ivantsoff *et al.*, 1991; Larson, 1995).

Considering the distribution pattern of the genus (Fig. 12), one might entertain the idea that the ancestor of the present *Dugesia* species dispersed to Australia from Asia, probably some time during the Pleistocene, when sea levels were much lower. Freshwater planarians need contiguous freshwater bodies in order to be able to survive and disperse. However, paleogeographical reconstructions reveal that the river systems of Asia on the one hand and Australia/New Guinea on the other hand, have not been in contact during the Pleistocene (cf. Voris, 2000). This will have effectively prevented *Dugesia* to spread from Asia to New Guinea (where it is represented by the species *D. novaguineana* Kawakatsu, 1976) and Australia.

There are two options to explain the occurrence of *Dugesia* in Australia. One explanation could invoke jump dispersal, long-distance dispersal, which seems unlikely in view of the ecology of the animals. Second, if we look at the paleogeographical evolution of this area, one could hypothesize that future paleogeographical studies will show that during at least one period during the past 250,000 years, the river systems of Asia and Australia/New Guinea have been in contact with each other, thus enabling *Dugesia* to spread from its Old World main massing onto the Australian craton. However, the latter hypothesis will not be considered favourably by most paleogeographers.

It is even more difficult to explain the occurrence of monotypic *Weissius* (Fig. 12) and the presumably closely related *Cura pinguis* (the sole representative of its genus on the Australian continent) in Australia. The latter occurs also in New Zealand and New Caledonia. Furthermore, other species of *Cura* are distributed in New Zealand (*C. fortis*), South Africa (*C. evelinae*), and the eastern half of North America (*C. foremanii*). This extensive biogeographic track for the genus (cf. Grant *et al.*, 2006, fig. 71) probably implies that *Cura* is an old group that radiated well before Pleistocene times. The same may apply to the presumably closely related *Weissius*.

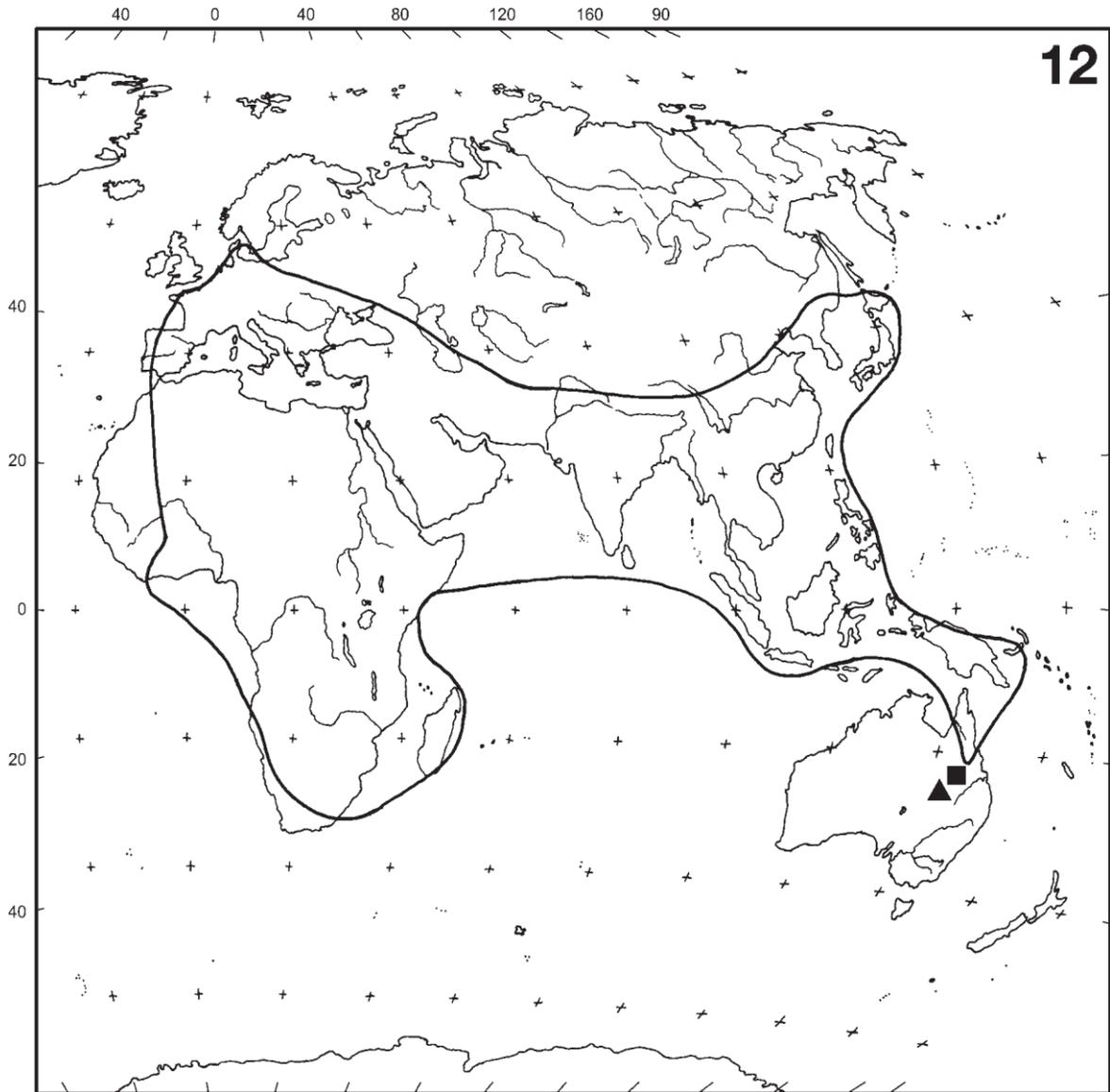


Fig. 12. Distribution of the genus *Dugesia* (extent of known range indicated by continuous line; after Sluys *et al.*, 1998), with the new localities for *D. artesia* (rectangle) and *W. capaciductus* (triangle).

### Acknowledgements

We are grateful to Dr. W.F. Ponder ( Australian Museum, Sydney) for making available to us the flatworm samples and for providing information on the sampling localities. The work of LJG on the freshwater planarians of Australia was supported by an ABRS grant for the project "Biodiversity, biogeography, and phylogeny of Australian aquatic planarians (Platyhelminthes, Tricladida, Paludicola)." Mr. J. van Arkel (IBED, University of Amsterdam) is thanked for the digital rendering of the illustrations.

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# **APPENDIX 1d: A contribution to the current species inventory for Australian Freshwater Triclad (Platyhelminthes, Tricladida)**

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**Lauryne Grant, Ronald Sluys and David Blair**

## **Introduction**

We recently provided a detailed account of an extensive collection of Australian freshwater triclads made by others many years ago (Grant et al. 2006). Apart from descriptions of new species, that study was the first to provide detailed GIS-based distribution maps for all Australian species known up to that moment. We now supplement that study with species descriptions, re-descriptions, and identifications based on newly and much more recently collected specimens. This new survey also was performed as part of LJG's PhD research project between 2002 and 2005. The states covered in this new investigation are New South Wales, Queensland, South Australia, Tasmania, Victoria, and Western Australia. The collection made represents the most wide-spread and thorough ever undertaken on the Australian continent. Please note, as this document is being prepared for publication, it does not incorporate any of the proposed changes to the classification as outlined in Chapter 3. As these changes will not be formalised at the time of publication it is thought that any reference to them would cause confusion.

## **Materials and methods**

Newly collected animals were sectioned at intervals of 5µm and stained with the trichrome stain Martius-Scarlet-Blue. A more detailed description of the materials and methods used to determine species identification can be found in Chapter 3. All histological material resulting from this thesis will be deposited in the Naturalis Biodiversity Centre, Leiden, The Netherlands (official registration numbers will be assigned in the published version of this manuscript). Molecular data were used in some cases to assist in confirming species identifications and often used to assign asexual, immature or non-sectioned individuals to a species group. In the 'material examined' section those species identified via molecular sequences have been identified as such. For details of the methods used for molecular identification, see Chapter 2 and Appendix 2c.

## APPENDIX 1d

A CONTRIBUTION TO THE CURRENT SPECIES INVENTORY FOR AUSTRALIAN FRESHWATER TRICLADS  
(PLATYHELMINTHES, TRICLADIDA)

**Abbreviations used in figures:** ad=adenodactyl, bc=bursal canal, br=brain, ca=copulatory apparatus, cat=common atrium, cb=copulatory bursa, cod=common oviduct, cp=ciliated pit, div=diverticulum, dg=diaphragm, e=eye, ed=ejaculatory duct, fgd=female genital duct, gd=gonoduct, gid=genito-intestinal duct, gl=glands, go=gonoduct, gp=gonopore, int=intestine, ma=male atrium, mc=muscular cavity, mo=mouth, od=oviduct, ol=oviducal loop, ov=ovary, pg=penial glands, ph=pharynx, pp=penial papilla, sf=sensory fossae, sg=shell glands, sp=sperm, spe=spermatophore, sph=sphincter, sv=seminal vesicle, te=testes, vd=vas deferens, vi=vitellaria.

## Systematic account

Order Tricladida Lang, 1884

Suborder Continenticola Carranza et al., 1998

Family Dugesiidae Ball, 1974

Genus *Cura* Strand, 1942

***Cura pinguis* (Weiss, 1909)**

## Material examined

### New South Wales:

LG22, Hunter River, New England Hwy, Fitzgerald Bridge, Aberdeen (32°09.532' S - 150°52.949' E), New South Wales, Australia, 27 January 2003, coll. L.J. Grant, sagittal sections, on 9 slides, sagittal sections on 8 slides, sagittal sections on 9 slides, horizontal sections on 4 slides, horizontal sections on 4 slides, sagittal sections on 6 slides.

LG131, Nepean River, Hawksbury road, just west of Agnes Banks, Yarramundi Bridge (33°36.782' S - 150°42.026' E), New South Wales, Australia, 26 January 2003, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 10 slides.

LG147, Euccumbene River, approximately 5km south of Kiandra on Snowy Mountain Highway (35°53.111' S - 148°30.806' E), New South Wales, Australia, 15 December

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2003, coll. L.J. Grant, sagittal sections on 10 slides, sagittal sections on 11 slides, sagittal sections on 15 slides, horizontal sections on 6 slides.

LG146, Jounama Creek, Snowy Mountain Highway, 40 km south of Tumut (35°33.928' S - 148°19.915' E), New South Wales, Australia, 15 December 2003, coll. L.J. Grant, sagittal sections on 4 slides.

LG28, Boorowa River, south Darby Falls, road off Frogmore - Graham road, Bennett Springs Bridge (34°01.454' S - 148°49.122' E), New South Wales, Australia, 24 January 2003, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 12 slides, horizontal sections on 7 slides, transverse sections on 12 slides, sagittal sections on 9 slides.

LG70, Billy's Creek, Armidale - Grafton Road, south of Nymboida (30°09.941' S - 152°34.611' E), New South Wales, Australia, 8 January 2003, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 7 slides, sagittal sections on 4 slides.

LG74, Bielsdown River, 2km north of Dorrigo above Dangar Falls (30°19.352' S - 152°42.846' E), New South Wales, Australia, 9 January 2003, coll. L.J. Grant, sagittal sections on 8 slides.

LG120, Shoalhaven River, Kings Highway, Warri Bridge, 15km west of Braidwood (35°20.621' S - 149°44.229' E), New South Wales, Australia, 18 January 2003, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 7 slides.

LG133, Burrawans Creek, Morton National Park, off Pearsons Road, on Belmore Falls Road, southwest of Robertson (34°37.139' S - 150°32.534' E), New South Wales, Australia, 22 January 2003, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 6 slides.

LG137, Wildes Meadow Creek, Wildes Meadow, Wildes Meadow Road (34°36.305' S - 150°31.155' E), New South Wales, Australia, 22 January 2003, coll. L.J. Grant, sagittal sections on 4 slides, sagittal sections on 10 slides, sagittal sections on 6 slides.

LG144, Three Mile Dam, 14km northeast of Cabramurra on Kiandra - Cabramurra Road (35°52' S - 148°27' E), New South Wales, Australia, 14 December 2003, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 8 slides, sagittal sections on 14 slides, horizontal sections on 4 slides.

LG148, Gang Gang Creek, Snowy Mountain Highway approximately 20kms south of Kiandra (35°56.006' S - 148°36.230' E), New South Wales, Australia, 15 December 2003, coll. L.J. Grant, sagittal sections on 6 slides.

LG150, Thredbo River, Thredbo (36°30.246' S - 148°18.300' E), New South Wales, Australia, 16 December 2003, coll. L.J. Grant, sagittal sections on 8 slides, sagittal

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sections on 8 slides, sagittal sections on 5 slides, horizontal sections on 3 slides,  
horizontal sections on 4 slides.

LG145, Ogilvies Creek, 40km from Khancoban on Khancoban - Caramurra road  
(36°02.240' S - 148°19.188' E, New South Wales, Australia, 14 December 2003, coll. L.J.  
Grant, sagittal sections on 7 slides, sagittal sections on 6 slides, sagittal sections on 7  
slides, horizontal sections on 3 slides, horizontal sections on 3 slides.

LG152, Khancoban Back Creek, near Murray Power Station, approximately 5km south of  
Khancoban on Alpine Way (36°15.264 - 148°11.589) New South Wales, Australia, 16  
December 2003, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 7 slides,  
sagittal sections on 6 slides, horizontal sections on 4 slides.

LG135, Lachlan River, southeast of Darby Falls on Darby Falls Road (33°56.932' S -  
148°51.717' E), New South Wales, Australia, 24 January 2003, coll. L.J. Grant, sagittal  
sections on 8 slides, sagittal sections on 6 slides, sagittal sections on 7 slides, horizontal  
sections on 5 slides.

LG20, Tuena Creek, south of Tuena on Junction Point Road (34°08.064' S - 149°19.914' E),  
New South Wales, Australia, 24 January 2003, coll. L.J. Grant, sagittal sections on 7  
slides, sagittal sections on 10 slides, sagittal sections on 10 slides, sagittal sections on  
11 slides, sagittal sections on 9 slides.

LG19, Abercrombie River, 2km north of Abercrombie at continuation of Point Junction  
road (33°57.316' S - 149°19.531' E), New South Wales, Australia, 24 January 2003, coll.  
L.J. Grant, sagittal sections on 10 slides, sagittal sections on 5 slides, sagittal sections on  
5 slides.

LG138, Macquarie River, Great Western Highway at Eglinton, north of Bathurst  
(33°23.052' S - 149°32.833' E), New South Wales, Australia, 25 January 2003, coll. L.J.  
Grant, sagittal sections on 10 slides, sagittal sections on 8 slides, horizontal sections on  
4 slides, sagittal sections on 8 slides, horizontal sections on 5 slides, sagittal sections on  
5 slides, sagittal sections on 6 slides, horizontal sections on 5 slides.

LG105, Gunny Bag Creek, Kangaroo Flat Road, south of Werrikimbe National Park  
(31°13.087' S - 152°05.434' E), New South Wales, Australia, 13 January 2003, coll. L.J.  
Grant, sagittal sections on 11 slides, sagittal sections on 7 slides, sagittal sections on 10  
slides, horizontal sections on 5 slides.

LG102, Gara River on Waterfall Way, east of Wollomombi (30°32.669' S - 151°47.835' E),  
New South Wales, Australia, 12 January 2003, coll. L.J. Grant, sagittal sections on 13  
slides.

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- LG153, Swampy Plain River at Geehis Campsite, on Alpine Road (36°23.090' S - 148°10.856' E), New South Wales, Australia, 16 December 2003, coll. L.J. Grant, sagittal sections on 11 slides, sagittal sections on 11 slides, sagittal sections on 5 slides, horizontal sections on 4 slides.
- LG126, Tributary of Mongarlow River, on River Road, near Monga State Forest, just off Braidwood Road (35°32.602' S - 149°55.823' E), New South Wales, Australia, 20 January 2003, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 6 slides, sagittal sections on 10 slides, horizontal sections on 5 slides.
- LG149, Yarrangobilly River, Snowy Mountain Highway 62km South Tumut (35°39.085' S - 148°27.797' E), New South Wales, Australia, 15 December 2003, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 7 slides, sagittal sections on 7 slides, horizontal sections on 3 slides.
- LG104, Tobins River, Tobins Road, Doyles River State Forest (31°21.397' S - 152°05.544' E), New South Wales, Australia, 13 January 2003, coll. L.J. Grant, sagittal sections on 14 slides, sagittal sections on 7 slides.
- LG117, Telegherry River, Chichester State Forest, near Barrington Tops National Park (32°13.385' S - 151°44.493' E), New South Wales, Australia, 15 January 2003, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 7 slides.
- LG122, Brogo River, 18km north of Bega on Pacific Highway, Greendale Road, Greendale Bridge (36°36.458' S - 149°50.830' E), New South Wales, Australia, 19 January 2003, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 7 slides, sagittal sections on 9 slides, horizontal sections on 4 slides, horizontal sections on 3 slides.
- LG151, Sandy Creek, at Tom Groggin Rest Area, Alpine Way (36°52.308' S - 148°08.056' E), New South Wales, Australia, 16 December 2003, coll. L.J. Grant, sagittal sections on 5 slides, sagittal sections on 7 slides.
- LG124, Lowdon Forest Park, Tallaganda State Forest, Lowdon Forest Road (35°30.632' S - 149°36.162' E), New South Wales, Australia, 30 January 2003, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 12 slides, horizontal sections on 4 slides.
- LG81, Basket Swamp Falls, Boona State Forest, approximately 23km north of Tenterfield (28°54.974' S - 152°10.472' E) New South Wales, Australia, 5 January 2003, coll. L.J. Grant, sagittal sections on 2 slides, sagittal sections on 13 slides, horizontal sections on 7 slides, horizontal sections on 8 slides.
- LG82, Carrolls Creek, Mt. Lindesay Road, approximately 30km north of Tenterfield (28°50.726' S - 152°06.036' E), New South Wales, Australia, 6 January 2003, coll. L.J.

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#### **A CONTRIBUTION TO THE CURRENT SPECIES INVENTORY FOR AUSTRALIAN FRESHWATER TRICLADS (PLATYHELMINTHES, TRICLADIDA)**

Grant, sagittal sections on 13 slides, sagittal sections on 11 slides, horizontal sections on 4 slides.

LG73, Deer Park Creek, Ebor - Dorrigo Road 25km west of Dorrigo (30°22.033' S - 152°31.490' E), New South Wales, Australia, 9 January 2003, coll. L.J. Grant, sagittal sections on 10 slides, sagittal sections on 10 slides, horizontal sections on 5 slides, sagittal sections on 8 slides, horizontal sections on 5 slides.

LG53, Styx River, Kempsey Road (bridge), just north of Styx State Forest (30°35.307' S - 152°09.906' E), New South Wales, Australia, 12 January 2003, coll. L.J. Grant, sagittal sections on 15 slides, sagittal sections on 7 slides, sagittal sections on 18 slides.

LG98, Gara River on Waterfall Way, east of Wollomombi (30°32.669' S - 151°47.835' E), New South Wales, Australia, 12 January 2003, coll. L.J. Grant, sagittal sections on 13 slides, sagittal sections on 12 slides, sagittal sections on 13 slides, horizontal sections on 8 slides, horizontal sections on 5 slides.

LG107, Gunny Bag Creek, Kangaroo Flat Road, south of Werrikimbe National Park (31°13.087' S - 152°05.434' E), New South Wales, Australia, 13 January 2003, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 7 slides, sagittal sections on 8 slides, horizontal sections on 4 slides, horizontal sections on 5 slides.

LG116, Dilgry River, Dilgry Creek Road, Barrington Tops National Park, (31°53.343' S - 151°32.288' E), New South Wales, Australia, 15 January 2003, coll. L.J. Grant, sagittal sections on 9 slides, sagittal sections on 9 slides, sagittal sections on 8 slides, horizontal sections on 4 slides.

LG119, Murrumbidge River, near bridge opposite Bungle Road, Gundagai (35°04.427' S - 148°06.735' E), New South Wales, Australia, 18 January 2003, coll. L.J. Grant, sagittal sections on 10 slides, sagittal sections on 9 slides, sagittal sections on 11 slides, horizontal sections on 4 slides, horizontal sections on 3 slides.

#### **Queensland:**

LG352, Birthday Creek, on road to Paluma Dam before Birthday Creek Falls, Paluma (18°58.568' S - 146°10.086' E), Queensland, Australia, 25 August 2003, coll. L.J. Grant, sagittal sections on 4 slides.

LG270, Morans Creek, Green Mountains, Lamington National Park (28°14'11.2" S - 153°08'01" E), Queensland, Australia, 24 August 2004, coll. A. Glaister, sagittal sections on 11 slides.

LG274, Coomera Rivulet, Coomera Rivulet Road, 20km southeast of Canungra (28°08'55.5" S - 153°09'48" E), Queensland, Australia, 24 August 2004, coll. A. Glaister, sagittal sections on 8 slides.

**APPENDIX 1d**A CONTRIBUTION TO THE CURRENT SPECIES INVENTORY FOR AUSTRALIAN FRESHWATER TRICLADS  
(PLATYHELMINTHES, TRICLADIDA)

LG275, Sandy Creek, 4km north of Conondale (26°42'20" S - 152°41'02.3" E), Queensland, Australia, 17 August 2004, coll. A. Glaister, sagittal sections on 6 slides, sagittal sections on 8 slides.

LG280, South Pine River, Mt. Glorious-Samford Road (27°21'59" S - 152°47'40.6" E), Queensland, Australia, 21 August 2004, coll. A. Glaister, sagittal sections on 7 slides.

LG281, West Gap Creek, Main Range National Park (28°03'49" S - 152°21'47.8" E), Queensland, Australia, 26 August 2004, coll. A. Glaister, sagittal sections on 6 slides, sagittal sections on 7 slides.

LG87, Mary Elizabeth Colemann Crossing, Bunya Road (26°50.256' S - 151°50.256' E), Queensland, Australia, 28 December 2002, coll. L.J. Grant, sagittal sections on 8 slides.

LG88, Barker Creek, first creek crossing road from Bunya Mountains to Nanango (26°49.793' S - 151°40.044' E), Queensland, Australia, 28 December 2002, coll. L.J. Grant, sagittal sections on 6 slides.

LG91, Yabba Creek, near Imbil (26°27.596' S - 152°39.793' E), Queensland, Australia, 30 December 2002, coll. L.J. Grant, sagittal sections on 9 slides, sagittal sections on 11 slides.

LG354, North Creek, Mt. Elliot, Mt. Elliot National Park (19°28' S - 146°58' E), Queensland, Australia, 21 July 2002, coll. L.J. Grant, sagittal sections on 2 slides, sagittal sections on 3 slides, sagittal sections on 4 slides, sagittal sections on 3 slides, sagittal sections on 3 slides.

LG355, Creek on Fredricksons Farm, Eungella (21°02.775' S - 148°36.172' E), Queensland, Australia, 7 September 2002, coll. L.J. Grant, sagittal sections on 3 slides, sagittal sections on 3 slides, sagittal sections on 3 slides.

LG80, Pike Creek, 41km southeast of Texas on Glenlyon Dam Road (28°59.385' S - 151°26.112' E), Queensland, Australia, 5 January 2003, coll. L.J. Grant, sagittal sections on 10 slides, sagittal sections on 8 slides, horizontal sections on 9 slides, horizontal sections on 5 slides.

**South Australia:**

LG177, Forth Creek, Morialta Conservation Park, (34°54.186' S - 138°47.117' E), South Australia, Australia, 31 December 2003, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 6 slides, sagittal sections on 6 slides, horizontal sections on 3 slides, horizontal sections on 2 slides.

LG170, Onkaparinga River (below Murray River inflow) (35°00.377' S - 138°47.964' E), South Australia, Australia, 30 December 2003, coll. L.J. Grant, sagittal sections on 3 slides, sagittal sections on 3 slides.

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- LG178, Spring at Wilpena Pound, Wilpena National Park (31°31.490' S - 138°36.335' E), South Australia, Australia, 2 January 2004, coll. L.J. Grant, sagittal sections on 5 slides.
- LG184, Meadows Creek, near Kyeema Conservation Park, north of Hope Forest (35°15.814' S - 138°39.122' E), South Australia, Australia, 4 January 2004, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 6 slides, sagittal sections on 7 slides, horizontal sections on 3 slides, horizontal sections on 3 slides.
- LG186, Hindmarsh River, near Hindmarsh Falls (35°28.031' S - 138°35.229' E), South Australia, Australia, 4 January 2004, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 7 slides, horizontal sections on 3 slides, sagittal sections on 8 slides.
- LG185, Parananacooka River in Second Valley at intersection with main road south (35°31.618' S - 138°13.680' E), South Australia, Australia, 4 January 2004, coll. L.J. Grant, sagittal sections on 8 slides.
- LG182, Deep Creek, Deep Creek Conservation Park (35°36.250' S - 138°14.723' E), South Australia, Australia, 4 January 2004, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 6 slides, sagittal sections on 7 slides, horizontal sections on 3 slides.
- LG171, Brownhill Creek, Brownhill Creek Road, Mitcham, Adelaide (34°59.314' S - 138°37.694' E), South Australia, Australia, 30 December 2003, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 6 slides, sagittal sections on 6 slides, horizontal sections on 3 slides, horizontal sections on 5 slides.
- LG173, Sturt River, Sturt Gorge Recreation Park (35°02.125' S - 138°34.499' E), South Australia, Australia, 30 December 2003, coll. L.J. Grant, sagittal sections on 5 slides, sagittal sections on 5 slides.
- LG263, River Torrens, Mahogany Avenue (34°51.942' S - 138°40.985' E), South Australia, Australia, 31 December 2003, coll. L.J. Grant, sagittal sections on 3 slides, sagittal sections on 3 slides, sagittal sections on 3 slides.
- LG183, Mt. Barker Creek, Mt. Barker (35°04.150' S - 138°51.479' E), South Australia, Australia, 4 January 2004, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 6 slides, sagittal sections on 6 slides, horizontal sections on 3 slides, horizontal sections on 3 slides.
- LG181, Victoria Creek on South Parra Road, Williamstown (34°40.647' S - 138°53.145' E), South Australia, Australia, 3 January 2004, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 7 slides, sagittal sections on 10 slides, horizontal sections on 4 slides, horizontal sections on 4 slides.

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LG179, Broughton River at Yacka (33°34.132' S - 138°26.675' E), South Australia, Australia, 2 January 2004, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 4 slides.

LG180, Mambray Creek, Mt. Remarkable National Park (32°50.023' S - 138°03.253' E), South Australia, Australia, 2 January 2004, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 7 slides.

**Tasmania:**

LG232, Carlton River, Arthur Highway, west of Copping, at intersection with small creek (42°48.922' S - 147°45.818' E), Tasmania, Australia, 25 January 2004, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 5 slides, sagittal sections on 6 slides, sagittal sections on 10 slides, horizontal sections on 5 slides.

LG234, Brushy Plains Rivulet, 10km southwest of Buckland (42°38.022' S - 147°36.283' E), Tasmania, Australia, 26 January 2004, coll. L.J. Grant, horizontal sections on 6 slides, sagittal sections on 7 slides, sagittal sections on 7 slides, horizontal sections on 6 slides, sagittal sections on 7 slides.

LG237, Prosper River, near Orford, next to Tasman Highway, (42°33.345' S - 147°49.835' E), Tasmania, Australia, 26 January 2004, coll. L.J. Grant, sagittal sections on 11 slides.

LG238, Brushy Creek, near Little Swan Port on Tasman Highway, (42°19.510' S - 147°56.902' E), Tasmania, Australia, 26 January 2004, coll. L.J. Grant, sagittal sections on 9 slides, sagittal sections on 8 slides.

LG240, Groom River, just east of Pyengana on Tasman Highway, (41°15.391' S - 148°03.212' E), Tasmania, Australia, 27 January 2004, coll. L.J. Grant, sagittal sections on 15 slides.

LG236, Coal River at Richmond (42°44.047' S - 147°26.342' E), Tasmania, Australia, 26 January 2004, coll. L.J. Grant, sagittal sections on 10 slides, sagittal sections on 7 slides, sagittal sections on 10 slides.

LG233, Sorrell Creek, creek from Mt. Wellington running north at Molesworth (42°48.211' S - 147°09.144' E), Tasmania, Australia, 25 January 2004, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 6 slides, sagittal sections on 7 slides.

LG241, North Esk, at Aplico road on the road to Ben Lomond National Park (41°29.775' S - 147°25.205' E), Tasmania, Australia, 27 January 2004, coll. L.J. Grant, sagittal sections on 19 slides, sagittal sections on 14 slides.

LG242, St Patricks River, next to Sidling State Forest (41°17.525' S - 147°24.429' E), Tasmania, Australia, 27 January 2004, coll. L.J. Grant, sagittal sections on 4 slides, sagittal sections on 5 slides.

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- LG252, Macquarie River, on road to Cressy approximately 10km southwest of Epping Forest (41°49.585' S - 147°15.588' E), Tasmania, Australia, 29 January 2004, coll. L.J. Grant, sagittal sections on 8 slides.
- LG227, Snug Falls, near Snug, approximately 8km south of Kingston (43°05.038' S - 147°13.380' E), Tasmania, Australia, 24 January 2004, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 7 slides, sagittal sections on 4 slides.
- LG230, "Fortescue Bay" Creek, at Fortescue Bay, Tasman National Park (43°08.752' S - 147°57.176' E), Tasmania, Australia, 25 January 2004, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 7 slides, sagittal sections on 6 slides.
- LG226, North West Bay River, at Longley, near Mt. Wellington (42°58.202' S - 147°11.841' E), Tasmania, Australia, 24 January 2004, coll. L.J. Grant, sagittal sections on 6 slides.
- LG218, Judds Creek at Judbury west of Huonville (42°59.587' S - 146°55.401' E), Tasmania, Australia, 22 January 2004, coll. L.J. Grant, sagittal sections on 5 slides, sagittal sections on 5 slides, sagittal sections on 6 slides, horizontal sections on 3 slides.
- LG217, Small creek in Geeveston (the more southern of 2 creeks that meet at Geeveston) (43°10.524' S - 146°55.423' E), Tasmania, Australia, 22 January 2004, coll. L.J. Grant, sagittal sections on 5 slides, coll. L.J. Grant, sagittal sections on 5 slides.
- LG228, Nicholas River, at Nicholas Rivulet on road between Cygnet Cove and Oyster Cove (43°08.931' S - 147°09.043' E), Tasmania, Australia, 24 January 2004, coll. L.J. Grant, sagittal sections on 5 slides.
- LG222, Lake Gordon, near Lake Gordon Dam at boat ramp, (42°44.019' S - 145°58.916' E), Tasmania, Australia, 23 January 2004, coll. L.J. Grant, sagittal sections on 6 slides.
- LG247, Meadowbank Lake, at "Meadowbank Bridge" (42°32.177' S - 146°44.478' E) Tasmania, Australia, 30 January 2004, coll. L.J. Grant, sagittal sections on 14 slides, sagittal sections on 11 slides, sagittal sections on 17 slides, sagittal sections on 6 slides, horizontal sections on 5 slides.
- LG239, Meridith River, just north of Swansee off Tasman Highway (42°06.982' S - 148°03.691' E), Tasmania, Australia, 26 January 2004, coll. L.J. Grant, horizontal sections on 5 slides, sagittal sections on 13 slides, sagittal sections on 6 slides, sagittal sections on 8 slides.
- LG224, Styx River, at Bushy Park (42°41.990' S - 146°53.467' E), Tasmania, Australia, 23 January 2004, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 8 slides, sagittal sections on 8 slides, horizontal sections on 4 slides.

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LG243, Murchinson River at Lake Rosebury (41°45.608' S - 145°37.405' E), Tasmania, Australia, 31 January 2004, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 6 slides.

LG259, Mersey River, approximately 2km west of Kimberley (41°23.881' S - 146°29.160' E), Tasmania, Australia, 1 February 2004, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 5 slides, sagittal sections on 5 slides.

LG250, Arthurs Lake, at Pumphouse Bay, just off Poatina Road (41°59.147' S - 146°51.617' E), Tasmania, Australia, 29 January 2004, coll. L.J. Grant, sagittal sections on 9 slides.

LG299, Sandy Bay Rivulet (42°54' - 147°19' E), Tasmania, Australia, 1 March 2003, coll. K. Richards, horizontal sections on 3 slides, sagittal sections on 8 slides.

LG248, Dee Lagoon (42°16.135' S - 146°34.981' E), Tasmania, Australia, 30 January 2004, coll. L.J. Grant, sagittal sections on 14 slides.

LG308, Approximately 15km northeast of Scottsdale, at intersection with Specs Road (04°33.41809' S - 147°39'46.99573" E), Tasmania, Australia, 4 June 2003, coll. K. Richards, sagittal sections on 5 slides.

**Victoria:**

LG190, Barham River on Barham River road approximately 6km from Appollo Bay (38°45.671' S - 143°47.804' E), Victoria, Australia, 8 January 2004, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 6 slides.

LG159, King River, Hamiltons Bridge, Upper King Valley road (36°49.265' S - 146°24.611' E), Victoria, Australia, 20 December 2003, coll. L.J. Grant, sagittal sections on 6 slides.

LG158, Dangdonvale River, Lake Buffalo - Whitfield road (36°48.332' S - 146°37.855' E), Victoria, Australia, 20 December 2003, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 7 slides, horizontal sections on 4 slides.

LG161, Eurobin Falls, Eurobin creek, Mt. Buffalo road, Mt. Buffalo (36°43.071' S - 146°50.539' E), Victoria, Australia, 21 December 2003, coll. L.J. Grant, sagittal sections on 5 slides, sagittal sections on 5 slides.

LG160, Steavensons River, in Buxton on Maroondah Highway (37°25.535' S - 145°42.423' E), Victoria, Australia, 20 December 2003, coll. L.J. Grant, sagittal sections on 7 slides.

LG209, Campaspe River in Redesdale near Lake Eppalock (37°00.973' S - 144°32.434' E), Victoria, Australia, 16 January 2004, coll. L.J. Grant, sagittal sections on 11 slides.

LG214, Sailors Creek near Lake Daylesford in Daylesford (37°20.968' S - 144°08.080' E), Victoria, Australia, 19 January 2004, coll. L.J. Grant, sagittal sections on 4 slides, sagittal sections on 4 slides, sagittal sections on 4 slides.

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- LG211, Wimmera River, on road between Elmhurst and Landsborough (37°09.305' S - 143°12.941' E), Victoria, Australia, 17 January 2004, coll. L.J. Grant, sagittal sections on 9 slides.
- LG169, Fyans Creek, at Borough Huts Camping Area, Central Grampians (37°13.392' S - 142°32.416' E), Victoria, Australia, 29 December 2003, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 7 slides.
- LG205, Olinda Creek, on Falls Road, Olinda State Forest (37°50.100' S - 145°21.998' E), Victoria, Australia, 14 January 2004, coll. L.J. Grant, sagittal sections on 5 slides.
- LG198, Sasafra Creek, along Monbulk Rd, Kays Picnic Ground (37°52.963' S - 145°23.335' E), Victoria, Australia, 14 January 2004, coll. L.J. Grant, sagittal sections on 4 slides.
- LG265, East Barwon River, downstream from Lake Elizabeth, Otways Ranges (38°33' S - 143°45' E), Victoria, Australia, 7 July 2003, coll. L.J. Grant, sagittal sections on 5 slides.
- LG192, Gellibrand River, Upper Gellibrand, just downstream of Steavenson Falls, west of Barramunga (38°34.306' S - 143°39.518' E), Victoria, Australia, 8 January 2004, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 6 slides.
- LG191, Aire River, at 'The Redwoods' on Aire Valley Road (38°40.128' S - 143°34.883' E), Victoria, Australia, 8 January 2004, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 6 slides.
- LG193, Erskine River, at falls, approximately 9km northwest of Lorne (38°30.474' S - 143°54.765' E), Victoria, Australia, 8 January 2004, coll. L.J. Grant, sagittal sections on 5 slides, sagittal sections on 5 slides.
- LG155, Tangil River, west branch on road to Mt Baw Baw, near Tangil Bren (37°48.891' S - 146°10.166' E), Victoria, Australia, 19 December 2003, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 5 slides, horizontal sections on 3 slides.
- LG166, Ovens River, Great Alpine Way, Harrietville (36°52.337' S - 147°03.796' E), Victoria, Australia, 22 December 2003, coll. L.J. Grant, sagittal sections on 5 slides, sagittal sections on 5 slides, horizontal sections on 4 slides.
- LG206, Upper Loddon - Guildford Creek, near Castlemain on Midland Hwy (37°08.839' S - 144°09.987' E), Victoria, Australia, 15 January 2004, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 7 slides, sagittal sections on 7 slides, horizontal sections on 7 slides, horizontal sections on 5 slides.
- LG196, Crystal Brook, Cardinia Reservoir, Narre Warren east (37°58.266' S - 145°23.594' E), Victoria, Australia, 14 January 2004, coll. L.J. Grant, sagittal sections on 9 slides, sagittal sections on 7 slides.

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- LG202, Monbulk Creek at intersection with Belgrave - Hallam Road, Selby Conservation Reserve (37°55.279' S - 145°21.340' E), Victoria, Australia, 14 January 2004, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 9 slides, sagittal sections on 7 slides.
- LG164, Crystal Brook, near Reservoir, Mt. Buffalo (36°33.366' S - 146°47.135' E), Victoria, Australia, 21 December 2003, coll. L.J. Grant, sagittal sections on 4 slides.
- LG199, Lerderderge River, in Bachus Marsh at intersection with Bachus Marsh - Gisborn Road (37°39.287' S - 144°27.054' E), Victoria, Australia, 14 January 2004, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 6 slides, sagittal sections on 7 slides, horizontal sections on 2 slides.
- LG163, Mountain Creek, Mountain Creek Road, east of Tawonga (36°42' S - 147°15' E), Victoria, Australia, 21 December 2003, coll. L.J. Grant, sagittal sections on 4 slides, sagittal sections on 4 slides.
- LG212, Avoca River, in Avoca (37°05.451' S - 143°28.325' E), Victoria, Australia, 17 January 2004, coll. L.J. Grant, sagittal sections on 5 slides, sagittal sections on 6 slides.
- LG278, Agnes River, at the base of Agnes Falls, north of Toora (38°38'39" S - 146°22'15.4" E), Victoria, Australia, 11 August 2005, coll. A. Glaister, sagittal sections on 12 slides.
- LG210, Bet Bet Creek, in Bung Bong (37°06.221' S - 143°33.589' E), Victoria, Australia, 17 January 2004, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 5 slides, sagittal sections on 7 slides, horizontal sections on 4 slides.
- LG156, Maroonda Dam, Healsville Outflow (37°38.624' S - 145°32.974' E), Victoria, Australia, 19 December 2003, coll. L.J. Grant, sagittal sections on 5 slides, horizontal sections on 5 slides, sagittal sections on 12 slides, horizontal sections on 5 slides, sagittal sections on 9 slides.
- LG194, Balcombe Creek, Mt. Eliza Regional Park (38°12.595' S - 145°06.068' E), Victoria, Australia, 11 January 2004, coll. L.J. Grant, sagittal sections, on 6 slides, sagittal sections on 7 slides, sagittal sections on 9 slides, horizontal sections on 4 slides, horizontal sections on 4 slides.
- LG200, Jacksons Road, Sunbury Road, Sunbury (37°35.071' S - 144°44.534' E), Victoria, Australia, 14 January 2004, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 6 slides, sagittal sections on 10 slides, horizontal sections on 3 slides.
- LG213, Leigh River, Shelford, near Geelong (38°00.853' S - 143°58.654' E), Victoria, Australia, 19 January 2004, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 7 slides.

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LG188, Curdies River, near Brucknell, west of Timboon (38°28.179' S - 142°56.562' E), Victoria, Australia, 7 January 2004, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 8 slides.

LG162, Buckland River, Buckland Valley Road, near Mt. Buffalo (36°47.699' S - 146°50.898' E), Victoria, Australia, 21 December 2003, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 5 slides, sagittal sections on 6 slides, horizontal sections on 3 slides.

LG266, Fern Tree Gully Creek, Sherbrooke Forest, Dandenong Ranges National Park (37°53' S - 145°21' E), Victoria, Australia, 13 October 2002, coll. L.J. Grant, sagittal sections on 3 slides.

#### **Western Australia:**

LG368, Beedelup Brook, outflow from small dam, below falls (34°25.294' S - 115°51.782' E), Western Australia, Australia, 9 October 2004, coll. L.J. Grant, and K. Evertz, sagittal sections on 3 slides, sagittal sections on 5 slides.

LG372, Circular Pool, Franklin River, Mt. Franklin National Park (34°56.634' S - 116 ° 48.013' E), Western Australia, Australia, 7 October 2004, coll. L.J. Grant and K. Evertz, sagittal sections on 5 slides.

LG369, "Scarpe Road Creek" at intersection with Scarpe Road, just off Pinjarra - Williams Road (32°42.253' S - 116°00.011' E), Western Australia, Australia, 13 October 2004, coll. L.J. Grant, and K. Evertz, sagittal sections on 4 slides.

LG364, Ellen Brook, Caves Road crossing, northwest of Margaret River (33°54.433' S - 115°01.832' E), Western Australia, Australia, 11 October 2004, coll. L.J. Grant, and K. Evertz, sagittal sections on 2 slides, sagittal sections on 6 slides, sagittal sections on 4 slides.

#### **Specimens Identified by Molecular Sequence Data**

##### **New South Wales:**

LJ277, Eucumbene River (35°53.111' S - 148°30.806' E), New South Wales, Australia, 15 December 2003, coll. L.J. Grant, identified via molecular samples.

LJ372, Guy Fawkes River, Ebor (30°24'20" S - 152°20'49.7" E), New South Wales, Australia, 31 August 2004, coll. L.J. Grant, identified via molecular samples.

##### **Queensland:**

LJ181, creek crossing on Mount Lewis Road, Mount Lewis State Forest (16°34.313' S - 145°15.893' E), Queensland, Australia, 31 August 2003, coll. L.J. Grant, identified via molecular samples.

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LJ194, Charmillain Creek, approximately 10km south of Ravenshoe on Tully Falls Road, Ravenshoe State Forest (17°41.965' S - 145°31.403' E), Queensland, Australia, 29 August 2003, coll. L.J. Grant, identified via molecular samples.

LJ210, Forbes River, Forbes River Road, near Birdwood (31°20.706' S - 152°20.453' E), New South Wales, Australia, 13 January 2003, coll. L.J. Grant, identified via molecular samples.

LJ265, creek at Mount Bartle Frere, Bobbin Bobbin Falls, just below west track (17°22' S - 145°47' E), Queensland, Australia, 6 September 2003, coll. L.J. Grant, identified via molecular samples.

MR, Mitchell River, approximately 5km south of Mount Carbine (16°34.591' S - 145°07.167' E), Queensland, Australia, 5 September 2003, coll. L.J. Grant, identified via molecular samples.

**Victoria:**

LG264, West Barwon River, just off Forrest-Apollo Bay Road (38°32' S - 143°42' E), Victoria, Australia, 7 July 2003, coll. L.J. Grant, identified via molecular samples (LJ100).

LG208, Trentham Falls, Upper Coliban River, near Trentham (37°22.211' S - 144°19.544' E), Victoria, Australia, 15 January 2004, coll. L.J. Grant, identified via molecular samples (LJ155).

LJ282, Maroondah Dam (37°37' S - 145°31' E), Victoria, Australia, 19 December 2003, coll. L.J. Grant, identified via molecular samples.

LG267, Main Creek, running through Greens Bush, Mornington Peninsula National Park (38°27' S - 144°54' E), Victoria, Australia, 11 October 2002, coll. L.J. Grant, identified via molecular samples (V1).

LJ150, Buckland River, Buckland Valley Road, near Mount Buffalo (36°47.699' S - 146°50.898' E), Victoria, Australia, 21 December 2003, coll. L.J. Grant, identified via molecular samples.

**Tasmania:**

LJ110, Prosper River, near Orford, next to Tasman Hwy (42°33.345' S - 147°49.835' E), Tasmania, Australia, 26 January 2004, coll. L.J. Grant, identified via molecular samples.

LJ144, Lake Burbury, near boat ramp at point where small creek enters (42°08.643' S - 145°39.029' E), Tasmania, Australia, 31 January 2004, coll. L.J. Grant, identified via molecular samples.

LG260, Leven River, at junction with small creek at Gunns Plains (41°16.146' S - 146°01.656' E), Tasmania, Australia, 1 February 2004, coll. L.J. Grant, identified via molecular samples (LJ30).

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LJ33, Ringarooma River, at intersection with Tasman Hwy, at Derby (41°08.900' S - 147°48.417' E), Tasmania, Australia, 27 January 2004, coll. L.J. Grant, identified via molecular samples.

#### **Western Australia:**

LJ125, Warren River, at Cascades, Pemberton (34°28.604' S - 116°01.784' E), Western Australia, Australia, 8 October 2004, coll. L.J. Grant, identified via molecular samples.

LG373, Deep Creek, Fernhook Falls, Mount Franklin National Park (34°49.127' S - 116°35.527' E), Western Australia, Australia, 6 October 2004, coll. L.J. Grant, identified via molecular samples (LJ172, LJ173).

LJ175, Shannon River, Shannon Dam, Shannon National Park (34°34.898' S - 116°24.771' E), Western Australia, Australia, 8 October 2004, coll. L.J. Grant, identified via molecular samples.

LJ177, Turtle Brook, flowing into the Canning River, Canning Dam (32°09' S - 116°07' E), Western Australia, Australia, 3 October 2004, coll. L.J. Grant, identified via molecular samples.

LJ53, Ellen Brook, at intersection with Caves Road, northwest of Margaret River (33°54.433' S - 115°01.832' E), Western Australia, Australia, 11 October 2004, coll. L.J. Grant, identified via molecular samples.

#### **South Australia:**

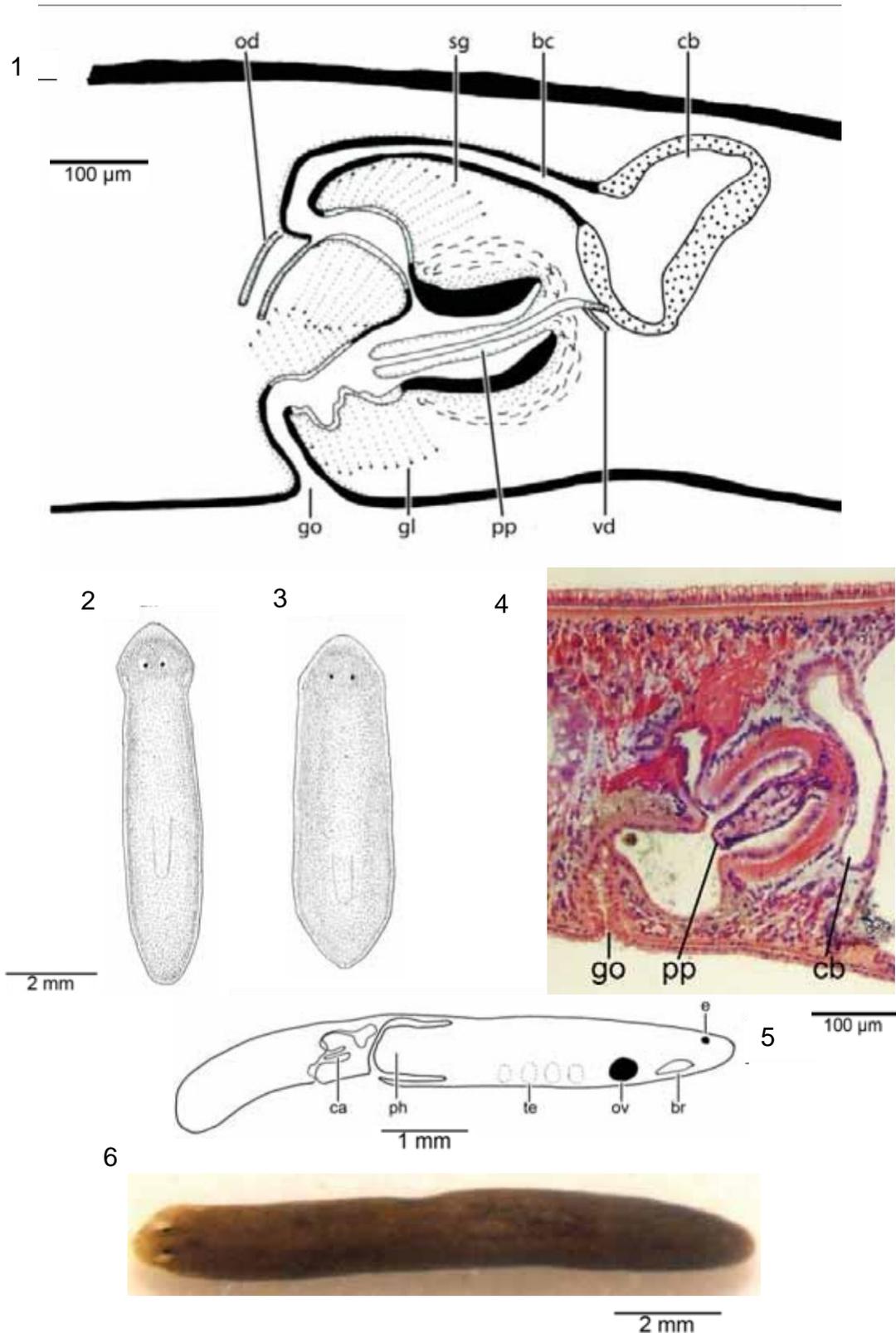
LG172, Onkaparinga River, upstream from Murray River inflow (35°00.377' S - 138°47.964' E), South Australia, Australia, 30 December 2003, coll. L.J. Grant, identified via molecular samples (LJ169).

## **Description**

This species is highly polymorphic in regards to external morphology and the below description is an attempt to create a generalised description of the external morphology from all the available data. The largest live specimens are 15mm x 3mm. Heads are triangulate with no (Figure 3) to small (Figure 2) rounded auricles, the tails are consistently rounded (Figure 6). Eyes are small or large, sitting in small or large pigment free patches, respectively, much closer to each other than to the lateral margins and approximately equal to auricular points. There are small pigment free patches on the lower portion of the auricles. The dorsal surface base colour was light brown/beige and covered with fine, dense black specks.

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**Figures 1-6** *Cura pinguis*. 1, LG124-3, Sagittal reconstruction of the copulatory apparatus; 2, LG145, external features of living animal; 3, LG153, external features of living animal; 4, LG222-1, microphotograph of a sagittal section of the copulatory apparatus; 5, LG124-3, sagittal reconstruction of the reproductive system; 6, LG194, photograph of live specimen.

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The overall colour impression ranged between light brown and black depending upon the density of the specs. The ventral surface is pigmented but to a lesser extent than the dorsal surface. There is evidence of two sets of very shallow sensory fossae found in most specimens. The pharynx is positioned medially and occupies approximately one-sixth of the total body length. Testes are positioned ventrally, pre-pharyngeal, and are not numerous. The large, irregular ovaries are located a short distance behind the brain (Figure 5). The internal morphology has been described many times and I have discovered no discrepancies to warrant another description here.

## Discussion

*Cura pinguis* has been described from Australia on many occasions and is easily identifiable owing to the “finger-shaped” penial papilla and the dorsal communication of the bursal canal (Figures 1, 4 & 6). The only feature within the copulatory apparatus that is still questionable is the presence/absence of a common oviduct (Grant et al. 2006). All of the specimens inspected above exhibit separate communication of the oviducts with the bursal canal (Figure 1). As these specimens have been collected from all over Australia I believe that this feature is consistent for all Australian *Cura pinguis*. Attempts to categorise the polymorphic external morphology according to specimen origin (e.g. Australia/New Zealand) have been made (Ball 1974b). It has been my observation that it is impossible to assign morphological types based on locality, as this species expresses polymorphism within drainage divides and often within populations. The morphological types illustrated above (Figures 2-3) represent the morphological extremes for *Cura pinguis* with many variations on this theme being expressed throughout Australia.

## Ecology and distribution

*Cura pinguis* is the most widespread of all Australian species having been collected from all Australia states and territories, excluding the Northern Territory, and is therefore found across a diverse range of both lentic and lotic habitats. This species is found most commonly on the off-channel area of streams and rivers on the underside of rocks. Worms are often associated with leaf litter and in the absence of rocks can be found on macrophytes. The substrates of the habitats are variable, including mud, sand, gravel, cobble, and bedrock. *Cura pinguis* appears to be adept at taking advantage of residual pools left in drying streambeds, which are a common feature of Australian freshwater systems.

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### Genus *Dugesia* Girard, 1850

#### *Dugesia notogaea* Sluys & Kawakatsu, 1998

#### Material examined

##### New South Wales:

LG102, Gara River on Waterfall Way, east of Wollomombi (30°32.669' S - 151°47.835' E), New South Wales, Australia, 12 January 2003, coll. L.J. Grant, sagittal sections on 11 slides.

LG67, Blaxlands Creek, Armidale-Grafton Road, southwest of Coult's Crossing (29°53.719' S - 152°47.411' E), New South Wales, Australia, 8 January 2003, coll. L.J. Grant, sagittal sections on 11 slides, sagittal sections on 10 slides.

##### Queensland:

LG347, Small unnamed creek, off Bruce Highway, approximately 10km north of Tully (17°51.798' S - 145°59.084' E), Queensland, Australia, 24 July 2003, coll. L.J. Grant, sagittal sections on 3 slides, sagittal sections on 2 slides, sagittal sections on 3 slides.

LG384, Jarrah Creek, Tully Gorge Road, approximately 10km west of Tully (17°53.824' S - 145°51.055' E), Queensland, Australia, 28 August 2003, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 4 slides.

LG348, Emmogen Creek, Cape Tribulation-Cooktown Road, approximately 10km north of Cape Tribulation, (16°02.375' S - 145°27.437' E), Queensland, Australia, 2 September 2003, coll. L.J. Grant, sagittal sections on 4 slides, sagittal sections on 5 slides, sagittal sections on 8 slides.

LG357, Ross River, near Rowing Club, Douglas, Townsville, (19°16' S - 146°50' E) Queensland, Australia, 25 October 2002, coll. L.J. Grant, sagittal sections on 2 slides,

LG283, Burnett River, Mt. Perry Road (25°23'54" S - 151°46'38.6" E), Queensland, Australia, 15 August 2006, coll. A. Glaister, horizontal sections on 4 slides, sagittal sections on 6 slides.

LG346, Mossman River, Mossman Gorge National Park (16°28.252' S - 145°19.853' E), Queensland, Australia, 31 August 2003, coll. L.J. Grant, sagittal sections on 3 slides, sagittal sections on 3 slides.

LG345, Creek flowing into Bloomfield River, approximately 5km north of Bloomfield (15°57.204' S - 145°20.934' E), Queensland, Australia, 2 September 2003, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 6 slides, sagittal sections on 6 slides.

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LG344, Dalrymple Creek, bridge on Hawkins Creek Road, 20km west of Ingham, (18°32.857' S - 146°02.457' E), Queensland, Australia, 27 August 2003, coll. L.J. Grant, sagittal sections on 5 slides, sagittal sections on 7 slides, sagittal sections on 8 slides.

LG341, Fletcher Creek, Lynd Highway (19°48.922' S - 146°03.240' E), Queensland, Australia, 23 November 2003, coll. L.J. Grant, sagittal sections on 4 slides, sagittal sections on 4 slides, sagittal sections on 4 slides.

LG383, Rocky River, Silver Plains, near campsite and at junction with Silver Plains Road (13°47' S - 143°32' E), Queensland, Australia, 9 November 2002, coll. L.J. Grant, sagittal sections, on 7 slides, sagittal sections on 7 slides.

LG342, Mc Cleod River, north of Mt. Carbine (16°31' S - 144°56' E), Queensland, Australia, 13 November 2002, coll. L.J. Grant, sagittal sections on 3 slides, sagittal sections on 6 slides.

LG65, Gregory River, Dingo Beach Road (20°18.018' S - 148°32.838' E), Queensland, Australia, 18 December 2002, coll. L.J. Grant, horizontal sections on 4 slides, horizontal sections on 4 slides, sagittal sections on 8 slides, sagittal sections on 9 slides, sagittal sections on 11 slides.

LG60, Cattle Creek, Mattie O'Niell Bridge near Eungella (21°093.91' S - 148°44.033' E), Queensland, Australia, 21 December 2002, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 9 slides, sagittal sections on 7 slides, horizontal sections on 6 slides, horizontal sections on 7 slides.

**Western Australia:**

LG363, Cherevondina Pool, Millstream National Park (21°35.360' S - 117°04.300' E), Western Australia, Australia, 11 September 2004, coll. L.J. Grant, sagittal sections on 4 slides, sagittal sections on 5 slides, sagittal sections on 4 slides.

LG371, Fortescue River (south branch), Hamersly Gorge (22°15.441' S - 117°59.173' E) Western Australia, Australia, 8 September 2004, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 7 slides.

LG323, Fortescue River, Fortescue River System (22°29' S - 118°33'6" E), Western Australia, Australia, 1 October 95, coll. C.A.L.M., sagittal sections on 10 slides, sagittal sections on 6 slides.

LG333, Fortescue River, Fortescue River System (31°34'46" S - 117°5'8" E), Western Australia, Australia, 2 October 95, coll. C.A.L.M., sagittal sections on 4 slides.

LG314, Weeli Wolli Creek, Fortescue River System (22°55' S - 119°12'3" E), Western Australia, Australia, 2 September 2001, coll. C.A.L.M. sagittal sections on 8 slides.

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### **Specimens Identified by Molecular Sequence Data**

#### **New South Wales:**

LG45, Nymboida River at Nymboida, Armidale-Grafton Road (29°57.285' S - 152°43.590' E), New South Wales, 8 January 2003, coll. L.J. Grant, identified via molecular samples.

#### **Queensland:**

LJ182, Hutchinsons Creek, near Cow Bay on Cape Tribulation Road (16°17.997' S - 145°25.366' E), Queensland, Australia, 1 September 2003, coll. L.J. Grant, identified via molecular samples.

LJ183, Oliver Creek, just south of Noah Head on Cape Tribulation Road (16°08.263' S - 145°26.458' E), Queensland, Australia, 1 September 2003, coll. L.J. Grant, identified via molecular samples.

LJ185, Kauri Creek, Mount Haig, Lamb Range, southwest of Cairns (17°05.952' S - 145°31.544' E), Queensland, Australia, 30 August 2003, coll. L.J. Grant, identified via molecular samples.

LJ186, Davies Creek, Davies Creek National Park, camping site approximately 22km east of Mareeba (17°00.300' S - 145°34.231' E), Queensland, Australia, 31 August 2003, coll. L.J. Grant, identified via molecular samples.

LJ189, Noel Creek, off Pullom Road, east Palmerston (17°35.236' S - 145°50.061' E), Queensland, Australia, 28 August 2003, coll. L.J. Grant, identified via molecular samples.

LJ264, Babinda Creek, at The Boulders, west of Babinda (17°20.502' S - 145°52.161' E), Queensland, Australia, 6 September 2003, coll. L.J. Grant, identified via molecular samples.

LJ267, Broadwater Creek, Broadwater State Forest, Broadwater Creek Camping Area, near Abergowie (18°24.966' S - 145°56.712' E), Queensland, Australia, 26 August 2003, coll. L.J. Grant, identified via molecular samples.

LJ278, Dalrymple Creek, just north of Ingham on Bruce Hwy (18°29' S - 146°04' E), Queensland, Australia, 27 August 2003, coll. L.J. Grant, identified via molecular samples.

LJ290, Harveys Creek, approximately 2km south of Deral on Bruce Hwy (17°15.620' S - 145°55.299' E), Queensland, Australia, 27 July 2003, coll. L.J. Grant, identified via molecular samples.

LJ291, Oliver Creek, just south of Noah Head on Cape Tribulation Road (16°08.263' S - 145°26.458' E), Queensland, Australia, 2 September 2003, coll. L.J. Grant, identified via molecular samples.

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- LJ4, Whynabeel Creek, approximately 1km east of Karnak on Whynabeel Road (16°23.313' S - 145°20.218' E), Queensland, Australia, 1 September 2003, coll. L.J. Grant, identified via molecular samples.
- LJ41, Burdekin River, and intersection with Flinders Hwy at McCrossin Bridge (19°59.920' S - 146°26.064' E), Queensland, Australia, 15 November 2003, coll. L.J. Grant, identified via molecular samples.
- LJ45, main creek through Ceder Bay National Park, at union of two creeks (15°48.497' S - 145°19.123' E), Queensland, Australia, 2 September 2003, coll. L.J. Grant, identified via molecular samples.
- LJ5, Little Crystal Creek, at intersection with Mount Spec Road, Paluma (19°00.941' S - 146°15.980' E), Queensland, Australia, 25 August 2003, coll. L.J. Grant, identified via molecular samples.
- LJ60, Jumrum Creek, Rob Veivers Drive Environmental Park, Kuranda, (16°49.177' S - 145°38.038' E), Queensland, Australia, 26 July 2003, coll. L.J. Grant, identified via molecular samples.
- LJ61, O'leary Creek, Old Culpa Road, Koombaloomba State Forest (17°56.962' S - 145°39.125' E), Queensland, Australia, 29 August 2003, coll. L.J. Grant, identified via molecular samples.
- LJ62, Small Creek on Kennedy Road, approximately 8km west of Kennedy (18°12.896' S - 145°52.189' E), Queensland, Australia, 27 August 2003, coll. L.J. Grant, identified via molecular samples.
- LJ7, union of tributary and Mulgrave River, Gouldsbrough Valley State Forest, just past camping ground (17°14.404' S - 145°46.468' E), Queensland, Australia, 4 September 2003, coll. L.J. Grant, identified via molecular samples.
- NQ10, stream leading into Rocky River, Silver Plains, Coen (13°48' S - 143°33' E), Queensland, Australia, 9 November 2002, coll. L.J. Grant, identified via molecular samples.
- NQ12, Nesbit River, Silver Plains, Coen (13°33' S - 143°35' E), Queensland, Australia, 11 November 2002, coll. L.J. Grant, identified via molecular samples.
- NQ15, Mary Creek, south of Mount Carbine, at intersection with Peninsula Developmental Road (16°36' S - 145°10' E), Queensland, Australia, 13 November 2002, coll. L.J. Grant, identified via molecular samples.
- NQ9, Dowlings Water Hole, Laura River, Lakefield National Park (15°04' S - 144°08' E), Queensland, Australia, 8 November 2002, coll. L.J. Grant, identified via molecular samples.

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LG90, Yabba Creek, at intersection with Yabba Creek Road, near Imbil (26°27.596' S - 152°39.793' E), Queensland, Australia, 30 December 2002, coll. L.J. Grant, identified via molecular samples (Q15).

LG26, Burdekin River, Macrossins Bridge approximately 20km east of Charters Towers (19°59.925' S - 146°26.241' E), Queensland, Australia, 16 December 2002, coll. L.J. Grant, identified via molecular samples.

#### **Western Australia:**

LG378, Palm Pool, Fortescue River, Millstream National Park (21°34.138' S - 117°03.200' E), Western Australia, Australia, 11 January 2004, coll. L.J. Grant, identified via molecular samples (LJ16).

LJ164, Joffre Falls, Joffre Gorge, Karijini National Park (22°23.495' S - 118°16.137' E), Western Australia, 7 September 2004, coll. L.J. Grant, identified via molecular samples.

LJ179, Tunnel Creek, Yandabiddy Pool on Turree Station, Newman (24°06' S - 118°24' E), Western Australia, 5 September 2004, coll. L.J. Grant, identified via molecular samples.

LJ255, creek flowing through Dales Gorge, Fortescue Falls, Karijini National Park (22°28.523' S - 118°33.086' E), Western Australia, Australia, 7 September 2004, coll. L.J. Grant, identified via molecular samples.

LG377, Kalamina Falls, creek flowing through Kallamina Gorge, Karijini National Park (22°25.025' S - 118°24.095' E), Western Australia, Australia, 7 September 2004, coll. L.J. Grant, identified via molecular samples (LJ256).

#### **Description**

Largest live specimen's 15mm x 3mm. There is some external polymorphism within *Dugesia notogaea*, but generalisations can be made. Specimens exhibit a triangulate head with well defined but small auricles and a pointed tail. Small eyes sitting in small pigment free patches located slightly anterior to auricular points and slightly closer to each other than to the lateral margins. There are small pigment free patches on the auricles, but there is no evidence of sensory pits or fossae. The base colour is light brown with fine, dark brown specks overlying this base. Many specimens have pigment interspersed with small pigment free patches throughout, giving a mottled appearance (Figures 13 & 14). However, some populations lack pigment-free patches (Figure 15). The presence of a pale or dark mid-dorsal stripe is commonly found in both mottled and non-mottled individuals (Figures 13 & 15). The ventral surface is light brown but does not exhibit any trace of the pigment free patches or dark pigment that is found on the dorsal surface.

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The pharynx is positioned medially and occupies approximately one-quarter of the total body length (Figure 16).

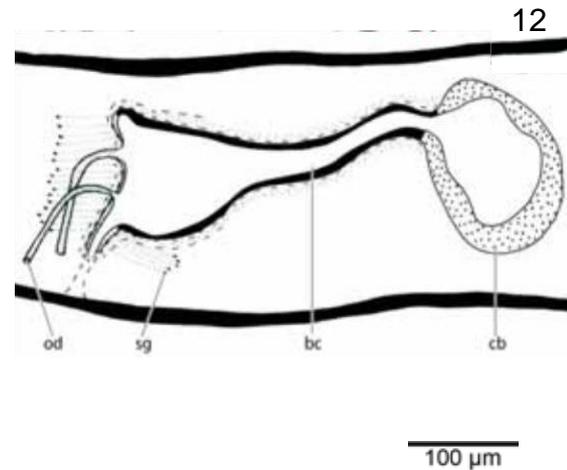
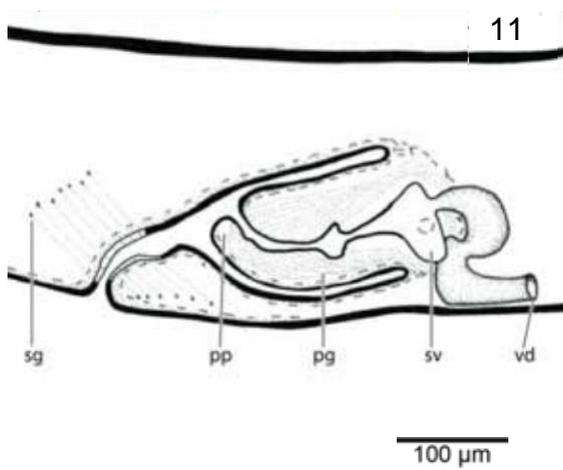
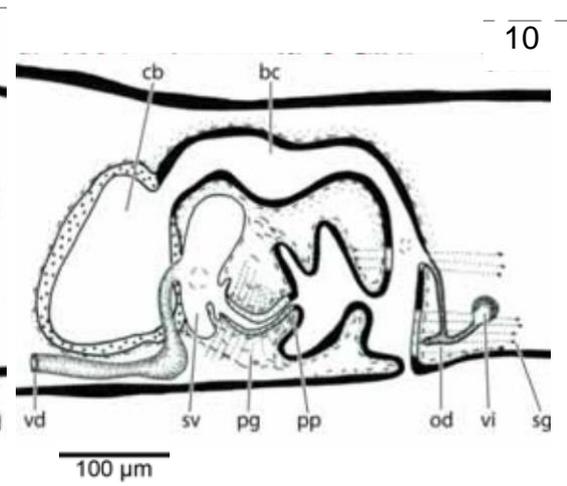
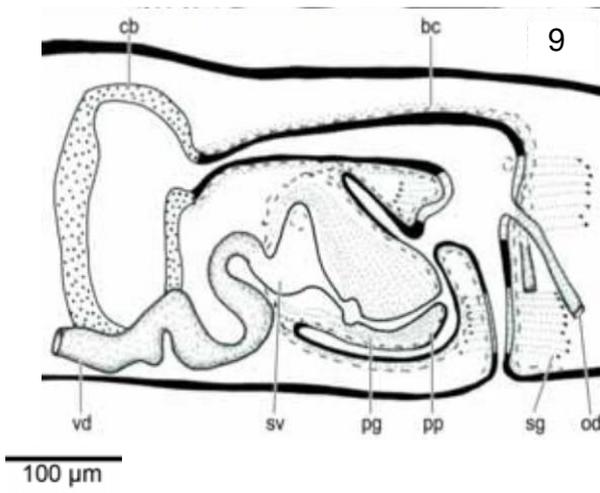
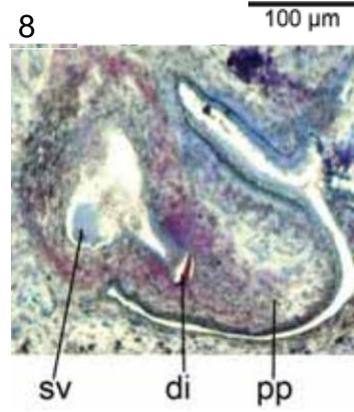
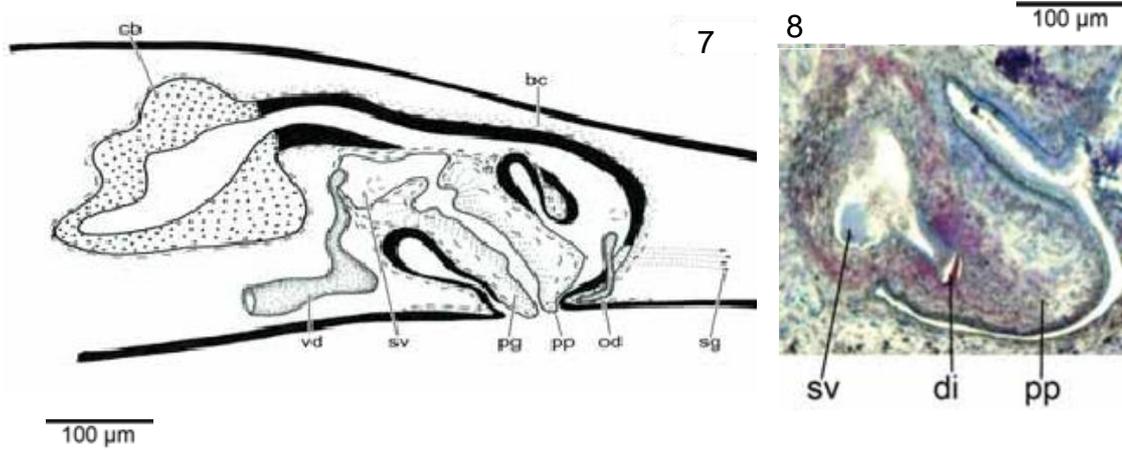
Testes are numerous, small, discrete follicles situated dorsally throughout the entire body length (Figure 16). The vasa deferentia are very broad ducts, which approach the penis bulb in a convoluted fashion. After penetrating the lightly muscled penis bulb the vasa deferentia communicate separately with a large seminal vesicle (Figures 7-11). This seminal vesicle then leads directly onto the narrow ejaculatory duct. Both the seminal vesicle and the ejaculatory duct have no associated musculature. Sitting closer to the seminal vesicle than to the papilla tip is a large, well defined diaphragm. The ejaculatory duct travels acentrally through the rounded penial papilla, creating a larger dorsal lip. There are many penial glands entering the ejaculatory duct, beginning at the level of the diaphragm and extending to the papilla tip. The male atrium is almost completely filled by the penial papilla and communicates with the female atrium via a narrow gap.

The nature of the bursal canal communication appears to vary between individuals, some show the female atrium at the same lateral plane as the male atrium (Figures 9-10), while in others it is positioned laterally (Figures 7 & 12); this could be due to minor variations in the way the individual was sectioned. In most cases the gonoduct is long, receives light glandularisation and is situated directly below the origin of the bursal canal. The female atrium is spacious and the dorsal surface gives rise to a broad bursal canal. The bursal canal has a short epithelium surrounded by a thin longitudinal muscle layer, followed by a thicker layer of circular muscle. A third layer of longitudinal muscular re-enforcement is visible around the lower portion of the bursal canal in many specimens; it is assumed that the absence of this feature in other specimens is a result of minor processing differences. The copulatory bursa is a large rounded structure with a thick epithelium occupying much of the dorso-ventral space.

The oviducts communicate separately with the copulatory apparatus and one communication is substantially further dorsal than the other (Figure 9). The difference is such that one appears to communicate with the female atrium while the other communicates with the most ventral section of the bursal canal. Glands enter the female atrium/bursal canal throughout from just below the lower oviduct to just above the higher oviduct. There is no caudal branch of the oviducts present. The ovaries are situated a short distance behind the brain; the oviducts communicate with the ovaries on the dorsal surface (Figure 16) and communicate also with many vitellaria throughout the body length (Figure 10).

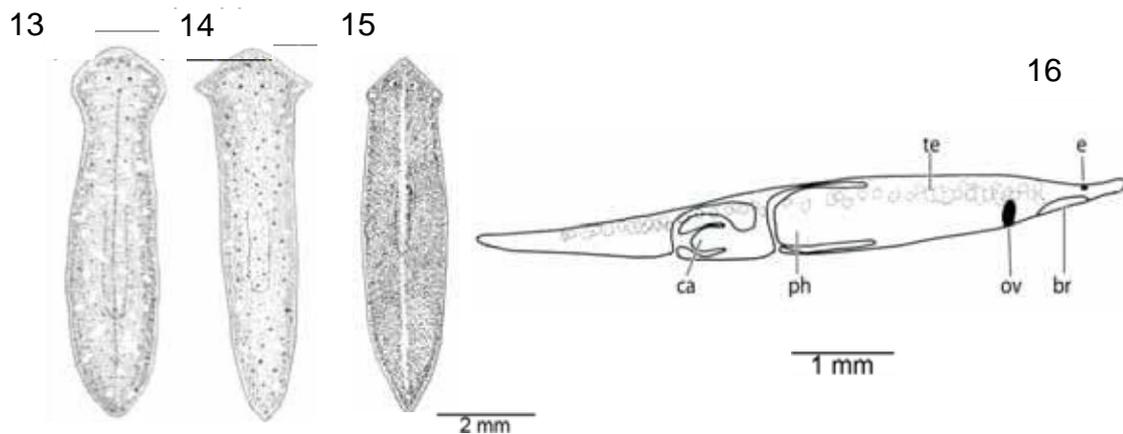
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**Figures 7-16** *Dugesia notogaea*. 7, LG314-1, Sagittal reconstruction of the copulatory apparatus; 8, LG344-2, microphotograph of a sagittal section of the penial papilla; 9, LG344-2, sagittal reconstruction of the copulatory apparatus; 10, LG363-3, sagittal reconstruction of the copulatory apparatus; 11, LG344-1, sagittal reconstruction of the male apparatus; 12, LG344-1, sagittal reconstruction of the female apparatus; 13, LG26, external features of living animal; 14, LG179, external features of living animal; 15, LG363, external features of living animal; 16, LG344-2, sagittal reconstruction of the reproductive system.

#### Discussion

While this description does not differ a great deal from existing descriptions, it is given here to demonstrate the variability within the species (Grant et al. 2006, Sluys et al. 1998). Intraspecific variations include the presence or absence of glands entering the gonoduct. Sluys et al. (1998) make no mention of glands entering the gonoduct in their original description, while this investigation suggests that they are visible in many but not all individuals (Figure 9 & 10). A similar situation is found with the ectal reinforcement of the bursal canal. It appears to be described irregularly in the literature and I have found its presence common but not constant (Figure 10).

So far the species has been described with hyperplastic ovaries (Grant et al. 2006, Sluys et al. 1998) yet this is not the case in these animals (Figure 16). I therefore suggest that this state is not diagnostic for the species. The lack of hyperplastic ovaries allowed for the identification of the dorsal communication between the ovaries and the oviducts, supporting Sluys' (2001) proposal that this feature is an apomorphy for the *Dugesia*.

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### Ecology and distribution

*Dugesia notogaea*, whilst generally inhabiting rivers and creeks, has been found thriving in isolated springs and billabongs. Specimens are commonly found on the underside of rocks or logs within riffles or the off-channel area. In the absence of any firmer substrate worms could be detected living within the macrophytes and leaf litter in the slower or stagnant parts of the stream. This species is found throughout coastal northern Australia, an area that is typified by non-permanent freshwater. During the summer months many creeks and rivers are reduced to residual pools, which often support populations of *Dugesia notogaea*. This species is found in northern Western Australia and northern Queensland extending to the north of New South Wales.

### *Dugesia orientoaustralis* Grant & Sluys sp. nov.

#### Material examined

##### New South Wales:

###### HOLOTYPE:

LG72, Guy Fawkes River, on the opposite side of the bridge to Ebor Falls, Ebor (30°24.291' S - 152°20.825' E), New South Wales, Australia, 9 January 2003, coll. L.J. Grant, sagittal sections on 11 slides, sagittal sections on 14 slides, sagittal sections on 15 slides, horizontal sections on 8 slides, horizontal sections on 7 slides.

###### PARATYPE:

LG71, Middle Creek at Coutts Water (30°20.970' S - 152°28.602' E), New South Wales, Australia, 9 January 2003, coll. L.J. Grant, sagittal sections on 10 slides, sagittal sections on 12 slides, sagittal sections on 11 slides, horizontal sections on 5 slides, horizontal sections on 5 slides, horizontal sections on 6 slides.

#### Other material examined

##### New South Wales:

LG25, Clyde River, on Carisbrook Road and Clyde Ridge Road, Flat Rocks State Forest northeast of Termeil (35°24.829' S - 150°14.263' E), New South Wales, Australia, 21 January 2003, coll. L.J. Grant, sagittal sections on 10 slides.

LG69, Orara River, bridge at Catts Crossing, Grafton - Armidale Road (29°49.548' S - 152°53.496' E), New South Wales, Australia, 8 January 2003, coll. L.J. Grant, sagittal sections on 5 slides.

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LG271, Bobo Creek, Lowanna-Dorrigo Road, Brooklana (30°16'06" S - 152°51'37.9" E), New South Wales, Australia, 30 August 2004, coll. A. Glaister, sagittal sections on 6 slides.

LG68, Blinks River, Armidale - Grafton Road, just before Tyringham (30°11.625 S - 152°32.798 E), New South Wales, Australia, 8 January 2003, coll. L.J. Grant, horizontal sections on 4 slides horizontal sections on 3 slides, sagittal sections on 8 slides, sagittal sections on 6 slides.

LG100, Tia River, upstream from Tia Falls, Oxley Wild Rivers National Park (31°09.607' S - 151°51.212' E), New South Wales, Australia, 12 January 2003, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 7 slides, sagittal sections on 7 slides, horizontal sections on 5 slides.

LG127, Shoalhaven River, Warri Bridge, Kings Highway, 15km west of Braidwood (35°20.621' S - 149°44.229' E), New South Wales, Australia, 20 January 2003, coll. L.J. Grant, sagittal sections on 12 slides, sagittal sections on 8 slides.

**Queensland:**

LG351, South Johnstone River, on Forestry Road off Palmerston Highway east of Karang Garee (17°38.746' S - 145°43.947' E), Queensland, Australia, 28 August 03, coll. L.J. Grant, sagittal sections on 4 slides, sagittal sections on 5 slides, sagittal sections on 7 slides

LG273, Dumaresq Rivulet, 60km west of Tenterfield (28°59'26.9" S - 151°31'23" E), Queensland, Australia, 28 August 2004, coll. A. Glaister, horizontal sections on 7 slides, sagittal sections on 7 slides, sagittal sections on 10 slides.

LG276, Canungra Creek, Sarabah Bridge, 10km southwest of Canungra (28°04'28.8" S - 153°06'43" E), Queensland, Australia, 22 August 2004, coll. A. Glaister, sagittal sections on 5 slides.

LG277, Bald Rock Creek, near Bald Rock camp ground, Girraween (28°49'59" S - 151°55'53.9" E), Queensland, Australia, 29 August 2004, coll. A. Glaister, sagittal sections on 4 slides.

LG63, Kangaroo Creek, Cathu State Forest (20°50.337' S - 148°32.561' E), Queensland, Australia, 19 December 2002, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 7 slides, sagittal sections on 10 slides, sagittal sections on 8 slides, horizontal sections on 4 slides.

LG282, Cedar Creek, Cedar Creek Road (27°53'50" S - 153°10'49.3" E), Queensland, Australia, 22 August 2006, coll. A. Glaister, sagittal sections on 4 slides.

**Victoria:**

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LG154, Murray River, Murray Valley Highway, approximately 10km north of Carryong (36°10.149' S - 148°01.551' E), Victoria, Australia, 17 December 2003, coll. L.J. Grant, sagittal sections on 5 slides, sagittal sections on 5 slides, sagittal sections on 7 slides, horizontal sections on 3 slides.

**Specimens Identified by Molecular Sequence Data****New South Wales:**

LG128, Kangeroo River, Kangeroo Valley Road, Hampden Bridge, Just north of Kangeroo Valley (34°43.752' S - 150°31.375' E), New South Wales, Australia, 21 January 2003, coll. L.J. Grant, identified via molecular samples (KR).

LG21, Hunter River, New England Hwy, Fitzgerald Bridge, Aberdeen (32°09.532' S - 150°52.949' E), New South Wales, Australia, 27 January 2003, coll. L.J. Grant, identified via molecular samples (LJ101)

LG27, Macquarie River, Great Western Highway at Eglinton, north of Bathurst (33°23.052' S - 149°32.833' E), New South Wales, 25 January 2003, coll. L.J. Grant, identified via molecular samples (LJ165).

LJ159, Murrumbidge River, Showgrounds, Gundagai, near bridge on Bungle Road (35°04.427'S - 148°06.735' E), New South Wales, Australia, 18 January 2003, coll. L.J. Grant, identified via molecular samples.

LG54, Styx River, at bridge on Kempsey Road, just north of Styx State Forest (30°35.307' S - 152°09.906' E), New South Wales, Australia, 12 January 2003, coll. L.J. Grant, identified via molecular samples (LJ209).

LG29, Nepean River, Hawksbury Road, Yarramundi Bridge, just west of Agnes Banks (33°36.782' S - 150°42.026' E), New South Wales, Australia, 26 January 2003, coll. L.J. Grant, identified via molecular samples (LJ213).

LG139, Peel River, Nundle Road, west of Nundle (31°27.631' S - 151°07.410' E), New South Wales, Australia, 28 January 2003, coll. L.J. Grant, identified via molecular samples (LJ240).

LG140, Burrows Creek, approximately 20km southeast of Tamworth (31°28.478' S - 151°11.354' E), New South Wales, Australia, 28 January 2003, coll. L.J. Grant, identified via molecular samples (LJ241).

LG114, Barrington River, Scone Road, Barrington (31°58.367' S - 151°54.156' E), New South Wales, Australia, 15 January 2003, coll. L.J. Grant, identified via molecular samples (LJ273).

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- LG3, Mulgum Creek, Cullen Bridge, Nimbin, on Lismore - Murwillumbah Road (28°35.506' S - 153°13.314' E), New South Wales, Australia, 29 January 2003, coll. L.J. Grant, identified via molecular samples (LJ42).
- LG142, Tweed River, just south of Terragon on Byrill Creek Road (28°26.228' S - 153°16.840' E), New South Wales, Australia, 29 January 2003, coll. L.J. Grant, identified via molecular samples (LJ73).
- LG109, Gloucester River, Barrington Road, Gloucester (31°59.907' S - 151°57.032' E), New South Wales, Australia, 14 January 2003, coll. L.J. Grant, identified via molecular samples (LJ80).
- LG42, Never Never Creek, near Gleniffer on Gleniffer Road (30°23.228' S - 152°53.677' E), New South Wales, Australia, 10 January 2003, coll. L.J. Grant, identified via molecular samples (LJ82).
- LG83, Iron Pot Creek, Toonumbar National Park, Murray Scrub Road (28°31.267' S - 152°44.940' E), New South Wales, Australia, 7 January 2003, coll. L.J. Grant, identified via molecular samples (N33).
- LG96, Bellinger River, just south of Gordonville, Gordonville Road (30°25.070' S - 152°50.822' E), New South Wales, Australia, 10 January 2003, coll. L.J. Grant, identified via molecular samples (N41).
- LG103, Hastings River, bridge on Forbes River Road, approximately 50km east of Wauchope (31°24.661' S - 152°20.690' E), New South Wales, Australia, 13 January 2003, coll. L.J. Grant, identified via molecular samples (N49).
- LG113, Karvah River, at Stroud Road, on road to Dungog (32°20.873' S - 151°55.524' E), New South Wales, Australia, 15 January 2003, coll. L.J. Grant, identified via molecular samples (N52).
- LG112, Cobark River, Scone Road, Cobark, (31°56.857' S - 151°42.641' E), New South Wales, Australia, 15 January 2003, coll. L.J. Grant, identified via molecular samples (N53).
- Q26, Oaky River, Kempsey Road, west of Jeogla (30°33.749' S - 152°05.350' E), New South Wales, Australia, 5 January 2003, coll. L.J. Grant, identified via molecular samples.

**Queensland:**

- LJ184, Lake Eacham National Park, small creek under bridge on side road (17°16.763' S - 145°37.925' E), Queensland, Australia, 30 August 2003, coll. L.J. Grant, identified via molecular samples.

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LJ187, Stoney Creek, Stoney Creek Road, Barron River National Park, near Redlynch (16°52.634' S - 145°39.977' E), Queensland, Australia, 4 September 2003, coll. L.J. Grant, identified via molecular samples.

LJ259, Gooligan Creek, off Palmerston Hwy, 30km southeast of Milla Milla (17°35.946' S - 145°45.507' E), Queensland, Australia, 29 August 2003, coll. L.J. Grant, identified via molecular samples.

LG272, Amamoor Creek, 8km southwest of Amamoor (26°22'12.5" S - 152°26'59" E), Queensland, Australia, 17 August 2004, coll. L.J. Grant, identified via molecular samples (LJ367).

LJ384, Running Creek, 7th bridge, 16km southeast of Rathowney (28°19'13.2" S - 152°55'39" E), Queensland, Australia, 25 August 2004, coll. L.J. Grant, identified via molecular samples.

LG37, Kinbombi Falls, off Wide Bay Hwy, east of Goomeri (26°13.280' S - 152°08.998' E), Queensland, Australia, 29 December 2002, coll. L.J. Grant, identified via molecular samples (Q11).

#### Victoria:

LG195, Tarwin River, near Leongatha at intersection with South Gippsland Hwy (38°34.945' S - 145°59.547' E), Victoria, Australia, 13 January 2004, coll. L.J. Grant, identified via molecular samples (LJ24).

#### Diagnosis

*Dugesia orientoaustralis* can be distinguished from its congeners by the combination of the following features: presence of a common oviduct; ectal re-enforcement of the entire length of the bursal canal; long gonoduct communicating with the common atrium.

#### Description

Largest live specimens measure 20mm x 2mm. Triangulate head with large pointed auricles and a short pointed tail. The eyes are small sitting in small pigment free patches positioned closer to each other than to the lateral margin and slightly anterior to the auricular points (Figure 21). Large pigment free patches are situated on the lower part of the auricles. There are no sensory pits or fossae visible in sections. The dorsal base colour is white/transparent overlain with many fine specs and sparser large dark specs throughout. Pigment free patches are present with the size varying between populations but giving all an overall brown mottled appearance. The ventral surface has no pigment and therefore appears white/transparent.

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The pharynx occupies approximately one-fifth of the total body length and the mouth is situated at the most posterior point of the pharyngeal pocket (Figure 19).

Testes are small, discrete follicles situated dorsally and extending from a long distance behind the brain to the tail of the animal (Figure 19). The broad vasa deferentia travel close to the ventral surface as they approach the muscular penis bulb. Upon reaching the level of the penis bulb the vasa deferentia turn dorsally, narrowing slightly before penetrating the muscular penis bulb. The vasa deferentia penetrate from opposing sides of the penis bulb and communicate separately with a large, thin walled seminal vesicle. The seminal vesicle communicates directly with a narrow ejaculatory duct, which is interrupted approximately half way along its length by a small diaphragm. The ejaculatory duct travels acentrally creating a much larger dorsal lip with the duct terminating close to the ventral surface (Figures 17-18 & 20). The ejaculatory duct is thin walled and receives penial glands along most of its length. The penial papilla appears rounded, mildly flexible, has light musculature and occupies most of spacious the male atrium. A constriction in the male atrium creates a common atrium, which receives the opening of a long, narrow gonoduct. The gonoduct has a short epithelium through which glands penetrate to open into the gonoduct.

The common atrium then communicates the broad origin of the bursal canal. This area receives the entrance of a short common oviduct with heavy glandularisation (shell glands) entering below the point of communication (Figures 17-18). The oviducts travel ventrally separately and do not turn anteriorly until reaching the ventral surface. The oviducts communicate with numerous small vitellaria along the animal's entire body length. Once reaching the level of the small ovaries the oviducts communicate with the dorsal surface. The ovaries are situated a long distance behind the brain (Figure 19).

Above the common oviduct the female bursal canal narrows slightly before turning anteriorly to travel over the penis bulb and communicate with a large copulatory bursa. The bursal canal in a few specimens is convoluted but this does not appear to be the common state (Figure 17). The bursal canal has a relatively short epithelium surrounded by reversed musculature with a narrow longitudinal layer and a thicker circular layer. This musculature has ectal reinforcement with an additional layer of longitudinal muscle along the canals entire length. The copulatory bursa is sitting directly behind the penis bulb, it is a large spacious bursa extending some distance into the anterior of the animal and occupying most of the dorso-ventral space (Figures 17-18). The epithelium of the copulatory bursa is tall and there is no overlying musculature.

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#### Discussion

The external features of this species are very similar to that of *D. notogaea* and I suspect that this species has been accidentally assigned to *D. notogaea* in the past. However there are several very important differences between *D. notogaea* and this species. The most useful diagnostic character is the common oviduct, (Figures 17-18) which is not present in *D. notogaea* (Grant et al. 2006, Sluys and Kawakatsu 2001, Sluys et al. 1998). The extension of the ectal re-enforcement to the termination of the duct was found without exception amongst these specimens (Figure 17-18). This feature has been described on rare occasions from *D. notogaea*. It remains to be examined in detail whether this presumed variability is based on a misidentification of a few animals. The long gonoduct and the ventral point of termination of the ejaculatory duct are additional features characteristic for this species. This species can also be separated from *D. notogaea* by the absence of ventral pigmentation, this pigmentation is consistently described as light brown in *D. notogaea* (Sluys et al. 1998) (this paper).

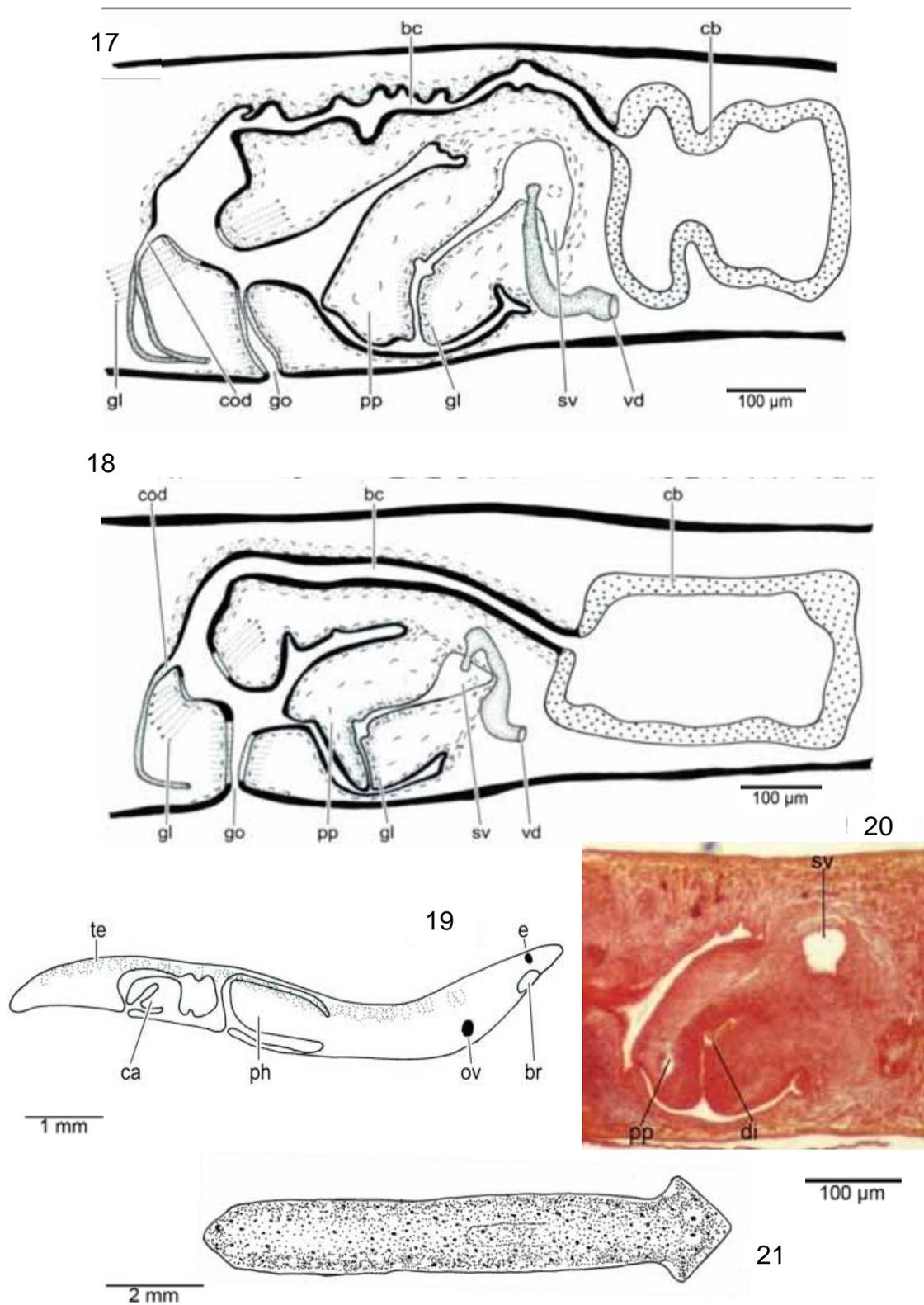
While comparison with *D. notogaea* is important owing to their distributional similarities there are other *Dugesia* species that have a common oviduct and therefore must be considered. *D. andamanensis* Kaburaki, 1925 from The Andaman Islands (India) exhibits many similar characters, however, its ovaries are situated directly behind the brain, in contrast to *Dugesia* sp. nov. *D. congolensis* Beauchamp, 1951 from the Congo also has a common oviduct (De Beauchamp 1951, Kaburaki 1925). Unfortunately, this species is poorly described and considered to be a species inquirenda (De Vries 1988). While *D. deharvengi* Kawakatsu & Mitchell, 1989 from Thailand, *D. mertoni* Steinmann, 1914 from Indonesia and *D. myopa* De Vries, 1988 from Madagascar, can be eliminated owing to, among other disagreements, little or no ectal re-enforcement of the bursal canal we believe that this new species exhibits a unique combination of characters and can therefore be considered distinct from all other *Dugesia*'s (De Vries 1988, Kawakatsu and Mitchell 1989, Steinmann 1914).

#### Ecology and distribution

Currently this species is known only from lotic environments most commonly in the off-channel area, but occasionally amongst the riffles. Found in creeks and rivers with variable substrates but always on the underside of rocks. This species is restricted to eastern Australia, extending from northern Queensland to northern Victoria.

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**Figures 17-21** *Dugesia orientoaustralis*. 17, LG71-1, Sagittal reconstruction of the copulatory apparatus; 18, LG72-1, sagittal reconstruction of the copulatory apparatus; 19, LG71-1, sagittal reconstruction of the reproductive system; 20, LG71-1, microphotograph of sagittal section of the penial papilla; 21, LG27, external features of living animal.

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### Etymology

This specific epithet is derived from the latin nouns '*oriento*' (orientation) and '*australis*' (south), referring to the orientation of the gonoduct at its termination point.

### *Dugesia* sp.

### Material examined

#### New South Wales:

LG85, Iron Pot Creek, Murray Scrub Road, Toonumbar National Park (28°31.267' S - 152°44.940' E), 7 January 2003, New South Wales, Australia, coll. L.J. Grant, sagittal sections on 7 slides.

#### Queensland:

LG59, Waterpark Creek, Byfield Road, Byfield State Forest (22°50.201' S - 150°40.193' E), 23 December 2002, Queensland, Australia, coll. L.J. Grant, on 5 slides sagittal sections, on 8 slides sagittal sections.

LG356, Big Crystal Creek, near camping ground, Paluma Ranges National Park (18°58.812' S - 146°15.346' E), 24 July 2003, Queensland, Australia, coll. L.J. Grant, sagittal sections on 4 slides, sagittal sections on 4 slides.

LG350, Nesbit River, near junction with Leo Creek, Silver Creek (13°32' S - 143°29' E), 11 November 2002, Queensland, Australia, coll. L.J. Grant, sagittal sections on 3 slides.

LG359, Chester River, Silver Plains (13°42' S - 143°32' E), 12 November 2002, Queensland, Australia, coll. L.J. Grant, sagittal sections on 3 slides, sagittal sections on 3 slides, sagittal sections on 3 slides.

### Specimens Identified by Molecular Sequence Data

#### New South Wales:

LG99, Little Nymboida River, Bindarri Nation Park, east of Ulong (30°13.684' S - 152°55.247' E), New South Wales, Australia, 9 January 2003, coll. L.J. Grant, identified via molecular samples (LJ76).

LG40, Macleay River, east of Sherwood on Sherwood Road (31°03.548' S - 152°43.913' E), New South Wales, Australia, 11 January 2003, coll. L.J. Grant, identified via molecular samples (N42).

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**Queensland:**

- LG282, Cedar Creek, Cedar Creek Road, Cedar Creek (27°53'50" S - 153°10'49.3" E), Queensland, Australia, 22 August 2006, coll. L.J. Grant, sagittal sections on 9 slides.
- LG57, Captains Creek, 15km southwest of Agnes Water (24°17.393' S - 151°47.021' E), Queensland, Australia, 24 December 2002, coll. L.J. Grant, identified via molecular samples (LJ20).
- LG56, Lenthall's Dam, approximately 20km north of Maryborough (25°24.186' S - 152°32.019' E), Queensland, Australia, 25 December 2002, coll. L.J. Grant, identified via molecular samples.
- LJ286, Alligator Creek, Bowling Green Bay National Park, approximately 28km south Townsville (19°26' S - 146°57' E), Queensland, Australia, 11 July 2002, coll. L.J. Grant, identified via molecular samples.
- LJ63, Murray River, Murray Falls, Girramay National Park, (18°09.125' S - 145°48.914' E), Queensland, Australia, 27 August 2003, coll. L.J. Grant, identified via molecular samples.
- LJ66, Alice River, Herveys Range Road, 25km west of Townsville (19°18.898' S - 146°35.783' E), Queensland, Australia, 15 November 2003, coll. L.J. Grant, identified via molecular samples.
- LG30, Petters Creek, 15km west of Proserpine, on Crystal Brook Road (20°21.448' S - 148°25.067' E), Queensland, Australia, 18 December 2002, coll. L.J. Grant, identified via molecular samples (LJ68).
- NQ8, Massy River at junction with main road (13°55' S - 143°36' E), Queensland, Australia, 8 November 2002, coll. L.J. Grant, identified via molecular samples.
- LG32, Teemburra Creek, Miama State Forest, Near Pinnacle (21°13.628' S - 148°41.129' E), Queensland, Australia, coll. L.J. Grant, identified via molecular samples (Q13).
- LG49, Thanos Creek, Thanos Creek Bridge, 40km west of Warwick (28°09.738' S - 151°42.307' E), Queensland, Australia, 4 January 2003, coll. L.J. Grant, identified via molecular samples (LJ244).
- LG61, Marion Creek, Henry De Costa Bridge, Bruce Hwy, south of Koumala (21°42.525 S - 149°21.658' E), Queensland, Australia, 21 December 2002, coll. L.J. Grant, identified via molecular samples (Q7).
- LG56, Lenthalls Dam, approximately 20km north of Maryborough (25°24.186' S - 152°32.019' E), 25 December 2002, Queensland, Australia, coll. L.J. Grant, identified via molecular samples.

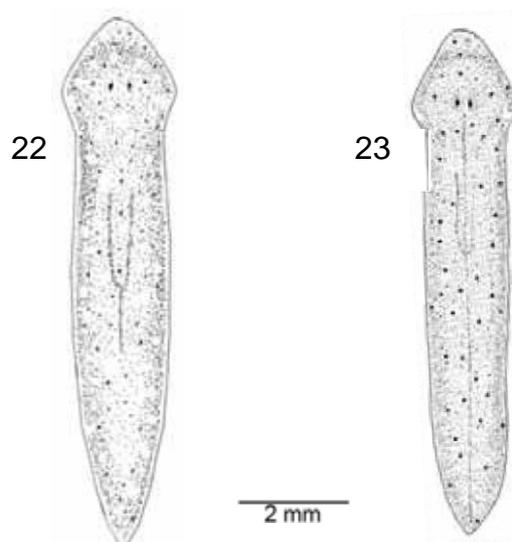
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#### Description

Largest live specimens measure 10mm x 1mm with a triangulate head, large rounded auricles and a rounded tail. Small eyes are sitting in small pigment free patches located closer to each other than to the lateral margins and positioned equal to or slightly anterior to auricular extremities. Small pigment free patches on auricular points are visible in most specimens. Dorsal base colour is white/transparent overlain with fine light and coarse dark brown specks creating, speckled effect (Figure 22). In addition to this pigmentation the dorsal surface is often, but not always, interrupted by small pigment free patches (Figure 23). The ventral surface is pigment free. There is no evidence of any sensory pits or fossae.

The pharynx is located posteriorly and is occupying approximately one-fifth of the total body length; the mouth is situated at the most posterior point of the pharyngeal pocket. There is no trace of any ovaries, testes or copulatory apparatus in any of the individuals.



**Figures 22-23** *Dugesia* sp. 22, LG56, external features of living animal; 23, LG59, external features of living animal.

#### Discussion

It is unknown whether this species is lacking sexual organs because it is asexual or simply immature at the time of collection. Both explanations are plausible as Australian species are often subjected to drying episodes, causing periods of starvation, known to be capable of initiating the re-absorption of sexual organs (Woollhead 1983). It has been well documented that Australian species are regularly found with immature or non-existent

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sexual apparatus (Grant et al. 2006, Sluys 1997, Sluys and Kawakatsu 2001)(this paper). Upon inspection of the field data pertaining to these specimens it should be noted that while the majority of collections were made from rivers and creeks with low or no flow, there are several examples of sites where the water level was normal or high. These populations were collected in the same asexual state as their presumably stressed counterparts, making it hard to discount the possibility that this is an asexual species reproducing solely via fission (similar to the many invasive populations of *Girardia tigrina*) (Ball and Reynoldson 1981).

Due to the lack of any diagnostic characters, this group has been identified as a separate species only via molecular data and consequently a complete species description cannot be provided. This species falls within the genus *Dugesia* when both the mitochondrial and the nucleic data are examined. The external morphology also supports this placement with *Dugesia* sp. displaying the characteristic mottled dorsal pigmentation. While this branch is close to the *Dugesia* sp. nov., in both trees, the molecular evidence suggests that this is a separate *Dugesia* species is compelling to the point of certainty (see Chapter 2 and Appendix 2c).

#### **Ecology and distribution**

This species has been found predominantly on the underside of rocks in the off-channel areas and residual pools of creeks and rivers. The distribution extends from far northern Queensland to northern New South Wales.

#### *Masaharus* Grant & Sluys, gen. nov.

Seminal vesicle absent or of the non-muscular type. Separate oviducal entries to the bursal canal with one species exhibiting a caudal branch. Bursal stalk musculature of inner circular muscles surrounded by longitudinal fibres or intermingled. Copulatory bursa often surrounding by musculature. Penial glands, entering the ejaculatory duct, are present in all species. Testes numerous, distributed throughout the body length and usually ventral. No pharyngeal pigmentation. Species within this new genus were formerly assigned, rather tentatively (owing to a lack of synapomorphies), to the genus *Girardia*. However, molecular data demonstrates that these species are not closely associated with the genus *Girardia* and strongly supports the erection of this new genus to accommodate them.

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### Etymology

In conformity with the fact that most of the current generic names in the Dugesiidae are derived from names of triclad workers, the genus is named for Masaharu Kawakatsu in recognition of his contribution to our knowledge of the Dugesiidae. Gender: masculine.

### *Masaharus extentus* Grant & Sluys, sp. nov.

### Material examined

#### Tasmania:

##### HOLOTYPE:

LG257, Iris River on road to Cradle Mountain, approximately 10km north of Cradle

Mountain (41°33.708' S - 145°56.994' E), Tasmania, Australia, 1 February 2004, coll. L.J.

Grant, sagittal sections on 5 slides, sagittal sections on 7 slides, horizontal sections on 4 slides.

##### PARATYPE:

LG246, Lake Sorrell at Interlaken (42°08.050' S - 147°10.135' E), Tasmania, Australia, 30

January 2004, coll. L.J. Grant, sagittal sections on 7 slides.

### Diagnosis

The caudal branch of the oviducts and lack of dorsal pigment differentiate this species from its congeners.

### Description

Largest live specimens measure 10mm x 1mm. Small eyes are sitting very close to each other and equal with the bottom of the auricles. There is one set of large sensory pits present on the lateral margin. Auricles are large and rounded and the triangular head comes to a point (Figure 25). Some individuals are lacking pigment altogether, presenting as white and slightly transparent, while others have extremely light brown pigment overlaying the white base. The pigment that exists is not continuous and many large pigment free areas exist. The ventral surface never has any pigment.

The pharynx is situated in the mid-region of the body and occupies approximately one-sixth of the total body length. The mouth is situated at the most posterior extent of the pharyngeal pocket (Figure 27).

The testes are large, extremely numerous ventral follicles often extending into the dorsal region; they extend throughout the body length (Figure 26). The vasa deferentia

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travel ventrally before turning dorsally at the level of the penis bulb. After penetrating the muscular penis bulb the ducts communicate with each other to form an acentral ejaculatory duct (Figures 24 & 26). The ejaculatory duct is a narrow, straight duct with a short epithelium. The duct receives the openings of glands along the terminal half of its length. The penial papilla is a lightly muscled, rounded structure that fills most of the male atrium.

The opening of the bursal canal communicates directly with the male atrium in a relatively dorsal position (Figures 24 & 26). The bursal canal runs anteriorly over the penis bulb to communicate with the large copulatory bursa situated just anterior to the penis. The canal has a short epithelium and is surrounded by weak intermingled musculature. This musculature weakens but continues over the copulatory bursa. The epithelium of the bursa seems unique, the cells are huge but there appears to be only one layer. The oviducts communicate separately with the bursal canal close to the level of communication with the male atrium. The oviducts enter from opposing lateral sides, just above the level of the shell glands entering at the base of the bursal canal. There is a caudal oviducal branch that extends far into the posterior of the animal and can be observed communicating with vitellaria in this region. The large but discrete paired ovaries are situated directly behind the brain and the communication of oviducts is on their ventral, posterior surface (Figure 27).

## Discussion

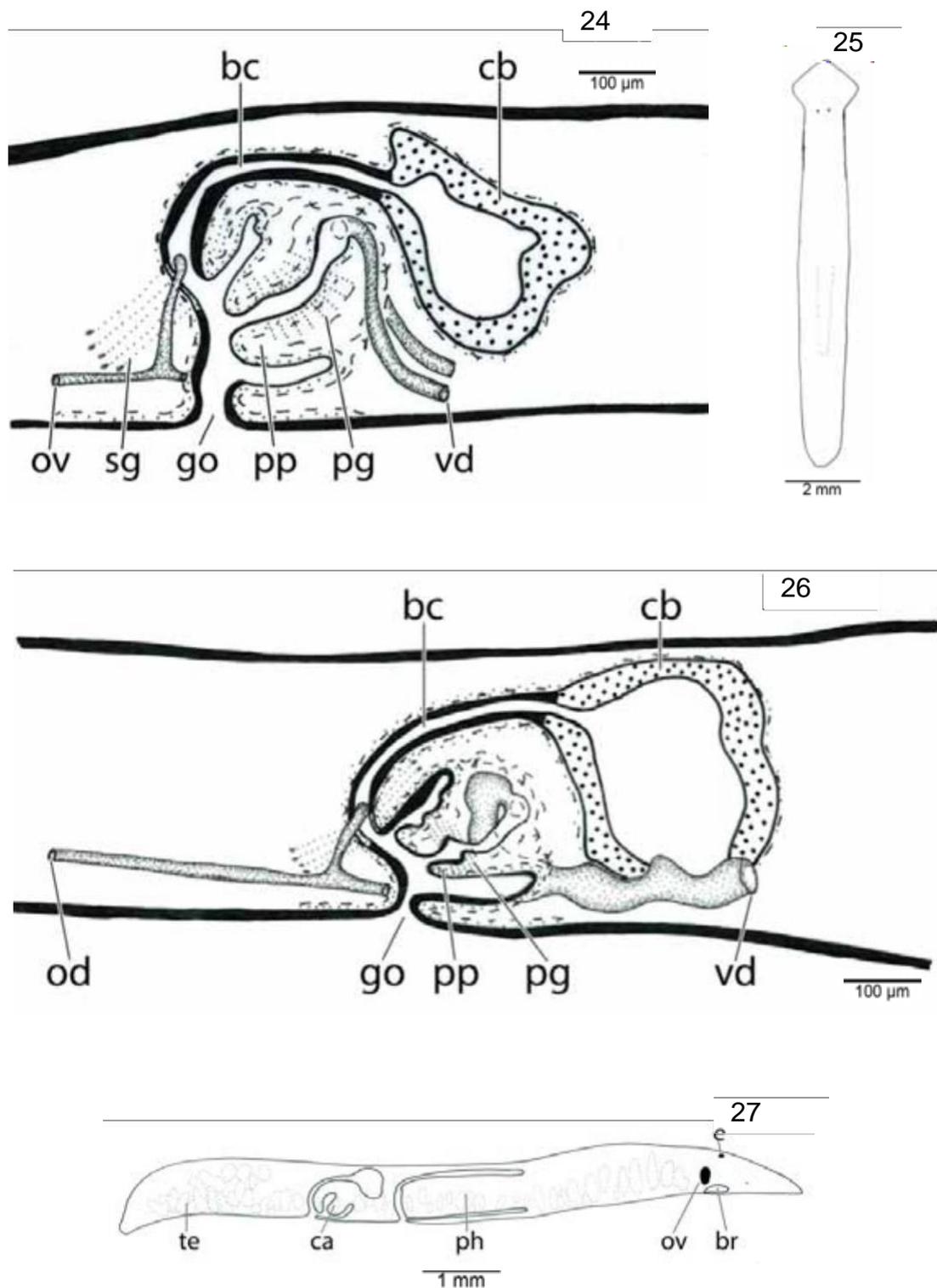
The separate entries of the oviducts to the bursal canal and the caudal branch should easily place this species within the genus *Spathula*. However, there are several features that complicate this assignment including the presence of intermingled musculature on the bursal canal and musculature surrounding the copulatory bursa.

The lack of external pigmentation has been recorded for many *Spathula* species (e.g. *Sp. foeni*, *Sp. tryssa*, *Sp. goubaultae*, and *Sp. agelaea*). However none of these species exhibit intermingled musculature on the bursal canal and muscularisation around the copulatory bursa.

*Spathula truculenta*, *Girardia tigrina* and *Masaharus informis* are the only Australian species for which intermingled musculature on the bursal canal has been reported. *Sp. truculenta* has dorsal testes, a common oviduct and no musculature surrounding the copulatory bursa (Grant et al. 2006). *Girardia tigrina* is invasive and possesses a pigmented pharynx, a feature lacking in this species. *Masaharus informis* has the intermingled musculature, the musculature surrounding the copulatory bursa and

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**Figures 24-27** *Masaharus extentus*. 24, LG257-1. sagittal reconstruction of the copulatory apparatus; 25, LG246, external features of a live animal; 26, sagittal reconstruction of the copulatory apparatus; 27, LG246-1, sagittal reconstruction of the reproductive system.

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separate oviducal entries. *Masaharus informis* differs due to its lunate head, dorsal testes and lack of a caudal branch of the oviducts. We are therefore unable to assign this specimen to any known species.

*Masaharus* has the most characters in common with this species and it is therefore proposed that this species belongs to this genus. It is presumed that the presence of a caudally branching of the oviducts in this species represents a link between this genus and the *Spathula*, *Reynoldsonia* and *Eviella* group (similar to that found in *Romankenkius*). The relevance of the caudally branching oviducts is discussed by Sluys and Rohde (1991) in relation to *Romankenkius* and the *Spathula* group where they propose that the lack of a caudal branch is the primitive state. While this is a possibility I believe that the relationship between these genera and the importance of the oviducts will be clarified with further phylogenetic analysis.

#### Ecology and distribution

Known only from the type locality, this species was collected from the underside of cobble in the off-channel area of a pristine Tasmanian river.

#### Etymology

The specific epithet was derived from the Latin adjective '*extentus*', meaning stretching out, extending.

### ***Masaharus informis* (Sluys & Grant, 2006)**

#### Material examined

##### Tasmania:

LG254, Lake St. Clair, near campground (42°06.925' S - 146°10.699' E), Tasmania,

Australia, 31 January 2004, coll. L.J. Grant, sagittal sections on 6 slides.

LG258, Iris River, on road to Cradle Mountain approximately 10km north of Cradle

Mountain (41°33.708' S - 145°56.994' E), Tasmania, Australia, 1 February 2004, coll. L.J. Grant, sagittal sections on 7 slides.

LG249, River Ouse, at junction with highway (41°59.329' S - 146°38.806' E), Tasmania,

Australia, 29 January 2004, coll. L.J. Grant, sagittal sections on 7 slides.

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#### **Description**

Largest live specimens measure 14mm x 2mm. The head is lunate in appearance and the eyes are positioned equal with the point where the head tapers to the width of the body (Figure 30). There are small pigment-free patches on the extremity of each auricle corresponding to a large sensory pit. The tail comes to a shallow point. The base colour of the dorsal surface is white/transparent, densely covered in dark pigment, the density of which is variable but specimens often appear black. The ventral surface is pigmented but to a slightly lesser extent than the dorsal surface.

Large discrete testes are positioned dorsally, extending from a short distance posterior to the brain throughout the body (Figure 29). The vasa deferentia are narrow ducts that run along the ventral surface of the body before rising gradually towards the penis bulb. The vasa deferentia enter the penis bulb from opposing sides, communicating inside the muscular penis bulb directly with the ejaculatory duct (i.e. no seminal vesicle). The ejaculatory duct is narrow and acentral, creating a substantially larger dorsal lip of the penial papilla (Figure 31). The short epithelium of the ejaculatory duct receives the openings of glands along its distal half (Figure 28). The penial papilla is muscularised and exhibits a standard rounded shape. The papilla is situated in a small male atrium, which communicates directly with the gonoduct/bursal canal.

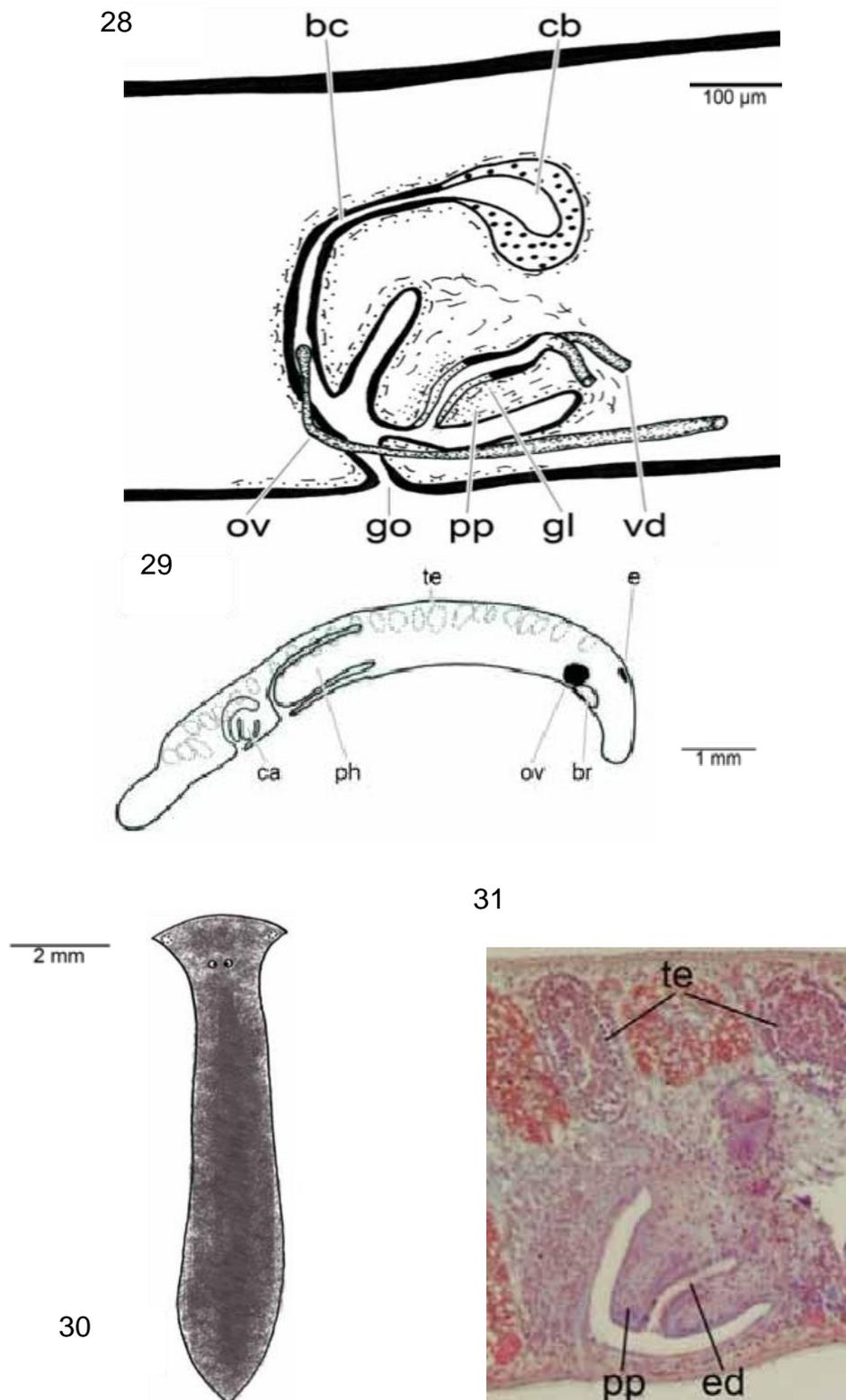
The bursal canal, whose short epithelium is surrounded by intermingled musculature, runs dorsally to the level of the top of the penis bulb, then turning anteriorly to communicate with a small copulatory bursa (Figure 28). The copulatory bursa, located just above the penial papilla, is covered with a weak layer of intermingled musculature. The oviducts enter the bursal canal at the same level but separately from opposing sides. The point of communication is only a short distance from the point where the bursal canal communicates with the atrium and, interestingly, there are no associated glands at this point. The oviducts run directly to the ventral surface before turning anteriorly, there is no caudal branch. Large paired ovaries are sitting directly behind the brain (Figure 29). The point of communication of the oviducts with the ovaries is untraceable in this specimen.

#### **Discussion**

The only discrepancy between this description and that of the holotype is the lack of the flexible penial papilla described in Grant and Sluys (2006). The external morphology was previously unknown and has proven to be interesting as the lunate head shape described is quite unique amongst the Australian Dugesiids (Figure 30). The molecular data for this species is interesting, as it has motivated the assignment of this species to the newly

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**Figures 28-31** *Masaharus informis*. 28, LG254-2, sagittal reconstruction of the copulatory apparatus; 29, LG254-2, sagittal reconstruction of the reproductive system; 30, LG258, external features of living animal; 31, LG254-2, microphotograph of a sagittal section of the penial papilla.

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formed *Masahrus*. This in turn has created significant doubt in relation to the natural presence of *Girardia* in Australia (i.e. there are currently no endemic Australian species assigned to *Girardia*). *Masahrus informis* does not sit near the *Girardia tigrina* branch; instead it is located separately but close to the *Spathula*'s.

#### Ecology and distribution

*Masahrus informis* has been found on the underside of rocks in the off-channel area of creeks, rivers and lakes. This species has been collected from cool water at high latitudes in the northwest of Tasmania in and around Cradle Mountain National Park.

#### *Girardia* Ball, 1974

#### *Girardia tigrina* (Girard, 1850)

#### Material examined

##### New South Wales:

LG55, Styx River, Kempsey Road, just north of Styx State Forest (30°35.307' S - 152°09.906'E), 12 January 2003, New South Wales, Australia, coll. L.J. Grant, sagittal sections on 10 slides.

LG2, Tweed River, Byrill Creek Road, just south of Terragon (28°26.228' S - 153°16.840' E), 29 January 2003, New South Australia, Australia, coll. L.J. Grant, sagittal sections on 5 slides.

LG46, Washpool Creek, Mt. Lindsay Road, approximately 10km south of Tenterfield (28°59.477' S - 152°03.744' E), 6 January 2003, New South Wales, Australia, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 8 slides.

LG111, Gloucester River, Barrington Road, Gloucester (31°59.907' S - 151°57.032' E), 14 January 2003, New South Wales, Australia, coll. L.J. Grant, sagittal sections on 7 slides.

LG84, Iron Pot Creek (dam outflow), Toonumba Dam (28°37.254' S - 152°47.727' E), 7 January 2003, New South Wales, Australia, coll. L.J. Grant, sagittal sections on 7 slides.

##### Queensland:

LG62, Teemburra Dam (21°10.870' S - 148°39.944' E), 20 December 2002, Queensland, Australia, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 8 slides.

LG58, Alligator Creek, approximately 30 km north of Rockhampton (23°07.575' S - 150°23.698' E), 23 December 2002, Queensland, Australia, coll. L.J. Grant, sagittal sections on 9 slides, sagittal sections on 9 slides, horizontal sections on 7 slides.

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LG380, Ross River, Ross River Road, Thuringower (19°16' S - 146°50' E), 28 January 2003, Queensland, Australia, coll. L.J. Grant, sagittal sections on 1 slide.

LG343, Gooligan Creek, Palmerston Highway, 30km southeast Milla Milla (17°35.946' S - 145°45.507' E), 28 August 2003, Queensland, Australia, coll. L.J. Grant, sagittal sections on 3 slides.

LG50, Emu Creek, New England Highway, north of Crows Nest, (27°08.581' S - 151°57.104' E), 1 January 2003, Queensland, Australia, coll. L.J. Grant, sagittal sections on 6 slides, horizontal sections on 4 slides.

LG36, Gordonbrook Dam (26°26.797' S - 151°45.205' E), 25 December 2002, Queensland, Australia, coll. L.J. Grant, identified via molecular samples.

**Tasmania:**

LG235, Coal River at Richmond (42°44.047' S - 147°26.342' E), 26 January 2004, Tasmania, Australia, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 8 slides.

**Western Australia:**

LG336, Lower Vasse River, Busselton (33°39.01.97' S - 115°20'49.31" E), 4 September 2001, Western Australia, Australia, C.A.L.M., sagittal sections on 6 slides.

**Specimens Identified by Molecular Sequence Data****New South Wales:**

LG29, Nepean River, Hawksbury Road, at Yarramundi Bridge, west of Agnes Banks (33°36.782' S - 150°42.026' E), New South Wales, Australia, 26 January 2003, coll. L.J. Grant, identified via molecular samples (LJ104).

LG134, Boorowa River, Boorowa - Crookwell Road, Boorowa (34°23.558' S - 148°46.129' E), New South Wales, Australia, 23 January 2003, coll. L.J. Grant, identified via molecular samples (LJ160).

LG23, Oxley River, McKenzies Bridge at Eungella, southwest of Murwillumbah on road to Eungella (28°21.236' S - 153°18.233' E), New South Wales, Australia, 30 January 2003, coll. L.J. Grant, identified via molecular samples (LJ202).

LG1, Tuntable Creek, Osborn Bridge, approximately 2km north of "The Channon", on road from Nimbin (28°39.079' S - 153°15.606' E), New South Wales, Australia, 29 January 2003, coll. L.J. Grant, identified via molecular samples (LJ203).

LG97, Bellinger River, just south of Gordonville, Gordonville Road (30°25.070' S - 152°50.822' E), New South Wales, Australia, 10 January 2003, coll. L.J. Grant, identified via molecular samples (LJ206).

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- LG41, Macleay River, interception with Grafton Road, Waterfall Way (30°49.161' S - 152°30.356' E), New South Wales, Australia, 11 January 2003, coll. L.J. Grant, identified via molecular samples (LJ207).
- LG110, Manning River, Gloucester Road, south of Wingham (31°55.014' S - 152°19.029' E), New South Wales, Australia, 14 January 2003, coll. L.J. Grant, identified via molecular samples (LJ211).
- LG130, Cox's River, Cox's River Road, southwest of Little Hartley (33°37.083' S - 150°09.653' E), New South Wales, Australia, 25 January 2003, coll. L.J. Grant, identified via molecular samples (LJ212).
- LG101, Georges Creek, Kempsey Road, north of Georges Junction (30°44.498' S - 152°11.479' E), New South Wales, Australia, 12 January 2003, coll. L.J. Grant, identified via molecular samples (LJ272).
- LG125, Shoalhaven River, Kings Hwy, Warri Bridge, 15km west of Braidwood (35°20.621' S - 149°44.229' E), New South Wales, Australia, 20 January 2003, coll. L.J. Grant, identified via molecular samples (LJ274).
- LG141, Mulgum Creek, Cullen Bridge, Nimbin, on Lismore - Murwillumbah Road (28°35.506' S - 153°13.314' E), New South Wales, Australia, 29 January 2003, coll. L.J. Grant, identified via molecular samples (LJ3).
- LG43, Never Never Creek, near Gleniffer on Gleniffer Road (30°23.228' S - 152°53.677' E), New South Wales, Australia, 10 January 2003, coll. L.J. Grant, identified via molecular samples (LJ83/LJ77).
- LG132, Wollondilly River, east of Wombeyan Caves Road, west of Bullio (34°38.624' S - 150°04.345' E), New South Wales, Australia, 22 January 2003, coll. L.J. Grant, identified via molecular samples (LJ84).
- LG47, Richmond Range, Summerland Way, Casino (28°52.176' S - 153°02.245' E), New South Wales, Australia, 6 January 2003, coll. L.J. Grant, identified via molecular samples (N30).
- LG57, Bielsdown River, 2km north of Dorrigo, above Dangar Falls (30°19.352' S - 152°42.846' E), New South Wales, Australia, 9 January 2003, coll. L.J. Grant, identified via molecular samples (N40).
- LG52, Apsley River, upstream from falls in Oxley Wild Rivers National Park, approximately 25km southeast of Walcha (31°03.055' S - 151°40.029' E), New South Wales, Australia, 12 January 2003, coll. L.J. Grant, identified via molecular samples (N44).

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LG106, Forbes River, Forbes River Road, near Birdwood (31°20.706' S - 152°20.453' E), New South Wales, Australia, 13 January 2003, coll. L.J. Grant, identified via molecular samples (N47).

LG108, Gloucester River, Barrington Road, Gloucester at bridge (31°59.907' S - 151°57.032' E), New South Wales, Australia, 14 January 2003, coll. L.J. Grant, identified via molecular samples (N50).

LG115, Barrington River, Scone Road, Barrington (31°58.367' S - 151°54.156' E), New South Wales, Australia, 15 January 2003, coll. L.J. Grant, identified via molecular samples (N54).

LG123, Brogo River, 18km north of Bega on Pacific Hwy, Greendale Road, Greendale Bridge (36°36.458' S - 149°50.830' E), New South Wales, Australia, 19 January 2003, coll. L.J. Grant, identified via molecular samples (N57).

LG121, Benboka River, Morans Crossing, 2km west Numbugga, Snowy Mountain Hwy (36°39.818' S - 149°38.774' E), New South Wales, Australia, 19 January 2003, coll. L.J. Grant, identified via molecular samples (N58).

LG44, Nymboida River at Nymboida, Armidale-Grafton Road (29°57.285' S - 152°43.590' E), New South Wales, Australia, 8 January 2003, coll. L.J. Grant, identified via molecular samples (LJ75).

LG24, Macquarie River, Great Western Hwy at Eglinton, north of Bathurst (33°23.052' S - 149°32.833' E), New South Wales, Australia, 25 January 2003, coll. L.J. Grant, identified via molecular samples (LJ166).

LG106, Forbes River, Forbes River Road near Birdwood (31°20.706' S - 152°20.453' E), 13 January 2003, New South Wales, Australia, coll. L.J. Grant, identified via molecular samples.

**South Australia:**

LG176, River Torrens, small pond to the side of river, Mahogany Ave, Dernancourt (34°51.942' S - 138°40.985' E), South Australia, Australia, 31 December 2003, coll. L.J. Grant, identified via molecular samples (LJ12).

LG175, Onkaparinga River, above Murray River inflow (35°00.377' S - 138°47.964' E), South Australia, Australia, 30 December 2003, coll. L.J. Grant, identified via molecular samples (LJ168).

LG174, Lake Playford, Belair National Park, near Adelaide (35°00.420' S - 138°38.117' E), South Australia, Australia, 30 December 2003, coll. L.J. Grant, identified via molecular samples (LJ44).

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**Western Australia:**

LJ176, Leschenaultia Lake, Wooraloo National Park, Perth (31°51' S - 116°15' E), Western Australia, Australia, 2 October 2004, coll. L.J. Grant, identified via molecular samples.

**Queensland:**

LJ198, Red Falls, turn off on Lynd Hwy, approximately 40km northwest of Charters Towers (19°55.635' S - 145°44.251' E), Queensland, Australia, 15 November 2003, coll. L.J. Grant, identified via molecular samples.

LG35, Mary River, west of Caloundra (25°42.885' S - 152°34.655' E), Queensland, Australia, 26 December 2002, coll. L.J. Grant, identified via molecular samples (LJ201).

LJ270, Birthday Creek, on road to dam before Birthday Creek Falls, Paluma (18°58.568' S - 146°10.086' E), Queensland, Australia, 28 October 2003, coll. L.J. Grant, identified via molecular samples.

LJ46, North Johnstone River, Malanda Falls, Mallanda (17°21.172' S - 145°35.298' E), Queensland, Australia, 20 August 2003, coll. L.J. Grant, identified via molecular samples.

NQ4, Alligator Creek, Bowling Green Bay National Park, approximately 28km south of Townsville (19°26' S - 146°57' E), Queensland, Australia, 24 August 2002, coll. L.J. Grant, identified via molecular samples.

LG86, Lake Barambah, Bjelke Peterson Dam, Barambah Road, south of Murgon (26°15.985' S - 151°59.046' E), Queensland, Australia, 27 December 2002, coll. L.J. Grant, identified via molecular samples (Q10).

LG93/LG89, Amamoor Creek, Amamoor Creek Road, Amamoor (26°20.530' S - 152°40.373' E), Queensland, Australia, 30 December 2002, coll. L.J. Grant, identified via molecular samples (Q12/Q14).

LG92, Kandanga Creek, Bunya Bridge, northeast of Kandanga on Mary Valley Road (26°22.744' S - 152°41.429' E), Queensland, Australia, 30 December 2002, coll. L.J. Grant, identified via molecular samples (Q16).

LG94, Baroona Lake, near Maleny (26°42.793' S - 152°52.319' E), Queensland, Australia, 31 December 2002, coll. L.J. Grant, identified via molecular samples (Q17).

LG38, Elaman Creek, south of Conondale on Kenilworth - Maleny Road (26°43.423' S - 152°46.126' E), Queensland, Australia, 31 December 2002, coll. L.J. Grant, identified via molecular samples (Q18).

LG77, Lake Wivenhoe, east of Esk (27°17.966' S - 152°30.954' E), Queensland, Australia, 1 January 2003, coll. L.J. Grant, identified via molecular samples (Q20).

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LG78, North Pine River, just South of Dayboro on Mount Samson Road (27°12.945' S - 152°49.983' E), Queensland, Australia, 2 January 2003, coll. L.J. Grant, identified via molecular samples (Q22).

LG39, Terrors Creek, in the township of Dayboro, Dayboro Road (27°11.734' S - 152°49.563' E), Queensland, Australia, 2 January 2003, coll. L.J. Grant, identified via molecular samples (Q23).

LG79, Leslie Dam, approximately 13km west of Warwick, Cunningham Hwy (28°13.204' S - 151°55.075' E), Queensland, Australia, 4 January 2003, coll. L.J. Grant, identified via molecular samples (Q24).

LG64, Kinchant Dam, near Eungella, 35km west Mackay (21°12.954' S - 148°53.844' E), Queensland, Australia, 19 December 2002, coll. L.J. Grant, identified via molecular samples (Q3).

LG33, Hedlow Creek, 30 minutes north of Rockhampton (23°08.503' S - 150°34.170' E), Queensland, Australia, 22 December 2002, coll. L.J. Grant, identified via molecular samples (Q33).

LG34, Lake Awoonga, 20km west of Gladstone (24°04.362' S - 151°18.075' E), Queensland, Australia, 24 December 2002, coll. L.J. Grant, identified via molecular samples (Q9).

LG36, Gordonbrook Dam, Kingaroy (26°26.797' S - 151°45.205' E), Queensland, Australia, 25 December 2002, coll. L.J. Grant, identified via molecular samples (LJ200).

**Victoria:**

LG167, Lysterfield Lake, Lysterfield (37°57' S - 145°17' E), Victoria, Australia, 27 December 2003, coll. L.J. Grant, identified via molecular samples (LJ222).

LJ94, Maroonda Dam, Healesville outflow (37°38.624' S - 145°32.974' E), Victoria, Australia, 19 December 2003, coll. L.J. Grant, identified via molecular samples.

LG268, West Barwon River, off Forrest - Apollo Bay Road (38°32' S - 143°42' E), Victoria, Australia, 7 July 2003, coll. L.J. Grant, identified via molecular samples (LJ98).

LG197, Monbulk Creek at intersection with Belgrave - Hallam Road, Selby Conservation Reserve (37°55.279' S - 145°21.340' E), Victoria, Australia, 14 January 2004, coll. L.J. Grant, identified via molecular samples (LJ221).

LJ113, King River, Hamiltons Bridge, Upper King Valley Road, Cheshunt (36°49.265' S - 146°24.611' E), Victoria, Australia, 20 December 2003, coll. L.J. Grant, identified via molecular samples.

LJ25, Lerderderge River, in Bachus Marsh at intersection with Bachus Marsh - Gisborn Road (37°39.287' S - 144°27.054' E), Victoria, Australia, 14 January 2004, coll. L.J. Grant, identified via molecular samples.

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LG143, Buchan River at Buchan (37°29.833' S - 148°10.482' E), Victoria, Australia, 11

February 2004, coll. L.J. Grant, identified via molecular samples (LJ88).

LG201, Mullum Mullum Creek, Currawong Bush Park at Reynolds Road, Donvale

(37°45.927' S - 145°11.068' E), Victoria, Australia, 14 January 2004, coll. L.J. Grant, identified via molecular samples (LJ92).

LG204, Jacksons Road, Sunbury Road, Sunbury (37°35.071' S - 144°44.534' E), Victoria,

Australia, 14 January 2004, coll. L.J. Grant, identified via molecular samples (LJ95).

## Description

Largest live specimens measuring 15mm x 1.2mm. Head is triangulate with large auricles and a pointed tail. Eyes are large sitting in large pigment free patches and are located closer to each other than to the lateral margins and equal to or slightly anterior to the auricular points (Figures 32-35). Large pigment free patches are located on the lower portion of the auricles. The dorsal surface has a white/transparent base with light and dark brown coarse specks throughout, interrupted by regular pigment free patches. This gives an overall brown, mottled dorsal surface, in some populations interrupted by a pale stripe running medially. The ventral surface is lacking all pigment. There is no trace of any sensory pits or fossae.

The pharynx is located in the posterior half of the animal and occupies approximately one-fifth of the total body length. The mouth is situated at the most posterior extreme of the pharyngeal pocket. The pharynx is clearly pigmented. There is no trace of any ovaries, testes or copulatory apparatus in any of the individuals; so, this species appears to be asexual.

## Discussion

The presence of a pigmented pharynx, the distinctive external morphology and the similarity of the molecular sequences with published *G. tigrina* sequences, allow no other conclusion than that these animals concern the invasive *G. tigrina*. The lack of sexual organs does not affect this reasoning as Ball and Reynoldson (1981) note that in this species it is normally the asexual or fissiparous forms that have become established outside of North America. The first confirmed record of this species within Australia was from Brisbane in 1995 when this *G. tigrina* was identified from external morphology alone (Sluys et al. 1995). Prior to this there had been tentative identifications from the Victorian Alps and Western Australia (Ball 1974a, Hay and Ball 1979, Sluys et al. 1995). With the

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present record we can confirm and also expand this distribution, as it appears that *G. tigrina* has been able to undertake another successful invasion.



**Figures 32-35** *Girardia tigrina*. 32, LG36, external features of living animal; 33, LG106, external features of living animal; 34, LG36, photograph of live specimen; 35, LG143, photograph of live specimen.

#### Ecology and distribution

As would be expected from a successful invader, this species is found in a wide variety of lentic and lotic environments. Living on the underside of rocks, leaf litter, and macrophytes in environments ranging from fast flowing riffles to residual pools. This species exhibits a remarkable thermal tolerance, with successful populations existing in temperatures ranging from 9°C to 33°C. *G. tigrina*'s Australian distribution can now be expanded to include all states and territories, excepting the Northern Territory (not sampled).

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### *Romankenkius* Ball, 1974

#### *Romankenkius cf. bilineatus* Ball & Tran, 1979

#### Material examined

##### Tasmania

LG304, Approximately 17km northwest of Sheffield, at intersection with Eastleys Road  
(41°20'44.55547" S - 146°07'49.50828" E), Tasmania, Australia, 15 March 2003, coll.  
K. Richards, sagittal sections on 4 slides, sagittal sections on 4 slides.

#### Description

There is no detail on the external morphology excluding that which can be gleaned from serial sections. Dorsal pigment present, some pigment present on the ventral surface but much lighter. One set of large sensory pits sitting on lateral margin, and at least one set of sensory fossae on anterior margin. Large eyes are also present, located medially.

The pharynx lies in the posterior part of the animal and occupies approximately one-half of the total body length; the mouth is located at the most posterior extent of the pharyngeal pocket. The copulatory apparatus is located remarkably close to the end of the pharyngeal pocket (Figure 37).

The testes, which show a tendency for fusion, are situated primarily ventrally; however, there are also a few located dorsally. The ventral testes extend to at least the level of the pharyngeal root, while the dorsal testes do not appear to extend this to this level (Figure 37). The broad vasa deferentia travel towards the penis bulb and fuse on its muscular margin (Figure 36). After fusion the duct widens slightly, forming a wide ejaculatory duct without forming a seminal vesicle. The penial papilla is orientated dorso-ventrally and consequently the acentral ejaculatory duct creates a slightly larger posterior lip. The ejaculatory duct narrows only slightly towards its termination and receives many glands along its length. The tip of the rounded penial papilla is distinctive due to the large fold at the point where the ejaculatory duct terminates. However, the latter may well be a fixation artefact and not represent the natural form of the papilla. The male atrium houses the penial papilla comfortably but cannot be described as spacious. There is a small constriction leading to a common atrium, which receives the opening of the broad gonopore.

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The relatively narrow bursal canal arises from a ventral point on the common atrium, rising sharply, travelling laterally to the penial papilla (Figure 36). The bursal canal has a tall epithelium surrounded by a thin layer of longitudinal musculature and a thin layer of circular muscle. Unfortunately in all specimens the bursal canal ends blindly which is probably a result of the relatively immature status of the individuals. In the most mature specimen parts of the epithelium of a copulatory bursa is visible but the detail is not clear enough for description.

There is a long diverticulum arising from so low on the bursal canal that the latter is more accurately described as the common atrium (Figure 36). The short epithelium of the diverticulum has some musculature surrounding it however its exact structure is indistinguishable. Glands enter along the diverticulum's entire length. The narrow oviducts communicate separately with the diverticulum and travel directly towards the anterior of the animal, a caudal branch is absent. The ovaries could not be identified in these animals.

## Discussion

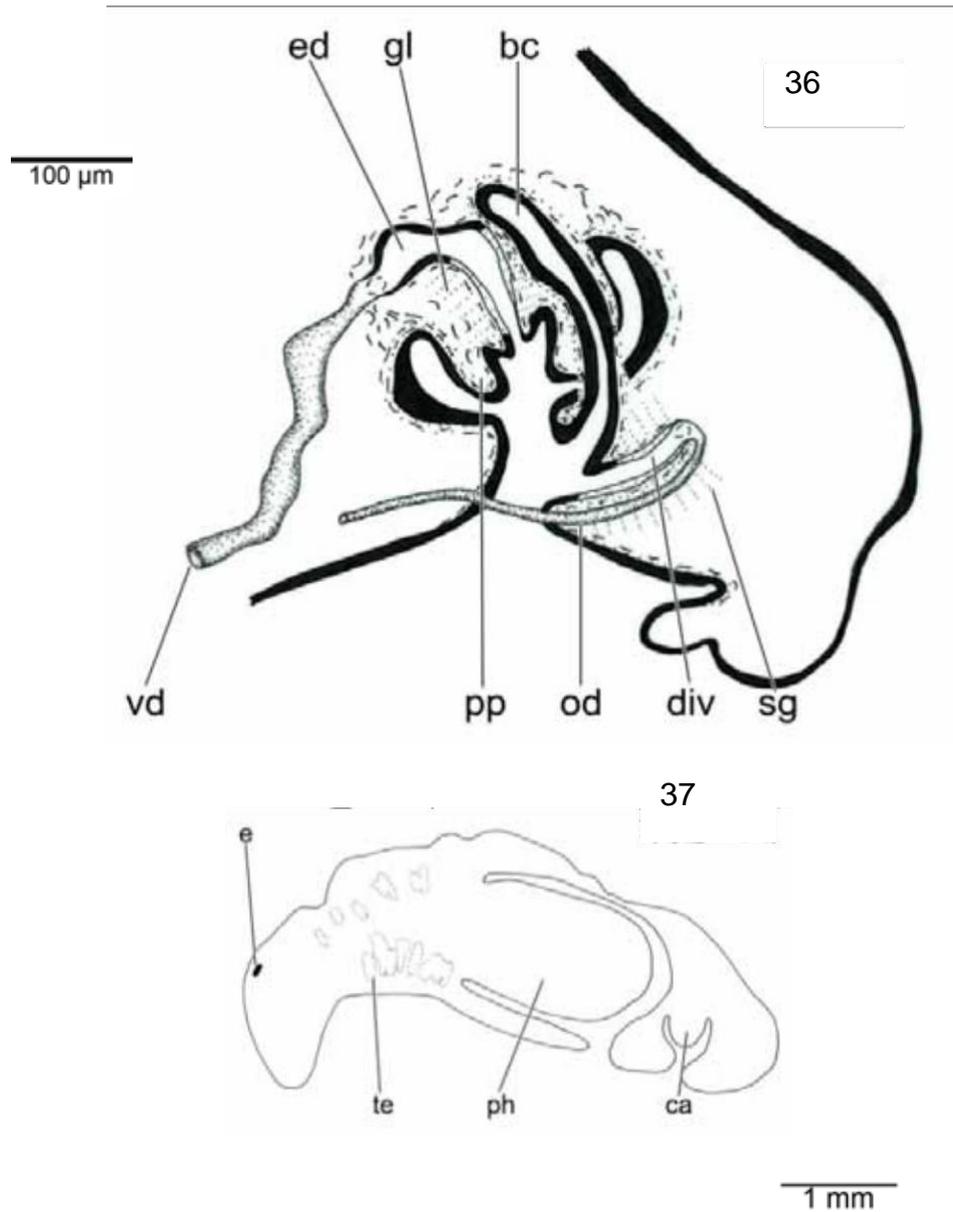
These specimens have been assigned to the genus *Romankenkius* due to the presence of a long diverticulum, receiving shell glands (Figure 36), making generic assignment straightforward. These specimens are only tentatively assigned to *R. bilineatus* due to the inability to identify copulatory bursa or ovaries.

Beyond these inadequacies, this description does not contradict the original description (Ball and Tran 1979) and many notable similarities can be identified. These similarities include: the presence of fusing ventral and dorsal testes, a feature lacking in all other species of *Romankenkius*; the lack of a true seminal vesicle, a rare state for this genus; the reduced musculature on the diverticulum (Figure 36).

Ball and Tran (1979) describe the copulatory apparatus as being remarkably variable, owing to a difference displayed in the orientation of the penial papilla, and suggest that their description may actually be dealing with two very closely related species. While the present new material does not resolve the question of this variability, the orientation of the penial papilla in these new specimens is dorso-ventral confirming at least that this state was not a result of fixation artefacts and may be a common feature of this species.

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**Figures 36-37** *Romankenkius cf. bilineatus*. 36, LG304-2, sagittal reconstruction of copulatory apparatus; 37, LG304-2, sagittal reconstruction of reproductive system.

**Distribution**

*Romankenkius bilineatus* has been described from central northern Tasmania in the Great Lake area. *R. cf. bilineatus* was collected from the slightly north of this site but still within the Tamar River basin.

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### **Romankenkius boehmigi (Weiss, 1909)**

#### **Material examined**

##### **Western Australia:**

LG366, Jane Brook, John Forest National Park (31°51' - 116°00'), Western Australia, Australia, 2 October 2004, coll. L.J. Grant, sagittal sections on 6 slides, sagittal sections on 8 slides, sagittal sections on 5 slides.

#### **Identified via molecular sequence data**

##### **Western Australia:**

LJ253, "Sandlewood Downs" Creek, 286 Bindoon - Dawards Pool Road, small creek along road, 1.3km southeast on Blackwattle Road (31°17' S - 121°09' E), Western Australia, Australia, 30 September 2004, coll. L.J. Grant, identified via molecular samples.

#### **Description**

Largest live specimens 12 x 2mm. Triangulate head with rounded auricles and a pointed tail (Figure 39). Eyes are small, sitting in medium sized, non-discrete pigment free patches. The eyes are closer to each other than to the lateral margins and are positioned anterior to the auricular extremities. While there are no pigment free patches sitting on the auricles, there are two large areas of very low pigment situated on the same level as the eyes but closer to the margins; these areas of low pigmentation do not appear to be associated with any pits or fossae. There is also a striking lack of pigment on the anterior margin but here it seems to be associated with several sensory fossae associated. Dorsal colouration consists of a light brown base colour with an overlying fine, dense dark brown pigment. The overall colouration is dark brown; however, many specimens exhibit a pale medial stripe. The ventral pigmentation is the same as the dorsal with slightly less overlying dark brown pigment.

The pharynx occupies approximately one-third of the total body length and the mouth is situated at posterior end of the pharyngeal pocket (Figure 40).

Testes are represented by non-discrete testicular tissue primarily located dorsally but often extending into the ventral body region. Traces of this testicular tissue can be identified throughout the body length (Figure 40). Vasa deferentia travel ventrally before penetrating the weakly muscled penis bulb to subsequently penetrate the lateral walls of a large seminal vesicle (Figures 38 & 42). This seminal vesicle communicates with a broad, irregular ejaculatory duct. This ejaculatory duct receives two sets of glands: one set opens

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at a small area close to the seminal vesicle and the second set opens at larger area towards the termination of the duct. The ejaculatory duct travels acentrally through the large rounded penial papilla, creating a larger dorsal lip. The flexible papilla is not overly muscularised and does not completely fill the modest male atrium.

Small, paired ovaries are located directly behind the brain; the communication with the oviducts is not traceable (Figure 40). The female atrium houses a large, muscular adenodactyl (Figures 38 & 41-42). The epithelium between the penis papilla and the adenodactyl receives over a small area the openings of glands. The adenodactyl exhibits two character states suggesting that this structure is quite flexible. In LG366-2 the adenodactyl appears to be retracted. What would normally form a papilla type structure is instead convex shaped, with glands exiting through what would normally be the "tip" of the adenodactyl. In LG366-1 the adenodactyl is more papilla-like in its appearance with a large rounded structure protruding into the female atrium. In both specimens the large muscular bulb, exhibiting mixed musculature, is orientated dorsally, reaching the dorsal body surface, and sits laterally to the bursal canal.

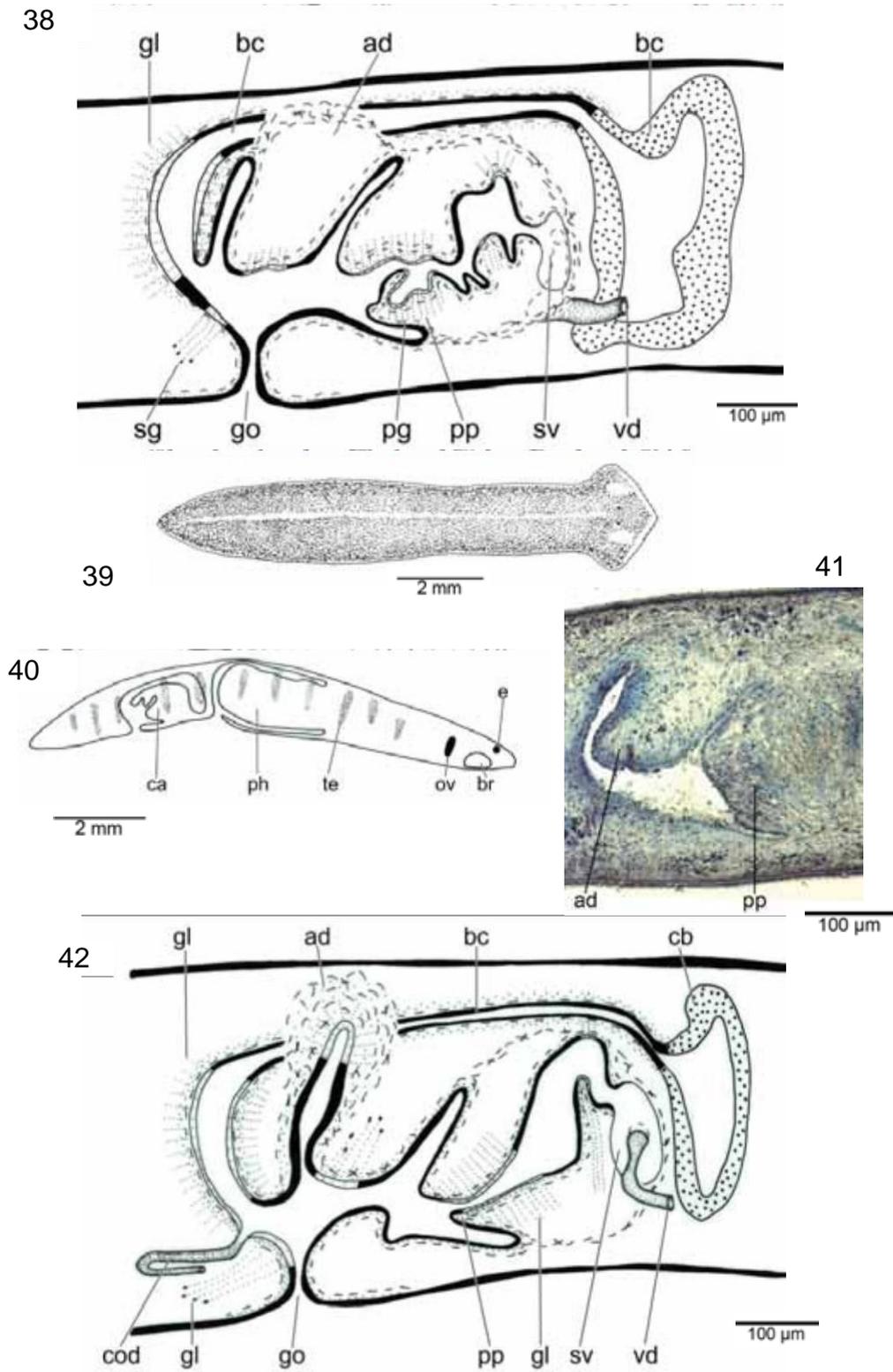
There is a short gonoduct opening into the female atrium just below the adenodactyl. The bursal canal communicates with the female atrium posterior to the adenodactyl (Figures 38 & 42). The canal begins quite broadly, however, as it travels anteriorly it narrows considerably. The narrow epithelium of the canal is covered with reversed musculature and receives the openings of glands from its origin to the point where it turns anteriorly. The bursa is located just anterior to the penial papilla and while the bursa does not extend far into the anterior of the animal it fills the entire dorso-ventral space. The bursa is not surrounded by musculature. The diverticulum communicates directly with the female atrium just below the point where the bursal canal originates. The narrow diverticulum/common oviduct travels a short distance posteriorly before communicating with the oviducts. After the communication the oviducts immediately travel anteriorly.

## Discussion

These specimens match very closely the re-description of *Romankenkius boehmigi* by Sluys (1997). This new material has allowed for the accurate description of the external morphology that, before now, had only been described from preserved specimens. Despite this, the similarities are remarkable, with the extreme paleness on the anterior margin and large pigment free areas close to the eyes already having been suggested in previous descriptions (Sluys 1997). This species is easily located within the genus *Romankenkius*

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**Figures 38-42** *Romankenkius boehmigi*. 38, LG366-1, Sagittal reconstruction of copulatory apparatus; 39, LG366, external features of living animal; 40, LG366-2, sagittal reconstruction of reproductive system; 41, LG366-1, microphotograph of a sagittal section of the penial papilla; 42, LG366-2, sagittal reconstruction of copulatory apparatus.

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due (1) to the long diverticulum of the bursal canal receiving the oviducts and (2) the large seminal vesicle. Within the genus *Romankenkius* there are five species with an adenodactyl, viz. *R. libidinosus*, *R. hoernesii*, *R. boehmigi*, *R. conspectus* and *R. impudicus* (Grant et al. 2006, Sluys 1997, Sluys and Rohde 1991). *R. hoernesii* exhibits many similar features to this specimen. However, the posterior orientation and lateral position of the adenodactyl eliminate this species from the discussion. If we pursue this process of elimination we discover that it is only *R. impudicus* and *R. boehmigi* that show an adenodactyl that is not orientated in line with the penial papilla. For *R. impudicus* this is where the similarity ends between the adenodactyls, as its orientation, which is directly opposing the penial papilla, and structure are markedly different from that of this specimen.

This leaves these specimens to be confidently assigned to *R. boehmigi*. The unique dorso-ventral orientation of the adenodactyl, large seminal vesicle, broad, irregular ejaculatory duct and non-folded penial papilla support this conclusion. The adenodactyl is extremely interesting as it exhibits a high degree of flexibility not reported in the original description (Sluys 1997). LG366-2 appears very similar to the original descriptions in which the adenodactyl probably has been contracted, while in LG366-1 we see the relaxed condition. Finally Sluys' (1997) description of *R. boehmigi* reports the presence of a diaphragm or fold in the ejaculatory duct. This new material suggests that this structure is simply a fold and subject to intraspecific variation.

### Ecology and distribution

Specimens were collected from the underside of rocks in the off-channel area of small streams. The location of this new specimen is 28km east of Perth and only 5km north of the type locality at Gooseberry Hill. Another specimen identified via molecular data was collected approximately 50km north of Perth.

### *Romankenkius glandulosus* (Kenk, 1930)

### Material examined

#### Western Australia:

LG367, Beyondrup Falls, small creek flowing into the Blackwood River (33°52.687' S - 115°51.587' E), Western Australia, Australia, 11 October 2004, coll. L.J. Grant and K. Evertz, sagittal sections on 3 slides, sagittal sections on 2 slides, sagittal sections on 3 slides.

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#### Description

Largest live specimens measure 12mm x 2mm. Head shape is “shovel”-like with rounded auricles and a rounded tail (Figure 46). Two small eyes, located closer to each other than to the lateral margins and posterior to the auricular points, are surrounded by large pigment free patches. The dorsal base colour is very light beige with densely spaced fine, dark grey pigment, giving a consistent dark grey appearance. The ventral surface exhibits the similar pigmentation, however the grey pigmentation is sparser, thus creating a marginally lighter appearance. One set of huge ciliated sensory pits exist on the lateral margin, while closer to the anterior margin there is another set of smaller ciliated sensory pits. Furthermore, there is also at least one set of sensory fossae positioned very close to the anterior extremity of the animal.

The pharynx is located posteriorly and occupies approximately one-quarter of the total body length the mouth is situated at the most posterior point of the pharyngeal pocket (Figure 45).

There are numerous large, discrete testicular follicles extending throughout the body and situated dorsally (Figure 45). The vasa deferentia are broad ducts travelling in a convoluted, but generally ventral, path towards the penis bulb. The ducts ascension to the level of the penis bulb is gradual and they penetrate the musculature of the penis bulb before penetrating the lateral surface of the seminal vesicle (Figures 43-45). The seminal vesicle could be described as a rounded swelling of the very broad ejaculatory duct. The ejaculatory duct travels directly to the end of the conical penial papilla, which is positioned slightly acentrally, thus creating a larger dorsal lip.

The most useful diagnostic feature of this species is the three types of penial glands penetrating the short epithelium of the ejaculatory duct (Figures 43-44). All three types of glands enter through the central section of the ejaculatory duct, yet their entries are clearly delineated; the glandular tissue of the middle type does extend into the penis bulb, unlike the two surrounding types, which are localised around the ejaculatory duct. The penial papilla fills the entire male atrium and even extends slightly past the constriction of the male atrium, which leads to a very narrow common atrium.

A short gonoduct leads to a small gonopore, with the bursal canal communicating with the common atrium at this same level but in a very dorsal position (Figures 43-44). The broad canal arcs gently over the penis bulb to communicate with a massive copulatory bursa situated anterior to the penis bulb. The short epithelium of the bursal canal is surrounded by a layer of longitudinal musculature, which is in turn surrounded by

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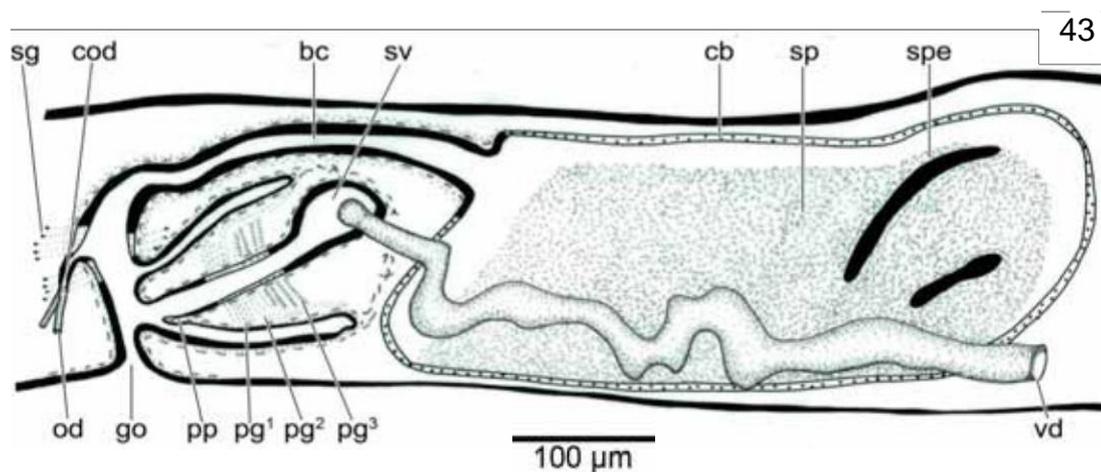
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a stronger layer of circular musculature. The bursa is not surrounded by any musculature and its epithelium is short. The copulatory bursa fills the entire dorso-ventral space and contains large amounts of sperm and, in this specimen, fragments of a spermatophore. The short diverticulum communicates with the most ventral section of the bursal canal. The shell glands enter the bursal canal above and below the level of this diverticulum with no entries into the diverticulum itself.

The oviducts appear to travel dorsally, communicating with the diverticulum from opposing sides. The oviducts can only be traced for a short distance but there is no evidence of a caudal branch. The very small ovaries are located at halfway between the brain and the root of the pharynx. The connection with the oviducts is ventral-posterior.

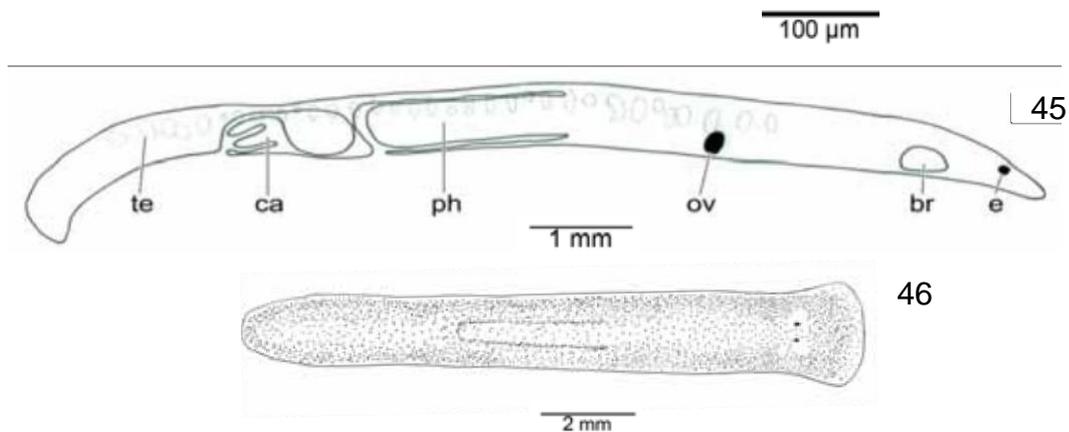
#### Discussion

This description does not differ substantially from the original description of *Romankenkius glandulosus* (Weiss 1910). Subsequent descriptions (Ball 1977, Grant et al. 2006) have noted that Weiss' original description of non-reversed bursal canal musculature was incorrect and this specimen supports this correction. The only minor deviation from previous descriptions is the presence of a clearly defined seminal vesicle receiving the vasa deferentia (Figure 43). This level of variation is not uncommon in other species and is likely to be due to intraspecific variation.



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**Figures 43-46** *Romankenkius glandulosus*. 43, LG367-2, sagittal reconstruction of copulatory apparatus; 44, LG367-2, microphotograph of a sagittal section of the copulatory apparatus; 45, LG367-2, sagittal reconstruction of reproductive system; 46, LG367, external features of living animal.

#### Ecology and distribution

These specimens were found in low numbers across a broad range of habitats including riffles, runs and off-channel areas in a large, predominantly sandy, river. Individuals were collected from the underside of rocks not associated with any macrophytes or leaf litter. This new site is located within kilometres of the type locality in the southern corner of Western Australia and yielded the second series of specimens that has become available after Weiss' (1910) first detailed description of the species.

#### *Romankenkius impudicus* Sluys & Grant, 2006

#### Material examined

##### Victoria:

LG209, Campaspe River in Redesdale near Lake Eppalock (37°00.973' S - 144°32.434' E), Victoria, Australia, 16 January 2004, coll. L. J. Grant, horizontal sections on 6 slides, sagittal sections on 10 slides, sagittal sections on 9 slides.

#### Description

Live specimens measure 2mm x 9mm. A mildly triangulate head with small auricles and small eyes sitting in large pigment free patches (Figure 54). The eyes are positioned at approximately equal distance between the lateral margins and the midline, equal to the auricular points. There are two sets of sensory fossae on the antero-ventral margin. Dorsal

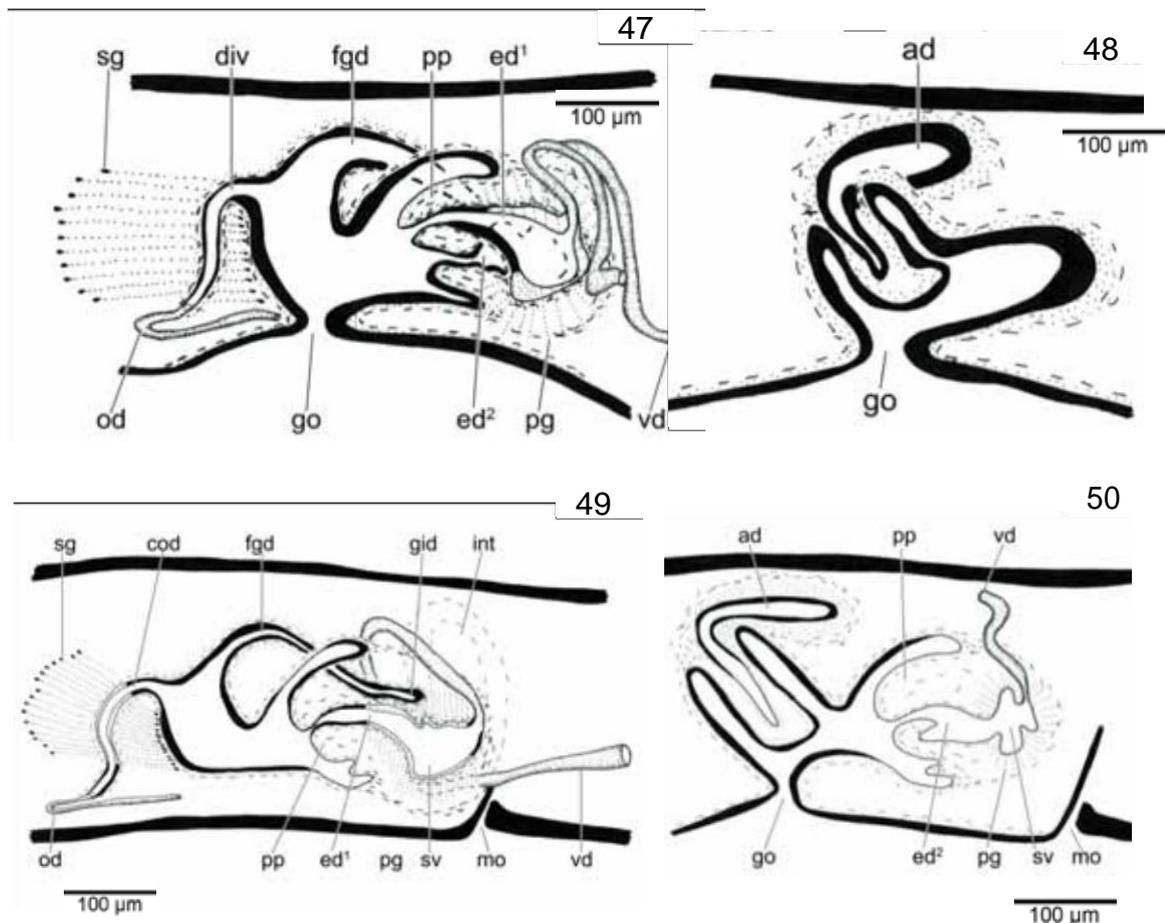
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colouration exhibits a light, almost transparent base with coarse light brown specks creating a slightly mottled effect. The ventral surface is lacking all pigment.

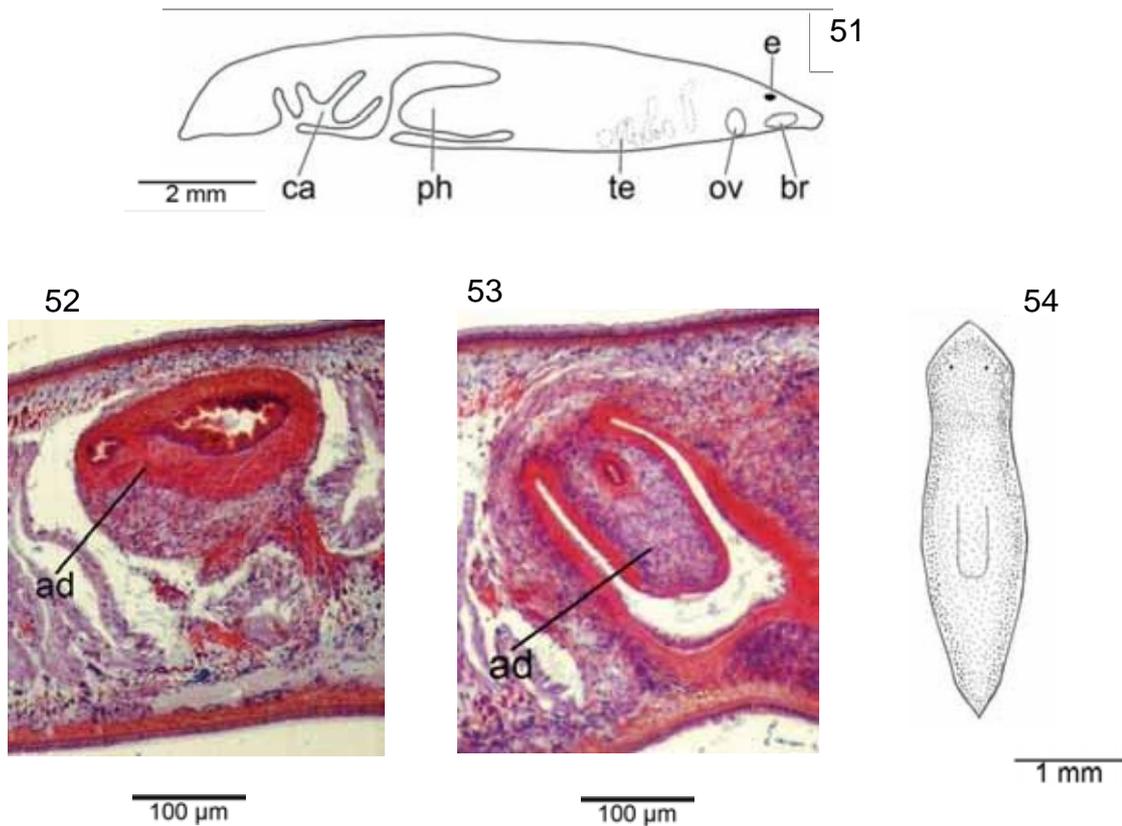
The pharynx is situated posteriorly and occupies approximately one-fifth of the total body length. The mouth is situated at the most posterior point of the pharyngeal pocket (Figure 51).

The testes are large pre-pharyngeal follicles positioned primarily ventrally, however follicles are often extending into the dorsal region. The vasa deferentia are broad, convoluted ducts which travel dorsally above the level of the penis bulb (Figures 47, 49 & 50). After passing the level of the penis bulb both ducts re-curve to communicate with separate seminal vesicles, inside the penis bulb. The two large, glandularised, seminal vesicles are located laterally to each other and give rise to two quite separate ejaculatory ducts. In both cases the ejaculatory ducts are broad narrowing slightly before terminating separately at the tip of the papilla. While the openings of the ejaculatory ducts are sitting laterally to each other one is more dorsal than the other. The penial papilla is a flexible, rounded structure with moderate levels of muscularisation. The penis bulb is modest with light intermingled muscularisation. The penial papilla is sitting in a spacious male atrium, which communicates with a huge common atrium (Figures 47, 49 & 50).



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**Figures 47-54** *Romankenkius impudicus*. 47, LG209-1, sagittal reconstruction of the copulatory apparatus; 48, LG209-1, sagittal reconstruction of the adenodactyl; 49, LG209-2, sagittal reconstruction of the copulatory apparatus showing one ejaculatory duct; 50, LG209-2, sagittal reconstruction of the second ejaculatory duct and adenodactyl; 51, LG255-3, sagittal reconstruction of reproductive system; 52, LG209-1, microphotograph of the root of the adenodactyl; 53, LG209-1, microphotograph of the papilla of the adenodactyl; 54, LG209, external features of a living animal.

The common atrium is large and houses the muscular adenodactyl (Figures 48, 50 & 52-53). The adenodactyl has a highly muscular root filling the space between the dorsal margin and the common atrium. This dorsal end of the adenodactyl has an extremely thick layer of circular muscle surrounding it, while the epithelium is quite short. There is a cavity that runs the length of the adenodactyl surrounded by light circular muscle and a thin epithelium, which narrows further before ending.

In both specimens examined, the cavity inside the adenodactyl appears to simply stop, however, it appears that this termination is in fact an opening that is not at the tip of the structure. The adenodactyl protrudes a considerable distance into the common atrium, orientated antero-ventrally, mirroring the penial papilla.

Another structure communicating with the common atrium is the narrow female genital duct (Figures 47 & 49). The canal communicates with the most dorsal part of the

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atrium, after which the canal quickly curves antero-ventrally. The duct narrows as it progresses towards a communication with a branch of the intestines lateral to the penis bulb. A thin layer of reversed musculature surrounds the female genital duct. The oviducts travel a considerable distance posterior to the common atrium before recurving to communicate at the same point with a broad, muscularised diverticulum. The diverticulum is long, travelling dorsally in order to communicate with the base of the bursal canal or possibly the common atrium. The other distinctive feature of this diverticulum is its high level of glandularisation. There is no evidence of a caudal branch of oviducts in these specimens. The ovaries are large follicles sitting a short distance behind the brain; the oviducts communicate with the mid-anterior surface of the ovaries (Figure 51).

## Discussion

This species possesses a long diverticulum and large seminal vesicles, indicating that these specimens are members of *Romankenkius*. The adenodactyl with its anterior orientation and the unique free papilla is reminiscent of the recently described *Romankenkius impudicus* (Grant et al. 2006)(Figures 48, 50 & 52-53). Other characters common to both *R. impudicus* and the present specimens include the dorsal communication of the bursal canal or female genital duct and the dorso-ventral nature of the testes. These undeniable similarities lead us to a re-examination and re-interpretation of the poorly stained type material. This examination allowed for no other conclusion than that these new specimens are *Romankenkius impudicus* and, therefore, a re-description of this species was necessary.

Prior to this re-description, *R. impudicus* was already well-defined by its unique adenodactyl. Now the animal appears to be even more aberrant than initially thought. The presence of two ejaculatory ducts opening independently at the tip of the penial papilla, only the second case in the triclads (first case being *Girardia biapertura* (cf. Sluys et al. 1997)) presents an excellent diagnostic character (Figures 47, 49 & 50). Another notable feature is the absence of a copulatory bursa, instead a female genital duct communicates directly with a branch of the intestine (Figures 47 & 49), the latter incorrectly considered to be a copulatory bursa by Grant et al. (2006).

## Ecology and distribution

This species was collected from the underside of cobble in the warm, off-channel area of a sandy river. Grazing activity had heavily disturbed the river, riparian zone and surrounding region, making suitable habitat sparse. *R. impudicus* is now known from two

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sites in southern Australia, the type specimen was collected from south of Adelaide while this new material was collected from northwest of Melbourne.

#### ***Romankenkius kenki* Ball, 1974**

#### **Material examined**

##### **Tasmania:**

LG231, Russel Falls, Mt. Field National Park (42°40.916' S - 146°43.004' E), Tasmania, Australia, 25 January 2004, coll. L. J. Grant, sagittal sections on 7 slides, sagittal sections on 9 slides, sagittal sections on 7 slides, horizontal sections on 3 slides.

LG227, Snug Falls, near Snug, approximately 8km south of Kingston (43°05.038' S - 147°13.380' E), Tasmania, Australia, 24 January 2004, coll. L. J. Grant, sagittal sections on 4 slides.

LG219, Arve River, on road to Hartz Mountain National Park west of Geeveston (43°09.520' S - 146°48.411' E), Tasmania, Australia, 22 January 2004, coll. L. J. Grant, sagittal sections on 12 slides.

LG220, Lake Pedder, near Serpentine Dam at junction with small cascade (42°46.273' S - 145°59.065' E), Tasmania, Australia, 23 January 2004, coll. L. J. Grant, sagittal sections on 7 slides.

LG255, Lake Burbury, near boat ramp (42°08.643 S - 145°39.029' E), Tasmania, Australia, 31 January 2004, coll. L.J. Grant, horizontal sections on 8 slides, sagittal sections on 16 slides, sagittal sections on 8 slides, horizontal sections on 10 slides.

LG225, Kallister Creek, on Gordon River Road (42°46.113' S - 146°34.061' E), Tasmania, Australia, 23 January 2004, coll. L. J. Grant, sagittal sections on 8 slides.

LG298, Approximately 38km east of Strathgordon, at intersection with Gordon River Road (42°44'38.35410" S - 146°30'51.59476" E), Tasmania, Australia, 1 July 2003, coll. K. Richards, sagittal sections on 6 slides, horizontal sections on 3 slides.

LG258, Iris River, on road to Cradle Mountain approximately 10km north of Cradle Mountain (41°33.708' S - 145 ° 56.994' E), Tasmania, Australia, 1 February 2004, coll. L. J. Grant, sagittal sections on 8 slides.

##### **Material identified via molecular sequence data:**

LG261, Wandle River, on Road to Hellyer Gorge approximately 60km south of Somerset (41°21.706 S - 145°34.882' E), Tasmania, Australia, 1 January 2004, coll. L.J. Grant, identified via molecular samples (LJ29).

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LJ299, small creek approximately 21km northwest of St Helens, near Anchor Road (41°15'38.780" S - 145°00'44.274" E), Tasmania, Australia, 11 September 1999, coll. K. Richards, identified via molecular samples.

#### Description

Largest live specimens measure 12 mm x 1.5 mm. This species is remarkable due to the high degree of variation in the external morphology. Throughout all populations all specimens have a triangulate head with well-defined pointed auricles and a tail tapering to a point (Figures 59-60). Small eyes, sitting in small pigment free patches are positioned close to the midline and approximately equal to the extremes of the auricular points. There a small pigment free patch sitting at on each auricle denoting a deep sensory pit, however no evidence of sensory fossae was found. The dorsal pigmentation ranges from a very pale, almost transparent, base colour to a yellow hue or light brown pigmentation. This base colour is overlain with variable densities of darker pigment. The overall appearance therefore ranges from light grey to almost black. Many populations exhibit a fine medial stripe exposing the base colour. The ventral pigmentation ranges from pigment free to exhibiting only slightly less pigment to the dorsal surface.

The pharynx occupies approximately one-quarter of the total body length and is located in the posterior half of the animal (Figure 62).

Numerous large irregular testes are situated dorsally and only in the pre-pharyngeal area (Figure 62). The vasa deferentia travel ventrally, turning abruptly at the level of the penis bulb before fusing to create a common intrabulbar vas deferens (Figures 55 & 57). This common duct travels some distance before communicating with an irregular seminal vesicle. The seminal vesicle receives the openings of many glands through its epithelium. The ejaculatory duct is also irregular in shape but always broad and appears to run centrally through the penis papilla; however, due to the nature of the duct it is difficult to accurately label this feature. The penial papilla is a flexible, rounded structure with some muscularisation. The muscular penis bulb is large, occupying much of the dorso-ventral space. The penial papilla nearly fills the large male atrium, which communicates via a broad opening to a common atrium.

The common atrium receives a short gonoduct arising from a distinct gonopore. The bursal canal also communicates with the common atrium, but on its lateral face. The narrow canal then runs dorsally before turning anteriorly in order to communicate with the copulatory bursa (Figures 56, 58 & 61). The bursal canal is surrounded by reversed musculature, with a thick layer of circular muscle surrounding a thin layer of longitudinal.

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The copulatory bursa is a large sac shaped structure filling almost the entire dorso-ventral space, the entire female apparatus sits laterally to the penial papilla and therefore much of the copulatory bursa is not positioned anterior to the penial papilla, as is common in Dugesiids. There is some light intermingled musculature surrounding the bursa, however, the most interesting feature is a clear connection with the intestine. A short duct arises from the bursa to communicate with a branch of the intestine. In all specimens this communication is on the opposite side of the bursa from the penial papilla.

The broad diverticulum communicates with the base of the bursal canal at a point that could easily be interpreted as the common atrium (Figures 55 & 58). The diverticulum is long and surrounded by indistinguishable musculature until the point where the oviducts communicate with it. Large amounts of glands also enter the diverticulum along its length. There is no evidence of a caudal branch of the oviducts, however, both oviducts travel beyond the copulatory apparatus before recurving to communicate with the diverticulum. Small discrete ovaries are situated ventrally and directly behind brain (Figure 62).

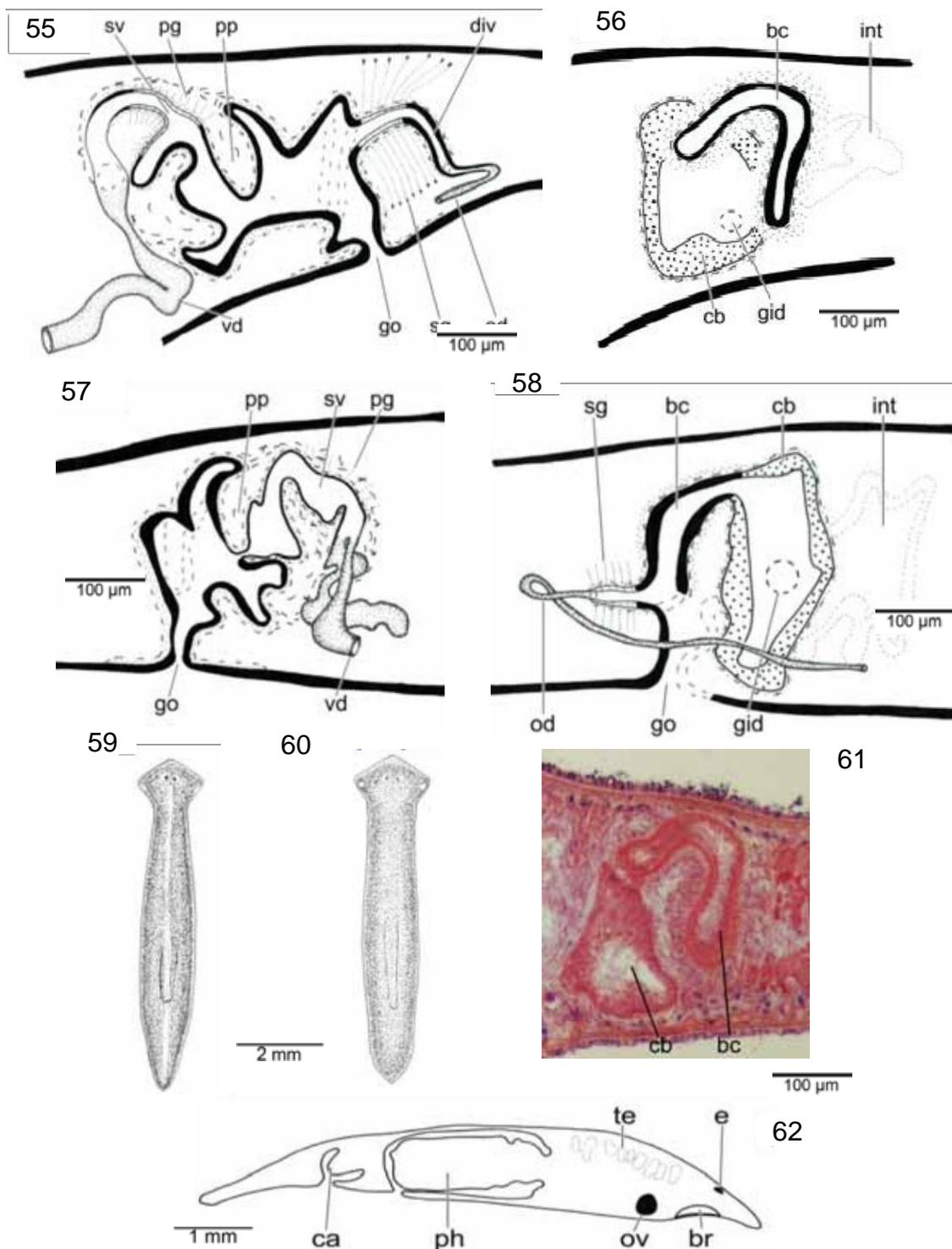
## Discussion

Ball's (1974b) original description of *Romankenkius kenki* mentions a double seminal vesicle and dorsal testes as being diagnostic characters for this species. Due to the increase in species assigned to this genus, these features are no longer very helpful in distinguishing *R. kenki* from the five other *Romankenkius*' with dorsal testes (Grant et al. 2006, Sluys 2001). The double seminal vesicle has also become somewhat redundant, as this character has proven to be influenced heavily by the highly flexible penial papilla. The most striking feature of the seminal vesicle is the extreme variability between representatives. However variability is not a very useful diagnostic character (Figures 55 & 57).

As has been noted in more recent studies, the most characteristic feature of *R. kenki* is the postero-lateral position of the copulatory bursa (Grant et al. 2006)(Figures 56, 58 & 61). Indeed this new material supports this feature as a defining character for the species.

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**Figures 55-62** *Romankenkius kenki*. 55, LG227-3, sagittal reconstruction of the male copulatory apparatus; 56, LG227-3, sagittal reconstruction of the female copulatory apparatus; 57, LG255-3, sagittal reconstruction of the male copulatory apparatus; 58, LG255-3, sagittal reconstruction of the female copulatory apparatus; 59, LG261, external features of living animal; 60, LG231, external features of living animal; 61, microphotograph of a sagittal section of the female apparatus; 62, LG255-3, sagittal reconstruction of reproductive system.

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The other interesting feature of *R. kenki* is the presence and occasional absence of the direct connection between the copulatory bursa and the intestine (Figures 56 & 58). This feature was described by Ball (1974b) in the initial description, however this feature was only recorded in one of the more recent specimens examined (Grant et al. 2006). All specimens examined in this study have a clear connection between the lateral side of the copulatory bursa and a branch of the intestine. This discrepancy may be due to the poor staining of previous specimens, preventing us from seeing this level of detail. Nevertheless, there is a possibility that the connection is absent in some specimens. This could be an independent state or may be related to the observed variability in the development of the copulatory bursa (Grant et al. 2006).

#### Ecology and distribution

*Romankenkius kenki* was collected from the underside of cobble on the shores of lakes or the off-channel area of rivers with muddy substrates. All specimens were collected from pristine, forested environments throughout the entire Tasmanian drainage division.

#### ***Romankenkius libidinosus* Sluys and Rohde, 1991**

*Romankenkius libidinosus* is considered something of an anomaly due to the non-reversed musculature of the bursal canal. At the time of description this character state was known only from *Schmidtea*, *Eviella* and a few species of *Girardia* (Sluys and Rohde 1991). More recently this character has also been described for *Masaharus* (this thesis). The presence of a short diverticulum of the bursal canal prompted Sluys and Rohde (1991) to assign this species to *Romankenkius* despite the presence of the long caudal branch of the oviducts, a synapomorphy of the genus *Spathula*. However, a similar length diverticulum is also present in *Sp. truculenta*, which was assigned to *Spathula* with little controversy (Ball 1977). Interestingly *Sp. truculenta* also has a unique bursal canal musculature described as reversed with ectal re-enforcement.

The presence of an adenodactyl was at the time of description thought rare in Dugesiidae, however several species have been described with this character from the genera *Romankenkius* and *Spathula*. Interestingly the size and orientation of the adenodactyl found in *R. libidinosus* closely resembles that of *Sp. musculosa*.

All of these morphological inconsistencies simply support the already convincing evidence provided by phylogenetic analysis for the re-assignment of this species. Both morphological and molecular analyses place *R. libidinosus* within the *Spathula* clade,

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considerably removed from other *Romankenkius* species. It is possible that this species does represent a new genus, as it often sits on the periphery of *Spathula* with other monotypic genera (*Eviella*, *Reynoldsonia*), however, for now I think it is satisfactory that it remain within *Romankenkius*, with further consideration to follow.

#### Ecology and distribution

Specimens found in cool temperature rainforest areas, with water percolating through the rock of an inland cliff to form runnels and trickles. In one such *Romankenkius libidinosus* (white) was found exclusively. Higher up it mixed with *Spathula simplex* (black), and 100 m down the path only *S. simplex* could be found. The specimens examined were collected from the type locality of this species in New England National Park in northeastern New South Wales.

#### *Romankenkius musculoglandulosus* Grant and Sluys, sp. nov.

Material examined

#### Western Australia:

##### HOLOTYPE:

LG328, Darkin River (32°4'58' S - 116°26'12" E), 29 August 1999, Western Australia, Australia, C.A.L.M., sagittal sections on 8 slides.

##### PARATYPE:

LG362, Small Creek flowing into Avon River on road into Northam coming from west just off Great Eastern Highway in Northam (31°38' S - 116°40' E), 2 October 2004, Western Australia, Australia, coll. L.J. Grant, sagittal sections on 5 slides, sagittal sections on 5 slides, sagittal sections on 6 slides.

#### Diagnosis

This species is distinguishable from its congeners due to the presence of a muscular adenodactyl opposing the penial papilla, pre-pharyngeal dorsal testes, antero-lateral copulatory bursa and glands exiting through the epithelium of the penial papilla.

#### Description

#### **APPENDIX 1d**

##### **A CONTRIBUTION TO THE CURRENT SPECIES INVENTORY FOR AUSTRALIAN FRESHWATER TRICLADS (PLATYHELMINTHES, TRICLADIDA)**

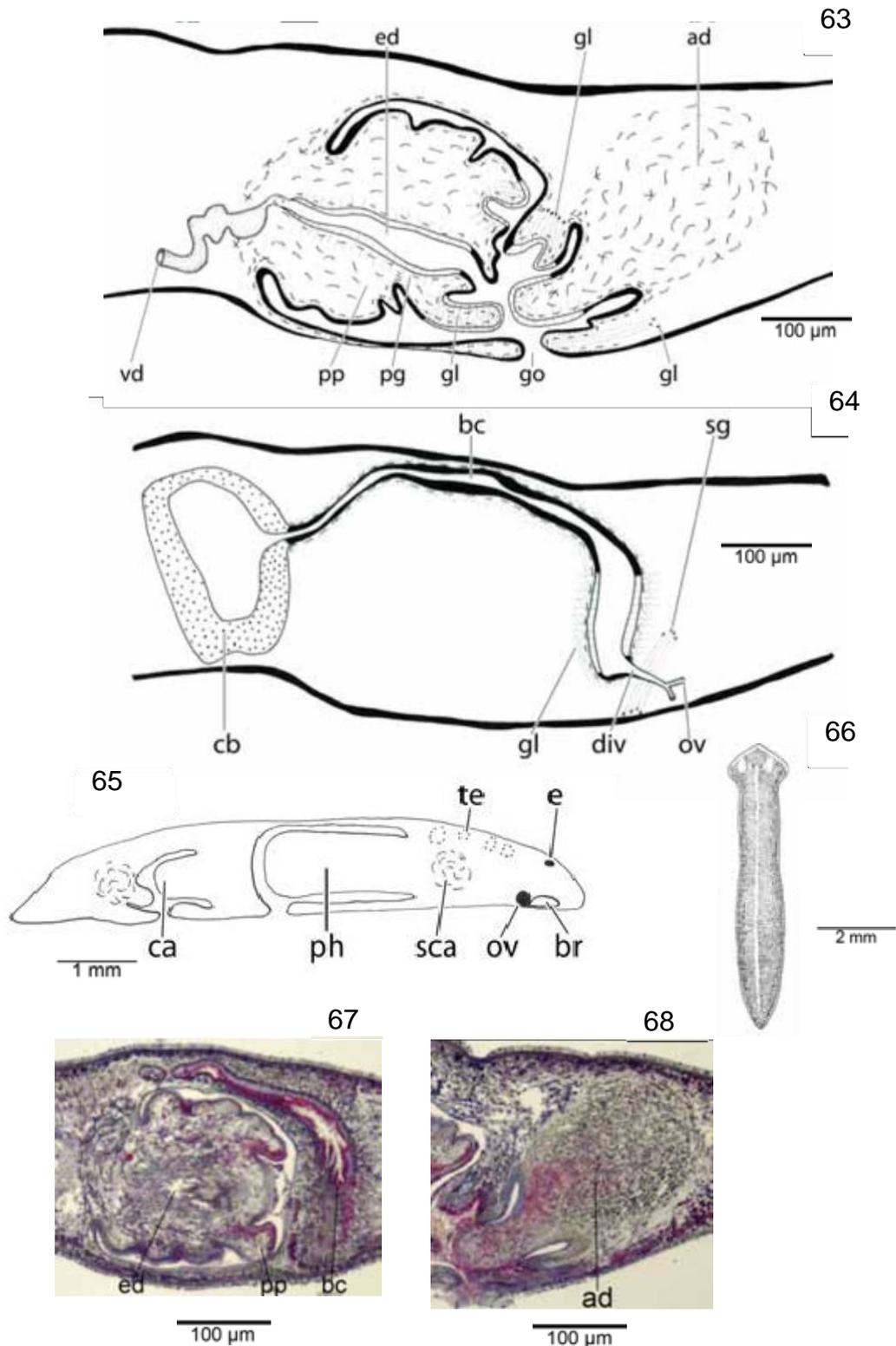
Largest live specimens are 13mm x 2 mm. The head is triangulate with rounded auricles and a pointed tail. The small eyes are sitting in large pigment free patches closer to each other than to the lateral margins and positioned anterior to the auricles (Figure 66). There are no pigment free patches on the auricles. However, large pigment free patches do exist between the auricles and the eyes. The dorsal surface is heavily pigmented with a light brown base and dense, fine dark pigment throughout. The pigment does lighten dramatically, however, on the anterior margin and medially to create a fine medial stripe. The ventral pigment less than the dorsal surface yet still heavily pigmented. There are several sensory fossae located on the anterior margin, the details of these sensory structures are difficult to determine due to the poor condition of the epithelium of the sections.

The pharynx occupies approximately one-quarter of the total body length and the mouth is located at the posterior end of the pharyngeal pocket (Figure 65). There are a low number of small testes, sitting dorsally and only identifiable in the pre-pharyngeal region (Figure 65). The vasa deferentia are broad ducts that narrow slightly before penetrating the muscular penis bulb (Figures 63 & 67). The vasa eiferentia communicate directly with a broad, straight ejaculatory duct, there is no trace of a seminal vesicle. The tall epithelium of this duct receives light glandularisation along its entire length. The duct runs slightly acentrally creating a larger dorsal lip in the large, muscular penial papilla. There are several folds in the papilla, suggesting that this structure is extremely flexible. The other remarkable feature of the papilla is the four areas of glandularisation exiting through the short epithelium. The first set is close to the tip of the penial papilla while the second set, which is clearly delineated from the first one, is found on the dorsal and ventral surfaces. The papilla occupies the majority of the male atrium, which occupies almost the entire dorso-ventral space. The male atrium communicates via a broad opening with a large common atrium.

A gonoduct is absent and therefore the gonopore communicates directly with the common atrium. The large, muscular adenodactyl occupies much of the space in the common atrium (Figures 63 & 68). The adenodactyl's orientation directly opposes the penial papilla with the muscular bulb orientated towards the posterior of the animal while the papilla of the structure extends anteriorly. The papilla is muscular and its short epithelium is heavily glandularised. In addition to this glandularisation, there are two large areas of glands exiting into the female atrium through the epithelium on either side of the adenodactyl. The large muscular bulb of the adenodactyl occupies the entire dorso-ventral space and also extends some distance into the posterior of the animal.

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**Figures 63-68** *Romankenkius musculoglandulosus* 63, LG328-1, sagittal reconstruction of the male copulatory apparatus; 64, LG328-1, sagittal reconstruction of the female copulatory apparatus; 65, LG328-1, sagittal reconstruction of reproductive system; 66, LG328, external features of living animal; 67, LG328-1, microphotograph of a sagittal section of the penial papilla; 68, LG328-1, microphotograph of a sagittal section of the adenodactyl.

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A broad bursal canal arises from the lateral surface of the common atrium (Figure 64). Light glandularisation penetrates the epithelium of the bursal canal from its point of origin to the point where it turns anteriorly. The canal then travels over the lateral extreme of the penial papilla to communicate with a large rounded bursa anterior to the penis bulb. The bursal canal is surrounded by a thin layer of longitudinal muscle and by a thicker layer of circular muscle. The communication of the oviducts with bursal canal is via a diverticulum. This narrow diverticulum arises from the most ventral extreme of the bursal canal. The diverticulum receives glands along its length as it travels further ventrally to communicate with the oviducts. The path of the oviducts is difficult to trace, however, there is no evidence of a caudal branch. Communication of the oviducts with vittellaria is evident. Small, paired ovaries are positioned directly behind the brain on the ventral surface (Figure 65).

## Discussion

Despite the lack of a seminal vesicle, the lengthy diverticulum suggests that this species is a member of *Romankenkius*. The presence of an adenodactyl and the nature of the glandularisation in the penial papilla invites comparison with *R. hoernesii* (Sluys 1997). Despite some remarkable similarities, *R. hoernesii* has a clear seminal vesicle, testes that extend up to the gonopore and ovaries located some distance behind the brain. In addition to all of these conflicts, the adenodactyl has an opposing orientation and a lateral position in *R. hoernesii*. It would be unlikely that the latter fact is a result of intraspecific variability or individual polymorphism, particularly as such variability has not been encountered in other species of *Romankenkius* with adenodactyls. All other *Romankenkius*' with adenodactyls can be discounted due to the conflicting orientation, excluding *R. impudicus* and *R. boehmigi*. But *R. impudicus* can be immediately discounted owing to the double ejaculatory duct and absence of a copulatory bursa (this paper).

The external morphology of *R. boehmigi* and this species are almost identical (Figures 36 & 66). Other similarities include the nature of the glandularisation on the adenodactyl and the extended glandularisation on the bursal canal (Figures 63, 67 & 68). Despite these similarities, *R. boehmigi* exhibits a seminal vesicle, non-folded papilla, an absence of glands exiting through the epithelium of the penial papilla and the bursal canal does not run laterally to the penial papilla, conflicting with observations for this species. Additionally the testes in *R. boehmigi* extend into the posterior of the animal. Therefore, a new *Romankenkius* species is here proposed.

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### Ecology and distribution

This species was collected from *Acacia* forest inland from Perth, Western Australia. Worms from site LG362 were found in large numbers gliding across granite bedrock and swimming freely in the water channel of a small stream.

### Etymology

The specific epithet is derived from the Latin noun '*musculo*' (muscle) and adjective '*glandulosus*' (glandular), referring to the highly muscularised and glandularised penial papilla.

### *Romankenius pedderensis* Ball, 1974

#### Material examined

##### Tasmania:

LG223, Lake Pedder, near Serpentine Dam at junction with small cascade (42°46.273' S - 145°59.065' E), Tasmania, Australia, 23 January 2004, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 7 slides, sagittal sections on 5 slides.

LG288, Approximately 20km east of Railton, creek intersects with Dolerite Road (41°20'37.02449" S - 146°05'34.06718" E), Tasmania, Australia, 30 March 2003, coll. K. Richards, sagittal sections on 5 slides, horizontal sections on 3 slides.

LG290, Approximately 20km west of Sheffield at intersection with Castra Road (41°21'04.31174" S - 146°04'59.26121" E), Tasmania, Australia, 30 March 2003, coll. K. Richards, horizontal sections on 4 slides, sagittal sections on 5 slides.

LG292, Approximately 19km west of Sheffield, at intersection with Gaunts Road (41°21'29.07136" S - 146°05'54.86374" E), Tasmania, Australia, 23 March 2003, coll. K. Richards, sagittal sections on 6 slides, sagittal sections on 4 slides.

LG294, Approximately 7km southeast of Ulverstone, at intersection with Thompsons Road (41°12'59.40254" S - 146°11'58.31991" E), Tasmania, Australia, 8 October 2003, coll. K. Richards, sagittal sections on 4 slides, sagittal sections on 3 slides.

LG297, Approximately 18km northwest of Sheffield, at intersection with Ghost Hole Road (41°20'50.54754" S - 146°06'44.88134" E), Tasmania, Australia, 22 March 2003, coll. K. Richards, sagittal sections on 4 slides, sagittal sections on 5 slides.

LG301, Approximately 27km south of Penguin, at intersection with Flints Road (41°20'59.83949" S - 146°05'48.81205" E), Tasmania, Australia, 30 March 2003, coll. K. Richards, sagittal sections on 4 slides, sagittal sections on 5 slides.

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LG302, Approximately 18km northwest of Sheffield, at intersection with Ghost Hole Road (41°20'50.54754" S - 146°06'44.88134" E), Tasmania, Australia, 22 March 2003, coll. K. Richards, sagittal sections on 5 slides, sagittal sections on 5 slides.

LG295, Approximately 16km northwest of Sheffield, also intersecting with Ghost Hole Road (41°20'42.89360" S - 146°07'44.15163" E), Tasmania, Australia, 15 March 2003, coll. K. Richards, sagittal sections on 4 slides.

LG340, Approximately 17km southwest of Burnie at Intersection with Prospect Road (41°09'04.54982" S - 145°45'05.89212" E), Tasmania, Australia, 5 August 2003, coll. K. Richards, sagittal sections on 4 slides, sagittal sections on 4 slides.

## Description

Largest live specimens measure 8mm x 2 mm. Head is triangulate with small, pointed auricles and a pointed tail (Figure 71). Small eyes are sitting in small pigment free patches, medially and approximately equal to auricular extremities. There are small pigment free patches on the lateral margin just below the auricles representing a pair of large ciliated pits, one set of sensory fossae is also present on the anterior margin. The dorsal pigmentation is a mildly transparent yellow hue, overlain with fine, brown, sparse pigmentation, giving an overall brown-yellow appearance. The ventral surface has none of the overlying brown pigmentation found on the dorsal surface, thus the transparent yellow base colour is exposed.

The pharynx is located in the posterior half of the animal and occupies approximately one-fifth of the total body length. The mouth is situated at the most posterior extreme of the pharyngeal pocket (Figure 72).

Testes are large, discrete, ventral follicles starting a long distance behind the brain, and found only in the pre-pharyngeal region (Figure 72). The vasa deferentia are broad ducts, travelling ventrally towards the penis bulb, each duct narrowing slightly before penetrating the musculature of the penis bulb (Figure 69). The vasa differentia communicate with a small seminal vesicle, which could realistically be described as a mild swelling of the ejaculatory duct. The narrow ejaculatory duct travels acentrally through the penial papilla creating a larger dorsal lip. The ejaculatory duct has a very short epithelium, surrounded by intermingled musculature, and receiving the openings of penial glands along its entire length. The large, rounded penial papilla almost fills the entire dorso-ventral space, a small fold in the ventral lip suggests that the papilla is flexible, yet there is a paucity of musculature. The papilla fills the entire male atrium, which receives the opening of a short gonoduct as well as the diverticulum (Figure 69). The diverticulum

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is a broad duct, which travels towards the ventral surface where the oviducts communicate separately with it. Shell glands enter the diverticulum along the proximal half of its length. After their communication with the diverticulum the oviducts travel a short distance caudally, to communicate with the resorptive vesicles of the vitellarian follicles.

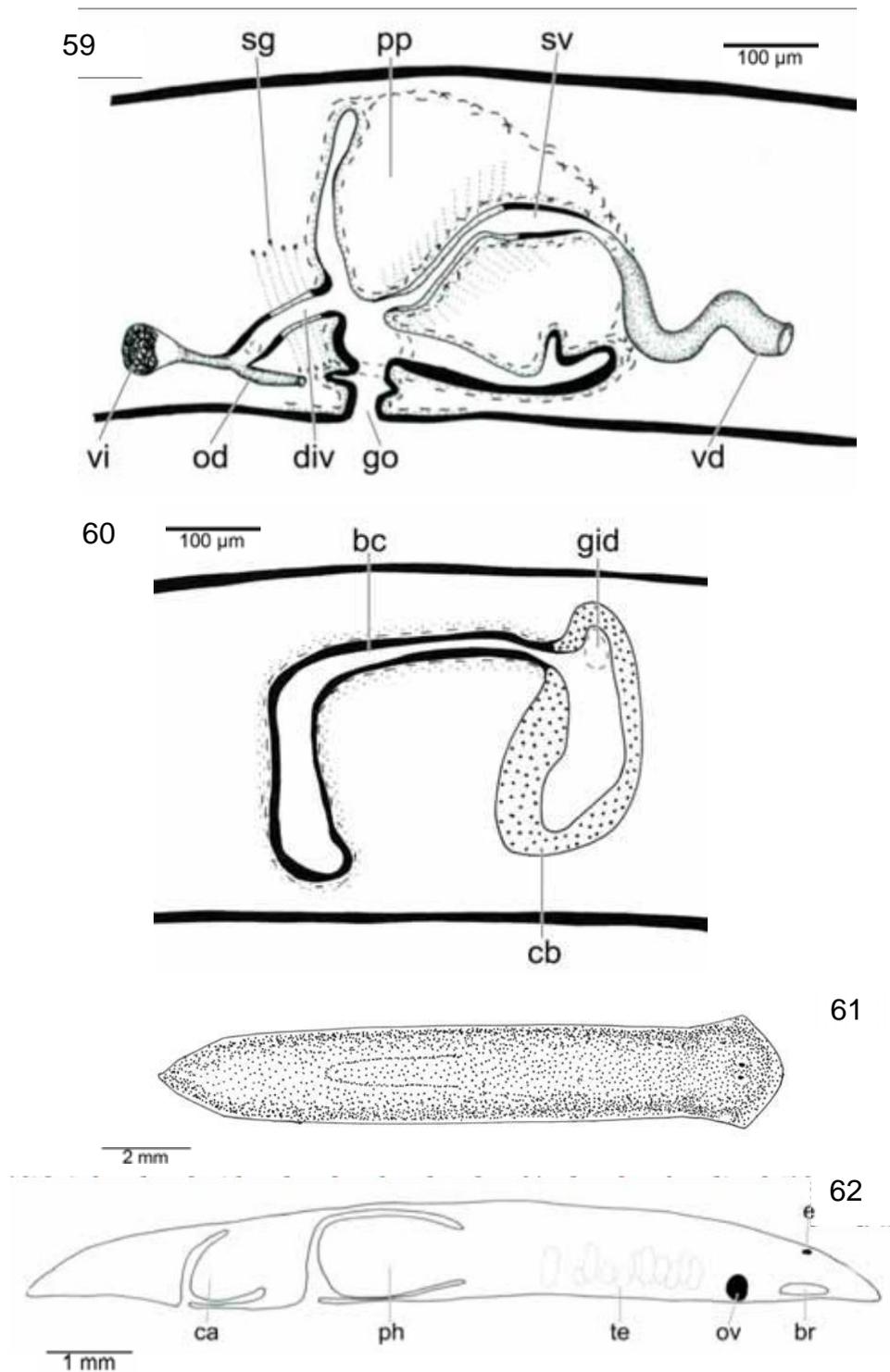
The bursal canal communicates with the male atrium on the atrium's lateral surface and travels dorsally before turning abruptly towards the anterior of the animal (Figure 70). The canal is broad with a short epithelium surrounded reversed musculature, the circular layer being substantially thicker than the inner longitudinal layer. The bursa has a tall epithelium and extends some distance ventrally and is positioned slightly anteriorly but to the side of the penis bulb. The intestine connects directly to the copulatory bursa on the lateral surface farthest from the penis bulb. The large ovaries are situated at a short distance behind the brain and the oviducts arise from the distal lateral surface.

#### **Discussion**

The long diverticulum receiving the oviducts and reduced seminal vesicle allow us to restrict our generic allocation to the genus *Romankenkius*. The lateral position of the bursa and the presence of a gastro-intestinal duct are both useful diagnostic characters for these specimens eliminating all other species excepting *R. kenki* and *R. pedderensis* (Figure 70). While the bursa is lateral in both species, the bursa in *R. kenki* is clearly positioned towards the posterior of the animal unlike these specimens where the bursa sits slightly antero-lateral to the penis bulb. The arrangement of the diverticulum provides the most conclusive argument for these specimens to be assigned to *R. pedderensis*. The diverticulum arises from a point clearly separate from the root of the bursal canal in the common atrium, a detachment not found in any of *R. pedderensis*' congeners (Sluys 1997). This feature has been discussed in the literature at some length and while not included in the original description of the type specimen is considered to be the most useful diagnostic character for this species (Ball 1974b, Grant et al. 2006, Sluys 1997).

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**Figures 69-72** *Romankenkius pedderensis*. 69, LG223-1, sagittal reconstruction of the male copulatory apparatus; 70, LG223-1, sagittal reconstruction of the female copulatory apparatus; 71, LG223, external features of living animal; 72, LG223, sagittal reconstruction of reproductive system.

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### **Ecology and distribution**

This species has been collected from the underside of rocks and logs in the off-channel area of cool lakes and streams. Prior to this investigation *R. pedderensis* was known only from Lake Pedder, however it appears that this species is quite widespread throughout Tasmania.

### ***Romankenkius rutrum* Grant & Sluys, sp. nov.**

### **Material examined**

#### **Western Australia:**

#### **HOLOTYPES:**

LG370, Falls Brook, flowing into Harvey Dam (33°02.990' S - 116°00.553' E) Western Australia, Australia, 13 October 2004, coll. L.J. Grant & K. Kassahn, sagittal sections on 2 slides.

#### **PARATYPES:**

LG361, Turtle Brook, Canning Dam, flowing into the Canning River (32°09' S - 116°07' E), Western Australia, Australia, 3 October 2004, coll. L.J. Grant & K. Kassahn, sagittal sections on 3 slides, sagittal sections on 3 slides, sagittal sections on 9 slides.

### **Other material examined**

#### **Identified via molecular sequence data**

LJ279, Mundaring Weir, seepage into outflow, Helena River Reservoir (31°57' S - 116°09' E), Western Australia, Australia, 2 October 2004, coll. L.J. Grant, identified via molecular samples.

LJ54, small creek running into Collie River, Wellington Dam (33°24.206' S - 115°58.318' E), Western Australia, Australia, 13 October 2004, coll. L.J. Grant, identified via molecular samples.

### **Diagnosis**

*Romankenkius rutrum* can be distinguished from its congeners by the combination of primarily dorsal testes extending throughout the body, small seminal vesicle, only one type of penial gland, and a short diverticulum entering the bursal canal receiving the opening of shell glands.

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#### Description

Largest live individuals measure 13mm x 1mm. This species has a very distinctive “shovel” shaped head with large auricles (Figure 75). The tail is long and mildly pointed. The small eyes, sitting in small pigment free patches, are situated posterior to the auricles and closer to the lateral margins than to each other. There are small pigment free patches sitting just anterior to the level of the eyes on the lower section of the auricles. Other external structures include two sets of ciliated sensory pits. The pits are in close proximity to each other on the lateral margin, one set is substantially larger than the other, more anterior pit.

The pharynx is located posteriorly and occupies approximately one-third of the total body length (Figure 76). The mouth is situated at the most posterior extent of the pharyngeal pocket. The large testes are not numerous but appear sporadically from a long distance behind the ovaries to the tail of the animal. Some of the follicles are situated dorsally whilst those in the anterior of the animal tend to be closer to the ventral surface. The vasa deferentia are broad ducts that travel medially through the animal (Figures 73 - 74). Upon approaching the muscular wall of the penis bulb the ducts narrow slightly. Almost immediately after having penetrated the penis bulb the vasa deferentia communicate separately with the most posterior point of a large seminal vesicle. This thin walled seminal vesicle communicates with a broad, straight ejaculatory duct. The ejaculatory duct has a short epithelium, which receives the entrance of penial glands from the base of the seminal vesicle to approximately half way along its length. The ejaculatory duct travels acentrally creating a slightly larger dorsal lip. The penial papilla is blunt, occupying most of the male atrium. The male atrium communicates with the common atrium via a large gap. The common atrium receives the entrance of a long, broad gonoduct.

The bursal canal originates from the dorsal surface of the common atrium (Figure 73). The broad duct travels almost immediately anteriorly, arcing over the penis bulb, to communicate with a large irregular copulatory bursa. The broad duct is lined with a short epithelium and surrounded by a thin layer of longitudinal muscle, followed by a slightly thicker layer of circular muscle. The copulatory bursa is lined with an extremely tall epithelium and extends a fair distance into the anterior of the animal and occupies most of the dorso-ventral space. There is a short diverticulum of the bursal canal arising from the base of the bursal canal. The oviducts communicate separately with this diverticulum; there is no trace of a caudal oviducal branch. The diverticulum receives the secretion of shell glands through this narrow epithelium. The ovaries are large and located at a short

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distance behind the brain; the connection with the oviducts cannot be traced in either ovary.

#### Discussion

The reversed bursal canal musculature, seminal vesicle, diverticulum and lack of a caudal branch of the oviducts eliminate all other genera excepting *Romankenkius* for this species. *Romankenkius* has several species that appear superficially similar to this species, including, *R. glandulosus*, *R. kenki*, and *R. pedderensis*. *Romankenkius rutrum* shares many features with *R. glandulosus* including the head shape, dorsal testes, short diverticulum, small seminal vesicle, and a large copulatory bursa. However, *Romankenkius rutrum* lacks the distinctive penial glands, conical papilla and shell glands entering the bursal canal characteristic of *R. glandulosus* (this paper). The polymorphic *R. kenki* was also investigated, however, presence of pre-pharyngeal testes, postero-lateral copulatory bursa, and genito-intestinal duct eliminate this species as a possible match; this paper). A similar case exists applies to *R. pedderensis* as the ventral testes and presence of genito-intestinal duct discount this possibility ((Ball 1974b, Grant et al. 2006); this paper). The only other possibility worth reviewing is the similarity of *Romankenkius rutrum* to the *Romankenkius* sp. (Grant et al. 2006). The dorsal testes, short diverticulum, simple penial papilla, and large copulatory bursa are all in accord between the two species. However, the lack of diagnostic characters, particularly the glands and the curious pharyngeal arrangement in *Romankenkius* sp. suspend any further comparison between the two taxa. By a process of progressive elimination we therefore arrive at the conclusion that this specimen represents a new *Romankenkius* species.

#### Ecology and distribution

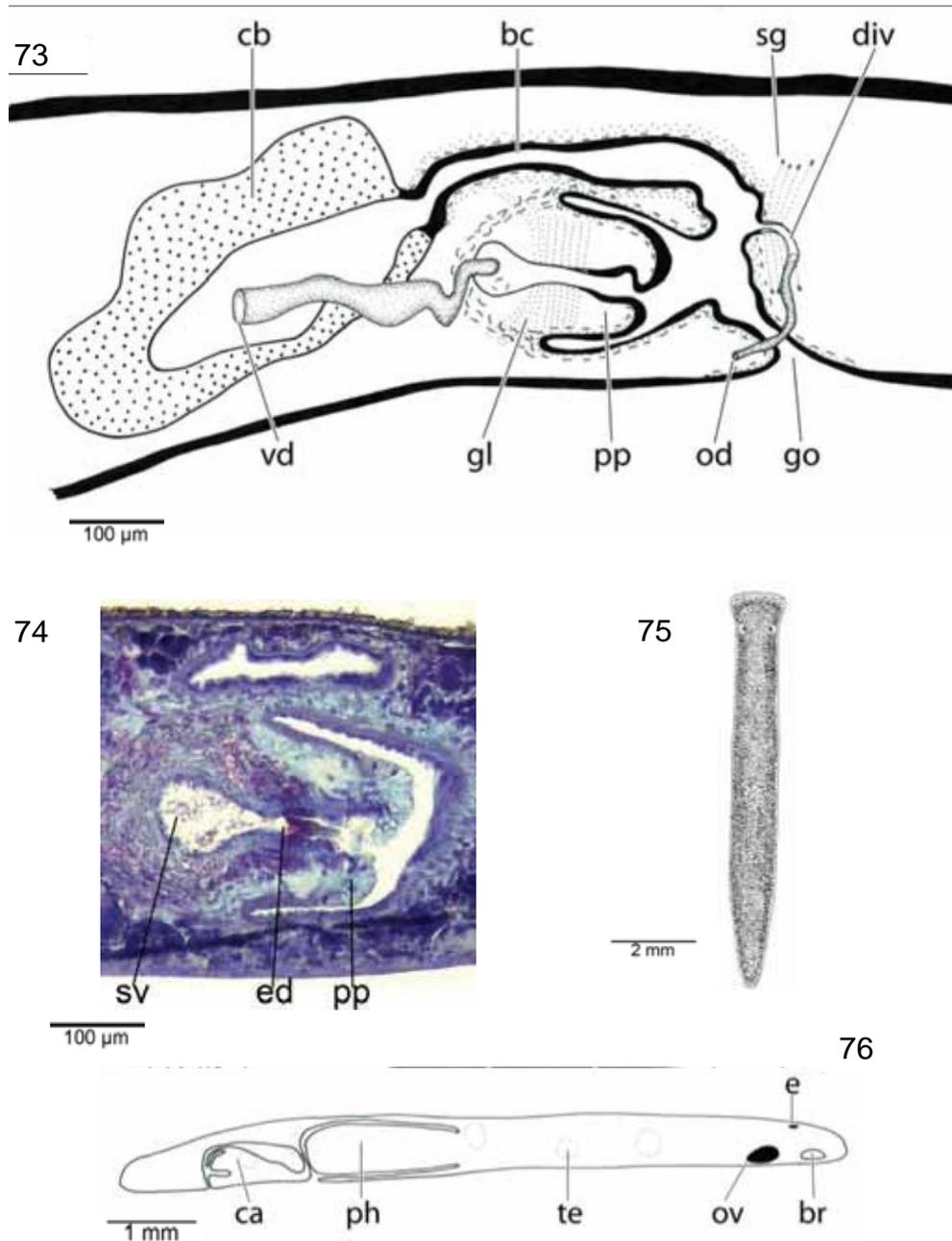
This species was collected from the underside of rocks in the off-channel area of small, sandy bottom creeks. All collecting sites were within 50 km south of Perth, Australia.

#### Etymology

This specific epithet is derived from the Latin noun '*rutrum*', meaning a shovel, in reference to the head shape.

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**Figures 73-76** *Romankenkius rutrum*. 73, LG370-1, sagittal reconstruction of the copulatory apparatus; 74, LG370-1, microphotograph of a sagittal section of the penial papilla; 75, LG370, external features of living animal; 76, LG370-1, sagittal reconstruction of reproductive system.

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#### **Romankenkius sp.**

#### **Material examined**

##### **Victoria:**

LG168, Wannon River, Wannon Crossing off Grampians Road, Grampians National Park (37°26.006' S - 142°28.547' E), Victoria, Australia, 29 December 2003, coll. L.J. Grant, sagittal sections on 5 slides.

LG189, Hoplins Falls, Hopkins River, approximately 13km southeast of Warnambool (38°19.983' S - 142°37.152' E), Victoria, Australia, 7 January 2004 coll. L.J. Grant, sagittal sections on 5 slides.

#### **Description**

Largest live specimens measure 9mm x 1mm. External morphology exhibits some variability, however, the following are features found in all. Triangulate head with large auricles and a pointed tail (Figure 77). The eyes are small, sitting in small pigment free patches, positioned closer to each other than to the lateral margins and equal with the auricular points. There are small pigment free patches sitting on the auricles. The base colour is white/transparent with coarse grey specks overlying this base; these specks are quite sparsely spaced, creating an overall grey slightly mottled appearance. The ventral surface is lacking all pigment. There are two large sensory pits present but no sensory fossae. The pharynx is located in the posterior half of the animal and occupies approximately one-fifth of the total body length.



**Figure 77** *Romankenkius sp.* LG168, external features of live animal.

#### **Discussion**

All specimens were asexual and therefore a complete description cannot be provided. These individuals have been identified as belonging to a separate species via the molecular data since a separate branch consisting of these specimens is positioned within the genus *Romankenkius* cluster. The location data does suggest that this species is quite possibly *Romankenkius conspectus* (Grant et al. 2006). Unfortunately the external

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morphology of *R. conspectus* is unknown, making comparison impossible. This species was initially found in the Grampians, Victoria and all three specimens of *R. sp.* were found in the same river system. The presence of the large sensory pits is consistent with the original description and *R. conspectus*, which is currently the only known Victorian species of *Romankenkius*.

## Spathula Ball, 1974

### *Spathula agelaea* Hay and Ball, 1979

#### Material examined

##### Victoria:

#### Identified via molecular sequence data

LG287, Brandy Creek, 5km west of Dinner Plain, Alpine National Park (37°00.86' S - 147°11.2' E), Victoria, 2 March 2004, coll. K. Richards, identified via molecular samples (LJ313).

#### Discussion

An individual collected from the type locality of *Sp. agelaea*, matching perfectly the description of external morphology for this species, was collected as part of this study. These undeniable coincidences have prompted the assignment of the individual to *Spathula agelaea*.

It is possible that this species reproduces purely via fission as many hundreds of specimens have been examined, none displaying any traces of a copulatory apparatus (Hay and Ball 1979, St. Clair et al. 1999). However, Hay and Ball (1979) did mention the presence of ventral testes in some specimens, suggesting that *Spathula agelaea* is capable of sexual reproduction. It may be that the harsh environments this species inhabits make resources scarce, forcing the reabsorption of the copulatory apparatus in most individuals (Connell and Stern 1969, Bowen et al. 1976, Romero and Baguna 1991, Baird et al. 2005). Additional to the question of the internal morphology of this species we have been unable to confirm its discovery during the course of our research. However, discussion regarding a potential description can be found in the musings surrounding *Spathula dupladiaphragma* (see below).

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### Ecology and Distribution

*Spathula agelaea* is the dominant species on Mt. Buffalo, although it is also abundant in the Falls Creek - Mt. Hotham area (Hay and Ball 1979). The creeks in which *Sp. agelaea* are commonly found are frequently covered by several feet of snow. Whilst, *Sp. agelaea* inhabits the same mountains as *Reynoldsonia reynoldsoni*, research suggests that the two species never occur together (Hay and Ball 1979). *Sp. agelaea* shows two very distinctive behaviours; the first being that they are most commonly found in large clumps of individuals, the second is the unusually high frequency of cannibalism found in this species (Hay and Ball 1979). Hay and Ball (1979) ponder the possibility that the two behaviours are linked, suggesting that the clumping behaviour may facilitate cannibalism during times when resources are limited.

### *Spathula dupladiaphragma* Grant & Sluys, sp. nov.

#### Material examined

##### Victoria:

##### HOLOTYPE:

LG207, Trentham Falls on Upper Coliban River, near Trentham (37°22.211' S - 144°19.544' E), Victoria, Australia, 15 January 2004, coll. L.J. Grant, sagittal sections on 6 slides.

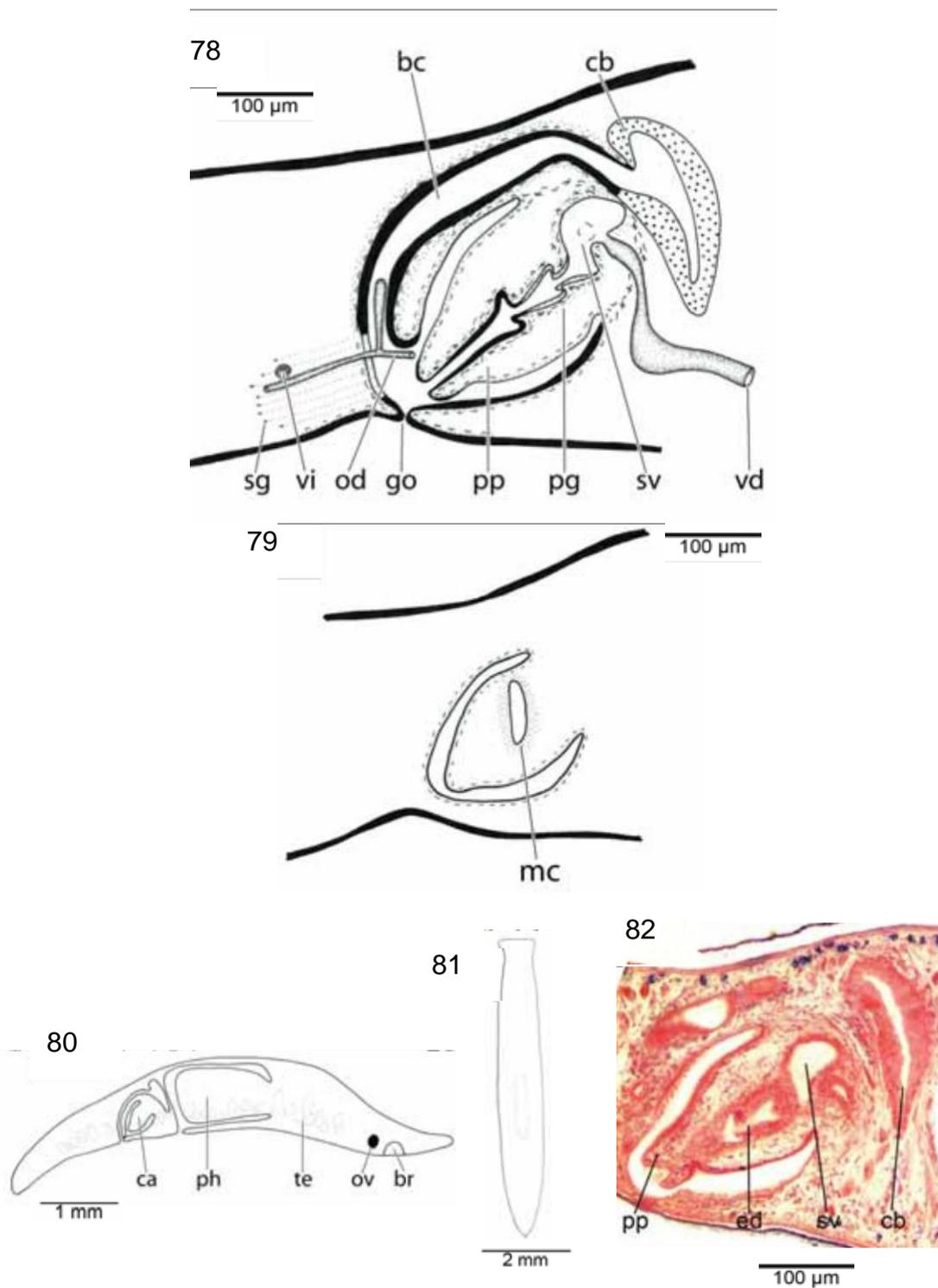
#### Description

Largest live specimens measure 14mm x 1mm. The head shape is square in most and mildly pointed in some, all with small auricles (Figure 81). The dorsal and ventral surfaces are lacking almost all pigment. Eyes are absent. Examination of the sagittal sections revealed at least two sets of sensory structures: (a) one large set of ciliated pits, (b) the other, smaller structures are situated anterior to the pits and can be described as small pits or large fossae.

The pharynx occupies approximately one-third of the total body length with the mouth situated at almost the most posterior end of the pharyngeal pocket (Figure 80).

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**Figures 78-82** *Spathula dupladiaphragma*. 78, LG207-4, sagittal reconstruction of the copulatory apparatus; 79, LG207-4, sagittal reconstruction of the adenodactyl; 80, LG207-4, sagittal reconstruction of reproductive system; 81, LG207, external features of living animal; 82, LG207-4, microphotograph of a sagittal section of the penial papilla.

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The testes are medium, discrete follicles, situated ventrally and extending from a short distance behind the ovaries to the tail of the animal (Figure 80). The broad vasa deferentia travel towards the muscular penis bulb and narrow slightly before penetrating it (Figures 78 & 82). The vasa deferentia communicate separately with a large seminal vesicle, which constricts before connecting with a curious ejaculatory duct. The broad ejaculatory duct appears to have two folds, constrictions or diaphragms along its length.

Both folds/diaphragms are approximately the same size and are separated by a short length of duct, both occur at the more anterior end of the ejaculatory duct. Beyond these folds the duct travels slightly acentrally to the tip of the penial papilla, thus creating a slightly larger dorsal lip. The seminal vesicle and the first section of the ejaculatory duct receive the entrance of penial glands through their short epithelium. This glandularisation is light, identifiable only by the slightly darkened granular quality to the tissue. The muscularisation surrounding the seminal vesicle and ejaculatory duct is extremely strong and intermingled.

The most unique feature of this papilla is the separate muscular cavity found in the penial papilla (Figure 79). This is not situated in a free papilla, but laterally to the ejaculatory duct. The cavity is a small oval space orientated dorso-ventrally and surrounded by an extremely thick layer of circular muscle. The male atrium comfortably houses the penial papilla and communicates via a large gap with a common atrium.

A gonopore opening up from the common atrium has no associated duct and is situated on the same level as the origin of the bursal canal (Figure 78). The broad bursal canal arises from the top of the small common atrium, which is still relatively close to the ventral surface. The duct then travels dorsally before arcing over the penial papilla to communicate with a copulatory bursa situated directly behind the penis bulb. The bursal canal has a tall epithelium and is surrounded by well defined but weak reversed musculature. The bursal canal travels a short distance ventrally before communicating with the long, narrow copulatory bursa. The latter is not large, occupying approximately one-half of the dorso-ventral space.

The oviducts open separately through the lateral surface of bursal canal via a long, medially directed branch. There is a large region of shell glands entering the common atrium below the point where the bursal canal communicates with the atrium. Both the long posterior oviducal branch and the anterior branch communicate with small vitellaria along its entire length. In the anterior end of the body the oviducts communicate with the antero-lateral surface of the small ovaries. The ovaries are situated at a short distance behind the brain (Figure 80).

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#### Discussion

The “folds or diaphragms” in the ejaculatory duct could indicate a possible *Dugesia*, however, there are currently no reported cases of a *Dugesia* with a caudal branch of the oviducts (Sluys et al. 1998). The seminal vesicle and muscularised cavity are reminiscent of a species of *Romankenkius*. *Romankenkius libidinosus* and *R. sinuousus* are the only Australian *Romankenkius* species with a substantial caudal branch of the oviducts, and this species is neither of these (Sluys and Kawakatsu 2001, Sluys and Rohde 1991). The caudal branch of the oviducts, ciliated pits and ventral testes extending throughout the body length suggest that this species belongs to *Spathula*.

The presence of the secondary chamber within the ejaculatory duct eliminates all known *Spathula*'s for which the copulatory apparatus has been described (Figures 78 & 82). *Sp. agelaea* has been described from Victoria, without eyes, with ventral testes throughout the body length and with light brown or dirty white with light sepia external pigmentation. All of these features are consistent with the above description. Unfortunately, the internal morphology has never been fully described (Hay and Ball 1979), making a definitive identification difficult. While it would appear possible that this species is *Sp. agelaea*, the situation is complicated by the fact that the external features are not rare in this part of Australia. Victoria is home to many other species with very little pigment and small to no eyes (i.e. *Sp. foeni*, *Sp. tryssa*, *Sp. goubaultae*, *Reynoldsonia reynoldsoni*). So it is not unreasonable to suggest that this species is another example of this morphological type. However, the body shape of *Spathula dupladiaphragma* is somewhat different from that reported for *Sp. agelaea*. Hay and Ball (1979) describe live specimens of *Sp. agelaea* with a distinct, bluntly pointed anterior end. Further, we have access to an unpublished drawing of a live specimen of presumed *Sp. agelaea* collected from Mt. Buffalo National Park (just above Tekal (unable to find this site, have converted the height from feet to metres) at 1554m). The body shape and other external features of this animal fully agree with the account of Hay and Ball (1979). In contrast, the anterior end of *Sp. dupladiaphragma* is truncated (Figure 81) and not bluntly pointed.

There are two other diagnostic features known for *Sp. agelaea*. The first concerns sensory structures, which are described similarly to what is described above. The second character, which has not been discovered in this specimen, is the presence of a small vesicle, somewhat similar to the statocyst of lower Turbellarians, above the brain.

In view of the fact that (1) several of the known features of *Sp. agelaea* do not fully agree with those of *Sp. dupladiaphragma* and (2) our specimen was not collected from the

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precise type locality (Mt. Buffalo National Park), we have refrained from assigning our specimen to the former and instead have chosen to erect a new species.

#### Ecology and distribution

*Spathula dupladiaphragma* has been collected from the underside of rocks in the off-channel area of cool rivers and streams. This species was found in the presence of *Spathula foeni* and distributed throughout the Victorian alpine region and central highlands.

#### Etymology

This specific epithet is derived from the Latin nouns '*dupla*' (pair) and '*diaphragma*' (diaphragm), in reference to the two diaphragms in the ejaculatory duct.

#### *Spathula camara* Ball, 1974

#### Material examined

##### Tasmania:

LG289, Approximately 1km north of Targa, at intersection with Targa Hill Road (41°18'15.39659" S - 147°21'55.91445" E), Tasmania, Australia, 6 November 2003, coll. K. Richards, sagittal sections on 7 slides, sagittal sections on 4 slides.

#### Description

There are no details on the external morphology, however, from inspection of the sagittal sections we can deduce that these animals have no eyes and no traces of pigmentation on either body surface. An inspection of the anterior margin revealed one set of large sensory pits and at least two sets of sensory fossae situated anterior to these pits.

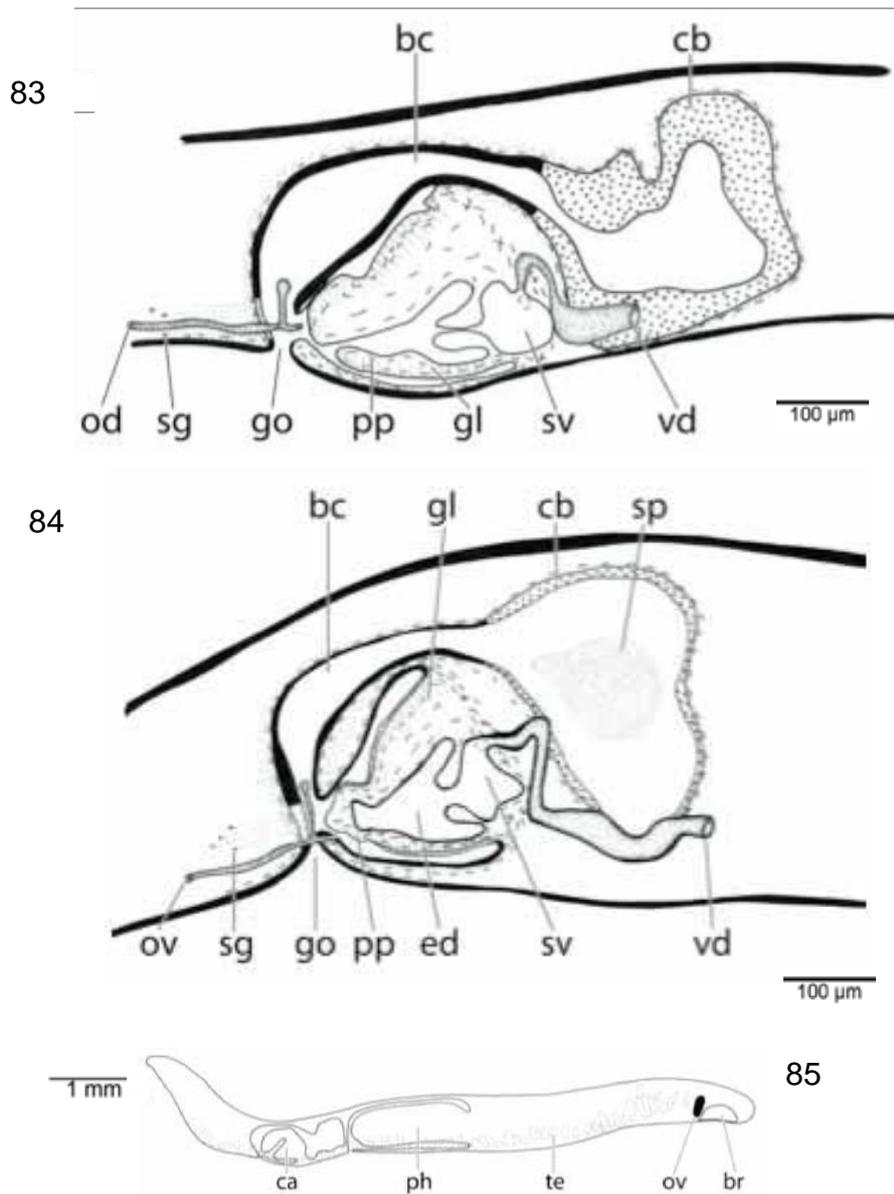
The pharynx occupies approximately one-fifth of the total body length and the mouth is situated at the most posterior point of the pharyngeal pocket (Figure 85).

Testes are large, discrete follicles, beginning at the same level as the ovaries and extending throughout the length of the body. While primarily ventral the follicles often extend into the dorsal region (Figure 85). Vasa deferentia narrow considerably before entering the seminal vesicle separately, inside the lightly muscled penis bulb (Figures 83-84). The seminal vesicle is pleated with two distinct chambers, which are separated by a distinct fold. The tissue between the two chambers appears sinuous and flexible in all specimens. The second chamber constricts to form a broad ejaculatory duct surrounded by a short epithelium and light musculature. The duct travels acentrally, creating a slightly

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larger dorsal lip for the rounded penial papilla. The papilla is a large, well-muscled and not overly flexible structure that fills the entire male atrium. In all specimens there is a thickening of circular muscle, particularly on the dorsal wall of the papilla; this area also receives light glandularisation.



**Figures 83-85** *Spathula camara*. 83, LG289-1, sagittal reconstruction of the copulatory apparatus; 84, LG289-2, sagittal reconstruction of the copulatory apparatus; 85, LG289-2, sagittal reconstruction of reproductive system.

The common atrium communicates with the ventral communication of the bursal canal (Figures 83-84). The broad bursal canal travels dorsally for some distance before

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turning anteriorly to communicate with the bursa. The canal is lined with a very short epithelium and surrounded by a thin layer of longitudinal muscle and a slightly thicker layer of circular muscle. This musculature thins but continues to surround the much thicker epithelium of the copulatory bursa. This bursa is volumous and occupies much of the dorso-ventral space. One specimen has a large clump of sperm inside the bursa.

The oviducts penetrate separately the opposing lateral surfaces of the bursal canal, at its most ventral point, from here the oviducts travel ventrally before sending a long branch both anteriorly and posteriorly (Figures 83-84). Shell glands enter the bursal canal just below the openings of the oviducts. The large ovaries are situated directly behind the brain and receive the oviducts on their latero-ventral surface (Figure 85).

## Discussion

The separate entries of the oviducts into the bursal canal and the caudal branch eliminate all genera from this investigation excepting *Spathula*. The most useful characters to use for the differentiation of this species are the absence of eyes, the pleated seminal vesicle, the musculature extending over the copulatory bursa, and the glands exiting through the epithelium of the penial papilla (Figures 83-84). Species in which the eyes are more or less absent are: *Sp. agelaea*, *Sp. camara*, *Sp. miserabile*, *Sp. goubaultae*, and *Sp. tryssa*. Within this group *Sp. goubaultae* can be easily eliminated as it has large dorsal testes and lacks a seminal vesicle (Ball 1977). It is difficult to assign any species to *Sp. agelaea* as very little is known of the reproductive system beyond ventral testes (Hay and Ball 1979). The presence of a small vesicle, somewhat similar to the statocyst of lower Turbellarians, above the brain is the most effective diagnostic character for *Sp. agelaea*, a feature not found in this species. Of the three remaining species all have a seminal vesicle. However, the vesicle in *Sp. miserabile* is singular and its bursa is lacking any surrounding musculature (Grant et al. 2006). Although *Sp. tryssa* does have a double seminal vesicle, the musculature surrounding the bursa is also lacking in this species (this paper). The remaining species is *Sp. camara* and closer inspection reveals a considerable amount of similarity between this species and the specimens examined. *Sp. camara* been described with no eyes, often lacking all external pigmentation, and with one set of large sensory pits and a smaller set of pits or fossae, all of which are consistent with the description above (Ball 1977, Grant et al. 2006). Other shared characters include, ventral testes extending throughout the body, folded seminal vesicle, penial papilla lacking flexibility, no penial glands, shell glands entering below the openings of the oviducts into the bursal canal and a spacious copulatory bursa surrounded by relatively thick musculature (Figures 83-85).

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The only real discrepancies exist in the presence of glands exiting the epithelium of the penial papilla and the larger chambers existing between the folds in the seminal vesicle in the specimens examined, as compared with the published descriptions of *Sp. camara*. However, the variation in folds may be due to polymorphism. It is debatable whether the same situation applies to the glands opening through the penial epithelium in the present specimens examined. However, we are of the opinion that these minor discrepancies are not substantial enough to argue against the assignment of these animals to *Sp. camara*.

### Ecology and distribution

*Spathula camara* is the most widespread species of the genus, having been collected in cool, creeks at high altitudes in southern New South Wales, Victoria and now also Tasmania.

### *Spathula dittae* Ball & Tran, 1979

### Material examined

#### Tasmania:

LG252, Macquarie River, on road to Cressy, approximately 10km southwest of Epping Forest (41°49.585' S - 147°15.588' E), Tasmania, Australia, 29 January 2004, coll. L. J. Grant sagittal sections on 3 slides, sagittal sections on 7 slides.

LG251, Great Lake at Miena (41°58.955' S - 146°43.032' E), Tasmania, Australia, 29 January 2004, coll. L.J. Grant, sagittal sections on 9 slides, sagittal sections on 10 slides.

LG245, Lake Sorrell at Interlaken (42°08.050' S - 147 ° 10.135' E), Tasmania, Australia, 30 January 2004, coll. L.J. Grant, sagittal sections on 11 slides, sagittal sections on 8 slides, sagittal sections on 17 slides.

LG221, Lake Pedder, near Serpentine Dam at Junction with small cascade (42°46.273' S - 145°59.065' E), Tasmania, Australia, 23 January 2004, coll. L.J. Grant, sagittal sections on 5 slides, sagittal sections on 5 slides, sagittal sections on 6 slides, sagittal sections on 5 slides.

LG250, Arthurs Lake, at Pumphouse Bay, just off Poatina Road (41°59.147' S - 146°51.617' E), Tasmania, Australia, 29 January 2004, coll. L.J. Grant, sagittal sections on 11 slides, sagittal sections on 9 slides, sagittal sections on 16 slides, horizontal sections on 7 slides.

LG229, Small trickle near summit of Mt. Wellington (42°53.389' S - 147°14.168' E), Tasmania, Australia, 24 January 2004, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 6 slides, sagittal sections on 6 slides.

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LG215, Macquarie River, on road to Cressy approximately 10km southwest of Epping Forest (41°49.585' S - 147°15.588' E), Tasmania, Australia, 29 January 2004, coll. L.J. Grant, sagittal sections on 7 slides, sagittal sections on 6 slides, sagittal sections on 4 slides, horizontal sections on 3 slides, horizontal sections on 5 slides.

#### **Identified via molecular sequence data**

LJ107, "Fortescue Bay" Creek, at Fortescue Bay, Tasman National Park (43°08.752' S - 147°57.176' E), Tasmania, Australia, 25 January 2004, coll. L.J. Grant, identified via molecular samples.

LG305, approximately 26km northwest of St. Helens, at intersection with Lotta road (41°12'56.309" S - 147°58'14.682" E), Tasmania, Australia, 13 February 1999, coll. K. Richards, sagittal sections on 3 slides, sagittal sections on 2 slides.

#### **Description**

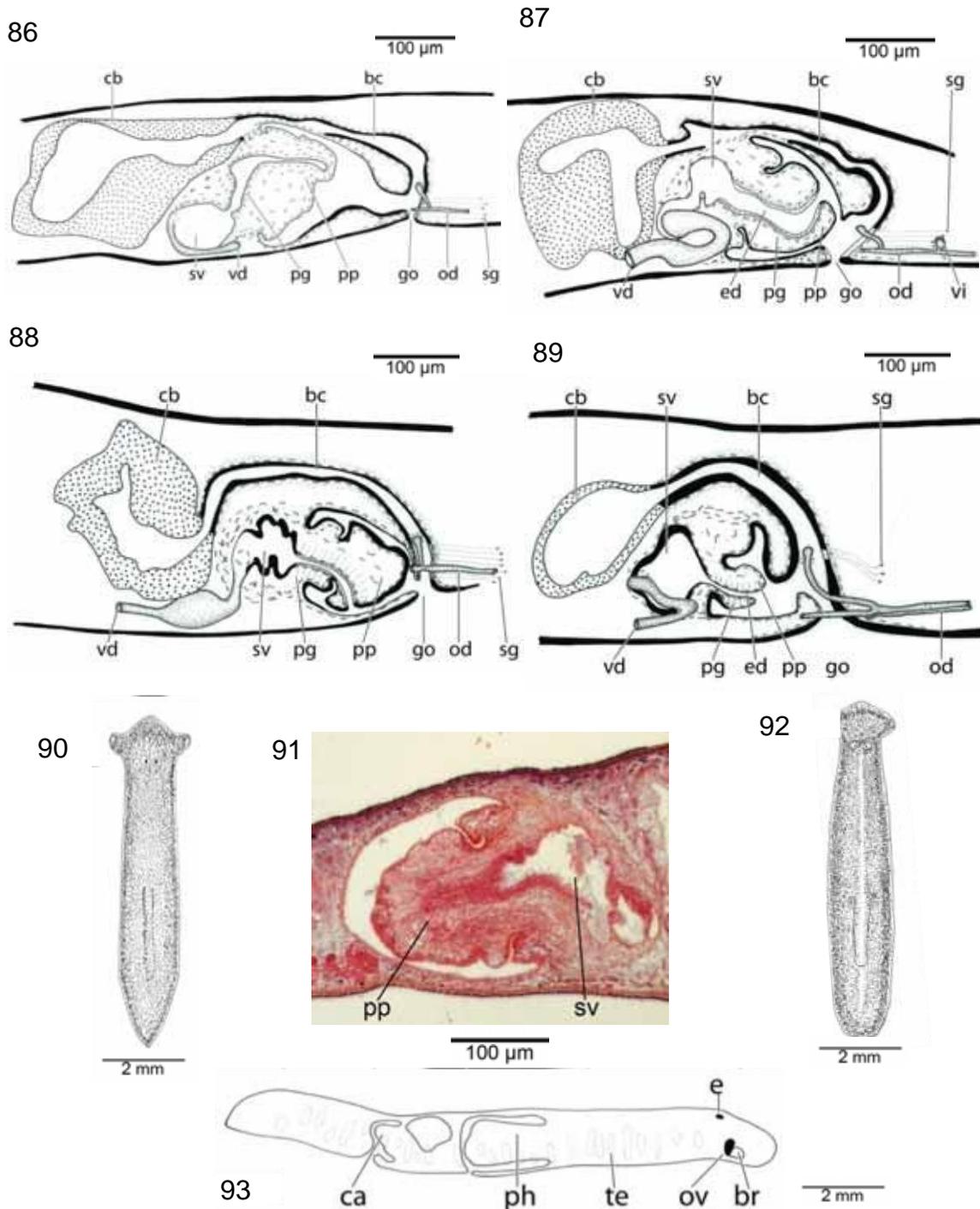
Largest live specimens measure 2mm x 12mm. External morphology highly polymorphic. All specimens exhibit a triangulate head with large auricles (Figures 91-92). Pigmented eyecups are situated in relatively small pigment free areas. Eyes are positioned medially and tend to be situated posteriorly to the auricular points. All of the specimens collected exhibit well-defined pigment free areas on the auricles. Large sensory pits are located on the lateral anterior margin, and several sensory fossae are situated anteriorly to these pits. While the base colour of all appears consistently "transparent", the hue varies between light brown and yellow. All specimens exhibit fine black pigmentation throughout, the density of which is extremely variable between collecting sites and individuals, some appearing light brown/yellow whilst others appear almost black. Some specimens also exhibit a pale mid-dorsal stripe. Ventral pigment is always present to varying degrees.

The pharynx occupies approximately one-quarter of the total body length and the mouth is sitting at the most posterior extent of the pharyngeal pocket (Figure 93).

The testes are discrete follicles situated ventrally but often extending into the dorsal region (Figure 93). The testes arise just behind the brain and are found throughout the entire length of the animal. The vasa deferentia swell, then narrow, prior to communicating separately with a lightly muscled seminal vesicle (Figures 86-90). The structure of the seminal vesicle is extremely variable, with double, singular folded or singular spacious seminal vesicles all being common states for this feature. In some individuals the seminal vesicle receives light glandularisation through its epithelium. This glandularisation is found penetrating the short epithelium of the ejaculatory duct in all

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**Figures 86-93** *Spathula dittae*. 86, LG221-2, sagittal reconstruction of the copulatory apparatus; 87, LG229-1, sagittal reconstruction of the copulatory apparatus; 88, LG250-3, sagittal reconstruction of the copulatory apparatus; 89, LG252-3, sagittal reconstruction of the copulatory apparatus; 90, LG251, external features of living animal; 91, LG229-1, microphotograph of a sagittal section of the penial papilla; 92, LG229, external features of living animal; 93, LG221-1, sagittal reconstruction of the reproductive system.

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individuals. The ejaculatory duct is surrounded by longitudinal muscle and travels acentrally, creating a larger dorsal lip of the penial papilla. The penial papilla is muscular and flexible, often exhibiting large folds. The male atrium communicates with the bursal canal via a small opening.

All specimens have a very short gonoduct below the point where the bursal canal originates (Figures 86-89). The bursal canal is broad with a short epithelium and reversed musculature. The inner longitudinal muscle layer is distinct but substantially thinner than the outer circular muscle layer. The bursal canal travels slightly laterally to the penis bulb before communicating with a copulatory bursa. The copulatory bursa is a large irregular structure with a tall epithelium, positioned anterior to the penis bulb and often filling the entire dorso-ventral space.

The oviducts penetrate separately the opposing lateral faces of the bursal canal (Figures 86-89). The communication point is ventral, close to the point of communication of the bursal canal with the male atrium. The caudal branch of the oviducts is traceable far into the posterior of the animal. The small ovaries are situated directly behind the brain; the oviducts communicate with the antero-dorsal surface of the ovaries (Figure 93).

## Discussion

These specimens align comfortably with the descriptions of both *Spathula ochyra* and *Sp. dittae*. That these two nominal species may be one and the same has been suggested previously (Grant et al. 2006). It is unfortunate that we have no new *Spathula* specimens from the type locality of *Sp. dittae*, Lake St. Clair, as this would enable a definitive answer to this question. Despite this there is a very strong case to be made for suppression/synonymization of *Sp. dittae*.

The type material of *Sp. dittae* is very poor and *Sp. ochyra* was described from just one specimen (Ball and Tran 1979). These initial restrictions have meant that as more material has become available, the morphological differences have been systematically discounted. So we are left with the current situation where the only dividing character between the two species is the presence or absence of folds in the papilla (i.e. those with folds are *Sp. ochyra*) (Grant et al. 2006) (Figures 86-89). This is not a very strong diagnostic character and would not be considered an acceptable differentiation for any new species. The use of this feature seems even more tenuous when the variability that *Sp. ochyra* displays throughout a range of other characters is considered. The present analysis has the added advantage of molecular data, which does not discriminate between those individuals with a folded papilla and those without (see Appendix 2c). Therefore, these

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two species are here considered to be one and the same and any specimens displaying these characters should be considered as *Sp. dittae*, in conformity with the previous suggestion by Grant et al. (2006)

#### Ecology and distribution

*Sp. dittae* has been collected from the underside of rocks in the off channel area of creeks and rivers and on the shores of lakes. This species is widespread throughout Tasmania and is found in a range of environments from pristine to highly disturbed areas, with water temperatures ranging from 9°C - 22°C.

#### *Spathula foeni* Ball, 1977

#### Material examined

##### Victoria:

LG207, Trentham Falls on the Upper Coliban River near Trentham (37°22.211' S - 144°19.544' E), Victoria, Australia, 15 January 2004, coll. L.J. Grant, sagittal sections on 7 slides, horizontal sections on 5 slides, sagittal sections on 4 slides.

#### Description

Maximum size of live specimens 14mm x 1mm. Small eyes are present. There is one set of large sensory pits present and a second, smaller set of sensory pits situated close to the midline on the anterior margin. Live specimens have exhibited white dorsal and ventral surfaces, but sections demonstrated that light pigmentation is present.

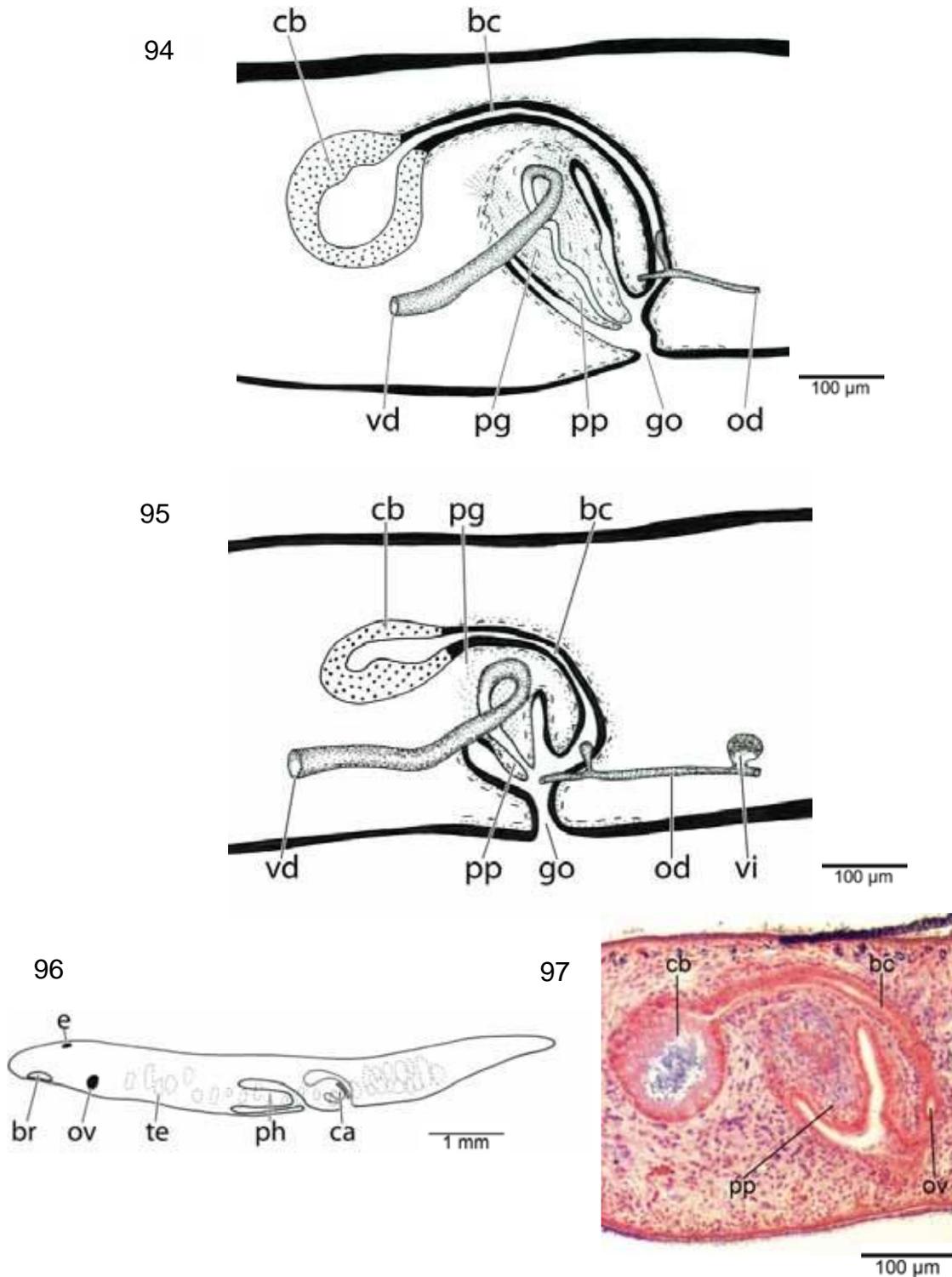
The pharynx is narrow and occupies approximately one-fifth of the total body length; the mouth is located at the most posterior extent of the pharyngeal pocket (Figure 96).

Discrete testicular follicles are situated ventrally but very often extend into the dorsal space, beginning from a long distance the brain and continuing throughout the length of the worm (Figure 96). The vasa deferentia travel dorsally and extend a short distance beyond the level of the ejaculatory duct before re-curving and penetrating the lightly muscled penis bulb (Figures 94, 95 & 97). The vasa deferentia communicate separately with the ejaculatory duct; a seminal vesicle is absent. The ejaculatory duct is a narrow, slightly acentral duct creating a somewhat larger dorsal lip of the penial papilla. The epithelium of the duct is short and receives the openings of penis glands along half of its length. The penial papilla has a conical shape and is orientated more dorso-ventrally than is the case in most dugesiids. The male atrium is spacious, easily housing the penial

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papilla and communicating directly with the top of a short gonoduct, communicating directly above this is the bursal canal, no real common atrium being present.



**Figures 94-97** *Spathula foeni*. 94, LG207-1, sagittal reconstruction of the copulatory apparatus; 95, LG207-3, sagittal reconstruction of the copulatory apparatus; 96, LG207-3, sagittal reconstruction of the reproductive system; 97, LG207-1, microphotograph of a sagittal section of the copulatory apparatus.

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The bursal canal is a narrow, gently curving duct, which extends over the penis bulb to communicate with a small, rounded copulatory bursa, situated anterior to the penis bulb (Figures 94, 95 & 97). The epithelium of the canal is short and surrounded by thin but well-defined reversed musculature. The tall epithelium of the copulatory bursa is not surrounded by any musculature. The oviducts penetrate separately, via a short medial branch, the opposing lateral faces of the base of the bursal canal. The oviducts then travel both anteriorly and posteriorly from the copulatory apparatus, communicating with vitellaria along the body length. The ovaries are situated a long distance behind the brain and the oviducts communicate with their lateral surface.

## Discussion

Undoubtedly a *Spathula*, these animals have a conical penial papilla, which is the most useful character in the species assignment. Australian *Spathula* species with comparable, in regards to shape or orientation, penial papilla's are *Sp. simplex*, *Sp. triculenta*, *Sp. tryssa*, *Sp. gorbaultae* and *Sp. foeni*. *Sp. triculenta* has dorsal testes instantly eliminating it as a potential species assignment. *Sp. simplex* has an oviducal loop and heavy external pigmentation, diagnostic characters that have not been detected in these specimens. In *Sp. tryssa* and *Sp. gorbaultae*, penial glands are present in the ejaculatory duct. However, both of these species are discounted due the absence of eyes.

This specimen is most suitably assigned to *Sp. foeni*. A difference concerns the absence of penial glands, which is most likely due to the poor staining of the preparations described by Ball (1977). The large bursa and the tendency of the testes to fuse in *Sp. foeni* are likely to vary depending upon maturity of the animals.

## Ecology and distribution

This species has been collected from the underside of cobble in the off-channel area of rivers in the relatively untouched central Victorian wilderness. Grant et al. (2006) reported a *Sp. cf. foeni* from northeastern Tasmania.

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### *Spathula oblongata* Grant and Sluys, sp. nov.

#### Material examined

Tasmania:

#### HOLOTYPE:

LG296, Approximately 37km east of Strathgordon, at intersection with Gordon River Road  
(42°46'22.04658" S - 146°30'39.78343" E), Tasmania, Australia, 8 October 2003, coll.

K. Richards, sagittal sections on 4 slides, sagittal sections on 9 slides.

#### Diagnosis

This species can be differentiated from its congeners by the presence of an elongated penis bulb.

#### Description

Details on the external morphology from the live specimens are not available. Details obtained from inspection of the sagittal sections include, one set of wide set pigmented eyecups, one set of extremely large ciliated pits and at least one set of sensory fossae.

The pharynx occupies approximately one-fifth of the total body length, with the mouth situated at the most posterior end of the pharyngeal pocket (Figure 101). The testes are small, discrete, dorsal follicles, extending from the level of the brain to well into the posterior of the animal (Figure 101). The follicles appear to be absent in the pharyngeal region. The vasa deferentia are relatively narrow ducts travelling a short distance beyond the penis bulb before heading dorsally and re-curving to penetrate the bulb. Once inside the penis bulb the vasa deferentia communicate separately with the ejaculatory duct (Figures 98-100). The most distinctive character of the male copulatory apparatus is the extended, highly muscular, penis bulb. The bulb is surrounded by a thick layer of predominately circular muscle and measures approximately three times the length of the short penial papilla. The bulb also appears quite flexible, appearing almost folded particularly in specimen LG296-2.

The broad ejaculatory duct originates at the most anterior point of the penis bulb and travels centrally through the penis bulb before narrowing slightly through the penial papilla (Figures 98-100). The long ejaculatory duct has a tall epithelium with small areas of three types of glands (two fine and one coarse grained) penetrating this epithelium at the origin of the duct. The penial papilla is a small, lightly muscled conical structure housed within a spacious male atrium. The male atrium communicates with a common

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atrium, which receives a short gonoduct and the entrance of a common oviduct. This duct is here labelled as a common oviduct due to the fact that a large area of shell glands enter the common atrium at the level of the common oviduct, no glands enter the duct itself as is the usual case in diverticula such as found in species of *Romankenkius*.

The common oviduct is short, originating close to the origin of the bursal canal (Figures 98-99). The two oviducts arise from the common oviduct and travel a short distance ventrally before sending a caudal branch far into the posterior of the animal. The anterior branches of the oviducts communicate with the anterior face of large paired ovaries situated a short distance behind the brain (Figure 101). The bursal canal arises from the most dorsal point of the common atrium and travels almost immediately anteriorly (Figures 98-99). The narrow canal travels laterally in a mildly convoluted fashion to communicate with the copulatory bursa situated anterior to the penis bulb. The canal is lined with a short epithelium surrounded by a thin layer of longitudinal muscle, which is in turn surrounded with a thicker layer of circular muscle. There is also a thin reinforcing layer of longitudinal muscle surrounding the bursal canal, covering one third of the canal's length. Musculature also extends over the thin epithelium of the large copulatory bursa; this musculature is thin and intermingled. The bursa is spacious and in specimen LG296-2 segments of a spermatophore are present.

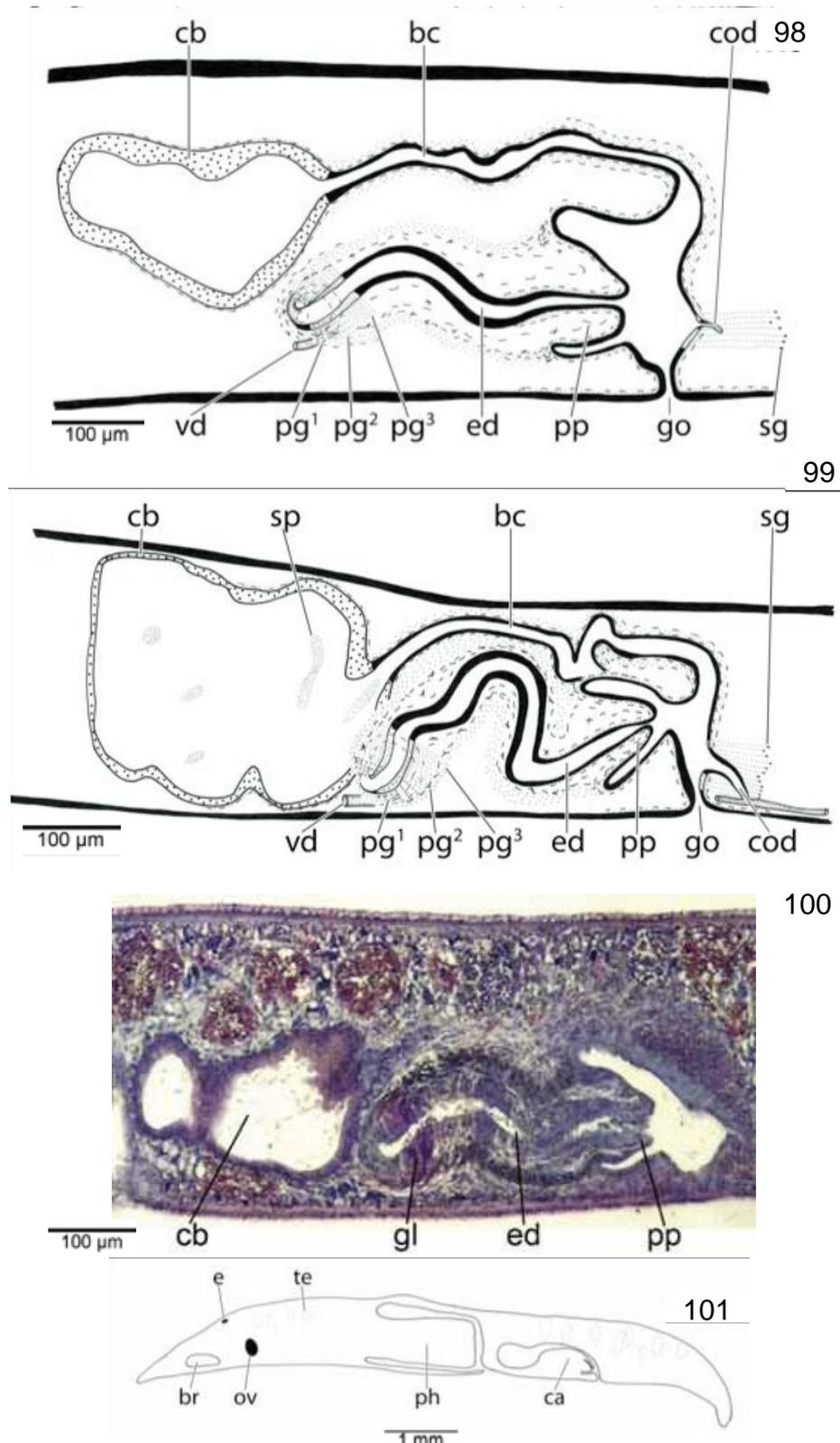
## Discussion

These specimens represent a new species not only because of the nature of the penis bulb but also due to the fact that the unique combination of features eliminates all other Australian dugesiids. A combination of characters not often seen in Australian dugesiids is the presence of a common oviduct and a caudal branch of the oviducts. All other species with both of these features have other characters precluding the assignment of these specimens to any of these species: *Romankenkius libidinosus* (has a seminal vesicle, adenodactyl and gastro-intestinal communications), *R. pedderensis* (the testes are ventral and pre-pharyngeal), *Sp. truculenta* (lacks the large muscular penis bulb, the three types of glands entering the broad ejaculatory duct and the massive copulatory bursa).

Perhaps the most important comparison is with *R. sinuosus*. This species exhibits a common oviduct and short caudal branch of the oviducts and, unlike other species with these features, also has an elongated penis bulb. *R. sinuosus* is easily discounted from this investigation, however, as it has a large seminal vesicle, no ciliated pits, no glandular differentiation in the penis bulb, a common oviduct receiving the openings of shell glands, and the entire copulatory apparatus is sinuous.

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**Figures 98-101** *Spathula oblongata* 98, LG296-1, sagittal reconstruction of the copulatory apparatus; 99, LG296-2, sagittal reconstruction of the copulatory apparatus; 100, LG296-1, microphotograph of a sagittal section of the copulatory apparatus; 101, LG296-1, sagittal reconstruction of the reproductive system.

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As these specimens cannot be easily assigned to any known species, the genus needs to be identified. This species is most likely a *Spathula* due to the presence of large sensory pits, shell glands penetrating the bursal canal and caudally branching oviducts. All of these characters are uncommon outside of *Spathula* and the combination of all three is quite conclusive (cf. Sluys 2001). This genus assignment allows us to be confident in proclaiming a new species as, within the *Spathula*, as an elongated penis bulb excludes many known species, excepting *Sp. alba* Allison, 1997, *Sp. fontinalis* Nurse, 1950, and *Sp. musculosa* Sluys & Grant, 2006. However, the former is a blind species with a bursal canal provided with a sphincter (cf. Allison 1957). And *Sp. fontinalis* does also possess a sphincter on the bursal canal, in contrast to *Sp. oblongata*. *Sp. musculosa* has a large adenodactyl, which is absent in *Sp. oblongata*.

#### Ecology and distribution

This species is known only from the type locality.

#### Etymology

The specific epithet is derived from the Latin adjective '*oblongata*', meaning oblong and referring to the shape of the penis bulb.

#### ***Spathula caeca* Grant and Sluys, sp. nov.**

#### Material examined

##### Tasmania

##### HOLOTYPE:

LG244, Westerway Creek, at intersection with Zeehan Highway (41°55.470' S - 145°25.783' E), Tasmania, Australia, 31 January 2004, coll. L.J. Grant, sagittal sections on 8 slides, sagittal sections on 4 slides.

#### Other material examined

##### Identified via molecular sequence data

LG253, Raglan Creek, at intersection with Lyell Hwy, Franklin - Gordon Rivers National Park (42°07.130' S - 145°47.815' E), Tasmania, Australia, 31 January 2004, coll. L.J. Grant, identified via molecular samples (LJ142).

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(PLATYHELMINTHES, TRICLADIDA)

### **Diagnosis**

This species can be distinguished from its congeners by its lack of eyes and pigment, lack of penial glands entering the ejaculatory duct, and presence of a double seminal vesicle.

### **Description**

Largest live specimens measure 10mm x 1mm. Head is shovel-shaped with moderate-sized rounded auricles (Figure 103). The only sensory structures present are two large ciliated sensory pits on the lateral margins. Both the dorsal and ventral surfaces are devoid of pigment.

The pharynx occupies approximately one-quarter of the total body length. The mouth is situated at the most posterior extent of the pharyngeal pocket (Figure 105).

The testes are small, discrete follicles, situated ventrally, beginning almost directly behind the brain and extending throughout the entire body length (Figure 105). The vasa deferentia narrow considerably before entering the muscled penis bulb (Figures 102, 104, 106 - 107). They communicate separately, but in the same region, with the seminal vesicle immediately after entering the bulb. The thin-walled seminal vesicle has two chambers similar sized chambers, which are separated by a constriction in the epithelium.

The second, posteriormost chamber communicates with the ejaculatory duct, which is much narrower than the seminal vesicle but still broad. The lining epithelium of the ejaculatory duct thickens along its length and is surrounded by non-reversed musculature (Figures 102, 104, 106 - 107). The ejaculatory duct travels acentrally through the blunt penial papilla, creating a larger dorsal lip. The almost cylindrical penial papilla is lightly muscled and occupies most of the male atrium; there is no evidence of any flexibility beyond a small fold in the ventral margin of specimen LG244-2.

The male atrium communicates via a narrow gap to the base of the bursal canal (Figures 102 & 104). There is no common or female atrium the bursal canal originates from the male atrium above the short gonoduct. The bursal canal is a broad duct travelling dorsally before turning towards the anterior of the animal. The duct travels above and slightly lateral to the penis bulb before communicating with the irregularly shaped copulatory bursa. The epithelium of the bursa is thick but appears incredibly flexible, the two specimens exhibiting vastly different forms. The bursal canal has a short epithelium and is surrounded by a thin layer of longitudinal muscle, which is in turn covered by a thick layer of circular muscle. The oviducts penetrate separately with lateral side of the most ventral section of the bursal canal. Shell glands enter the bursal canal below the level of the oviducal communications. From hereon the oviducts travel ventrally for a short

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### A CONTRIBUTION TO THE CURRENT SPECIES INVENTORY FOR AUSTRALIAN FRESHWATER TRICLADS (PLATYHELMINTHES, TRICLADIDA)

distance before branching and travelling a long distance into the posterior of the animal. The other branch travels to the anterior of the animal to communicate with the dorsal lateral face of the paired ovaries. The ovaries are small follicles that are located directly behind the brain (Figure 105). Both branches of the oviducts communicate with the many large vitelaria along the body length.

## Discussion

This species is clearly a *Spathula* and has many characters in common with *Sp. dittae*, albeit that there are a few important differences that exclude it from this assignment. The external morphology, lacking pigment and eyes, cannot be ignored (Figure 103). Although *Sp. dittae* displays considerable variability in external morphology, to suggest that some populations may lack eyes and pigment is difficult to justify. The penial papilla of *Sp. caeca* is a completely different shape to that found commonly in *Sp. dittae* and displays none of the characteristic irregularity or folding found in the latter. The ejaculatory duct is lacking penial glands altogether and the copulatory bursa is not volumous, both of which are features consistently described for *Sp. dittae*.

Another potential species assignment is the blind *Sp. tryssa*, which has a similar external morphology, while specimen LG244-2 has a similar penial papilla shape. However, *Sp. tryssa* can be discounted due to the lack of a seminal vesicle, the presence of penial glands and two distinct pits on the frontal margin. Furthermore, the head shape of *Sp. tryssa* is rounded and not shovel-shaped (cf. Ball 1977, Hay & Ball 1979).

*Sp. miserabile* is the only remaining possibility for these specimens to be assigned to a known species. The virtual absence of eyes described for *Sp. miserabile* could be interpreted as consistent with the state in these specimens. However, the presumed absence of ciliated pits in *Sp. miserabile* is in direct contradiction to the situation described above. The double seminal vesicle and narrow, muscularised ejaculatory duct support the notion that these specimens cannot be assigned to *Sp. miserabile*. The only remaining conclusion is that these specimens represent a new *Spathula* species.

## Ecology and distribution

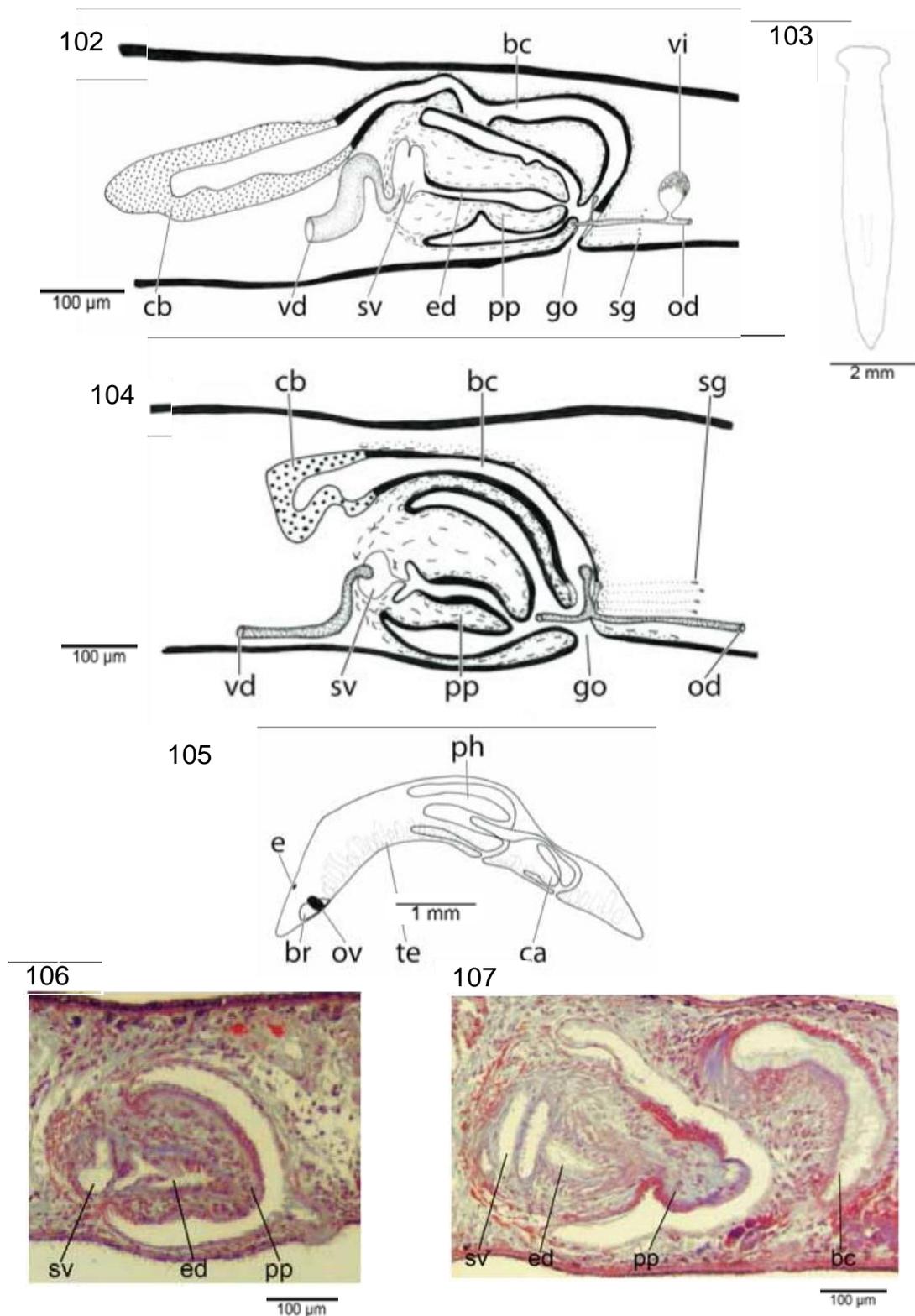
Both populations were collected from the underside of rocks in the off-channel area of cool creeks. This species has been collected from two sites in central western Tasmania.

## Etymology

This specific epithet is derived from the Latin noun '*caeca*', meaning blind gut.

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**Figures 102-107** *Spathula caeca* 102, LG244-2, sagittal reconstruction of the copulatory apparatus; 103, LG244, external features of a live animal; 104, LG244-1, sagittal reconstruction of the copulatory apparatus; 105, LG244-1, sagittal reconstruction of the reproductive system; 106, LG244-1, microphotograph of a sagittal section of the penial papilla; 107, LG224-2, microphotograph of a sagittal section of the penial papilla.

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***Spathula sp. 1***

**Material examined**

**Tasmania:**

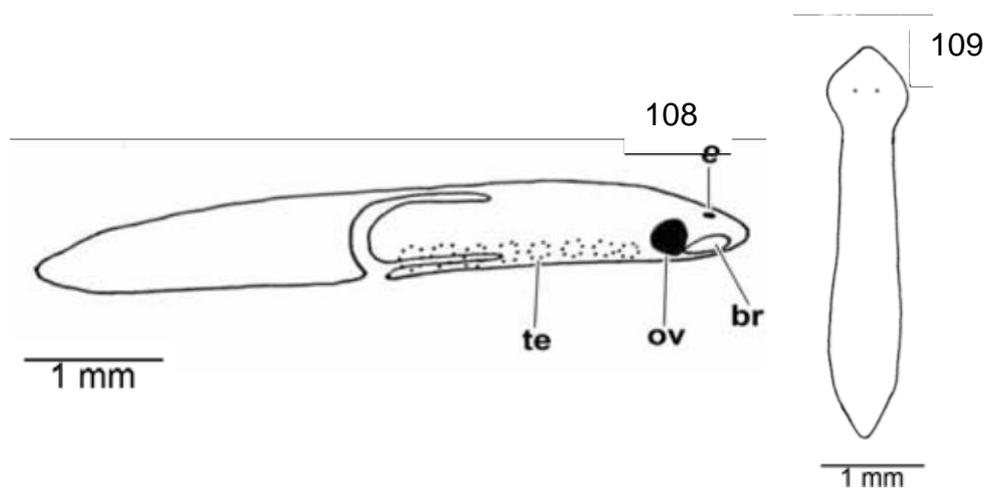
LG256, Franklin River at intersection with Lyell Highway, Franklin - Gordon Rivers National Park (42°12.933' S - 146°01.210' E), Tasmania, Australia, 31 January 2004, coll. L.J. Grant, sagittal sections on 3 slides.

**Description**

Very small specimens with the largest measuring approximately 5mm x 0.5mm. Triangulate head with small, broadly rounded auricles. Eyes are small and were unidentifiable in the field (Figure 108). No other sensory structures are identifiable, however, the histological sections were distorted. Dorsal and ventral pigmentation is absent, giving the species a completely white appearance.

These individuals were immature, without any trace of a copulatory apparatus. However, testes are situated ventrally, extending at least to the level of the pharynx and the large ovaries are located directly behind the brain (Figure 109).

The pharynx occupies approximately one-fifth of the total body length and the mouth is located at the most posterior extent of the pharyngeal pocket (Figure 109).



**Figures 108 -109** *Spathula sp. 1* 108, LG256, External features of a live animal; 109, LG256 - 1 sagittal reconstruction of the reproductive system.

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#### Discussion

Most likely, this individual represents a new species, which unfortunately cannot be described fully from the current material. Molecular data unmistakably identifies this species as a *Spathula* (see Chapter 2 and Appendix 2c), and the morphological data allows all other *Spathula* species to be eliminated. The only other *Spathula* recorded with little pigment and with eyes is *Spathula foeni* (Ball 1977). While this specimen could represent the first confirmed Tasmanian record (a heavily pigmented *Spathula* cf. *foeni* was recorded from Tasmania, cf. Grant et al. 2006), the molecular data suggests that this species is separate from *Sp. foeni*. Unfortunately, in the absence of more information this species will have to remain *Spathula* sp. 1.

#### *Spathula* sp. 2

#### Material examined

##### Victoria:

LG153, Steavensons River, Buxton, at intersection with Maroondah Highway (37°25.535' S - 145°42.423' E), Victoria, Australia, 20 December 2003, coll. L.J. Grant, molecular specimen only.

#### Description

Live specimens 8mm x 0.5mm. Specimen is slender with a truncated head and a pointed tail (Figure 110). The eyes are small, situated approximately equal distance between each other and the lateral margin and located at a considerable distance from the anterior margin. There are no details on any other sensory structures. The most distinctive character is the lack of pigment throughout the specimen. Both the dorsal and ventral surfaces are lacking all traces of pigment, presenting the body surface as white.



**Figure 110.** *Spathula* sp. 2 LG153, External features of living animal.

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### A CONTRIBUTION TO THE CURRENT SPECIES INVENTORY FOR AUSTRALIAN FRESHWATER TRICLADS (PLATYHELMINTHES, TRICLADIDA)

#### Discussion

Although the internal anatomy of this specimen remains unknown, its discovery presents a potential opportunity and is worth examining. Ball and Hay (1979) describe *Reynoldsoni reynoldsonia* as a “Slender species up to 7.5 mm long. Head spathulate with two close set eyes. Usually white or yellowish”. Our description of the external morphology of *R. reynoldsoni* is nearly identical with the description given above. This specimen was found at the base of the Victorian Alps close to the type locality of *R. reynoldsoni*. A specimen very similar to *Spathula sp. 2* (LG165) was collected from the type locality of *R. reynoldsoni* (Mt. Buffalo, Victoria), and can therefore be confidently assigned to this species. Unfortunately only one specimen was collected and DNA extraction was unsuccessful. Recent morphological phylogenies have suggested that *R. reynoldsonia* is just an aberrant *Spathula* (Sluys 2001). With this in mind one would expect *R. reynoldsonia* to sit close to the *Spathula*'s in any molecular analysis. The molecular analysis of the current study places this *Spathula sp. 2* within the *Spathula*'s, thus providing further support for the potential assignment of this specimen to *R. reynoldsoni*. The only other species resembling *Spathula sp. 2* (LG153) are *Spathula foeni* and *Romankenkius libidinosus*, both of which are pigment free with eyes. However, the size and head shape discrepancies, eliminates both of these alternatives.

So we are left with two scenarios. If we assume that these specimens are the same, then we can confidently assign them to *R. reynoldsoni*. This assignment would be significant since it would imply that we have the molecular data for *R. reynoldsoni* with all the resulting implications. Alternatively, if these specimens are not the same we are left with a specimen superficially very similar to *R. reynoldsonia* that is a new Australian *Spathula sp. 2*.

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## APPENDIX 1e: Taxonomy of the Australian DugesIIDae s.l.: Methods

### 1. Site Data Details

**Table 1:** Details of site data scored.

<b>Data Type</b>	<b>Specifics</b>	<b>Examples/Options</b>
<b>Location</b>	Latitude, longitude and altitude via Geographical Positioning System (GPS)	S 28°54.674 E 152°10.472 907.2 m
<b>Location</b>	Universal Transverse Mercator (UTM) via Geographical Positioning System (GPS)	56J 0419545 6801573
<b>Temperature</b>	Degrees Celsius	24 °C
<b>Freshwater Environment</b>	Lotic	Stream, river
<b>Freshwater Environment</b>	Lentic	Lake, small pond, swamp, spring, rock pools, lagoon, billabong, marsh, salt lakes, cavesystems, ground water/cast, dam, reservoir, ditch.
<b>Water System</b>	Width and Depth (m)	Active width: 30m Current Width: >5m Depth: 0.5m
<b>Habitat Present</b>	Potential habitats present at the site	Riffles, runs, off channel area, residual pools, backwater
<b>Vegetation Type</b>	Riparian Zone/ Outside Riparian Zone	Crop land, pasture, urban sites, clear cutting, rainforest and vine thickets, eucalypt tall open forest, acacia shrublands, tussock grasslands etc.
<b>Dominant Vegetation Form</b>	Riparian Zone	Trees>30m, trees 10-30m, trees<10m, woody shrubs, vines, rushes & sedges, herbs/non-woody, grasses, tree ferns, ferns/bracken, mosses, etc.
<b>Canopy Cover</b>	% cover in riparian zone Left Bank/Right Bank	5%, 10%, 15%, 25% 50%, 75%, 90%
<b>Riparian Buffer</b>	Width (m)	Extensive (>20m), Wide (10-20m), Moderate (5-10m), Narrow(<5m)
<b>Shading of water body</b>	% of water body shaded by riparian vegetation	5%, 10%, 15%, 25% 50%, 75%, 90%
<b>Bank Slope</b>	Degree of incline	<10°, 10°-20°, 20°-60°, 60°-80°, 80°-90°
<b>Bank Stability</b>	% of erosion or bank failure	Stable (<10%), moderately stable (10-29.9%), moderately unstable (30-50%), unstable (>50%)
<b>Aesthetics of Reach</b>	Level of disturbance to surroundings	Wilderness, natural area, common setting, offensive
<b>Substrate Type</b>	Substrate type ranked according to occurrence	Bedrock, boulder, cobble, pebble, coarse gravel, fine gravel, sand, mud/silt

<b>Channel flow/lentic water status</b>	Water level in relation to the banks	High, moderate, low, no flow/fill
<b>Flow Severity</b>	Rate of flow in lotic systems	Dry, no flow, low, normal, high, flood
<b>Water Colour</b>	Colour of water	Brown, reddish, green, black, blue, clear
<b>Water Clarity</b>	Clarity of water	Excellent, good, fair, poor
<b>Macrophytes</b>	Abundance and Type	Abundant, common, rare, absent: floating attached, submerged feathery, submerged not feathery, emergent narrow leaf, emergent broad leaf
<b>Algae</b>	Abundance	Abundant, common, rare, absent
<b>Triclad Habitat</b>	Location of the specimen	Riffles, runs, off channel area, residual pools, backwater
<b>Triclad Microhabitat</b>	Location of the specimen	Underside of rocks, logs, aquatic vegetation, leaf litter, substrate
<b>Triclad Abundance</b>	Estimation based on ease of discovery	Abundant, common, rare, absent
<b>Dominant associated fauna</b>	Fauna found regularly in the same location as specimen	Coleoptera, Ephemeroptera, Hemiptera, Odonota, Trichoptera, Hirudinea, Diptera, Mollusca, Lepidoptera, Plecoptera, Megaloptera, Crustacea

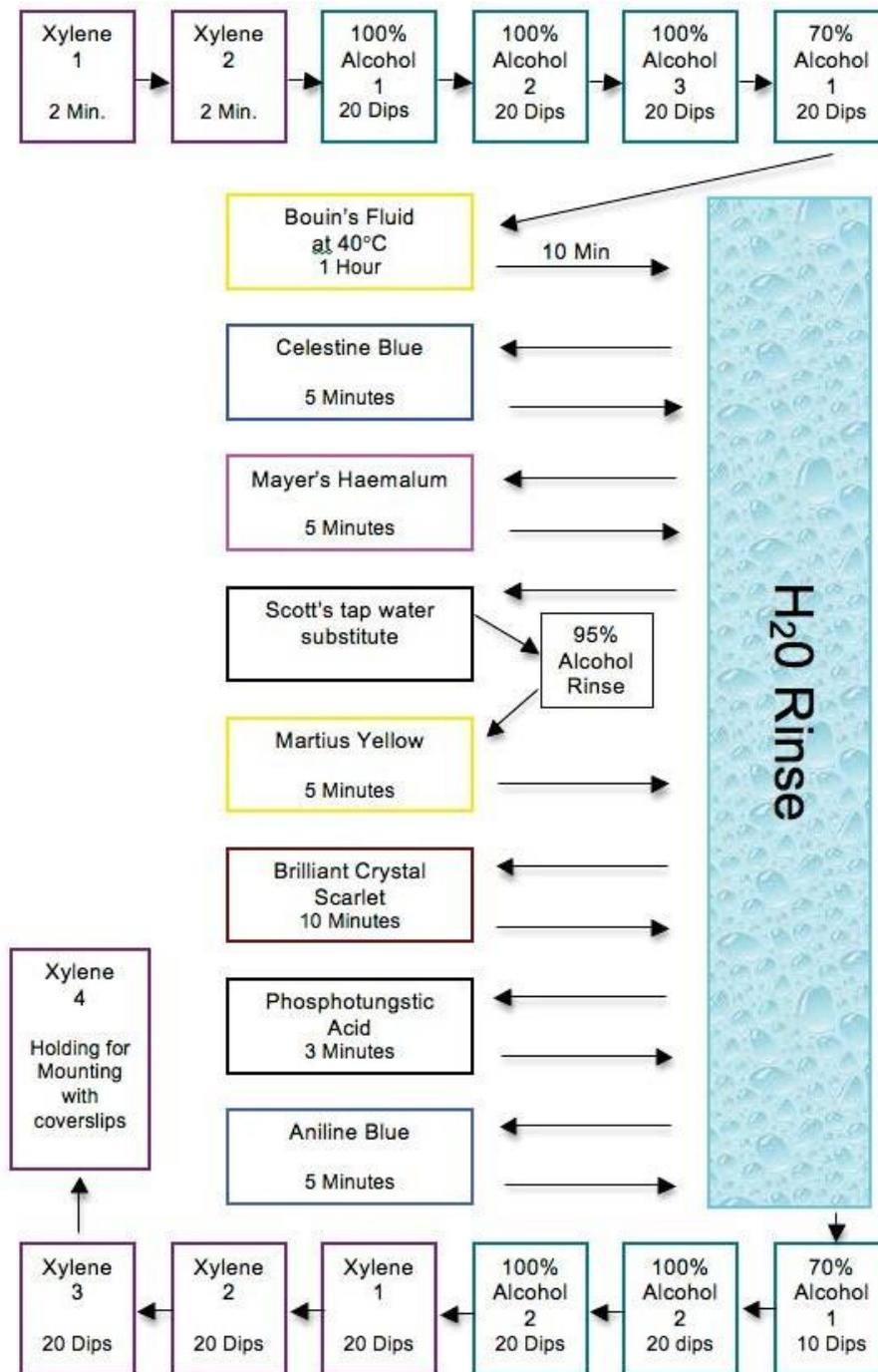
## 2. Embedding and Staining Procedures

1. 70% Ethanol (1 hour)
2. 70% Ethanol (1 hour)
3. 80-90% Ethanol (1 hour)
4. 100% Ethanol (30 minutes)
5. 100% Ethanol (30 minutes)
6. 100% Ethanol (30 minutes)
7. 100% Xylene (30 minutes)
8. 100% Xylene (30 minutes)
9. 100% Liquid Paraffin Wax (45 minutes)
10. 100% Liquid Paraffin Wax (45 minutes)
11. 100% Liquid Paraffin Wax (45 minutes)
12. Vacuum (30 minutes)

Xylene also acts as a tissue clearant and thus specimens could be inspected at this stage for the presence/absence of the copulatory apparatus.

**Figure 2:** Specimen Embedding Process

**APPENDIX 1e**  
**TAXONOMY OF THE AUSTRALIAN DUGESIIDAE S.L.: METHODS**



**Figure 3.** MSB staining protocol as used in this study.

Please note: unless otherwise indicated the rinse steps in water were one or two dips to rinse off excess stain. After the staining is complete the sections must be dehydrated in order to mount the coverslip using Dibutyl Phthalate Xylene (DPX) as the mountant. After cover slipping the slides were placed in a 40°C oven for three days.

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## APPENDIX 2a: Dugesiid Phylogeny: Molecules, Morphology and a Working Hypothesis – Methods

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### 1. DNA Extraction

DNA is extracted from the tissue of whole specimens using an ammonium acetate protocol with minor alterations (Sambrook and Russell 2001).

1. To a 1.5 ml eppendorf tube add:  
250 $\mu$ L of Digsol  
A whole worm  
5 $\mu$ L of 10mg/mL Proteinase K
2. Vortex samples
3. Digest at 55°C with agitation for a minimum of 4 hours (can leave up to 24 hours)
4. Add 5 $\mu$ L of RNase
5. Vortex, let stand for 10 minutes, vortex
6. Add 255 $\mu$ L of Ammonium Acetate solution to each sample (equal volume to step 1)
7. Vortex for 10 seconds
8. Leave at room temperature with agitation for 15 minutes
9. Cool sample to room temperature for 10 minutes
10. Spin samples at 13,000 rpm for 10 minutes
11. Pipette 400 $\mu$ L of supernatant (contains DNA) into a clean eppendorf tube being careful to leave precipitate behind.
12. Add 800 $\mu$ L of 100% ethanol (double the amount of supernatant) using a pipette
13. Vortex for 10 seconds thoroughly to mix
14. Spin samples at 13,000 rpm for 8 minutes
15. Place in a freezer for 1 hour (can be left overnight)
16. Spin samples at 13,000 rpm for 8 minutes
17. Pour off supernatant being careful to leave behind pellet (contains DNA)
18. Pipette 500 $\mu$ L of 70% ethanol into the tube, centrifuge for 5 minutes on maximum setting before pouring off ethanol
19. Place tube upside down on rack to air-dry for 30 minutes (or until completely dry)
20. Pipette 30 $\mu$ L of nuclease-free H<sub>2</sub>O
21. Store in at 4°C until use

## 2. Sequencing Protocol

### Step 1: Perform cycle sequencing

1. Prepare a forward or reverse sequencing reaction mix in a tube on ice:

Components	Volume for each reaction
BigDye® Direct Sequencing Master Mix	2.0 µL
One sequencing primer: <ul style="list-style-type: none"> <li>• BigDye® Direct M13 Fwd Primer or</li> <li>• BigDye® Direct M13 Rev Primer</li> </ul>	1.0 µL
<b>Total volume for each reaction</b>	<b>3.0 µL</b>

2. For each sequencing reaction, add 3 µL of the sequencing reaction mix to the appropriate well in the respective forward or reverse reaction plate.
3. Seal the reaction plate with adhesive film or caps, then spin the plate briefly.
4. Run the reactions in a thermal cycler:

Stage	Veriti® thermal cyclers		9700 thermal cycler	
	Temp	Time	Temp	Time
Hold	37°C	15 min	37°C	15 min
Hold	80°C	2 min	80°C	2 min
Hold	96°C	1 min	96°C	1 min
Cycle (25 cycles)	96°C	10 sec	96°C	10 sec
	50°C	5 sec	50°C	5 sec
	60°C	75 sec	60°C	4 min
Hold	4°C	∞	4°C	∞

5. After the cycle sequencing reactions are complete, spin the plate briefly.  
 STOPPING POINT. (Optional) Store the reaction plate at 4°C overnight or at -15°C or -25°C for long-term storage.

## Step 2: Purifying Sequencing products

1. Spin the reaction plate at 100 x g for 1 minute, then remove the seal.
2. Prepare a premix with SAM™ Solution and XTerminator® Solution in an appropriately sized tube:

Component	Volume for 1 well	Volume for 96 wells
SAM™ Solution	45 µL	4752 µL
XTerminator® Solution	10 µL	1056 µL
<b>Total volume</b>	55 µL	5808 µL

- a. Add the SAM™ Solution to the tube using a conventional pipette tip.  
**Note:** Make sure there are no particulates in the SAM Solution before pipetting. If there are particulates, heat the SAM Solution to 37°C and mix to resuspend. Cool to room temperature before using.
  - b. Vortex the XTerminator® Solution bulk container at maximum speed for at least 10 seconds, until the solution is homogeneous.
  - c. Using a wide-bore pipette tip, aspirate the XTerminator® Solution.  
**IMPORTANT!** Avoid pipetting from the top of the liquid.
  - d. Mix the reagents until homogeneous.
3. Add 55 µL of SAM™ Solution/XTerminator® Solution premix to each well.
  4. Seal the plate using one of the following methods, then verify that each well is sealed:
    - MicroAmp® Clear Adhesive Films  
*or*
    - A heat seal at 160°C for 1.5 seconds
  5. Vortex the reaction plate for 20 minutes, using the following conditions:

Vortexer	Speed
Digital Vortex-Genie® 2	1800 rpm
IKA MS3 Digital	2000 rpm <sup>†</sup>
IKA Vortex 3	Setting 5 <sup>‡</sup>
Taitec MicroMixer E-36	Maximum
Union Scientific Vertical Shaker	Setting 100 <sup>§</sup>

<sup>†</sup> Set the vortexer to Mode B.

<sup>‡</sup> Use the maximum setting without allowing the vortexer to move across the bench.

<sup>§</sup> Add more plates, if necessary, to meet mass requirements.

6. In a swinging-bucket centrifuge, spin the plate at 1000 × g for 2 minutes.  
**STOPPING POINT.** If you plan to store the plate before proceeding with capillary electrophoresis, store the sample plates sealed with heat seal film or adhesive film for up to 48 hours at room temperature (20 to 25°C) or up to 10 days at 4°C or –20°C.

### **Step 3: Perform capillary electrophoresis**

Refer to instrument user guide for instructions on setting up and performing capillary electrophoresis run.

## **3. Additional Tree building methods**

### **Neighbour Joining**

In order to determine whether the data was suitable for estimating a neighbour-joining tree the average pairwise Jukes-Cantor (JC) distance needed to be determined. This was done in MEGA4 (Tamura et al. 2007) and all alignments were shown to be suitable candidates with all distances being less than 1.0. Neighbour joining trees were also inferred in MEGA4 (Tamura et al. 2007) with the following parameters. Due to the presence of missing data in some sequences a pairwise deletion method was selected so that gaps would be removed from consideration only as the need arises. The substitution model selected was a Maximum Composite Likelihood model, which the author of MEGA4 suggests should be used for the inference of all Neighbour Joining trees (Hall 2007). The substitution included transitions and transversions, and the pattern among lineages was treated as homogeneous. Finally, the rates among sites parameter was treated as different (Gamma distributed). This requires that the  $\alpha$  parameter must be estimated, which was obtained in BEAST (Drummond and Rambaut 2007) using a General Time Reversible (GTR) model for both genes and a Gamma and Invariant Sites site heterogeneity model. The log file was read in Tracer v 1.4 (Drummond and Rambaut 2007)(Chapter 2: Table 2.5). In order to judge the strength of support for nodes on trees a bootstrap (Felsenstein 1985) analysis was performed using 2000 replicates. A 50% majority rule consensus tree was inferred in MEGA4. Trees were viewed in Tree Explorer (Tamura et al. 2007) where the root could be designated and image manipulated.

### **Parsimony (molecular)**

Maximum Parsimony trees were inferred in MEGA4 (Tamura et al. 2007). A Close-Neighbour-Interchange (CNI) (level = 3) search method was chosen using Random Addition (10 replications) to infer initial trees. All sites were used in the analysis. In order to judge the strength of support for nodes on trees a bootstrap (Felsenstein 1985) analysis was performed using 2000 replicates. A bootstrap 50% majority rule consensus tree was inferred in MEGA4. Trees were viewed in Tree Explorer (Tamura et al. 2007), which allows the computation of a bootstrap consensus tree and the correct designation of the root.

Trees were also inferred in TNT (Goloboff et al. 2008). The tree-collapsing algorithm selected was Heuristic, tree bisection and reconnection (TBR). The alignment was analysed via a New Technology Search with trees obtained via random addition sequences (1,000). A sectorial search was implemented using the random sectorial search option (default settings used), tree fusing was also selected, starting from best tree and swapping after exchanging (every 3 rounds). Only the most parsimonious trees were kept, from which a 50% majority rule consensus tree was inferred. The matrix was re-sampled using bootstrap (Felsenstein 1985) with Poisson independent reweighting (1000 replicates). For this analysis a New Technology Search (settings as above) was implemented using groups from a 50% majority rule consensus tree (if the bootstrap was allowed to run independently of the consensus tree groups, most groups were collapsed, offering no information about relationships). Consistency Index (CI) and Retention Index (RI) for each analysis were obtained in TNT (Goloboff et al. 2008), using a script available from the TNT wiki site (<http://tnt.insectmuseum.org/index.php/Scripts>).

### **Maximum Likelihood**

Maximum likelihood trees were inferred in PHyML (Guindon and Gascuel 2003) using the following parameters. For those alignments specifying a TVM or TIM model the GTR model was used as these models are special cases of the GTR model and are not yet implemented in PHyML. In order to judge the strength of support for nodes on trees a bootstrap analysis was performed using 500 replicates. While the  $p_{inv}$  and Gamma parameter could be estimated from the data during this analysis it was thought that the BEAST (Drummond and Rambaut 2007) results obtained earlier for these alignments presented a more accurate value. The Transition/Transversion ratio was estimated and four substitution rate categories were used for all analysis. A bootstrap (Felsenstein 1985) 50% majority rule consensus tree was inferred using Geneious tree builder (Drummond et al. 2009), using a Jukes-Cantor genetic distance model and a neighbour joining tree building algorithm. Trees were read and manipulated in Dendroscope (Huson et al. 2007). Maximum likelihood trees were also inferred in Paup (via Geneious) using GTR, fixed proportion of invariable sites (Chapter 2: Table 2.5), and Gamma distribution, a heuristic search and Neighbour Joining tree searching was used. An outgroup was chosen depending upon the alignment (*Procerodes* for large and *Crenobia* for smaller alignments). Bootstrapping was performed using a random seed, 100 replications and heuristic search method. Concatenated molecular alignments were not processed using PAUP, as the process took many days and results were not substantially different from the PHyML results. A bootstrap (Felsenstein 1985) 50% majority rule consensus tree was inferred

using Geneious tree builder (Drummond et al. 2009) using a Jukes-Cantor genetic distance model and a neighbour joining tree building algorithm. Trees were read and manipulated in Dendroscope (Huson et al. 2007).

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## References

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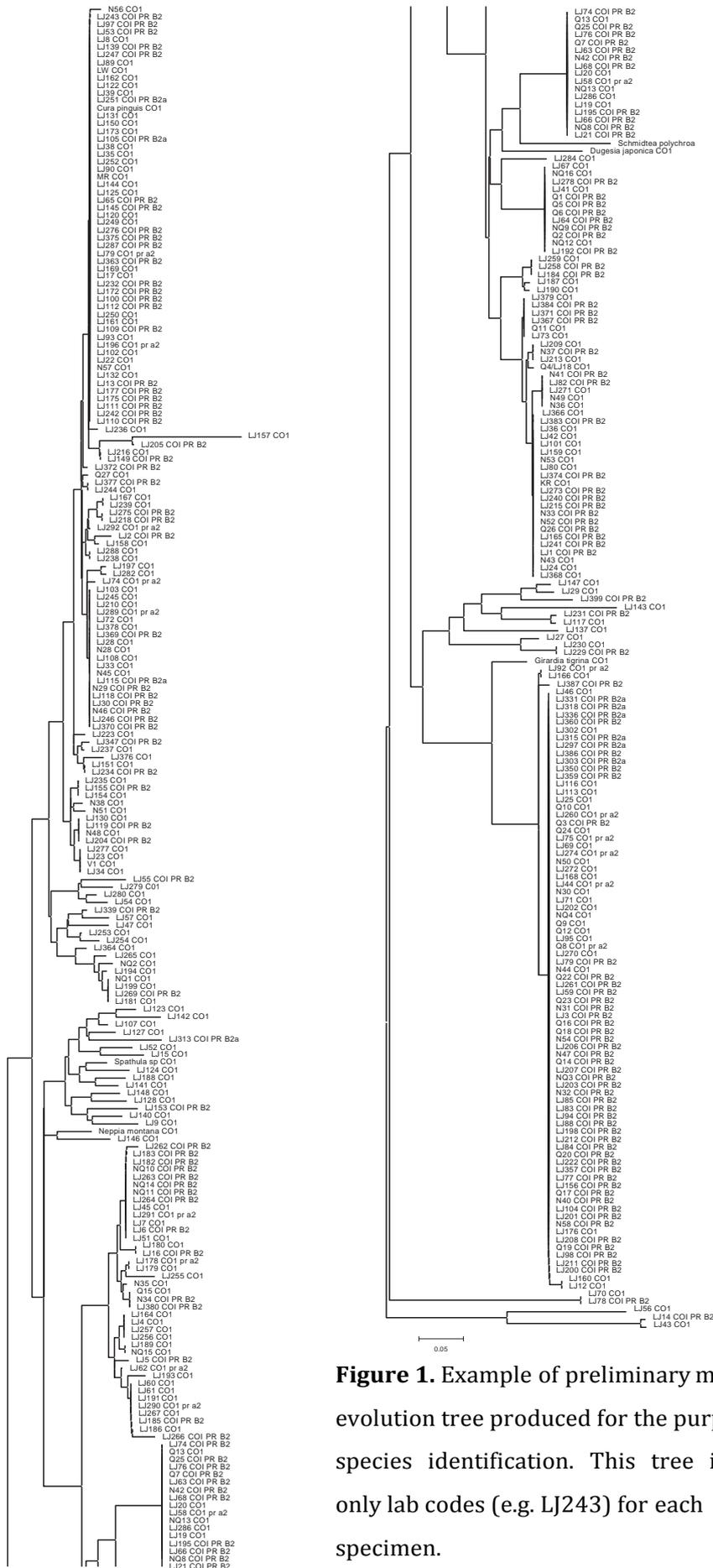
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## APPENDIX 2b: Molecular Identification

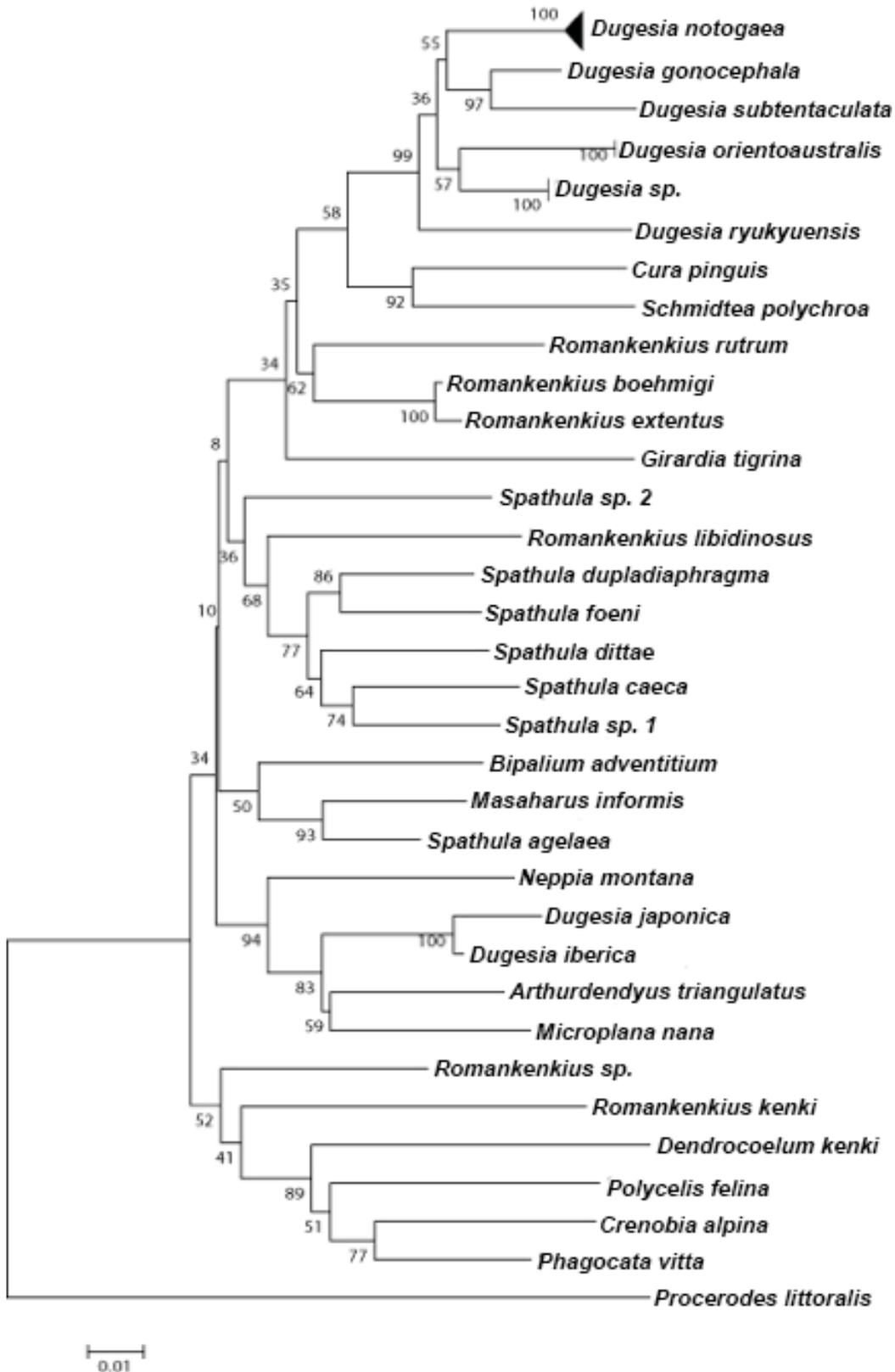
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This appendix contains a series of phylogenetic trees that were used in the preliminary stages of species identification. They allowed for the identification of those species for which taxonomic information was not available (Figures 1-4), to provide assistance with genus assignment for enigmatic species (Figures 1-4) and to review population structure and to avoid the unnecessary sectioning of repetitive species (Figures 1 & 5-8). Figure 1 is an example of the trees created to make the initial species assignments. This information was then used to create trees 2-8, which refine the species details. The *cox1*, minimum evolution trees produced here were created in MEGA4 (Tamura et al. 2007). Additional details around methods used for the molecular analysis can be found in Chapter 2.

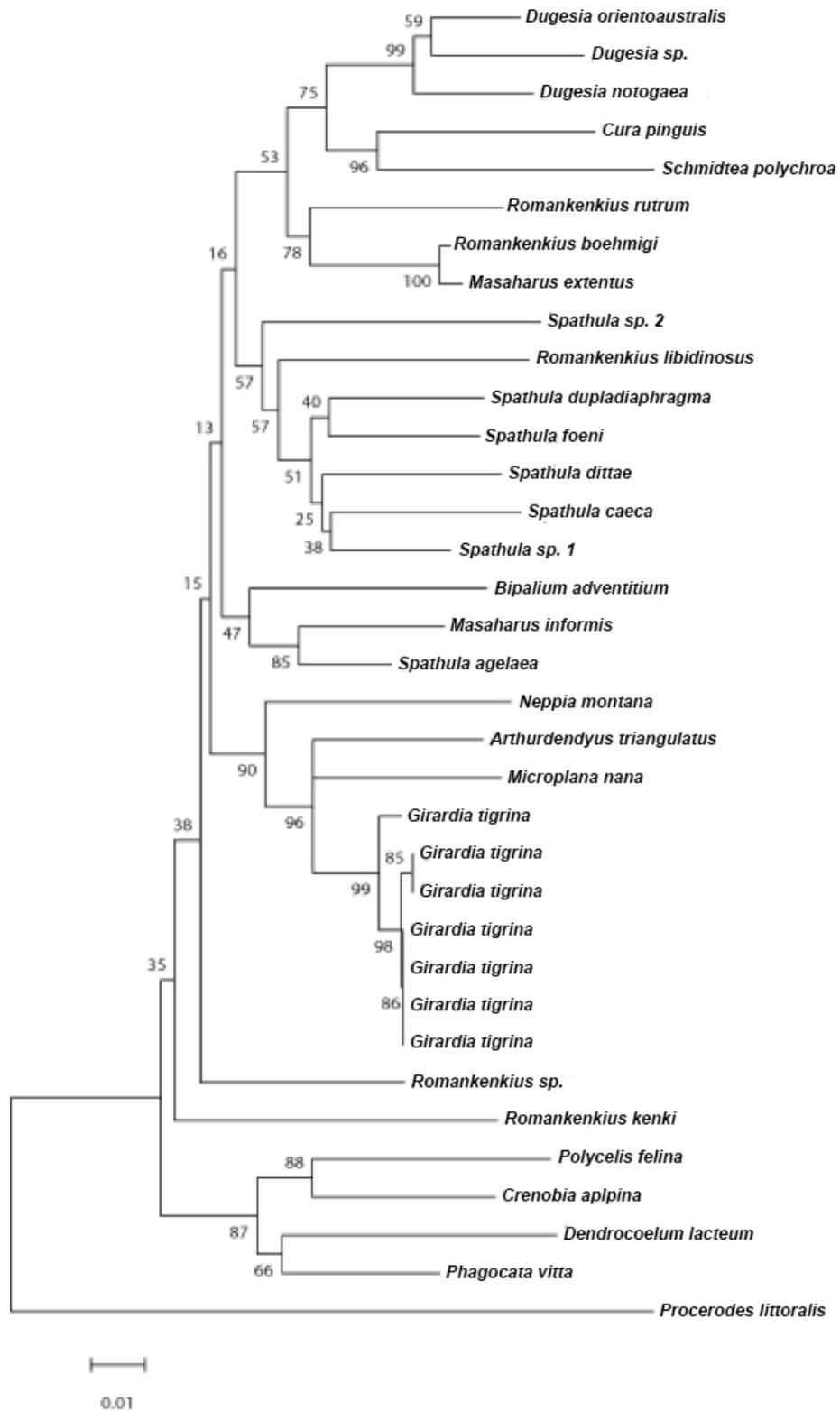
APPENDIX 2b  
Molecular Identification



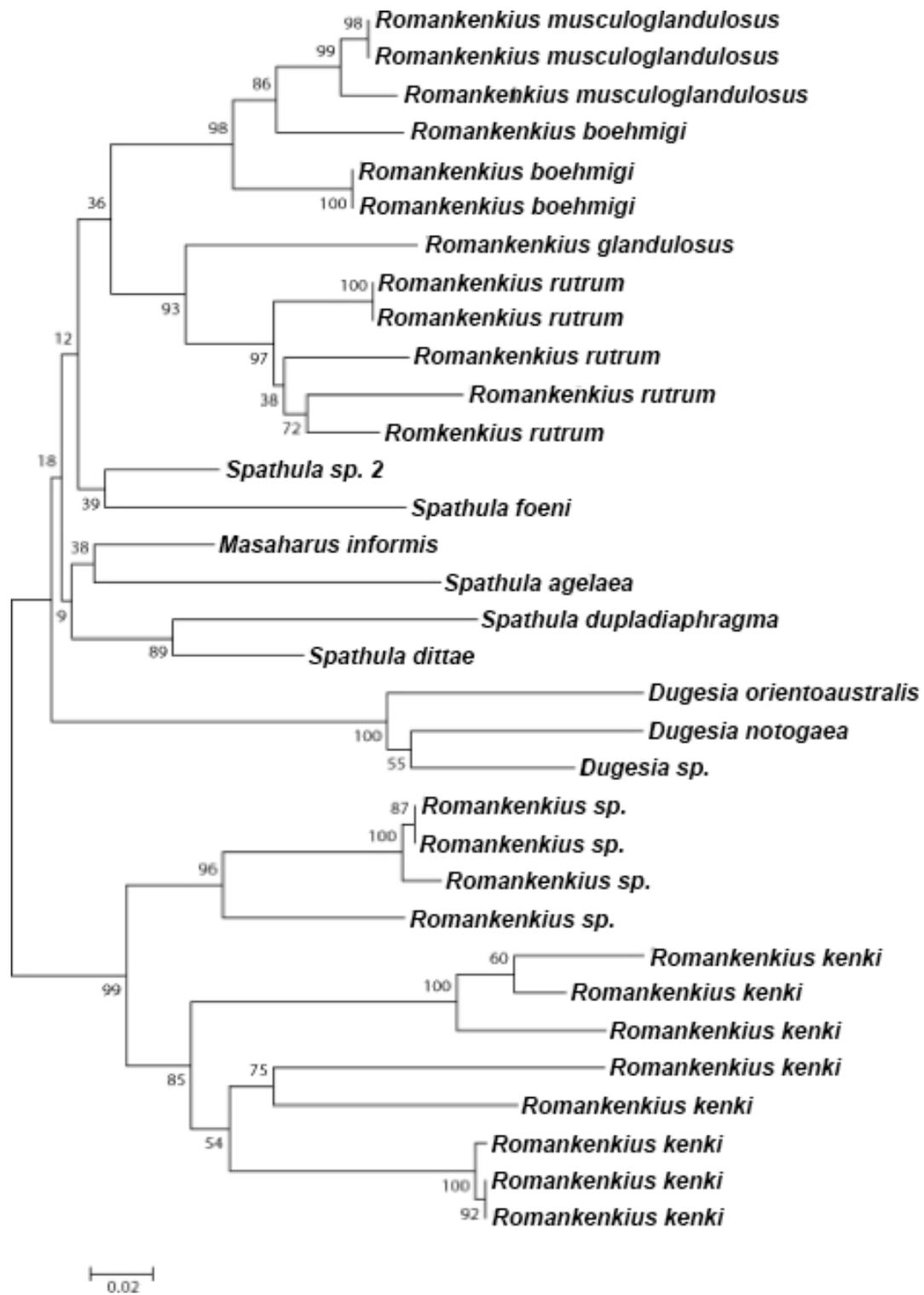
**Figure 1.** Example of preliminary minimum evolution tree produced for the purposes of species identification. This tree includes only lab codes (e.g. LJ243) for each specimen.



**Figure 2.** *cox1* consensus tree based on a minimum evolution analysis with most dugesiid genera represented focusing on species within the genus *Dugesia*, with representatives of the Planariidae, Dendrocoelidae, Geoplanidae and Maricola for comparison.



**Figure 3.** *cox1* consensus tree based on a minimum evolution analysis with most dugesiid genera represented, focusing on the relationship between *Girardia tigrina* and *Masaharus*, with representatives of the Planariidae, Dendrocoelidae, Geoplanidae and Maricola for comparison.

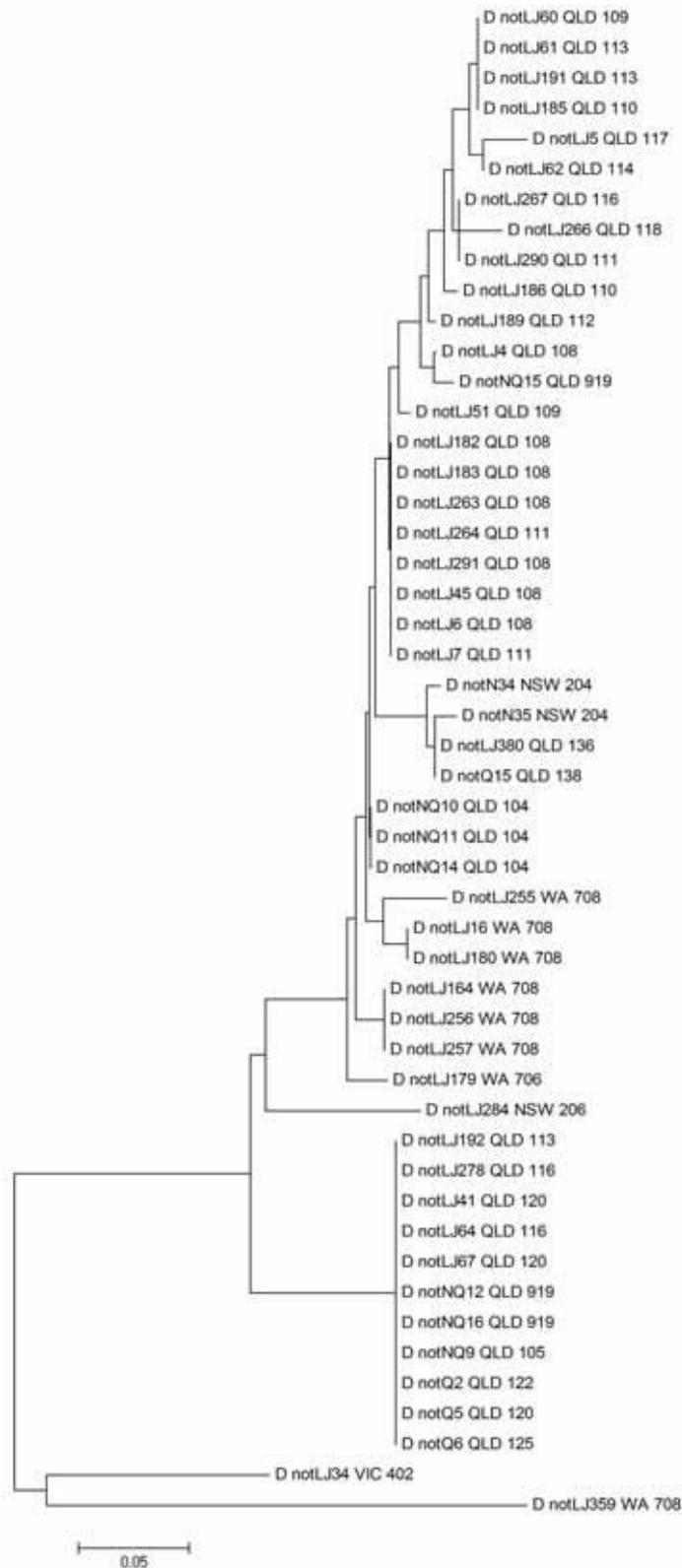


**Figure 4.** *cox1* consensus tree based on a minimum evolution analysis with most dugesiid genera represented, focusing on the relationship within the genus *Romankenkius*, with representatives of the Planariidae, Dendrocoelidae, Geoplanidae and Maricola for comparison.

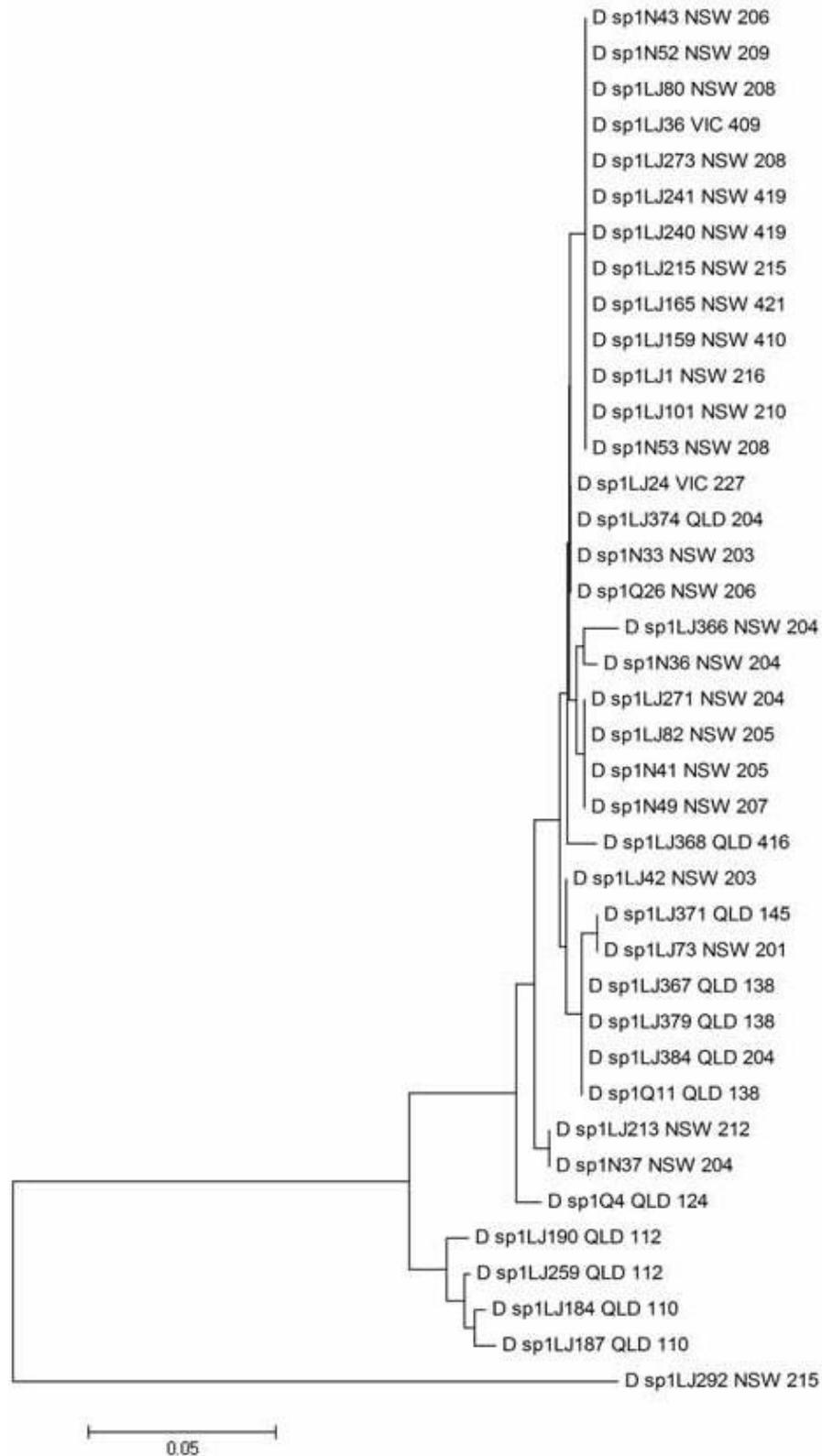
APPENDIX 2b  
Molecular Identification



**Figure 5.** *cox1* consensus tree based on a minimum evolution analysis, providing identification of unsectioned individuals and presenting the population structure for *Cura pinguis*. “C pin” represents the species name, *Cura pinguis*; the lab code, state of origin and Australian drainage division code is also included.



**Figure 6.** *cox1* consensus tree based on a minimum evolution analysis, providing identification for unsectioned individuals and presenting the population structure for *Dugesia notogaea*. “D not” represents the species name, *Dugesia notogaea*; the lab code, state of origin and Australian drainage division code is also included.



**Figure 7.** *cox1* consensus tree based on a minimum evolution analysis, providing identification of unsectioned individuals and presenting the population structure for *Dugesia sp.* “D sp1” represents the species name, *Dugesia sp.*; the lab code, state of origin and Australian drainage division code is also included.

APPENDIX 2b  
Molecular Identification



**Figure 8.** *cox1* consensus tree based on a minimum evolution analysis, providing identification of unsectioned individuals and presenting the population structure for *Girardia tigrina*. “G tig” represents the species name, *Girardia tigrina*; the lab code, state of origin and Australian drainage division code is also included.

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## References

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Tamura, K., J. Dudley, M. Nei and S. Kumar. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology And Evolution* **24(8)**: 1596.

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## APPENDIX 3a: Details of Characters for Morphological Phylogeny

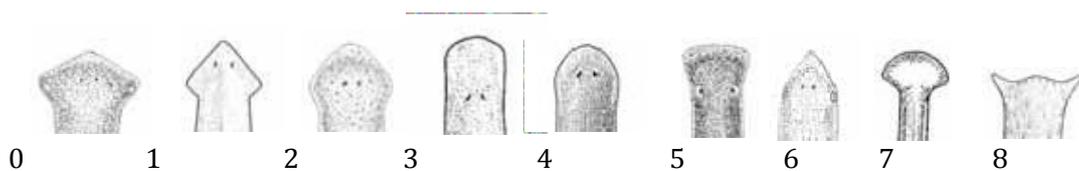
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Abbreviations: te=testes, br= brain, ov=ovary, cp=ciliated pit, od=oviduct, cod=common oviduct, sg=shell glands, bc=bursal canal, cb=copulatory bursa, pg=penial glands, vd=vas differens, ed=ejaculatory duct, pp=penial papilla, ol=oviducal loop, dg=diaphragm, sv=seminal vesicle, gl=glands, div=diverticulum, sph=sphincter, gd=gonoduct, ca=copulatory apparatus, ph=pharynx, ad=adenodactyl, int=intestine, ca=common atrium, go=gonoduct, ol=oviducal loop, sf=sensory fossae, ma=male atrium, gonopore=gp

**Note:** All images not referenced were created as part of this thesis.

### External Morphology

1. Head shape: Low triangular (0), high triangular (1), bluntly triangular (2), rounded (3), spatulate (4), truncate (5), pointed (6), lunate (7), convex (8).



Due to the flexible nature of triclads the process of fixation distorts the external morphology of specimens. All external features must therefore be recorded from live specimens. States 0-6 of the above head shapes are found (with minor variations) within the Australian freshwater Geoplanoidea. The lunate state is found in other Turbellarians and included here due to the terrestrial outgroup.

2. Auricles: Absent (0), short (1), long (2)

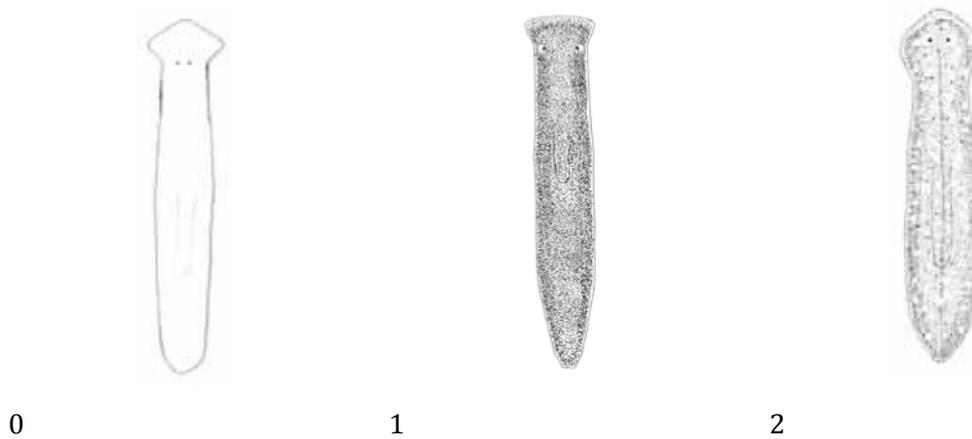


3. Pigmented eye-cup: absent (0), multicellular (1), singlecellular (2)

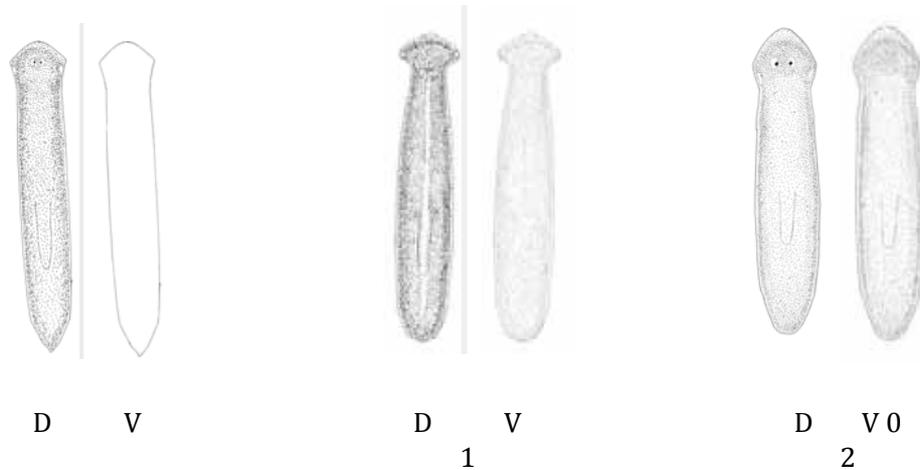


The presence and nature of the pigmented eyecup is identifiable in the serial sections. Pigment-free patches that surround the eyes are present in all species, however they are not identifiable in pigment free species. The multicellular eye-cup has generated much discussion and is now thought to represent a synapomorphy uniting the Dugesiidae and Geoplanidae clades (Carranza et al. 1998a, Carranza et al. 1998b). For a detailed discussion on eye structure and its implications see Sluys (2001).

4. Dorsal pigmentation: Absent (0), solid (1), mottled/patterned (2)



5. Ventral pigmentation: Absent (0), less than dorsal (1), same as dorsal (2)



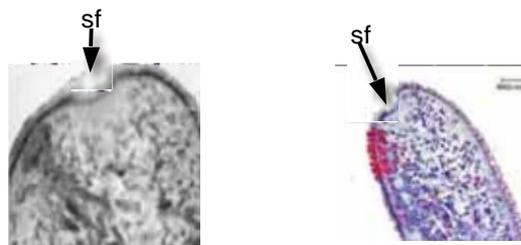
While the level of dorsal pigment is impossible to score owing to its subjectivity, the ventral pigmentation, relative to the dorsal is very consistent within species, thus constituting a useful character.

6. Ciliated pits (cp): Absent (0), Present (1)



Sensory ciliated pits are commonly situated either side of the head in Australian dugesiids (image adapted from Ball 1974a). These pits differ from the much shallower sensory fossae that are often present along the anterior margin; the number of fossae is variable (Sluys 2001).

7. Sensory fossae (sf): absent (0), present (1)



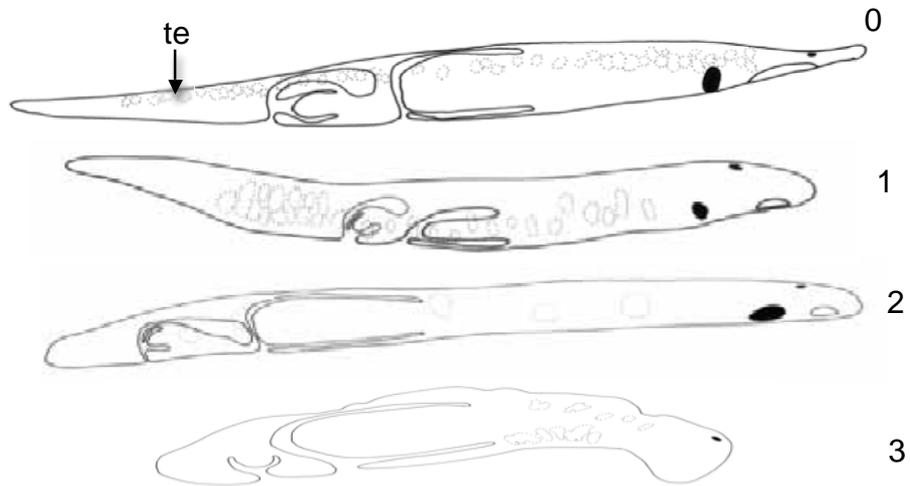
### Internal Morphology

8. Position of Mouth: At the hind end of the pharyngeal pocket (0), at 1/5 of the distance between the hind end of the pharyngeal pocket and the root of the pharynx (1), at 1/3 (2), halfway the distance between the hind end and the root of the pharynx (3), at 1/4 (4)



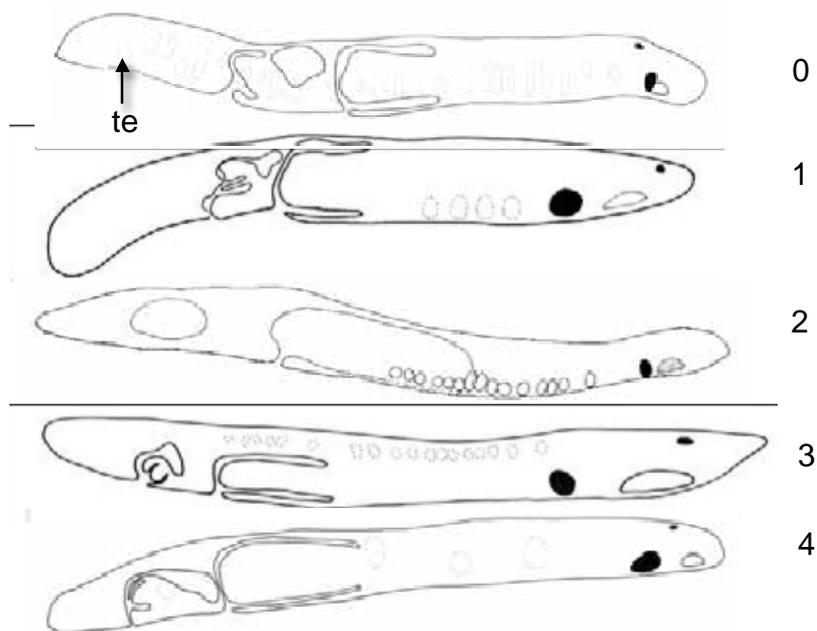
Mouths of most Australian dugesiids are situated at the hind end of the pharyngeal pocket, *Romankenkius* is the only genus to exhibit any other state. The additional states have been included to facilitate the terrestrial outgroup.

9. Position of testes (te): Ventral (0), dorsal (1), situated in the middle of the body (2), situated dorsally and ventrally (3), extending from dorsal to ventral surface (4)

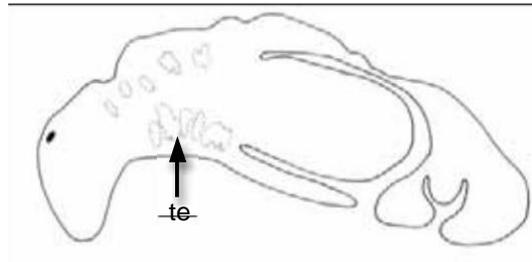


States 0-3 are found in the Australian dugesiidae. Testes filling the entire dorso-ventral space is a state found only rarely in the Geoplanidae.

10. Extension of testes (te): Throughout body length (0), prepharyngeal (1), to about halfway along the pharyngeal pocket (2), to the mouth at the posterior end of the pharyngeal pocket (3), to the copulatory apparatus (4)

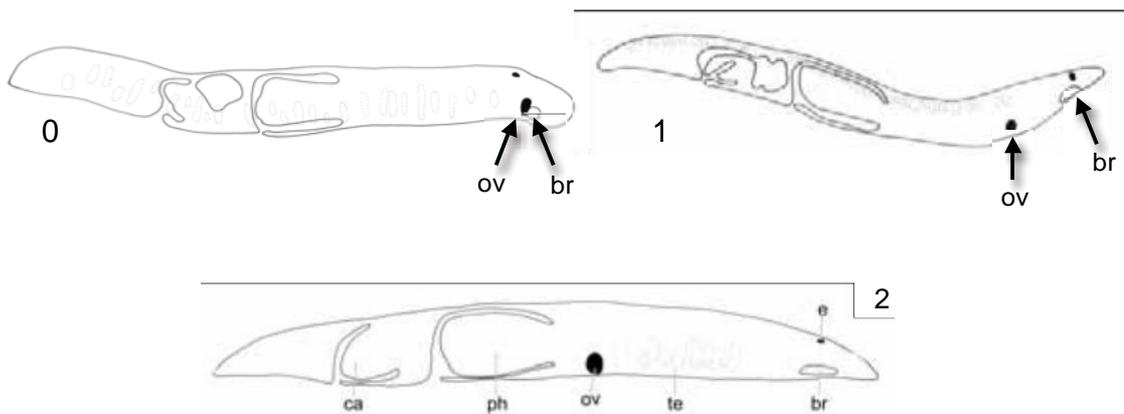


11. Fused Testes (te): Absent (0), present (1)



This condition, rarely found in freshwater Geoplanoids, differs from the normal state where testes are discrete follicles.

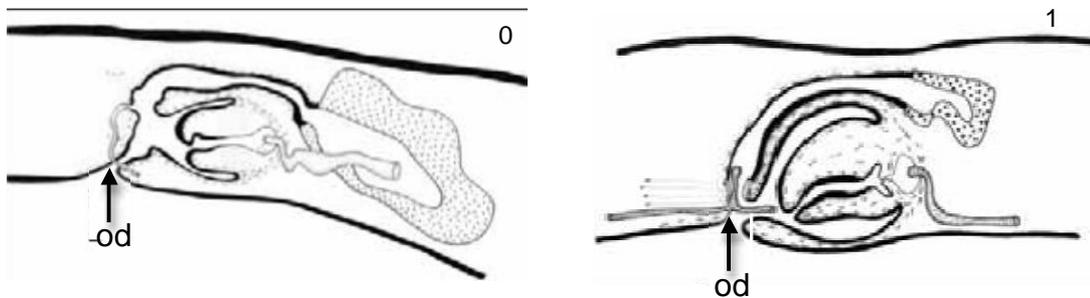
12. Position of ovary (ov): Directly behind the brain (br) (0), somewhat removed from the brain (1), a long distance behind the brain (2)



If the ovary is situated further than the width of the ovary behind the brain it is considered to be sitting “somewhat removed from the brain”. The character state “a long distance behind the brain” is included to accommodate the outgroups where the ovary can be sitting posterior to the pharynx.

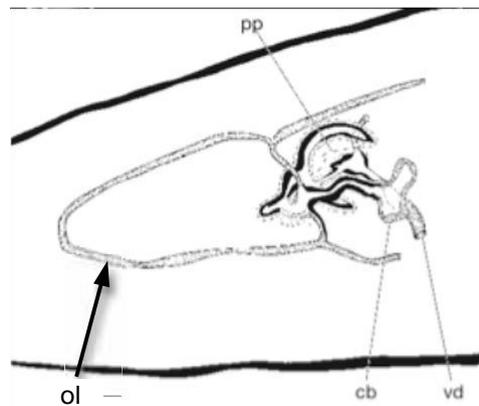
### Female Copulatory Apparatus

13. Oviducts (od) with caudal branch: Absent (0), present (1)

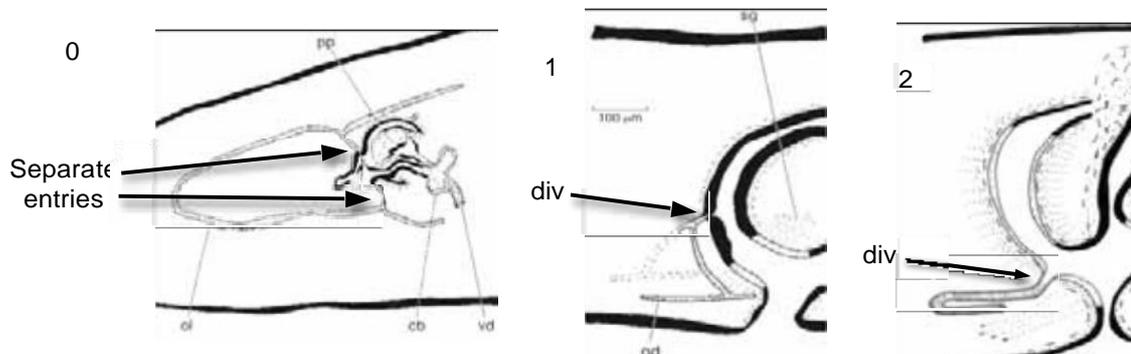


All Australian *Spathula* spp exhibit caudally branching oviducts, i.e. the oviducts extend beyond the copulatory apparatus into the posterior region of the specimen. This condition is not exclusive to *Spathula* as several other genera have representatives with, generally less extensive, caudal branches.

14. Oviducal loop (ol): Absent (0), present (1)

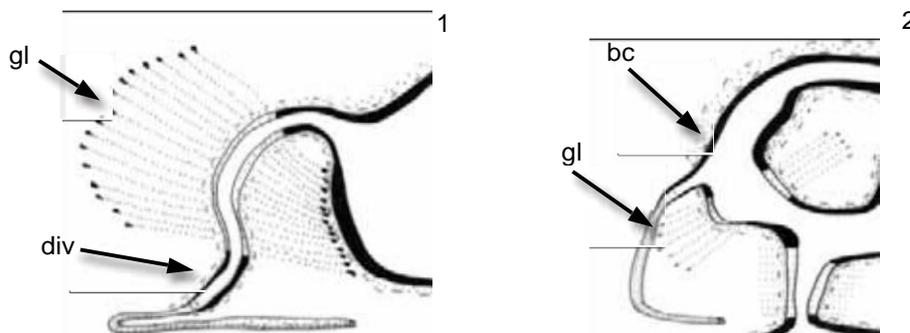


**15. Common oviducts/diverticulum (div):** absent (0), opening well into bursal canal (1), opening at the most posterior-ventral section of the bursal canal or even into the common atrium (2)



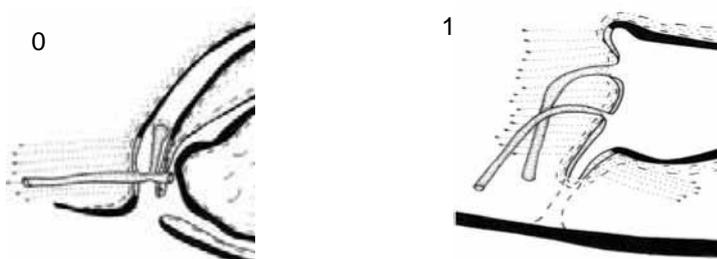
Ball's (1974b) description of the genus *Romankenkius* notes the diverticulum of the bursal canal as being morphologically and histologically different from a common oviduct. While the diverticulum had not been described outside of the freshwater Geoplanoidea, common oviducts are found regularly within the Planariidae and Dendrocoelidae. Ball's suggestion was that a diverticulum has an independent origin and can be easily differentiated from the common oviduct as it receives the entrance of shell glands (Ball 1974b). Unfortunately since this description the terms "common oviduct" and "diverticula" appear to have been used interchangeably and differentiation between the two structures has become difficult. Both terms refer to a broad duct-like structure that receives both oviducts then connects to the bursal canal. The difficulty arises from the fact that while common oviducts are never described with musculature, there are examples of "diverticulum's" being described with no surrounding musculature. Similarly, glands have been described as entering through the epithelium of both common oviducts and diverticula. While it is certainly possible that these structures have different origins, for the purposes of this study these features will be considered as a single character. Diverticula are found, without exception, within Australian *Romankenikus* spp. and occur rarely outside of this genus. The alternate condition is that of separate oviducal entries (image adapted from Grant et al. 2006).

**16. Shell glands (gl):** Absent (0), entering the diverticulum (div) (1), entering the bursal canal (bc) (2)



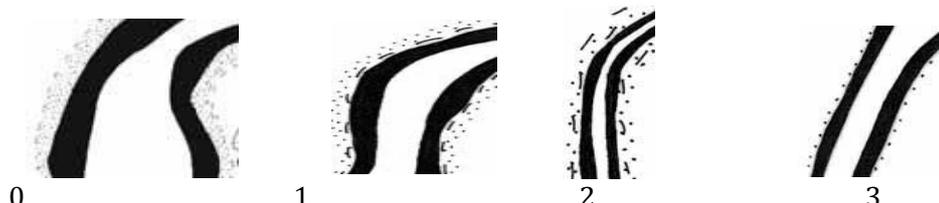
Shell glands, which secrete a substance important in eggshell production (Ishida and Teshirogi 1986), are consequently very common across all Australian genera. While these glands most regularly enter through the bursal canal, in species with a diverticulum of the bursal canal, these glands can penetrate this zone.

**17. Entrance of oviducts into bursal canal:** Symmetrical (0), asymmetrical (1)



Species with a diverticulum were scored as symmetrical.

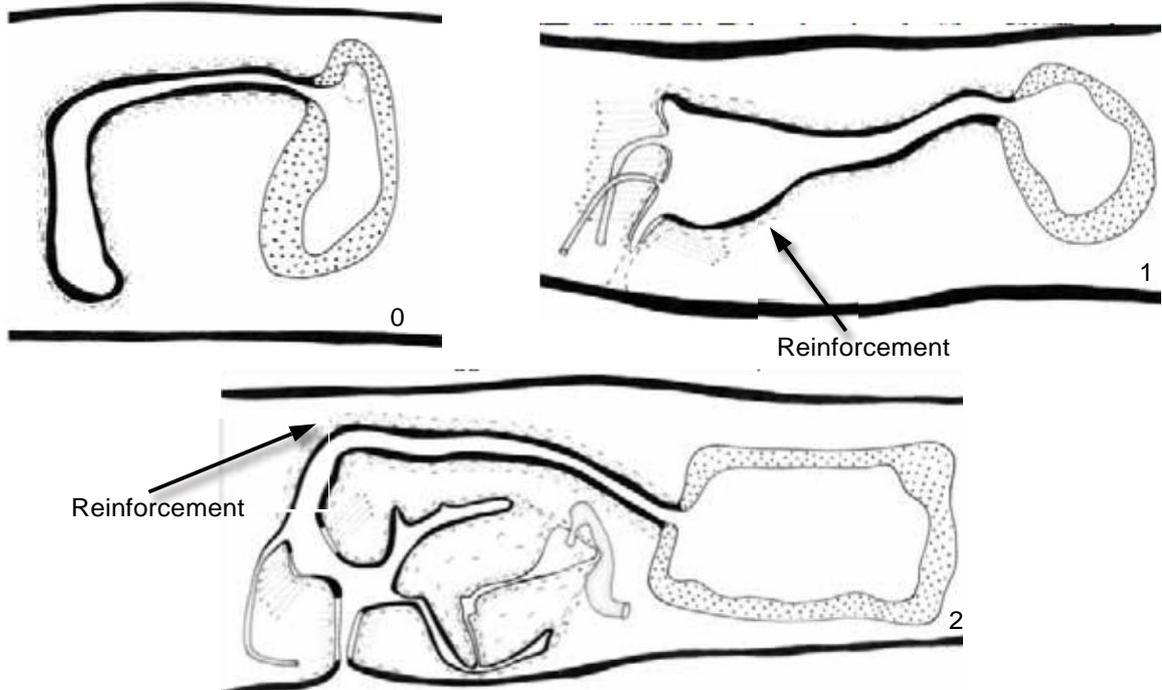
**18. Musculature of bursal canal:** Non-reversed (0), reversed (1), mixed (2), circular only (3), canal absent (4)



In the above images the dashes refer to longitudinal muscle and the dots to circular muscle. The most common state in dugesiids is reversed (1). The terminology is simply referring to the fact that this musculature structure is reverse in relation to the rest of the

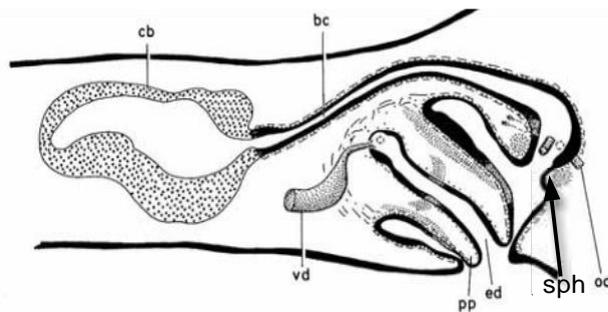
copulatory apparatus (circular surrounded by longitudinal i.e. non-reversed). The character state “canal absent” is included to accommodate the outgroups. All currently described freshwater geoplanoids have a bursal canal.

**19. Ectal reinforcement on bursal canal: Absent (0), present (1), extended (2)**



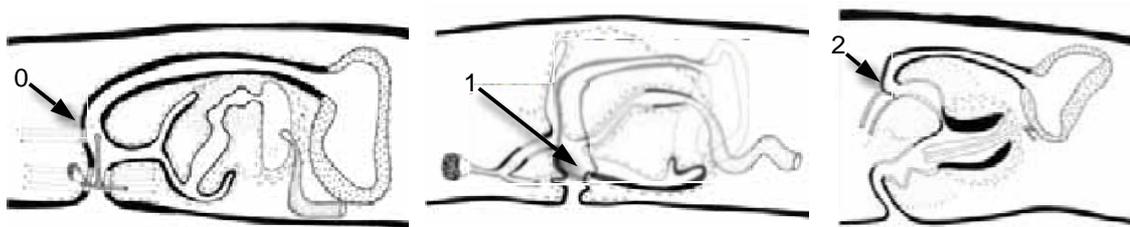
Some dugesiids with reversed musculature have a third layer of longitudinal muscle surrounding the circular muscle, reinforcing the muscle coat. The extra layer may be confined to the ventral zone of the bursal canal (1), however it can occasionally extend much further, even as far as the copulatory bursa (2).

**20. Sphincter (sph) on bursal canal: Absent (0), present (1)**



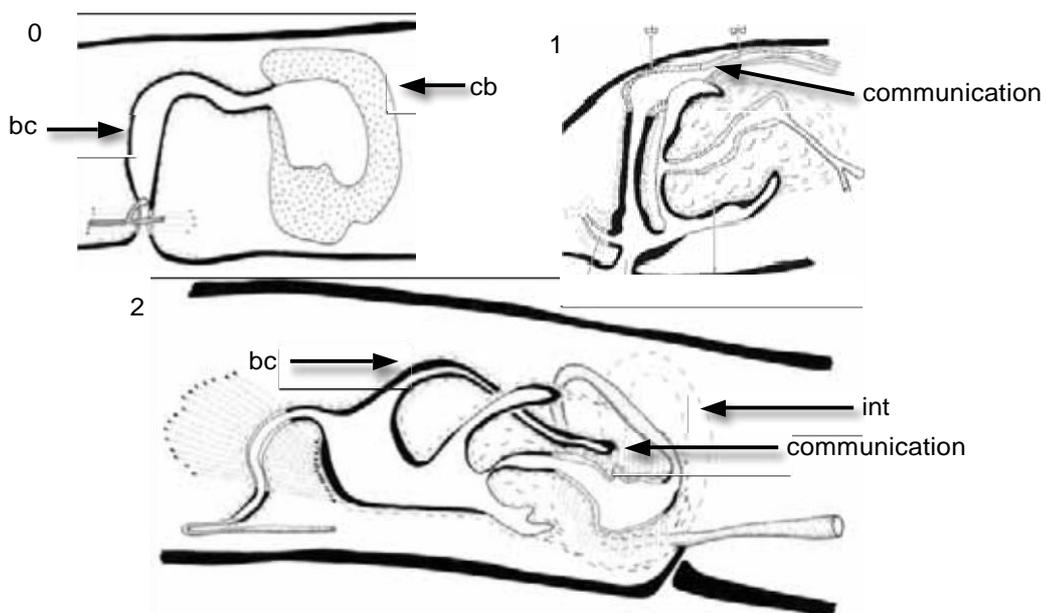
Two genera (*Girardia* and *Spathula*) within the freshwater Geoplanoidea have representatives with a highly developed muscular sphincter on the bursal canal. The only species within the Australian freshwater Geoplanoidea to exhibit this state is *Girardia sphincter* (image adapted from Sluys and Kawakatsu 2001).

**21. Bursal canal opening into atrium: Posterior (0), lateral (1), dorsal (2), absent (3)**



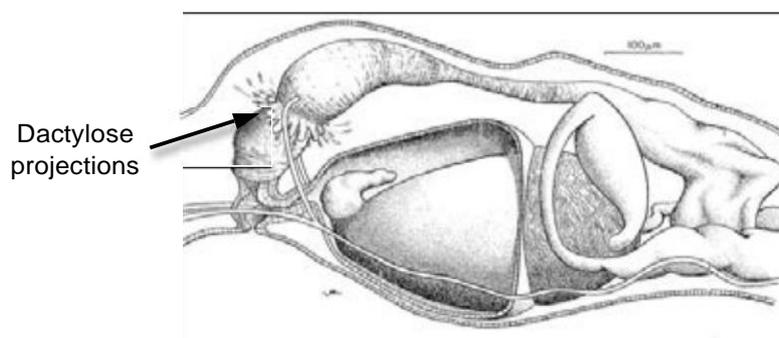
The most usual point of communication for the bursal canal and the atrium is postero-ventral (0), however in some cases the connection has shifted anteriorly. The most extreme case is that of *Cura pinguis* in which the bursal canal communicates directly with the male atrium (2), this state appears to be restricted to this species, the lateral communication is a more common exception with species across several genera exhibiting this state (1).

**22. Bursal canal (bc) connects to: Copulatory bursa (cb) (0), intestine (int) via copulatory bursa (1), intestine (2), expansion (3), common oviduct/oviducts (4)**



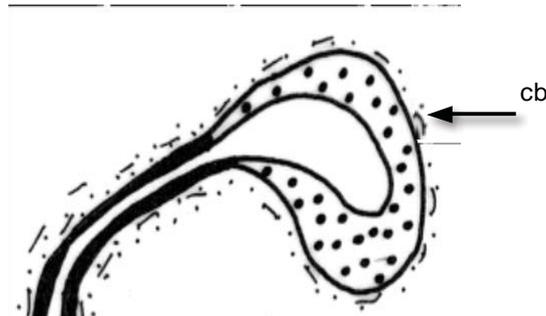
In sexual forms, triclads produce large amounts of sperm, which is generally traded. Excess sperm is digested (reabsorbed) via the copulatory bursa and represents an important energy source (Michiels and Bakovski 2000). Direct communication between the copulatory apparatus and the intestine is thought to aid this digestion and has currently been described within *Romankenkius* and *Spathula*. The character state “expansion” is included to accommodate the monotypical genus *Eviella*, which exhibits a large expansion of the bursal canal in the place of a bursa. Terrestrial triclads also lack a freshwater triclad style bursa, instead the common oviduct communicates directly with the female atrium. The presence of a bursa is uncommon in terrestrial species and the oviducts often simply communicate with a glandular duct arising from the common atrium, this structure will be treated as a bursal canal in this analysis.

**23. Dactylose projections on bursal canal: Absent (0), present (1)**



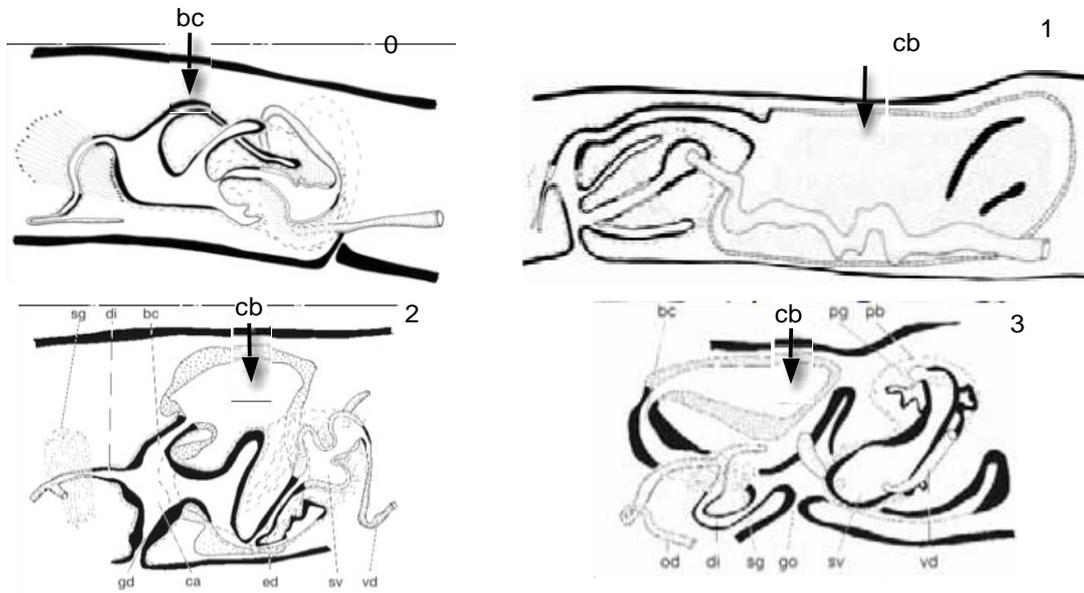
The presence of a ring of blind finger-like projections or papillae is confined to the monotypical genus *Reynoldsonia* (Ball 1974a).

**24. Musculature around bursa (cb): Absent (0), present (1)**

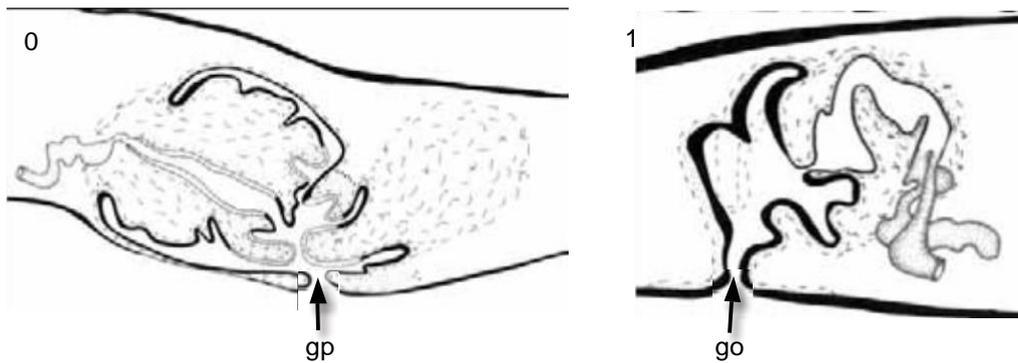


This character can only be scored for species with a bursa.

25. Bursa (cb): Absent (0), sitting anterior to penis bulb (1), sitting lateral to penis bulb (2), sitting posterior to penis bulb (3)

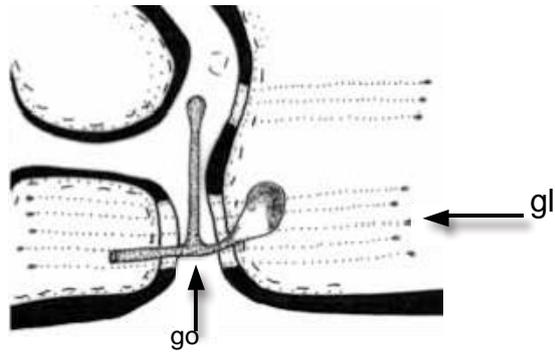


26. Gonoduct (go): Absent (0), present (1)



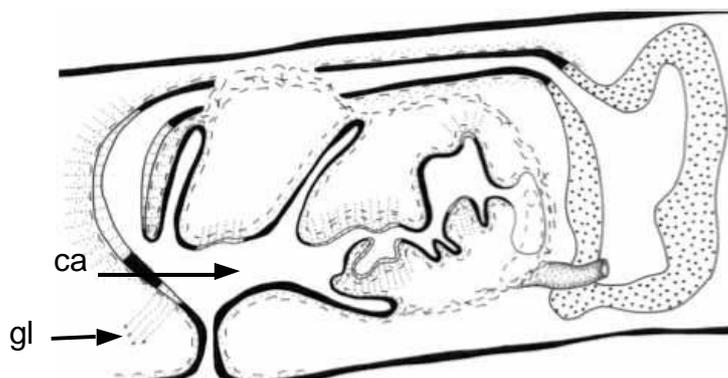
Only species where all individuals exhibit a definite gonoduct were scored as “present” for this character.

27. Glands (gl) entering directly into the gonoduct (go): Absent (0), present (1)



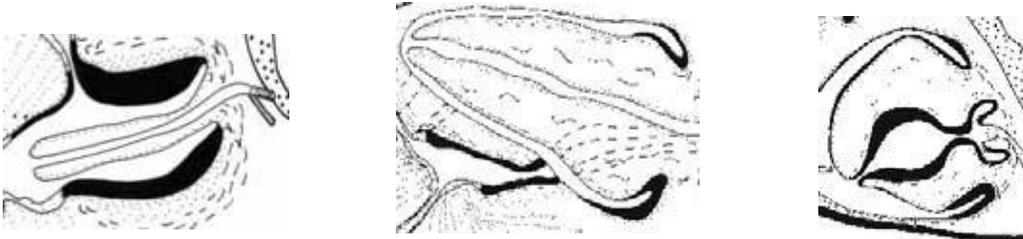
This character can only be scored for species with a gonoduct (character 26).

28. Glandularisation (gl) of common atrium (ca): Absent (0), present (1)



**Male Copulatory Apparatus**

**29. Penial papilla shape:** Finger-shaped (0), conical (1), rounded (2), plug (3), irregular/flexible (4), absent (5)



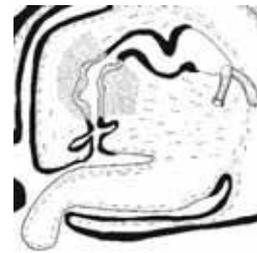
0

1

2



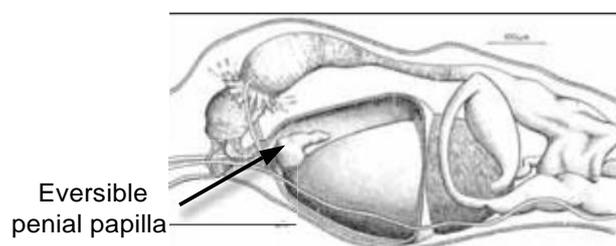
3



4

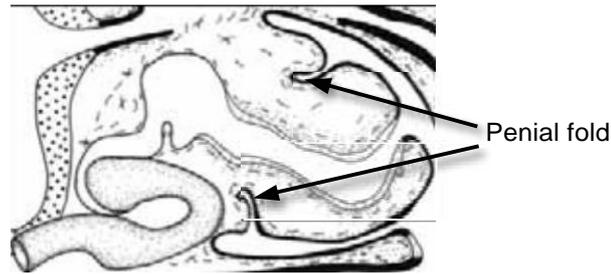
Those species that do not show consistent intra-specific penial papilla structure have been described as irregular/flexible (4). Despite the issues associated with the fixation of such a flexible structure, there are many examples of species that show consistency in the general shape (0-3). The most pronounced example of this is the “finger-shaped” penial papilla, found exclusively in *Cura pinguis*, the only representative of its genus found on the continent.

**30. Eversible penial papilla:** Absent (0), present (1)



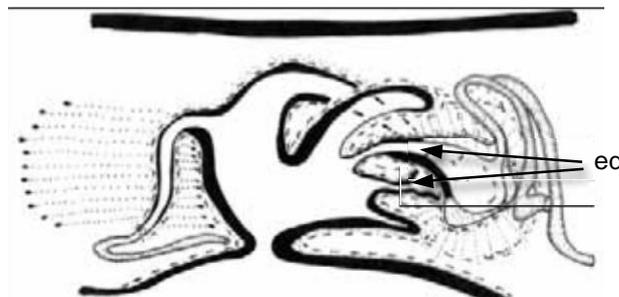
A rare state in dugesiid species is the ability to evert the penial papilla i.e. turn the tip of the papilla inside out. This state is recognisable, as the tip appears histologically distinct from the rest of the organ, lacking a defined epithelium and musculature (image adapted from Ball 1974a).

**31. Penial folds: Absent (0), present (1)**

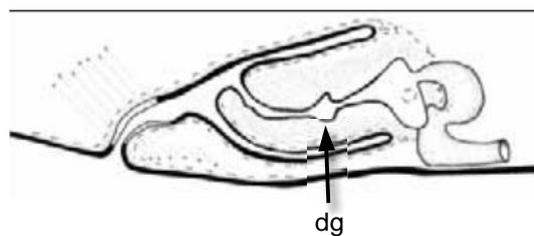


This state refers, not to small irregularities in the penial papilla margin, but to well developed folds of the entire structure (identified as shown above). This feature is very consistent within species, despite the flexibility of the organ and fixation issues.

**32. Separate ejaculatory ducts (ed): absent (0), present (1)**

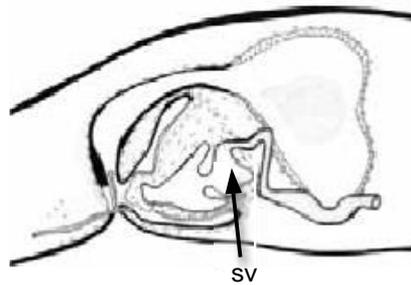


**33. Diaphragm (dg): Absent (0), present (1)**



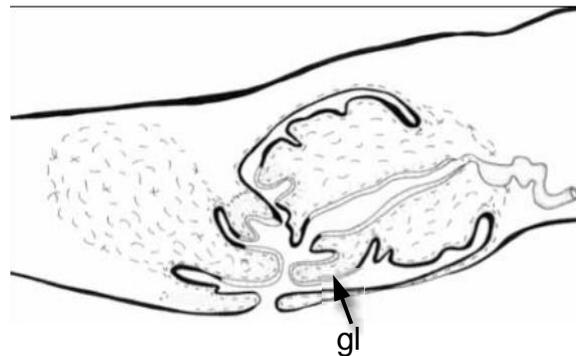
The diaphragm refers to a small chamber in the proximal section of the ejaculatory duct (Sluys et al. 1998). The diaphragm is a diagnostic feature of the genus *Dugesia*, which currently has three described Australian representatives, *Dugesia notogaea*, *Dugesia artesiana* and *Dugesia orientoaustralis*.

**34.** Pleated ejaculatory duct/seminal vesicle (sv): Absent (0), present (1), pleated (2), double (3)



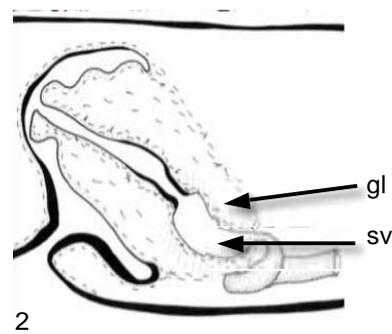
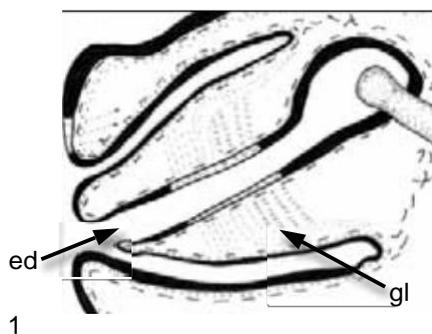
Many Australian species exhibit a fold or swelling of the proximal section of the ejaculatory duct. This feature can be a simple expansion of the ejaculatory duct or be pleated and appearing to have several chambers (Ball 1977, Sluys and Kawakatsu 2001).

**35.** Penis glands (gl) opening through the epithelium of the penial papilla: Absent (0), present (1)

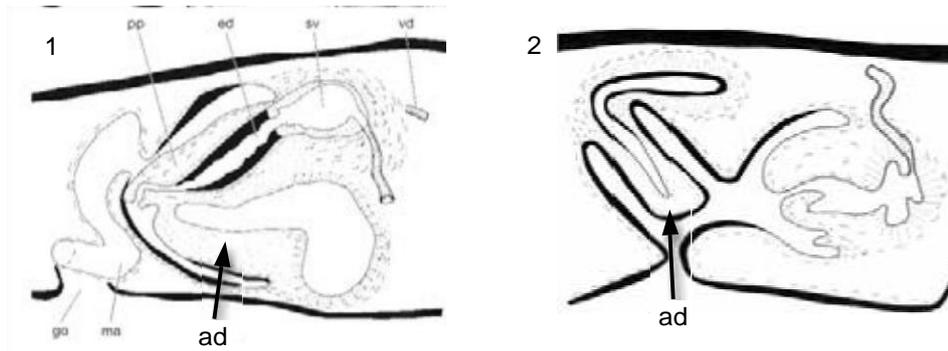


While penial glands entering into the ejaculatory duct are common, the above state, where the glands open to the exterior, is much less so (Leal-Zanchet and Hauser 1999, Sluys 2001).

**36.** Penial glands (gl): Absent (0), opening into ejaculatory duct (ed) (1), opening into seminal vesicle (sv) (2), opening into both the ejaculatory duct and seminal vesicle.

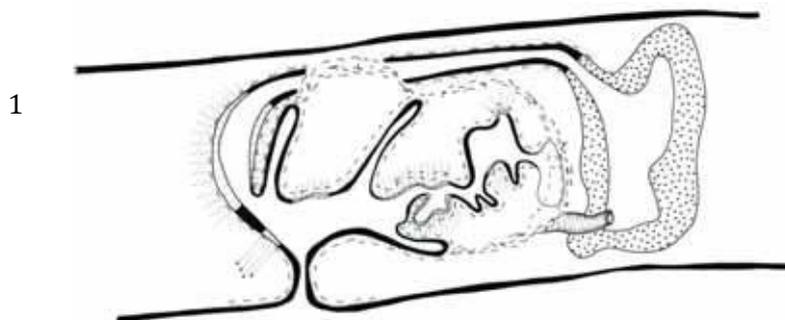


37. Adenodactyl (ad): absent (0), orientated posteriorly (1), orientated anteriorly (2), orientated ventrally (3), orientated laterally (4).



There are currently four species for which an adenodactyl with a large free papilla has been described. Three species exhibiting this structure are representatives of the genus *Romankenkius*, while there is one example from amongst the *Spathula*, however the orientation of the papilla does vary. An adenodactyl with the same orientation as the penial papilla is considered to be orientated posteriorly while anything opposing the papilla is considered to be orientated anteriorly.

38. Pleated ejaculatory duct: absent (0), present(1)



In some dugesiids the ejaculatory duct is not the usual straight tube with a smooth wall but consists of a duct with a pleated wall.

**39.** Pigmented pharynx: absent (0), present (1)



**40.** Posterior sucker: absent (0), present (1)



In some representatives of the Planarioidea, a sucker (or adhesive disk) is present at the posterior end of the ventral side.

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APPENDIX 3a  
DETAILS OF CHARACTERS FOR MORPHOLOGICAL PHYLOGENY

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## APPENDIX 3b: Detailed Character Analysis

### *Eviella* and *Reynoldsonia*

*Reynoldsonia* and *Eviella* are defined by an apomorphy, but this is a result of their monophyletic status. The revised phylogeny (Chapter 2) places both of these genera amongst the *Spathula*. While both species will remain in their current genera at this point, both assignments need review (particularly *Reynoldsonia reynoldsoni*).

**Diagnosis:** *Eviella* is a dugesiid identifiable by non-reversed muscular that extends of over a bursal canal that expands in place of a copulatory bursa.

**Diagnosis:** *Reynoldsonia* is a dugesiid identifiable by a large and muscular penis with an eversible tip. Bursal stalk very muscular with ectal papillae.

### *Schmidtea*

*Schmidtea* exhibits a double seminal vesicle (character 34, state 3: Table 1). Seminal vesicles are extremely common throughout the DugesIIDae, and in fact in the proposed ancestral Maricolans (Sluys 1989a, Sluys and Kawakatsu 2001), however, the clear double seminal vesicle, present in all described species of *Schmidtea*, represents a clear apomorphy (Figure 1).

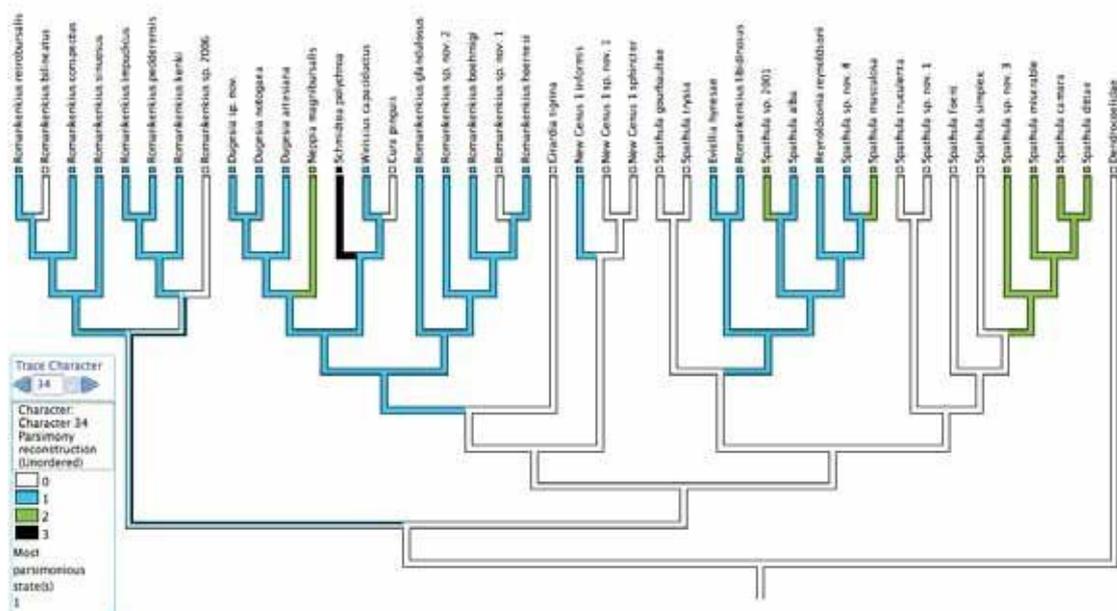


Figure 1 Seminal vesicle character trace.

**Diagnosis:** *Schmidtea* may be described as a Dugesiidae exhibiting a double seminal vesicle.

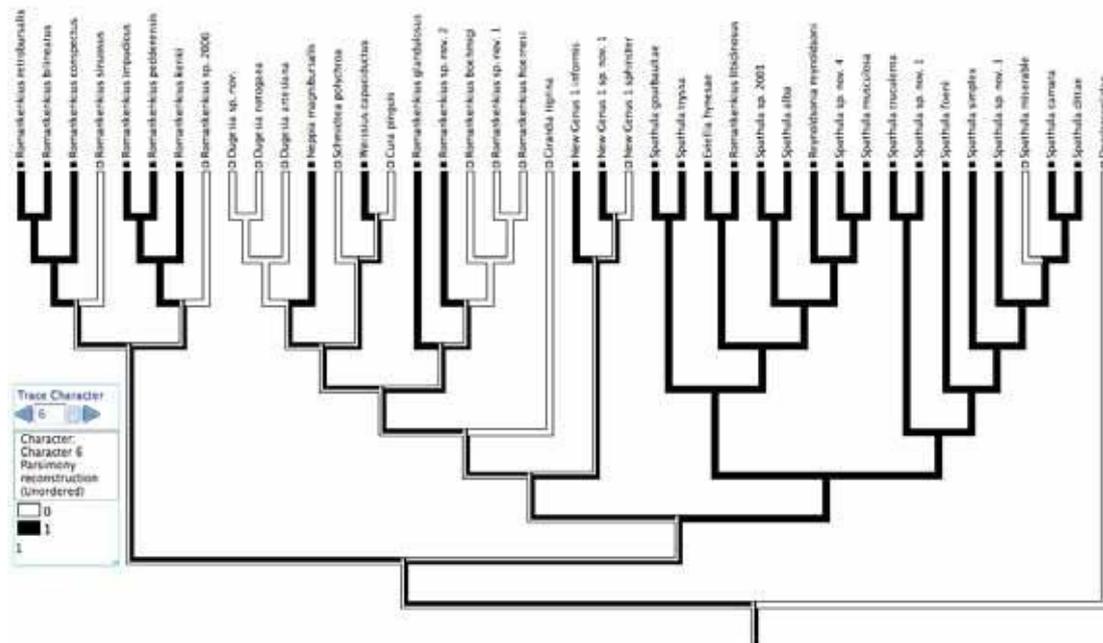
Prior to this analysis, intermingled musculature on the bursal canal (character 18, state 2) was also postulated as an apomorphy for *Schmidtea* (Sluys 2001). The most common character state for musculature on the bursal canal in the freshwater Geoplanoidea is reversed; the ancestral state found in Maricolans is non-reversed, which is also found in *Girardia* and the eccentric species *Eviella hynesae*, *Romankenkius libidinosus* and *Masaharus sphincter* (a more detailed analysis of this character will follow). The suggestion that intermingled musculature is an apomorphy for *Schmidtea* is now obsolete due to the description of a new genus, *Masaharus*, which houses two species with intermingled musculature, yet lacking the double seminal vesicle characteristic of the *Schmidtea* (Figure 1).

### ***Dugesia***

The *Dugesia* group also exhibits a clear apomorphy, a diaphragm in the ejaculatory duct (character 33, state 1: Table 1). There are exceptions to this rule, being the diaphragms found in several *Romankenkius* species, specifically, *R. libidinosus*, *R. boehmigi* and *R. patagonicus*. However, it is considered unlikely that the diaphragm of the *Romankenkius* species is homologous with that found in *Dugesia* (Sluys 1997). In species of *Dugesia* the diaphragm receives the openings of glands, in *Romankenkius* these glands do not open into the diaphragm but into the most proximal section of the ejaculatory duct. Sluys and Rohde (1991) therefore argue that the diaphragms found in *Romankenkius* and *Dugesia* species do not satisfy the principle of connectivity, one of the most important criteria for homology assessment. An extensive discussion of this character can be found in Sluys et al.'s (1998) analysis of the genus *Dugesia* in Australia. Sluys' (1989a) monograph of the marine triclads does not describe any species with a diaphragm in the ejaculatory duct; we can therefore assume that this feature is a uniquely derived character for the *Dugesia*.

Table 1 demonstrates that the Australian *Dugesia* also share several other characters. This group appears to lack external sensory structures with no ciliated pits or sensory fossae (character 6, state 0 & character 7, state 0), a theme that continues throughout the *Dugesia* group worldwide (Sluys 2001). While the lack of ciliated pits is not uncommon in other groups, in those cases, their absence is more likely to be a derived state for a given group or individual (Figure 2). While sensory pits are not present in the Maricola, the character trace indicates that the ancestral group possessed sensory pits.

Homologous sensory structures are also found in the Terricola (Meixner 1928, Von Graff 1912-1917). Sensory fossae are not so common amongst freshwater Geoplanoidea, but do appear in most genera. This character is not overly useful due to its sporadic appearance across the tree. It is unclear whether the presence of sensory fossae is an ancestral or derived state and the fact that they are often difficult to identify in preserved specimens complicates the matter. As a consequence this character will not be considered in further discussions.



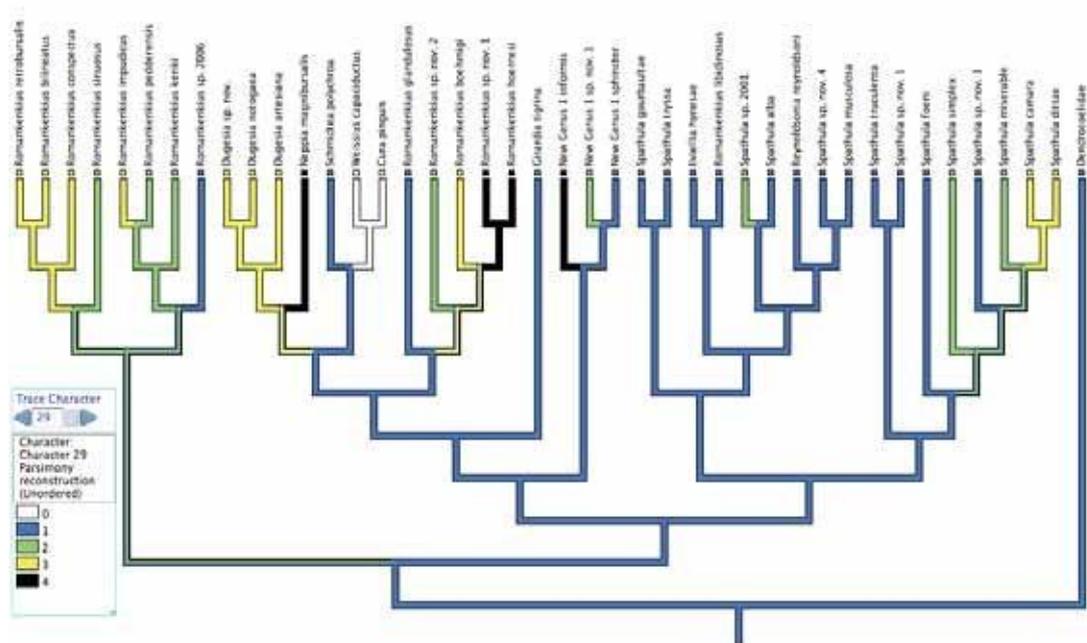
**Figure 2** Sensory pits character trace.

The Australian *Dugesia* share the dorsal testes state (character 9, state1) with the remainder of the international *Dugesia* species (Sluys 2001). *Dugesia* is quite unique in that most genera exhibit variation in the position of the testes, whilst dorsal is the only character state found in *Dugesia*. *Dugesia's* proposed closest relative *Neppia* shows considerable variation and it is not at all clear, from research or from the character trace, what the ancestral state may be. The Maricola and Planariidae exhibit only ventrally located testes while the Dendrocoelidae and Geoplanidae show similar variability to the freshwater Geoplanoidea (Sluys 1989a, Sluys 2001, Sluys and Kawakatsu 2006). This character appears to be quick to adapt after a speciation event. An idea supported by the fact that testes position also shows a large degree of phenotypic plasticity, more so than any other trait currently used in dugesiid analysis (Sluys 2001).

While a clear gonoduct (character 26, state1; Table 1) is evident in all Australian *Dugesia*, this character is not found consistently across the genus as a whole. This character is a highly polymorphic one, with various conditions shown within species. It is

also true that the description of a gonoduct can often depend upon the condition of the preserved specimen. While this character state may suggest a relatively recent divergence of these three species, due to the inconsistencies between descriptions, the presence of a gonoduct must be viewed with some scepticism.

The description of a new *Dugesia* species in Australia has revealed an interesting development. *Dugesia orientoaustralis* exhibits a short common oviduct (character 15, state 1). There will be a detailed analysis of the character states relevant to the entrance of the oviducts into the bursal canal to follow in the discussion surrounding *Romankenkius* and *Spathula*. For the purposes of this *Dugesia* discussion it is relevant to say that: (1) this feature is a common oviduct and not a diverticulum (2) has not been described in *Dugesia* previously, and (3) common oviducts do appear in several other genera (Figure 7). It is therefore proposed that this feature has arisen independently in this species and does not represent the ancestral state. This conclusion is supported by the presence of shell glands entering the bursal canal, a character state consistent with all other *Dugesia*'s and not a feature of species with true diverticula (character 16, state 2; Table 1 and Table 2).



**Figure 3** Penial papilla shape character trace.

Finally, while a plug-shaped penial papilla is a common feature amongst *Dugesia*'s worldwide, it is by no means the only character state (character 29, state 3). Penial papilla shape is another highly variable character within genera (Figure 3) and therefore the fact that all Australian *Dugesia*'s share a common shape again suggests a relatively recent divergence from a common ancestor.

The interesting feature of the *Dugesia* is presence of a set of very consistent characters throughout the over 70 species currently described. This exemplified by the fact that Sluys (2001) was able to keep it as a single operational unit in his analysis of the freshwater Geoplanoidea. This would not be a unique feature in many other taxa, however, amongst the notoriously difficult to define freshwater Geoplanoidea, the *Dugesia* represent something of an anomaly.

**Diagnosis:** *Dugesia* may be identified as a Dugesiidae with a diaphragm that receives the opening of glands in the ejaculatory duct.

### ***Neppia***

The two characters proposed as representing defining characters for *Neppia*, convoluted ejaculatory duct and thick circular muscle on the bursal canal, were not included in my analysis. They were excluded as both are extremely subjective and, in general, do not occur in Australian species. A discussion surrounding the apomorphies for this genus can be found in the chapter introduction with the pertinent point being that *Neppia* is in dire need of review. The only Australian representative of the genus is *Neppia magnibursalis*, which was assigned to this genus via a process of elimination (Sluys and Kawakatsu 2001). In doing so one of the proposed apomorphies had to be ignored, that of the convoluted ejaculatory duct. This is justified by the suggestion that many other species assigned to *Neppia* do not exhibit this character, meaning either that *Neppia* needs new defining characters or that several representatives are incorrectly assigned (Sluys and Kawakatsu 2001). *Neppia magnibursalis* does have a reasonably thick layer of circular muscle surrounding the bursal canal and if this character could be defined with more rigour it is a possible apomorphy for the group (Sluys 2001). It appears that many species have been placed in this genus due to the vague defining characters allowing easy allocation for difficult yet unexceptional species. For the purposes of this study it is important to note that a *Neppia-like* species does exist in Australia, however, until *Neppia* has a more satisfying definition it would be unwise to infer too much from this.

**Possible Diagnosis:** *Neppia* may be identified as a Dugesiidae with an exceptionally thick layer of circular muscle surrounding the bursal canal.

APPENDIX 3b  
DETAILED CHARACTER ANALYSIS

**Table 1** Characters shared by all members of each genus, characters highlighted in blue represent proposed apomorphies, from current analysis and Sluys (2001).

Genus	Informative Characters	Uninformative Characters
<i>Dugesia</i>	6 (0) - ciliated pits absent 7 (0) - sensory fossae absent 9 (1) - dorsal testes 16 (2) - shell glands entering the bursal canal 26 (1) - gonoduct present 29 (3) - plug shaped penial papilla 33 (1) - diaphragm	3, 10, 13, 18, 22, 25, 28, 31, 34, 38
<i>Neppia</i>	From Sluys (2001): • thick circular muscle on the bursal canal present • convoluted ejaculatory duct present	
<i>Schmidtea</i>	From Sluys (2001): 34 (3) - double seminal vesicle present 18 (2) - intermingled musculature on bursal canal	
<i>Weissius</i>	From Sluys <i>et al.</i> (2007): 18 (3) - circular muscle around bursal canal 29 (0) - finger shaped penial papilla 21 (0) - posterior point of communication between the atrium and the bursal canal 15 (0) - common oviduct/diverticulum absent	
<i>Cura</i>	From Sluys (2001), Sluys and Kawakatsu (2001) and Kawakatsu and Mitchell (1982): 6 (0) - ciliated pits absent 15 (0) - common oviduct/diverticulum absent 18 (3) - circular muscle around bursal canal 29 (0) - finger or thumb shaped penial papilla 21 (1 & 2) - point of communication of the atrium and the bursal canal anterior or middle	
<i>Romankenkius (Western)</i>	4 (1) - solid dorsal pigmentation 5 (1) - less than dorsal 15 (2) - common oviduct present opening into the most postero-ventral section of the bursal canal or even into the common atrium 16 (1) - shell glands entering the diverticulum (all except <i>R. glandulosus</i> ) 24 (0) - musculature around the bursa absent • common oviduct lacking surrounding musculature	3, 13, 17, 18, 22, 27, 33
<i>Girardia</i>	From Sluys (2001) and Sluys <i>et al.</i> (2005): 2 (2) - long auricles 6 (0) - ciliated pits absent 18 (0 & 2) - non-reversed and intermingled musculature on bursal canal 39 (1) - pigmented pharynx present (except several species including three polymorphic for this character)	
<i>Masaharus</i>	2 (2) - long auricles 7 (0) - sensory fossae absent 10 (0) - testes situated throughout the body length 16 (2) - shell glands entering the bursal canal 18 (0 & 2) - non-reversed and intermingled musculature on bursal canal 24 (1) - muscle surrounding bursa (excluding <i>Masaharus sphincter</i> ).	15, 17, 19, 22, 25, 26, 27, 28, 31, 33, 36, 37
<i>Eviella</i>	From Ball (1977b): 25 (0) - Bursal canal connected to expansion 11 (1) - Fused testes	
<i>Reynoldsonia</i>	From Ball (1974a): 23 (1) - Dactylose projections on bursal canal 30 (1) - Eversible penial papilla	
<i>Spathula</i>	6 (1) - ciliated pits present (except <i>Sp. miserabile</i> ) 13 (1) - caudally branched oviducts present 16 (2) - shell glands entering the bursal canal (excluding <i>R. libidinosus</i> ) 21 (0) - bursal canal communication with atrium posteriorly. 25 (1) - bursa sitting anterior to penis bulb	17, 22, 28, 31, 33, 35
<i>Romankenkius (Eastern)</i>	4 (1) - solid dorsal pigment 15 (2) - diverticulum present opening into the most postero-ventral section of the bursal canal or even into the common atrium 16 (1) - shell glands entering the diverticulum (all except the underdeveloped <i>R. sp</i> (2006)) 18 (1) - reversed musculature on the bursal canal • musculature surrounding the diverticulum	3, 17, 27, 28, 31, 33
Dendrocoleidae		

### ***Cura/Weissius***

*Weissius* is currently represented by a single species, *Weissius capaciductus*. This is an interesting species, as it possesses several characters that are reminiscent of *Cura*, specifically *Cura pinguis*, the only Australian *Cura* species. The presence of a finger-shaped penial papilla (character 29, state 0; Table 1), the circular muscle around the bursal canal (character 18, state 3) and the broad zones of circular and longitudinal muscle surrounding the atrium (Sluys et al. 2007), create a superficial resemblance to *Cura pinguis*. The anterior point of communication of the bursal canal and the common atrium (character 21, state 0) and the absence of the short common oviduct (character 15, state 0) are both features that are proposed to distinguish this species from *Cura* (Sluys et al. 2007). The more conventional communication of the bursal canal with the atrium certainly separates this species from *Cura pinguis*, however, some doubt remains whether this is a strong enough character to preclude the assignment of this species to the genus *Cura* (e.g. the position of this communication varies within the *Cura*). The most “reliable” (i.e. not positional) character Sluys et al. (2007) suggest as eliminating *Cura* as a potential genus for the new species is the absence of a short common oviduct that is described as a characteristic feature of this genus. The difficulty with this lies in the fact that this is not a consistent character across the genus. Whilst *C. foremanii* is always described with a clear short common oviduct (Kawakatsu and Mitchell 1982), the character appears to be polymorphic in *C. pinguis*, which is often described as having separate entries of the oviducts into the bursal canal (Sluys and Kawakatsu 2001, Grant et al. 2006)(Appendix 1d). It is for this reason that I question the diagnosis of this species and suggest that it should sit within *Cura*. It should be noted that *Weissius* also closely resembles *Schmidtea*, however, *W. capaciductus* lacks the double seminal vesicle (character 34, state 1).

A clear set of characters for the genus *Cura* has only recently been established (Sluys and Kawakatsu 2001). With the description of *Cura fortis* Sluys (Sluys and Kawakatsu 2001) sighted the finger or thumb shaped penial papilla (character 29, state 0) and the opening of the bursal canal into the mid-dorsal or even anterio-dorsal section of the common atrium as describing a *Cura* (character 21, state 1 & 2). The reliability of using positional characters is discussed above and I believe that, in the light of new information (i.e. *Weissius capaciductus*) the defining characters of *Cura* need to be reviewed. I suggest the use of bursal canal musculature (character 18) and penial papilla shape (character 29) should be used to diagnose a *Cura*. All *Cura*'s, except *Cura fortis*, have a unique (amongst Dugesiiids) condition of circular musculature surrounding the bursal canal (character 18, state 3). This character is also present in *Weissius capaciductus*. Additionally, Sluys and Kawakatsu (2001) describes *Cura fortis* as having a “thumb-shaped” penial papilla,



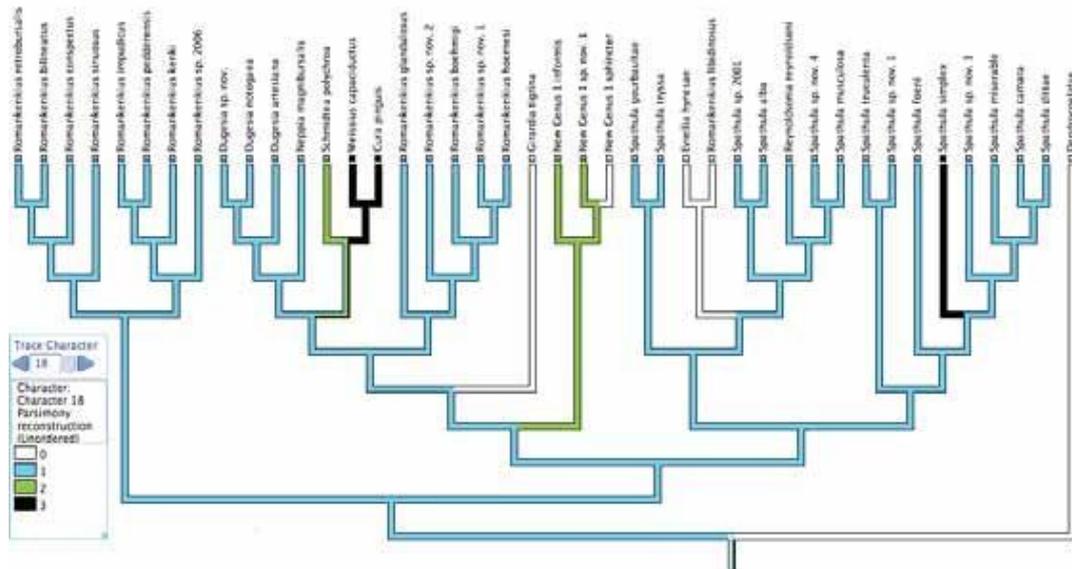
**Diagnosis:** *Cura* is defined as a Dugesiidae with only circular muscle on the bursal canal and a finger-shaped penial papilla.

### ***Girardia/Masaharus***

The status of *Girardia* is unclear despite the abundance of two relatively unique character states within the group. A pigmented pharynx (character 39, state 1) is often used to unequivocally place a species in this genus (Sluys 2001). However, this character is not present in several species that have been assigned to this genus (eg. *Girardia canali* (Sluys 2001)) and is polymorphic in others (eg. *Girardia tigrina* (Grant et al. 2006)). It should be noted that many species are poorly described and the state of their pharynx unknown. Until recently the musculature on the bursal canal had provided a useful tool in identifying a *Girardia* (character 18, state 0). Sluys (2001) described all *Girardia*'s as having non-reversed musculature, a relatively unique character amongst the freshwater Geoplanoidea, this character state otherwise appearing only sporadically amongst other genera, interestingly all other examples are within the more difficult to diagnose specimens (eg. *Romankenkius libidinosus*, Figure 5). This distinction has changed in recent times, however, as several species have been re-described and many others assigned to this genus, effectively eroding the presumed apomorphy. Several species have now been described with intermingled musculature and even with reversed musculature on the bursal canal (eg. Sluys et al. 2005). Pointed auricles were also suggested (character 2, state 2), yet for similar reasons to those described above, this feature is now inconclusive (Sluys 2001, Sluys et al. 2005). This group is consistent in a few features, for example the absence of ciliated pits (character 6, state 0) and the absence of caudally branching oviducts (character 13, state 0), yet these are features found throughout the freshwater Geoplanoidea. While they may serve to exclude a species from assignment to this genus, they are not apomorphic for the group and so cannot be used to place a species within *Girardia*. Currently there is not one single character that we could hypothesise as diagnostic for all of the currently known *Girardia*'s. This group is invasive in Australia and therefore a resolution to this issue, does not form part of my remit. For this reason the diagnosis for this species will remain unresolved.

**Diagnosis:** A *Girardia* is a Dugesiidae with or without a pigmented pharynx, possibly with non-reversed musculature on the bursal canal, possibly with pointed auricles and an angled bursal canal.

APPENDIX 3b  
DETAILED CHARACTER ANALYSIS



**Figure 5** Character trace of the bursal canal musculature.

The genus *Girardia* was formerly presumed to exist in Australia (Ball 1974b, Grant et al. 2006, Sluys 1997, Sluys and Kawakatsu 2001), however, it has become increasingly evident that there was no evidence for the natural occurrence of *Girardia* within Australia (i.e. *Girardia tigrina* maintains its invasive status). Several species were removed from this proposed Australian *Girardia* group when the genus *Romankenkius* was erected (*R. glandulosus*, and *R. hoernesii* (Ball 1974b)). Despite this, there were still several native Australian species assigned to *Girardia* (*G. graffi*, *G. informis*, *G. sphincter*, *G. sp.*). The description of a new Australian genus, *Masaharus* (Appendix 1d), prompted a review of the so-called Australian *Girardia*'s. As discussed above *Girardia* has no unequivocal diagnostic characteristics, and, there is no indisputable evidence to suggest that any Australian species are representatives of *Girardia*.

There are certainly characteristics of *Masaharus* species that resemble *Girardia*. These similar characteristics include, pointed auricles (character 2, state 2) and the musculature on the bursal canal being intermingled or non-reversed (character 18, state 0 & 2). The disparate features of the Australian species include: the lack of a pigmented pharynx (character 39, state 0), the presence of ciliated pits, a feature absent in all *Girardia* (character 6, state 1), and the absence of the angled bursal canal. All Australian species assigned to *Girardia* have at least one of these characters. The assignment of these species has profound biogeographical implications and so it is important that they are not incorrectly allocated. Upon weighing up the evidence it was determined that until a more definite diagnosis of *Girardia* was available, the Australian species should not be assigned to it. While they possess several characteristics that would not preclude them from being assigned to this genus (i.e. bursal canal musculature), there is nothing specifically

suggesting this assignment either (i.e. pigmented pharynx, angled bursal canal, ciliated pits). One of the only features that is consistent across all *Girardia*'s is the absence of ciliated pits, a feature that appears in several of the Australian species previously assigned to it. One feels that they were placed in this genus, as they did not fit comfortably within any other genera. In the light of the description of *Masaharus extentus*, and with it the acquisition of molecular data (see Chapter 2), it was deemed necessary to erect a new genus to house these somewhat ambiguous species. This being said it is likely that this genus shares a close relationship with *Girardia*, and in fact the molecular tree supports this notion.

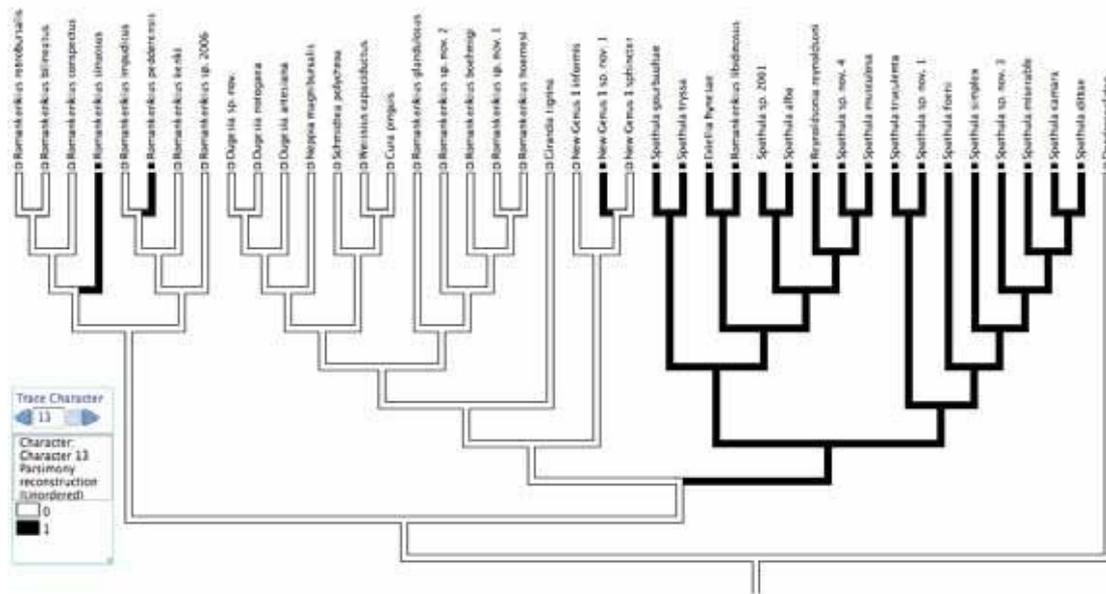
Unfortunately, this new genus, in its current form, does not possess an apomorphy that easily diagnoses its members. The common characters are as listed in the Table 1. It is true that this is a fairly weak set of characters to define a genus, however, musculature on the bursal canal precludes assignment to any other currently known genus (character 18, state 0 & 2, Figure 5). The issues with assigning these species to *Girardia* are outlined above and they lack the double seminal vesicle of *Schmidtea*. It should be noted that the inclusion of *Girardia* sphincter in this group precludes the description of several more shared characters. For example, intermingled musculature on the bursal canal (character 18, state 2) and musculature on the bursa itself (character 24, state 1), are all shared characters for *Masaharus extentus*, and *Masaharus informis*. This obviously throws into question the validity of placing *Masaharus sphincter* in this new genus. *Masaharus sphincter* was initially placed amongst the *Girardia* by Sluys and Kawakatsu (2001) due to the non-reversed musculature on the bursal canal, however, as stated above this musculature arrangement is not exclusive to *Girardia*, nor is it an essential character for assignment to this genus. *Masaharus sphincter* is also lacking the pigmented pharynx, angled bursal canal and the head shape is unknown. However, until more examples of this group are discovered, it would be ambitious to describe two new genera from this set of reasonably weak characters. For now, *Masaharus sphincter* must remain assigned to *Masaharus*, however, for the purposes of future research it is useful to examine the diagnoses for this new genus both with and without *Masaharus sphincter*.

#### **With *Masaharus sphincter***

**Diagnosis:** *Masaharus* may be differentiated from its congeners by the presence of long auricles, a lack of sensory fossae, testes situated throughout the body length and either intermingled or non-reversed musculature on the bursal canal.

**Without *Masaharus sphincter***

**Diagnosis:** *Masaharus* may be differentiated from its congeners by long auricles, sensory pits but a lack of sensory fossae, intermingled musculature on the bursal canal, musculature surrounding the bursa and testes situated throughout the entire body length.



**Figure 6** Character 13 caudally branched oviducts.

***Spathula/Eviella***

In Nurse's (1950) description of the genus *Spathula* she describes a dugesiidae whose "oviducts continue posteriorly beyond the genital region and a duct from each runs into the basal position of the bursal stalk". This currently holds true, however, this character is no longer an apomorphy with several species being described with branching oviducts that have been assigned to other genera (Figure 6). The relevance of the caudally branching oviducts is discussed by Sluys and Rohde (1991) in relation to *Romankenkius* and the *Spathula* group, where they propose that the lack of a caudal branch is the primitive state. The molecular data suggests a close relationship between Eastern *Romankenkius* and *Spathula* (See Chapter 2). Eastern *Romankenkius* (for the most part lacking caudally branching oviducts) is the more primitive of the genera and so Sluys and Rohdes' (1991) hypothesis maintains credibility.

In the case of *R. sinuosis* and *R. pedderensis* it is assumed that the branches observed in these species are either individually derived, represent an intermediary stage between the genera or that they have been incorrectly assigned (a distinct possibility for *R. sinuosis* as this species displays several other unique characteristics - see *Romankenkius*

discussion). In the case of *Masaharus extentus* the short branching oviducts did initially confuse this species diagnosis as the authors instinct was to place this species amongst the *Spathula*. Upon detailed inspection, however, it became obvious that this species possessed too many aberrant features to be placed assigned to *Spathula* (as well as some convincing molecular evidence) and the parallels with *Masaharus informis* became blaring (see above discussion). While it is not possible to present a single defining apomorphy for *Spathula*, once bursal canal musculature, and the diverticulum (see *Romankenkius* discussion) are considered, it is possible to present a reasonably useful diagnosis for this genus (Sluys 2001)(Figure 5 and Figure 7).

**Diagnosis:** *Spathula* is a dugesiidae with caudally branching oviducts but lacking a true diverticulum, most likely possessing reversed musculature on the bursal canal.

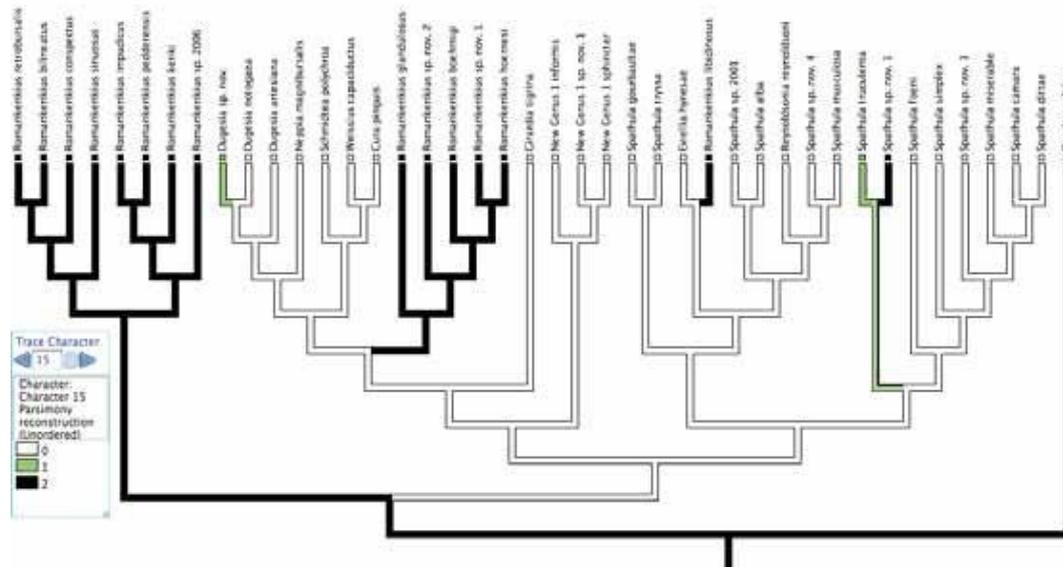
Naturally, there are exceptions to this rule. *Spathula simplex*, possesses only a layer of circular muscle on its bursal canal (Figure 5), it should be noted that, this species was described from a single specimen and future research may shed more light on this species (Grant et al. 2006). When *Reynoldsonia reynoldsoni* was described, Ball (1974a) deemed it necessary to erect a new genus in order to house this unique species. While this is an understandable impulse considering the dactylose projections on the bursal canal and the eversible penial papilla, the remaining characters fit clearly into our current diagnostic framework for *Spathula* (please note that *R. reynoldsoni* has been described with both non-reversed and intermingled musculature of the bursal canal)(Ball 1974a, Hay and Ball 1979). I therefore concur with Sluys' (2001) musing that this species is in fact an aberrant *Spathula*.

While I had intended to also place the monotypical genus *Eviella* amongst the *Spathula*, detailed character analysis reveals that this approach is too simplistic and that this genus is indeed a unique entity. The type specimen for *Eviella* has: (1) non-reversed musculature (character 18, state 0), (2) fused testes (character 11, state 1), (3) asymmetrical entrance of the testes into the bursal canal (character 17, state 1), (4) the expansion of the bursal canal (character 22, state 3), (5) absence of a bursa (character 25, state 0) and (6) the glands entering directly into the gonoduct (character 27, state 1). And while this genus does share the caudally branched oviducts with *Spathula*, along with several other characters, it does not fit comfortably within this genus. These same unique set of characters also preclude it from being assigned to any other genus, *Eviella* must therefore remain a monotypical genus (see initial diagnosis).

The question remaining to be answered is, if *Eviella* is no *Spathula* where does it sit on our tree? In Balls (1977b) original description of the genus, he comments that *Eviella* is “undoubtedly” an old and primitive form representing a direct descendant of marine ancestors. This is evidenced by, amongst other features, the loss of the primary bursa, common in marine planarians (e.g. *Procerodes variabilis*) and the non-reversed musculature of the bursal canal, also common feature of marine triclads (Ax 1956). He also comments on the genus’ similarity to *Rhodax* (Cavernicola) and *Opisthobursa* (Cavernicola) and that the presence of ciliated pits and fossae give it clear ties to Australiasian freshwater Geoplanoidea. I agree with Ball’s (1977b) conclusion, and due to the similarity with *Spathula* believe this genus should be located outside, but at the base of the *Spathula* group.

The final anomaly in the *Spathula* clade is the presence of *R. libidinosus* within it. This species has always been a little difficult to place owing to the combination of caudally branched oviducts (character 13, state 1) and a “diverticulum” (Character 15, state 2). However, the acquisition of molecular data strongly suggests that *R. libidinosus* sits firmly within the *Spathula*, and when its characters are analysed, it is easy to reconcile oneself to this positioning.

In the discussion on *Romankenkius* (below) the nature of the diverticulum is examined. It is established that the diverticulum of *R. libidinosus* differs from that of “true” *Romankenkius*. In their original description of *R. libidinosus* Sluys and Rhode (1991) describe the diverticulum as being “devoid of any surrounding muscle” and therefore could reasonably be described as a short common oviduct. Short common oviducts are not unknown amongst the *Spathula* with *Spathula truculenta* and *Spathula oblongata* possessing a clear, unmusclcd common oviduct (Figure 7)(Ball 1977a). The non-reversed musculature (character 18, state 0) of the bursal canal would also be considered very unique if this species were placed amongst the *Romankenkius*, yet this feature invites parallels with *Eviella* (Figure 5). Finally, the presence of caudally branching oviducts supports its inclusion in the *Spathula* group (Figure 6). This being said, using the same rational as that described above for *Eviella*, it is clear that this species has a set of unique characters and that these are more than likely due to a close relationship to a common ancestor to both *Romankenkius* and *Spathula*. However, it has not been deemed necessary to construct a new genus to house this species. In the short term this species will remain a *Romankenkius*, however, I am confident that further molecular evidence will support a reassignment to *Spathula*.



**Figure 7** Character 15 - Diverticulum/common oviduct

### ***Romankenkius***

The currently accepted diagnosis for *Romankenkius* includes: the presence of a seminal vesicle (character 34, state 1), reversed musculature of the bursal canal (character 18, state 1) and a diverticulum of the bursal canal (character 15, state 1). The seminal vesicle and the reversed musculature of the bursal canal are not useful diagnostic characters as they are found throughout the freshwater Geoplanoidea (Figure 1 and Figure 5). There is confusion surrounding the use of the diverticulum for diagnosis. As we have already noted, several non-*Romankenkius* species possess a “diverticulum” or “common oviduct” (see *Spathula* discussion). It would be convenient if *Romankenkius* could be recognised via the presence of a diverticulum and the exclusion of a caudal branch of the oviducts. However, several *Romankenkius* species possess a caudal branch of the oviducts: a defining feature of *Spathula* (Figure 6).

A short caudal branch of the oviducts has been described for three species outside of *Spathula*, *Masaharus extentus*, *Romankenkius sinuosis* and *Romankenkius pedderensis*. The presence of this character in *Masaharus extentus* is discussed above (see *Spathula* discussion) and it is assumed that the presence of the caudal branch represents an ancestral relic or an autapomorphy for this species. *Romankenkius pedderensis* was one of the first *Romankenkius* described and its positioning in this group appears justified (see discussion on diverticulum below). This species is described as “extending a very short ventral branch (of the oviducts) towards the posterior vitellaria (a group of glands)” (Ball 1974b). While many planarians possess oviducts that communicate with vitellaria, the unique nature of this arrangement suggests that the caudal branch described for *R. pedderensis* may not be homologous with that found in *Spathula* species.

*Romankenius sinuousus* was placed in this genus by Sluys and Kawakatsu (2001) based on the presence of a “diverticulum”. However, they commented at the time that this was a unique species possessing many characters that separated it from its congeners. For example, the mouth is positioned half way between the hind end and root of the pharynx (character 8, state 3) and the presence of ectal reinforcement of the bursal canal (character 19, state 1), none of these characters being diagnostic. Of course the presence of a long caudal branch of the oviducts is a diagnostic character and one that would suggest a relationship with *Spathula*. We must therefore look in detail at the diverticulum and see if this species has a “true” diverticulum.

The definition of such important diagnostic characters has been a topic of discussion for many years (e.g. Ball 1974b, Meixner 1928, Sluys 1997). The presence of common oviducts (anatomically very similar structures) within the freshwater Geoplanoidea has created much confusion. For example, when Weiss (1909) initially described *Romankenius boehmigi* (formerly *Planaria boehmigi*), she describes a common oviduct, which receives the opening of shell glands. When Sluys (1997) reassigns this species to *Romankenius*, he also uses the term common oviduct to justify placing this species into a genus for which a “diverticulum” is a pre-requisite. The width of the lining of the structure and the width of the canal itself had been proposed as indicators of the structure’s origin, however, these characters were proven to be unreliable as more species were discovered (Ball 1974b). Sluys (1997) currently differentiates a diverticulum from a common oviduct using its point of origin, musculature and the location of the opening of the shell glands. The use of “point of origin” as a criterion is extremely difficult to justify as there appears to be substantial intraspecific variation for this feature and consequently not a great deal of consensus within genera (Table 2) (Sluys 1997).

The musculature surrounding the duct/diverticulum does appear to provide a great deal of information. Table 2 provides a summary of the musculature and position of the shell glands for all Australian dugesiids possessing a duct/diverticulum. The shell glands, whilst commonly described as entering what has previously been described as a diverticulum, do not always do so (e.g. *R. boehmigi*) and therefore again make a weak diagnostic character. The musculature surrounding the diverticulum is used as evidence as to the true origins of this, and any other diverticula found in Platyhelminth anatomy (Winsor et al. 1998). The logic being that the musculature simply represents the continuation of the bursal canal musculature, a premise that has a great deal of merit.

When the details of the musculature are examined a clear pattern emerges. All “Eastern” *Romankenius* possess a muscled diverticulum, excluding *Romankenius sinuousus* and *Romankenius sp.* I believe *Romankenius sp.* can be ignored to a certain

extent as its description was extremely tentative and many features were not present. The lack of musculature on *Romankenkius sinuousus*, on the other hand, warrants further discussion. It is clear from Table 2 that there are two different types of duct. These ducts can now easily be divided into muscled diverticulum or non-muscled common oviducts.

*Romankenkius sinuousus* was described by Sluys and Kawakatsu (2001) as lacking a muscular coat of the diverticulum. Based on our recent supposition, this structure may therefore be described as a common oviduct. While “common oviducts” are also found in the “Western” *Romankenkius*, the presence of a long caudal branch of the oviducts advocates for the movement of this species to the genus *Spathula*. Placing it comfortably amongst several other *Spathula* species possessing common oviducts (eg. *Spathula libidinosus* and *Spathula truculenta*) (Figure 6).

**Table 2** Details the diverticulum/common oviducts of Australian dugesiids.

<b>Species</b>	<b>Muscled</b>	<b>Glands Entering</b>	<b>Point of Origin</b>
<i>R. bilineatus</i>	+	Duct	Ventral (Bursal Canal)
<i>R. kenki</i>	+	Duct	Ventral (Bursal Canal)
<i>R. pedderensis</i>	+	Duct	Ventral (Bursal Canal)
<i>R. conspectus</i>	+	Duct	Common Atrium
<i>R. impudicus</i>	+	Duct	Ventral (Bursal Canal)
<i>R. retrobursalis</i>	+	Duct	Ventral (Bursal Canal)
<i>R. sp.</i>	-	?	Common Atrium
<i>R. sinuousus</i>	-	Duct	Ventral (Bursal Canal)
<i>R. boehmigi</i>	-	Common Atrium	Common Atrium
<i>R. glandulosus</i>	-	Bursal Canal	Common Atrium
<i>R. hoernesi</i>	-	Duct	Common Atrium
<i>R. musculoglandulosus</i>	-	Duct	Ventral (Bursal Canal)
<i>R. rutrum</i>	-	Duct	Ventral (Bursal Canal)
<i>R. libidinosus</i>	-	Duct	Ventral (Bursal Canal)
<i>S. truculenta</i>	-	Bursal Canal	Ventral (Bursal Canal)
<i>S. oblongata</i>	-	Common Atrium	Common Atrium
<i>D. orientoaustralis</i>	-	Bursal Canal	Dorsal (Bursal Canal)

While the problem of caudally branching oviducts amongst the *Romankenkius* has been tackled (above), it does not help when attempting to determine diagnostic characters. This process has, however, introduced a possibly invaluable diagnostic character, the diverticulum (with muscle) or the common oviduct (lacking muscle). The molecular data demonstrates a clear division between the eastern and western *Romankenkius* species (See Chapter 2). This character may provide a method of diagnosing the two different groups. Thus far there has been little said as to the relative evolutionary relevance of the two structures, however, as the common oviducts are found in the presumed more advanced groups (Figure 6), one may assume that this character is the more derived of

the two.

In the Maricola the oviducts generally open separately or combined into the bursal canal, providing a possible origin for the common oviduct (Sluys 1989a). A structure extremely reminiscent of the Eastern *Romankenkius* muscled diverticulum can be found in the Maricolan genus *Procerodes* Girard, 1850 (Ball 1977c, De Vries and Sluys 1991). While more research would be necessary to determine whether either of these structures are homologous, it provides a possible independent origin for the structures, ergo the species. The most parsimonious theory is that the diverticulum has lost its musculature in the more advanced groups and that these structures are homologous (in the case of *Dugesia orientoaustralis* I would suggest an independent origin).

There is only one *Romankenkius* species outside of Australia and the question presents itself, to which group does this species belong? After inspection of the original descriptions of *R. patagonicus* there is no evidence to suggest that this species is not a *Romankenkius* (Böhmgig 1902, Borelli 1901). Further inspection also suggests that this species is most likely more closely related to the eastern *Romankenkius* group due to the fact that the diverticulum is described as being surrounded by musculature (Böhmgig 1902). This discovery has obvious biogeographical implications that are discussed in Chapter 4.

**Diagnosis:** *Romankenkius* can be distinguished from its congeners by the presence of a muscled diverticulum of the bursal canal that receives the entrance of shell glands.

**Diagnosis:** *Western Romankenkius* can be distinguished from its congeners by the presence of a non-muscled common oviduct and the absence of a caudal branch of the oviducts.

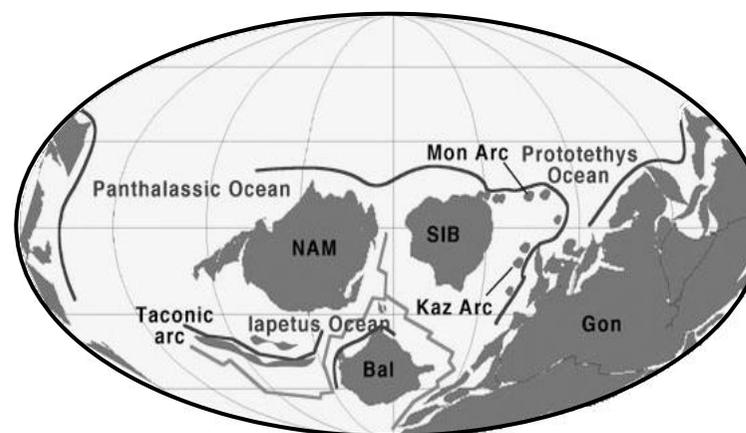
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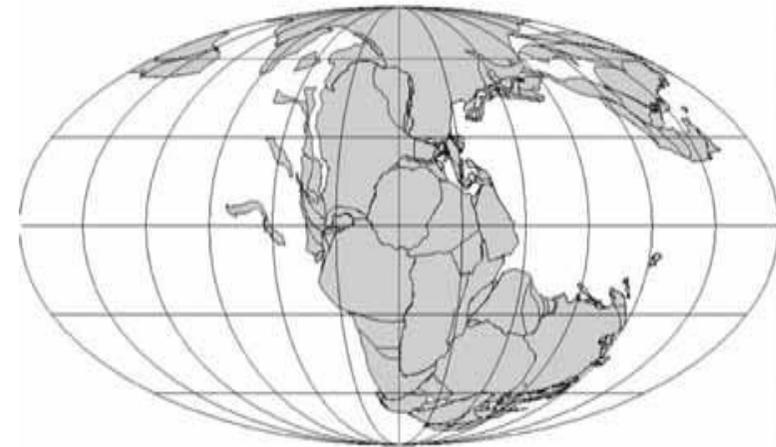
## Appendix 4: Summary of the relevant continental movements during the Phanerozoic (excluding the Cenozoic)

<b>Cambrian 542 - 488 mya</b>	Continents were positioned at low latitudes and the southern continents were loosely clustered together (Windley 1995) (Figure 4.1) to form Gondwana, which was made up of Australia, South America, Antarctica, Africa and India (Cox and Moore 2000, Sanmartin and Ronquist 2004). Australia was positioned on the northeastern margin of Gondwana communicating with Antarctica (Johnson 2004). Geological and fossil evidence suggests that parts of Asia (including India) and Australia were adjacent to each other (Stauffer 1983, Huang et al. 2000, McLoughlin 2001, Metcalfe 2001).
<b>Ordovician and Silurian 488 – 416 mya</b>	Movement within Gondwana was fairly limited but the super continent as a whole was slowly drifting south (Huang et al. 2000, Metcalfe 2001).



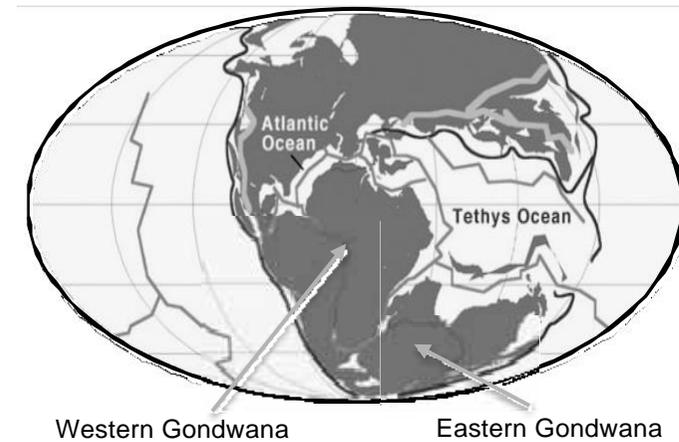
**Figure 4.1** Proposed continental configuration during the Cambrian (Gon = Gondwana)  
(Image adapted from: [http://ffvishnu.glg.nau.edu/frcbfEar\\_Camb.jpg](http://ffvishnu.glg.nau.edu/frcbfEar_Camb.jpg).)

<p><b>Devonian and Carboniferous</b> <b>415 – 300</b> <b>mya</b></p>	<p>During the Devonian, Australia was residing in low southern latitudes. The Carboniferous saw Australia still attached to northeast Gondwana and moving from low southern latitudes to high southern latitudes (Metcalf 2001, Johnson 2004).</p>
<p><b>Permian</b> <b>299-250 mya</b></p>	<p>By the early Permian, Gondwana had united with the northern continents to form the supercontinent Pangaea (Cattermole 2000) (Figure 4.2). The northern continents consisted of North America, Europe and northern Asia and were referred to as Laurasia (Cattermole 2000). From a biogeographic point of view Pangea represented a “superhighway” for the dispersal of flora and fauna.</p>



**Figure 4.2** Proposed fit of the modern continents in Pangaea (Image adapted from: Schettino and Scotese 2005).

<p><b>Triassic</b> 251 - 199 mya</p>	<p>Australia, maintained its position within east Gondwana and low sea levels provided access for organisms to every corner of Pangea via that emergent land area (Figure 4.3)(Scotese 2004).</p>
<p><b>Jurassic</b> 199 – 146 mya</p>	<p>Sea levels rose and oceans were opening up within Pangaea. Although the oceans were relatively narrow they created a formidable barrier to overland migration bringing about the end of the “Pangean Superhighway” (Scotese 2004). Eastern and western Gondwana began separating around 180 mya, when rifting began between Laurasia and Gondwana (Figure 4.3)(Cattermole 2000).</p>



**Figure 4.3** Proposed separation of eastern and western Gondwana (Image adapted from: [http://ffvishnu.glg.nau.edu/frcbfEar\\_Camb.jpg](http://ffvishnu.glg.nau.edu/frcbfEar_Camb.jpg).)

**Cretaceous  
145 – 65 mya**

Rifting continued in Gondwana as the Indian sub-continent separated from western Australia around 140 mya, subsequently detaching from Antarctica (120 mya) then finally Madagascar in the Late Cretaceous (85 – 90 mya) (Storey et al. 1995, Ali and Aitchison 2005). Africa began a slow severing of its connection with South America, a process that was complete by the mid Cretaceous (120 – 100 mya) (Jokat et al. 2003, Scotese 2004, Upchurch 2008). By the end of the Cretaceous very little trace of the once massive continents of Gondwana and Laurasia remained, rifting between Australia and Antarctica was underway (Veevers and Eittreim 1988, Scotese 2004).

**Box 1.1:** The exact sequence of fragmentation for Gondwana is the subject of much debate. Theorists have proposed a series of models for the break-up sequence that are diverse and contradictory (Upchurch 2008). One theory proposes that Africa was in fact the first continent to become isolated from Gondwana 140–120 mya, while South America remained in contact with East Gondwana until around 80 mya (Jokat et al. 2003, Gheerbrant and Rage 2006). Another model proposes that Gondwana actually remained connected until approximately 80 mya, at which time the continents separated simultaneously (Serenio et al. 2004, Upchurch 2008).

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