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Studies on the Systematics and Biogeography of Terrestrial Flatworms (Platyhelminthes: Tricladida: Terricola) of the Australian Region.

Thesis submitted by
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in March 2003

for the degree of Doctor of Philosophy
in the Discipline of Zoology and Tropical Ecology
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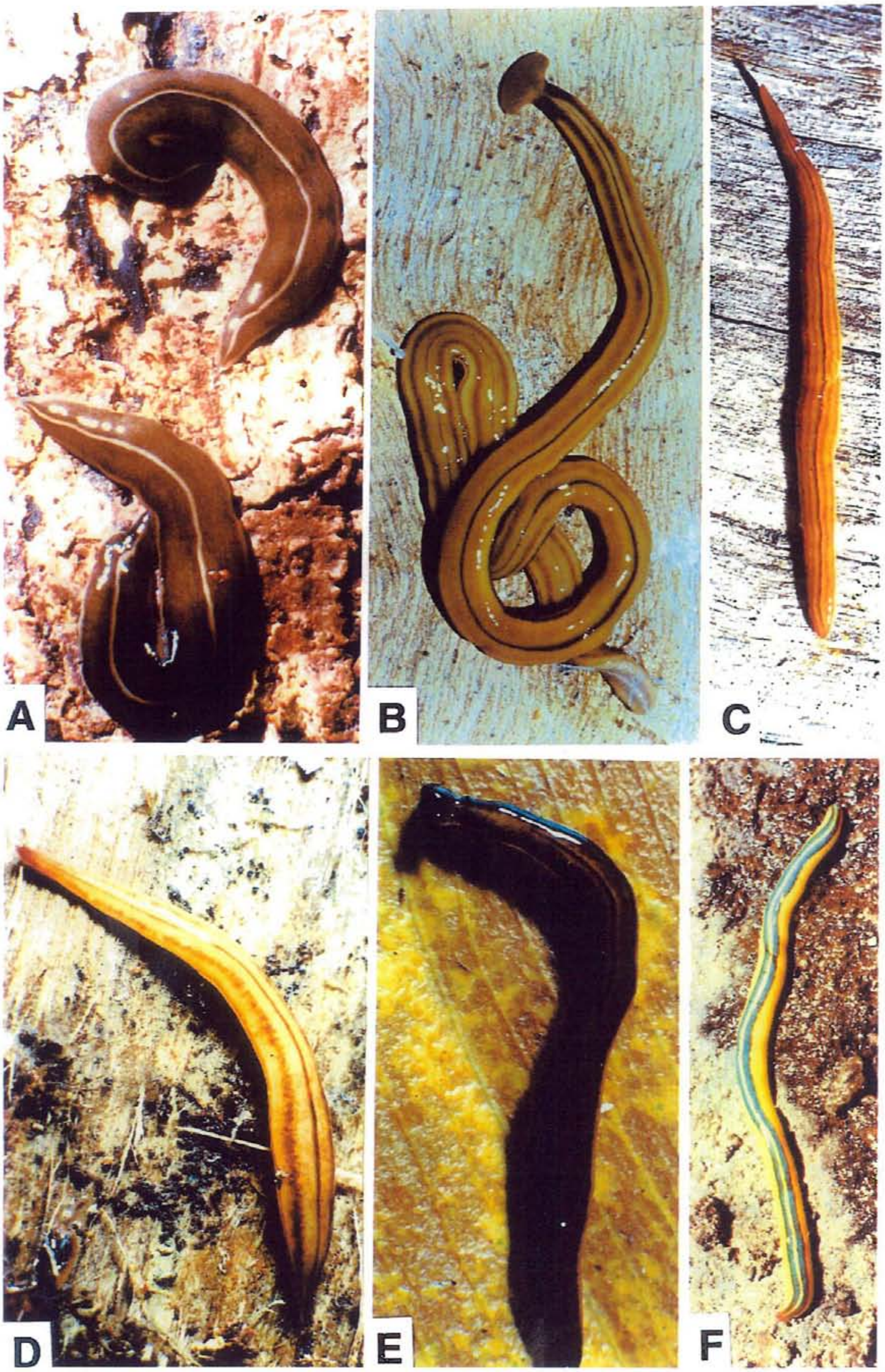
at James Cook University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Frontispiece

A. *Platydemus manokwari* Beauchamp, 1962 (Rhynchodemidae: Rhynchodeminae), 40 mm long, urban habitat, Townsville, north Queensland dry tropics, Australia. A molluscivorous species originally from Papua New Guinea which has been introduced to several countries in the Pacific region. Common. (photo L. Winsor).

B. *Bipalium kewense* Moseley,1878 (Bipaliidae), 140mm long, Lissner Park, Charters Towers, north Queensland dry tropics, Australia. A cosmopolitan vermivorous species originally from Vietnam. Common. (photo L. Winsor).

C. *Fletchamia quinquelineata* (Fletcher & Hamilton, 1888) (Geoplanidae: Caenoplaninae), 60 mm long, dry Ironbark forest, Maryborough, Victoria. Common. (photo L. Winsor).

D. *Tasmanoplana tasmaniana* (Darwin, 1844) (Geoplanidae: Caenoplaninae), 35 mm long, tall open sclerophyll forest, Kamona, north eastern Tasmania, Australia. This was the first described Australian species, collected by Darwin during the voyage of the Beagle. Common. (photo L. Winsor).

E. *Cotyloplana sp.* (Rhynchodemidae: Rhynchodeminae), 25 mm long, tropical rainforest, near Millaa Millaa, north Queensland wet tropics, Australia. Uncommon.
(photo L. Winsor).

F. *Caenoplana dubia* (Dendy, 1892a) (Geoplanidae: Caenoplaninae), 50 mm long, tall open sclerophyll forest, Gembrook, Victoria, Australia. Uncommon. (photo L. Winsor).

STATEMENT ON ACCESS TO THIS THESIS

Nomenclature in this thesis complies with the *International Code of Zoological Nomenclature* 4th edition, effective from 1 January 2000. However the new taxa described in this thesis are *nomina nuda* as the provisions of Article 11 of the *International Code of Zoological Nomenclature* (ICZN 1999) are not met. For this reason an embargo is placed on access to this thesis for a period of two years. This should allow sufficient time to formally publish the descriptions of new taxa.

After the embargo period, I, the undersigned, the author of this thesis, understand that James Cook University will make it available for use within the University Library and, by microfilm or other photographic means, allow access to users in other approved libraries. All users consulting this thesis will have to sign the following statement:

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29.03.2003

L. Winsor Date

2/4/03

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Abstract

This study contributes to knowledge of the systematics and biogeography of terrestrial flatworms (Platyhelminthes: Tricladida: Terricola) of the Australian region. Terrestrial flatworms are carnivorous soil animals some of which are important commercially as biological control agents for the Giant African snail, or as introduced pest species preying upon earthworms in Europe.

The total Australian terrestrial flatworm fauna is estimated to be in excess of 300 species. At present only 137 species are named of which three-quarters have been described solely from external features. Taxonomy of the Terricola is based on a combination of external features and internal anatomical characters and remains in a state of flux. Recent molecular studies of the Tricladida have cast doubt on the monophyly of the Terricola. The austral fauna is poorly known anatomically, and taxonomy of the group further hampered by the existence of numerous species complexes which are poorly understood.

The objectives of this study are to locate and examine type and supplementary specimens, review the functional anatomy of austral terrestrial flatworms, identify reliable taxonomic characters and states, revise certain taxa, and develop identification keys. Furthermore, efforts have been made to determine the distribution of terricolan taxa of the region, identify the biogeographical components of the austral terricolan fauna and to identify the occurrence of introduced, threatening and vagrant terrestrial flatworms. Cladistic and molecular analyses of the taxa have not been undertaken.

Fixation and parasite-induced artifacts can cause mis-interpretation of anatomical characters. A suite of taxonomic characters and states is used to provide standardized diagnoses for genera. Included in the characters assessed for their application in taxonomy are colour and pattern of markings, pharyngeal musculature, configuration of the nephridial ducts, dorsoventral distribution of testes, penis type, resorptive organs, and musculoglandular organs (adenodactyls). Seven types of the hitherto enigmatic adenodactyls are identified, some of which function to secrete sclerotins which form the cocoon wall. The mechanism of cocoon formation in the Terricola involves the deposition of sclerotins, derived from antral secretions or adenodactyls, on the outer pre-cocoon membrane. This mechanism differs from the generally accepted Triclad model based upon the Paludicola, in which the cocoon is formed from within the pre-cocoon membrane by shell-globule components of the vitellocytes.

Representatives of all three terricolan families are present in the Australian region. A new subfamily is erected in the Geoplanidae, and of the twenty-seven genera considered in the study, ten are new. The problematic *Australoplana* complex is resolved into five genera and two sub-genera. Field keys are provided for the Terricola genera of the Australian region. This study supports the view that the Terricola are polyphyletic. Anatomical data suggest a possible phylogenetic relationship between some austral caenoplaninid and maricolan taxa.

The Australian flatworms mostly occur within hyper-humid to sub-humid moisture regions, and are assigned to two principal families. In the Rhynchodemidae: Rhynchodeminae 45 species are accommodated within *Cotyloplana, Digonopyla, Dolichoplana, Platydemus*, *Rhynchodemus* and in new genera not considered in this study. In the Geoplanidae: Caenoplaninae, 80 species are assigned to *Artioposthia, Australoplana, Caenoplana, Fletchamia, Lenkunya, Parakontikia, Reomkago, Tasmanoplana* and additional new genera considered here. Four species are assigned to two new genera in a new subfamily of the Geoplanidae. Eight introduced species are identified, none of which at present poses an ecological or commercial threat in the Australian region.

Two major flatworm faunal units are recognized in Australia. A northern element dominated by rhynchodemid genera including taxa which also occur in Papua-New Guinea – Irian Jaya and Indonesia. The southern element is dominated by geoplanid genera including some with currently poorly-defined Gondwanan affinities. These flatworm faunal units broadly accord with the Torresian (northern) and with the Tasmanian, South-western, and Bassian or Kosciuskan (southern) zoogeographic subregions.

STATEMENT ON SOURCES

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references given.

28.03.2003

L. Winsor   Date

Preface and Acknowledgments

My fascination with flatworms began when I was a young boy interested in all manner of invertebrate animals. My father Keith (1911-1950) a research engineer, died when I was not quite four years old. Sadly I have few recollections of him. By all accounts a clever and likable man, his library contained many books encompassing a wide variety of subjects including books on zoology and collecting methods for invertebrates. My favourite book was the excellently illustrated *Animals without backbones* by Ralph Buchsbaum, 1938, the hard covered copy of which has a large and engaging line drawing of a planarian on the cover. Early access to these books, and also to my father's hand lenses and stereo microscope, made knowledge readily accessible to a growing and curious lad, as they still do for me now. The completion of this thesis is in some small way a tribute to my mother Lesley (1920-1998), for the sacrifices she made, opportunities she presented, and for her love, encouragement and support over fifty two years. The thesis is dedicated to the memory of my late parents.

My earliest recollection of flatworms was as a child of five to six years old. A long thin lemon yellow, and black mono-striped species, probably *Fletchamia mediolineata*, lived along with centipedes and other interesting beasties behind the wooden border of my sandpit in semi-rural Watsonia, in the outer eastern suburbs of Melbourne, Victoria. These worms were difficult to handle, stuck to my fingers, and fragmented, the separate pieces crawling off into cover. They made a lasting impression on me.

My next memorable encounter with flatworms was in 1970 in the Field Survey Group (FSG), Field Naturalists' Club of Victoria. Having completed seven years of study at night school, I finally had time to renew my long-standing interest in natural history; in particular spiders and the odds and sods, such as flatworms and nemerteans in which nobody else was particularly interested. I became rapidly disenchanted with taxonomy of spiders. However the land planarians and nemerteans fascinated me. My life long love affaire with the flatworms began with the discovery of a paper on *The Victorian land planarians* by Arthur Dendy (1891) that had colour illustrations of the species I had fortuitously collected some fourteen kilometres from the Type locality. My subsequent collections of specimens 1970-74 were made with the able assistance and wonderful companionship of FSG members, especially Arthur Brook and Ros St Clair.

Members of the FSG were fortunate in having as their mentor Dr Brian Smith, at the time Curator of Molluscs, Museum of Victoria. Brian's enthusiasm, encouragement, and support of natural history groups are well known. It was Brian who remarked to me that as far as he was aware, little had been done on the histology of Australian Terricola, and suggested that as a histologist interested in these flatworms I was well placed to rectify this deficiency. Little did I realize just what I was letting myself in for. To Brian I am deeply grateful for setting me on this path, for his practical support, encouragement over many years, and especially for successfully arguing on my behalf the case for sectioning Holotypes of Terricola held by the Natural History Museum – a major policy shift for the NHM.

My journey of discovery of the Terricola is life-long. Companionship and cooperation with colleagues overseas, especially Eudoxia Froehlich, and Ana-Maria Leal Zanchet and her team, have made for a challenging and stimulating journey. The good advice of Laurence Richardson (1913-1989), and empathy and support of my taxonomic endeavours by Marion Fyfe (1894–1986), have been most helpful. Both taxonomists had successfully grappled with the tyranny of distance working on unfashionable, relatively obscure and poorly known groups in the Australian region.

Support for equipment, consumables and facilities from the Australian Biological Resources Study, CSIRO Science and Industry Endowment Fund, and from Rhondda Jones, Howard Choat, and Richard Pearson, Heads of the Schools within Biological Sciences, is gratefully acknowledged. Special thanks to Ted Dews, and recently Peter Hill, Directors, Central Services, for their tolerance of a zoologist in their midst, and for facilitating my studies after my moving to Central Services. For access to collections, information, and for facilitating and extending specimen loans, I am most grateful to museum curatorial staff, in particular Carden Wallace, Peter Arnold, Lester Cannon, Pat Hutchings, Penny Berents, Brian Smith, Tim Stranks, Tammy Scarborough, Rod Bray, and Ricardo Palma. My thanks to the numerous people who kindly forwarded to me specimens of flatworms for identification.

My part-time PhD has taken some nine years to complete. My heartfelt thanks to Rhondda Jones for her initial encouragement and backing to undertake a PhD. Without the support and encouragement of many friends and colleagues over the nine years, particularly during the very difficult times of personal problems and change of vocation, this thesis would never have been completed. It is in this sense very much a collaborative effort. Your odd comment, joke, shared experience, practical help, IT support, technical and other advice, and empathy, have all helped get me through: my heartfelt gratitude to all these friends, especially to Chris Alexander, Orpha & David Bellwood, Gillian Brodie, John Collins, Jim, Margaret and Martin Darley, Rachel Groves, Zoli Florian, Savita Francis, Ray Gibson, Betsy & Mick Jackes, Michelle Keeler, Helene Marsh, Di McNamara, Bob Mesibov, Laurie & Sue Reilly, Peter & Ann Renton, Jason Symonds, and all my workmates in the Central Services team.

To my supervisor David Blair I owe a special debt of gratitude. Despite an impossibly crowded schedule of teaching, administration, and research, David always made time for me to discuss progress with my project, and promptly returned drafts with constructive comments. For these, his sound advice, and long association as a colleague and enthusiastic fellow taxonomist of the Platyhelminthes, my deep appreciation.

Completing this thesis is but one small way in which I can express my deepest gratitude to my family Heather, Keith and Lara who over many years provided me with practical help, support and encouragement with my private madness. I am especially appreciative of their long suffering and forbearance with the fieldwork… *we'll just stop here for ten minutes to quickly check these logs …* in reality usually half an hour or more ….. and our camping holidays and trips to places which just happened to be to type localities. I am particularly grateful to my extended family in Melbourne, especially Meg, Hilary, and Michele for their emotional support. Last but definitely not least, a very special thanks to Kay Cameron for her love, understanding, support, and faith in me that this task would eventually be completed.

Contents

Chapter 1. Introduction to the Terricola: world perspective and systematics 1

Chapter 2. Studies on Australian Terricola: the historic background 10

Chapter 3. Materials and Methods 32

Chapter 4. Functional anatomy and taxonomic characters of Terricola 46

Appendices

List of Figures

List of Plates

List of Tables

Publications arising from this thesis

Publications in refereed journals and book:

Winsor, L. 1997. The biodiversity of terrestrial flatworms (Tricladida: Terricola) in Queensland - a preliminary report. *Mem. Mus. Vict.* **56**: 575-579.

Winsor, L., Johns, P.M., and Yeates, G.W. 1998. Introduction, and ecological and systematic background, to the Terricola (Tricladida). *Pedobiologia* **42:** 457-463.

Winsor, L. 1998. Collection, handling, fixation, histological and storage procedures for taxonomic studies of terrestrial flatworms (Tricladida: Terricola). *Pedobiologia* **42:** 405-411.

Winsor, L. 1998. Aspects of taxonomy and functional anatomy in terrestrial flatworms (Tricladida: Terricola). *Pedobiologia* **42:** 412-432.

Winsor, L. 1998. The Australian terrestrial flatworm fauna (Tricladida: Terricola). *Pedobiologia* **42:** 457-463.

Winsor, L. 1998. Flatworm infestation of commercial earthworm farms in Australia (abstract). *Pedobiologia* **42**: 573

Jones, H.D., Johns, P.M., and Winsor, L. 1998. The proposed synonymy of *Parakontikia ventrolineata* (Dendy, 1892) and *Kontikia mexicana* (Hyman, 1939): what is a penis papilla. *Hydrobiologia* **383**: 91-96.

Cannon, L (Ed). 2000 *Wildlife of Tropical North Queensland.* Worms - text and illustrations for terrestrial and freshwater triclads by L. Winsor. Brisbane: Queensland Museum.

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Winsor, L. 1997. Terrestrial flatworms INFOSHEETS No.1. The collection, handling and preservation of specimens for general identification.

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Winsor, L. 1998. Terrestrial flatworms INFOSHEETS No.3. Prevention and control of flatworm infestation of earthworm beds. Revised edition.

Winsor, L. 1997. Terrestrial flatworms INFOSHEETS No.4. The Long Wanderers – *Dolichoplana* species: earthworm predators.

Winsor, L. 1998. Terrestrial flatworms INFOSHEETS No.5. Predators in earthworm beds – flatworms or leeches?

Winsor, L. 1999. Terrestrial flatworms INFOSHEETS No.6. The New Guinea flatworm – *Platydemus manokwari*: predator of land snails.

Winsor, L. 2003. Terrestrial Flatworms – Facts Sheet. James Cook University: Townsville.

Author's note:

New genera are described in Chapter 5, and new taxa are described in Appendix 8.

In order to improve the readability of the thesis, the decriptors *gen. nov, sub. gen. nov,* and *sp. nov.,* apart from the formal descriptions, are not used with the new names throughout the text.

The nomenclature of new taxa will be treated in the recognized formal manner in any publications arising from this thesis.

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The nomenclature of new taxa will be treated in the recognized formal manner in any publications arising from this thesis.

Chapter 1

Introduction to the Terricola: world perspective and systematics.

> *The existence of a division of the genus Planaria, which inhabits the dry land, interested me much.... (they) were found in the drier parts of the forest beneath logs of rotten wood .. in general form they resemble little slugs, but are much narrower in proportion, and several of the species are beautifully coloured with longitudinal stripes.... I found no less than twelve different species of terrestrial Planariae in different parts of the southern hemisphere.*
>
> Darwin, 1845, in *Journal of Researches*, April 1832 at Rio.

1.1 INTRODUCTION

Terricola - terrestrial flatworms or land planarians - live in damp soil and similar cryptic habitats and prey on other soil animals. They range between 1 and 20 cm in length and are often brightly coloured and patterned. Known species are allocated to three families and five subfamilies (Table 1.1). Although known since the voyage of H.M.S. *Beagle* in 1831 – 1836 and despite subsequent systematic activity around the turn of the century, they attracted little interest outside the southern hemisphere. The impact of the New Zealand terrestrial flatworm *Arthurdendyus triangulatus* (Dendy, 1895a) preying on native earthworm populations in Europe (Blackshaw and Stewart 1992) stimulated a resurgence of research on the Terricola. Recent contributions to our knowledge of the basic biology, behaviour and ecology of these flatworms are particularly important (Alford *et al* 1998). Yet much remains to be done.

Little is known of the ecology of the Terricola. What factors control the natural distribution and dispersal of a flatworm species, and what factors allow the species, as an exotic in another country, to successfully exploit similar habitats? Can we predict which group of species is likely to become a threat to the natural environment or agriculture? In order to answer these questions significant progress must be made in increasing our knowledge of the taxonomy and biogeography of the Terricola. This is the over-arching objective of this thesis with respect to the Terricola of the Australian region.

1.2 ECOLOGICAL PLACEMENT OF THE TERRICOLA

For over a century since their discovery in 1831 the Terricola were recognized as land-living flatworms. Soil animals can be classified on the basis of size, whether they are temporary or permanent members of the soil fauna, their habitat preferences (Wallwork 1970), and their feeding preferences (Birch and Clarke 1953). On these bases, the terrestrial flatworms are active geophilic macro carnivores. Most are predators, though some species are necrophagic (Winsor 1977). Cannibalism has been observed, though in artificial circumstances (Froehlich 1956, Winsor pers.obs). Surprisingly, European biologists rarely placed the Terricola within any of the terrestrial ecological groupings, such as the soil fauna. Dendy (1890), from his zoological experiences in the tall wet eucalypt forests of Walhalla in Victoria, recognised and coined the term *cryptozoic* for a fauna he defined as "all that assemblage of animals which is found living habitually under logs and stones and under the rotten bark of trees." He considered that the terrestrial flatworms were a characteristic and distinct group within the cryptofauna.

The terrestrial flatworms are rarely mentioned in standard texts on litter or soil fauna (e.g.Wallwork 1970), possibly reflecting a boreal bias (Platnick 1991), though there are exceptions (e.g. Ball & Sluys 1990). Recent experiences with *Arthurdendyus triangulatus* and other antipodean taxa in Europe have emphasised the importance of the flatworms as predators within the soil community. Austral zoologists have long recognised the flatworms as an important component in the cryptofauna (Steel 1901a, Williams 1941, Lawrence 1953), litter fauna (Grimmett 1926, Springett 1976) and the soil fauna (Steel 1901a, Birch and Clarke 1953). The niche occupied by the cryptofauna is continuous at one extreme with those of the soil and litter faunas, and at the other extreme with the faunas of rocks and trees (Wallwork 1976).

In terms of microclimate preferences the Terricola have been defined as stenohygric hygrocoeles (Froehlich 1956) – animals living in damp or moist habitats, and tolerant of a narrow range of atmospheric humidity. They have practically no water-saving adaptations, being dependent upon moisture in their microhabitat, yet are sensitive to too much water and thus generally avoid saturated places. They range through mesophile to xerophile habitats; from alpine herbfields to sandy semi-desert, subantarctic rata forest to tropical rainforest.

Flatworms are mostly strongly seasonal in occurrence. This probably reflects attempts to maintain themselves within a constant microclimate by vertical migration through the continuum of habitat niches. When the moisture conditions are optimal they will occupy the cryptozoic and litter microhabitats on the soil surface. If conditions are humid or too wet they may move higher, for example up trees and walls. When conditions are too dry for them they retreat into the soil. In the absence of surface cover terrestrial flatworms can live entirely in soil as permanent members of the soil fauna and can be regarded as facultative soil animals. Most species appear not to burrow, but make use of existing pore spaces to migrate within the soil.

1.3 SYSTEMATIC PLACEMENT OF THE TERRICOLA

The Terricola have a long and chequered systematic and nomenclatural history, reflecting in part the attempts of early zoologists to grapple with the classification of vermiform animals. Originally included with the freshwater and marine flatworms in the genus *Planaria* of the Dendrocoela (Müller 1774), terrestrial flatworms were subsequently classified by Diesing (1850) on the basis of eye number and placed in *Polycladus* (no eyes), *Planaria* (two eyes), and *Polycelis* (multiple eyes). The terricolous flatworms were later split from the water-living forms and placed in *Geoplana* (Schultze & Müller 1857) in which was included the genus *Rhynchodemus* Leidy. Stimpson (1858) erected two families to accommodate the terrestrial flatworms within the suborder Turbellaria Dendrocoela: the Geoplanidae which included the genera *Geoplana, Rhynchodemus* now reinstated, plus a new genus *Bipalium* and the Polycladidae with the sole genus *Polycladus*.

Schmarda (1859), apparently unaware of Stimpson's classification, placed the terrestrial flatworms in five genera within two principal divisions in the Dendrocoela based on the distinction of the head from the body: Acarena (head indistinct): *Polycladus (*= Stimpson's *Polycladus* + *Geoplana* in part), *Polycelis* (= *Geoplana* in part), *Prostheceraeus* (= Leimacopsis), and *Planaria* (= *Rhynchodemus* in part), and Carenota (head distinct): *Sphyrocephalus* (= *Bipalium*).

Particular interest in the Turbellaria and numerous reports of new species prompted a major reclassification of the Turbellaria Dendrocoela by Diesing (1861). He listed sixteen families and assigned existing genera of terricolous flatworms to the Anocelidea - no eyes (*Polycladus*, *Geobia*), Planaridea - two eyes (*Rhynchodemus*), Polycelidea - multiple eyes (*Geoplana*, *Bipalium*), and Limacopsidea - tentaculate head (*Leimacopsis*).

At this time the paucity of external morphological characters was a serious constraint in further developing the taxonomy of terrestrial flatworms. The potential usefulness of microanatomical characters for classification in the group was highlighted by Moseley (1875). His publication of generic diagnoses incorporating characters derived from both external and internal morphology of new species and genera (Moseley 1877b) was a watershed in the classification of terrestrial flatworms. The use of microanatomical characters for classification of flatworms gained acceptance, and these were increasingly incorporated in generic diagnoses. In his classification of the triclads, Hallez (1892) established the Terricola (terrestrial triclads) as one of three supposedly natural tribes (= suborders), the others being the Maricola (marine triclads) and the Paludicola (freshwater triclads). A new infra-order, the Cavernicola (cave dwelling triclads), has recently been added to the Tricladida (Sluys, 1990; refer to Table 1.1).

Within his Order Tricladida, Tribe III (= suborder) Terricola, Hallez (1893) established three families (Table 1.2). The austral genera *Geobia* and *Caenoplana* were subsumed within his *Geoplana*. Graff (1896b, 1899) revised this classification and divided the Terricola into three families accommodating eleven genera (Table 1.3). This classification of the Terricola provides the basis for the present arrangement of the group (Table 1.4). Since 1987 Ogren and various co-workers, notably Kawakatsu, have produced and continue to emend the *Land Planarian Indices of the World* and this is taken as the starting point for this thesis.

Over the last two decades the availability of molecular and ultrastructural techniques, coupled with cladistic analysis, has resulted in the demise of the taxon Turbellaria (Ax, 1984, Ehlers 1985, Soppot-Ehlers 1985), and the constructions of new phylogenies of the phylum Platyhelminthes (Rohde *et al.* 1995, Carranza *et al* 1997; Littlewood *et al* 1999). As a consequence, the phylogenetic relationships of the suborder Tricladida with other Platyhelminthes has received increasing attention, in particular the relationships among the triclad infraorders (Ball 1981, Sluys 1989) and the phyletic position of the terrestrial flatworms within the Tricladida (Carranza *et al.* 1998; Baguñà 2001).

1.4 PRESENT STATE OF TERRICOLAN TAXONOMY

Taxonomic studies of the Terricola are presently in a state of flux. They are primarily concerned with determining relationships at species level and those among the higher taxa. The taxonomy of the Terricola is based upon a combination of characters derived from external morphology and from internal microanatomy, especially the copulatory organs. The histology of the copulatory organs is known for only 55% of described species, the most poorly known groups of which are those of Australia and New Zealand (Ogren *et al.* 1997b). It is estimated that published anatomical data are available for only some 12% of the 90 named Australian Terricola (Winsor 1991c) within a fauna estimated in excess of 300 species (Winsor 1997a).

Caenoplaninid species that could not be confidently assigned to current genera were placed by Ogren *et al* (1991) in *Australopacifica*, a collective group. Many of these species will be accommodated in new genera (this thesis) and in a new subfamily foreshadowed in recent studies of Terricola from Australia and New Zealand (Johns, 1998; Winsor 1998c).

Emphasis by taxonomists, particularly during the mid 20th century, on the broad structure of the copulatory apparatus at the expense of other anatomical structures now constrains the taxonomy of the Terricola. The suite of characters and character states available for classification must be broadened. It is clear that the means to resolving many taxonomic problems lies with the availability of species descriptions and generic diagnoses with comparable treatment of comparable characters (Winsor, 1998b). Apart

Table 1.1 Generalized position and classification of terrestrial flatworms

Platyhelminthes	
"Turbellaria"	
Seriata	
Tricladida	
Infraorder Maricola (marine triclads)	
Infraorder Cavernicola (cave-dwelling triclads)	
Infraorder Paludicola (freshwater triclads)	
Infraorder Terricola (terrestrial triclads) –	(815 named species)
Family Rhynchodemidae	
Subfamily Rhynchodeminae	(5 genera)
Subfamily Microplaninae	(7 genera)
Family Geoplanidae	
Subfamily Caenoplanidae	(15 genera)
Subfamily Geoplaninae	(14 genera + 2 subgenera)
Subfamily Pelmatoplaninae	(2 genera)
Family Bipaliidae	(4 genera)

Table 1.2 Classification of the Tricladida: Terricola (Hallez, 1893)

	Families		
	Limacopsida	**Geoplanida**	**Polycladida**
Genera	*Limacopsis*	*Geoplana*	*Polycladus*
		Sphyrocephalus	
		Geodesmus	
		Rhynchodemus	
		Dolichoplana	
		Microplana	

Table 1.3 Classification of the Tricladida: Terricola (Graff 1896b)

	Families				
	Leimacopsididae	Geoplanidae	Bipaliidae	Cotyloplanidae	Rhynchodemidae
Genera	*Leimacopsis*	*Geoplana*	*Bipalium*	*Cotyloplana*	*Rhynchodemus*
		Groups a & b	Groups a & b		
		Pelmatoplana	*Perocephalus*	*Artiocotylus*	*Dolichoplana*
		Choeradoplana	*Placocephalus*		*Platydemus*
		Polycladus			*Microplana*
		Artioposthia			*Amblyplana*
		Geobia			*Nematodemus*
					Othelosoma

Table 1.4 A synopsis of the families, subfamilies and genera of the Terricola, based upon Graff 1899; Ogren *et al* 1987-2001; Kawakatsu *et al* 2000-2002

Family **BIPALIIDAE**
Body elongate and flattened, with a semilunate head; with numerous small marginal eyes, and sensory groove around head; creeping sole begins at the base of the headplate. With 169 named species

Genera: *Bipalium, Diversibipalium, Humbertea, Novibipalium*

Main distribution: the majority occur in the Indonesian archipelago. Also reported from Madagascar, India/Nepal, Japan, Malaysia/Singapore, Sri Lanka, Vietnam, China/Hong Kong/Taiwan, Philippines, United States (introductions), and Korea. Many of the species recorded in Indonesia were obtained from the Botanic Gardens at Buitenzorg (now Bogor) in Java, where some species are probably exotics.

Family **RHYNCHODEMIDAE**

Terrestrial flatworms of elongate flattened to cylindroid form with two eyes near the simple tapered or blunt anterior end; no tentacles or headplate; with a well defined creeping sole occupying part of the ventral surface. May have an antero-ventral sucker. Divided into two subfamilies on the basis of cutaneous musculature. With 206 species.

Rhynchodeminae:

Rhynchodemidae generally of elongate, flattened form, anterior end often tapered and elongate; with conspicuous eyes; body wall with strong cutaneous musculature, the longitudinal fibres of which are grouped into large well developed bundles; penis papilla absent or greatly reduced.

Genera: *Cotyloplana, Digonopyla, Dolichoplana, Platydemus, Rhynchodemus.*

Main distribution: Worldwide. United Kingdom, central Europe, eastern United States, central and eastern Africa, Madagascar, Sri Lanka, India, Japan, Malaysia, Indonesia, Philippines, Irian Jaya - Papua - New Guinea, New Caledonia, Pacific Islands, eastern Australia, Central America, Colombia, Peru, Brazil, Paraguay and Argentina.

Microplaninae:

Rhynchodemidae of generally short, plump, cylindroid form, anterior end often blunt; eyes often small or may be regressed; with weak cutaneous musculature in which the longitudinal fibres are poorly developed and not aggregated into large bundles; male copulatory organ often complicated with well developed penis papilla; female copulatory organs simple or complex, may have genito-intestinal connection; with or without seminal bursa with one or more exits.

Genera: *Amblyplana, Diporodemus, Geobenazzia, Incapora, Microplana, Othelosona, Pseudartiocotylus.*

Main distribution: United Kingdom, Europe, eastern United States, western central and southern Africa, Madagascar, India, Sri Lanka, Vietnam, Japan, Central America, Peru, Surinam.

Family **GEOPLANIDAE**

Terricola with numerous small eyes concentrated around the anterior, and along the sides, sometimes located dorsally, or without eyes; without auricular or tentacular organs, or semilunar headplate. With 442 named species.

Divided into three subfamilies Geoplaninae, Caenoplaninae and Pelmatoplaninae chiefly on the basis of the width of the creeping sole, the position of the testes, and the organisation of the cutaneous musculature.

Geoplaninae:

Creeping sole 50% or more of body width, testes dorsal, cutaneous musculature in well developed bundles.

Genera: *Geoplana (with two subgenera Geoplana and Barreiana), Gigantea, Pasipha, Notogynaphallia, Amaga, Choeradoplana, Geobia, Issoca, Gusana, Liana, Enterosyringia, Xerapoa, Polycladus, Pseudogeoplana*

Main distribution: South and Central America, and the south-eastern United States.

Caenoplaninae:

Creeping sole 30-50% or more of body width, testes ventral, cutaneous musculature in well developed bundles.

Genera: *Caenoplana, Artioposthia, Australoplana, Kontikia, Parakontikia, Fletchamia, Tasmanoplana, Lenkunya, Reomkago, Timyma, Pimea, Endeavouria, Coleocephalus, Newzelandia, Australopacifica.*

Main distribution: Australia, Papua-New Guinea, Pacific Islands, New Caledonia, New Zealand, Peru, Argentina, Madagascar, India, Sri Lanka, Philippines, Indonesia, Japan, and a few species introduced into many countries including the United Kingdom and the United States.

Pelmatoplaninae:

Creeping sole 25% or less, testes ventral, cutaneous musculature weak, in poorly developed bundles.

Genera: *Pelmatoplana, Beauchampius*

Main distribution: Madagascar, India, Sri Lanka, Philippines, Thailand, Cambodia, Vietnam, Malaysia, Indonesia, Colombia, and New Caledonia. Introduced into the United Kingdom and Australia.

from constraining the taxonomy, the dearth of anatomical data also hampers understanding of the functional morphology and biology of these flatworms.

In addition to the difficulties in locating, accessing, preparing and describing type and supplementary specimens, there is the bequest of earlier taxonomists - the taxonomic impediment. Similarities in the external morphology of particular taxa has resulted in the establishment of species complexes such as that of *Artioposthia subquadrangulata* (Dendy, 1895c) *sensu lato* (in Johns, 1998), and comprehensive catch-all genera such as *Diversibipalium, Artioposthia* and *Australoplana* the last two of which are heterogeneous and comprise several distinct lineages (Winsor 1983b,1991c). Even the original workers unwittingly confused species, as specimens forming some syntype series have been found to represent two distinct species (Fyfe, 1946, Winsor pers.obs). Taxonomic work on New Zealand Terricola is further hampered by the absence of illustrations accompanying the original species descriptions, and the redescription of some species from non-type or mixed material (Johns, 1998). Rationalising the complex synonymies and comprehensive genera is difficult. Specimens that formed the basis for taxonomic decisions must be traced and examined histologically, then related to topotypic specimens recently obtained, photographed and sectioned. Only then are the taxonomic problems likely to be resolved.

1.5 OBJECTIVES OF THIS STUDY

The purpose of this study is to develop the alpha and beta taxonomy of some key taxa of the Terricola fauna in the Australian region (Australia, Lord Howe Island, New Zealand, and the subantarctic islands of Australia and New Zealand). The study is largely based upon existing collections of formaldehyde-preserved specimens that unfortunately precluded complementary molecular-based phylogenetic studies. Through the identification of reliable taxonomic characters, and provision of data on the distribution of taxa, it is hoped that this study will provide the foundations for subsequent cladistic biogeographical analysis of the Terricola of the region. The research was divided in two integrated sections, with the following aims:

1.5.1 Systematics

- To locate Type and other specimens of terrestrial flatworms of the Australian region that are held in museums worldwide;
- To review aspects of the functional anatomy of Terricola with particular reference to Australian taxa, and from this,
- To identify reliable taxonomic characters and states, and establish which of the latter are plesiomorphic, and which are apomorphic conditions;
- To undertake taxonomic revisions of some Caenoplaninid taxa; and
- To develop simple taxonomic keys, field keys, and other aids to make the austral Terricola accessible to the scientific and lay communities

1.5.2 Biogeography

- To determine the distribution of key taxa of the Australian region;
- To identify and describe the biogeographical components the Terricola fauna of the Australian region;
- To determine the identity and occurrence of rare and endemic taxa; and
- To determine the identity and occurrence of introduced, threatening and vagrant taxa.

Chapter 2.

Studies on Australian Regional Terricola: the historic background

But one thing is certain, namely, that in a general way the question of the whereabouts of Australian zoological types is a much more complicated and perplexing one than the botanist interested in the Australian flora can ever have to battle with. (Fletcher 1901)

2.1 INTRODUCTION

The present binominal system of zoological nomenclature is underpinned by the Type concept (ICZN 2000). Thus the location and examination of the relevant Type specimens was crucial to this study of the systematics of terrestrial flatworms of the Australian region. The first problem was to locate these Types. This task necessitated a considerable amount of detective work in Australia and overseas, aided by a thorough appreciation of the historical background research into Australian terrestrial flatworms. The results of this search are provided in the Catalogue of Types of Australian Terricola (Appendix 1).

This chapter provides an historical overview of research on terrestrial flatworms of the Australian region and attempts to place this present study in context. Names of taxa used in this chapter are those given in the original publications.

2.2 PRE-HISTORY

It is uncertain whether or not indigenous Australians were, and are, aware of terrestrial flatworms. Aboriginal knowledge of native plants and animals is extensive and detailed, especially with regard to species valued for food and medicine. This knowledge is revealed in aboriginal art and in spoken and signed aboriginal languages (Stanbury 1987). Given the conspicuous appearance of many species of terrestrial flatworms, and their occurrence in habitat probably searched for food, it is difficult to believe that flatworms would not be known to aborigines - particularly those people of tribes frequenting the forests of south-eastern Australia and Tasmania. Some aboriginal language dictionaries and vocabularies of tribes in eastern Australia (Papps 1965; Dixon 1991) contain words for worm. In the context of this study, no evidence has been found suggesting that aboriginal Australians were specifically aware of terrestrial flatworms or differentiated them linguistically from polychaetes, earthworms, or leeches. This lack of linguistic evidence may also indicate that the compliers of the vocabularies themselves did not fully differentiate terrestrial vermiform invertebrates. Non-zoologists, on the basis of appearance, frequently confuse terrestrial flatworms with terrestrial leeches (Winsor, unpublished observations).

From a biological aspect one of the most detailed and well researched Aboriginal language vocabularies is that of Yidiny, the language of the Cairns-Yarrabah region (Dixon 1991). In the Yidiny language seven species of worm are recognized, and are differentiated from three species of leech. The "worms" include a big dark coloured swamp worm used for bait, a brown ground worm, a big black rock worm, a little grass worm, a big blue worm, a large worm, and a parasitic hookworm. Of these only the last can be confidently separated taxonomically from the other species' names. Whether these worms are all oligochaetes, or whether they include flatworms is uncertain. For example the big blue worm could refer to one of the large blue caenoplanid flatworms, or more probably refers to the uncommon Turquoise earthworm (*Terrywalkerius terrareginae*); both taxa are found in upland areas of the wet tropics.

Assessment of aboriginal knowledge of the Terricola must therefore await collaboration with linguists and members of indigenous communities on this subject. There is a long chronology of discovery and knowledge of the gross anatomy and ecology of numerous species of Australian animals by pre-European aboriginals (Stanbury 1987), and figured in their art. The recorded discovery of terrestrial flatworms in Australia, however, presently appears to have been made by Europeans.

2.3 EUROPEAN EXPLORATION AND DISCOVERIES

Discovery of the Australian fauna began with early sightings and collections by Dutch mariners from 1611 onwards, though it was not until James Cook's 1768 voyage that the first scientific collections of the Austral flora and fauna were made (Stanbury 1987). The description of the European species *Fasciola terrestris* by O. F. Müller in 1774 (now *Microplana terrestris*) was the earliest report in Western literature of a terrestrial flatworm; an Australian terricolan was not discovered and described for another 68 years.

2.3.1 Charles Darwin

Charles Darwin (Plate 2.1), naturalist on voyage of the *Beagle* (1831-1836), was the first person to describe Terricola from Australia. The *Beagle* visited Tasmania in February 1836 and remained in Hobart for ten days. It was here that Darwin obtained one species, *Planaria tasmaniana.* It was found on his first full day, in considerable numbers *beneath decayed trees in the woods of Van Diemen's land* (Darwin 1844; Nicholas & Nicholas 1989); the precise location in the open forest from which the flatworms were collected is unknown.

The specimens were retained alive on board ship for some two months for observation. During this time, as part of experiments on regeneration, one flatworm was bisected (Fig. 2.1). Kept in a box together with decayed wood for twenty-one days, the specimens increased in size, leading Darwin to believe that the flatworms *live on decayed vegetable matter, namely decayed wood* (Darwin 1844). As the *Beagle* approached the equator all the specimens perished from a combination of neglect, experimental treatment and the heat in the warmer latitudes where ….*as soon as the cessation of life allows the ordinary laws of change to act, their entire bodies become soft and fluid, with a rapidity which I have never seen equalled* - a classic description of the death and autolysis of a terrestrial flatworm. (Darwin 1844 p242, 1845 p 27, and Darwin's *Beagle Zoology Notes*, courtesy of Prof R.D. Keynes).

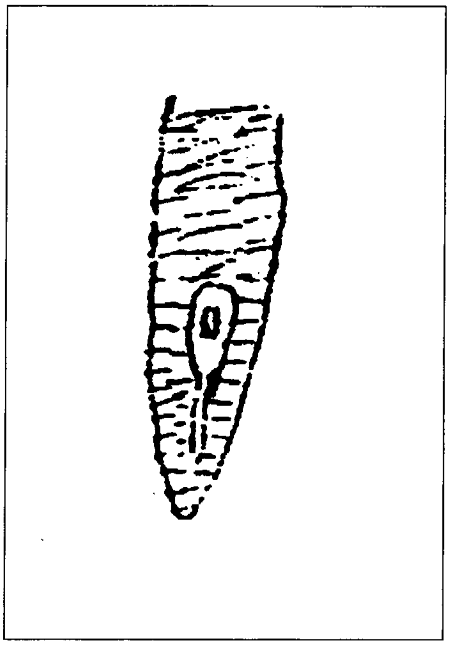


Figure 2.1 Charles Darwin's notebook, page 366. 31st March. Sketch of posterior half of Planarian collected from near Hobart, Tasmania. The species was later described by Darwin (1844) as *Planaria tasmaniana* (courtesy, Prof Richard Keynes, Cambridge).

2.3.2 Henry Moseley

The next episode in the discovery of Australian Terricola came with the appointment in 1872 of Henry Nottidge Moseley (1844 - 1891) as the naturalist on the scientific staff of the *Challenger* expedition (1872 -1876). Moseley had a particular interest in terrestrial flatworms having earlier described the anatomy and histology of a number of species he collected whilst a member of the Government Eclipse Expedition to Ceylon in 1871 (Moseley 1875).

During the visit to Sydney by the *Challenger* in April to June 1874, Moseley collected four species of Terricola which he later accommodated in a new genus *Caenoplana*. The species *Caenoplana coerulea, C. sanguinea, C. subviridis* and *Caenoplana sp.* ?var of *subviridis*, were obtained at Parramatta, and *C. subviridis* at Sir William McArthur's 10,000 acre property at Camden Park. The generic diagnosis (Moseley 1877b) of *Caenoplana* is notable as being the first to include histological characters in the taxonomy of Terricola. This was the last work on the Terricola undertaken by Moseley. Following his appointment as the Linacre Professor of Human and Comparative Anatomy at Oxford, Moseley's interests increasingly inclined towards anthropology.

2.3.3 Non-British expeditions

Terricola are conspicuous by their absence from published reports and collections of non-British expeditions to Australia in the early nineteenth century. For example none of the eight French expeditions to Australia between 1792 and 1832 (Fletcher 1901) appear to have yielded collections of terrestrial flatworms. However this may be an artefact as the collections in the Museum D'Histoire Naturelle, Paris, are arranged by expeditions, collectors or workers rather than organized along taxonomic lines; specimens of particular taxa must be searched for in each collection (B.J. Smith, pers. comm). Whatever the cause, it is notable that despite numerous and well organized French expeditions world-wide there is a complete absence of French museum collections noted in the list of sources of Terricola specimens in Graff 's monograph of the Terricola (Graff 1899).

Some specimens of Australian flatworms presently held in museums in Germany and Poland were collected on early expeditions by German scientists or professional collectors. An unknown collector obtained specimens of *Artioposthia grubei*, labelled "Australia", now in the Wrocklaw (Breslau) University Museum of Natural History. The collections in the Hamburg and Berlin Natural History Museums include *Geoplana daemeli* obtained from Rockhampton by Rolle, from Sydney by Dämel, and *Rhynchodemus putzei* from Rockhampton collected by an "E.K". Krone collected *Geoplana moebiusi* Graff, now lodged in the Zoologisches Museum, Berlin, during the German Transit of Venus Expedition of 1874 to Auckland Island (Fraser 1986).

These Australian flatworms in British and European collections represent the only Terricola material deposited in overseas institutions by early international collectors and scientists. From the 1880s onwards the Australian and New Zealand Terricola faunas were to be largely discovered and described by naturalists and scientists living in Australia and in New Zealand.

2.4 THE COLONIAL PERIOD

In the period 1888 until Federation in 1901, zoologists in Australia (Plate 2.1) produced numerous publications in which were described a total of 77 new species and sub-species of terrestrial flatworms from the Australian colonies. The influence of the Linnean Society of New South Wales, the Royal Society of Victoria, and the Field Naturalists' Club of Victoria can be readily measured in the number and quality of their publications. The membership of these institutions comprised a synergetic mix of professional and skilled amateur biologists with a sense of purpose, enthusiasm and vitality in describing the Austral flora and fauna.

In this period the descriptive taxonomy of terrestrial flatworms undertaken by Australian-based workers was largely based upon external characters. Early investigations the Australian Terricola by Dendy and Spencer were deliberately suspended in favour of forwarding specimens of Australian taxa to

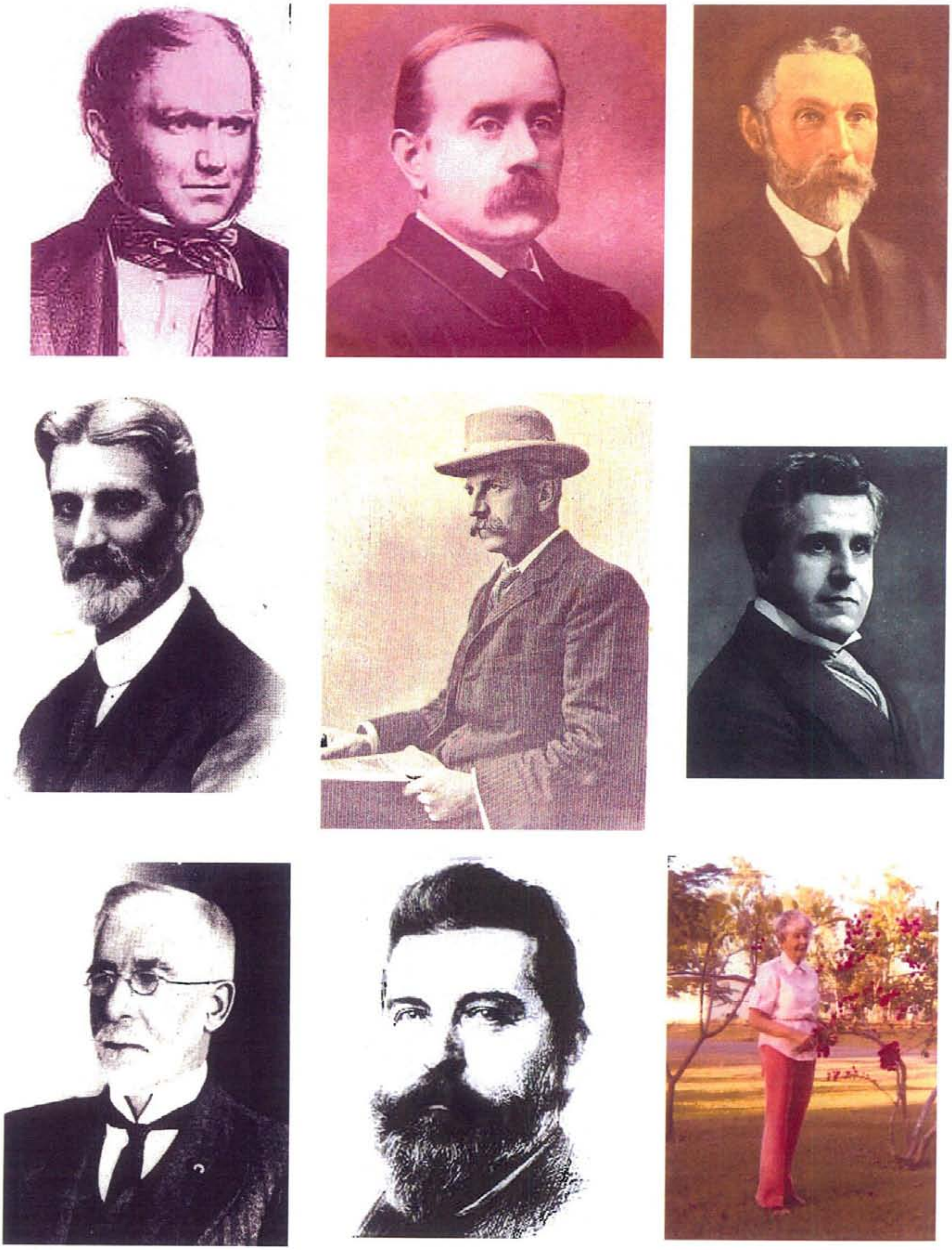


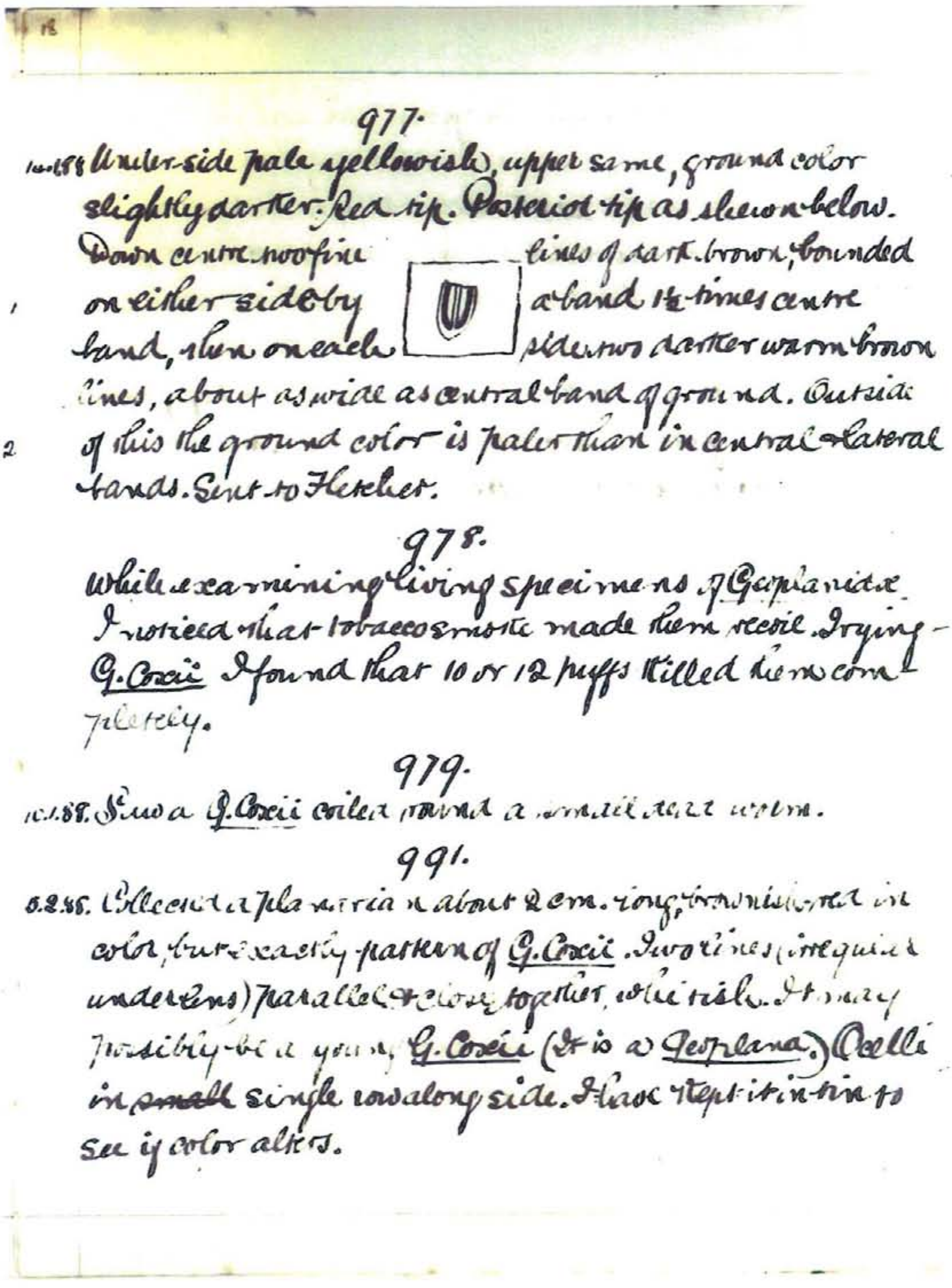
Plate 2.1 Former discoverers and taxonomists of the Australian Terricola.
Top Row: Charles Darwin, Henry Moseley, Joseph Fletcher.
Middle Row: Alexander Hamilton, Walter Baldwin-Spencer, Arthur Dendy
Bottom Row: Thomas Steel, Ludwig von Graff, Marion Fyfe.

Ludwig von Graff in Austria for detailed microanatomical investigations. Towards the close of the century research on the Terricola worldwide culminated in the publication of the *Monographie der Turbellarien II. Tricladida Terricola (Landplanarien)* (Graff 1899).

2.4.1 James Fletcher and Alexander Hamilton

The first report of Terricola in Australia by a local biologist, J. J. Fletcher, concerned the occurrence of the cosmopolitan species *Bipalium kewense* in Sydney (Fletcher 1887). James Joseph Fletcher (1850 - 1926), Secretary of the Linnean Society of New South Wales, was a former science teacher, and an avid biologist. He collaborated with Alexander Greenlaw Hamilton (1852-1941), a school teacher, keen naturalist, and personal friend living at that time in Hartley in the Mudgee district north west of Sydney. In a paper on *Notes on Australian land-planarians, with descriptions of some new species. Part I* (Fletcher and Hamilton 1888), they described and provided ecological notes on six new species of geoplanid flatworms, and six species of rhynchodemids representing the first records of the latter family in Australia. Most of these specimens, from the Mudgee district and Blue Mountains areas, have been lost. A consequence of their observations on living geoplanids was the subsumption of Moseley's new genus *Caenoplana* within *Geoplana*.

Anticipation of a second paper on Australian land planarians, foreshadowed in the first paper (page 349, Fletcher and Hamilton 1888), led Alex Hamilton to keep a note book *Planarians. Notes since publication of first paper* (Mitchell Library catalogue ML MSS 2009/108, in McCarthy 1991, erroneously attributed to J.J. Fletcher). These unpublished notes (Fig. 2.2) contain observations on terrestrial flatworms obtained in the Mudgee district, June 1887 - September 1897, and on those found in the Illawarra district, November 1887 - April 1891. Hamilton provides details of the microhabitat, locomotion, and prey of flatworms, and mentions some new species which he describes with the aid of small sketches of body markings. Unfortunately none of this described material is extant, and none of these species has been subsequently encountered. Some of these observation on flatworms received brief mention in *Bush Rambles* (Hamilton 1937), a collection of essays on natural history originally published around the 1880's in the *Sydney Quarterly Magazine*. Following their 1888 paper, neither Fletcher or Hamilton individually or



18

977.

10.1.88 Under-side pale yellowish, upper same, ground color
slightly darker. Red tip. Posterior tip as shewn below.
Down centre two fine lines of dark brown, bounded
1 on either side by a band 1½ times centre
band, then on each side two darker warm brown
lines, about as wide as central band of ground. Outside
2 of this the ground color is paler than in central & lateral
bands. Sent to Fletcher.

978.

While examining living specimens of Geoplanidae
I noticed that tobacco smoke made them recoil. Trying
G. Coxii I found that 10 or 12 puffs killed them com-
pletely.

979.

10.1.88. Saw a G. Coxii coiled round a small earth worm.

991.

8.2.88. Collected a planarian about 2 cm. long, brownish red in
color, but exactly pattern of G. Coxii. Two lines (irregular
underlines) parallel & close together, whitish. It may
possibly be a young G. Coxii (It is a Geoplana.) Ocelli
in ~~small~~ single row along side. I have kept it in tin to
see if color alters.

Figure 2.2 An extract from A.G. Hamilton's note book *Planarians. Notes since publication of first paper* (June 1887 – 22 April 1891). Note 978 records the fatal effects of passive tobacco smoke on *Geoplana coxii*.

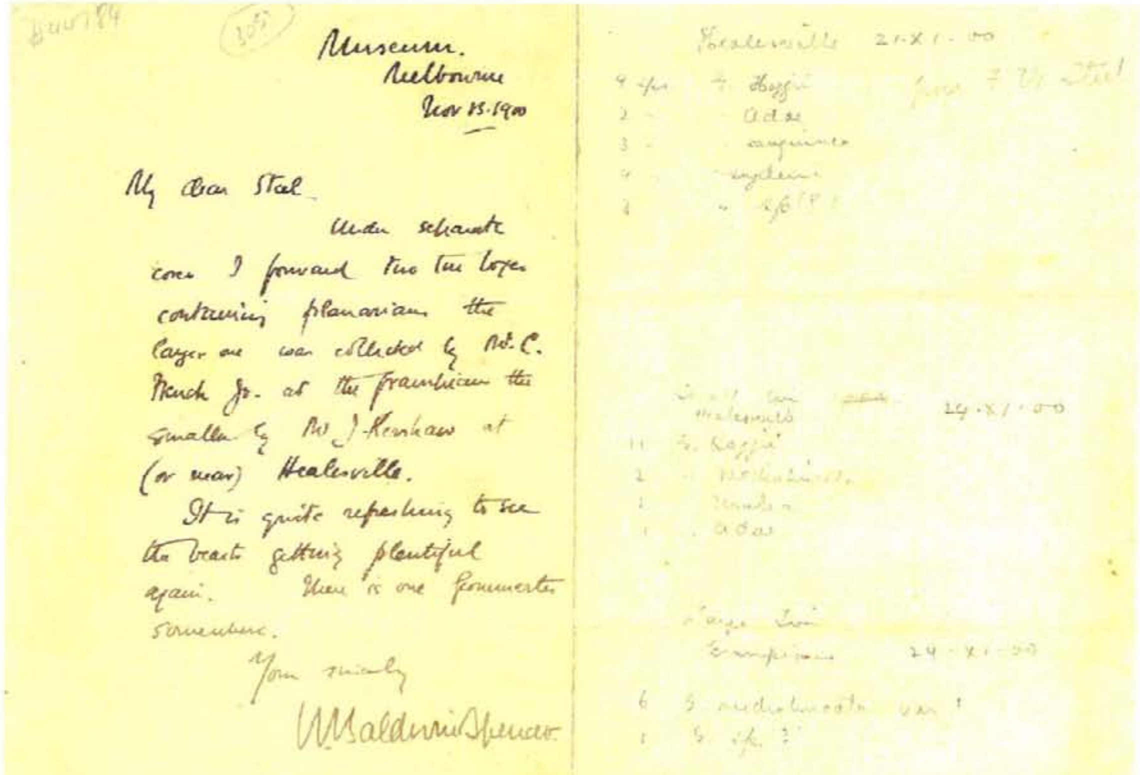
collaboratively published further scientific papers on flatworms. In Australia the centre of research on terrestrial flatworms shifted from Sydney to Melbourne following the appointment in 1887 of W. Baldwin Spencer to the newly founded Chair of Biology, University of Melbourne.

2.4.2 Walter Baldwin Spencer

Walter Baldwin Spencer (1869-1929) was a former assistant to Henry Moseley, the Linacre Professor of Human and Comparative Anatomy, Oxford. It was from Moseley that Spencer gained knowledge and considerable experience in the then new histological techniques, and developed a lasting interest in anthropology and museum work. Following his arrival at the University of Melbourne in March 1887 Spencer set about developing the new Department of Biology. One of his earliest acts was to appoint Arthur Dendy of the British Museum and a fellow graduate of Owens College, Manchester, as demonstrator and assistant lecturer in Biology. Thus began one of the most remarkable professional associations in early Australian science in the Colony of Victoria (Smith *et al* 1991). Both men sought and studied terrestrial flatworms, and started the vogue for digging under rotten wood in search of new specimens – *"already the Biology School has developed into a society of log-rollers"* observed the *Melbourne University Review* (Mulvaney and Calaby 1985). Each named a flatworm in the other's honour: *Geoplana spenceri* (by Dendy 1890), and *Geoplana dendyi* (by Spencer 1891). It was a time of new paradigms in evolution and systematics, flourishing scientific societies, and exciting discoveries, and Spencer's enthusiasm was manifested in *"founding scientific clubs and vacationing in the wilds - always full of that urge to scientific endeavour"* (Mulvaney and Calaby 1985).

Spencer contributed two papers on Australian terrestrial flatworms, and also provided Dendy with flatworm specimens collected during his field work in Victoria, Tasmania, and Queensland. He described ten new species of flatworms collected on the field trip of the Victorian Field Naturalists' Club to the Upper Yarra district, Victoria (Spencer 1891).

His second paper concerned the terrestrial flatworms collected by Mr Thomas Whitelegge during the 1887 Australian Museum Expedition to Lord Howe Island (Etheridge 1889). These were entrusted to Spencer on the recommendation of



Museum.
Melbourne
Nov 15.1900

My dear Steel

Under separate cover I forward two tin boxes containing planarians the larger one was collected by Mr C. French Jr. at the Grampians the smaller by Mr J. Kershaw at (or near) Healesville.

It is quite refreshing to see the beasts getting plentiful again. There is one fomenter somewhere.

Yours sincerely
W. Baldwin Spencer

Healesville 21-XI-00
4 spn G. Hoggii   from ? W. Steel
2 " adae
3 " sanguinea
4 " [illegible]
1 " sp (?)

Small tin [illegible]
Healesville 29.XI.00
11 G. Hoggii
2 " mediolineata
1 [illegible]
1 " adae

Large tin
Grampians 24-XI-00
6 G. mediolineata var ?
1 G. sp. ?

Figure 2.3 A note written on 15 November, 1900, by W. Baldwin Spencer to Thomas Steel concerning a consignment of terrestrial flatworms being forwarded to Steel. On the reverse of the letter Steel has pencilled a list of the species and number of specimens received. (Letter in Author's collection).

Fletcher to whom the collection was first offered by the museum trustees (Spencer 1892). Eight species were described, two of which were accommodated in the new genus *Cotyloplana* erected by Spencer. The remaining six species were placed in *Rhynchodemus*. Spencer indicated (1892 p43) that this account was the first part of a two part study, the second part of which was to be a description of the anatomy of the species. This paper never eventuated. Carmine-stained sections of the two *Cotyloplana* species prepared by Spencer, now in the Natural History Museum, Vienna, were forwarded to Graff for investigation and description (Graff 1899). Spencer increasingly became involved in anthropological research, for which he is best known, and led a successful multifaceted scientific and public career (Mulvaney and Calaby 1985), though maintained an interest in the Terricola and directed specimens to other workers (Fig. 2.3).

2.4.3 Arthur Dendy

Arthur Dendy (1865 - 1925) described 34 new species and subspecies of Australian Terricola in 12 papers (Dendy 1890a,b; 1891; 1892a,b,c,d,e,f; 1894a,b; 1895) whilst at the University of Melbourne, and later (Dendy 1915) following the meeting of the British Association for the Advancement of Science in Western Australia and Tasmania in 1914. His first paper concerned a detailed anatomical and histological description of *Geoplana spenceri*, though he subsequently adopted the practice of describing external characters in scientific publications and then forwarding named specimens to Professor L. von Graff for histological examination, comparison with other taxa from other parts of the world, and incorporation in his monograph of the terrestrial flatworms (Dendy1892f; 1894a). Dendy was a fine draughtsman, and species descriptions in five of these early papers are illustrated by beautiful coloured lithographs of the flatworms (see Plate 4.1).

In 1894 Dendy moved to New Zealand where he took charge of the Department of Biology at Canterbury College, Christchurch. Over the next seven years he described 43 new species and subspecies from New Zealand in a series of seven papers (Dendy 1895a,c,d; 1896; 1897; 1901, 1904; 1909; 1911). The absence of illustrations accompanying these original descriptions has hampered subsequent taxonomic work on New Zealand Terricola (Winsor *et al* 1998). Only the first three of these papers were published in time for the new species to be incorporated in Graff's Monograph of the Terricola.

Dendy was an excellent science communicator, and radical in scientific thought especially in the field of evolutionary biology. Although best known for his extensive high quality research on sponge taxonomy (Smith *et al* 1991), Dendy had a long standing interest in the animals habitually found under logs, stones, and the rotten bark of trees, for which he coined the term *cryptozoic fauna* (Dendy 1890). His scholarship can best be summed up as *veracity in record, swift work, accurate observation, clear description, untiring industry, and enthusiasm for biological knowledge* (G.P. Bidder in McLachlan 1935).

2.4.4 Ludwig von Graff

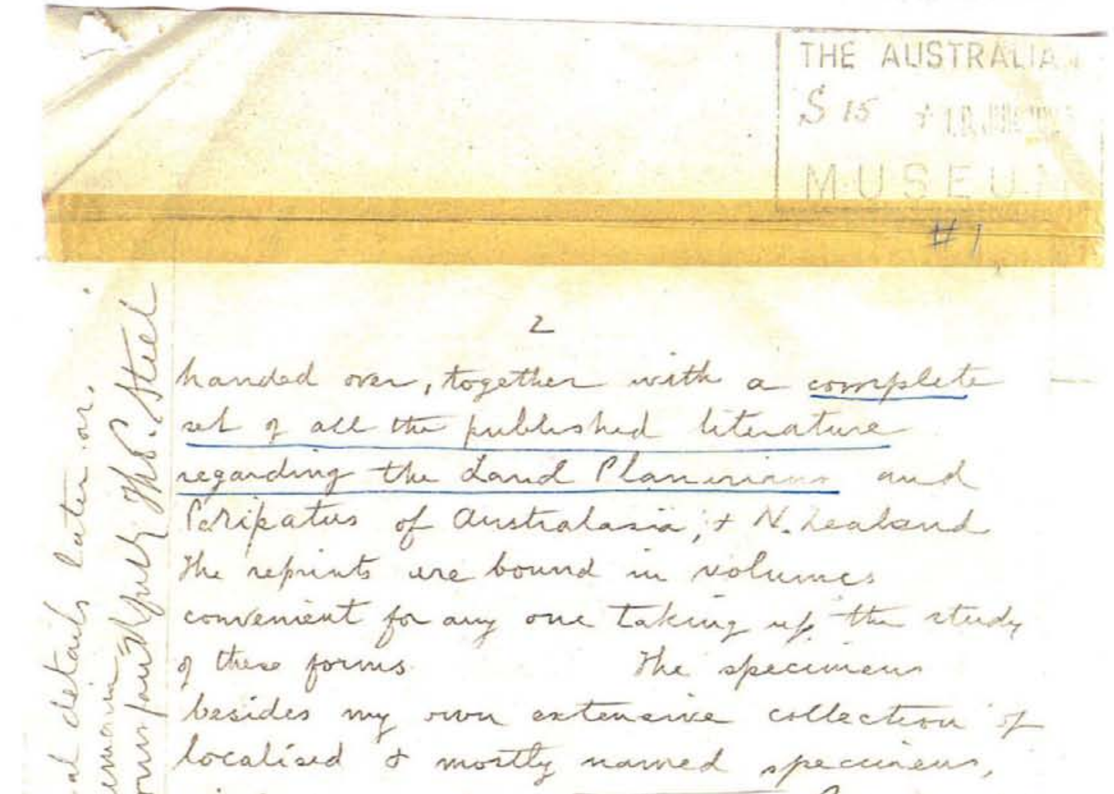
Pre-eminent in Turbellarian research, Ludwig von Graff (1851 - 1924) of the K.K. Carl-Franzens University, Graz, Austria, proposed the preparation of a monograph on the Terricola (*in litt* to Dendy, Meeting of the Royal Society of Victoria, Thursday 11 December 1890, in *Proc. Roy. Soc. Vict.1891, 3:161*) following the successful publication of his monograph on the Acoela in 1891. In Australia this project was actively championed by Arthur Dendy (Ibid; Dendy 1892f, page 369) who over some five years provided Graff with the bulk of specimens of Australian terrestrial flatworms. Publication of the *Monographie der Turbellarien II. Tricladida Terricola (Landplanarien)* (Graff 1899) consolidated knowledge of the Terricola world-wide up to 1895. Four new Australian flatworm species, and two new species from New Zealand were described in this monograph. Graff (1896b) had earlier erected the genus *Artioposthia* to accommodate certain Australian geoplanid taxa, and established the family Cotyloplanidae to accommodate Spencer's genus *Cotyloplana*. The *Monographie*, and subsequent reviews (Graff 1912 - 1917) set a standard rarely attained since, and has provided the framework and stimulus for further morphological, histological and systematics research following into the next century (Rieger 1998).

2.4.5 Thomas Steel

Thomas Steel (1858-1925), an industrial chemist and highly respected authority on terrestrial flatworms and *Peripatus*, published four papers (Steel 1897; 1901a, b, c) in which he described sixteen new flatworm taxa collected in New South Wales, Tasmania, South Australia, Queensland, and Western Australia, and also a new species from Fiji. He laid firm foundations for future studies on Australian Terricola with accurate and illustrated species descriptions, and in systematically investigating killing and preservation reagents and methods. He was the only worker of his time to designate Type specimens and provide their museum registration numbers in his species descriptions.

Frustrated in being unable to readily access specimens of Australian Terricola deposited in the Museum of Natural History, London, and unable to obtain vital scientific literature, Steel lodged all his Types and a reference collection of typical specimens of previously described Australian forms in the Australian Museum, Sydney, where he knew they would be readily available to Australian workers (Steel 1897 p115). He also sent similar reference sets of specimens to the Museum of Natural History, the Smithsonian Institution, and the Swedish Museum of Natural History. This was in sharp contrast to his contemporary workers who failed to designate Types in their descriptions, and who sent their collections to the Museum of Natural History, London, from where they can still now only be retrieved with some difficulty. In the weeks preceding his death Steel bequeathed his entire specimen collection, reprints and reference library on the Terricola to the Australian Museum (Steel, *in litt* 17 June 1925, Australian Museum Correspondence S15, 19 June 1925; Steel, *in litt* circa 2 August 1925, Australian Museum Correspondence 4 August 1925).

The Steel collection included *....a set of Co-types of Prof Dendy's species from Australia, Tasmania & New Zealand, which I got direct from him & are practically complete. There will thus be no serious difficulty for any one taking either group* (viz. Onychophora or Terricola) *seriously in working through the collections for Aust, Tasmania & New Zealand. Since then Prof Dendy has presented to the British Museum his entire collection of Land Planarians from Australasia and New Zealand with the exception of a typical series of mine which I gave him & which he*



THE AUSTRALIA

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MUSEU

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handed over, together with a complete set of all the published literature regarding the Land Planarians and Peripatus of Australasia, & N. Zealand. The reprints are bound in volumes convenient for any one taking up the study of these forms. The specimens besides my own extensive collection of localised & mostly named specimens, include a set of Co-types of Prof. Dendy's species from Australia, Tasmania & New Zealand which I got direct from him & are practically complete. There will thus be no serious difficulty for any one taking either group up seriously in working through the collections for Aust. Tasmania & N. Zealand. Since then Prof. Dendy has presented to the British Museum his entire collection of Land Planarians from Australasia & New Zealand with the exception of a typical series of mine which I gave him & which he has kept for demonstration purposes, they being well preserved. Trusting that this will make the position clear, but that I will have an ~~opportunity~~ opportunity to fix up final details later on.

I remain

Yours faithfully

Thos. Steel

Figure 2.4 Extract of the letter of 17 June 1925 from Thomas Steel to the Director of the Australian Museum, concerning the presentation of his literature and specimen collections to the museum. On this page Steel advises that his collection contains an almost complete set of Co-Types of Dendy's species, given by Dendy to Steel.

has kept for demonstration purposes, they being well preserved. (Steel, *in litt* 17 June 1925, Australian Museum Correspondence S15, 19 June 1925) (Figure 2.4). Steel died at his home on 17 August 1925.

2.5 THE POST-FEDERATION PERIOD

By the early 1920s all the early zoologists who had worked on Australian terrestrial flatworms during the late 19th century had died or were no longer active in research. Continuity of systematic and microanatomical research on Terricola by the Graff School was maintained at the Zoological Institute in Graz, and in other countries including France and South America. However zoologists in Australia and New Zealand working on the Terricola did so in relative isolation, and without the benefits of a solid local tradition of anatomical research on terrestrial flatworms. As a consequence, the Austral terrestrial flatworm fauna is the most poorly anatomically-known worldwide. Present Austral and Antipodean taxonomists of the Terricola are now grappling with the practical consequences of the "tyranny of distance" on these early workers.

2.5.1 Lucy M. Wood

A Masters degree student at the University of Sydney, Lucy Wood described four new species of flatworms - *Geoplana citrina, G. barringtonensis, Artioposthia harrisoni,* and *Platydemus assimilis* - collected from Barrington Tops at the southern end of the Mt Royal range, New South Wales by a University party led by Prof. L. Harrison in January and February 1925 (Wood 1926). She provided an succinct account and figure of the copulatory apparatus of *A. harrisoni*, and also a new record of *Geoplana coerulea.* Three of these taxa are further considered in this thesis.

2.5.2 The Graz School - Hans Tavorik and Lothar Heinzel

Professors Böhmig and Meixner succeeded Graff at the Zoological Institute of the University, Graz. Under their supervision named specimens of Australian Terricola forwarded by Dendy to Graff in preparation for the *Monographie* were subsequently further studied by postgraduate research students.

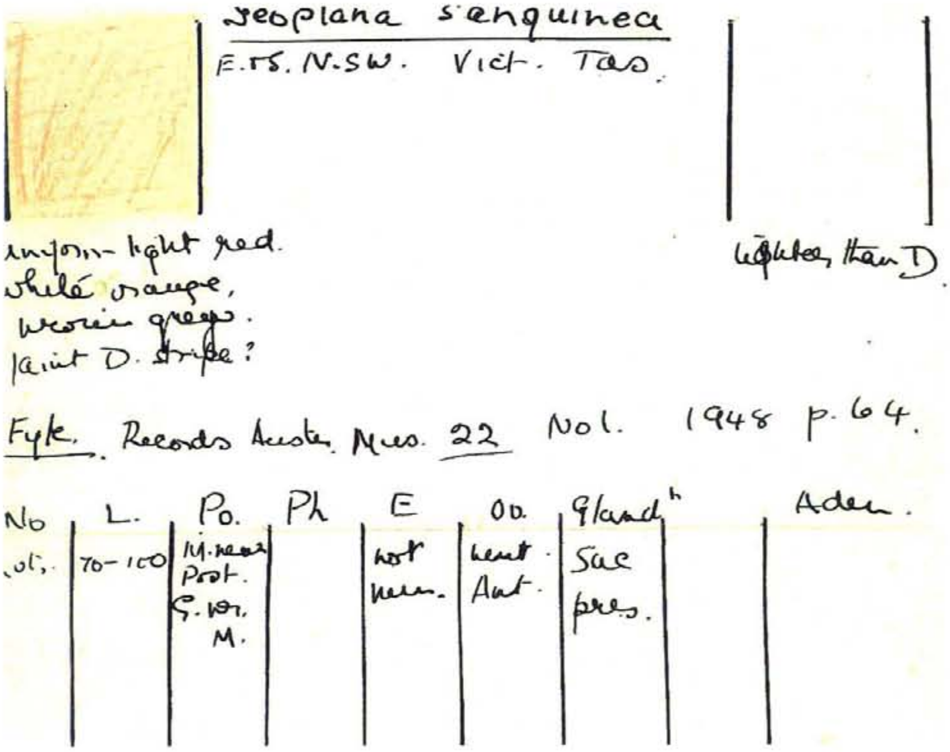
Hans Tavorik submitted a PhD dissertation in 1928 on *Untersuchungen an Landplanarien Viktorias* (*Investigations in respect to Victorian land planarians*), a study of ten flatworm species based upon non-Type material (Tavorik's thesis is in the O.O. Landesmuseum, Linz, Austria. A copy was kindly obtained for LW by Dr Artur Neboiss, formerly of the Museum of Victoria). Unfortunately there are no illustrations in the handwritten thesis, and none of the results were published nor subsequently used here. The sectioned material is not in the Graff Collection donated by the University to the NMW in 1978 and is considered lost.

Lothar Heinzel (1929) published his general anatomical and histological investigations of several species of rhynchodemids including the Victorian species *Platydemus victoriae*. The material for this study was the original specimen from Croajingalong (east Gippsland), Victoria, sent by Dendy to Graff (Graff 1899 p525), now in the NMW. The species is the Type of the genus *Platydemus*, and Heinzel (1929) provided a succinct description of the histology including that of the copulatory apparatus of this species.

2.5.3 Marion Fyfe

Marion Fyfe (1897-1986) of the Otago University, New Zealand, was the first Antipodean zoologist to undertake an anatomically-based taxonomic revision of the regional Terricola. Shortcomings in her published anatomical work, when compared to that of the Graz School, do not detract from her pioneering work in difficult times and under difficult circumstances. As part of her taxonomic revisions of New Zealand terrestrial flatworms, Fyfe prepared flatworm identification cards (Fig. 2.5), and examined Dendy's New Zealand specimens of Terricola in the British Museum. Specimens identified by Dendy as *Geoplana sanguinea*, thought to be introduced from Australia, were found anatomically to be *Artioposthia australis*. To compare the latter species "with the real *Geoplana sanguinea…*" (in Fyfe 1948 page 64), she examined a series of specimens of *G. sanguinea* from Australia, and described the reproductive organs of non-Type specimens collected from Victoria (Fyfe 1948); this work and material shall be considered further in this thesis.

Apart from taxonomic revisions and descriptions of new taxa from New Zealand (Fyfe 1937; 1944; 1946; 1947; 1956), she also described three new species



Geoplana sanguinea

E.S. N.S.W. Vict. Tas.

uniform light red.
while orange,
brown green.
faint D. stripe?

lighter than D.

Fyfe. Records Austr. Mus. 22 No 1. 1948 p. 64.

No	L.	Po.	Ph	E	Od.	Glandn		Aden.
[illegible]	70-100	M. near Post. G. br. M.		not seen.	unit. Ant.	Sac pres.		

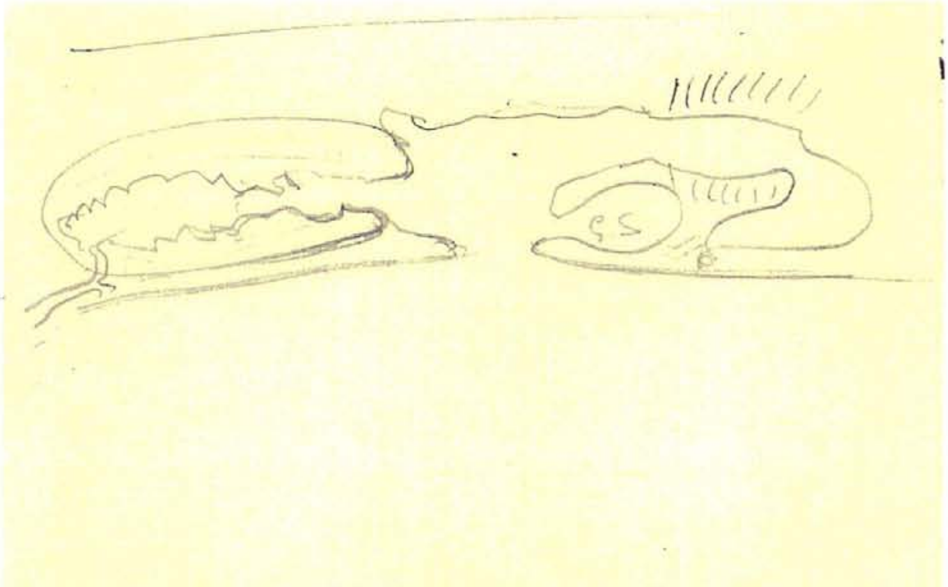


Figure 2.5 An example of the flatworm identification cards prepared by Marion Fyfe, in which the colour and pattern and other external features were noted. On the reverse of the card for *Geoplana sanguinea* shown here, is a simplified line drawing of the copulatory apparatus of the species.

from the New Zealand subantarctic islands *Cape Expedition* (Fyfe 1953). This latter collection, made during World War II by coast watching parties in these strategically important islands, has been re-examined in this study, together with other specimens of Terricola collected from New Zealand subantarctic islands and now held by the Museum of New Zealand. Earlier, in 1907 the Philosophical Institute of Canterbury mounted an expedition to Auckland and Enderby Islands during which George Marriner collected three species of flatworms later described by Dendy (1909), the Type specimens of which are lost. Sporadic scientific visits to Macquarie Island (Australia), situated 54°35' S 158°55' E, began in 1820, but planned long term scientific investigations did not commence until 1911 (Selkirk *et al* 1990). Two species of terrestrial flatworms were found for the first time on Macquarie Island in 1998 by Dr R. Blakemore during a survey of earthworms in 1998. One of these species of flatworms is described in this thesis.

2.6 RECENT RESEARCH AND COLLECTIONS

2.6.1 Contributions by the present author

During the period 1970-1990 there was a strong demand by State Government conservation and wildlife agencies for data on the distribution of flora and fauna for specified study areas in Victoria and Tasmania. In Victoria this demand stimulated the formation of the Field Survey Group (FSG) of the Field Naturalists' Club of Victoria (FNCV), a group of amateur botanists and zoologists which developed close working relationships with the staff of the State Herbarium and the Museum of Victoria in surveying study sites. These opportunities and associations, together with mentoring by museum scientists, stimulated a serious interest and commitment to distributional and taxonomic studies of terrestrial flatworms by myself. At this time the Terricola faunas of Australian and New Zealand were the most poorly known in the world, with only some 12% of the 82 species investigated anatomically, thus setting the stage for the present study.

My research on the Australian Terricola included faunal surveys and new records (Winsor, 1973a, b, c; 1977; 1979; 1984b; 1985;1986, 1997a), establishing an extensive collection of Terricola mainly from eastern Australia (Fig. 2.6), the development of collecting and microscopical techniques (Winsor 1978, 1991a; 1997b,c; 1998a), aspects of terricolan biology (Winsor 1980; 1983a; 1995; 1998e,f; Winsor *et al* 1998), information sheets (Winsor 1997a, b, c, d, e; 1998f; 1999; 2003), and biogeography (Winsor 1977a; 1998c). Taxonomic research included a revision of *Bipalium kewense*, at one time considered to be native to Australia (Winsor 1981, 1983b, 1985), the exotic species *Kontikia orana* and *Platydemus manokwari* (in Winsor 1986, 1990), description of *Pimea monticola* from New Caledonia (Winsor 1991b), and aspects of terricolan taxonomy and functional anatomy (Winsor 1998b, d). The genera *Australoplana, Lenkunya, Reomkago, Tasmanoplana* and *Fletchamia* were erected in a provisional classification of Australian geoplanid flatworms (Winsor 1991c). *Caenoplana* was redefined, and five groups were identified within the heterogeneous genus *Artioposthia.*

2.6.2 Research overseas

Recent taxonomic investigations on adventive Austral flatworms in the United Kingdom, North America, South Africa and Pacific include *Australoplana sanguinea alba* Auctorum from the Scilly Isles, (Jones 1981), *Arthurdendyus albidus* Jones & Gerrard (Jones & Gerrard 1999), *Caenoplana vaga* (Hyman) from California, conspecific with the Australian species *Caenoplana coerulea* (Moseley) (Ogren 1989), and the Australian species *Parakontikia ventrolineata* (Dendy) considered conspecific with *Kontikia mexicana* (Hyman) by Jones *et al* (1998). Taxonomic and distributional studies on New Zealand terrestrial flatworms are presently being undertaken by Johns (1993; 1998; Johns *et al* 1998) and the present author.

Nomenclatural changes to Terricola, based upon existing published data, were made in a series of publications forming the Index to Land Planarians of the World (Ogren *et al* 1987; 1988a; 1988b; 1989; 1990; 1991; 1992a,b; 1993a,b; 1994; 1995; 1996; 1997a,b; 1998; 1999; Kawakatsu *et al* 2000; 2000; 2002). The changes included the formation of new subfamilies and genera, necessary because of the exclusion of many Austral geoplanid taxa from revised, mostly Neotropical, geoplanid genera.

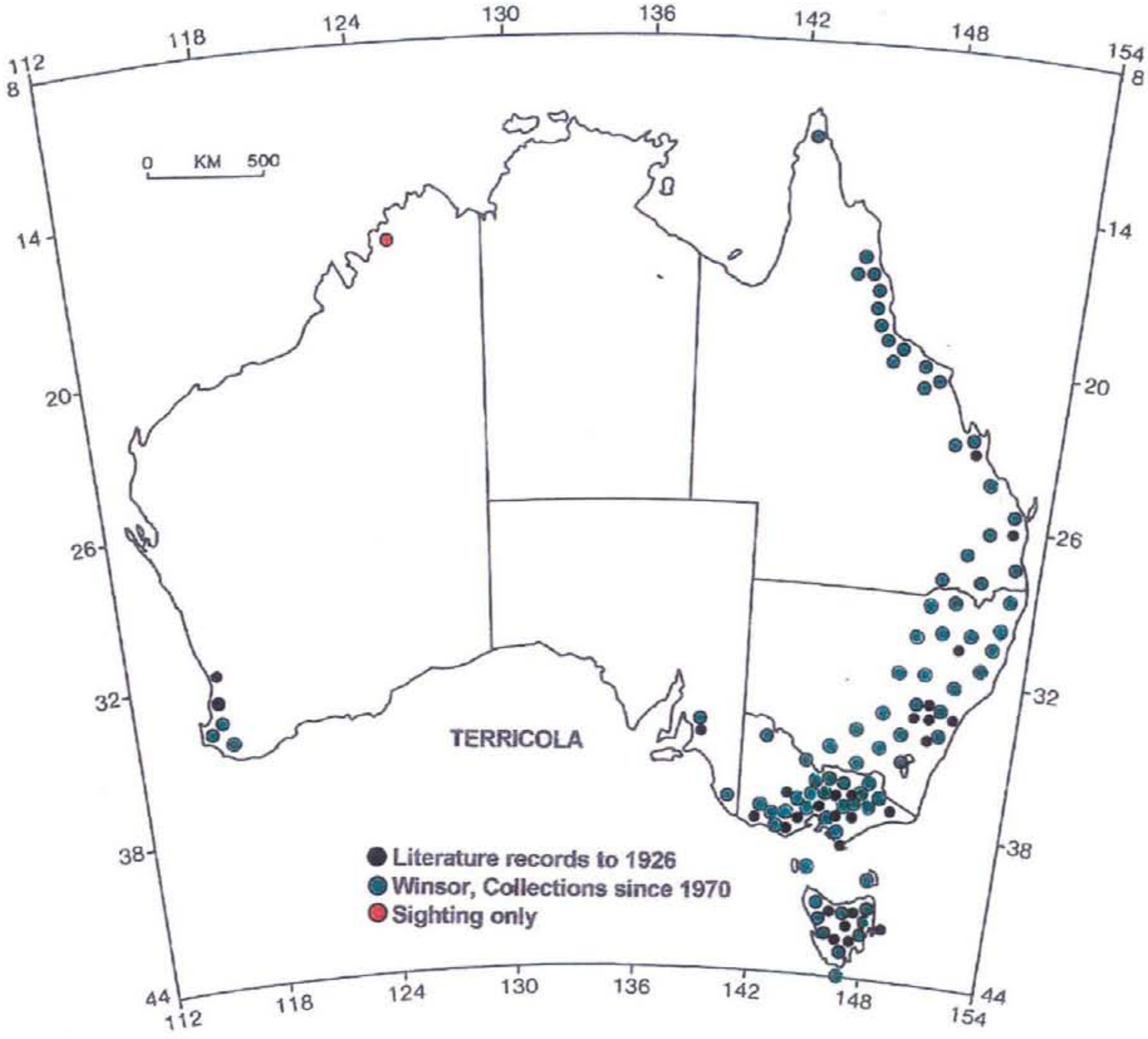


Figure 2.6 Literature records and collections of Terricola in Australia to 1926, and approximate areas of subsequent collections made by, or reported to the author.

The research forming the basis for this thesis builds directly on the foundations laid by the early Austral workers, in particular the work of Thomas Steel whose foresight in retaining Terricola Type specimens in Australia, and lodging reference works in the Australian Museum, greatly facilitated this study. In order to provide a sound taxonomy, the next step is the investigation of the microanatomy of the Australian Terricola and the identification of reliable taxonomic characters.

Chapter 3

Materials and Methods.

The parenchymatic Terricola cannot be dissected ...they must be microtomized, and as many of them are big, their sections require dozens of slides. For that reason the taxonomy of the Terricola has not yet attained the degree of security as that of the earthworms.

Marcus (1953)

In flatworms and nemerteans, detailed analyses of internal morphology is now routine practice, despite the tedious histological preparations or stained mounts which have to be prepared. Minelli (1993)

Attempts to section this specimen failed due to hardening. Jones & Gerard (1999)

3.1 INTRODUCTION

Improvements in the systematics of the Tricladida have largely come about through the more thorough and extensive examination of traditional, morphological characters (Sluys 1989a; 1990; 2001; Sluys *et al* 1998; Minelli 1993). Combined with molecular studies, this provides a *total evidence* approach to the systematics of the component taxa (Baguñà *et al* 2001).

Terricola are difficult animals with which to work. They can decompose rapidly, contract strongly during fixation causing anatomical artefacts, and can, because of their muscularity, cause technical difficulties during their preparation for microscopical examination. Techniques advocated in various texts and specialist papers for Triclads - chiefly the Paludicola - are often unsatisfactory for the generally larger more muscular Terricola. On the basis of comparative trials, the following detailed procedures have been established for the collection, handling, fixation, histology and storage of terrestrial flatworms for taxonomic research. The account includes an historical review of methods used on Terricola, a rationale of available techniques, and the methods used in this study.

3.2 HISTORICAL OVERVIEW OF METHODS

General information on microhabitat and histological methods prior to 1897 is summarised by Graff 1899. Specific information on microhabitat, and collection of Terricola by hand picking, and methods for killing and preservation of these flatworms is provided by several authors (Steel 1897; 1901a, b; Gelei 1929; Baylis & Munro 1941; Ogren 1955, 1981; Froehlich 1956; Ball & Reynoldson 1981; Ball & Sluys 1990; Winsor 1991a; 1996a,b; 1998). Trapping, and soil and litter extraction methods appear to be seldom used for taxonomic purposes, mostly being employed as sampling methods in ecological and related studies of exotic pest species of terrestrial flatworms.

3.2.1 Trapping methods

Flatworm "traps" essentially mimic the natural refugia beneath which flatworms are frequently found – leaf litter, fallen timber, rotting logs and stones. Trapping techniques include plantain leaves laid on the ground (Moseley 1875), small boards laid directly on the ground (Ogren 1955), and wetting areas of dry soil with water and covering with a damp board (Ogren 1955). Of the wood, ceramic tile, corriboard plastic and polystyrene or ceramic tile refuge traps assessed for efficiency in catching the feral species *Arthurdendyus triangulatus* in the United Kingdom, ceramic tile with a 5 mm thick polystyrene sheet caught the greatest numbers of flatworms and had the highest number of positive counts (Blackshaw 1990). Heavy-duty polythene bags, 22 cm square each filled with 1.5 kg of sand were successfully used as traps in studies on Terricola in modified habitats in New Zealand (Yeates *et al* 1998). Conventional pitfall traps have proved successful for flatworms in Button grass moorland sites in Tasmania (Michael Driessen *in litt*).

3.2.2 Extraction methods

Terricola can be extracted from soil samples using a 100 Watt light bulb as a heat source (Ogren 1955), but generally are not collected from soil or litter using dry extraction methods such as Berlese-funnel extractors. Success in obtaining Terricola from soil samples using wet extractors such as those of Macfayden or Kempson (Southwood 1966) appear not to have been reported. On-site formalin extraction (application rate of 4.5 litres of 0.2% formaldehyde per 1.2

square metre quadrat, after Raw 1959) is effective to a depth of at least 30 cm, the irritant bringing to the surface all specimens of *A. triangulatus* (Blackshaw & Stewart 1992).

3.2.3 Anaesthesia and general preservation

Terricola contract strongly when placed in a fixative. To minimise agonal contractions and preserve the specimens narcotization was achieved by gradually increasing the strength of the reagent in which the flatworms were immersed (e.g. in Moseley 1875), or by rapidly killing the flatworm by applying fixative, preferably hot, over the fully extended specimen (e.g. in Graff 1899). In an attempt to maintain the external morphology, markings, and especially the brilliant colours of Australian terrestrial flatworms, a variety of substances were investigated (Steel 1897,1901a), and narcotization of the worms using approximately 6% aqueous methylated spirit, and preservation in 1.2% formaldehyde solution or 80-85% spirit recommended. The concentration of formaldehyde recommended by Steel is sufficient to act only as a preservative, not as a histological fixative.

3.2.4 Taxonomic histology

Few workers provide details of, or references for, their methodology. The earliest histological investigations of the Terricola appear to have been undertaken by Moseley (1875) leading to the first use of histological characters for taxonomy of this group (Moseley 1877b). Using a razor wetted with absolute alcohol, Moseley cut sections from specimens fixed in strong alcohol or chromic acid and embedded in a mixture of sweet oil and white wax (?methyl salicylate and purified beeswax). The resulting sections were stained with ammoniacal carmine; none of his sections of Terricola are extant. Preserved specimens Moseley collected from Australia, now in the Museum of Natural History are excellently fixed and stain well.

Graff (1899) serially sectioned specimens previously bulk-stained with Borax Carmine, and mounted them in Canada balsam. He also stained sections with picrocarmine, Van Gieson's stain, Thionin, and Bismark brown. Graff considered Erlich's haematoxylin and eosin to be particularly useful in differentiating glandular secretions. Heidenhain's and Benda's iron haematoxylins were used to investigate the structure of flatworm eyes. Graff's sectioned material, now in the Naturhistorisches Museum, Vienna, is mostly intact and the stains are well conserved. Most of Graff's colleagues and students continued to use these methods into the early part of the twentieth century. Beauchamp (1926) used haemalum and eosin as a general stain, with a preference for the inclusion of orange G to provide optimal differentiation of glands. Unfortunately Beauchamp discarded all but the median series of slides of serially sectioned Terricola, seriously limiting any revisionary work using his material to provide the necessary data required by a modern whole-morphological approach. His sectioned material at the Muséum National d'Histoire Naturelle, Paris, exhibits marked stain fading.

Gelei (1929) provided an exhaustive account of investigative methods for research on Turbellaria. However he appears to have had little direct experience with methods for Terricola, relying instead upon personal communications from Reisinger and Bresslau: they recommend Carnoy's, Gilson & Preneyi, and Steinmann & Bresslau's fixatives, all of which, because of their poor penetration rates, are generally unsatisfactory for all but the smallest Terricola. For staining tissues they recommended Heidenhain's iron haematoxylin, Delafield's haematoxylin, Mayer's haemalum, and Haematein IA after Apathy, and the counterstain aqueous orange G or eosin. Gelei also mentioned the usefulness of trichrome methods of Mallory, van Gieson, Apathy and Flemming, but not specifically associated with the histology of land planarians.

Fyfe (1937) used Carleton's method for trematodes in which the specimen was sandwiched between glass plates then immersed into hot acetic sublimate. She found picro-nigrosin to be the most successful staining method, later adopted for most of her taxonomic work. Unfortunately picro-nigrosin has poor keeping qualities. Her extant sectioned material in the Museum of Natural History, and in the Museum of New Zealand, Wellington, shows evidence of poor histological technique, reflecting fixation and processing difficulties, with excessively thick and poorly conserved stained sections.

Froehlich (1955) fixed extended Terricola with hot Heidenhain's SUSA, and used haematoxylin and eosin, Heidenhain's AZAN and iron haematoxylin to stain sections. Ball & Reynoldson (1981) recommend methods generally applicable to Triclads with an emphasis on the Paludicola. Methods evaluated and adopted for taxonomic studies of Terricola (Winsor 1990; 1991a; 1998) follow.

3.3 COLLECTING TECHNIQUES

Details of habitats favoured by Terricola, collecting and handling methods for the flatworms, labelling and transport of specimens are provided by Winsor (1997c). Specimens of terrestrial flatworms forming the basis of this study were collected prior to the introduction of recent legislation which requires permits to collect. The Australian Biogeographical Integrated Grid System (Brooks 1976; 1977) provided the basis for the collection, analysis and presentation of the distribution of Terricola in Australia.

3.4 ANAESTHESIA

Living Terricola strongly contract when directly immersed in a fixative. Agonal contractions may cause a variety of fixation artefacts, here termed *contraction artefacts*, which have been mistaken for characters present in the living animal (Winsor, this thesis). Contraction artefacts were minimised by anaesthetising specimens prior to fixation. The most effective anaesthetic, established by comparative trials using a variety of agents (Appendix 2), was an aqueous solution of 10% ethanol (Winsor 1990; 1991a).

3.5 FIXATION

Fixation, which is the process of stabilisation of flatworm tissues at a microscopic level, is critical for taxonomic purposes. An evaluation of fixatives used for Terricola is provided in Appendix 3. There is a conflict between preserving external morphology, and providing the best microanatomical fixation for histological studies. To this can be added further conflict in the need for fixation in 100% ethanol for molecular studies. Many excellent traditional microanatomical fixatives used for tissues intended for paraffin wax embedding, such as Steinmann & Bresslau's sublimate-nitric acid fixative, Heidenhain's SUSA and Bouin's picroformaldehyde fluid are generally unsuitable for taxonomic work as they destroy important external features (Winsor 1991a). These fixatives, especially SUSA, were used here where multiple specimens were available for investigation. Ideally a fixative employed for terrestrial flatworms maintains external characters as well as providing good fixation at a histological level. A good compromise is Tyler's formaldehyde - calcium chloride - cobaltous nitrate fixative (Winsor 1990; 1991a) originally designed to preserve the delicate epidermal features of anuran tadpoles. Most of the fresh terrestrial flatworm material obtained for this study was fixed in this fixative. Cobalt forms an insoluble complex with phospholipids, and tissue biopolymers are further stabilised by calcium and formaldehyde. Body form, colour and pattern of markings are well conserved, and internal anatomy well fixed.

3.6 LONG TERM STORAGE OF SPECIMENS

Although preservation of Terricola in spirit (80% ethanol) has its drawbacks, specimens can remain in it indefinitely without undue harm (Winsor 1991a). Fixed specimens forming the basis of this study are preserved in 80% ethanol, and stored using a standard museum method: specimens and labels are placed in glass vials of alcohol, stoppered with a tight plug of cotton wool; the vials are inverted within large glass storage jars sealed with rubber gaskets and spring clips.

The inclusion of 5% glycerol in preservatives is not recommended. Glycerol may prevent the specimen from drying out; however it may grow mould and this can destroy the specimen. Other wide mouthed universal jars can be used providing that the screw lids are sealed with tape. Polypropylene-acrylic adhesive tape (PPA), available from 3M Industrial Tape Division, reduces evaporative loss considerably when compared to using simply the lid and lid liner only (Steigerwald & Laframboise 1996). Vinyl electrical insulating tape, used for some specimens jars in this study, was satisfactory for a year, but deteriorated over a longer period. Fluid levels and state of material in collections were checked once or twice annually. Conservation methods for spirit specimens are provided by Moore (1989), and methods for recovery of dried specimens are available (Moore 1989, Winsor 1994) and have been used successfully.

An alternative to long term preservation in alcohol is the use of Steedman's propylene phenoxetol - propylene glycol preservative (Steedman 1976). This reagent was combined with Tyler's fixative to provide good histological fixation as well as good preservation of external morphology (Winsor 1991a). Following fixation the specimen was simply transferred to Steedman's fluid. Anecdotal evidence suggests that this preservative is not widely employed in museums, possibly for lack of evidence about its long term effectiveness, and cost. Tissues preserved in Steedman's fluid were softened but not to the point of dissociation (Moore 1989, Winsor, unpublished observations). This raised doubts about the long term maintenance of external morphology of preserved Terricola in this preservative under working conditions in museum collections. Formulae of fixatives and methods suitable for Terricola are provided in Appendix 4. Specimens should be stored in the dark to avoid fading.

3.7 HISTOLOGICAL TECHNIQUES

3.7.1 Specimen subdivision

Specimens for histological studies were generally divided into at least three portions (Appendix 5), and following processing to paraffin wax ideally embedded in a single block so as to provide:

- sagittal sections of the anterior end, sectioned from the left side, or
- transverse sections of the anterior end in taxa with specialized structures of the anterior end, and
- sagittal sections of the posterior portion from just in front of the pharynx to the tip, sectioned from the left side, and
- transverse sections of the prepharyngeal region sectioned front to back.

3.7.2 Tissue processing

Steedman (1960) provided an excellent detailed account of tissue processing, microtomy and serial sectioning, and Sanderson (1994) a good modern general reference book on biological microtechnique. Cooper (1988) formerly of the Worms Section (Zoology) of the Museum of Natural History provided an excellent account of histological techniques for parasitic helminths, which apply equally to terrestrial flatworms. For this study, tissue blocks for histological studies of terrestrial flatworms were dehydrated in an ascending ethanol series, or in isopropanol. When examination of eye pattern and internal structures under a stereomicroscope was necessary tissues were cleared in terpineol or other medium (Appendix 5); otherwise a hydrocarbon transition solvent such as toluene was used prior to infiltration and embedding in Tissue Prep or similar proprietary histological paraffin wax with melting point of 56°C. Choice of reagent and duration of each step was varied with the size and nature of the specimen; representative processing schedules suitable for Terricola are provided by

Winsor (1994) and in Appendix 5. Highly muscular species such as some *Artioposthia* spp. can be particularly difficult to section and special attention must be paid to not over-dehydrate the specimens. For this type of material techniques minimising hardening in muscular mammalian tissues were used to advantage (Winsor 1994).

3.7.3 Sectioning and mounting

Paraffin blocks were generally sectioned at 8μm using a rotary microtome (Reichert, Jung or Leitz). Using a waterbath or hotplate, ribbons of sections were floated onto glass slides pre-treated with Mayer's egg albumen or similar section adhesive. Completed slides were dried in an oven at 60°C overnight. An overview of microtomy and slide preparation is provided in Appendix 6.

3.7.4 Mordants

Prior to staining, dewaxed sections were treated by a mordant to ensure crisp staining. This procedure was particularly useful for old spirit fixed and formaldehyde fixed specimens. Mordants included acid dichromate, alcoholic dichromate, Mallory's mordant, Heidenhain's SUSA fixative, Bouin's picroformaldehyde-acetic acid fluid, and saturated aqueous mercuric chloride solution (Cooper 1988, Drury & Wallington 1980, Humason 1972). Tissue acidophilia characterised mordants containing ferrous ammonium sulphate such as that of Schoobridge (1983). Zinc chloride, substituted for mercuric chloride on environmental and safety grounds in mordant reagents, resulted in mordanted tissues exhibiting overall basiphilia and is not recommended. All these mordants improved stain uptake and fidelity. Mallory's mordant resulted in particularly crisp and vivid AZAN staining. Acid dichromate (Appendix 7), whilst providing balanced though slightly less intense staining to Mallory's reagent, was the method of choice where large series of sections were to be stained. The reagent is non-corrosive and does not attack stainless steel slide racks, unlike Mallory's mordant which contains highly metal-corrosive and environmentally harmful mercuric chloride.

3.7.5 Staining methods

Good differentiation of stained tissues in Terricola is essential for the identification and interpretation of taxonomically important histological characters. Two main factors influence the choice of staining technique for taxonomic histology of flatworms: the ability of a method to provide consistently good colour contrast and differentiation of tissues fixed in a variety of different fixatives, and stability of the dyes in the stained preparations over a long period. An assessment of past and present methods using these two criteria is provided by Winsor (1991a).

Oversight or topographical stains which provide optimum differentiation of the various types of secretions and connective tissues are preferred. Traditional staining techniques such as haematoxylin and eosin methods recommended by Graff (1899), or the haematoxylin-Van Gieson technique, and Masson and similar bichrome methods restrict differentiation of secretory elements to broadly acidophil (erythrophil) and basiphil (cyanophil) tissue entities. Haematoxylin counterstained with a mixture of eosin and orange G, favoured by Beauchamp (1926), provides better differentiation of the acidophil elements than eosin when used as the sole counterstain; however differentiation of acidophil connective tissues from secretory elements remains problematic. Polychrome methods employing three, four or five differently coloured dyes are the most valuable staining techniques for use in histology of the Terricola as they tinctorially differentiate tissue entities within broadly acidophil and basiphil structures. For example, using haematoxylin and eosin, possibly three or four groups of erythrophil secretions can be recognised on the basis of granule size and relative acidophilia; when stained with a polychrome technique the same structures may be differentiated into six or more secretion types thus providing additional potential taxonomic characters.

3.7.5.1 Haematoxylin and eosin

The haematoxylin and eosin technique, despite its limitations in Terricolan histology, is safe and simple. There are a number of different haematoxylin and eosin formulations. Mayer's Haemalum and Young's eosin-erythrosin (Appendix 7) used in this study provided stable, consistent results for a wide variety of tissues including those of Terricola. The method involves short immersion times, and does not employ heat. For these reasons it is preferred where there is uncertainty about section adhesion to slides, and for use with delicate, poorly fixed and friable tissue sections. In these "at risk" situations small series of sections taken at intervals through a specimen can be stained using polychrome techniques under carefully controlled conditions, thus not putting at risk a whole set of serial sections.

3.7.5.2 Polychrome methods

The Martius Scarlet Blue (MSB) method with a celestine blue – haemalum or iron haematoxylin nuclear stain (Drury & Wallington 1980; Pasey & Edwards 1978), or Heidenhain's AZAN (Heidenhain 1916, Gabe 1976, Romeis 1968, Culling, 1974) are the most reliable and informative of the polychrome techniques suited to flatworm taxonomic histology. These two techniques (Appendix 7) used in this study provided excellent differentiation of connective tissue, muscle, erythrophil, cyanophil and xanthophil secretions. When used with neutral synthetic mountants both exhibited good resistance to oxidative fading. The Mallory-Heidenhain method, standardised by Schoobridge (1983) for histopathological applications, did not give as good results for flatworm material as the MSB and AZAN methods where the emphasis is on differentiation of secretions. A rationale for the practical application of polychromes together with a useful trouble-shooting guide, albeit with a histopathological bias, was provided by Schoobridge (1983).

3.7.5.3 Other staining methods

Steedman's *para*-toluene sulphonic acid fixation - triacid staining technique (Steedman 1970) is a simple automatic trichrome method which employs very light-fast dyes, and for this reason ideal for taxonomic histology. Unfortunately the dyes required for this method are now difficult to obtain in Australia (though a good supply of the dye is available at James Cook University). Additional staining methods that can be used to highlight specific structures include phosphotungstic acid haematoxylin for connective tissues, and alcian blue-periodic acid Schiff (AB-PAS) for acid and neutral mucopolysaccharides (Cook 1974).

3.7.6 Oxidative fading

Sectioned flatworm Type and voucher specimens are taxonomically significant and are stored in museum collections indefinitely. Thus staining and mounting reagents which are stable over long periods, exhibit good light-fastness, and which resist oxidative changes should be used. Haematoxylin and eosin stains exhibit good resistance to oxidization, though Sirius Red F3B (C.I. 35780) has better light-fastness than eosin and may be substituted for the latter (Winsor 1991a). Gabe (1976) reports excellent conservation of stains in tissues stained by himself, and also material stained by M. Heidenhain by the AZAN technique, which when examined some 30 years later showed no diminution of stain brightness. Acid fuchsin (C.I. 42685), used in the Mallory Heidenhain and similar trichrome methods can be highly variable and exhibits very poor light fastness, sections fading within 8 years (Winsor 1991a). If these techniques must be used it is recommended that Ponceau S (C.I. 27195) which has superior light fastness is substituted for the acid fuchsin (Curtis 1905). The light-fastness of aniline blue (C.I. 42755) is best ensured with formulations incorporating phosphotungstic or phosphomolybdic acids (Puchtler *et al* 1989). Sirius supra blue GL (C.I.23160) has good light-fastness and can be substituted for methyl blue in polychrome methods (Humason 1972; Lillie 1997).

3.7.7 Mountants

Sections are most conveniently mounted in synthetic resin-based mounting media such as DPX (DPX - Kirpatrick & Lendrum, 1941 formulation marketed by Merck, not DePeX Gurr which is problematic) from which they are readily recovered should restaining or remounting be necessary. Material prepared and mounted in DPX some 30 years ago and used in this study shows no sign of shrinkage or deterioration.

Canada balsam is considered by some (Cooper 1988 for sections; Brown 1997 for entomological wholemounts) to be the only safe mountant for taxonomic material and reject the use of Gurr's DePeX for these purposes; they do not consider DPX. A natural resin, Canada balsam contains acids and other unsaturated reducing agents which cause fading of dyes, and darkening of the resin. Sections stained with haematoxylin or aniline dyes, and mounted in balsam, may be bleached. Balsam is generally thinned with xylene; safer substitute diluents such as Histoclear might hasten darkening of the resin (Brown 1997). A further disadvantage of Canada balsam is its slow drying properties and difficult slide cleanup, making this an inconvenient mountant to use on sections, especially when large numbers of slides are produced.

Inhibition of oxidative fading at the periphery of stained mounts, and the formation of annual rings in mounts is achieved by the incorporation of 1% w/v of the antioxidant 2,6,di-*tert*-butyl-*p*-cresol (Hollander and Frost 1971) in the mountant. Whilst this substance was added to some batches of DPX mountant used in this study, formal trials to evaluate the effectiveness of the antioxidant were not undertaken. Peripheral oxidative fading appears to be accelerated in sectioned material stored in the tropics (Winsor pers.obs).

3.7.8 Old spirit-preserved museum specimens

On the basis of personal experience it cannot be emphasised too strongly that Type and other museum specimens on loan automatically invoke Murphy's Law - *if anything can go wrong, it will.* Old spirit-preserved museum specimens of Terricola frequently presented a number of difficult, often multiple technical problems to the investigator. These included tissue brittleness, and tissues with a tendency to over-process resulting in inferior sectioning properties, chiefly cracking and crumbling. These problems were partly overcome by careful choice of reagent and reduced duration in processing fluids (Winsor 1994). Sections often adhered poorly to microslides necessitating the use of an adhesive such as Mayer's glycerol-albumen (Appendix 6), or celloidinization (Culling 1974); even this treatment did not always prevent sections folding and lifting from slides, especially where fine strands of tissue lay adjacent to anatomical cavities. Receptiveness to dyes was also frequently impaired, reflecting previous exposure of the tissue to particular fixatives, fixation and storage conditions such as the presence of metals (container lids and metal labels), and tannins (cork). Mordanting sections just prior to staining was found to be more effective and convenient in restoring stain receptiveness to tissues than secondary fixation using microanatomical fixatives.

3.8 MICROSCOPY AND PHOTOMICROGRAPHY

Sections were examined under brightfield using a Leitz DIALUX microscope, and oblique illumination and Nomarski differential interference contrast microscopy (Winsor 1978) were employed for faded or poorly stained Type and other museum material. Eye patterns and other features were drawn with the aid of a camera lucida attached to a Bausch and Lomb stereomicroscope. Photomicrographs, using Ilford Pan F black and white film, and Ektachrome 64 Tungsten colour negative film, were taken using an Olympus BH photomicroscope. Drawings were done with the aid of a drawing tube fitted to the Leitz DIALUX microscope, and for reconstructions of internal anatomy the method of best fit or contour tracing using coloured pencils to simultaneously trace different structures was employed (Gaunt 1971).

3.9 MORPHOMETRY

For measurement whole fixed specimens were laid ventral side uppermost in a shallow groove cut into dense polystyrene foam. Body dimensions were then measured using a steel rule; a calibrated micrometer eyepiece was used to measure small specimens examined with a dissecting microscope. Dimensions recorded were total length, width over the mouth, distance from the anterior tip to the mid mouth, and anterior tip to the mid gonopore. Body height, width of creeping sole, length of pharynx, and pharyngeal pouch, and other microanatomical dimensions were established from sections using a calibrated micrometer eyepiece. The values cited for dimensions at a microscopical level are generally the average of three determinations.

To facilitate comparisons between taxa the positions of body apertures were also expressed as percentages of the total body length. For most taxa considered here there was insufficient data available for developing regression formulae for the ratios of their body dimensions.

Calculation of the *Cutaneous Musculature Index* (CMI) follows Froehlich (1955) where the CMI is the height of the cutaneous musculature expressed as a percentage of the body height (mc:h ratio). Normally these measurements were made in the midline. However in some taxa cutaneous musculature thinned in the median ventral region and it was appropriate to take measurements between the midline and body margin; a range is then provided. The parenchymal musculature index (PMI) was similarly calculated from the sum of the heights of the dorsal and ventral longitudinal ring-zone musculature, or other designated set of parenchymal muscles where appropriate eg. ventral plate, and expressed as a percentage of the body height. Unless otherwise stated, values for CMI and PMI are for the pre-pharyngeal region.

3.10 TAXONOMY

In the following taxonomic descriptions, only the principal synonymies are provided for taxa. I subscribe to the view that ".. the exhaustive preparation not only of a complete synonymy but also of a listing of all references to previous publications with all possible binomial combinations (in case of generic transfers) has become the misplaced ideal of scholarship for some taxonomist "(Mayr and Ashlock 1991, p360). Full synonymies for taxa are available in the series *Land Planarian Indices of the World* by Ogren *et al*, which at the time of writing is updated annually, and is the starting point for the taxonomy in this thesis. Nomenclature here complies with the *International Code of Zoological Nomenclature* 4th edition, effective from 1 January 2000. However the new taxa described in this thesis are *nomina nuda* as the provisions of Article 11 of the *International Code of Zoological Nomenclature* (ICZN 1999) are not met.

3.11 THE AUSTRALIAN REGION - DEFINED FOR THIS STUDY

The Australian Region, as defined by Graff (1899) comprises the Austromalaysian, Australian, Polynesian, and New Zealandian subregions. This thesis considers flatworms from the Australian and New Zealandian subregions. The Australian subregion includes the Australian mainland, Tasmania, Bass Strait Islands, and other adjacent continental islands. The New Zealandian subregion includes Lord Howe Island, Norfolk Island, the north and south islands of New Zealand, Chatham Island, and the subantarctic islands. The term "Australia" includes Tasmania. Australian oceanic islands, such as Lord Howe, Norfolk and Macquarie islands, are considered separately.

3.12 Definitions and abbreviations used in the text:

Anatomical terms are defined in the Glossary. Abbreviations for anatomical figures follow the Glossary.

Distributional data were derived from literature and specimen records. Location of Type specimens and sources of specimen records, together with appropriate alphanumeric registration identifiers, are indicated by the following abbreviations: Australian Museum, Sydney (AM); American Museum of Natural History, New York (AMNH); Zoologisches Museum der Humboldt-Universitit, Berlin (BZM); the author's collection (LW); Museum d'Histoire Naturelle, Geneve (MHN); Museum of Natural History, London (MNH); Museum of Victoria, Melbourne (MV); Queensland Museum, Brisbane (QM); National Museum of Natural History, Smithsonian Institution, Washington (USNM); Naturhistorisches Museum, Wien (W) and Western Australian Museum, Perth (WAM).

Chapter 4

Functional anatomy and taxonomic characters of Terricola

On the whole, it is evident that a close study of the anatomy of Land-Planarians cannot fail to lead to interesting results .... Henry Moseley (1875)

The chief difficulty in judging these [taxonomic] problems besides scarcity of data remains in the lack of a clear understanding of what characters are to be considered plesiomorphic, apomorphic or convergent in the Terricola.

Eudoxia M. Froehlich (1978)

A classification based on poorly analysed characters is like a building without a foundation. Component-function analysis is yet one more touchstone for analysing characters, one more means for strengthening the foundations of phylogenetic systems.

Seth Tyler (1988)

4.1 INTRODUCTION

The taxonomy of terrestrial flatworms is presently based upon a combination of external morphological characters, and internal anatomical characters revealed by histological investigations. It is in great need of revision (Cannon 1986), and the three families are all insecurely based. Understanding the relationships between taxa at all levels in the Terricola is largely hampered by the unevenness of the taxonomic treatment of the various groups. Descriptions frequently lack comparable anatomical data, a problem addressed in this thesis by the adoption of a common format. Intraspecific variability in colour and stripe patterns and in the morphology of copulatory organs during maturation are further sources of taxonomic confusion.

Uncertainties regarding the function of various anatomical, histological and cytological elements, particularly those of the reproductive system, have hindered full taxonomic exploitation of available characters. The copulatory apparatus of planarians is considered the most conservative structure for evolutionary changes, and therefore reliable as a basis for taxa (Ogren & Sluys 1998). However heavy reliance on the copulatory apparatus for classification in the Terricola has been demonstrably limiting – a whole-animal approach is required (Winsor *et al* 1998).

Taxonomic descriptions reflect the knowledge, understanding, insights and taxonomic mores of the investigator at the time they were written, and are thus necessarily incomplete. New techniques and methodologies facilitate the discovery and interpretation of characters and their states, and may also improve understanding of the functional anatomy of Terricolan organ systems. There is a dearth of knowledge of the Terricola at an ultrastructural level. Molecular and phylogenetic studies which include the Terricola, and which are based on modern phylogenetic analysis of classic morphological, ultrastructural, and molecular characters, have only recently begun (for example Rohde *et al* 1995; Ogren & Sluys 1998; Baguñà *et al* 2001; Sluys 2001).

At a practicable taxonomic level, a major challenge is the selection of reliable taxonomic characters and their use in keys. Reliable characters are those significantly stable characters derived from traceable developmental paths, and not those considered temporary, sexual, physiological or developmental conditions (Ogren & Sluys 1998).

This chapter attempts to recapitulate, update and augment the early consolidated accounts of the general anatomy and histology of Terricola (Graff 1899, 1912-1917). Examples are largely drawn from the flatworms of the Australian region, previously largely unknown anatomically. Taxonomic characters and states are identified and evaluated for the cladistic analysis of taxa, to be undertaken subsequent to this thesis. The general morphological external and internal features of a caenoplaninid terricolan are provided (Fig. 4.1) together with a glossary of terms (Glossary). Whilst every attempt has been made to provide full data sets for all characters and states for all taxa considered here, this has not always been possible. Unsupported statements and assertions are based upon unpublished observations by the author.

SECTION A: EXTERNAL MORPHOLOGY

Important external morphological taxonomic characters in the Terricola, in particular form and markings, are best observed in living animals. Flatworm behaviour

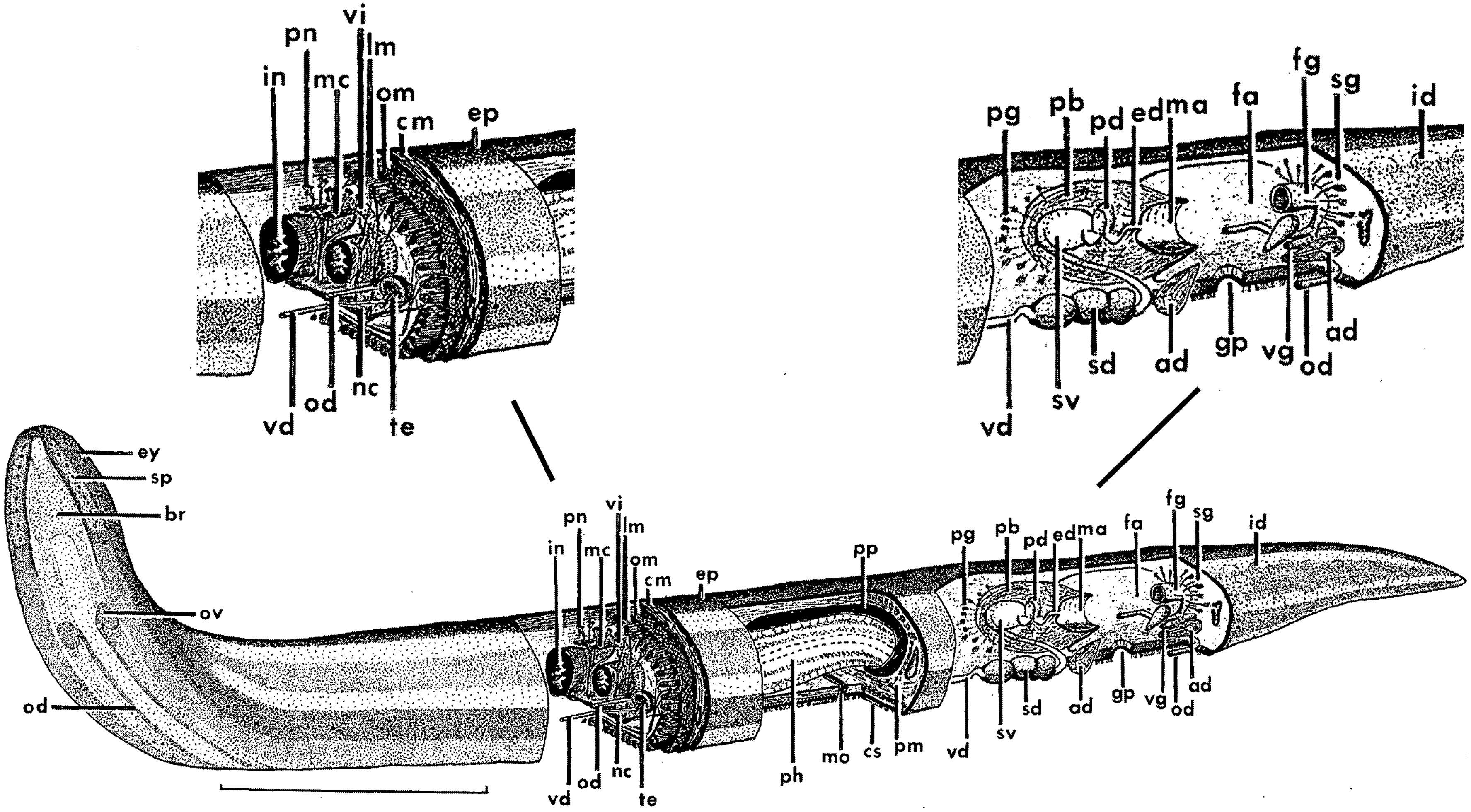


Figure 4.1. A diagrammatic representation of a composite caenoplaninid terrestrial flatworm. Abbreviations: ad adenodactyl, br brain, cm cutaneous circular muscles, cs ciliated creeping sole, ed ejaculatory duct, ep epidermis, ey eye, fa female antrum, fg female glandular canal, gp gonopore, id intestinal diverticulum, in intestine, lm cutaneous longitudinal muscle, ma male antrum, mc main nephridial canals, mo mouth, nc nerve cord, od ovovitelline duct, om cutaneous oblique muscles, ov ovary, pb penis bulb, pd prostatic duct, pg penial glands, ph cylindrical pharynx, pp pharyngeal pouch, pm parenchymal muscle, pn protonephridia, sd spermiducal vesicle, sg shell glands, sp sensory pit, sv seminal vesicle, te testis, vd vas deferens, vg viscid gland, vi vitellaria. The brain, ovaries and ovovitelline ducts are all internal structures. The scale bar represents 5 mm.

such as the way in which it glides, how it carries the anterior end, hunting behaviour, escape reactions and sensory movements, can only be determined from living specimens.

Agonal contraction, distortion, colour and pigment loss often severely limit the usefulness of external morphology in preserved specimens. Examples of fixation contraction artefacts include the generic character of the "hood" over the anteroventral sucker of *Coleocephalus*, the "quadrangulate" cross sectional shape of fixed specimens of *Reomkago* and *Coleocephalus*, and the ridging and bulging of the creeping sole in various taxa (illustrated in Graff 1899 page 15).

External characters include colour and pattern; body shape and size; cross-sectional shape; shape of the head and posterior end; adhesive suckers; sensory structures; the relative size, pattern and distribution of eyes; dimensions of the body, number and relative position of body apertures, and the width and form of the creeping sole. The relative positions of body apertures, together with eye size and pattern, body shape and size are valuable indicators in the rapid sorting of specimens into Recognisable Taxonomic Units (RTUs).

4.2 COLOUR AND PATTERN

The markings of Terricola comprise two elements: colour and pattern. Markings are cryptic: the grey, brown, and yellow colours, with patterns of mottling, dark stripes and striae, as in many rhynchodemids and geoplanids. Presumed aposematic (warning) colouration such as the bright yellow of some *Fletchamia* species is exhibited by species often found moving about by day; the possible toxicity of these species is assumed, not proven. Numerous species are longitudinally striped, and the nomenclature for stripes and body zones is provided in Fig. 4.2. Fewer species exhibit transverse banding, present in the markings of a few geoplaninids from Chile, several species of Bipaliidae from Indonesia (Sumatra and Sulawesi) and Philippines, two caenoplaninids from New Caledonia, and a cotyloplanid, a platydemid and a caenoplaninid from north Queensland, Australia. Some geoplaninids and caenoplaninids exhibit unpigmented zones around their eyes. This type of characteristic marking is termed a halo. A number of Australian Terricola are non-pigmented or exhibit dark green – blue-green

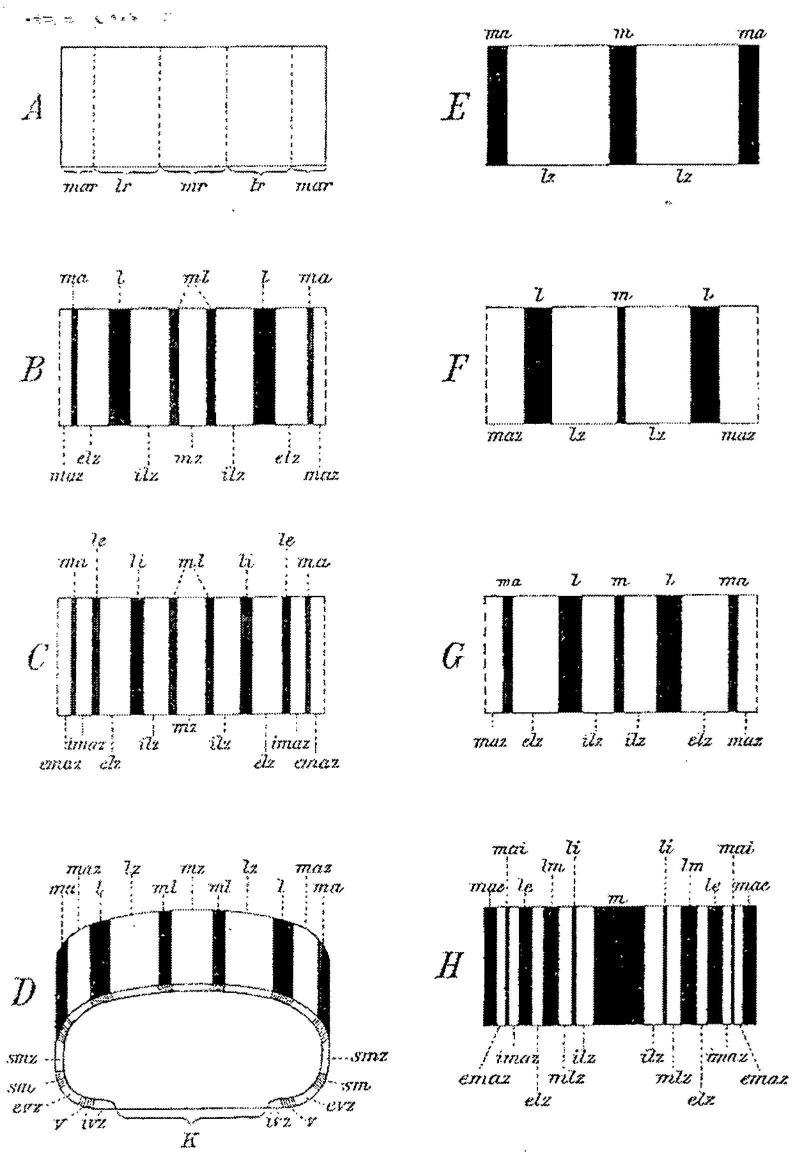


Figure 4.2 Nomenclature of body zones and longitudinal stripes
(copied from Graff, 1899, Fig 2 page 25)

A.	Division of the dorsal surface into regions.
B, C.	Nomenclature for paired dorsal stripes and zones.
D.	Nomenclature of dorsal and marginal stripes and intervening zones.
E - H.	Nomenclature for unpaired stripes and intervening zones.

Key to abbreviations.

elz- outer lateral zone, emaz- outer marginal zone, evz- outer ventral zone, ilz- inner lateral zone, imaz- inner marginal zone, ivz- inner ventral zone, k- creping sole, l- lateral stripe, le- outer lateral stripe, li- inner lateral stripe, lm- midlateral stripe, lr- lateral region, lz- lateral zone, m- median stripe, ma- marginal stripe, mai- inner marginal stripe, mae- outer marginal stripe, maz- marginal zone, ml- paired median stripes, mz- median zone, sm- submarginal stripe, smz- submarginal zone, v- ventral stripe.

colouration, in common with many other soil and cryptofaunal animals (Lawrence 1953). Colour and pattern characters are not described in a consistent manner in the literature, and are difficult to code.

4.2.1 Variation and polymorphism

In general, juveniles exhibit the same colour and basic pattern as the adult but are clearer and more brightly coloured (Fletcher & Hamilton 1888). The markings may however alter considerably as the worm matures. Stripes may broaden, and mottlings coalesce to form irregular lines or stripes. In the variations in markings seen in taxa with longitudinal stripes, the central stripe is the most conservative and varies least (Winsor 1983b; F. Carabayo *in litt*), as do the markings at the anterior end. Often the only suggestion of median and lateral stripes is at the anterior and posterior ends (Dendy 1891; 1892e). The greatest variations are seen in the lateral and marginal stripes that may broaden, narrow, become discontinuous or be present only as the margins of stripes (as in *Bipalium kewense* in Winsor 1983). Transverse dislocations (faults) in the longitudinal dorsal stripes are indicative of rupture of a cocoon through the dorsum (Winsor 1998b) or some other injury.

Colour and dark general pigmentation generally increase as the individual flatworm matures, with the final appearance of the markings coinciding with the patency of the gonopore (E. M. Froehlich 1955) though the latter is not necessarily synonymous with sexual maturity. In some taxa, for example *Geoplana josefi*, the colour and pattern can continue to change after the gonopore is patent giving rise to ontogenetic polymorphism - differences in appearance of juvenile, sexual and mature individuals (Carabayo & Leal-Zanchet 2001).

Some taxa exhibit polymorphism in which there is the co-occurrence of differently marked forms, for example as demonstrated in *Rhynchodemus bromelicola* (in Beauchamp 1912), and in *Newzealandia moseleyi* and *Arthurdendyus australis* (in Fyfe 1944, 1947). In this context Dendy (1894b, reproduced in Graff 1912, and Plate 4.1 this thesis) illustrated the graded variations in markings of *Artioposthia fletcheri*. A number of these "polymorphs" are now known to be different taxa and Dendy's rationalization of markings of some flatworm species is undoubtedly the reason why some of his Syntype series include more than one species (Fyfe 1946; Johns 1998; Winsor *et al* 1998). Conversely, Beauchamp (1926) observed that infrequently, taxa that

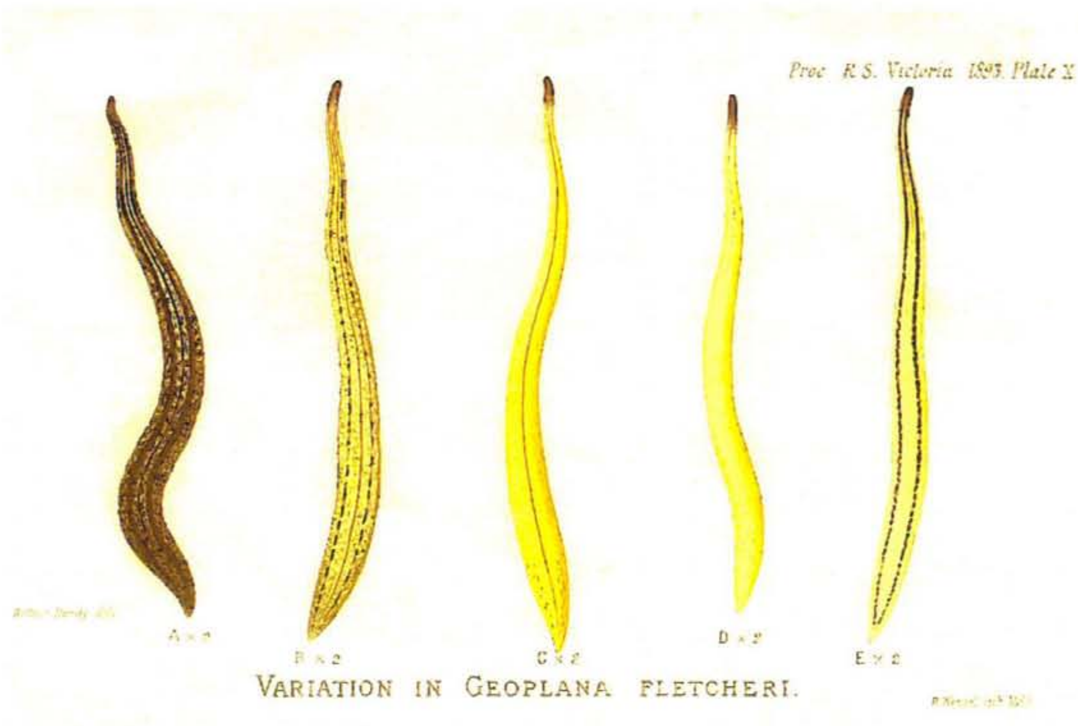


Plate 4.1 An illustration showing graded variations in *Artioposthia fletcheri* (Dendy, 1894b). Figure D is the original specimen. A number of the other variants illustrated are different species. Dendy's rationalization of markings of some flatworm species is undoubtedly the reason why some of his syntype series include more than one species.

(This plate is copied from Dendy 1894b, Plate X) were similar externally could differ anatomically, clearly illustrated in the confusion between *Australoplana* and *Nelipoplana* which has persisted for over a century (refer to *Australoplana* and *Nelipoplana* Appendix 8).

4.2.2 The nature of colouration in Terricola

The identification and nature of colour and pigments in the Terricola has received little attention. General accounts of colouration in animals (Fox & Vevers 1960, Fox 1976, Vevers 1982) make no mention of the Terricola. Investigations of pigments in the Terricola, and in particular the Australian taxa, are confined to those of Moseley (1877a) and the unpublished observations on colour of the author (in the following section, this thesis). Colour can be due to the presence of pigments, or can be structural colour due to the scattering of light (Tyndall scattering), to interference, or to diffraction (Vevers 1982). Interference and diffraction effects produce iridescent colours.

Non-pigmented terricolans of the Australian region are few. They include *Apogaioplana paluma, Bassioplana typhlops, Nelipoplana N. alba, N. minor* and *Sokoplana kanangra.* All are taxa that appear to be subterranean or largely saproxylic, and eyeless or with minute eyes. Some of these species may exhibit very pale light fawn to pale orange colouration, ranging through apricot and pink to red, probably largely due to ingesta.

White colour is generally simply due to unpigmented integument. However there are some taxa where the white colour is particularly striking, as in *Artioposthia scaphoidea* from northern New South Wales, and in some Tasmanian and New Zealand species. This white colour may have a structural or pigmentary origin.

Red pigmentation is present in *Australoplana sanguinea* and species of *Arthurdendyus*, in the red stripes in species of *Bassioplana* and *Nelipoplana,* and in the pale reddish-orange tip described in many species. It is not known just how much of the red pigment is endogenous, and how much is exogenous, attributable to pigments present in ingesta such as earthworms or crustaceans. The red pigment in *A. sanguinea* has not been identified. It is insoluble in acid alcohol, unreactive to dilute acids and alkalis, and shows no spectral absorption bands (Moseley 1877).

Brown and yellow colour and pigments characterise the majority of Australian Terricola. Some yellow and orange pigments, and all the brown and black pigments are melanins. These yellows and pale browns are probably phaeomelanins as they exhibit dim yellowish fluorescence, whereas the darker browns and near-black pigments are eumelanins which do not fluoresce. Yellow pigments in some taxa such as in *Fletchamia species*, are fugitive, soluble in water and alcohol, and do not fluoresce.

Green and blue-green colour in the stripes of some *Caenoplana* and *Fletchamia* species are due to sparsely or thinly distributed melanin granules in the outer integument. These granules scatter and reflect shorter wavelengths of light preferentially (Tyndall scattering) to give a blue colour which, when overlaid on the yellow ground colour, gives a green coloured stripe or green ground colour. Darker green species such as *C. walhallae* have heavier concentrations of melanin around the cutaneous musculature in addition to the more superficial sparse melanin deposits. In the black-grey species *Parakontikia coxii* the colour of the paired dorsal azure stripes is due to Tyndall scattering of light by sparse melanin granules within otherwise unpigmented dorsal zones; the rest of the dorsal integument is heavily pigmented with melanin granules. Similarly in the region of the lateral stripes in *Lenkunya adae* which can appear dark green or dark brown with bluish flecks (Dendy 1892e), a thin layer of melanin granules is present in the epithelium, and a thick layer of melanin granules is present around the cutaneous musculature. The nature of the clear green colour in the New Zealand species *Australopacifica tenuis,* and in an arboreal rhynchodeminid from Eungella, Queensland (Winsor 1997a) is unknown.

Blue colouration may also be due to blue pigment. In the *Caenoplana* species *C. coerulea* and *C. spenceri* the rhammites contain an unidentified blue pigment (Dendy 1890). This pigment is insoluble in alcohol but soluble in acid alcohol, is pH sensitive, changing to red in dilute acid, reverting to blue in alkali, and has no spectral absorption bands (Moseley 1877). It is probably not a porphyrin or carotenoid-protein. The blue pigment is absent from rhammites in pale whitish juveniles of these species and in some specimens from open woodland.

Iridescence observed in some species (by Dendy 1892e; Winsor pers. obs.) is possibly due to the closely packed transparent proteinaceous rhabdoids in the epithelium acting as a diffraction grating. Similarly the point flecks of colour in the epithelium of some live flatworms observed using a hand lens might be due to diffraction effects from superficial secretory granules, or rhammites just protruding through the epithelium.

4.2.3 Melanism

Brown melanin entirely overlying normal markings, giving some specimens a "dirty appearance", has been observed in young and adult specimens of taxa from upland closed forests (Winsor 1983b; F. Carabayo *in litt*), and absent in the same taxa in open forest. Contrary to the opinion of Hyman (1951) and Beauchamp (1961), chromatophore-like cells, with ultrastructural characteristics of chromatophores, containing melanin-type granules are present in the paludicolans *Dugesia gonocephala* (Palladini *et* al 1979), and in *Polycelis nigra* and *Dugesia lugubris* and these cells have been shown to be reactive to light (Cloudsley-Thompson 1945). Whilst chromophoric activity has not been observed in the Terricola, the generalized melanism observed in the upland flatworms may be a physiological response of the pigmentary system to the low light in closed forest. A less likely explanation is melanism in response to a relatively cooler upland environment. In some invertebrates melanin is cycled (Vevers 1982): melanin synthesis increases at low temperatures and melanin breakdown decreases with decrease in temperature. Thus at low temperature melanin synthesis can exceed breakdown, resulting in melanin accumulation. Equilibrium between synthesis and breakdown occurs at higher temperatures, present in open forest. If this mechanism operates in Terricola, taxa in cold climates would be expected to exhibit seasonal melanism; this has not been reported. Similarly melanism in response to solar radiation to protect the photosensitive uroporphryin-containing epidermis of some taxa that move about by day has not been observed.

Progressive melanism occurs with increasing maturity in many taxa, though it has not been established whether the pigment concerned is a melanin or possibly a cumulative age-related lipofuscin (a chromolipid).

4.3 BODY MORPHOLOGY

4.3.1 Dimensions and the relative positions of apertures

The relative positions of the body apertures are important at the level of genus. Included are mouth, or mouths in polypharyngeous taxa, the gonopore(s), and other cutaneous apertures as in *Diporodemus, Incapora,* and *Enterosyringia.* Despite sources of error such as damaged anterior and posterior tips, coiling, and uneven contraction in fixative, these dimensions are valuable indicators in the rapid sorting of specimens into Recognisable Taxonomic Units (RTUs). For example, in some taxa the mouth and gonopore are close together, with both in the hind third of the body (e.g. *Kontikia, Parakontikia, Nelipoplana*). In other taxa the mouth may be central with the gonopore closer to the hind end (e.g. in *Caenoplana*). In some taxa the mouth is in the anterior half with the gonopore centrally placed (e.g. in *Artioposthia polyadoides*).

The dimensions and positions of apertures on the body presently used are (in millimetres except where stated otherwise):

1. Total length;
2. Width at the mouth (which is generally the maximum width);
3. Distance from anterior tip to the mouth;
4. Distance from the anterior tip to the gonopore;
5. Distance from the mouth to gonopore;
6. Height of body (measured in µm from sections);
7. Width of body (measured in µm); and
8. Width of creeping sole (measured in µm from same section as 7 previously).

The positions of the mouth, gonopore and mouth-gonopore are also generally expressed as percentages of the total length of the specimen. Of these values, the most reliable is the distance between the mouth and gonopore, which exhibits the lowest standard deviation of these values (Winsor 1983b). Where multiple specimens of a species are available, the dimensions can be treated statistically to provide data on range, mean and standard deviation (Winsor 1983b), and a regression formula determined, for example for the distance of the mouth from the anterior tip correlated with the body length for *Bipalium kewense* is y = 1.7x + 16.3, and distance from mouth to gonopore correlated with length y = 6.3x + 19.8 (Winsor, unpublished results). Plotting the distance between mouth and gonopore against total length of body has been helpful in identifying RTUs as this process can assist in the separation of taxa of similar external appearance and in assigning unidentified bleached museum specimens to an RTU. Where there is non-isometric growth in a species, there are limitations in using ratios of the distance of body apertures from the anterior end, to length, plotted against length, (Jones *et al* 1995).

4.3.2 Body shape and size

4.3.2.1 Head shape

The shape of the anterior (head) end is a useful taxonomic character, particularly in identification of specimens into RTUs. The anterior end may be elongate and snout-like (rhynchodeminids), blunt and rounded (microplaninids), anteroventrally puckered (amblyplanids and othelosomids), tapered (most geoplanids and pelmatoplanids), spatulate (a few caenoplaninids), bulbous (cotylate taxa), semilunate, or fan-shaped (bipaliids) (Fig. 4.3a). The anterior end may bear a adhesive pad(s) as in *Choeradoplana, Isocca* and *Thosteelia,* sensorial zones, or sensory papillae as in *Xerapoa* (all discussed later, this chapter).

4.3.2.2 Body shape

At the extremes of morphology the Terricola may be very long and thin with high length: width ratios, for example *Rhynchodemus, Pelmatoplana* and *Bipalium* species (30:1 - 20:1), or relatively short and broad with low length: width ratio, for example *Polycladus gayi* (2.5:1). Austral caenoplaninids have length-width rations of 10:1 (*Lenkunya*) to 20:1 (*Caenoplana*), and rhynchodeminids 30:1 (*Dolichoplana*) to 8:1 (*Platydemus*).

Contrary to their name, many species of terrestrial flatworms are far from flat in cross-sectional shape (Fig. 4.3b). Cylindroid taxa includes *Endeavouria, Kontikia,* and *Parakontikia*, typically with low width: height ratios (1.1-1.8:1). Living *Caenoplana* and many *Artioposthia* species at rest are strongly dorsally keeled, and flattened ovate to

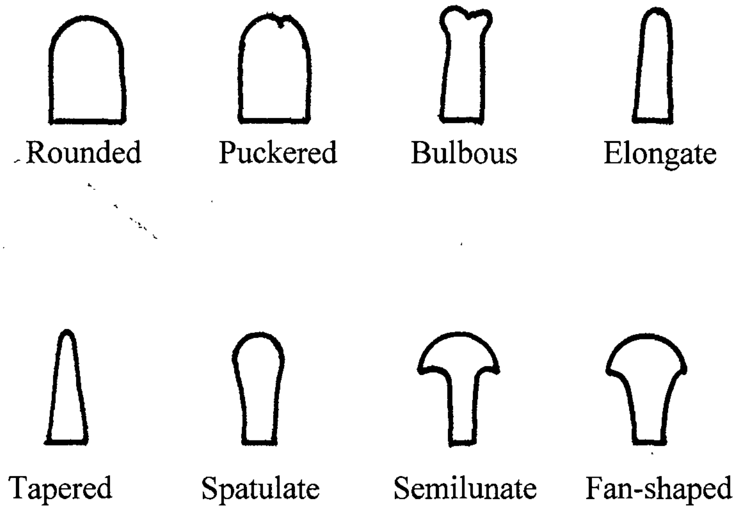


Figure 4.3a Head shapes

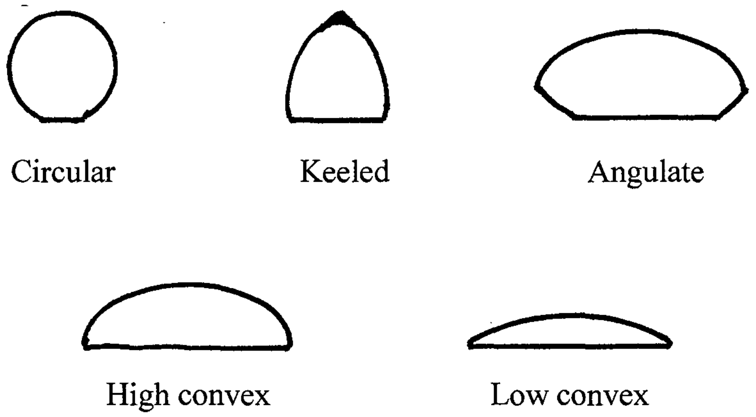


Figure 4.3b Body cross-sectional shapes. The flat side is the ventral surface.

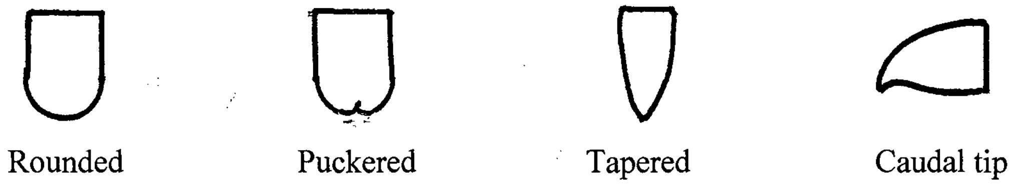


Figure 4.3c Posterior end shapes

broadly triangulate in cross section (typically medium width: height ratio 1.2:1-1.8) when crawling. *Australoplana* and *Nelipoplana* are flat forms, typically with a high width: height ratio (2.3-3.7:1). Thin, elongate taxa such as many bipaliids and dolichoplanids, and many Neotropical geoplanids are very flat in cross section. The ratios of body height to width at the pre-pharyngeal region have recently been used to provide an index for the degree of flatness (Carbayo & Leal-Zanchet 2001).

4.3.2.3 Posterior end shape

The shape of the posterior end is a useful taxonomic character, particularly in identification of specimens into RTUs. The posterior end may be rounded, tapered, pointed with a distinct caudal tip, or puckered. Blunt rounding and puckering of the posterior tip is generally indicative of regeneration following architomy (reproduction by fission), or regeneration following accidental damage (Fig. 4.3c).

4.3.2.4 Creeping sole

The present or absence of a ciliated creeping sole (termed *kriechleiste* -creeping ridge - by Graff 1899), and the relative width of this as a proportion of the ventral surface are important taxonomically. The creeping sole is evident in many taxa as a distinct median ventral zone of variable width. It may be delineated from the surrounding surface by the presence or absence of pigmentation, and it may be flat, or sometimes in the fixed specimen raised as a ridge or series of longitudinal ridges (Graff 1899 page 15 Fig. 1) generally reflecting contraction of the underlying specialized musculature.

Macroscopically it is generally difficult to differentiate between a ventral surface with no creeping sole (*Nelipoplana* and others) and taxa with a broad creeping sole occupying most of the width of the ventral surface. Creeping sole characters are normally determined at a microscopical level and will be further considered under internal anatomy.

In summary, with regard to external morphological characters and their states, Meixner (1928) considered that small body size, sub cylindrical shape, narrow creeping sole, simple (muted) colours and simple patterns of longitudinal stripes, were primitive (plesiomorphic) states. Large and long body size, with broad, flat shape, broad creeping sole, bright colours and mottling, lively stripes and bands were advanced (apomorphic) states. On the basis of material examined in this study, I concur with Meixner's opinions.

SECTION B: INTERNAL ANATOMY

In the taxonomic descriptions in this thesis, the internal anatomy of the Terricola is considered under four main headings: (a) The head, with details of adhesive organs, eyes, and chemo- and mechanosensory structures, (b) the pre-pharyngeal region, with features of the body wall, (c) the alimentary system, and (d) the reproductive organs. The following general account of the internal anatomy follows a more traditional systems approach similar to that of Graff (1899; 1912-17) and Sluys (1989a).

4.4 ARTEFACTS INFLUENCING INTERNAL CHARACTERS

4.4.1 Fixation artefacts

Longitudinal contraction of the flatworm body may cause superficial transverse wrinkling of the dorsal epithelium that in histological sagittal sections mimic cell nests. It may also cause changes in the morphology of the copulatory organs. Important taxonomic characters such as the posterior extent of the testes, asymmetric penis bulb, invaginated penis papilla and relative positions of female ducts could possibly be influenced by prolonged agonal contractions resulting from slowly acting fixative (Winsor 1983b). Difficulties in the interpretation of histological preparations exhibiting contraction artefacts are exemplified by the present confusion and debate (Jones *et al.* 1998) concerning protrusible and eversible penes in the copulatory organs of species of the caenoplaninid genera *Kontikia* and *Parakontikia*.

4.4.2 Artefacts due to parasites

Terricola are subject to parasitization by sporozoa, ciliates, nematodes, trematodes, and arthropods (Graff 1899; 1912-1917; Fuhrman 1917b). Numerous specimens of taxa examined in the course of this study were infected with gregarines, possibly resulting from the flatworms feeding on earthworms. The parasite load varied from light, with gamonts generally confined to the gut, to heavy, with infiltration of gamonts and presence of zygocysts within the parenchyma and gonads. Light peri-intestinal concentrations of gregarine forms were noted in specimens of *Lenkunya munda* and *Fyfea carnleyi.* Severe infection leading to frank tissue damage, termed here *gregarine-induced peri-intestinal histolytic vacuolation*, was observed in specimens of *Danidoplana palatris* and *Caenoplana coerulea.* In these cases the parenchyma surrounding the gut was highly vacuolated to the extent that initially it appeared to be a new anatomical structure. Some of the vacuoles contained zygocysts in varying stages of development. Heavy parasitic loads, and the presence of gregarines in the testes could result in the inhibition of gonadal development or parasitic castration, as described in the nemertean *Macobdella grossa* infected with *Haplosporidium malacobdellae* (see Jennings & Gibson 1968).

Phoretic nematode larvae were frequently encountered in the musculature and outer parenchyma, rarely in reproductive organs, in a variety of taxa. A nematode-induced neuroma in a specimen of *Bipalium kewense* was the only nematode-host reaction observed.

Graff (1899) described what was thought to be a mite or chalcid wasp larva in *Polycladus gayi.* Hickman (1964) described, and gave an account of the life history of the Tasmanian mycetophilid fly *Planarivora insignis* (Diptera: Mycetophylidae) that parasitizes *Tasmanoplana tasmaniensis* (28% infection of the population studied), and less commonly *Artioposthia mortoni* and *A. diemenensis.*

4.5 EPIDERMIS

The outer body wall is the key to the survival of terrestrial flatworms. Secretions from the epithelium of Terricola are principally concerned with adhesive, locomotory, prey capture, repugnatorial and homeostatic functions. Septate junctions of the triclad epithelium probably contribute to minimising water loss through the epithelium. Pleated-type septate junctions are present in the epithelium of *Bipalium kewense* and

Bipalium sp. (in Storch & Welsch 1977), and *B. adventitium* (in Curtis *et al.* 1983). These junctions appear to be analogous to the zonulae occludens (tight junctions) of vertebrate epithelia, and may seal off intercellular spaces from the environment (Curtis *et al* 1983).

4.5.1 Basement membrane

The basement membrane is a product of the epithelium, and attaches the epithelium to the underlying connective tissue. Depending upon the quality of fixation, the basement membrane generally appears as a single or bifibrillar lattice. It is composed of inextensible reticulin fibres arranged as a lattice composed of left- and right-handed spirals and is present in all triclads (Clarke & Cowey 1958). The tinctorial characteristics of the basement membrane in *Geoplana carinata* are provided by Mombrum de Carvalho *et al* (1975).

4.5.2 Thickness of the epidermis

Character: *Epidermis: thickness*

0. dorsal epithelium thicker than ventral epithelium
1. dorsal and ventral epithelia same thickness
2. ventral epithelium thicker than dorsal epithelium

The general situation in the "Turbellaria" is that the dorsal epithelium is thicker than the ventral epithelium. This character and its states should be used with caution. The relative thickness of the dorsal and ventral epidermis may depend upon the technique of specimen handling and the histological procedures employed, for example fixatives that cause swelling of the rhabdoids and epithelium. Whilst the thickness of the dorsal and ventral epithelium are often provided in species descriptions, the character and states appear to be rarely used in a differential diagnosis.

4.5.3 Epitheliosomes

The epithelium, particularly over the creeping sole, secretes *epitheliosomes,* also known as dermal rhabdites (Tyler 1984) or microrhabdites. Typically they are sharply fusiform, about a quarter or less of the cell height, and produced by rhabditogen cells in the mesenchyme. They contain basic protein and may function in a number of ways: regulation of the micro-environment of the epidermal surface, producing repugnatorial substances, or strengthening elements of the cell web in areas of mechanical stress (Tyler 1984) such as the creeping sole. The character epitheliosomes, with states presence or absence, has not been used in terricolan taxonomy.

4.5.4 Infranucleate (insunk) epithelium

An infranucleate epithelium is found in areas of mechanical stress such as the creeping sole and pharyngeal lumen. This condition may protect the cells from total destruction and may facilitate regeneration when cellular damage is sustained (Tyler 1976). Insunk secretory cells containing glycogen occur in the ventral ciliated epidermis of *B. kewense* (Hauser 1966), and *B. adventitium* (in Curtis *et al* 1983). As well as protection from mechanical stress the insunk condition facilitates the accumulation of glycogen by allowing a greater increase in cell size than can be accommodated in a normal epithelium limited by a basement membrane (Curtis *et al* 1983). Insunk epithelial cells are also found in ciliated sensory pits (Storch & Abraham 1972; Curtis *et al.* 1983) where this condition may facilitate close packing of receptors by having the nuclei of epithelial supporting cells located deep within the mesenchyme (Tyler 1984). The infranucleate condition is often used as a character state for particular organs, and will be considered further in these contexts.

4.5.5 Cilia

The extent of epidermal ciliation varies between taxa. In the rhynchodemid *Microplana terrestris* cilia are largely-confined to the creeping sole, which has a normal epithelium (Bautz 1977). Dorso-ventral epithelial cilia and an insunk epithelium on the creeping sole are found in *Bipalium adventitium* (in Curtis *et al* 1983) and in *B. kewense* (in Winsor 1983b). *Timyma* is entirely ciliated, with cilia longest over the creeping sole

(EM Froehlich 1978). *Arthurdendyus triangulatus* is apparently entirely ciliated, though it lacks a differentiated ciliated creeping sole (McGee *et al* 1995). *Geobia* is sparsely ciliated and has no ciliated creeping sole. Cilia are generally absent from sensorial zones. Cilia other than those on the creeping sole are often difficult to reliably differentiate from epidermal secretions. The character cilia: with states present or absent, and with details of length, are used to a varying extent in terricolan taxonomy. Use of the character is largely hampered by lack of data.

4.5.6 Creeping sole

The creeping sole provides a number of important taxonomic characters and states, best determined microscopically. In many microplaninids the sole is narrowed and extends almost to the anterior tip. In other taxa the creeping sole begins farther back from the tip. Cilia of the creeping sole are generally longer than other external cilia in taxa exhibiting general body ciliation. Exceptions include *Cotyloplana whiteleggi* where the body cilia are longer than those on the sole (Graff 1899 page 52). Cyanophil glands are generally most numerous over the creeping sole. Comparative data on creeping sole characters and states are provided in Table 4.1, Fig. 4.4.

***Character:** Creeping sole presence and width:*

0. With narrow creeping sole, less than 30% of the ventral surface.

1. With medium width creeping sole, from 30% to 50% of the ventral surface;

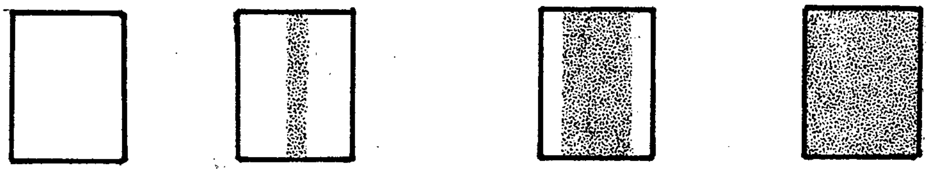
2. With broad creeping sole, more than 50% and up to 100% of the ventral surface

3. Creeping sole absent.

Meixner (1928) regarded a narrow creeping sole (State 0), present in the Rhynchodemidae (Microplaninae), and Pelmatoplaninae as primitive (plesiomorphic). A medium to broad creeping sole (States 1 & 2), and loss of a ciliated creeping sole (State

Creeping Sole and Ventral surface Table 4.1

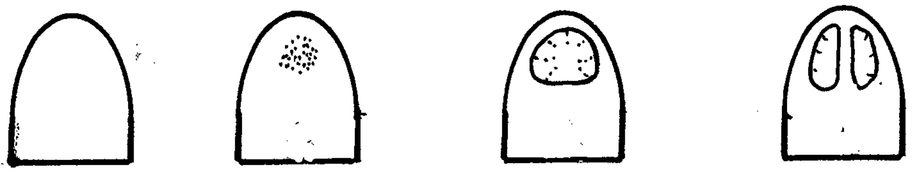
Taxon	Ciliated creeping sole present			Ciliated creeping sole absent	
	Broad >50%	Medium 30-50%	Narrow < 30%	without rhammites	with rhammites
Geoplanidae: Geoplaninae					
Geobia					yes
Gigantea		?			
Pasipha		?			
Notogyanaphallia		?			
Choeradoplana		?			
Geoplana		>30%			
Issoca		33			
Enterosyringia		50			
Xerapoa		50			
Liana	>50%				
Gusana	>50%				
Amaga	?100				
Polycladus	100				
Geoplanidae: Anzoplaninae					
Artioposthia ventropunctata					yes
Anzoplana	60				
Fyfea	70				
Geoplanidae: Caenoplaninae					
Apogaioplana					yes
Artioposthia spp (lucasi)					yes
Nelipoplana					yes
Arthurdendyus				yes	
Sokoplana				yes	
Bassioplana			15		
Australoplana			16		
Pimea			27		
Reomkago		49			
Endeavouria		48			
Timyma		?			
Kontikia	50-75				
Parakontikia	54-82				
Danidoplana	60				
Thosteelia	60				
Fletchamia	60-70				
Caenoplana	62-79				
Tasmanoplana	67-69				
Artioposthia howitti	69				
Coleocephalus	70				
Lenkunya	70-80				
Kynagoplana	76				
Barringtoplana	99				
Newzealandia	?				
Rhynchodemidae: Rhynchodeminae					
Dolichoplana	58-62				
Cotyloplana	60-70				
Rhynchodemus	72-95				
Platydemus	73-85				
Digonopyla	?				



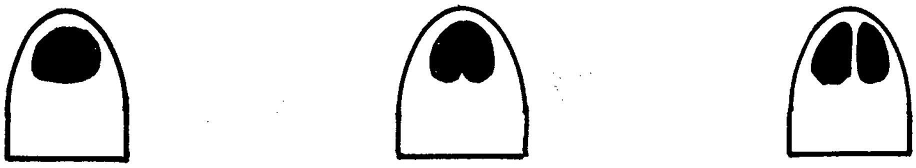
Nil Narrow Medium Broad

Figure 4.4 Creeping sole width.

Nil, ciliated sole absent. Narrow, creeping sole <30% of body width. Medium, creeping sole >30%, <50% of body width. Broad, creeping sole >50% of body width. Refer to text for explanation.



(a)Sucker absent (b) Adhesive zone (c) Adhesive sucker (d) Adhesive suckers



(e) Adhesive pad (f) Adhesive pad (g) Adhesive pads

(h) Adhesive patch (i) Epidermal adhesives (j) Haftpapillen

Figure 4.5

Anterior adhesive structures

Adhesive zone (b), suckers (c, d), and pads (e-g). Pad partly (f) and completely divided (g). Adhesive systems: (h) cutaneous erythrophil secretions (i) rhabdoids and erythrophil secretions (j) Haftpapillen. Refer to text for explanation.

3) are derived states (apomorphies). These states may represent a transformation series. It is suggested here that the narrow creeping sole in the caenoplaninids represents partial loss of the ciliated sole, moving towards an unciliated ventral epithelium. The state possibly arose separately from the development of the narrow creeping sole in the bipaliids, microplaninids and pelmatoplaninids, and may represent convergence.

There is a general paucity of quantitative data on the width of the creeping sole in the literature. The percentage widths of the creeping sole are those commonly used in the literature; exact and range of widths of the creeping sole in various taxa are also provided (Table 4.1, data derived from published figures and own measurements). A broad creeping sole (>50% of body width) is present in rhynchodeminids, Chilean geoplaninids, and in most Australian caenoplanids and anzoplaninids. Taxa with a creeping sole of medium width (30-50% of body width) include a number of South American geoplaninids, *Endeavouria* and *Reomkago.* A narrow creeping sole (<30% of body width) is characteristic of the Bipaliidae, Microplaninae, and Pelmatoplaninae. In these taxa the creeping sole is usually ridged or bordered by a furrow. A narrow creeping sole is also present in the caenoplaninids *Pimea, Australoplana* and *Bassioplana.* The creeping sole in these taxa is obvious only in the fixed specimen, and differs from the bipaliid type in being nucleate. Also in the last two genera erythrophil or xanthophil secretions, together with rhammites, are present over the ventral surface and creeping sole, though not as abundant as they are dorsolaterally.

Character: *Creeping sole epithelium:*

0. Creeping sole epithelium nucleate

1. Creeping sole epithelium infranucleate

A nucleate creeping sole appears to be the general situation for the Terricola. An infranucleate creeping sole epithelium is present in some bipaliids, microplaninids and pelmatoplaninids.

Character: *Ventral surface differentiation (in taxa without a ciliated creeping sole):*

0. With rhammites sparse or absent throughout the ventral epithelium, erythrophil secretions abundant dorsally and ventrally; ventral epithelium differentiated from the dorsal epithelium at outer ventral transition zone.
1. With rhammites and erythrophil secretions abundant dorsally and ventrally; ventral and dorsal epithelium the same, transition zone absent.

The numerous rhabdoids in the dorsolateral epithelium usually sharply diminish in density in the outer ventral zone, thus differentiating the dorsolateral epithelium from the ventral epithelium.

The loss of the ciliated creeping sole is an apomorphic condition, possibly related to habitat. Taxa without a creeping sole (0) live in damp subterranean habitats such as earthworm burrows, or are saproxylic where they inhabit interstices such as ant tunnels in wet-rotting logs. Locomotion is achieved by probably bracing and extension within microhabitat interstices using the powerful cutaneous musculature present. Taxa without a ciliated creeping sole, and in which the ventral surface is differentiated from the dorsal surface chiefly by the absence of rhammites, include *Arthurdendyus* and *Nelipoplana.* In these, rhammites are present dorso-laterally, generally terminating at the ventral zone. Otherwise the ventral parenchyma and epithelium are similar to that dorsally, charged with erythrophil or xanthophil glands and secretions. A similar situation exists in *Beauchampius trimeni* (*=Pelmatoplana trimeni*) but in this case both cyanophil and erythrophil glands open through the narrow creeping sole (Graff 1899 page 66). Ventral cyanophil glands and secretions may be sparse or about the same abundance as those dorsally. In *Nelipoplana (Nelipoplana) alba* rhammites are absent from the ventral epithelium. However *Nelipoplana (Peokrypta) enigma* occupies a position between state (0) and state (1). In this species rhammites continue over the ventral surface, but are less numerous than those dorsolaterally.

Taxa exhibiting little differentiation between the dorsal and ventral epithelia in which rhammites are distributed equally over the entire surface include *Geobia* (Brazil), *Apogaioplana, Sokoplana*, and some *Artioposthia* species including the "bootstrap stickies" of Johns (1998) present in Australia and New Zealand. They are extremely sticky when touched due to the secretion of rhammites. Dorsal and ventral epithelia are about the same thickness. Species such as *A. ventropunctata* are very sluggish and move slowly, and have weak cutaneous and weak parenchymal musculature, the sum of the indices (CMI + PMI – refer to Section 4.7) that generally equate to the muscularity of the other taxa in this group. The only evident differentiation between dorsal and ventral surfaces is that the latter is paler. *Geobia, Apogaioplana,* and *Sokoplana* have strong cutaneous musculature with similar cutaneous muscular indices.

4.6 SUBEPIDERMAL GLANDS

Characters and states derived from subepidermal glands generally relate to the types of secretions, their presence or absence, distribution of the secretory elements, and areas from which the secretions are passed. Secretory elements are mainly used as characters at the species level, as there can be considerable variation in the type and distribution of secretory elements within a genus. These characters are best exploited when the histological preparations have been stained using polychrome techniques. Characters and states derived from secretory elements will be considered where they are relevant elsewhere in this thesis.

Secretions from the Terricolan epidermis are mostly derived from long-necked sub-epithelial unicellular glands. In addition to rhabdoids, four types of glandular secretion are recognised on the basis of secretion morphology, polychrome tinctorial reactions, and histochemistry (Winsor 1983b; 1990; 1991a). At an ultrastructural level six types of secretions are recognised in *Arthurdendyus triangulatus* (in McGee *et al* 1995), and three in *Bipalium adventitium* (in Curtis *et al* 1983). A chymotrypsin-like serine protease, and the molecular weights of four other proteinases have been deduced from electrophoresis of *A. triangulatus* mucus (Mc Gee *et al* 1995). The usefulness of glandular secretions for classification may be limited by the histological procedures employed, as for example tinctorial differentiation of granular secretions.

Certain Australian live and preserved caenoplaninid species examined macroscopically (this study) under a Wood's ultra-violet light exhibited autofluorescence of the epithelium and mucus trail. This suggests the presence of compounds that may have a repugnatorial or toxic function (A. Flowers, personal communication). Severe paralysing effects noted in earthworms when touched by *Bipalium kewense* (in Johri 1952) and strong reactions provoked in earthworms by contact with *Arthurdendyus triangulatus* (in Blackshaw & Stewart 1992), suggest a neurotoxic effect, possibly due to substances present in epidermal secretions. Arndt (1925) isolated a cardiotoxin with physiological effects similar to those of cardiac glycosides from the slime of *B. kewense*, and the mucus of *A. triangulatus* contains a high concentration of neuropeptide (Curry *et al* 1991).

4.6.1 Rhabditiform glands

Rhabdoids of various types are secreted by rhabditogen cells lying in the mesenchyme below the cutaneous musculature. At the light microscopy level there are three main types of rhabdoids: rhabdites with length the same or less than the height of the epithelium, rhammites longer than the height of the epithelium, and chrondrocysts which are uncommon large cigar-shaped rhabdoids found in some bipaliids and rhynchodemids. At an ultrastructural level rhabdoids may be lamellate or non-lamellate, though the rhabdoid types present in the few species of terricolans investigated have not been specified (by McGee *et al* 1995 and Curtis *et al* 1983).

The commonest rhabdoid type is the rhammite - a sinuous rhabdoid longer than the height of the epithelium. They are usually distributed dorsolaterally, and extend ventrally to the margins of the creeping sole. In many taxa they are most numerous laterally. In taxa without a creeping sole such as Neotropical *Geobia,* and in *Artioposthia lucasi,* and *A. ventropunctata* they occur over the entire body surface. The austral species are extremely sticky to handle.

The functions of rhabdoids in the Terricola are uncertain, as it is difficult to isolate their effects from those of granular erythrophil secretions with parallel distribution over the dorso-lateral surface. When secreted from the epidermis the rhabdoids swell with moisture to form slime. They may have a repugnatorial function, as they form a very viscous layer over the dorsum and contain substances that taste unpleasant (Winsor pers. obs) or are toxic. They may also provide a protective proteinaceous coat over the flatworm to prevent desiccation, and they may serve as a means of excretion of unwanted metabolites. In the Paludicola it has been proposed that rhabdite-forming cells participate in the cellular succession of the epidermis, and in wound healing (Hori 1978).

Strongly acidophil, rhabdoids contain proteins with a disulphide component (Curtis *et al* 1983) and porphyrins. In deparaffinized sections of *Platydemus manokwari* the rhabdoids fluoresce a strong crimson colour indicative of the presence of uroporphyrins when examined by ultraviolet fluorescence microscopy (Winsor 1990). This phenomenon was originally observed in rhabdoids in Paludicola by McRae (1961). She noted that the rhabdoids in living worms did not autofluoresce, possibly because of elimination of the double bond structure in porphyrin by a protein or metal linkage with uroporphyrin; alternatively the uroporphyrin may be present in rhabdoids as non-fluorescing porphyrinogen. Porphyrins in other taxa are associated with respiratory pigments such as haemoglobin, and enzymes, and form chelates with metal ions. Haemoglobins reported from the parenchyma of enterosymbiotic rhabdocoeles (Umagillidae) are considered to facilitate diffusion of oxygen, and probably store oxygen during periods of hypoxia (Jennings & Cannon 1987). Whether the presence of porphyrins in *Platydemus* is evidence of a relict or novel biochemical respiratory pathway is uncertain. In humans high concentrations of cutaneous uroporphyrin result in photosensitivity because of the reaction of the substance with sunlight to form peroxides which destroy lipid and protein membranes (Bloomer & Straka 1990). Whether a similar situation occurs in terricolans, possibly related in some way to the marked photophobia exhibited by most terrestrial flatworms, has yet to be established

4.6.2 Cyanophil glands

Cyanophil glands (blue or basiphil; the mucus glands or Schleimdrüsen of Graff 1899) provide the mucus upon which the flatworm glides using the cilia of the creeping sole. The glands are distributed evenly dorsolaterally, but are more numerous ventrally over the creeping sole. Two types are presently recognised, one containing fine granules of glycogen (Hauser 1966), and the other secreting an amorphous acidic mucopolysaccharide (Winsor 1983b). In *Caenoplana coerulea*, it has been postulated that the high concentration of glutamine may relate to its involvement in the biosynthesis of glucosamine-6-phosphate and mucopolysaccharides and the production of mucus (Bryant & Janssens 1969). This pathway may also be one route by which excess nitrogen is excreted in *C. coerulea* in which the ornithine-urea cycle does not operate (Bryant & Janssens 1969). The character cyanophil glands: presence or absence is frequently provided in species descriptions. Little attention is paid to differentiating between the two types of cyanophil glands.

4.6.3 Erythrophil glands

Erythrophil glands (red, eosinophilous or acidophil; or erythrophil granular glands – the Körnerdrüsen of Graff 1899) are distributed evenly dorso-laterally and ventrally to the edge of the creeping sole. They are present ventrally in certain taxa (*Pelmatoplana, Xerapoa* and others), particularly those taxa without a ciliated creeping sole (*Nelipoplana* and others). They are often concentrated along the body in a marginal adhesive zone, and contrary to Graff's remarks (1899 page 66) there is no mutual exclusion between the presence of marginal adhesive zone and epidermal erythrophil glands (Froehlich 1955 and this thesis).

Erythrophil secretions are broadly divided into fine (Type I) and coarse granules (Type II). The fine granule secretions are probably heterogeneous. Some may contain proteins that may be proteolytic enzymes or enzyme precursors. Others may have adhesive properties, or protect the epithelium against moisture loss. The coarse granules are composed of basic proteins and are xanthophilic when stained with polychrome methods. Erythrophil secretions have an ultrastructurally characteristic lamellate appearance (Curtis *et al* 1983) also reported in the Paludicola (Pedersen 1965). The character erythrophil glands: presence or absence is frequently provided in species descriptions. Generally little attention is paid to differentiating between the types of erythrophil glands.

4.6.4 Xanthophil glands

Xanthophil (yellow-orange) coarse granular secretions have an adhesive function, and are only differentiated from other generally acidophil (erythrophil) secretions by using a polychrome staining technique incorporating both the usual red dye together with an orange or yellow acid dye. They occur along the marginal adhesive zone in geoplanids, dorsolaterally as basic protein secretions containing arginine in *Bipalium kewense* (in Winsor, 1983b), *Bassioplana* and *Coleocephalus*, on the creeping sole of *Bassioplana* and *Nelipoplana*, and are present in viscid secretions of the anterior adhesive suckers of *Pimea monticola* (in Winsor, 1991) and *Coleocephalus fuscus*. Arginine present in these secretions is an end product of the urea cycle present in the

Terricola. It is possibly one way in which terrestrial flatworms deal usefully with nitrogenous waste products. The character xanthophil glands: presence or absence is increasingly being provided in species descriptions.

4.6.5 Marginal adhesive zone

The marginal adhesive zone present in some Terricola, generally the broad geoplanids (Froehlich 1955), forms an outer ventral ring around the body. It is a rhabdoid-free marginal to submarginal longitudinal zone through which secretions pass directly from subepithelial long-necked unicellular glands. Generally erythrophil and xanthophil secretions predominate. In *Coleocephalus fuscus* the marginal secretory ridge is complex. Xanthophil and cyanophil secretions, suggestive of a viscid and releasor secreting combination of the duo-gland adhesive system (Tyler 1976, 1988) pass from the dorsal side of the ridge. Intermixed erythrophil and xanthophil secretions pass from the ventral side of the ridge suggesting a different function. Australian taxa with a prominent marginal adhesive zone include *Artioposthia diemenensis*, *A. howitti,* and *Platydemus* species. The character: marginal adhesive zone: presence or absence, is generally reported in species descriptions.

The marginal adhesive zone must have a function unrelated to movement, as the Terricola have a specialized creeping sole for locomotion (Graff 1912-1917). In the Paludicola secretions from the marginal adhesive zone may act as a lubricant upon which the flatworm glides (Tyler 1976). From my observations, in the Terricola the marginal adhesive zone and its secretions seem to function as a moisture-retaining sealant between the flatworm body, and the substratum and microenvironment in the resting animal. It is possibly analogous to the epiphragm in aestivating terrestrial molluscs. Silvery mucus outlines of flatworms are commonly observed under logs where worms have been resting. The marks are particularly evident in relatively dry microhabitats. Hardened subepidermal secretions probably also protect the whole flatworm against desiccation when the worms are buried deep in the soil during the dry summer months.

4.6.7 Adhesive suckers

Anterior adhesive suckers in the Terricola are used to capture prey. There appear to be no records of observations of the use of the sucker for locomotion. The term sucker is used here in a generic sense and includes glandulo-epidermal and glandular-muscular adhesive organs. True suckers in which adhesion is obtained from a vacuum principle rather than by adhesive secretions, as defined by Hyman (1951), are found only in some species of Paludicola from Lake Baikal. Characters provided by the sucker include presence or absence, type (Fig. 4.5), nature of the adhesive system, and specialized musculature.

Character: *Presence and type of an anterior sucker*

0. anterior sucker absent
1. with anteroventral glandulo-epithelial adhesive zone
2. with glandulo-muscular cup-shaped sucker(s) (Saugnäpf of Graff 1899), which may be:
    a. with single sucker
    b. with paired suckers
3. with glandulo-muscular pad(s) (Drüsenpolster, the glandular cushions of Graff 1899), which may be:
    a. with pad undivided
    b. with pad incompletely divided
    c. with pad wholly divided

The majority of Terricola, other triclads and "Turbellaria" do not have an anterior sucker, a condition that is probably plesiomorphic. The remaining states are apomorphic, and are analogous. Adhesive zones are present in species of *Amblyplana, Bipalium, Microplana, Othelosoma,* and *Rhynchodemus.* Single cup-shaped suckers are found in *Beauchampius spp., Cotyloplana,* and *Coleocephalus*; paired cup-shaped suckers are present in *Otheloma* species (formerly *Artiocotylus*). An undivided pad is present in *Pimea*, a pad partly divided occurs in *Thosteelia* and *Issoca*, and a wholly divided pad occurs in *Choeradoplana.*

Within each of the states (2 a-b) and (3a-c), the conditions are probably homologous, and have probably independently arisen in each of the genera; these may represent convergence in taxa occupying similar ecological niches, though at present little is known of the ecology of the non-geoplaninid taxa.

4.6.7.1 Sucker adhesive systems

***Character**: Anterior sucker adhesive system* (Fig. 4.4):

0. with erythrophil secretions, (with or without xanthophil secretions as these may not be revealed by the staining method used), with few or no rhammites
1. with rhammites, and with erythrophil (and / or xanthophil secretions)
2. with adhesive papillae (haftpapillen)

Epidermal erythrophil secretions are encountered in the majority of Terricola but can show considerable intrageneric variation, and should be used cautiously as diagnostic characters in supra-specific units (Froehlich 1955 page 244). The presence of numerous rhammites over the entire body surface reflects a loss of elements that differentiate dorsal and ventral surfaces, and is an apomorphy. Haftpapillen are specialized structures found only in the Maricola, and in the adhesive pad of *Pimea* in which a duo-gland adhesive system possibly operates (Winsor 1991b page 23). The presence of haftpapillen in *Pimea* is a plesiomorphy. It might be indicative of some common lineage of the genus with the Maricola. Alternatively it may represent convergence. It is uncertain whether the genus is a relatively young one, phylogenetic survivor or refugional relict (Winsor 1991b). Based upon the hypothesis that haftpapillen were present in ancestral seriates Sluys (1989a) considered that the absence of these structures in the Paludicola and Terricola represented a secondary loss, hypothesized to be a synapomorphy. For the Paludicola, the absence of haftpapillen is an apomorphy.

Anterior suckers require a temporary adhesion system for the capture of the prey, followed by release of the prey to pass it into the pharynx. It is uncertain whether a duo-gland adhesive system operates in adhesive suckers (and also in adhesive margins) in all the Terricola, or whether single-gland adhesion systems, operating by a viscoelastic adhesive mechanism (Hermans 1983, Tyler 1988), are present in some taxa.

4.7 MUSCULATURE

Terrestrial flatworm musculature is composed of smooth muscle. In *B. kewense* the muscle fibres are made up of thick and thin filaments and an additional intermediate filament which forms a network connecting “dense bodies”, considered analogous to Z lines of striated muscle. This suggests some degree of order in the grouping of the contractile elements of the smooth muscle cells (Sun *et al* 1979).

4.7.1 Muscle fibre types

Ultrastructural studies of muscles of *Bipalium kewense, Geobia subterranea,* and *Pasipha pasipha* revealed that all the muscles examined were of the slow fibre type, and related to the dimensions of the worm and energy requirements; both *B. kewense* and *G. subterranea* have strong muscles with thick myofilaments, extended Z-elements and voluminous mitochondria, compatible with systems requiring high energy for burrowing underground and also for catching and holding the prey (Silveria 1998). Unfortunately it is not stated from which region of the specimens the tissue blocks were taken, and thus whether the observations apply to all muscles in these taxa.

At the light microscopy level there are different forms of muscle fibres (Graff 1899, page 86, Fig. 4), and cross sectional appearance of cutaneous longitudinal muscle bundles may exhibit both solid and annulate fibres, suggestive of the fast and slow fibres seen in squid (personal observations). The rapid extension or retraction of some taxa when capturing prey, for example *Microplana termitophaga* (in Jones, & Cumming 1998), or in avoidance behaviour (*Nelipoplana, Parakontikia* species, personal observations) suggests the presence of fast-fibre muscles in certain muscle groups.

4.7.2 Terricola as muscular hydrostats

Muscular hydrostats, which include the Terricola, are characterized by a closely packed three-dimensional array of musculature. Individual muscle fibres or muscle bundles may be oriented in three general directions,

(1) perpendicular to the long axis,

(2) parallel to the long axis and

(3) helical or oblique around the long axis of the body (Kier & Smith 1985).

These muscle layers are all generally present in the cutaneous musculature, and when present the parenchymal musculature of the Terricola. Factors controlling the shape, movement, and locomotion of flatworms and muscular hydrostats have been extensively studied (Pantin 1950, Clark & Cowey 1958, Pfitzner 1958, Jones 1978a, b, Minelli 1981, Kier & Smith 1985). Characters provided by the body musculature are very important in terricolan taxonomy.

4.7.3 Cutaneous muscles

Character: Cutaneous musculature – structure (Fig. 4.6a)

0. Cutaneous musculature bipartite, with outer circular to circulo-helical (circulo-oblique) and longitudinal muscle layers.
1. Cutaneous musculature tripartite, with outer circular, helical (oblique or diagonal, generally decussate), and longitudinal muscle layers.

The cutaneous muscles in the Tricladida were regarded by Meixner (1928) as the primary organs of locomotion. In the "Turbellaria" the cutaneous musculature in its simplest form consists of an outer layer of circular fibres and an inner layer of longitudinal fibres (Bresslau 1928-1933; Hyman 1951, Sluys 1989a). In most Terricola the cutaneous musculature is generally tripartite, comprising an outer layer of circular muscles, mid layer of decussate helical (diagonal or oblique) fibres, and an inner layer of longitudinal muscles (Graff 1899). The simple two-layered state is less common, and is found in the microplaninids (definition of the Microplaninae, Ogren & Kawakatsu 1988a page 46), in the caenoplaninids *Barringtoplana,* the Caenoplana *coerulea* group (Geoplanidae), and *Kynagoplana* all of which have only single outer circulo-helical and longitudinal layers in the cutaneous musculature (Winsor 1991c), and in species of the bipaliid *Bipalium virgatum* group (Winsor 1983b). The Paludicola have four layers in the cutaneous musculature: an outer layer of circular muscle underlain by a thin ental layer of longitudinal muscle, then a layer of helical muscles, and an innermost layer of longitudinal muscles (Meixner 1928, Sluys 1989a). In the Maricola, the cutaneous musculature is tripartite although the diagonal layer is very thin and is generally difficult to discern (Sluys 1989b). It is considered here that in the Terricola the bipartite state is plesiomorphic, and the tripartite state of cutaneous musculature apomorphic. In

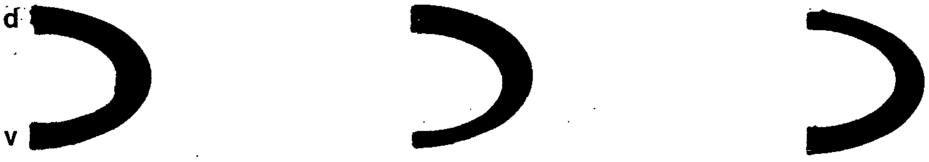
Bipartite Tripartite

Figure 4.6a

Cutaneous musculature - structure

Bipartite - with outer circulo-helical muscles, inner longitudinal muscles

Tripartite - with outer circular, mid helical, inner longitudinal muscles



(a) (b) (c)

Figure 4.6b

Cutaneous musculature - thickness

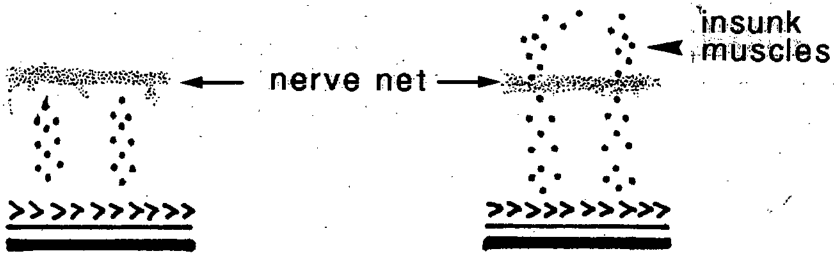
(a) dorsal and ventral same (b) dorsal > ventral (c) dorsal < ventral

(a) (b)

Figure 4.6c

Cutaneous musculature – development

(a) With longitudinal muscles not in bundles, (b) with longitudinal muscles in bundles



Normal Insunk

Figure 4.6d

Cutaneous musculature – specialization

Cutaneous longitudinal muscles fibres are present entally (insunk) to the cutaneous nerve net. Insunk muscles are often found in taxa with anterior suckers. Here the insunk muscles form part of the sucker retractor musculature.

Sokoplana there is an additional helical layer intermingled with the cutaneous longitudinal muscles possibly reflecting a derived paludicolan-type musculature, an apomorphy in this terricolan.

Character: *Cutaneous muscles – thickness* (Fig. 4.6b)

0. Dorsal and ventral musculatures about the same thickness.
1. Dorsal cutaneous musculature thicker than ventral cutaneous musculature
2. Ventral cutaneous musculature thicker than dorsal musculature.

The relative thickness of dorsal and ventral cutaneous musculatures is used here as a character with three states. The general state in the "Turbellaria" is equal development of the musculature dorsally and ventrally (considered here to be the plesiomorphic state), with strengthening and thickening ventrally with the development of the creeping sole (Bresslau 1928-1933) regarded here as the apomorphic state. Care must be exercised in interpreting this character and its states, as they can be strongly influenced by the use of relaxants, contraction on fixation, and by handling, such as stretching and squashing the specimen. For example different specimens of *Nelipoplana (Nelipoplana) alba* from various sources exhibited cutaneous muscle thickness such that dorsal and ventral musculatures were equal, ventral thinner than dorsal, and dorsal thinner than ventral. After examining a number of specimens it was decided that the last situation probably represented the norm. Similarly, in *Endeavouria*, the type species of which is *E. septemlineata* from Hawaii. Here the generic diagnosis states "subepithelial longitudinal muscle fibres in bundles which form a thick dorsal layer, but ventrally the layer is about half the thickness" (Ogren & Kawakatsu 1991 page 32). Specimens of *E. septemlineata* from Hawaii in the author's collection show the ventral musculature to be the same thickness in most parts, and only thinner than the dorsal musculature by 8%, and not the "half thickness" indicated in the generic diagnosis.

Character: *Cutaneous musculature – development* (Fig. 4.6c)

0. Cutaneous musculature generally weak, with longitudinal muscles weak, not present in bundles.
1. Cutaneous musculature generally well developed, with longitudinal muscles strong, present in well-defined bundles.

Weak cutaneous musculature with the longitudinal muscles reduced to a few fibres and not in bundles is the plesiomorphic state, and a well-developed cutaneous musculature with longitudinal muscles in well-defined bundles the apomorphic state. The arrangement of the cutaneous longitudinal muscles is used to divide the Rhynchodemidae into two subfamilies: strong muscles grouped in bundles (Rhynchodeminae) and weak muscles not in bundles (Microplaninae). Similarly in the Geoplanidae, where the cutaneous longitudinal muscles are coupled with the position of testes and creeping sole width to differentiate the three subfamilies Geoplaninae, Caenoplaninae and Pelmatoplaninae. An illustrated key incorporating these characters for the identification of families and subfamilies in the Terricola is provided by Ogren *et al* (1992b). However in the Bipaliidae, despite the availability of data and successful use of these characters in the two other families of the Terricola, taxonomists have shown a curious reluctance to use musculature to split the obviously heterogeneous *Bipalium*, until recently the sole genus in the family.

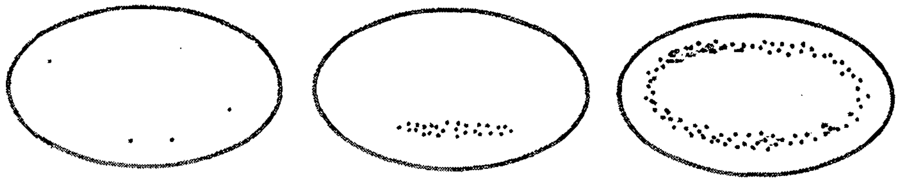
Ventral cutaneous longitudinal muscles extending entally into the parenchyma beyond the cutaneous nerve net, termed *insunk cutaneous longitudinal muscles* (Fig. 4.6d), may be encountered in taxa with anterior specializations, such as adhesive suckers. The insunk longitudinal muscles, generally in conjunction with ventral parenchymal longitudinal muscles, act as retractors for these specializations, e.g. in *Pimea* and *Thosteelia*. Such characters are used at species and genus levels in cotylate taxa.

4.7.4 Parenchymal muscles

Character: Parenchymal musculature – development (Fig. 4.7a)

0. Parenchymal longitudinal muscles weak as scattered single fibres, or absent
1. Parenchymal longitudinal muscles concentrated in a ventral (subneural) plate
2. Parenchymal longitudinal muscles present in ringzone

Parenchymal muscles in the Terricola are an adaptation to the terrestrial environment (Meixner 1928). In general there are longitudinal, transverse and dorsoventral muscles, peri-intestinal, and circulo-helical parenchymal muscles. Nomenclature of the main groups of parenchymal muscles is provided in Fig. 4.7b. Parenchymal musculature exhibits varying degrees of differentiation (organization) in



(a) (b) (c)

Figure 4.7a
Parenchymal musculature – development (transverse section)

(a) weak, with longitudinal muscles absent, or present as sparse single fibres
(b) longitudinal muscles present as a ventral plate (c) longitudinal muscles present in ring-zone and ventral plate

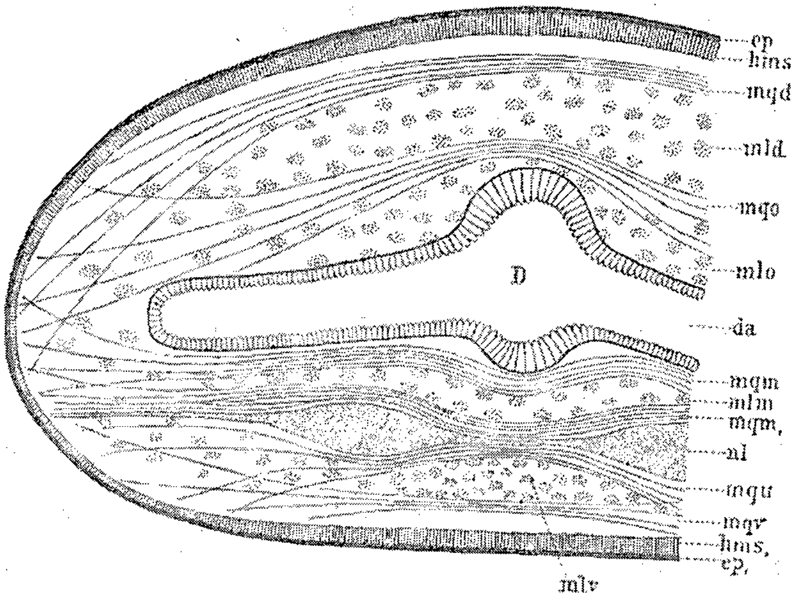


Figure 4.7b
Parenchymal musculature (transverse section) – nomenclature
(figure from Graff 1899, Fig 3, page 78).

Key to abbreviations
Ep- epidermis, hml- cutaneous musculature, mqd- dorsal transverse muscles, mld- dorsal longitudinal muscles, mqo- supraintestinal transverse muscles, mlo- supraintestinal longitudinal muscles, da- intestine, mqm- infraintestinal transverse muscles, mlm- supraneural longitudinal muscles, mqm'- supraneural transverse muscles, nl- longitudinal nerve cord, mqu- infraneural transverse muscles, mqv- ventral transverse muscles, mlv- ventral longitudinal muscles.
(terminology does not strictly follow that of Graff 1899)

much the same way as the cutaneous musculature. For example I regard the presence of compact circulo-helical parenchymal muscles in a ring zone as probably indicative of a more primitive level of organization than a ring zone largely comprising longitudinal muscles. Particular taxonomic significance is attached to the presence of longitudinal muscles, and also to the arrangements of these muscles, for example a ring zone (annulus) of longitudinal muscles as in *Kontikia*, or sub-neural ventral plate of longitudinal muscles as in *Endeavouria*, is used as characters at a genus level.

4.7.5 Quantitation of muscle strength

***Character:** Strength of cutaneous musculature*

Cutaneous Muscular Index (CMI) (Table 4.2)

***Character:** Strength of parenchymal musculature*

Parenchymal Musculature Index (PMI) (Table 4.2)

Quantitation of the previously subjective assessment of muscle layer strength is facilitated by the musculo-cutaneous index (mc:h index, the thickness of the cutaneous musculature as a percentage of the specimen height) introduced by Froehlich (1955), termed the Cutaneous Musculature Index (CMI) by Winsor (1983b). The lowest CMI value of the taxa studied was 2.7% in *Thosteelia*, and the highest value 22-24% in *Coleocephalus.* The principle of this index was extended to quantify parenchymal musculature (Winsor, 1983b), termed the Parenchymal Musculature Index (PMI), and generally applied to the relative thickness of the ventral plate, ring zone, or other prominent parenchymal muscle layer. The rhynchodeminids generally have the weakest parenchymal musculature (PMI 1.5-3.7%) with the exception of some *Cotyloplana* species (13-25%) where the musculature is modified as retractor muscles for the anterior sucker; the highest value was in *Kynagoplana* with PMI 38%. Comparative data on musculature is provided in Table 4.2.

Patterns of overall muscular strength can be identified, and generally correlate with Graff's (1899) groups (Table 4.3) within the Geoplanidae and Rhynchodemidae.

(1) The rhynchodeminids have three groups:

a. *Rhynchodemus simulans*, without parenchymal longitudinal muscles;

Cutaneous and Parenchymal Musculature

Table 4.2

Taxon	CMI%	PMI%
Geoplanidae: Geoplaninae		
Xerapoa	2-7	
Gusana	<4	
Geoplana	4-18	
Geobia	8.5	
Choeradoplana	9-15	
Liana	10	
Pasipha	12-17	
Gigantea	15	
Notogyanaphallia	15	
Enterosyringia	?	
Amaga	?	
Polycladus	?	
Geoplanidae: Anzoplaninae		
Anzoplana	22	10.8
Fyfea	9.4-10.6	9
Artioposthia spp (ventropunctata)	2.3-6.2	2.3-4
Geoplanidae: Caenoplaninae		
Thosteelia	2.7	
Timyma	3.3-4	
Danidoplana	4	
Barringtoplana	5.2-6.3	
Australoplana	8.7-15.6	
Apogaioplana	9	
Nelipoplana	9-18	
Artioposthia howitti	10.4-11.1	
Endeavouria	11	
Pimea	11.5	
Bassioplana	13	
Arthurdendyus	14	
Newzealandia	18	
Coleocephalus	22-24	
Sokoplana	11	1.2-1.4
Tasmanoplana	9-18	8.5-15
Reomkago	7	11.5-20
Caenoplana	6.7-13	5.5-13.8
Fletchamia	6.6	5.6
Lenkunya	6.5-7.6	8.2
Kontikia	3.6-5.5	12-18
Kynagoplana	2.5-3.3	38
Parakontikia	1.6-4.5	20-28.5
Rhynchodemidae: Rhynchodeminae		
Dolichoplana	14-19	1.5-3.6
Cotyloplana megalophthalma, sharpi	11-14	22
Platydemus	7-9	2.3-3.3
Rhynchodemus guttatus	6.2-6.9	2.4-3.7
Cotyloplana punctata, whiteleggi	3.8-8	13-25
Rhynchodemus simulans	5-7	
Digonopyla	?	

    b. the majority of the taxa where the cutaneous musculature is stronger than the parenchymal musculature (platydemids, dolichoplanids), and
    c. another group where the parenchymal musculature is 30-50% stronger than the cutaneous muscles largely due to the role of these muscles as sucker retractors (cotyloplanids). The microplaninids are not represented.

(2) In the caenoplaninids there are four groups:
    a. taxa corresponding to Graff's *Geoplana* Group (b) (*Thosteelia, Timyma, Danidoplana, Barringtoplana*);
    b. taxa corresponding to Graff's *Geoplana* Group (a) (*Australoplana, Apogaioplana, Nelipoplana, Artioposthia howitti, Endeavouria, Pimea, Bassioplana, Arthurdendyus, Newzealandia, Coleocephalus*);
    c. taxa with parenchymal longitudinal musculature broadly corresponding to Graff's *Geoplana* Group (c) where the cutaneous and parenchymal musculatures are approximately equal (*Artioposthia* sp., *Fletchamia, Lenkunya, Fyfea, Caenoplana, Tasmanoplana*), and
    d. those taxa where the parenchymal musculature is between 2 and 15 times the strength of the cutaneous muscles (*Anzoplana, Kontikia, Reomkago, Parakontikia, Kynagoplana*).

Meixner (1928) regarded the cutaneous musculature as the primary "motion organs" and the parenchymal muscles as an adaptation to the terrestrial environment. He considered a weak cutaneous musculature with cutaneous longitudinal muscles as thick fibres, or thin fibres grouped in small bundles, as simple (plesiomorphic); strong cutaneous muscles, but with weak parenchymal muscles, as advanced (apomorphic). It follows that a well-developed parenchymal musculature is an apomorphic condition.

4.7.6 Musculature and classification

With regard to the body musculature of the Terricola, Graff (1899) identified the following groups (Table 4.3) based on the longitudinal muscles. These are emended using standardized descriptors, with the genera placed in current families. These groups

Body musculature:

Geoplanidae:

- *Geoplana* (a) with very strong cutaneous musculature, parenchymal longitudinal muscle bundles absent. Typical of this groups are the Neotropical geoplanids
- *Geoplana* (b) with cutaneous musculature loose and not as strong as in Group (a), parenchymal longitudinal muscle bundles absent. Typical of this group are the numerous elongate planoconvex species mostly from Australia.
- *Geoplana* Group (c) with relatively strong cutaneous musculature, relatively as strong as in Group (a), with parenchymal longitudinal muscle bundles present in a loose ring zone. Typical of this group are the small cylindroid species.

Pelmatoplana: with cutaneous musculature weaker than in Group (a), with dense parenchymal longitudinal muscles dispersed throughout the parenchyma. *Choeradoplana*: with cutaneous musculature of Group (c), particularly strong ventrally (forms part of the sucker retractors). *Polycladus*: with massive cutaneous longitudinal muscle bundles, parenchymal longitudinal muscle bundles absent, parenchymal musculature generally developed weakly. *Artioposthia*: with cutaneous longitudinal musculature similar to Group (b) or possibly weaker, with parenchymal longitudinal muscle bundles very strong though not united in bundles.

Bipaliidae:

- *Bipalium*: Group (a) with well developed, through not strong cutaneous longitudinal musculature, with parenchymal longitudinal muscle bundles present in a weak ring zone.
- *Bipalium*: Group (b) with cutaneous longitudinal musculature reduced to small fibre bundles, with parenchymal longitudinal muscle bundles present in a broad ring zone and compacted into a ventral plate.

Bipaliids formerly placed by Graff in *Placocephalus* have cutaneous longitudinal musculature equally developed as in *Bipalium* Group (a), with parenchymal longitudinal muscle bundles present as loose fibres, not compacted into layers.

Rhynchodemidae:

- *Rhynchodemus*: Group (a) with very weak cutaneous longitudinal musculature, in parts reduced to single fibres, with parenchymal longitudinal muscle bundles present, often concentrated into a layer, now the Microplaninae.
- *Rhynchodemus*: Group (b) with strong cutaneous longitudinal muscles in bundles, as in *Geoplana* Group (c), parenchymal longitudinal muscle bundles weak, with muscles present as scattered fibres, or absent (in which case the cutaneous longitudinal musculature is particularly strong), now the Rhynchodeminae.

Dolichoplana: With extremely thick, strong cutaneous longitudinal musculo bundles, with parenchymal longitudinal muscle bundles present in a ventral plate. *Amblyplana*: With cutaneous longitudinal musculature reduced to single fibres and with parenchymal longitudinal muscle bundles close to the musculature of *Rhynchodemus* Group (a). *Platydemus*: With musculature essentially the same as Geoplana Group (a).

Cotyloplana: With well developed cutaneous musculature, with parenchymal longitudinal muscle bundles present, very strong (involved as retractors for the anterior sucker). *Artiocotylus*: With very weak cutaneous longitudinal muscle bundles reduced to a few fibres, with parenchymal longitudinal muscle bundles present, very strong; massive anteriorly, dorsally and laterally. Both *Cotyloplana* and *Artiocotylus* were placed by Graff in the family Cotyloplanidae, now subsumed within the Rhynchodemidae.

Table 4.3. Graff's (1899) classification of the Terricola based on body musculature

correlate well with the species, their morphology and their cutaneous and parenchymal musculature which formed the basis of his system of classification, and which in part form the basis of the present classification, to be discussed in Chapter 5.

4.8 EXCRETORY SYSTEM

The protonephridial system in the Terricola, as in other triclads, is presumed to be concerned with the ultrafiltration of parenchymal fluid, followed by resorption of all or part of the filtrate, and the excretion of wastes. Definitive experimental evidence for these functions of the protonephridia in the Terricola is lacking. However there is strong circumstantial evidence of an osmoregulatory function of the protonephridial system. This is provided by comparative ultrastructure of nephridial elements of Triclads with those of other taxa in which their osmoregulatory function has been established for the nephridial elements (McKanna 1968a,b). Terrestrial flatworms have few moisture-saving adaptations and live in humid microhabitats. However they generally avoid wet conditions, and have a narrow tolerance range for moisture levels (Froehlich 1955). Thus their excretory system is of critical importance for water regulation and conservation. For terrestrial nemerteans it has been suggested that maximal osmoregulation will follow flooding of the environment, and cessation of flame cell activity will occur when water conservation is vital (Gibson 1972). These suggestions probably hold equally for the Terricola. Protonephridial elements are particularly conspicuous and extensive in species such as *Lenkunya munda,* and in some species of *Parakontikia* and *Dolichoplana.* These taxa commonly occur in riparian habitats or low-lying country subject to periodic flooding. Their prominent nephridial systems may reflect a greater osmoregulatory requirement, compared to those required by species common in drier upland environments.

Most of the knowledge of protonephridial systems in Triclads comes from investigations of Maricola (summarized in Sluys 1989b), and Paludicola (McKanna 1968a, b; Wilson & Webster 1974; Ishii 1980a, b). The fragmentary knowledge of the excretory system in the Terricola at the light microscopical level (Graff 1912-17) largely concerns descriptions of flame cells and fine convoluted ducts and canals. The protonephridial system in Terricola is difficult to trace for any distance, and at a light microscopical level the fine ducts can be readily confused with those of secretory elements. Good fixation and staining are essential for light microscopical studies, and the MSB staining method used for mammalian nephridial studies is particularly useful in providing good colour contrast of the fine nephridial elements. The use of thin resin sections examined by light microscopy may facilitate reliable recognition of protonephridial elements and allow their use in taxonomy. Ultrastructural characters of the flames cells and protonephridial capillaries in Terricola (Silveira 1970, Silveira & Corinna 1976, Rohde & Watson 1992) are important in developing an ultrastructurally based morphological phylogenetic system within the Phylum Platyhelminthes (Rohde & Watson 1992).

4.8.1 Terricolan protonephridial system

The essential elements of the terricolan protonephridial system include a *terminal complex* with one or more flame bulbs joining a protonephridial tubule termed the *end-canal.* The end canals anastomose with ducts traversing the body laterally and dorso-ventrally which eventually open into the unciliated *main canals* passing longitudinally along the body (this thesis). End canals also communicate with *convoluted ciliated canals*, found to be rich in alkaline phosphatase suggesting resorption of water (Danielli & Pantin 1950) and hydrolysis of sugar phosphates prior to the uptake of sugar and phosphate moieties separately by the tubule cells (Wilson & Webster 1974). The convoluted canals join *final ducts* which open via *nephridiopores* through the pharyngeal outer epithelium, directly into the pharyngeal pouch diverticulum (this thesis), or through the epidermis, especially the ventral surface and ciliated creeping sole (Danielli & Pantin 1950). Ishii (1980a,b), in his ultrastructural studies of *Bdellocephala* (Paludicola), simplified the confusing nomenclature of canals, ducts and tubules (exclusive of the main longitudinal trunks or canals), reducing them to two types:

a) *proximal tubules* nearest the flame cells, up to 8µm diameter with ciliated epithelium except in the trunk, with cilia without accessory rootlets, tubules often branched, with epithelial cells containing organelles concerned with the reabsorption of proteinaceous substances, tubules always surrounded by *fixed parenchymal cells (Athrocytes or paranephrocyte)*; and

b) *distal tubules,* 10-15μm diameter, unciliated, tubule cells with organelles suggestive of active reabsorption of substances from the filtrate, evidenced by numerous pinocytotic vesicles within the cytoplasm, terminate with reduced calibre at the nephridiopore.

4.8.2 Taxonomic characters of the protonephridial system

At the light microscopy level there are few potential taxonomic characters afforded by the protonephridial system. The position, arrangement and number of the longitudinal main canals in Terricola, best determined from the pre-pharyngeal transverse sections, were investigated (this thesis) for use as possible taxonomic characters. Data are provided in Table 4.4:

***Character**: Protonephridial system, vertical configuration of main canals* (Fig. 4.8a)

1. Main canals are situated dorsally only

2. Main canals are situated dorsally and ventrally

3. Main canals are situated ventrally only

***Character**: Protonephridial system, transverse configuration of main canals* (Fig. 4.8b)

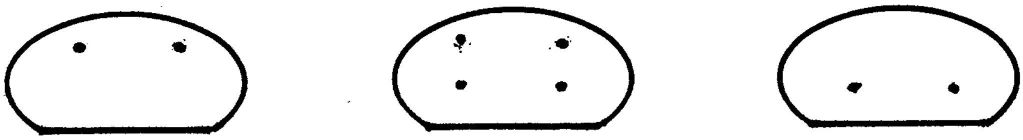
1. Main canals are situated only in the midline

2. Main canals are situated in the midline and bilaterally

3. Main canals are situated only bilaterally

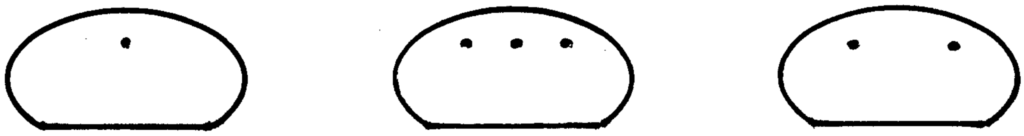
Table 4.4 Protonephridial Main Canals

Taxon	Position of canals			Arrangement of canals			Number of canals			Canal internal diameter um
	dorsal only	dorsal & ventral	ventral only	mid only	bilateral only	mid & bilateral	single	double	multiple/ grouped	
Rhynchodemidae: Rhynchodeminae										
Dolichoplana Norfolk Island	Y				Y		Y			3.6
Dolichoplana Lissner Park LW1212	Y				Y			Y		
Dolichoplana Condon #1 LW1432	Y				Y				Y	
Dolichoplana Iron Range LW 1089	Y				Y				Y	
Dolichoplana Kirrama LW 1085	Y				Y				Y	
Cotyloplana "erica" LW 1489	Y				Y				Y	
Rhynchodemus Pitcairn	Y				Y				Y	
Rhynchodemus simulans LW48	Y				Y				Y	
Platydemus manokwari	Y					Y	Y			
Platydemus Malanda LW 1148	Y					Y	Y	Y		4.5-7.2
Geoplanidae: Anzoplaninae										
Anzoplana	Y				Y			Y		
Artioposthia ventropunctata	Y				Y			Y		
Fyfea carnleyi			Y		Y				Y	
Geoplanidae: Caenoplaninae										
Artioposthia dovei	Y				Y		Y			3.4
Nelipoplena Nelipoplana alba W1992	Y				Y			Y		
Sokoplana W10216	Y				Y		Y			11.3
Kontikia orana	Y				Y		Y			
Parakontikia ventrolineata LW254	Y			Y				Y		
Parakontikia coxii LW1582	Y			Y				Y		3.6
Parakontikia "ourapilla" LW1551	Y			Y					Y	
Parakontikia atrata LW1285	Y				Y				Y	2-3.6
Fletchamia m'mahoni LW377	Y				Y				Y	
Tasmanoplana lyra QVMAG 19-3605	Y				Y				Y?	
Tasmanoplana tasmaniana	Y				Y			Y		6.3
Lenkunya adae	Y				Y		Y		Y	
Lenkunya munda LW378	Y				Y		Y			5.4
Lenkunya munda LW179, LW379	Y				Y		Y			3.6
Thosteelia	Y				Y?		Y			
Parakontikia "ardesia" LW1308	Y					Y			Y	
Caenoplana subviridis	Y					Y	Y			2.7
Endeavouria septemlineata LW1497	Y					Y			Y	5.4
Bassioplana typhlops		Y			Y		Y	Y		3.6-5.4
Australoplana sanguinea W1857		Y			Y		Y		Y	4.5-9.9
Reomkago quadrangulata LW926		Y			Y		Y		Y	5.4-8.1
Fletchamia sugdeni LW1039		Y			Y		Y			7.2
Fletchamia mediolineata LW458		Y			Y		Y			3.6-5.4
Caenoplana hoggii LW456		Y			Y				Y	2.7-5.4
Caenoplana variegata LW745		Y			Y			Y	Y	1.8-4.5
Fletchamia quinquelineata LW354			Y		Y		Y		Y	4.5
Caenoplana coerulea LW103			Y		Y			Y		
Artioposthia howitti			Y		Y					5.4
Caenoplana spenceri			Y			Y	Y		Y	7.2-9
Caenoplana spenceri QVMAG			Y			Y			Y	6.3-7.2
Kynagoplana										
Barringtoplana	unable to be reliably determined									
Apogaioplana	unable to be reliably determined									
Nelipoplana Peokrypta enigma	unable to be reliably determined									
Danidoplana	unable to be reliably determined									
Arthurdendyus	unable to be reliably determined									
Coleocephalus	unable to be reliably determined									
Bipaliidae										
Bipalium kewense	Y			Y			Y			



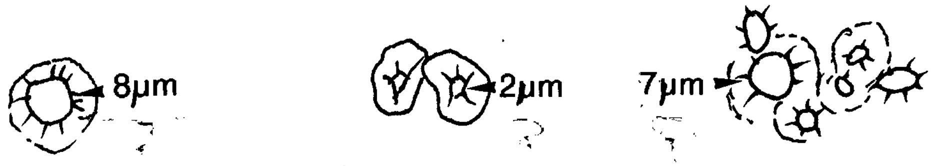
(a) (b) (c)

Table 4.8a
Protonephridial system – Configuration of the main canals in the dorsoventral plane
(a) dorsal only (b) dorsal and ventral (c) ventral only



(a) (b) (c)

Table 4.8b
Protonephridial system – Transverse configuration of main canals
(a) midline only (b) midline and bilaterally (c) bilaterally only



(a) (b) (c)

Table 4.8c
Protonephridial system – Grouping of main canals
(a) single canals (b) paired canals (c) grouped canals

Transverse section Longitudinal section

Table 4.8d
Protonephridial system – Main canals
(refer to text for explanation)

***Character**: Protonephridial system, grouping of main canals:* (Fig. 4.8c)

1. Main canals present singly
2. Main canals present in pairs
3. Main canals present in groups (for example in groups of 3, 4 or 6 canals)

4.8.3 Main Canals

The configurations of main canals are well known for the Maricola and Paludicola, but there are few comparative data for the Terricola. Hyman (1951) stated that "in Triclads there are one to four on each side and these are commonly anastomosed into a network or if single broken up into a network, with numerous nephridiopores. There may be two dorsal and two ventral pairs of tubules, or one dorsal and one ventral pair, or one to two dorsal pairs without ventral pairs". The main canal in *Rhynchodemus sylvaticus* described by Mecznikow (1866, page 562, Fig. 12) is probably an end canal. A possible main canal in *Dolichoplana feildeni* is figured by Graff (1899, Plate 47, Fig. 9, ex3, and Fig. 10).

The peri-intestinal and sub-intestinal regions of many taxa are highly vascular, comprising vitelline, secretory and protonephridial ducts. In some gregarine-induced pathologically vacuolate material, the vacuoles resemble ducts. It is extremely difficult at times to reliably differentiate a main protonephridial canal or secondary canal from the other vessels or lesions in these areas, and it is suspected that for many taxa there are more main canals present than have been identified here.

Main canals are the largest of the unciliated longitudinal protonephridial vessels, or may consist of a group of bundled smaller vessels. In the taxa studied, the main canals are 3.0μm – 11.0μm internal diameter with characteristic transverse appearance (Fig. 4.8d): circular to ovate in cross-section, with a thickened radially-striate wall with what appears to be short radial outward projections up to 2.8μm long. In longitudinal sections, the walls of the main ducts, and also those of the large secondary vessels running at right angles to the main ducts, are finely longitudinally striate. The "striae" are cyanophil with beaded appearance, separated by a distance of 0.7 – 1.0μm (in *Dolichoplana* sp. Norfolk Island). This appearance is similar to that of the ultrastructural appearance of the main canals described in *Temnocephala novazelandiae* by Williams (1981). Here the tubular epithelial cells of the canal are honeycombed by extensive tubular invaginations of the basal plasmalemma, with numerous oval mitochondria closely associated with the basal infoldings, the mitochondria being aligned with their long axes parallel to the basal infoldings giving a beaded appearance. The infoldings appear to be 0.2μm – 0.3μm wide (from measurements of Fig. 8, Williams 1981). The cyanophil beaded appearance of the "striae" noted in the main canals of Terricola is consistent with the structure of the main canals described in *Temnocephala*, the main difference being the width of the pale zones between the striae (= infoldings in *Temnocephala*) 0.7– 1.0μm wide in *Dolichoplana* sp.. It has not been established how, other than by changes in cross-sectional area, main ducts accommodate to the extension and contraction of the flatworm; the ducts do not appear to be helical or to have coiled zones.

Configurations of main canals in the Terricola generally follow the Triclad pattern though are more complex. The main canals can occur dorsally only, ventrally only, and both dorsally and ventrally. They may occur in the midline, bilaterally, or both midline and bilaterally, and as single canals, paired canals, or groups of multiple canals. Comparison of main canal characters and states between taxa is presently chiefly limited by the absence of comparable data for non-Australian taxa, especially those like the microplaninae considered "primitive" in other aspects of their anatomy. The potential usefulness of these characters for taxonomic purposes is uncertain: the main canal characters and states are not clearly aligned with present taxonomic groupings at subfamily and genus level. Furthermore it is not clear what would be considered the plesiomorphic and which the apomorphic states. Main canals occur bilaterally in a number of "Turbellaria" (Hyman 1951) and this may represent the plesiomorphic condition; the presence of a median canal, which might be a fusion of bilateral tubules, an apomorphy.

With the exception of *Platydemus,* in the Rhynchodeminae the main canals only occur dorsally and bilaterally. In *Platydemus* main canals are present bilaterally and in the median position as in the case of some species in *Parakontikia* (Caenoplaninae). Within the Caenoplaninae there is greater variation, with main canals occurring dorsally (some *Artioposthia* species, *Endeavouria, Kontikia,* Parakontikia, *Nelipoplana, Thosteelia, Tasmanoplana*, and *Lenkunya*; *Fletchamia mcmahoni* and *Caenoplana subviridis*) both dorsally and ventrally (*Bassioplana, Coleocephalus* and *Reomkago*; *Fletchamia mediolineata* and *sugdeni; Caenoplana hoggii* and *variegata*), and ventrally only (*Caenoplana coerulea, C. spenceri, Fletchamia quinquelineata* and *Fyfea*). In *Bipalium kewense* (Bipaliidae) the main canals occur as bilateral groups, each group comprising three canals, dorsally. Main ducts occur singly, in pairs, and as multiples in both Rhynchodeminae and Caenoplaninae.

4.8.4 Flame bulbs and proximal tubules

Flame cells and proximal tubules appear to have little taxonomic application at the light microscope level. The flask-shaped flame bulbs with cilia (flames) have been described in *Dolichoplana feildeni, Geoplana rufiventris,* and *Bipalium kewense* (Graff 1914), *Rhynchodemus terrestris* (by Danielli & Pantin 1950), *Bipalium kewense* (in Winsor 1983b), *Platydemus manokwari* (in Winsor 1990), and *Pimea monticola* (in Winsor, 1991b). The ultrastructure of the flame bulbs (cyrtocytes) has been described for *Pasipha pasipha* (Geoplaninae) (Silveira & Corinna 1976), and *Artioposthia* sp. (*Anzoplana trilineata* this thesis) (Rohde & Watson 1992). There appears to be no taxonomic significance in the number of flame bulbs present in the terminal complex, as at a light microscopical level they here may be one, two, or more flame bulbs present, and they can vary in number in the individual (personal observations). In *Artioposthia* sp. both single and twin terminal cells occur, and the flame bulb, some 14μm long, comprises a single cell with numerous interdigitations of cytoplasmic strands forming the slits in the weir in the proximal terminal cell, with cilia largely arising from the basal walls of the flame bulbs (Rohde & Watson 1992).

Ultrastructurally the flame bulbs of the Terricola are similar to those described in the Paludicola, though there remains some uncertainty about the commonality of the structure of the weir in the three species studied to date (Rohde & Watson 1992). These terminal organs differ from those in *Bipalium kewense* in which the end canals appear to lie within a syncytium of a finely granular cyanophil, sometimes vacuolated, cytoplasm (Winsor 1983b). This syncytial structure resembles the athrocyte or paranephrocyte of Bresslau (1928-1933 page 109; Hyman 1951, page 111), termed by Pedersen (1961a,b) *fixed parenchyma cells* the ultrastructural features of which, especially the presence of lysosomes, suggest a function as a mediator in the transfer of metabolites.

4.8.5 Distal tubules

The distal tubules terminate at the nephridiopore. They include, in part, the *collecting region*, and all the *osmoregulatory region* of *Pasipha pasipha* (Geoplaninae) mentioned by Silveira (1976). In *Dugesia tigrina* they also include the osmoregulatory duct characterized by an amorphous luminal coat, cytoplasmic vesicles, and by the presence of basiphil paracrystalline granules (McKanna 1968b) that are rhombic in shape (McKanna 1968b, Fig. 7). This suggests that the cells of the distal tubules selectively accumulate and probably excrete insoluble solids. Supporting evidence for this view is provided in the following section.

4.8.6 Nephridiopores and excretory products

Nephriopores open through the outer epithelium, probably over the entire body, though in *Rhynchodemus terrestris* appear to be concentrated over the ventral surface, especially the creeping sole (Danielli & Pantin 1950). In the caenoplaninids Australoplana, *Bassioplana, Danidioplana* and *Nelipoplana* the nephridiopores open into a very elongate diverticulum of the pharyngeal pouch. The diverticulum passes between the posterior rami of the gut, almost to the copulatory apparatus, and is lined by a syncytial-like epithelium with a cyanophil granular cytoplasm with ragged, finger-like processes on the lumenal margin. Fine cyanophil granular matter, present in the distal nephridial ducts, appears to pass into the diverticular epithelium where it is secreted in an apocrine-like matter: granules concentrate in the cytoplasm along the lumenal margin, then blebs of cytoplasm "pinch off" and move into the lumen of the diverticulum. It is postulated that the cyanophil granular material is insoluble waste. The pharyngeal diverticula act as a mid-posterior sump for waste excreted by the nephridiopores. Discharge of the excretory ducts into the pharyngeal cavity was not observed in other taxa studied here. This arrangement may be the re-emergence of an ancestral character, present in other "Turbellaria" where the excretory tubules empty into the pharyngeal cavity (Bresslau 1928-1933, pages 110-111; Hyman 1951 page 109).

Minute rhombic crystals of calcium carbonate excreted within the mucus from the dorsum of *Rhynchodemus britannicus* (now *Microplana scharffi)* were described by Percival (1925). The crystals also appeared to be present in the dorsal and dorsolateral epidermis and underlying parenchyma. Given the close parallels between the protonephridial systems within the Platyhelminthes, and especially between those of the Paludicola and Terricola, it is tempting to speculate that the crystals reported in the distal tubule cells of *Dugesia* are similar to those observed from *Rhynchodemus britannicus* by Percival (1925), and that they are also similar to the granular cyanophil matter excreted into the pharyngeal diverticula in certain caenoplaninids. Similar substances differentially vitally stained with alizarin are also present in the protonephridial canals of the trematode *Galactosomum lacteum* (Westblad 1924, in Wilson & Webster 1974); alizarin uptake is indicative of the presence of calcium salts, though the stain specificity for calcium was not recognized in the original paper. Concretions of calcium carbonate with some phosphate are also reported in the lumen of canals in other trematodes (Wilson & Webster 1974).

Percival (1925) speculated that the presence of the crystals observed from *Rhynchodemus britannicus* was associated with respiration. Carbon dioxide is a product of cellular respiration: it must be utilized biochemically or excreted by the body. Some Platyhelminthes, including the terricolan *Bipalium kewense* are ureotelic. In the urea cycle carbon dioxide is essential to the biosynthesis of arginine and urea from ammonia, the nitrogenous end product of protein metabolism (Campbell, 1965). In addition, carbon dioxide, probably in the form of bicarbonate, as frank carbon dioxide, or as carbamino compounds in body fluids, is probably precipitated as calcium carbonate in the distal tubule cells. The crystalline calcium carbonate is then eventually excreted without water loss, consistent with water conserving strategies of a stenohygric hydrocoele.

Whilst the excretory system provides some potentially useful characters and states for taxonomy, absence of data from taxa outside the study region, and apparent intra-generic variation make it difficult to determine at what level these characters are most useful. For the latter reason, characters and states of the excretory system are excluded from generic diagnoses in this thesis. Usefulness of these characters is also limited by the difficulties in clearly identifying these structures in paraffin sections, particularly in indifferently fixed material. Taxonomic characters of the excretory system provided by ultrastructural features appear to be applicable above an ordinal level; neither data nor techniques are practicable for routine taxonomic purposes at family, genus and species levels.

4.9 NERVOUS SYSTEM

The nervous system of the Terricola consists of a large anterior ganglion ("brain"), paired ventrolateral nerve cords or plate, nerve trunks, submuscular nerve net, and peripheral (subepidermal) nerve net. Reduction in the number of nerve cords to two is considered to be a synapomorphy in the Paludicola and Terricola (Sluys 1989a). The shape of the brain and ventral nerve cords generally reflect the shape of the head and body. The ventrolateral nerve cords are linked by a series of transverse commissures. Accounts of the nervous system in the Terricola are provided by Graff (1899, 1912-1915; Sluys 1989a). Apart from the eyes and sensory receptors, characters provided by the nervous system are presently seldom used below the ordinal level in the taxonomy of the Terricola, and will not be considered further in this thesis.

4.9.1 Eyes

The histology and taxonomic significance of eyes and eye characters in the Tricladida has recently been reviewed (Sluys 1989b, 2001) and will not be considered in detail here. Critical studies on eye histology require thin sections. Sluys (2001) rightly remarks on the constraints imposed by conventional serial sections at 7-8μm in elucidating details of eye histology at the light microscopical level, especially in often indifferently fixed specimens. This limits the availability of characters and states provided by the histology of the triclad eye. The following comments are taken from Sluys (1989b, 2001) unless otherwise indicated.

4.9.1.1 Eye types

Within the Tricladida there are two broad types of eyes: the unicellular type and the multicellular type. Each eye type can be further subdivided on the basis of differences in retinal clubs and the presence of "accessory" eye structures. Within the unicellular eye type (Sluys Type A) there are three subtypes of eyes: the *Planaria torva* type (A1), reported for *Bipalium kewense*; the *Dendrocoelum lacteum* type (A2); and lensed eyes (A3 and A3') restricted to the Maricola, though it is considered here that *Fyfea* may have this eye type (this thesis). The multicellular eye type (Sluys Type B) can be divided into the *Dugesia gonocephala* type (B1); the *Microplana terrestris* type (B2) that is present in species of rhynchodeminids, microplaninids, geoplaninids and caenoplaninids; and the *Platydemus grandis* type (B3) found only in rhynchodeminids. In the descriptions of taxa in this thesis, eyes have only been categorized as pigment-cup type, or lensed type of eyes. No attempt has been made to determine whether the eyes are uni- or multicellular. Sluys highlights the need for more comparative data and rechecking old data using modern techniques before plesiomorphic and apomorphic conditions for eye structure in planarians can be properly determined.

4.9.1.3 Eye number

Characters afforded by the eyes in Terricola include eye number, and the anterior, dorsoventral, and horizontal distribution of the eyes. These characters are most useful in sorting specimens into RTUs.

***Character**: eyes, presence or absence* (Fig. 4.9a)

0. eyes present
1. eyes absent

The presence of eyes in the Tricladida and other "Turbellaria" is the general (plesiomorphic) condition. Eyes are secondarily lost, an apomorphy, in a number of genera (*Apogaioplana, Bassioplana, Danidoplana, Geobia*) occupying subterranean and saproxylic niches. Very small uniformly sized and sparse eyes are present in some taxa (*Australoplana, Nelipoplana*), and secondary loss of eyes in taxa occupying these niches, akin to loss of eyes in cavernicolous animals, is not difficult to envisage.

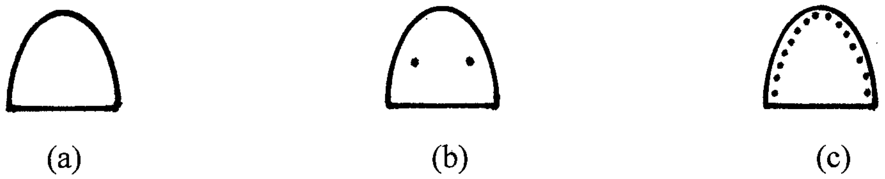

(a) (b) (c)

Table 4.9a
Eyes- absence or presence
(a) absent (b) with two eyes (c) with multiple eyes

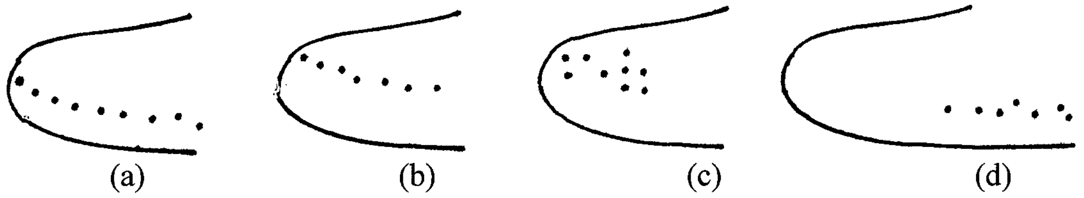

(a) (b) (c) (d)

Table 4.9b
Eyes – distribution of multiple eyes anteriorly
(a) eyes skirt anterior tip margin (b) eyes pass over anterior end (c) eyes scattered over anterior end (d) eyes do not extend to anterior tip

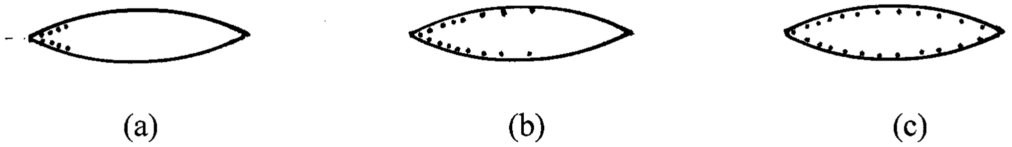

(a) (b) (c)

Table 4.9c
Eyes – distribution of eyes along body
(a) eyes anterior or extending a short distance anterolaterally (b) eyes extend to mid body only (c) eyes extend entire body length

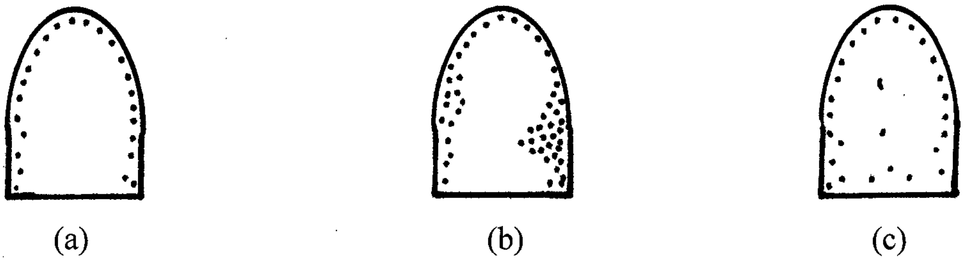

(a) (b) (c)

Table 4.9d
Eyes – distribution of eyes dorsally
(a) eyes absent dorsally (b) eyes extend dorsally anterolaterally, often in characteristic shaped patch (c) eyes scattered sparsely dorsally along body.

Character: *eyes, number* (Fig. 4.9a)

0. with two eyes
1. with multiple eyes

The presence of one pair of eyes in the Tricladida and other "Turbellaria" appears to be the general (plesiomorphic) condition. The multiplication of eyes beyond a single pair has been attributed to post-embryonal development and increase in eye number (Meixner 1928). In caenoplaninids such as *Caenoplana* and *Lenkunya*, eye number increases with age. It is not known whether the number of eyes plateau. In many geoplanids there may be populations of eyes of different sizes and orientation. Often both large and small eyes appear to be randomly mixed. Sometimes the anterior eyes are particularly large, with smaller eyes anterolaterally (eg. as in *Geoplana cassula* (in E.M. Froehlich 1955, plate 3, Fig. 17, and some subantarctic species).

Given close parallels in the ground plans of the copulatory organs in Rhynchodemid and Geoplanid taxa, Beauchamp (1939 page 337) questions the separation of these two families based solely upon eye number.

Relatively large eyes are present in some caenoplaninids such as *Kontikia* and *Parakontikia*, and are also present in taxa phenetically similar to members of these genera in which there are only a few eyes anteriorly, often with an uneven number on each side (e.g. *Parakontikia chapmani* with 6-8 eyes on each side). This suggests possible secondary loss of eyes. A consequence of this could be non-rhynchodemid two eyed taxa. The determination of eye type could be significant in tracing lineage in two-eyed taxa.

4.9.1.4 Eye distribution

Character: eyes, distribution of multiple eyes anteriorly (Fig. 4.9b)

0. with eyes skirting the anterior tip margin (single row)
1. with eyes passing over the anterior tip (single row)
2. with eyes over the anterior end (scattered, multiple)
3. with eyes absent around and over the anterior tip, but present in rows or patches anterolaterally

It is uncertain which of these conditions is plesiomorphic, and which are apomorphic. In the majority of geoplanids the eyes pass around the anterior end. In the bipaliids and some caenoplaninids (*Timyma,* and in *Kontikia cookiana* and *K. forsterorum* - which are not really *Kontikia*) the eyes pass over the anterior margin of the headplate or anterior end. Taxa with eyes over the anterior end include some pelmatoplaninids, and undescribed species from the subantarctic islands of New Zealand. Eyes are absent from the anterior tip, but present anterolaterally in *Pimea, Endeavouria* and *Choeradoplana*, and some pelmatoplaninids.

Character: eyes, distribution of multiple eyes horizontally (Fig. 4.9c)

0. with eyes anterior, or extending a short distance anterolaterally
1. with eyes extending only to the mid body (pharyngeal region)
2. with eyes extending along the entire body length

The presence of eyes anteriorly as in most non-terricolous triclads and other "Turbellaria", or extending for a short distance anterolaterally as in *Polycelis* (Paludicola) appears to be the plesiomorphic condition. The two states of further extent of eyes posteriorly are apomorphic conditions. Taxa with eyes extending only to the region of the mouth include the pelmatoplaninids and caenoplaninid *Pimea*, and possibly other taxa – it is difficult to ascertain the extent of eyes in dark coloured forms. The eyes continue almost to the posterior end, generally as a staggered single row, sometimes crowded along the row, in most of the Caenoplaninae and Geoplanidae.

Character: eyes, distribution of multiple eyes dorsolaterally (Fig. 4.9d)

0. with eyes absent dorsally
1. with eyes extending dorsally anterolaterally, often in a characteristically shaped patch
2. with eyes scattered sparsely dorsally, along most of the body length

The general condition in the Geoplanidae appears to be the absence of eyes dorsally. Anterolateral crowding with dorsal extension is present in the caenoplaninids *Fletchamia* and *Lenkunya* as well as in some geoplaninids (eg. *Pasipha*) and pelmatoplaninids. Eyes may be scattered sparsely dorsolaterally along the body length, as in *Geoplana chiuna* and other species. The presence of dorsal eyes is difficult to determine in heavily pigmented species.

4.9.2 Sensorial zones, sensory receptors and sensory pits

Sensorial zones, sensory pits, and sensory receptors in the Terricola, including the eyes, can only be correctly interpreted at an ultrastructural level, together with supporting evidence derived from the use of novel molecular probes and confocal microscopical techniques. The few ultrastructural studies of terricolan sensory structures are largely confined to the Bipaliidae, particularly one species, *Bipalium kewense* (in Storch and Abraham 1972; Curtis *et al* 1983; Fernandes *et al* 2001). It is not known to what extent these findings are representative of the other Terricola. Thus characters provided by these sensory structures at the macroscopical and light microscopical levels are limited to the type and extent of the sensorial zone, and types of the associated sensory receptors and sensory pits.

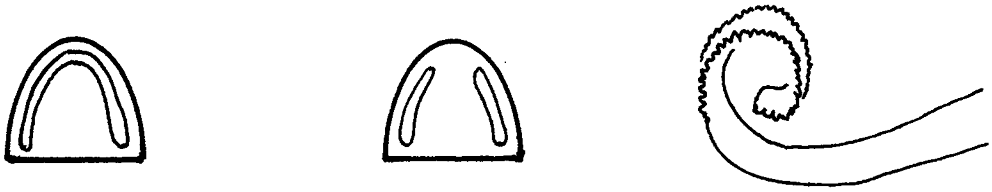
Ultrastructural studies of the sensorial zone of *Bipalium kewense* (in Storch and Abraham 1972; Curtis *et al* 1983; Fernandes *et al* 2001) reveal papillae around the outer margin of the headplate upon which are situated microvilli, considered to have a tactile function (mechanoreceptors). They are associated with multipolar receptor cells, whereas bipolar receptor cells are associated with the ciliated pits (Storch and Abraham 1972, Fernandes *et al* 2001). The ciliated pits have a presumed olfactory or taste – chemosensory - function (Dendy 1892b, Graff 1899, Storch and Abraham 1972; Curtis *et al* 1983; Fernandes *et al* 2001). The neuropeptide, substance P, is present around these structures, the eyes and ciliated pits, indicating that the papillae of the sensorial zone integrate a sensory organ (Fernandes *et al* 2001). Similar types of receptor cells have been found in the epidermis of various proseriates (Curtis *et al* 1983).

4.9.2.1 Sensorial zone extent

***Character**: Sensorial zone, extent* (Fig. 4.10a)

0. Sensorial zone extends around the anteroventral margin, or anterior margin of the headplate
1. Sensorial zone absent around anterior tip; sensorial zones present ventrolaterally

The ciliated pits are generally located around the anteroventral margin of the head in a non-pigmented, non-ciliated sensorial zone beneath the eyes. In geoplanids



(a) (b) (c)

Table 4.10a
Sensorial zone – extent
(a) extending around anteroventral margin (b) absent around tip, present ventrolaterally (c) sensory papillae (*Xerapoa*)

(a) (b)

Table 4.10b
Sensory pits -morphology
(a) simple invaginations (b) branched

and rhynchodemids the sensorial zone is transversely furrowed (Dendy 1892b), and obliquely furrowed in *Timyma* that has a wide, almost bipaliid, anterior extremity (E.M. Froehlich 1978 page 14). Between the furrows, the epithelium may be slightly raised or papillate, which in the living animal forms the serrated projecting papillate appearance of the anterior margins observed in foraging flatworms (for example observed by Fletcher & Hamilton 1888 page 358, and others). In bipaliids, in *Timyma* (Caenoplaniniae), and in *Liana* (Geoplaninae) the sensorial zone skirts the anterior margin of the headplate and appears vertically papillate, where the openings of the ciliated pits between the papillae appear as fine oval slits (Moseley 1875; Graff 1899; Storch and Abraham 1972; Curtis *et al* 1983; Fernandes *et al* 2001). The anterior margin of the headplate of living foraging bipaliids also exhibits downward projecting papillae that move sequentially in a beautifully delicate tactile manner rather like a pianist's fingers when playing an arpeggio. In some bipaliid taxa the auricles of the falciform headplate are held almost vertically, probably to increase the area of humid atmosphere just above the ground sampled by the chemoreceptors.

Generally the sensorial zone skirts the anteroventral tip and extends to varying distances ventrolaterally (Geoplanidae and Rhynchodemidae) or passes around the anterior margin of the headplate (Bipaliidae), or less commonly is confined to the antero ventrolateral margins as in *Pimea* and *Thosteelia.* In *Xerapoa* the ciliated pits are located on extensible papillae.

The presence of sensory "tentacles" was originally a defining characteristic of *Cotyloplana* (Graff 1899 page 477). The 'tentacles" were lateral wrinkled enlargements of the anterior (*Cotyloplana whiteleggi* see Spencer 1892 Plate 5, Figs. 1-4 and Graff 1899 Plate LI Figs. 2,3; *C. dugesi,* and possibly *C. sharpi*), reminiscent of the sensorial margin of a bipaliid headplate, or more rudimentary small anterodorsal protuberances (*C. punctata* Graff 1899 Plate XI, Figs. 9-10). The presence and histology of these structures have yet to be confirmed and investigated in the type material. The papillate, ridged, and "tentacular" features of the sensorial zone described by Graff (1899) appear to be largely influenced by fixation, and are presently difficult to assess.

4.9.2.2 Sensory pit morphology

***Character**: Sensory pit morphology* (Fig. 4.10b)

0. Sensory ciliated pits simple invaginations, not branched (without diverticula)
1. Sensory ciliated pits branched (with diverticula)

At a light microscopical level the ciliated sensory pits (Sinnesgrübschen of Graff 1899), present in all Terricola, are generally simple ciliated invaginations of the epidermis, with a nerve communicating with the blind end, and in caenoplaninids is often present together with fine erythrophil or cyanophil secretory granules in the surrounding parenchyma. In some taxa the cells of the pits may be infranucleate. The pits themselves are generally simple invaginations or pits (plesiomorphic), or may be branched or diverticulate (apomorphic) as in *Gusana* (in E.M. Froehlich 1978).

4.10 ALIMENTARY TRACT

The alimentary tract of the Terricola generally conforms to the Triclad *bauplan* of a gut with anterior ramus, and two posterior rami arising at the pharyngeal – intestinal junction (intestinal mouth), with a single pharynx lying within an oesophageal pouch, the floor of which contains the mouth (= the peripharyngeal opening of early authors).

4.10.1 Gut

The surface area of the gut is greatly increased by lateral intestinal branches (=diverticular roots of Graff 1899; lateral caecae of Jennings 1959). The lateral intestinal branches may bifurcate or trifurcate to form simple diverticula (terminal branches of Graff 1899), and these diverticula may further ramify and anastomose in a complex manner (see illustrations in Bresslau 1928-1933). Little appears to be known of the interspecific variation in these characters, and of the effect of nutrition on gut morphology. A gut fully distended with digesta shows little evidence of diverticula, and also compresses and distorts other taxonomic characters such as the musculature of the body wall. Characters and states provided by the division of the rami into branches and diverticula have been rarely described and seldom used for taxonomic purposes in the Terricola.

4.10.2 Pharynx

The pharynx is of mesenchymal origin (Hyman 1951), and provides a number of taxonomic characters and states: single or multiple pharynges, pharynx type, attitude of the pharynx within the pharyngeal pouch, relative positions of the dorsal and ventral insertions at the root of the pharynx, musculature of the inner and outer walls and thickness of the component muscle layers, and the lining epithelia of the inner and outer pharynx, in particular the presence of an infranucleate epithelium. To these can also be added pharyngeal secretions and areas of the pharynx from which they are discharged. The general situation in the Terricola is that cyanophil secretions are discharged from the outer pharynx and tip, possibly acting as a sealant, lubricant, and transport fluid for food particles, and erythrophil and / or xanthophil proteolytic secretions discharged from the tip of the pharynx.

Character: *Numbers of pharynges*

0. With a single pharynx and mouth

1. With multiple pharynges and mouths

A single pharynx is the general plesiomorphic state for most "Turbellaria" and Triclads. Polypharyngy is a derived, apomorphic condition, rare in the Terricola. The presence of numerous cylindrical pharynges and mouths, together with separate male and female gonopores, characterize the Indo-Australian genus *Digonopyla.* This genus was regarded by Meixner (1928) as a highly differentiated descendent of the rhynchodemids. An undescribed dolichoplanid with two mouths and two pharynges, one behind the other, has been found in botanic gardens in Townsville and Charters Towers, north Queensland (author's collection). In *Digonopyla* and the *Dolichoplana* species, polypharyngy seems fixed within populations. Polypharyngy can be teratological in origin. Steel (1901b, page 626, Plate 41, Fig. 4) described and figured paired cylindrical pharynges protruded through a single mouth in a specimen of *Tasmanoplana tasmaninana*, from Trevallyn Hills, northern Tasmania. Similarly a specimen of *Parakontikia atrata* (LW298, urban garden, Montague, Tasmania) was found to possess two pharynges and a single mouth. In this specimen there is a teratological malformation and rotation of the intestinal mouth, resulting in duplicate pharynges and duplicate pharyngeal pouches. The dorsal pharynx is the smaller of the two, is non-functional, and is contained within an imperforate pouch. The ventral pharynx is functional, with mouth present in the floor of the pouch. There are two hypotheses concerning the origin of polypharyngy (Curini-Galletti & Cannon 1997): Polypharyngy may be as a result of a lesion or loss of the pharynx whilst feeding, with abnormal regeneration of supernumerary pharynges, and this occasional polypharyngy may eventually become genetically fixed through reiteration. Alternatively polypharyngy is a process analogous to strobilization of the Cestoda.

4.10.3 Pharynx type

There are three main morphological types of pharynx (Graff 1899) (Fig. 4.11a).

***Character**: Pharynx - types*

0. Cylindrical pharynx present, which can be further divided into:
    - those that lie horizontally in the pharyngeal pouch,
    - those that are inclined within the pharyngeal pouch, and
    - those in which the dorsal insertion lies posterior to the intestinal mouth
1. Bell-form (glockenförmig) pharynx present, in which the dorsal insertion is in the mid roof of the pharyngeal pouch, and
2. Collar-form (kragenförmig) pharynx present, in which the dorsal insertion is in the posterior third of the roof of the pharyngeal pouch.

Meixner (1928) considered that the cylindrical pharynx, found in the Rhynchodemidae, pelmatoplaninids, some caenoplaninids and some geoplaninids was the most primitive (plesiomorphic) form, and that the bell and collar types of pharynges found in caenoplaninids, geoplaninid, and the Bipaliids are definitely acquired (apomorphic), a view with which I concur.

4.10.4 Pharyngeal musculature

Pharyngeal musculature is of considerable phylogenetic value in the Maricola and Paludicola, in which two main types of pharynx, based on the musculature of the inner and outer pharyngeal walls, are recognized (Fig. 4.11b):

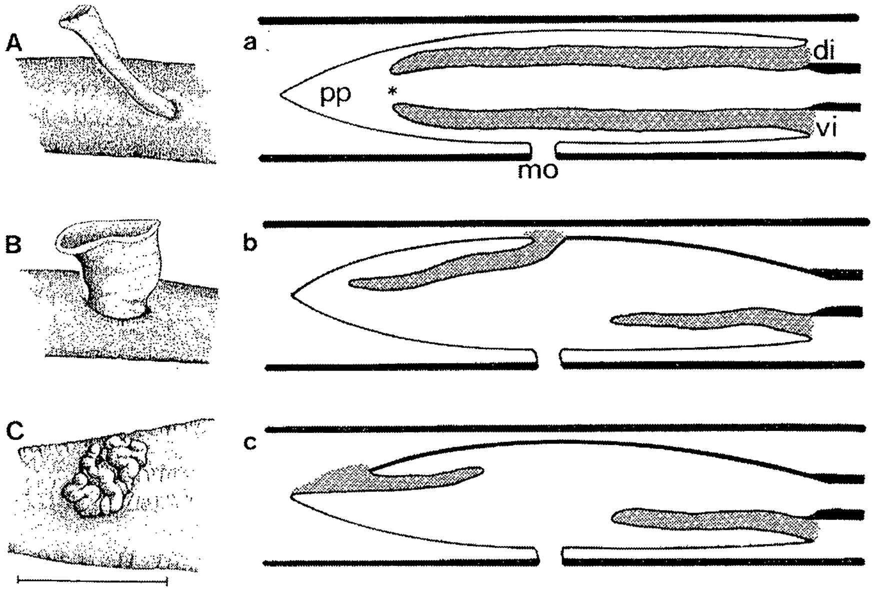


Figure 4.11a
Principal types of pharynx in the Terricola.

A, B, C illustrate the ventral aspect of preserved terrestrial flatworms. In each figure the pharynx is protruded through the mouth. Scale bar = 5mm.
a, b, c are diagrammatic representations of sagittal sections of the various types of pharynx. Not to scale. Abbreviations: di dorsal insertion of pharynx, mo mouth, pp pharyngeal pouch, vi ventral insertion of pharynx, * posteriad end of pharynx.

A, a: cylindrical pharynx. To feed, the flatworm protrudes the pharynx *into* the prey.
B, b: bell-form (glockenförmig) pharynx. To feed, the flatworm extends the pharynx *over* the prey.
C, c: plicate (kragenförmig) pharynx. In life this type of pharynx resembles a translucent veil with a slightly thicker white margin. To feed, the flatworm extends the pharynx *over* the soft bodied prey.

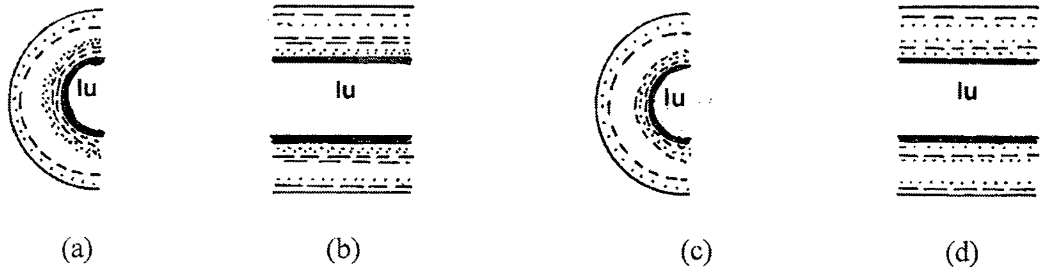


Figure 4.11b
Pharynx – musculature of pharyngeal wall
Diagrammatic transverse and sagittal sections through a planariid (a, b) and dendrocoelid (c, d) type of pharynx, showing the arrangement of circular and longitudinal muscles Abbreviation lu – lumen (after Sluys 1989).

Pharyngeal type and musculature

Table 4.5

Taxon	Origin	FAMILY / subfamily	Pharynx type	Diverticulum	Oesophagus	Inner musculature	Outer musculature
? Planariid type - derived, with possible loss & reversal of, and / or additional, muscle layers							
Digonopyla (tetracelis)	Indo	r	Cm			cm	lm cm lm
Geoplana josephi	Brazil	g	C		Yes	cm	lm cm lm
Notogyanaphallia guaiana	Brazil	g	PC		Yes	cm lm	cm lm
Newzealandia angusta, iris	NZ	c	C	Yes	?Yes	cm?	lm?
Artioposthia Group 4 (gramnicola)	Aust	c	C			cm ?	lm cm
Nelipoplana (Nelipoplana / Peokrypta)	Aust	c	B	Yes		lm cm lm	lm cm lm
Sokoplana kanangra	Aust	c	B			lm cm lm	lm cm lm
Arthurdendyus vegrandis	NZ	c	PC		Yes	lm cm lm	cm lm cm
Timyma juliae	Chile	c	C	Yes		cm lm	cm lm
Bipalium kewense	Vietnam	B	PC			lm cm lm	lm cm lm
Microplana purpura	Abyssinia	m	C			cm lm	lm cm lm
Planariid-type pharyngeal musculature							
Australoplana sanguinea	Aust	c	Cf	Yes	Yes	cm lm	lm cm
Thosteelia mesibovii	Aust	c	C	Yes	Yes	cm lm	lm cm
Dendrocoelid-type pharyngeal musculature							
Geoplana franciscana	Brazil	g	C		Yes	mixed	lm cm
Coleocephalus fuscus	Subantarctic	c	PC			mixed	lm cm
Kontikia andersoni	UK intro	c	C			mixed	lm cm
Microplana henrici	C. Europe	m	C			mixed	lm cm
Rhynchodemus richardi	Monarco					mixed	lm cm
? Dendrocoelid - derived, with possible loss & reversal of, and / or additional, muscle layers							
Cotyloplana punctata, whiteleggi	Aust	r	C			lm mixed lm	lm cm lm
Cotyloplana sharpi	S. Sulawesi	r	B			lm mixed lm	lm cm lm
Dolichoplana spp	Aust	r	C			lm mixed lm	lm cm lm
Platydemus victoriae, manokwari, sp	Aust	r	C			lm mixed lm	lm cm lm
Platydemus laterolineatus	Aust	r	C	Yes		lm mixed lm	lm cm lm
Rhynchodemus guttatus	Aust	r	C			lm mixed	lm cm lm
Rhynchodemus simulans	Aust	r	C			mixed	mixed
Microplana britannicus	Europe	m	C			mixed	lm cm lm
Microplana terrestris	Europe	m	C			lm mixed lm	lm cm lm
Microplana teres	Sri Lanka	m	C	Yes		mixed	mixed
Fyfea carnleyi	Subantarctic	a	C			lm mixed	lm cm mixed
Anzoplana trilineata	Aust	a	C			lm mixed	lm cm mixed
Endeavouria septemlineata	Pacific	c	C			mixed	lm cm lm
Kontikia fosterorum	New Cal	c	C			mixed	lm cm lm
Lenkunya adae	Aust	c	C			mixed	lm cm lm
Parakontikia ventrolin, melanochroa	Aust	c	C			mixed	lm cm lm
Reomkago quadrangulata, sp	Aust	c	C			mixed	lm cm lm
Pimea monticola	New Cal	c	C	Yes		mixed	cm lm
Bassioplana typhlops	Aust	c	B	Yes		cm mixed	cm lm
Kontikia orana	Intro	c	C			lm mixed	lm cm lm
Lenkunya munda	Aust	c	C			lm mixed	lm cm lm
Artioposthia Group 5 (howitti)	Aust	c	C			lm mixed	lm cm mixed
Artioposthia Group 2 (lucasi)	Aust	c	B			lm mixed	lm cm mixed
Caenoplana coerulea,bicolor,hoggii	Aust	c	C			mixed	lm cm mixed
Fletchamia sugdeni	Aust	c	C			mixed	lm cm mixed
Kontikia marrineri	Subantarctic	c	C			mixed	lm cm mixed
Apogaioplana paluma	Aust	c	C	Yes		cm mixed	lm cm mixed
Tasmanoplana lyra	Aust	c	C			cm mixed	lm cm mixed
Danidoplana palatris	Aust	c	C	Yes		cm mixed	lm cm
Barringtoplana harrisoni	Aust	c	C		Yes	cm mixed	lm mixed
Caenoplana dendyi, spenceri	Aust	c	C			cm mixed	lm mixed
Fletchamia quinquelineata	Aust	c	C			cm mixed	lm mixed
Kynagoplana citrinus	Aust	c	C	Yes		cm mixed	lm mixed
Amaga	Col'bia/Peru	g	?PC				
Choeradoplana iheringi, langi	Arg/Brazil	g	PC				
Choeradoplana marthae	Brazil	g	C				
Enterosyringia	Brazil	g	C				
Geobia	Brazil	g	PC		Yes		
Geoplana argus	Brazil	g	C	Yes			
Geoplana chanca	Chile	g	B	Yes			
Gigantea	Panama	g	C				
Gusana	Chile	g	C				
Issoca	Brazil	g	C				
Liana	Chile	g	C				
Pasipha	Brazil	g	C				
Polycladus	Chile	g	Cb				
Xerapoa	Brazil	g	C				
Othelosoma fuscum	S.Afr	g	C	Yes			

Key to abbreviations:

Family / subfamily

m microplaninae
r rhynchodeminae
a anzoplaninae
c caenoplaninae
g geoplaninae
B Bipaliidae

Pharnx Type

C cylindrical
Cf cylindrical folded
B bell form
PC plicate collar form

Musculature

cm circular muscles
lm longitudinal muscles
mixed circular & longitudinal

- the **dendrocoelid** type, characteristic of the Dendrocoelidae, with inner musculature from the lining epithelium comprising alternating rows of longitudinal and circular fibres, and an outer musculature consisting of an ectal sheath of longitudinal fibres underlain by a layer of circular fibres. The situation is more complex in some dendocoelids that exhibit variations from the main dendrocoelid condition (Sluys 1989; Sluys *et al* 1998). In the Paludicola the dendrocoelid type of musculature is postulated as the apomorphic condition, thus supporting the monophylly of the Dendrocoelidae (Sluys 1989b, 2001).
- the **planariid** type, found in the Planariidae and Dugesiidae and also all Maricola (Sluys 1989b), in which the inner musculature beneath the epithelium comprises a sheath of circular muscles ental to which is a layer of longitudinal muscles. The outer musculature is the same as the main dendrocoelid type, consisting of an ectal sheath of longitudinal fibres underlain by a layer of circular fibres.

Sluys (1989b) argued that comparative studies of pharyngeal musculature in the Paludicola should take into account the musculature of both the inner and outer pharynx – previously attention had largely been focused on the inner pharyngeal musculature. Little attention has hitherto been paid to the musculature of terricolan pharynges. It is highly likely that pharyngeal musculature of the Terricola may also be useful in comparative morphological phylogenetic studies. Data on pharyngeal type, and both inner and outer pharyngeal musculature for mainly Australian and Indo-Pacific taxa are provided in Table 4.5. Patterns of pharyngeal musculature in the Terricola appear to be more numerous and more complex than in the other Tricladida. Characters and states of pharyngeal musculature used in phylogenetic analysis of the Dugesiidae (Sluys 2001) have limited application in the Terricola.

Four main groupings of pharyngeal musculature types, which transect present subfamily divisions and some genera, are identified:

1. Taxa with Planariid-type musculature: *Australoplana, Thosteelia* (Australia).
2. Taxa with possibly derived Planariid-type musculature with possible loss and reversal of, and / or additional muscle layers (with six sections within two main subgroups). This broad group includes *Arthurdendyus, Bipalium, and*

Nelipoplana, which based on 18SrDNA gene sequencing, are placed together within the Terricola sister group to the Dugesiidae (Baguñà *et al* 2001). *Caenoplana coerulea,* also placed in this sister group, has? derived Dendrocoelid-type musculature.

   a. With an ectal longitudinal muscle layer in the outer pharyngeal wall: Bipaliidae: *Bipalium*, Microplaninae: *Microplana purpurea,* Rhynchodeminae: *Digonopyla;* Caenoplaninae: *Artioposthia Group 4, Nelipoplana, Sokoplana,* Geoplaninae: *Geoplana (part),* (Vietnam, Indonesia, Australia, Brazil)
   b. With an ectal circular muscle layer in the outer pharyngeal wall: *Arthurdendyus, Timyma, Notogynaphallia* (NZ, Chile, Brazil)

3. Taxa with Dendrocoelid-type musculature: *Geoplana (part), Coleocephalus, Kontikia* (one species, UK introduced, in my view possibly subantarctic in origin), *Microplana henrici, M. richardi* (in Bendl 1909, Sluys 1989a) (Europe, Brazil, subantarctic islands of New Zealand).
4. Taxa with possibly derived Dendrocoelid-type musculature with possible loss and reversal of, and / or additional muscle layers (with 11 sub-sections within two main subgroups).
   a. With mixed musculature in the inner pharyngeal wall: Rhynchodeminae: *Cotyloplana, Dolichoplana, Platydemus, Rhynchodemus* (part), Microplaninae: *Microplana* (part), Caenoplaninae: *Endeavouria, Kontikia, Parakontikia, Bassioplana, Pimea, Lenkunya and Reomkago* (Indopacific, Australia, New Caledonia, Europe).
   b. With mixed musculature in both inner and outer pharyngeal walls: Rhynchodeminae: *Rhynchodemus* (one species, Australia), Microplaninae: *Microplana* (one species, Sri Lanka), Caenoplaninae: *Artioposthia* Groups 2 & 5, *Anzoplana, Caenoplana, Danidoplana, Fletchamia, Fyfea, Kynagoplana, Tasmanoplana* (NZ, Australia, subantarctic islands).

Further analysis of these data, comparison with other paludicolan genera, and identification of characters and states will be undertaken outside of this thesis.

4.10.5 Oesophagus

In some geoplanid taxa, mainly those with planariid-type and possibly derived planariid-type pharyngeal musculature, the proximal region of the pharynx leading to the intestinal mouth is differentiated from the distal pharynx, and is regarded as the oesophagus. Generally the musculature in this region is considerably thicker and stronger than in the distal pharynx, and the epithelium mostly non-secretory, thicker and more cuticularized, possibly reflecting a function in the physical dissociation of the ingesta before it passes into the gut. The ratio of the length of the oesophagus to the length of pharynx expressed as a percentage has been used as a character for the Geoplaninae (Carabayo & Leal-Zanchet 2001).

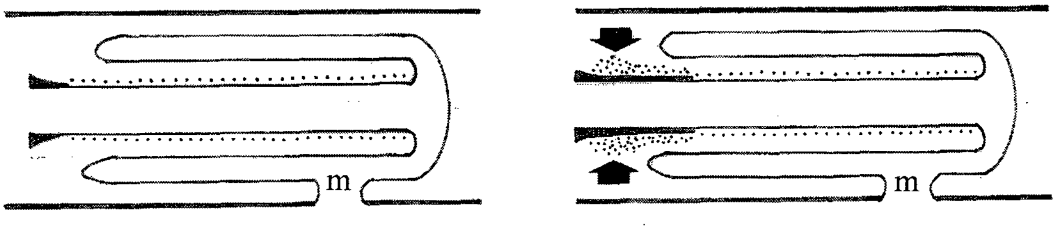
Character: *Oesophagus* (Fig. 4.12a)

0. Oesophagus absent
1. Oesophagus present.

Probably the first state, which is the general situation in the Tricladida, is plesiomorphic, and the second, uncommon state, apomorphic. The occurrence of this character in "Turbellarian" taxa outside of the Tricladida has not been investigated in this thesis. The presence of an oesophagus appears to be less common in caenoplaninid than geoplaninind taxa, though this may be simply due to the fact that less is known anatomically of members of the former subfamily. Australian genera with species with an oesophagus include Arthurdendyus, *Australoplana, Barringtoplana,* and *Thosteelia.*

4.10.6 Pharyngeal pouch

The pharyngeal pouch surrounds the pharynx, and is generally lined by flattened non-ciliated epithelium of ectodermal origin that may be syncytial (Hyman 1951). Near the root of the pharynx, pouch musculature generally reflects the musculature of the outer pharyngeal wall, and may attenuate rapidly to a very thin musculature, or may have a solid block of muscle dorsally. The mouth is situated in the floor of the pouch, though it is not a mouth in the usual sense, as the pharynx is protruded through this opening. Characters provided by the pharyngeal pouch include its length as a percentage of body length, the relative position of the mouth in the floor of the pouch, and the



(a) (b)

Figure 4.12a
Pharynx – oesophagus
(a) oesophagus absent (b) oesophagus present (arrows)
m = mouth

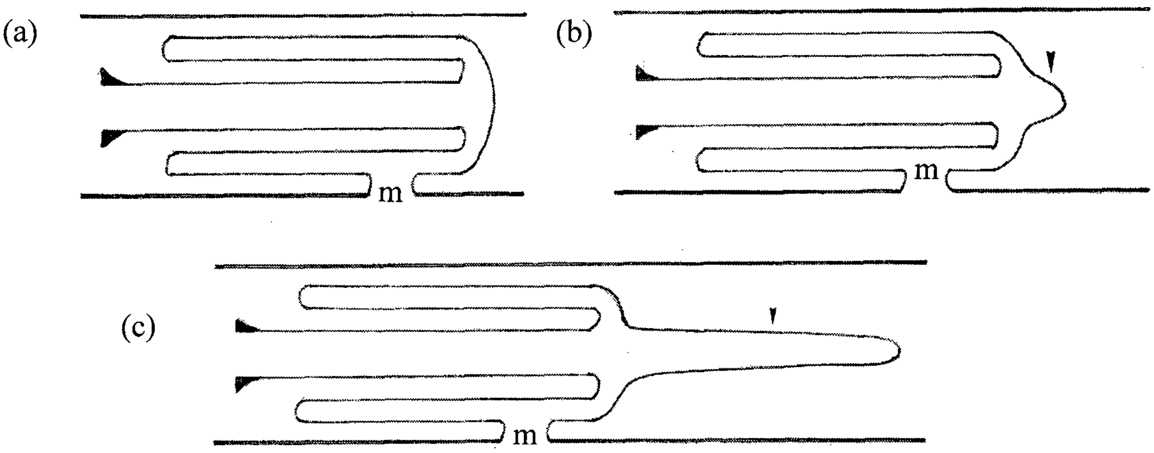


Figure 4.12b
Pharynx – Pharyngeal pouch – diverticulum (arrowed)
(a) diverticulum absent (b) small diverticulum present (c) elongate diverticulum
m = mouth

presence or absence of a pharyngeal diverticulum. The type of epithelial lining, and secretions passing into the pouch may also provide additional characters.

Character: *Pharyngeal pouch diverticulum* (Fig. 4.12b)

0. Pharyngeal pouch diverticulum absent

1. Pharyngeal pouch diverticulum present.

In the Tricladida the pharyngeal pouch generally has no diverticulum and this probably is the plesiomorphic state. Diverticula of the pharyngeal pouch are uncommon, and are probably apomorphic conditions. They have been described in the Brazilian species *Geoplana argus* and *G. rufiventris* (Geoplaninae), the rhynchodeminid *Platydemus laterolineatus* (Lord Howe Island), the microplaninids *Amblyplana fusca* (from Cape of Good Hope, South Africa) and *A. teres* (from Ceylon), and a hair-thin diverticulum present in *Microplana purpurea* (Abyssinia). In the Chilean caenoplaninid species *Timyma juliae* the diverticulum extends dorso-posteriorly to the hind end, and is lined by a cuboidal epithelium of variable height (E.M. Froehlich 1978). Pouch diverticula are also present in the rhynchodeminid *Cotyloplana,* and the caenoplaninids *Apogaioplana, Australoplana, Bassioplana, Danidoplana,* and *Nelipoplana* (this thesis). In these Australian caenoplaninids the pouch diverticulum serves as an enclosed mid-posterior or inter-ramial sinus into which solid waste products are excreted via nephridiopores; it may also in some way be a moisture conserving adaptation as the nephridiopores are protected from the external environment.

4.11 REPRODUCTIVE SYSTEM

The Terricola are hermaphroditic. Thus the reproductive system comprises the male and female gonads, efferent ducts, and copulatory apparatus, together with the vitellaria, and accessory genital organs. The copulatory organs are generally enclosed within a loose stroma or muscularis and lie ventro-posteriorly to the pharynx. The elements of the reproductive system are very important in triclad taxonomy and presently provide the majority of characters used for phylogenetic analysis.

4.11.1 Gonopore

The gonopore is single, and generally a round pore or a transverse slit in the Terricola, though the rhynchodeminid *Digonopyla* has separate male and female pores. The relative lengths and positions of male and female organs and associated structures relative to the gonopore are sometimes mentioned in descriptions. This is generally only where structures in a given species are larger or smaller than what is considered in the authors' opinion as "normal" for that taxon.

Male Reproductive System

4.11.2 Testis

In the Terricola characters provided by the testes include the follicular nature and number of the testes, arrangement along the body in one or two rows, position of the rows of testes laterally across the body, extent of the testes horizontally along the body, and position of the testes in the vertical plane between dorsal and ventral surfaces.

4.11.2.1 Follicular nature and number of the testes

In most terricolan taxa the testes are follicular, though partial fusion of the testes has been noted in some subantarctic taxa, and in *Othelosoma notabile* (in Graff 1899 Plate 54 Fig. 6). Testes may be spheroidal, or more inverted tear-shaped and ellipsoidal, tapered slightly where the efferent duct passes from the ventral testis. They are covered by a very thin, flattened nucleate tunica propria underlain by a distinct basement membrane to which the gonial cells are attached within the testis. In the majority of preparations the spermatogonia have contracted away from the basement membrane, thus giving the impression that a fibrous connective tissue sheath covers the testes. However the cytology of the tunica and its relationship may be more complex as observed in some Paludicola (Hendelberg 1983).

The absolute number of testes in all but small specimens is not easily determined and consequently is seldom reported. With respect to the Paludicola, the number of testes has been used as a character, with two states: *few* or *numerous* (Ball 1974). Number of testes per unit body length is dependent upon the maturity and diameter of the testes, and the distance between adjacent testes. Testes may range in number from 2 pairs (*Microplana humicola*) to 230 pairs (*Pseudogeoplana nephelis*) or more (Graff 1899). In the Terricola the testes in a particular specimen generally exhibit equal development (synchronous maturation). In mature specimens of Coleocephalus and other NZ sub-Antarctic Island species, the testes sometimes exhibit different degrees of development or sequential maturity from fully ripe with mature sperm to what appears to be a resting stage. This may be an energy-conserving strategy complimenting the donor sperm resorption structures in these taxa. Alternatively it might reflect a stage in the male maturation of the specimen that would result in all testes ripening. It has not yet been established whether the Terricola are generally protanderous or protogynous.

4.11.2.2 Arrangement and lateral position of the testes

The testes are generally arranged below the gut and between gut diverticula in (a) a single row on either side of the body (uniserial) as in most Rhynchodemids, Geoplanids (mostly caenoplaninids and pelmatoplaninids), and Bipaliids, (b) arranged laterally or in a slightly dorsoventrally staggered in a uniserial arrangement as in some caenoplaninid taxa such as *Nelipoplana*; or (c) arranged in two rows (biserial) as in many geoplaninid and adenodactylate caenoplaninid taxa. The arrangement of testes does not appear to have been used as a taxonomic character. In taxa with ventral testes, the testes are generally situated over the outer half of the nerve cords, or displaced sideways to varying degrees until they are lateral to the nerve cords. The reliability of this character and states for taxonomic purposes has not yet been fully assessed.

4.11.2.3 Extent of the testes along longitudinal body axis

Within the austral taxa considered in this thesis seven conditions for the extent of the testes along the body axis are identified, and rationalized into four principal states (Table 4.6), with testes.

Table 4.6
Testes, efferent ducts and penis type

Taxon	Dorso-ventral position			Horizontal extent				vas deferens		Penis type
	Dorsal	Dorso-ventral	Ventral	Ant - post	Ant– cop org	Ant - oral	Ant - Pre-pharynx	separately enter bulb	unite, then enter bulb	
Geoplanidae: Geoplaninae	Y									
Geoplanidae: Anzoplaninae										
Anzoplana		Y		Y				Y		apapillate
Artioposthia ventropunctata		Y		Y				Y		papillate
Artioposthia subquandrangulata		Y		?				Y		apapillate
Fyfea carnleyi		Y		Y				Y		invert pap
"Kontikia" ashleyi ("Parafyfea")		Y		Y 1				Y		invert pap
Australopacifica aucklandica		Y		Y				Y		invertpap?
Artioposthia alfordensis		Y			Y			Y		papillate
Geoplanidae: Caenoplaninae										
Arthurdendyus			Y	Y				Y 6		apapillate
Artioposthia lucasi			Y	Y				Y		papillate?
Artioposthia hamiltoni			Y	Y				Y		apapillate
Coleocephalus			Y	Y					Y	apapillate
Lenkunya			Y	Y					Y	papilate
"Kontikia" ranuii			Y	Y					Y	apapillate
Artioposthia garveyi			Y	Y 2				Y		apapillate
Artioposthia howesi			Y		Y			Y		papillate
Kontikia			Y		Y			Y		papillate
Parakontikia			Y		Y			Y		apapillate
Artioposthia glandulosa			Y		Y				Y	papillate
Artioposthia gramnicola			Y		Y				Y	apapillate
Newzealandia moseleyi, iris			Y		Y				Y	apapillate
Artioposthia angusta			Y		Y				Y	apapillate
Artiopothia langii			Y		Y				Y	papilate
Danidoplana			Y		Y				Y	papilate
Caenoplana			Y			Y		Y		apapillate
Australoplana			Y			Y		Y		intra-ant p
Barringtoplana			Y			Y			Y	papilate
Kynagoplana			Y			Y			Y	apapillate
Pimea			Y				Y		Y	intra-ant p
Endeavouria			Y				Y		Y	papilate
Artioposthia mariae			Y				Y		Y	apapillate
Fletchamia			Y				Y		Y	papillate
Thosteelia			Y				Y	Y		papillate
Reomkago			Y				Y	Y		papillate
Timyma			Y				Y	Y		apapillate
Bassioplana			Y				Y	Y		papillate
Nelipoplana			Y				Y	Y		apap/pap
Sokoplana			Y				Y	Y		papillate
Apogaioplana			Y				Y	Y		intra-ant p
Tasmanoplana			Y				Y	Y		papillate
Rhynchodemidae: Rhynchodeminae										
Cotyloplana			Y		Y 3			Y		papillate
Platydemus			Y		Y			Y		apap /pap
Dolichoplana			Y		Y			Y		apapillate
Digonopyla			Y		?			Y		papillate
Rhynchodemus			Y			Y		Y		papillate
Bipaliidae										
Bipalium kewense			Y			Y		Y		papillate

1. Mouth to posterior tip
2. Copulatory organs to posterior tip
3. Mouth to copulatory organs

Character: *Testes, extent along longitudinal body axis*

0. With testes extending from just behind the ovaries almost to the posterior end (and from mouth to posterior end, and from copulatory organs to posterior end).

1. With testes extending from just behind the ovaries to the copulatory organs, with testes generally up to or beside the penis bulb (and from the mouth to the copulatory organs).

2. With testes extending from just behind the ovaries to the oral region (and with testes beside the pharynx up to the mouth).

3. With testes extending from just behind the ovaries to just before or to the root of the pharynx (pre-pharyngeal).

Testes throughout the body length are regarded as plesiomorphic (in the phylogenetic analysis of the Dugesiidae by Sluys 2001), and the other conditions (anterior–copulatory organs, oral, and pre-pharyngeal) apomorphic. In the Terricola, taxa with testes extending almost the entire body length are restricted to anzoplaninids, and caenoplaninids from New Zealand, the subantarctic islands, and a few Australian taxa (*Lenkunya*). This state includes testes extending from the copulatory organs to the posterior end (subantarctic island taxa), and from the mouth to the posterior end (subantarctic island taxa). Taxa with testes extending from the anterior to the copulatory organs include *Kontikia, Parakontikia, Danidoplana* and some *Artioposthia* species, and the rhynchodeminids *Dolichoplana* and *Platydemus*. The state includes testes extending from the mouth to the copulatory organs (rhynchodeminid *Cotyloplana*). In the NZ species *Artioposthia polyadoides* the testes are described as being small, oval, ventral, restricted to the central region (Fyfe 1956 page 511); it is not known whether the testes in the species are pre- or post-oral. The state with anterior – oral extent of testes is restricted to the Australian taxa *Australoplana, Barringtoplana, Caenoplana,* and *Kynagoplana.* The condition with testes anterior – pre-oral occurs in mostly Australian taxa, and Pacific (*Endeavouria, Pimea*) and Chilean (*Timyma*) taxa.

4.11.2.4 Dorsoventral extent of the testes

***Character**: Testes, dorsoventral extent* (Fig. 4.13)

0. With testes ventral, lying above or to the side of the ventral nerve cords, with ventral efferent ducts.

1. With testes continuous dorsoventrally, extending from the ventral nerve cords to the dorsal parenchymal musculature, and with ventral efferent ducts.

2. With testes dorsal, lying just below the dorsal parenchymal musculature, with dorsal efferent ducts,

Ventral testes and efferent ducts, regarded as the primitive or plesiomorphic condition (Meixner 1928, Sluys 2001), are present in the Planariidae and Dendrocoelidae (in part) (Paludicola); Maricola (mostly); and in the Terricola, the Rhynchodemidae, Bipaliidae and Geoplanidae (Caenoplaninae, Pelmatoplaninae). The dorsoventral state for extent of the testes (Winsor *et al* 1998) is an apomorphy restricted to anzoplaninid taxa (this thesis). Dorsal testes and efferent ducts are present in the Dendrocoelidae (part) (Paludicola), and in the Terricola only in geoplaninid taxa, and are considered to be a derived (apomorphic) condition (Meixner 1928; Sluys 2001).

In respect of the horizontal and dorsoventral extent of the testes, these characters and states in the Terricola largely parallel those in the Paludicola and Maricola. All Terricolan taxa with testes extending from anterior to almost the posterior, those with testes extending from mouth to posterior, and most taxa with testes extending from anterior to the copulatory organs are adenodactylate or have musculoglandular organs. Most of the taxa with pre-oral testes are non-adenodactylate.

Within the Geoplanidae, the dorsoventral position of the testes, together with other characters, has provided the basis for the erection of the subfamilies Caenoplaninae and Pelmatoplaninae with ventral testes, Geoplaninae with dorsal testes

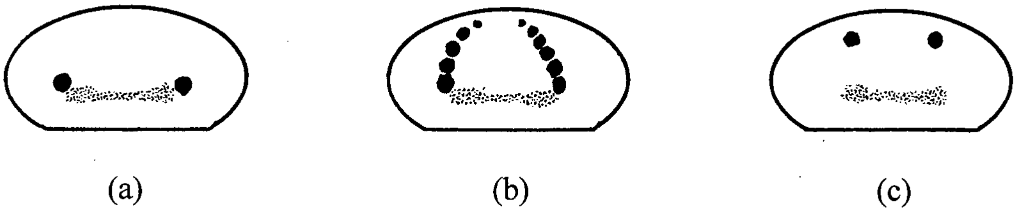


Figure 4.13
Testes – extent in dorsoventral plane
(a) Ventral (Caenoplaninae) (b) Dorsoventral (Anzoplaninae)
(c) Dorsal (Geoplaninae)

(Ogren & Kawakatsu 1990; 1991), and Anzoplaninae with dorsoventral testes (this thesis).

4.11.3 Efferent ducts

Generally a sperm ductule (vas efferens) passes from the testis to the sperm duct. The simplest arrangement of efferent ducts in the Terricola is where a sperm ductule passes from the lower pole of each testis to communicate directly with the sperm duct (vas deferens). In more complex arrangements the sperm ductule may open into an inter-testicular duct, then via a transverse intermediary duct to a longitudinal collecting duct, thence via another transverse intermediary duct (vas intermedium) to the vas deferens. In practice this arrangement is difficult to trace and determine, as the efferent duct system leading to the vas deferens is generally comprised of an irregular network of fine, thin-walled ducts lined by a flattened to low cuboidal nucleate non-ciliated epithelium.

The vas deferens is lined by a nucleate epithelium, which appears to be ciliated in only a few taxa of the caenoplaninids examined here (*Artioposthia langii, Barringtoplana, Endeavouria*). The vasa deferentia in *Arthurdendyus, Fyfea,* "Parafyfea" and other taxa from the subantarctic islands are extremely fine, thin walled, lined by a very thin nucleate epithelium, and pass posteriorly as a network above the nerve cords.

The main characters of the vasa deferentia considered here are their position dorsal or ventral to the lateral nerve cords, whether those dorsal to the nerve cords lie entally or ectally to the oviducts, and whether the vasa deferentia form an extra-bulbar common sperm duct, or separately enter the copulatory organs.

Character: *Position of the vasa deferentia in the body* (Fig. 4.14a)

0. With vasa deferentia below the nerve cords
1. With vasa deferentia above the lateral nerve cords

In the rhynchodeminids *Cotyloplana, Platydemus*, and some *Rhynchodemus* species, and in most Caenoplaninae, the efferent ducts and vas deferens lie below the nerve cords. Generally the vas deferens lies below and more-or-less in line with the ovovitelline duct, but may be displaced laterally as in *Bassioplana* and *Platydemus*. In

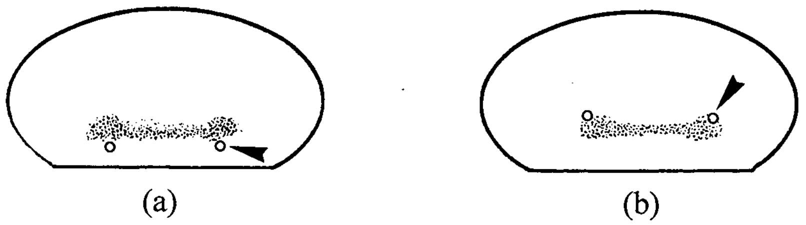


Figure 4.14a
Vas deferens – position
(a) ventral to the nerve cords (b) dorsal to the nerve cords

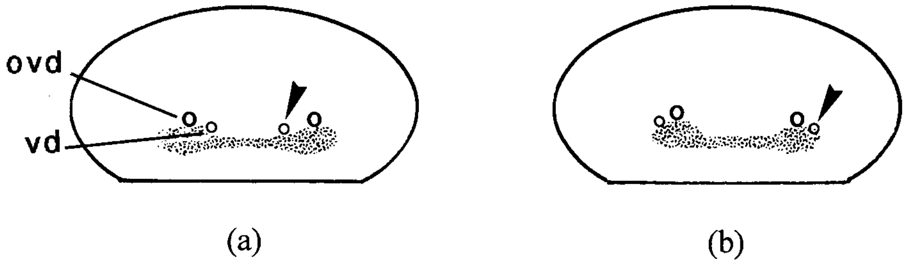


Figure 4.14b
Vas deferens – dorsal position relative to ovovitelline ducts
(a) between ovovitelline ducts (b) outside ovovitelline ducts

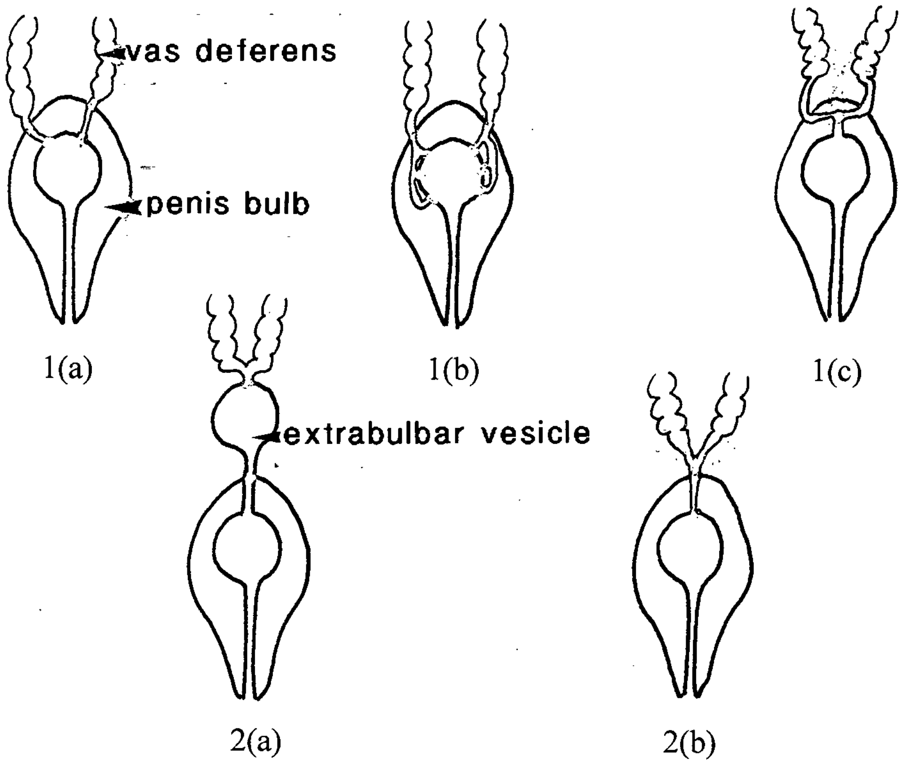


Figure 4.14c
Vas deferens – approach to copulatory organs
1. separately enter bulb 2. unite before entering bulb
Refer to text (4.11. .4.1) for explanation

caenoplaninid taxa with testes lying immediately above the nerve cords, the sperm ductules pass through the nerve tissue to join the vas deferens; in taxa with testes lateral to the nerve cords, the efferent ducts pass between the lateral nerves to communicate with collecting and sperm ducts.

In most Anzoplaninae, and in *Lenkunya* (in which the testes extend dorsally to the mid body) the vasa deferentia lie above the nerve cords, but the sperm ductules, intermediary and collecting ducts pass through and lie beneath the nerve cords. The Bipaliidae, the rhynchodeminids *Dolichoplana* and *Rhynchodemus*, and Geoplaninae (dorsal testes) have vasa deferentia that lie above the nerve cords.

Character: *Position of vasa deferentia relative to the oviducts, dorsal to the nerve cords* (Fig. 4.14b)

0. With vasa deferentia dorsal to the nerve cords lying between the ovovitelline ducts
1. With vasa deferentia dorsal to the nerve cords lying outside the ovovitelline ducts

Data on these states is surprisingly difficult to find in the literature, and the significance and usefulness of the character are unable to be fully evaluated here. The vasa deferentia lie between (mediad) the ovovitelline ducts in the rhynchodeminids *Dolichoplana* and *Rhynchodemus* (part), microplaninid *Othelosoma,* and *Lenkunya, Anzoplana,* and *Artioposthia fletcheri.* In the Bipaliidae and *Pimea*, the vasa deferentia lie outside (lateral) to the ovovitelline ducts.

4.11.4 Spermiducal vesicles

The vasa deferentia pass posteriorly and generally expand to form capacious spermiducal vesicles as they approach the copulatory organs. The presence or absence of spermiducal vesicles may be a reflection on the maturity of a specimen. For this reason there is little taxonomic significance in the character spermiducal vesicle, with states present or absent. The vesicles may be located beside the pharyngeal pouch, between the pouch and copulatory organs, or alongside the anterior penis bulb. Spermiducal vesicles are generally simple expansions of the vasa deferentia with continuation of the same lining as the vasa. However in some taxa they possibly have a secondary resorptive function suggested by the presence of a lining epithelium with vacuolate cytoplasm

(*Anzoplana trilineata*), facilitate intestinal digestion of excess sperm via vasa-gastrointestinal anastomoses (*Artioposthia langii*), and receive cyanophil secretions and possibly act as seminal vesicles in the absence of a distinct seminal vesicle (*Nelipoplana (Nelipoplana) alba*).

4.11.4.1 Approach of efferent ducts into penis bulb

Approaching the copulatory organs, the vasa deferentia, with or without spermiducal vesicles, may then either unite to form a common sperm duct, or separately enter the penis bulb.

Character: *Vasa deferentia: approach to the copulatory organs* (Fig. 4.14c)

1. with vasa deferentia separately entering the penis bulb, and
    a. separately pass into the seminal vesicle, prostatic duct or ejaculatory duct within the penis bulb,
    b. ramify and enter the seminal vesicle at multiple points, or
    c. join to form a common sperm duct within the penis bulb before passing into the seminal vesicle, prostatic duct or ejaculatory duct.
2. with vasa deferentia united to form a common sperm duct of variable length which then:
    a. enters an extra-bulbar seminal vesicle, or
    b. passes directly into the seminal vesicle, prostatic duct or ejaculatory duct within the penis bulb.

In taxa where the testes are distributed throughout the length of the body, anterior and posterior vasa deferentia join on each side generally near the point where the anterior vasa expand to form spermiducal vesicles.

Taxa in which the vasa unite before entering the copulatory organs include *Pimea* and *Endeavouria*, *Coleocephalus* and another species from the NZ subantarctic islands, and about equal numbers of taxa from Australia and New Zealand. Taxa in which the vasa deferentia separately enter the copulatory apparatus include many bipaliids, all the austral rhynchodeminids and anzoplaninids, *Timyma* (Chile), *Kontikia*, and almost two thirds of the Australian caenoplaninids studied.

The common sperm duct, or vasa deferentia generally enter the anterior end of the seminal vesicle. Where the vasa deferentia separately enter the bulb they may enter the seminal vesicle anteriorly, dorsally, ventrally, or may ramify and enter the vesicle at multiple points as in *Bipalium moseleyi* and in species of *Arthurdendyus.* Entry of the sperm duct(s) into the seminal vesicle provides an additional character and states for the male copulatory organs, but has not been further investigated in this thesis.

These two character states of the vasa deferentia – separately entering, or first uniting then entering the copulatory organs, are present in all suborders of the Tricladida (Sluys 1989a). According to Sluys (1989a) in the Proseriata the vasa deferentia generally separately enter the seminal vesicle forming part of the copulatory organ. He suggested (Sluys 1989a) that the ancestral condition for the Tricladida consists of the vasa deferentia separately communicating with the male copulatory apparatus, and argued that for the Maricola (Cercyroidea) an extra-bulbar vas deferens constituted the apomorphic condition. Undoubtedly a similar argument can be advanced for groups within the Terricola.

4.11.5 Male copulatory apparatus

Characters provided by the male copulatory apparatus include extra-bulbar structures such as a seminal vesicle, penis bulb, prostatic duct, ejaculatory duct, penis papilla, penis type, and male antrum

4.11.5.1 Extra-bulbar seminal and prostatic vesicles

***Character**: Extra-bulbar seminal and prostatic vesicles:*

0. Extra-bulbar seminal vesicle and prostatic duct absent
1. Extra-bulbar seminal vesicle and prostatic duct present

Most Terricola (and Tricladida) do not have extra-bulbar structures, and this state is probably plesiomorphic. The presence of an extra-bulbar seminal vesicle and prostatic duct is probably an apomorphy, and is found mostly in the pelmatoplaninids, a few geoplaninids, and the caenoplaninids *Kynagoplana* and *Reomkago*. The character has been used in the generic diagnoses of the latter two taxa, though the usefulness and significance of the character have yet to be assessed.

4.11.5.2 Penis bulb

In the Terricola, as in the majority of triclads, the penis comprises a muscular bulb with papilla, together with a seminal vesicle, prostatic duct, and ejaculatory duct.

The penis bulb, usually invested by a weak stromal sheath or muscularis, is generally muscularised to a greater or lesser extent. The bulb contains secretions from penial glands usually located in the surrounding parenchyma, destined for the seminal vesicle, prostatic duct, ejaculatory duct, or penis papilla. It may also contain accessory genital structures such as musculoglandular organs. The penis bulb provides two main characters: extent of muscularization, and degree of differentiation of the musculature into layers.

***Character**: Penis bulb musculature: Development:*

0. With penis bulb strongly muscularised
1. With penis bulb musculature weak, comprising stroma with sparse muscle fibres, with seminal vesicle, prostatic duct, and with ejaculatory duct often strongly muscularised

The penis bulb of the majority of Triclads is well muscularised, and possibly represents the plesiomorphic condition. The weakly muscularised state is possibly apomorphic, and found in a variety of taxa in all families.

***Character**: Penis bulb musculature: Differentiation*

0. Musculature poorly differentiated, with intermingled circular and longitudinal fibres, rather like yarn in a ball of wool

1. Musculature well differentiated, exhibiting well defined, often alternating layers, like an onion.

Poorly differentiated musculature in the penis bulb generally reflects the poor differentiation of body musculature overall, as in the caenoplaninid *Caenoplana spenceri* (in Dendy 1890). The poorly differentiated state is regarded here as probably a plesiomorphy, consistent with the assessment of the poorly differentiated state of the body musculature characters considered previously (Section 4.7.3 and 4.7.4.). Well-differentiated musculature is a more advanced state, considered here an apomorphy as in many bipaliids and rhynchodemids, and caenoplaninids *Arthurdendyus, Sokoplana.*

The penis bulb generally lies horizontally, but may be steeply angled pointing dorsally, as in *Tasmanoplana*, and in the inverted penis of *Fyfea* and "Parafyfea", or ventrally as in many bipaliids. The potential usefulness of the angle of the penis has not been explored here, though similarities between some Tasmanian and subantarctic taxa are noted.

4.11.5.3 Seminal vesicle

The seminal vesicle is generally a thin walled, simple tubular or sacciform structure, sometimes strongly coiled as in *Lenkunya.* Within the bulb the seminal vesicle may be located antero-dorsally, mid-anteriorly, or antero-ventrally.

The seminal vesicle is generally lined by a cuboidal epithelium that receives cyanophil, erythrophil, and sometimes xanthophil secretions as in *Arthurdendyus*. The secretions of the seminal vesicle are similar to those in mammalian reproductive systems, and comprise acid phosphatase, disulphide proteins, and neutral lipids, and are derived from glands in the parenchyma and adjacent to the penis (Winsor 1998b). The intra-penial seminal vesicle may be present or absent.

***Character**: Seminal vesicle: presence or absence*

0. Intra-penial seminal vesicle present

1. Intra-penial seminal vesicle absent

A seminal vesicle appears to be present in the majority of Triclads, and is probably the plesiomorphic state. In taxa with an inverted type of penis (*Fyfea* and "Parafyfea"), the sperm are received in the anterodorsal end of the bulb cavity. Histologically this region is the seminal vesicle; the more distal part of the cavity, differentiated on the basis of cell type and secretions, has the prostatic function. When a seminal vesicle is absent (the apomorphic condition) the function and secretions of the seminal vesicle appear to be taken over by the spermiducal vesicles as in *Nelipoplana (Nelipoplana) alba*. This state, coupled with poorly developed bulbar musculature, may represent a stage in the development of extra-bulbar structures. Mature terricolans generally have both spermiducal vesicles and a seminal vesicle.

4.11.5.4 Prostatic duct

The prostatic duct (= glandular part of the ejaculatory duct, Graff 1896a; 1899) is continuous with the seminal vesicle. The intra-penial prostatic vesicle is generally characterized by an increase in surface area of the duct, achieved by increased tortuosity of the duct as in *Lenkunya* or often a pronounced sinusoidal loop as in *Platydemus manokwari* and in *Australoplana*, or by a highly folded epithelium (*Nelipoplana Nelipoplana alba; Sokoplana*). The prostatic duct may be extra-bulbar and muscular (*Reomkago quadrangulata*).

The prostatic duct is lined by nucleate cuboidal to low columnar epithelium with an outer layer of circular muscles. The epithelium is sometimes ciliated, the cilia having either a mixing or absorptive function. Erythrophil secretions, derived from glands generally situated well anterior to the penis bulb, discharge through the epithelium into the prostatic duct. The prostate secretions contain acid phosphatases, tryptophane, neutral and phospholipids, and basic protein (Winsor 1998b). The secretions provide an energy source for the sperm, add to the bulk of the ejaculate, aid in dissolution of the ejaculate within the recipient, and may participate in the activation of the female copulatory organs during copulation to facilitate copulation and sperm transport (Guraya & Parshad 1988; Leal-Zanchet & Hauser 1999).

4.11.5.5 Ejaculatory duct

The ejaculatory duct is the terminal part of the male duct, sometimes eversible, which carries sperm together with seminal and prostatic secretions through the distal copulatory organ. It has also been defined as the duct within the penis papilla by which the sperm is discharged during copulation (Ball & Reynoldson 1981). It is generally lined by a low cuboidal epithelium which may receive secretions, or it may be lined by a glandular columnar epithelium as in *Fyfea* and *Arthurdendyus* in which the long-necked ducts of cyanophil gland cells terminate with characteristic swollen tips.

Characters provided by the ejaculatory duct have been used sporadically in the Terricola, mainly as diagnostic characters at species level. Potentially useful characters include:

***Character**: Ejaculatory duct: epithelium*

0. With ejaculatory duct epithelium nucleate

1. With ejaculatory duct epithelium infranucleate

***Character:** Ejaculatory duct: secretions*

0. Ejaculatory duct does not receive secretions

1. Ejaculatory duct receives secretions

***Character**: Ejaculatory duct: Pleated wall*

0. Ejaculatory duct with smooth unfolded wall

1. Ejaculatory duct with wall longitudinally folded (pleated)

***Character**: Ejaculatory duct: eccentric course in papilla*

0. Ejaculatory duct passes centrally through the penis papilla

1. Ejaculatory duct passes through the lower half of the papilla (eccentric)

The epithelium of the ejaculatory duct in the Tricladida is generally nucleate, (plesiomorphic)(Sluys 2001). In the Maricola (Sluys 2001) and Terricola both states occur. The infranucleate condition (an apomorphy), as in *Pimea monticola,* is uncommon in the Terricola. In some terricolan taxa the ejaculatory duct receives secretions, as in the penis papillae of *Lenkunya munda* and *Fyfea*; the general (plesiomorphic) condition is the absence of secretions. In the majority of Triclads the wall of the ejaculatory duct is smooth (plesiomorphic state), but in some dugesiids (Sluys 2001), and in *Lenkunya munda* the wall is thrown into longitudinal folds or pleats (apomorphic state). The ejaculatory duct passes more-or-less through the centre of the penis papilla in the majority of papillate taxa (plesiomorphic state), but in some taxa passes eccentrically through the ventral half of the papilla (apomorphic state), as in some species of *Geoplana*, for example *G. (G). metzi* Fuhrmann, 1914.

4.11.5.6 Penis Papilla

The penis in the Terricola can be papillate (with papilla), or apapillate (without a papilla) (Table 4.6). The penis papilla character is important in terricolan taxonomy, but not without problems of interpretation. There is some overlap between the penis papilla character and states (below), and the penis-type character, the latter being a functional approach incorporating additional characters from associated structures, considered later on. The penis papilla character originally considered by Ogren & Sluys (1998) for the

Bipaliidae, had four states, now slightly modified for the Geoplanidae and Rhynchodemidae, and including a new state (1) (Fig. 4.15)

0. With papilla of the fixed conical type enclosing a large part of the ejaculatory duct

1. With papilla inverted, enclosing a large part of the ejaculatory duct

2. With very small papilla, enclosing little or none of the ejaculatory duct

3. Intra-penial papilla present

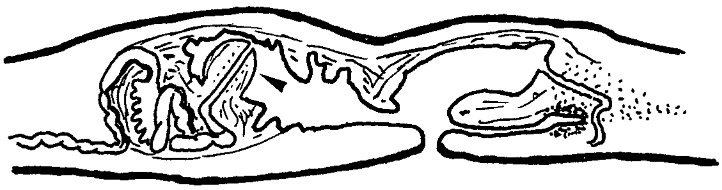
4. Papilla absent

A fixed conical papilla (State 0) is present in many Bipaliidae and microplaninae, geoplaninids, and pelmatoplaninids and some caenoplaninids such as *Kontikia* and *Lenkunya.* The inverted papilla (State 1) is a highly derived form apparently restricted to subantarctic taxa such as *Fyfea* and "Parafyfea". A very small papilla (State 2) is present in *Amaga, Bassioplana, Fletchamia,* and *Nelipoplana.* An intra-penial papilla (State 3) is uncommon, and is present in *Bipalium ephippium, Kontikia bulbosa, Pimea monticola, Reomkago quadrangulata,* and *Endeavouria septemlineata.* A papilla is absent (State 4) in certain Geoplanid and Rhynchodemid taxa including *Anzoplana, Caenoplana, Coleocephalus, Dolichoplana, Kynagoplana, Newzealandia, Notogynaphallia, Parakontikia, Pasipha, and Timyma.*

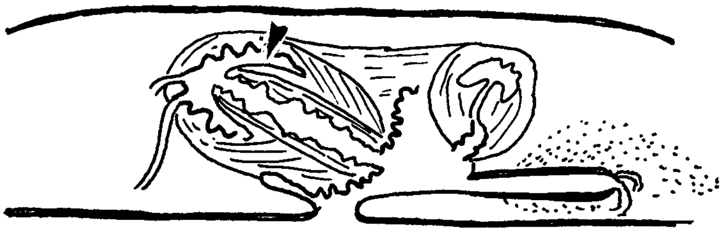
States (3) papilla fixed conical type, and (2) papilla small, can be difficult to assess in some geoplanids. In specimens of a given species the papilla may be contracted to a greater or lesser extent at the time of fixation. For this reason, it is probably better for phylogenetic analysis to simply have a character state *papilla present*, and note the size or size range and type of the papilla in the species description.

State (1), the presence of an intra-penial papilla, was originally termed an intra-antral papilla (Ogren & Sluys 1998). However the papillae in states (0), (1) and (2) are

(a) *Tasmanoplana*

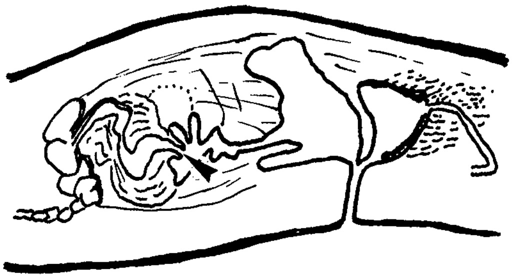


(b) *Fyfea*



(c) *Nelipoplana*

(d) *Pimea*



(e) *Caenoplana*

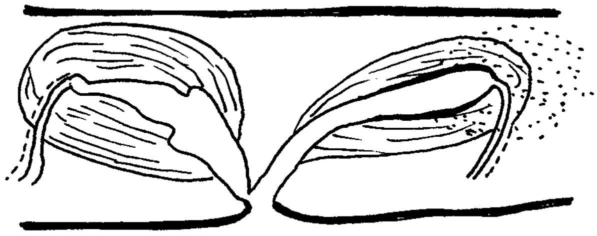


Figure 4.15

Penis papilla

(a) papilla fixed conical type (b) papilla inverted type (c) papilla small type (d) intrapenial papilla (e) papilla absent. Refer to the text for an explanation.

all intra-antral. A more accurate term is intra-penial papilla, as in the maricolan genera such as *Bdelloura, Miava, Palombiella, Synsiphonium,* and *Micropharynx* (in Sluys 1989b), and the caenoplaninids *Kontikia bulbosa, Pimea* and *Reomkago*. The bipaliid *Bipalium ephippium* has a small stumpy intra-penial papilla within a fixed papilla. In *Kontikia bulbosa* and *Reomkago* the intra-penial papilla is elongate, and more like those figured for some maricolans (Sluys 1989b). In *Pimea* the intra-penial papilla is closer to the situation in *Nelipoplana (Peokrypta) enigma* than to *Reomkago.* The penis papilla (= intra-antral papilla of Ogren & Kawakatsu 1990) of *Amaga* is regarded by me as a small papilla, and not an intra-antral papilla. E.M. Froehlich (in Ogren & Kawakatsu 1990, page 87) "is of the opinion that the intra-antral papilla of *Amaga*, which appears more like folds of the atrial wall, is not homologous with the penis papilla of the genus *Geoplana*." The illustrations of the copulatory apparatus of *Amaga amagensis* (in Fuhrmann 1914, page 775) bear a striking resemblance to those in the series of the copulatory organs of *Nelipoplana* figured (Appendix 8.12). The penis papilla in *Geoplana* is of the fixed type, State (0).

A papilla of the fixed conical type (State 0) was considered to be plesiomorphic for the Bipaliidae (Ogren & Sluys 1998), based upon comparisons with the outgroup in which all four states were represented. They regarded State (4), papilla absent, as apomorphic, presumably representing secondary loss of the papilla that is entirely feasible.

Character state (4) can also be regarded as a plesiomorphic condition - the persistence of juvenile (primitive or ancestral) characters in the mature worm. This state is illustrated in figures showing the differentiation of the atrial space in the mature copulatory organs of the Terricola (in Graff 1896a, 1899, 1911-1917, Fig. 135). This condition is present in mature specimens of *Geoplana (Geoplana) burmeisteri* (in Graff 1912-17 page 3018), in *Caenoplana spenceri*, and in *Rhynchodemus* and *Dolichoplana.* In the case of *Caenoplana spenceri* additional support for this view comes from the plesiomorphic states of its body musculature – it is a primitive terricolan. From this perspective, the penis papilla states could be reversed, with the presence of a conical papilla as an apomorphic condition. Whether the absence of a penis papilla in different genera is a homology or represents convergence has yet to be ascertained.

Penis papilla - ducts and secretions. The penis papilla may contain ducts through which the penial glands discharge through the epithelium of the papilla. The scant knowledge of penial glands in the Paludicola has recently been reviewed (Leal-Zanchet & Hauser 1999). Histochemical characterization of these glands can contribute to the characterization in some paludicolan taxa (Leal-Zanchet & Hauser 1999, Souza & Leal-Zanchet 2002), and may have application in the Terricola.

***Character**: Penis glands opening through the penial papilla:*

0. Without penis glands opening through the penis papilla,

1. With penis glands opening through the penis papilla

The penis papilla character does not include the musculoglandular organs present on the penes of some taxa (considered later, this chapter). The general (plesiomorphic) case is the absence of glands opening through the papilla (Sluys 2001). Penial glands normally discharge into the ejaculatory duct or peri-penial region of the male antrum. The presence of glands opening through the papilla, with secretions as in *Lenkunya* and the unusual papilla in *Barringtoplana,* is an apomorphy.

4.11.5.7 Penis type

Two functional types of penis are recognised (Winsor 1998b): a *protrusible* penis and an *eversible* penis. The highly derived penis with an inverted papilla is intermediate between these two types; it exhibits anatomical features that align it more with the protrusible penis than the eversible type.

The size and shape of a penis papilla can vary according to the maturity of the specimen (Ogren 1991), or to the circumstances present at the time of fixation. A taxonomic problem arises where agonal contractions during the preservation of a mature specimen of a species with eversible-type penis results in the formation of a temporary penis papilla; or where the use of relaxants seemingly prevents this occurrence, thus making interpretation of the penis type for a given species difficult (Jones *et al* 1998).

The penis type character is a partial amalgamation of the penis papilla character and male antral wall character (Fig. 4.16). It has three states:

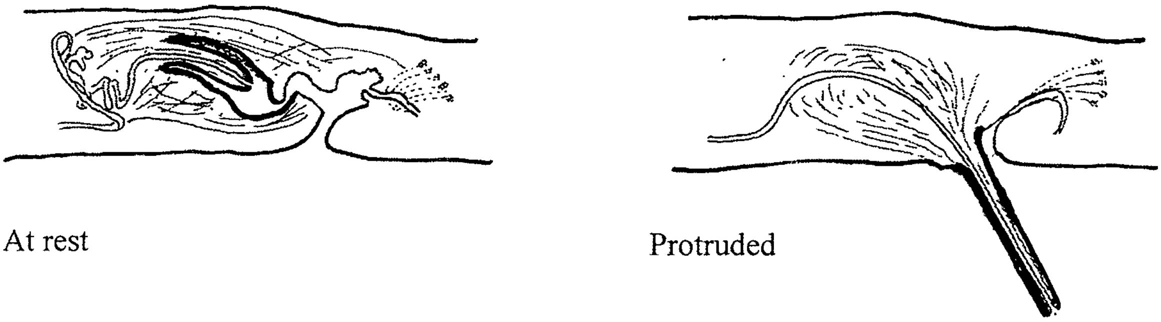
0. a *protrusible* penis: with fixed papilla extending into the antrum; papilla covered by low facing non-specialised epithelium. Musculature of bulb generally strong, differentiated into layers, or with intermixed longitudinal and circular muscles. Ejaculatory duct smooth without cocoon-forming epithelial elements. Length during intromission is largely due to elongation of the papilla and bulb.

1. an *inverted papilla* with ejaculatory duct without cocoon-forming elements, and powerful longitudinal and circular muscles differentiated in layers. The bulb is largely comprised of strong longitudinal muscles, and the distal papilla protruding into the antrum, and surrounded by strong circular muscles. The area of bulb below what would be the external papilla when everted, and the area at the crease (transverse axis of eversion), is open and sponge-like. This vacuolate tissue possibly allows compression of the tissues, thus facilitating the eversion process. Length during intromission is due to eversion of the inverted papilla plus elongation of the distal papilla and bulb.

2. an *eversible* penis or pseudophallus, formed from the ejaculatory duct which is sheathed in the everted male antrum: with a very small papilla or no papilla, with reduction of the facing epithelium, or with a specialised secretory epithelium, generally that of the ejaculatory duct or antrum, which may be infranucleate. Musculature of antrum strong, that of bulb weak apart from strong outer longitudinal musculature. Longitudinal muscles may also be attached to ejaculatory duct. Length during intromission due to the pseudophallus formed largely from the male antral wall supported by the elongated bulb.

The *protrusible* penis, common in bipaliids and microplaninids, is generally characterised by a conspicuous permanent papilla and muscular bulb which, through

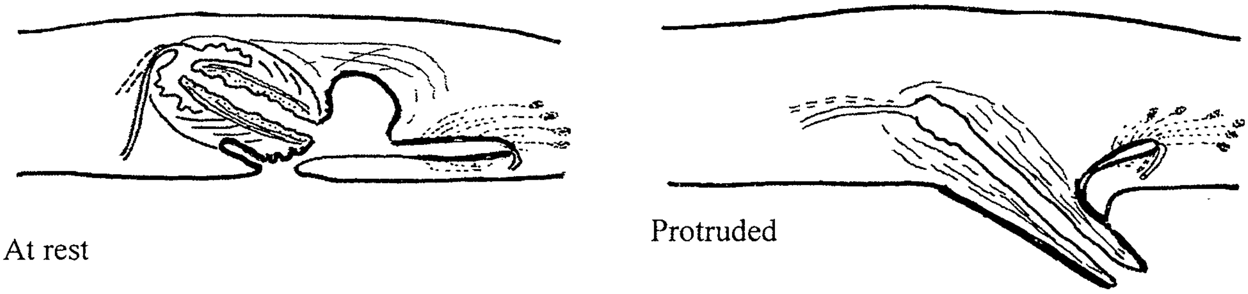
(a) Protrusible penis



At rest

Protruded

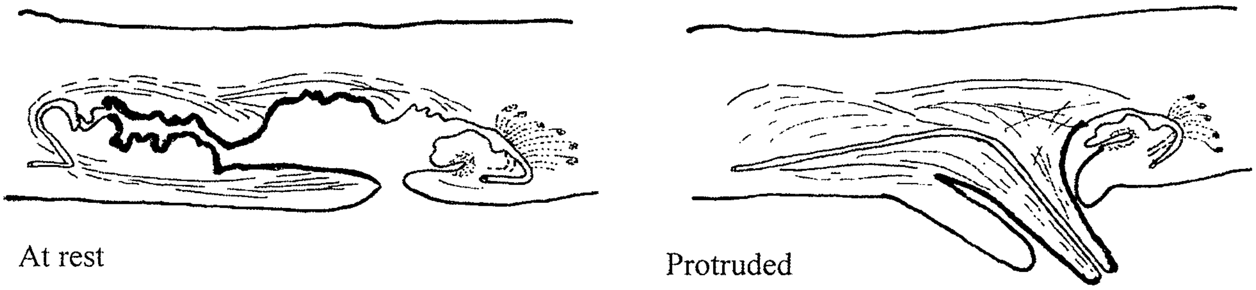
(b) Inverted penis



At rest

Protruded

(c) Eversible penis



At rest

Protruded

Figure 4.16
Penis type
Diagram illustrating protrusion of a penis with a fixed papilla, with an inverted papilla, and an eversible penis with pseudophallus. Refer to text for discussion. Red – facing epithelium of papilla, Blue – antral epithelium

contraction of circular muscles with consequent narrowing of the bulb and relaxation of longitudinal muscles, is protruded through the gonopore. The *inverted* penis type is uncommon, though it occurs in the Maricola (*Nexilis epichitonius* and *Centriovarioplana tenuis*, in Sluys 1989b), and in the terricolan *Fyfea* and other subantarctic taxa.

The *eversible* penis or pseudophallus is present in many rhynchodeminids and geoplanids, especially caenoplaninids. Extra-bulbar and bulbar structures generally exhibit coiling or folding when not extended. Eversion is achieved by contraction of the circular muscles in the bulb, around the ejaculatory duct, and antrum. It is facilitated by areas of compressible tissue at the pinch points. Compression tissue has been found in *Parakontikia ventrolineata, Arthurdendyus triangulatus, A. vegrandis,* and *Coleocephalus fuscus.* The outer longitudinal muscles surrounding the bulb facilitate bending of the pseudophallus. A temporary papilla appears to be formed only during eversion. An everted pseudophallus of *Newzealandia moseleyi* is figured by Fyfe (1956), and temporary papilla formation by the antrum is illustrated and discussed by Hyman (1940) in *Microplana atropurpurea* and by Marcus (1951) in *Notogynaphallia abundans.*

What is often regarded as an ejaculatory duct may be an antrum lined by a sclerotin-forming secretory epithelium. This is evident in *N. abundans* where the two sections of the male antrum have different types of epithelium, the proximal region forming the papilla. However caution must be exercised in interpretation as sclerotin-forming secretions occur in the ejaculatory duct of the penis papilla of *Sabussowia dioica* (Maricola) where they coat the spermatophore (Tekaya *et al* 1997), although a similar arrangement has not yet been encountered in the Terricola.

4.11.5.8 Ejaculate and spermatophore

Components of the ejaculate are primarily concerned with sperm delivery and protection in the viscid seminal secretions, and dissolution of the ejaculate by prostatic secretions. They, or substances present in the organs into which they are deposited, may capacitate the sperm (Leal-Zanchet & Hauser 1999).

The components of the ejaculate are similar to those reported from mammalian ejaculates (Mann, 1954; Mann and Lutwak-Mann, 1981). Within the ejaculate of *P. manokwari* sperm are surrounded by disulphide proteins derived from the seminal vesicle, mixed with proximal prostatic secretions comprising tyrosine, lipids and a secretory acid phosphatase, together with basic proteins of the distal prostatic region. The ejaculate breaks down and sperm are free to move anteriorly along the female glandular canal and ovovitelline duct or through the mesenchyme towards the ovary.

Not all ejaculates are surrounded by penial secretions. Incompletely enclosed ejaculates have been described from the geoplaninids *Amaga righii*, *Choeradoplana iheringi, Notogynaphallia müLleri* and *N. sexstriata.* These ejaculates may function as spermatophores, possibly with some important role in sperm transfer during copulation in these apapillate species (A-M. Leal-Zanchet, *in litt.*).

Spermatophores in the Terricola are uncommon. A spermatophore has been reported in the rhynchodeminid *Platydemus victoriae* (in Heinzel 1929) and in the copulatory bursa of the caenoplaninid *Pimea monticola* (in Winsor 1991b).

4.11.5.9 Sperm Infiltrate:

The presence of frank spermatozoa in the parenchyma is a rare condition in the Terricola, and may be due to a number of circumstances observed in Maricola and Paludicola (Cernosvitov, 1931):

- rupture of a sperm-containing structure such as a seminal vesicle during the process of fixation of the specimen;
- leakage of sperm from a testis with subsequent atrophy of the latter;
- rupture of the walls of a seminal vesicle, with subsequent formation of sperm masses which subsequently degenerate;
- hypodermic impregnation of one individual by another, or self-fertilization, giving rise to the presence of sperm in tissues.

1. *Rupture of spermiducal vesicle*: In the material examined in this study, two examples of sperm infiltrate were identified: in *Artioposthia langii* (AM W1995), rupture of one of a pair of large stalked spermiducal vesicles into the adjacent intestinal diverticula. It could not be determined whether the rupture

was natural (for sperm digestion) or an artefact of agonal contractions during fixation.

2. *Possible hypodermic impregnation:* In *Coleocephalus fuscus* (MONZ ZW1370) in the area immediately adjacent to the ovaries, just anterior of the pharynx, sperm were present throughout the parenchyma in such density as to appear on first glance to be a mass of collagen fibres. They were present in the immediate ventral epithelium and had infiltrated between ductules, vitellaria and muscle fibres, but had not extended beyond the dorsal cutaneous longitudinal muscles. There was no evidence of rupture of sperm-containing structures in this area. Sperm were also present throughout the ventral body wall immediately below the ovaries. It is tempting to infer that hypodermic injection took place in this area. Hypodermic impregnation has been postulated (Bohmig 1906 and Wilhelmi 1909 in Sluys 1989b, page 50) as the cause of similar sperm infiltrates exhibited by specimens of the *Cercyra hastata* and *Sabussowia dioica* (Maricola).

4.11.5.10 Male antrum

Characters proposed for the male antrum (slightly modified from Ogren & Sluys 1998, for the Bipaliidae) are concerned with the antral cavity and nature of the antral walls. Refer to Figs. 4.15, 4.16 for examples from the Caenoplaninae:

***Character**: Male antral wall*

0. posterior antrum wall consists of a broad open passage into which the ejaculatory duct opens directly, penis sheath not evident (Fig. 4.15a);

1. muscular walls form a prominent, often narrow penis sheath, that separates the male antrum from the common antrum (Fig. 4.15d);

2. the male antrum wall is very muscularised to form the eversible pseudophallus, the male antrum lining this muscular organ serves as the

distal ejaculatory duct, and the penis papilla is very small, not typically shaped or is absent (Fig. 4.15c,e).

A hemi-circular or circular muscular lip of varying length, often partly underlain by spongy compressible tissue, may wholly or partly encircle the distal male antrum (Winsor pers. obs).

The lip in some taxa has previously been considered to act as a papilla (Jones *et al* 1998). However, at least in some species, it does not appear to form a false papilla; rather, it appears to act as a basal sheath for a pseudophallus, and possibly acts as the pinch point or lock during eversion of the antrum to form a pseudophallus. As in the inverted penis, the spongy compression tissue probably facilitates eversion. In *Kynagoplana citrinus* beneath a thin antral lip is a strongly muscular lip, thought to act as a bracing or anchor point to facilitate eversion of the penis (Appendix 8.10). In apapillate taxa, structures at the distal part of the antrum appear to remain close to that point (refer to *Newzealandia moseleyi*, Fig. 1in Fyfe 1956) and do not appear to form a false papilla (refer to Fig. 4.16).

The proximal male antrum, adjacent to the ejaculatory duct or penis papilla, is generally lined by a nucleate unspecialised cuboidal epithelium, and the distal male antrum is generally lined by a tall columnar cocoon-making epithelium. This specialized type of epithelium may be absent from adenodactylate taxa, as in *Artioposthia hamiltoni* in which the antrum is lined by a non-specialized facing epithelium traversed by lines of low secretory nipples. Species descriptions should be consulted for the many variations on this theme. The secretions of the male antral epithelium of *Platydemus manokwari* (in Winsor 1998b) include leucine aminopeptidase, polyphenol oxidase, basic proteins, diphenols and polyphenols, and glycosaminoglycans. All these substances are involved in cocoon-making.

4.11.6 Common Antrum

There are presently no clear anatomical criteria for the differentiation of male, female and common antra in the Terricola, though in some taxa the male and female antra can be differentiated histochemically (Winsor 1998b page 418; Leal-Zanchet *in litt*). The term antrum where used here refers to both antrum and atrium. Cannon (1986) defines the term *antrum* as the terminal cavity of male or female systems when opening separately. For example in the bipaliids a muscular flap, fold or pad separates the male and female terminations. Such designations may cloud understanding of the antral functions in individual taxa, and it is essential to use histological criteria such as the presence or absence of certain cell types to identify antral functions. The *atrium*, defined as the space into which both the male and female systems open before expulsion of sex products via a common gonopore (Cannon 1986), should be similarly defined by histological criteria.

The antrum has several functions related to copulation, resorption, cocoon formation, and possibly capacitation of sperm (Schilke 1970 in Hendelberg 1983, Winsor 1998b). The antral lining epithelium and underlying musculature will reflect these activities. During copulation the antrum receives the penis, and possibly other structures, such as the accessory glands in *Newzealandia moseleyi illustrated* by Fyfe (1957). In certain taxa the antrum provides adhesion of the genital regions of copulating flatworms during penial intromission, suggested by Dendy (1891) following observations of copulating *Fletchamia mediolineata.* The interatrial muscular genital pad in *Bipalium kewense* and other bipaliids secretes an adhesive substance that may be involved during copulation. The antrum may also function as a bursa receiving and possibly nourishing the ejaculate, as in some geoplaninids sperm have been observed superficially penetrating the antral wall (E. Froehlich, pers. comm.*)*. In many species this function is performed by a specialised structure such as a copulatory or seminal bursa.

A summary of the histochemical findings for the shell glands, female genital (= glandular) canal, and epithelium of the male and female atria of the rhynchodeminid *Platydemus manokwari* (from Winsor 1990; 1998b), and caenoplaninid *Coleocephalus fuscus* is provided in Table 4.7.

Table 4.7

Copulatory organs: A summary of histochemical findings in the shell glands, female genital canal, female antral epithelium and male antral epithelium of *Platydemus manokwari.* Details of the full histochemical tests and methods and references are provided in Winsor (1998).

Tissue substance	Shell glands	Female genital canal	Female antrum	Male antrum
ENZYMES				
acid phosphatase		+		
leucine aminopeptidase		+	+	+
polyphenol oxidase	+		+	+
PROTEINS				
general protein	+	+	+	+
basic protein	+	+	+	+
tryptophane		+		
amino groups	+		+	
monophenols	+		+	
diphenols	±		±	+
polyphenols	±	+	±	+
aromatic amines	+		+	
CARBOHYDRATES				
neutral carbohydrates	+	+	+	
glycosaminoglycans		+		+

4.11.6.1 Resorption of sperm and gestational products in the antrum.

Degradation of surplus sperm and other copulatory and gestational products occurs in the antrum. In *Platydemus manokwari* a resorptive function of the antrum is suggested by the presence of erythrophilic secretions shown to contain acid phosphatase, together with leucine amino peptidase in the atrial epithelium and wall (Winsor 1990; 1998b). The acid phosphatase is secreted into the antrum from glands concentrated on the atrial floor surrounding the inner lips of the gonopore. This enzyme may intraluminaly digest unwanted sperm and yolk as occurs in the copulatory bursa of some Paludicola (Farnesi *et al* 1979; Sluys 1989b).

This situation is analogous to the vesicula resorbiens (bursa copulatrix) in *Temnocephala* species. Here sperm and yolk degrade autolytically in the lumen, the products then being absorbed, and digested by the enzymes present in the vesicle walls (Jennings 1968). Recent studies (Moretto 2001) of *Temnocephala jheringi* suggest that the bursal epithelium becomes a secretory syncytium, the merocrine secretions of which lyse sperm in the lumen, and the resulting products are resorbed.

How the degraded products are absorbed in the antrum of *Platydemus manokwari* is uncertain but may be through what appear to be stereocilia (large microvilli) in the atrial epithelium. Vacuolate cells of the type commonly associated with resorptive structures (Sluys 1989b) have not been found in the antrum of *P. manokwari.*

Atrial acid phosphatase secretions may also facilitate sclerotin production for cocoon-making. Substances that bind sulphydryl groups inhibit polyphenol oxidase (Hackman 1953) but are themselves inhibited by acid phosphatase (Mann and Lutwak-Mann 1981). In the Tricladida cocoon development is regarded as generally occurring in the common antrum or male antrum (Hyman 1951). As this function requires specific cell types, the decision as to where cocoons are formed must be based upon histological evidence: the presence of the necessary cell types, or presence of a developing cocoon.

In addition to cocoon-forming secretions, the antrum also produces an acid mucopolysaccharide which is localised on the lumenal margin of the atrial epithelium. This mucin possibly protects the epithelium from harmful intermediaries during sclerotin formation, and may also act as a lubricant or non-sticking surface.

Female Reproductive System

4.11.7 Ovary

In the Terricola, potential characters provided by the ovaries include the number of ovaries, position of the ovaries laterally and horizontally along the body, and presence of "parovarian" tissue (discussed in the following section).

Triclads have a single pair of ventrally located ovaries (Hyman 1951 page 151), each of which is covered by a thin, flattened nucleate tunica propria and a fine network of nerve cells and nerve fibres derived from the initial tract of the posterior ventral nerve cords (Gremigni 1983). The ovaries of Terricola are similarly invested by a tunica (the spindle-shaped parietal cells of Graff 1899; pavement cells of Fyfe 1937). Ovaries may be spheroidal (*Endeavouria, Kontikia*, *Parakontikia*), ellipsoidal (*Caenoplana*), trigonial or elongate (as in many of the adenodactylate caenoplaninids). Trigonial and elongate ovaries often exhibit slight lobation to one side, generally where the oviduct communicates with the ovary (*Pimea monticola, Newzealandia inaequiabilis*). Oocytes in various stages of development are present, together with stromal and nurse cells. A small area of undifferentiated cells – the germ centre or Keimlager, is sometimes evident along the membrane bounding the ovary, especially ventrolaterally in the lobulate area near the anastomosis between oviduct and ovary. This is similar to the situation in the Maricola (in Sluys 1989b). Descriptions are available of the general development of oocytes in the Tricladida (Graff 1912-17), and in the austral taxa *Caenoplana spenceri* (in Dendy 1890) and *Arthurdendyus triangulatus* (in Fyfe 1937). Ultrastructural studies of oogenesis in the Paludicola have some taxonomic and systematic benefits (Gremigni 1992), but have not yet been undertaken in the Terricola.

4.11.7.1 Numbers of ovaries

***Character**: Number of ovaries*

In this study, the majority of taxa investigated had a single pair of ovaries generally embedded in the dorsal side of the lateral nerve cords. This is regarded as the plesiomorphic state. Some specimens were found to have more than two ovaries, regarded here as an apomorphy. Taxa with supernumerary ovaries included *Kontikia orana* (LW1429) with three ovaries on the left and two ovaries on the right side, *Anzoplana tristriatus* (LW1755) with two ovaries on the right side, and *Endeavouria septemlineata* with a supernumerary ovary between mouth and copulatory organs, on the left side under the nerve cord just above the ventral cutaneous musculature; there was no connecting ovovitelline duct.

In *Kynagoplana citrinus* multiple ovaries (six to eight pairs) were found in both Type specimens, with fewer ovaries (six pairs) in the immature specimen. The presence of supernumerary ovaries in two specimens from the same site is highly unusual. As it is uncertain whether this condition could be a generic or specific state, it is presently included in the generic diagnosis of *Kynagoplana* (this thesis). In *Arthurdendyus* the ovaries communicate directly with elongate lateral bursae, and in *A. triangulatus* the ovaries have multiple germaria spaced at regular intervals along the length of the lateral bursae, though only the anterior-most germaria communicate with the oviducts (Fyfe 1937). A similar arrangement is present in *"Kontikia" marrineri* where there is a germarium at the anterior pole behind which are two regularly spaced branches from the oviduct. Fyfe postulated that germaria might develop at these sites at different times. These branches have been found by to lead from the oviduct to the lateral bursae, though they may be a developmental relict and indication of multiple germaria (Winsor, pers obs).

In the foregoing examples, the anterior-most ovaries on each side of the body were larger than the subsequent supernumerary ovaries, the latter diminishing in size passing posteriorly. The ovaries were regularly separated from each other. An explanation for the histogenesis of supernumerary ovaries, and branches from the oviducts as in *K. marrineri,* may be similar to that advanced by Moseley (1875) to explain the presence of small, branched, lateral diverticula in the ovovitelline ducts between the end of the pharynx and female copulatory organs in *Bipalium diana* – they may be "rudiments of the branched ovary possessed by lower planarians such as *Dendrocoelum lacteum*". Molecular evidence (Carranza *et* al 1998; Baguñà *et al* 2001) and pharyngeal muscular characters (this thesis) suggest planariid – dugesiid affinities for *Arthurdendyus*; on the basis of pharyngeal musculature, the other taxa exhibiting supernumerary ovaries have possible affinities with dendrocoelids.

4.11.7.2 Position of ovaries

Character: *Position of ovary on nerve cord:*

The ovaries are generally embedded in the dorsal surface of the lateral nerve cords. They may be located on the dorsomedian edge, mid-dorsal cord or dorsolateral edge, as reported in the Maricola (Sluys 1989b). Unfortunately few descriptions of Terricola record the precise position of the ovary on the lateral nerve cords, thus presently limiting the potential of this character and states.

Character: *Position of ovary along the body axis*

With regard to the taxa studied here, three states for the position of the ovaries along the body axis were recognized (Table 4.8, Fig. 4.17):

0. With ovaries located at a short distance behind the brain in the anterior third of the body

1. With ovaries located just anterior of the pharynx

2. With ovaries behind the mouth but before the gonopore.

Most taxa in this study, including some anzoplaninids, all but one caenoplaninid, all rhynchodeminids, and *Bipalium kewense* have ovaries located at a short distance behind the brain in the anterior third of the body. Given the anterior position of the ovaries in most Tricladida, this state is probably plesiomorphic and the more posterior locations of the ovary apomorphic states. *Coleocephalus fuscus* from the NZ subantarctic Islands appears to be the only terricolan presently described with ovaries located just anterior of the pharynx. Taxa with ovaries situated behind the mouth but before the gonopore, are all from the NZ subantarctic islands and New Zealand and include *Arthurdendyus* where the ovaries are located beside the penis bulb. The supernumerary ovary in a specimen of *Endeavouria* is also situated behind the mouth.

Table 4.8
Ovaries, ovovitelline ducts, and accessory genital organs

Taxon	ovaries - position		ovo-vitelline duct		parovarian resorptive tissue	MGO	Viscid gland
	anterior third	post oral pre g'pore	separately join fgc	unite, then join fgc			
Geoplanidae: Geoplaninae							
Geoplanidae: Anzoplaninae							
Anzoplana	Y		Y		Y	Y	
Artioposthia ventropunctata	?		Y			Y	
Artioposthia subquandrangulata	Y			Y		Y	
Fyfea carnleyi		Y	Y		Y	Y	
"Kontikia" ashleyi ("Parafyfea")		Y		Y	Y		
Australopacifica aucklandica		Y		Y	Y		
Artioposthia alfordensis		Y		Y		Y	Y
Geoplanidae: Caenoplaninae							
Arthurdendyus		Y		Y	Y	Y	
Artioposthia lucasi	?		Y			Y	
Artioposthia hamiltoni	Y		Y			Ynz	
Coleocephalus	Y 4			Y	Y	Y	
Lenkunya	Y			Y		Ypre-ad	
"Kontikia" ranuii	Y			Y			
Artioposthia garveyi	Y			Y		Y	
Artioposthia howesi	Y			Y		Y	
Kontikia	Y			Y			
Parakontikia	Y			Y			
Artioposthia glandulosa	Y			?		Y	?Y
Artioposthia gramnicola	Y			Y		Y	
Newzealandia moseleyi, iris	Y			Y		Ynz	
Artioposthia angusta	Y		Y			Ynz	
Artiopothia langii	Y		Y			Y	
Danidoplana	Y		?				
Caenoplana	Y		Y			(Y)	
Australoplana	Y 7			Y			
Barringtoplana	Y 7			Y			
Kynagoplana	Y 8			Y			
Pimea	Y 7			Y			
Endeavouria	Y7			Y			
Artioposthia mariae	Y		Y			Y	
Fletchamia	Y		Y			Y	
Thosteelia	Y		Y				
Reomkago	Y		Y				
Timyma	Y						
Bassioplana	Y		Y5				Y
Nelipoplana	Y 7						Y
Sokoplana	Y 7			Y			Y
Apogaioplana	?			Y			Y
Tasmanoplana	Y			Y			
Rhynchodemidae: Rhynchodeminae							
Cotyloplana	Y			Y			
Platydemus	Y			Y			Y
Dolichoplana	Y			Y			Y
Digonopyla				Y			
Rhynchodemus	Y			Y			
Bipaliidae							
Bipalium kewense	Y		Y				

4. Just anterior to pharynx
5. Shell glands open into ovovitelline ducts
7. Tuba present, proximal end of oviduct
8. Multiple ovaries present
pre-ad = pre-adenomuralia
nz = MGO of Newzealandia type

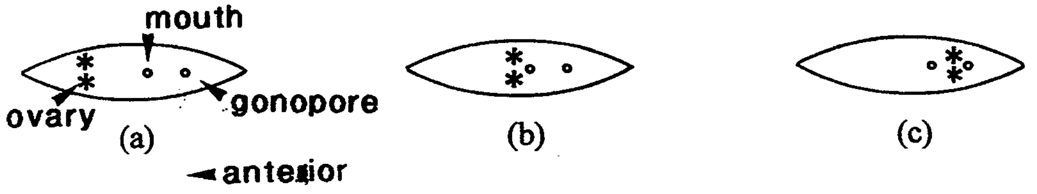


Figure 4.17
Ovaries – Position along the body axis
(a) ovaries in anterior body third (b) ovaries located just anterior to pharynx (c) ovaries located behind pharynx but before gonopore.

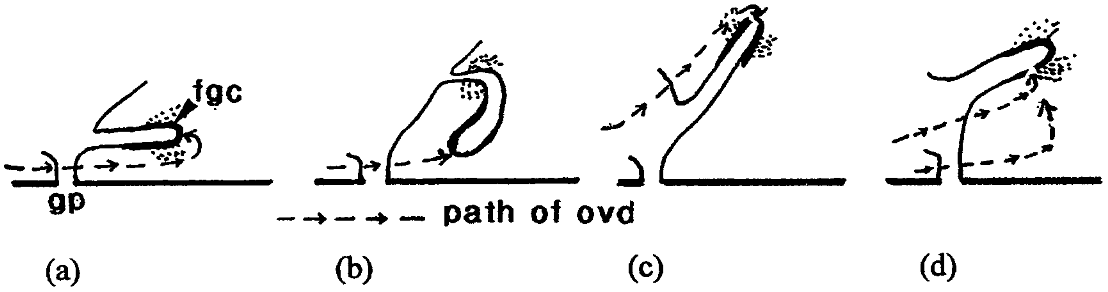


Figure 4.18a
Ovovitelline ducts – approach to female genital canal
(a) postflex (posterior approach (b) proflex condition with anterior approach (c) proflex condition with dorsal approach (d) mid ventral approach to female genital canal.

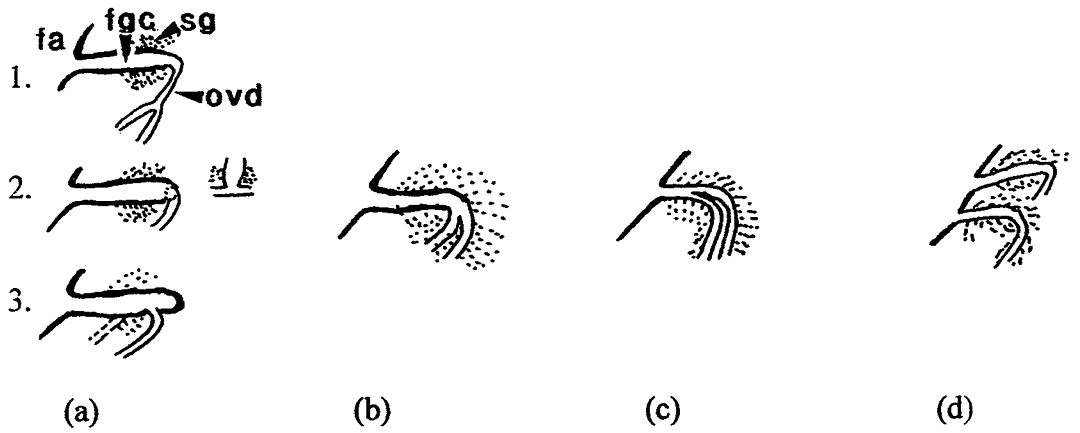


Figure 4.18b
Ovovitelline ducts – communication with female genital organs
(a) single female genital canal (fgc) receiving shell glands (sg) communicating with 1. common ovovitelline (ovd) duct, 2. common ovd opening, 3. separate ovd (b) ovd receive shell glands, fuse to form common fgc (c) distal ovd receive shell glands, share common opening into female antrum (fa) (d) ovd receive shell glands, open separately into female antrum. Refer to text for explanation.

Figure 4.19
Vitellaria – location
(a) peri-intestinal (b) subintestinal, intertesticular

4.11.7.3 Parovarium

The term *parovarium* refers to a structure appended to the ovary (Graff 1899; 1912-17). Parovaria are uncommon in the Terricola, and presently only serve as diagnostic characters at species level. One function of parovaria and stromal sacs is the storage of sperm in close proximity to the ovary. Whether all these structures also have a sperm resorptive function is uncertain. Parovaria include:

1. clumps of vitelline-like cells connected to the ovary, or joined by a short duct to the oviduct or ovovitelline duct behind the ovary, as observed (Graff, 1899) in *Geoplana (Geoplana) ladislavii*, *Bipalium sikori*, *Othelosoma speciosum*, by Moseley (1876) in *B. diana*, and in the paludicolan *Phagocata woodworthi* (mentioned by Ball & Reynoldson, 1981).
2. a spheroidal diverticulum, as in *Bipalium hilgendorfi* immediately adjacent to the posterior of the ovary (Graff 1899);
3. a sacculate lobe of the ovary, as in *Geoplana (Geoplana) rufiventris sensu* Graff 1899 page 152, and in *Geoplana (Geoplana) argus*, though the latter was unilateral and considered to be an abnormality (Graff, 1899). In the Maricola saccate appendages attached to ovaries termed *stromatic sacs* (Sluys 1989b) have been described in species of the cercyrid genera *Oregoniplana* and *Pacifides* and the meixnerid *Jugatovaria*. In the first two genera the oviducts first enter the sac, and in the last the ducts connecting the sacs to the ovaries are muscularised, and the oviducts communicate directly with the ovaries instead of the sacs.
4. parovarian tissue and parovarian cells *sensu* Fyfe (1937) as in *Arthurdendyus triangulatus* and some subantarctic island taxa (in Fyfe 1953) (Table 4.8) are considered here to be sperm resorptive tissues, to be further discussed within this chapter.

4.11.7.4 Ovovitelline ducts

Characters provided by the oviducts and ovovitelline ducts include the point on the ovary with which the oviducts communicate, presence of tubae at the proximal (ovary end), path posteriorly to the copulatory organs, approach of the ovovitelline ducts to the female copulatory organs, communication of the ovovitelline ducts with the female copulatory organs, caudal branching (distal end), communication with bursal canal, and communication with a genito-intestinal canal.

In the general case, at the point where the ovary and oviducts unite, occlusive cells may be present. These prevent sperm passing into the ovary. The oviduct passes posteriorly, usually along the top of the lateral nerve cords. From the point where it receives vitelline secretions, usually close to the ovary, it then becomes the ovovitelline duct. Approaching the hind end of the copulatory apparatus the ducts rise, turn inwards and communicate with the female glandular canal. In all taxa examined in this study, the ovovitelline duct was lined by a nucleate ciliated cuboidal epithelium.

***Character**: Communication between duct and ovary.* Few descriptions of Terricola indicate the precise point on the surface of the ovaries from which the oviducts arise. In the Maricola the oviducts can arise from the ovarian ventral or ventrolateral surface, anterior ovarian wall (anterodorsal, anterolateral, anteroventral) or posterior ovarian wall (posterolateral, posteroventral) (Sluys 1989b); similarly in the Terricola. This character and possible states, whilst potentially very useful, has not been investigated in this study.

***Character:** Presence of tubae:* The term *tuba*, which originally referred to the proximal ends of the oviducts in contact with the ovaries irrespective of morphology (Graff 1912-17 page 2983), is now restricted to pronounced expansions of the proximal oviducts (Sluys, 1989b). The tubae are surrounded by a fine muscularis underlying the tunica propria, and are lined by a tall columnar non-ciliated epithelium with basal nuclei and pale cyanophil cytoplasm. They generally communicate with the ovaries via a short, narrow duct, or through a pronounced and usually muscularized constriction (Sluys, 1989b). In this study, tubae were only found in the caenoplaninids *Australoplana sanguinea, Barringtoplana citrinus, Endeavouria septemlineata, Nelipoplana (N) alba, Pimea monticola* (incorrectly termed seminal *receptacles* by Winsor 1991b), and *Sokoplana kanangra*.

***Character**: Path posteriorly to the copulatory organs.* Few descriptions of Terricola indicate the precise path of the ovovitelline duct posteriorly. In the Maricola most ovovitelline ducts run laterally along the nerve cords, dorsally to the cords, or laterodorsally, dorsomedially or medially to the ventral nerves cords (Sluys 1989b). In the Terricola the ovovitelline ducts generally run along the mid dorsal surface of the lateral nerve cords. This character and possible states, whilst potentially very useful, has not been investigated in this study.

***Character**: Approach of the ovovitelline ducts to the female genital canal* (Fig. 4.18 a) Three states for the character of the approach of the ovovitelline ducts to the female genital canal – posterior, anterior, and ventral approaches - have been recognized in the Terricola, with emphasis on the Bipaliidae (Ogren & Sluys 1998), emended as follows:

0. with the postflex (posterior approach) condition, with ovovitelline ducts continuing posterior to the gonopore, turning dorsomedially to enter the female glandular canal posteriorly;
1. with the proflex condition, with the ovovitelline ducts turning dorsomedially anterior to the gonopore with dorsal approach; and
2. with ovovitelline ducts having mid-ventral entrance.

To these three states is added a fourth, intermediate between the proflex and postflex conditions:

3. with the postflex (anterior) the ovovitelline ducts continuing posterior to the gonopore, turning dorsomedially to enter the female canal anteriorly

The postflex (posterior approach) condition is present in all families of the Terricola, and is probably the plesiomorphic state; Ogren & Sluys (1998) consider it plesiomorphic in the Bipaliidae. The other states are probably all apomorphic conditions. Most taxa in the Rhynchodemidae exhibit the postflex (posterior) state, an exception being *Platydemus fasciatus* (Lord Howe Island) that has the postflex (anterior) state. In the Geoplanidae, most caenoplaninids, and some highly derived geoplaninids (*Xerapoa*) exhibit the postflex (posterior) state; most geoplaninids exhibit either the proflex or postflex (anterior approach) states, the latter including taxa with anterior suckers (*Choeradoplana, Issoca*).

***Character**: Communication of the ovovitelline ducts with the female copulatory organs.* This character concerns the immediate entry of the ovovitelline ducts into the female genital canal (= female glandular canal) (Table 4.8), analogous to the entry of the vasa deferentia into the male copulatory organs. In some states the terminal portion of the ovovitelline duct receives shell glands. Graff (1899 page 153) recognized four main conditions, slightly modified and given here in reverse order to Graff (Fig. 4.18b):

0. With single female genital canal (= female glandular canal), with the various modifications of the canal according to available space, and where the ovovitelline ducts may enter the glandular canal
    a) through a common ovovitelline duct (unite to form common duct) receiving few or no shell glands, or
    b) through a common opening (anastomose at the point of entry), or
    c) separately
1. With the distal parts of both ovovitelline ducts receiving shell glands, and with these glandular ducts fused to form a common female genital (glandular) duct that enters the female antrum.
2. With the distal parts of both ovovitelline ducts receiving shell glands, and with the mouths of both glandular canals sharing a common opening into the antrum.
3. With ovovitelline ducts separately directly entering the end part of the female antrum; with the terminal part of the ovovitelline duct altered and receiving shell glands, giving the effect of two glandular canals directly entering the antrum.

The first of these four states occurs in all three families of the Terricola, and all subfamilies (including the Anzoplaninae, this thesis) with the exception of the Geoplaninae. It also is present in the Maricola (Sluys 1989b). *Endeavouria* (Pacific) and *Pimea* (New Caledonia) both have state 1a, and this group also includes the majority of taxa from the NZ subantarctic islands. In *Timyma* (Chile) and *Nelipoplana* the blind end of the glandular canal is expanded ("ootype" of Froehlich 1978) and may act as a seminal receptacle into which the ovovitelline ducts separately enter. The first condition here possibly represents the plesiomorphic state, with the other states (1,2, and 3) apomorphic conditions. State 2 is present in a few geoplaninids, some microplaninids, especially South African species of *Othelosoma* and *Microplana* (see Jameson 1907), and in the caenoplaninid Bassioplana. States 3 and 4 occur only in geoplaninids.

***Character**: Caudal branching of ovovitelline duct.* The ovovitelline duct may divide caudally with one branch passing dorsomedially to enter the female canal, the other branch continuing posteriorly. This character, present in some Paludicolans and in a Cavernicolan (Sluys 1989a) is uncommon in the Terricola. It appears confined to species of *Kontikia* and *Parakontikia*, present as a closed loop (*Kontikia orana, Parakontikia coxii),* as a simple loop in the common ovovitelline duct (*Parakontikia melanochroa*) and as straight blind posterior ovovitelline duct (*Parakontikia ventrolineata*).

***Character**: Communication of ovovitelline duct with bursal canal.* Communication of the ovovitelline ducts with the bursal canal is complex in the Terricola (Sluys 2001 page 63). The ovovitelline ducts, separately or combined, may open into the antrum, or into structures that can be considered homologues of the bursal canal, viz. the female genital canal, the *canalis anonymous*, and Beauchamp's canal. This character and possible states, whilst potentially very useful, is not relevant to taxa considered in this study and therefore has not been further investigated here.

***Character**: Communication of ovovitelline duct with a genito-intestinal canal.* The genitointestinal canal occurs widely in several "turbellarian" orders and in monogeneans, and its origin is controversial (Gremigni 1983). It may connect with the gut, seminal or copulatory bursae, female glandular canal, or ovovitelline ducts. With respect to the bursa-intestinal duct, found in the Paludicola, Maricola and Terricola, Ball (1974) commented that it was of little phylogenetic significance and probably represents a recurrent ancestral character. The communication between the ovovitelline ducts and a genitointestinal canal is not relevant to taxa considered in this study and therefore has not been further investigated here.

4.11.8 Vitellaria

Vitelline cells are derived from neoblasts (Gremigni 1983), initially scattered throughout the parenchyma, aggregating to form follicles. In contrast to the Maricola, the vitellaria of Terricola are bound by a thin tunica with flattened nuclei, with the vitellocytes unbound within the follicle. The vitellocytes pass via fine vitelline tubules to enter the ovovitelline duct through vitelline funnels. At this point modified vitellocytes may have a sperm and yolk resorptive function, and they are termed resorptive vesicles (Sluys 1989c). Vitelline cells of freshwater triclads contain elements of a phenoloxidase system, yolk globules, lipid droplets, and glycogen (Gremigni 1983). Those of the rhynchodeminid *Platydemus manokwari* contain polyphenol oxidase, basic proteins, neutral carbohydrate, mono- and diphenols, diazo-reactive polyphenols, aromatic amines and lipids (Winsor 1998b), and are principally involved in the nutrition of the developing embryos; there appears little evidence of their direct participation in the development of the outer cocoon wall (Winsor 1998b).

The vitellaria do not appear to have been used as taxonomic characters in the Terricola. Two potentially useful characters provided by the vitellaria are location and abundance. Both characters are inter-related and can be influenced by the maturity of the specimen, as observed in specimens of *Caenoplana spenceri* collected at different times of the year (Dendy 1890).

***Character**: Vitellaria: location* (Fig. 4.19)

0. with vitellaria surrounding the diverticula of the gut and often filling all available space in the parenchyma; including the space in the parenchyma between the gut branches
1. with vitellaria below the gut, sometimes only between the testes.

Vitellaria may be present beyond the ovary and behind the copulatory organs. Exactly how vitelline cells anterior and posterior to the ovovitelline ducts enter the latter is uncertain. Fyfe (1937 page 112) considered that the vitelline follicles were interconnected, and transfer of mature vitellocytes largely achieved through general motion of the gut and body musculature. Given the wide occurrence of the first character state throughout all families and subfamilies, it is possibly the plesiomorphic condition, with the limited distribution of vitellaria below the gut possibly an apomorphic condition. In the Maricola the distribution of the vitellaria may be assessed with reference to the position of the testes (Sluys 1989b): vitellaria may be situated laterally or medially to the testes, or may occur at the same level as the testicular follicles. This character and states have not been used in the Terricola, and have not been explored in this thesis.

***Character**: Vitellaria: abundance*

The abundance of the vitellaria is a subjective assessment. They can be sparse, or abundant. Generally, but not always, these two states correspond to the location of the vitellaria – sparse in taxa with vitellaria below the gut (*Barringtoplana, Kynagoplana, Fyfea*), numerous in taxa with vitellaria between the gut diverticula (*Apogaioplana, Caenoplana, Nelipoplana (Nelipoplana), Tasmanoplana, Anzoplana*), and abundant in taxa where the vitellaria surround the gut (*Cotyloplana, Platydemus,* Rhynchodemus, *Arthurdendyus, Australoplana, Bassioplana, Coleocephalus, Fletchamia, Lenkunya, Nelipoplana Peokrypta, Reomkago, Sokoplana, Thosteelia, Bipalium kewense,* and present in geoplaninids, pelmatoplaninids and microplanids).

4.11.9 Female antrum

The female antrum is generally lined by a tall columnar cocoon-making epithelium. However species descriptions should be consulted for the many variations on this theme. A pattern repeated in a number of caenoplaninid taxa, for example in *Barringtoplana,* is for the dorsal half of the antrum to be lined by a cocoon-making epithelium, and the ventral half to be lined by a less specialized epithelium. An unusual multilayered antral lining, the function of which is unknown, is present in species of *Notogynaphallia* (Leal-Zanchet & Carabayo 2001). The secretions of the female antral epithelium of *Platydemus manokwari* (in Winsor 1998b) differ from that of the male antrum by the presence of proteins with amino, monophenol, and aromatic groups, and neutral carbohydrates. Di-and polyphenols are not as prevalent (Table 4.7). These substances are involved in cocoon-making.

The antrum may contain various flaps, folds, and small diverticula, in the sulci of which atrial glands may discharge secretions. Pronounced secretory ridges are present in the antrum of *Kynagoplana.* Narrow, elongate diverticula are present in *Australoplana* and *Apogaioplana* and though not differentiated histologically from the rest of the antrum may be relict ducts eg. genito-intestinal duct. These features are generally used to characterize species.

4.11.10 Female copulatory organs

The female copulatory organs and characters include the female copulatory apparatus profile, the female genital canal, and its position in the female antrum relative to other structures.

***Character**: Female copulatory apparatus sagittal profile (angle from horizontal)*

0. Female organs horizontal or slightly inclined

1. Female organs are vertical

2. Female organs are tilted anteriorly

This character has been used for analysis of the Bipaliidae (Ogren & Sluys 1998) for which it is particularly suited. Most of the taxa studied here have State (0), considered to be plesiomorphic. State (2) was originally "Female organ prolapsed or tilted forward". Here the term *prolapsed* has not been used as it normally refers to a pathological condition of female organs. Lengthwise contraction artefact may complicate interpretation of this character and states.

4.11.10.1 Female Genital Canal

Generally the female genital canal (= the vagina and glandular canal of Graff 1899 and others) is in-line with the female antrum. It is lined by a tall columnar epithelium heavily charged with brownish-erythrophil shell gland secretions. The genital canal is responsible for the secretions that form the inner membrane of the cocoon surrounding the embryos and associated vitelline cells. The shell glands, the secretions of which pass into the glandular duct, contain polyphenol oxidase, basic proteins, amino groups, monophenols, diphenols, polyphenols, aromatic amines and neutral carbohydrates. The secretions of the glandular duct epithelium contain acid phosphatase, leucine aminopeptidase, basic proteins, tryptophane, polyphenols, neutral carbohydrates, and glycosaminoglycans (Winsor 1998b). In *Australoplana* the glandular canal lies ventrally to the posterior strongly pleated extension of the female antrum; the epithelium in this region is the same as the rest of the female antrum. A similar antral extension, regarded as a bursa copulatrix (Riester 1938 page 10) is present in the geoplaninid *Notogynaphallia biseminalis*. The antral extension in *Australoplana* may be a structural relict of a bursa or bursal duct, though there is no histological evidence to support this view.

Accessory Genital Organs

Accessory genital organs include atrial diverticula that may be viscid glands, resorptive vesicles, copulatory bursae, lateral resorptive bursae, and musculoglandular organs.

4.11.10 Viscid gland

The viscid gland, previously known as the posterior atrial diverticulum (or in some cases uterus or atrial diverticulum) produces a sticky collagen-like fibrous glycoprotein secretion that cements the cocoon to the substratum (Winsor 1998b). The glands are characterised by a profuse coarse granular cyanophil secretion and fine granular erythrophil secretion. There are two useful viscid gland characters: presence / absence of the gland, and gland type.

***Character**: Viscid gland: presence*

0. Viscid gland in antral wall absent
1. Viscid gland present in antral wall

Viscid glands in the atrial wall are uncommon. They are present in the Australian region rhynchodeminids *Platydemus bivittatus, P. pindaudei, P. van-heurni,* and *P. victoriae*, and in *Bipalium pseudophallicum* from Sarawak. Examples of viscid gland diverticula, generally located on the posterior atrial wall below the female glandular canal, are found in the rhynchodeminid genera *Platydemus* and *Dolichoplana,* and in the caenoplaninid genera *Apogaioplana, Bassioplana, Nelipoplana, Reomkago, Sokoplana,* and *Tasmanoplana*. The viscid glands in these taxa are not necessarily homologous. In *Apogaioplana* and *Sokoplana,* the diverticulum is horizontal below the glandular canal, then bent dorsally passing to the left of the female genital canal. The diverticula of the other taxa are horizontal and below the female genital canal. In an undescribed species there is an indication of viscid glands on both anterior and posterior walls of the antrum just inside the gonopore. The accessory gland of *Artioposthia glandulosa* described by Fyfe (1956), and some of the musculo-parenchymatic glands and musculo-glandular organs of Maricola (Sluys 1989b) may also be viscid glands.

***Character**: Viscid gland type*

0. Viscid gland present a glandular patch on the antral wall
1. Viscid gland present as a diverticulum in the antral wall

On the basis of development of copulatory organs in *Platydemus* it has been suggested that the glandular patch is the plesiomorphic state, and the diverticulum the apomorphic state (Winsor 1998b).

4.11.11 Sperm resorptive organs

Sperm resorption in Triclads has been reviewed by Cernosvitov (1931; 1932) and Sluys (1989c). Sperm resorptive organs in the Paludicola and Maricola include lateral bursae and spermiduct, oviducal tubae and two other types of resorptive vesicle (Sluys, 1989b). Resorptive structures are characterised by the presence of large vacuolated phagocytic cells, and the presence of acid phosphatase and leucine amino peptidase below the epithelium (Jennings 1968). To these can be added the presence of acid phosphatase in the ovovitelline duct (Winsor, 1990), and presence of lysocytes - lysozyme-secreting cells found in resorptive bursae.

In many species of Terricola resorption of sperm occurs in the genital antrum (Winsor, 1998c), and in the intestine via the genito-intestinal duct as in *Microplana terrestris, M. scharffi* and *Incapora weyrauchi*, or possibly by the cutaneo-intestinal duct present in *Enterosyringia.* Specialized sperm-resorption organs found in Terricola include resorptive vesicles, copulatory bursae, seminal receptacles, lateral bursae, and tubae, together with various structures termed “uterus” by Graff (1899; 1912-1917; and others). These organs provide the character, with states present or absent.

4.11.11.1 Resorptive Vesicles

***Character**: Resorptive vesicles*

0. Resorptive vesicles absent
1. Resorptive vesicles present

The vitelline follicles of many freshwater and terrestrial flatworms have a dual function: the production of yolk, and also resorption of excess sperm. In some species the vitellaria have been completely altered to serve the latter function. The term *resorptive vesicle* has been coined for that part of the vitelline follicle involved with sperm resorption (Sluys 1989c), and four types of vesicle are identified in Terricola:

1. The ventral portion of the vitellaria, where they communicate with the ovovitelline duct, are devoid of vitelline cells, and comprise a few vacuolated cells which may form a distinct vesicle the lumen of which communicates with the ovovitelline duct. Sperm are destroyed in the lumen, and may also be phagocytosed (Cernosvitov 1931). This type of vesicle is found in *Kontikia orana* (in Froehlich 1955).

2. Vitelline follicles adapted solely for the resorption of sperm, opening into a complex of ovovitelline ducts in the region where the ducts ascend and join to form the common ovovitelline duct, occurring in *Kontikia orana* (in Froehlich 1955,Winsor 1986), in *K. whartoni* (in Beauchamp 1956), and in *K. insularis* (as a single large vesicle considered by Prudhoe (1949b) to be a seminal receptacle). There are similarities between this arrangement and that described by Sluys (1989b; 1990) in the paludicolan *Bdellasimilis barwicki.* In *Kontikia orana*, masses of excess sperm are extravasated into the parenchyma adjacent to openings in a posterior loop of the ovovitelline duct. The excess sperm are sequestrated and the masses gradually absorbed into the adjacent gut (Froehlich 1955 page 206).

3. Vesicles surrounding the ovovitelline duct along its length, present in the terricolan *Anzoplana* (this thesis). The vesicles are lined by a few cells with pale cyanophil cytoplasm, and the lumen is vacuolate or contains sparse pale cyanophil mostly amorphous material. Some vesicles contain what appears to be very pale-staining sperm. Another vesicle contained an oocyte. Each vesicle communicates with the

ovovitelline duct via a short ciliated duct. Normal vitellaria are in close proximity to these vesicles and probably discharge into the ovovitelline duct via the vesicle duct. This type of vesicle has not previously been found in Terricola. However a similar type of resorptive vesicle occurs in the paludicolans *Dendrocoelum maculatum* and *D. adenodactylosum*, both endemic to the Yugoslavian Lake Ohrid and surroundings (Sluys 1989c).

4. Lateral diverticula arising from the ovovitelline ducts. In *Bipalium diana* six pairs of small, branched, lateral diverticula arise in the ovovitelline ducts between the end of the pharynx and female copulatory organs. Moseley (1876) considered the histogenesis of these diverticula to be "rudiments of the branched ovary possessed by lower planarians such as *Dendrocoelum lacteum*". There are similarities between these diverticula (Moseley 1876, Plate 13, Fig. 9), which may have a resorptive function, and the vesicles previously described in *Anzoplana*.

4.11.11.2 Copulatory bursa

Character: *Copulatory bursa*

0. Copulatory bursa present

1. Copulatory bursa absent

In the Terricola copulatory bursae are all located posterior to the penis. They are found in species of all three families.

Within the Geoplanidae copulatory bursae may arise from a lateral atrial wall, as does the saccate bursa in *Pimea monticola* and in *Beauchampius,* or more commonly from the atrial walls dorsal or dorso-posterior to the female genital canal as in species of *Artioposthia, Othelosoma, Beauchampius,* and *Rhynchodemus*. These copulatory bursae are generally lined by tall, non-ciliated vacuolate cells with a demonstrated resorptive function (Cernosvitov 1931).

In the copulatory bursae arising as a continuation of the female genital canal, present in microplaninids such as *Microplana terrestris*, the bursal stalk and bursa are generally lined by ciliated, non-glandular cuboidal epithelium, and where there is a genito-intestinal connection, there is a transition from the ciliated cuboidal epithelium to non-ciliated columnar gastrodermis (Ogren 1984).

Muscular sacculate copulatory bursae occur in the Bipaliidae. In *Bipalium penrisse* the bursa arises from the lower left antrum immediately adjacent to the opening of the female glandular canal. The bursa is situated dorso-laterally to the female canal, and is lined by a low cyanophil epithelium (Beauchamp 1926). A similar situation exists in *Bipalium sudzuki*. Here the bursa arises from the lower third of the female genital canal, and is situated to the mid left of the canal. The tall nucleate epithelium receives weakly erythrophil secretions (Kawakatsu 1986). Copulatory bursae may also occur below the female genital canal as posterior atrial diverticula.

4.11.11.3 Posterior antral bursa

*Character: **Posterior antral bursa***

0. Posterior antral bursa absent

1. Posterior antral bursa present

A posterior antral bursa, or diverticulum, is essentially an invagination of the atrial wall and contains most of the tissue elements of the antrum. These diverticula differ histologically from other copulatory bursae in having a ciliated epithelium. The diverticulum is lined by a ciliated cuboidal epithelium with scattered cyanophil glands (Prudhoe 1949b; Froehlich 1955; Beauchamp 1956). Examination of the antral diverticulum of *K. orana* (this thesis) reveals that it receives the secretions of cyanophil glands and xanthophil glands located in the surrounding parenchyma. It is lined by ciliated cuboidal cells, some cilia of which, from their peripheral distribution around the lumenal cell margin, are probably stereocilia (giant microvilli) with an absorptive function. The presence of non-motile stereocilia in atrial epithelia has been suggested previously (Winsor 1998b). Also present are cuboidal cells with vacuolate epithelium.

The presence of these tissue elements strongly supports intra-lumenal digestion of sperm and a resorptive function for these posterior atrial diverticula.

Posterior antral diverticula with an observed sperm receptacle function and presumed associated resorptive function occur in *Kontikia nasuta, K. orana, K. whartoni, K. sp. (Samoa)*, and *K. insularis*. In the latter there is a clear anastomosis with the ends of the posterior branches of the intestine (Prudhoe 1949b) that would facilitate intestinal resorption of excess copulatory products. A sub-terminal or terminal cutaneo-diverticular opening is present in some specimens of K. *whartoni* (in Graff, 1899 page 235, and Beauchamp, 1956), though there is some uncertainty as to whether this is a natural structure or simply a damaged specimen.

4.11.11.4 Lateral bursa

***Character**: Lateral bursa*

0. Lateral bursa absent
1. Lateral bursa present

Lateral bursae, all arising from the female genital organs, occur in Australo-Pacific taxa. All bursae have a similar internal structure comprising thin, irregular arborizing folds that may fuse to appear honeycomb-like. Lysocytes and phagocytes are present, and sperm are digested intraluminaly.

Three types of lateral bursae are recognized:

1. Lateral bursae embedded in the atrial musculature; the short bursal stalks of which arise at the entrance of the female genital canal in *Australopacifica willeyi* (Busson 1903) which has anterior-facing, inequi-bilobed, bilateral bursae;
2. Lateral bursae situated either side of the pharynx; the short bursal stalks arise from the ovovitelline ducts. Present in *Anzoplana.*
3. Lateral bursae situated at the proximal end of the oviducts and ventrolateral to the ovaries. Present in species of *Arthurdendyus* (*triangulatus, vegrandis)* and subantarctic taxa. The bursae in A. *triangulatus, "Kontikia" marrineri, "Kontikia" ashleyi* and *Fyfea carnleyi* were previously regarded as "parovarian tissue" (Fyfe 1937; 1953).

4.11.12 Musculoglandular organs (MGOs)

The term musculoglandular organs as used here include adenodactyli (finger shaped, referred to here as adenodactyls), adenochires (hand-like), pyriform glands, glandular papillae, and adenomuralia that are adenodactyl-like structures largely embedded in the atrial wall. Characters provided by musculoglandular organs are presence or absence, and type of MGO. At species level the number and type of MGOs is important.

***Character**: Musculoglandular organs: presence*

0. Musculoglandular organs absent in copulatory organs
1. Musculoglandular organs present in the copulatory organs

Musculoglandular organs are found in the Paludicola, Maricola, and in all three families of the Terricola. Musculoglandular organs are uncommon, and are absent in the majority of taxa (plesiomorphic). I concur with Meixner's (1928) view that they are acquired (apomorphic).

Adenodactyli were first discovered in the Paludicola, where their functions were variously regarded as contributing in the development of the cocoon shell, the secretion of slime thread, attachment of the cocoons to the substratum, stimulatory organs in reproduction, and a seminal receptacle or a copulatory bursa (Graff 1899). In erecting the genus *Artioposthia* (Geoplanidae) to accommodate Terricola bearing adenodactyli in the copulatory apparatus, Graff (1899) was no nearer to establishing the function of these enigmatic structures. In addition to the foregoing possibilities he considered that they might augment the slime produced by the creeping sole, or augment pharyngeal glands by producing a toxic secretion for prey capture. He also conjectured that adenochires interlocked to provide a firm support for some particular copulatory action possibly facilitated by their glandular secretions.

Fyfe (1937) in considering the adenodactyli of *Artioposthia triangulata* stated that "one can only conjecture from the small size of the penis that the adenodactyli with their great muscularity do in some way supplement the function of the penis." She later stated (Fyfe 1944) "The reservoir, duct and glands of adenodactyli are related to cocoon formation" and suggested that the intramural accessory glands (adenomuralia) of

Newzealandia graffii and other species had the same function. Unfortunately Fyfe did not give reasons or provide evidence for her insight. Recent investigations of *Coleocephalus fuscus* and *Artioposthia* species confirm that the adenodactyli, at least in some species, are partly responsible for the development of the cocoon outer wall (Winsor 1998b).

4.11.12.1 Types and functions of musculoglandular organs

Whether all adenodactyli are cocoon-forming has not been yet established. Unfortunately most descriptions of adenodactyli lack detail of their cytology necessary to elucidate function. Some seven types of adenodactyli have been recognised (Table 4.9; Fig. 4.20). The number of adenodactyli in a particular species is constant and is not seasonal (Fyfe 1944; 1956).

A species may have more than one type of MGO present in the copulatory organs. This applies not only to the form of the adenodactyl, but also the nature of the secretions. For example in *Coleocephalus fuscus* protein is secreted by some adenodactyls, and a glycoprotein by others. It may be that some secretions have an adhesive function, suggested by the protrusion during copulation of the adenomuralia in *Newzealandia moseleyi*, though this latter phenomenon during copulation may simply represent an anatomical rather than a functional situation. The adenomuralia of *Newzealandia graffii* appear to have a cocoon-forming function, with the reservoir and duct providing one sclerotin element, and the muscular vesicle secreting the sclerotin strands, possibly mistakenly identified as sperm by Fyfe (1944).

The genus *Artioposthia* is heterogeneous (Winsor 1991c) and undoubtedly musculoglandular organs have arisen separately in a number of taxa. The multiple discrete glands in the atria of *Artioposthia hamiltoni, Geoplana multipunctata, Pelmatoplana glandulosa* and *Bipalium graffi*, and the pyriform glands in the penis papillae of *Gigantea idaia, G. montana, G. picadoi*, *G. sandersoni*, and *Geoplana vongunteri* and in the antrum of *Pasipha ercilla* are similar to adenodactyli embedded in adenochires in some *Artioposthia* species and may well have a cocoon-forming role. From the description of the secretions of the glandular papillae in *Gigantea idaia* (in Du Bois-Reymond Marcus 1951) these are probably cocoon wall-forming glands. Glandulomuscular organs are also found in the Bipaliidae, and the organs in *B. rigaudi*

Table 4.9

A Classification of Musculoglandular Organs

Cone-shaped muscular glandular organs, generally embedded in an antral flap or wall, or penis

Type I	Small conical strongly muscular organs, with short central canal lined by a columnar epithelium with basal nuclei. The margin of the epithelium is charged with strongly erythrophilic granules; cyanophilic granules present in the musculature of the MGO are secreted through the epithelium as sclerotin strands; large globular masses of erythrophilic material, secreted by unicellular glands within the surrounding muscle of the organ, pass through the duct epithelium into the lumen. Secretions form the cocoon outer wall. The single or multiple *drüsenkegel* of von Graff (1899). Typically present on muscular antral flaps termed **adenochiren**, as in *Artioposthia diemenensis* (in von Graff 1899).
Type II	Small glandular papillae, with short central canal (duct) of lamellar cytoplasm the nuclei of which lie at the base of the canal as a bud-like group; the canal receives cyanophilic secretions from extra-bulbar glands; erythrophil glands are situated around the central canal, and open onto the surface of the papilla. The pyriform glands of Prudhoe (1949) and glandular papillae of Du Bois-Reymond Marcus (1951) present on the penis papilla in species of *Gigantea.* Secretions are possibly related to formation of the cocoon outer wall.
Type III	Small spherical weakly muscularised organs with a short simple canal almost entirely surrounded by glandular tissue which secretes erythrophilic granules. The *adenocysten* of Müller (1902), present in the inner antral wall in *Bipalium graffi, B. böhmigi, Geoplana multipunctata* and *Bipalium glandulosa.* The functions of adenocysts are uncertain. It is probable that in some species these structures are viscid glands the secretions of which adhere the cocoon to the substratum.

Adenodactyli, strongly muscularised glandular organs which mostly project into the antral lumen. Function related to formation of cocoon outer wall.

In general the erythrophilic granules are derived from glands external to the MGO and are abundant in tissues of the proximal end of the organ. Cyanophilic granules are generally secreted into the proximal duct of the MGO. It is uncertain whether Typ23es IV and V have cyanophilic secretions.

Type IV	Simple weakly muscularised duct; without proximal reservoir; with glandular cavity near duct papilla, as in *Artioposthia howesi* (in Fyfe 1944).
Type V	Simple weakly muscularised duct; with proximal reservoir the same or larger diameter than the duct lumen, as in *Artioposthia subquadrangulata* (in Fyfe 1946).
Type VI	Strongly muscularised duct, with distinct proximal reservoir the diameter of which is greater than that of the duct, as in *Artioposthia exulans*, (in Fyfe 1946).

Adenomuralia, strongly muscularised glandular organs which are recessed within the antral wall. Function related to formation of cocoon outer wall.

Type VII	Muscular duct often with valves or sheath; with proximal reservoir the diameter of which is greater than that the duct. Duct opens into an antrum which is within the adenomuralium musculature, as in *Newzealandia graffii* (in Fyfe 1944), and *Bipalium rigaudi* (in Beauchamp, 1939).

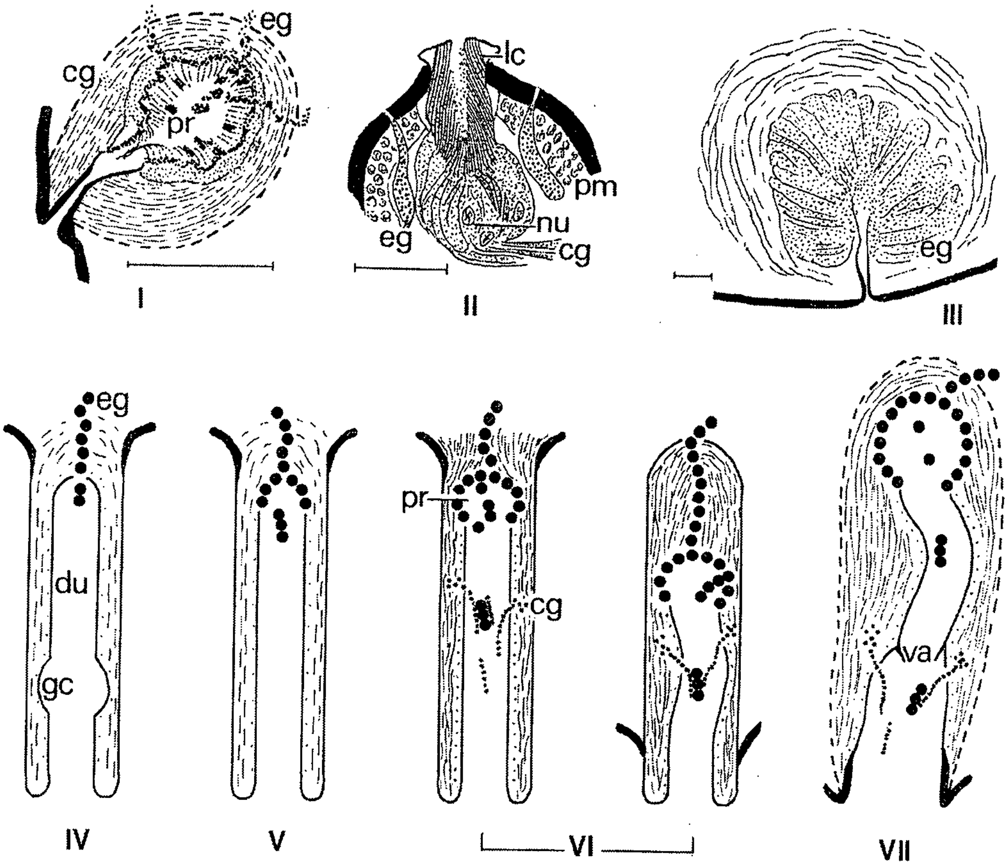


Figure 4.20. Types I – VII of musculoglandular organs (MGOs) in terrestrial flatworms. Refer to Table 4.9 for descriptions.

Types I – III: Conical musculoglandular organs, generally embedded in antral structures. Type I. Drüsenkegel – type of MGO on the adenochire of *Artioposthia lucasi*; Type II. Glandular papilla-type of MGO on the penis of *Gigantea idaia* (redrawn from E. Du B-R Marcus, 1951); Type III. Adenocysten- type MGO on the genital pad of *Bipalium graffi* (redrawn from Müller, 1907).

Types IV-VII. Adenodactyli and adenomuralia musculoglandular organs (diagrammatic). The abundance of erythrophil and cyanophil granules present in the MGOs has been reduced for clarity.

Abbreviations: cg- cyanophil granules, du- duct, eg- erythrophil granules, gc- glandular cavity, lc- laminate cytoplasm, nu-nucleus, pm- penial musculature, pr- proximal reservoir, va-valves. Scale bars in figures I, II and III represent 25µm. The other diagrams are not to scale.

appear close to the Type I MGOs found in some *Artioposthia* species. However given the number, complexity and arrangement of musculoglandular organs in a wide range of taxa it is uncertain whether all these structures are homologous or analogous.

SECTION C: COCOON FORMATION AND DEVELOPMENT

4.12 COCOON FORMATION

Cocoon formation in the Terricola has received little attention, apart from noting the presence of cocoons in the copulatory organs in a few taxa (as in Graff 1899). Regarding the development of cocoons in triclads, based on observations of Paludicola, Hyman (1951) states that the *eggs and yolk collect in the male antrum where a capsule (also called a cocoon) is formed around them from droplets present in the yolk cells.* Studies on the rhynchodeminid *Platydemus manokwari* showed that cocoon formation differs from this process, and involves three main stages (Winsor 1990; 1998b; Fig.4.21):

4.12.1 Mechanism of cocoon formation in non-adenodactylate taxa

Stage 1. *In female genital canal: formation of a very thin inner cocoon membrane.*

Fertilised ova intermixed with vitelline cells migrate together along the ovovitelline duct into the female genital canal. Here they are enclosed within a viscid membrane formed by shell gland secretions. The pre-cocoon mass then passes into the female antrum.

Stage 2. *In the female antrum: formation of the outer cocoon.*

The stage broadly follows the mechanism of sclerotin formation shown in Fig. 4.2.1. It requires three interrelated steps:

(a) apocrine secretion by erythrophil atrial cells (globule cells) of globules containing an amino acid-rich protein together with polyphenol oxidase. The protein is probably derived from tryptophane-containing granules localised below the atrial basement membrane. The polyphenol oxidase or its precursor is identified as fine erythrophilic granules derived from glands scattered throughout the atrial musculature. The

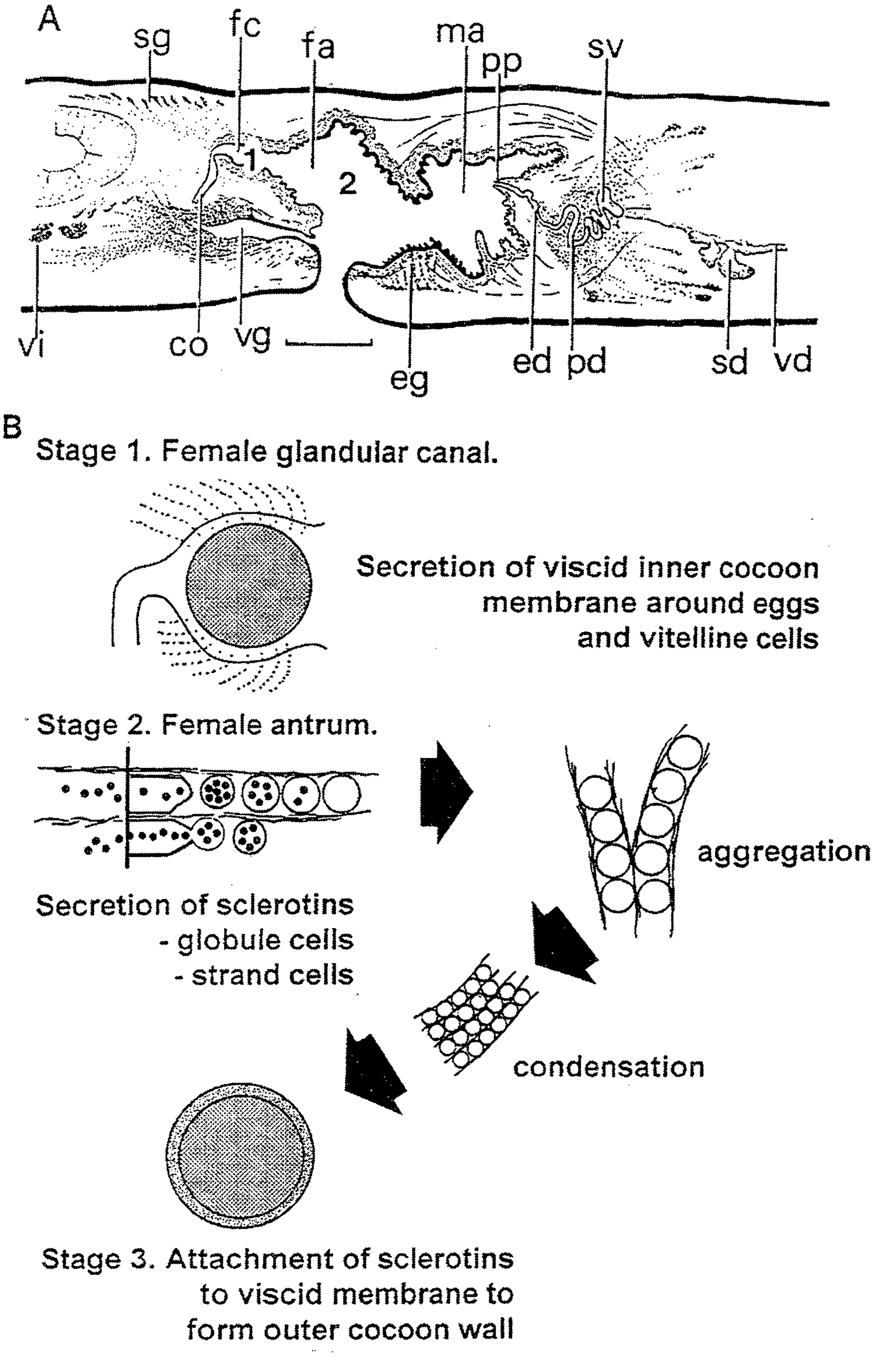


Figure 4.21 *Platydemus manokwari.* A schematic representation of the copulatory organs (A) and mechanism (B) for the formation of the inner membrane and outer cocoon wall. co - common ovovitelline duct, ed - ejaculatory duct, eg - erythrophilic glands, fa - female antrum, fc - female glandular (genital)canal, ma - male antrum, pd - prostatic duct, pp - penis papilla, sd - sperimducal vesicle, sg - shell glands, sv - seminal vesicle, vd - vas deferens, vg - viscid gland, vi - vitellaria. Scale bar 500μm. 1, 2 - sites of stages 1 and 2 of cocoon formation.

protein and enzyme react within the membrane-bound globules to form a sclerotin, indicated by the loss of the fine granules and chromophobicity of the globule

(b) at the same time, either side of the atrial cell, phenolic proteins are secreted from insunk cyanophilic glands (strand cells) situated within the atrial musculature. Secretions enter the antrum via elongate cell necks passing between the atrial cells. This protein is acted on by polyphenol oxidase present along the luminal margin of the atrial epithelium to form sclerotin strands. The sclerotin strands immediately aggregate with the sclerotin globules to form a sheet. At this stage the sheet resembles plastic "bubble" packaging material.

(c) The aggregated mass condenses and thins, possibly indicative of initial cross-linking and stabilisation. Whether this step is mediated by enzyme activity has yet to be determined.

Stage 3. *In female antrum: sclerotin sheet attaches to and covers the pre-cocoon mass.*

The exact assembly mechanism for this step is uncertain. The inner membrane is probably viscid. Attraction between outer wall components and the inner membrane may be due to hydrophobicity of the constituent proteins with subsequent aggregation.

The completed cocoon is then expelled through the gonopore together with secretions from the viscid gland that cement the cocoon to the substratum. In some species such as *Lenkunya munda* the cocoon is expelled through the dorsal body wall. In this situation the number of cocoons laid by an individual are indicated by repairs to the dorsal body wall, manifested as transverse dislocations (faults) in the longitudinal dorsal stripes.

4.12.2 Adenodactyli and cocoon-wall formation

The cocoon wall requires at least two major components: sclerotin globules and sclerotin strands (Winsor 1990; 1998b). The antrum of *C. fuscus* and some *Artioposthia* species lacks the apocrine secretions normally associated with the sclerotin globules. However it does have the sclerotin strand secretions. The adenodactyli produce a sclerotin protein - initially in erythrophilic globules formed in the adenodactyl reservoir through apocrine secretion from nucleate columnar cells. The fine secretion is derived from glands in the stroma outside the adenodactyl. Coarse xanthophilic proteinaceous

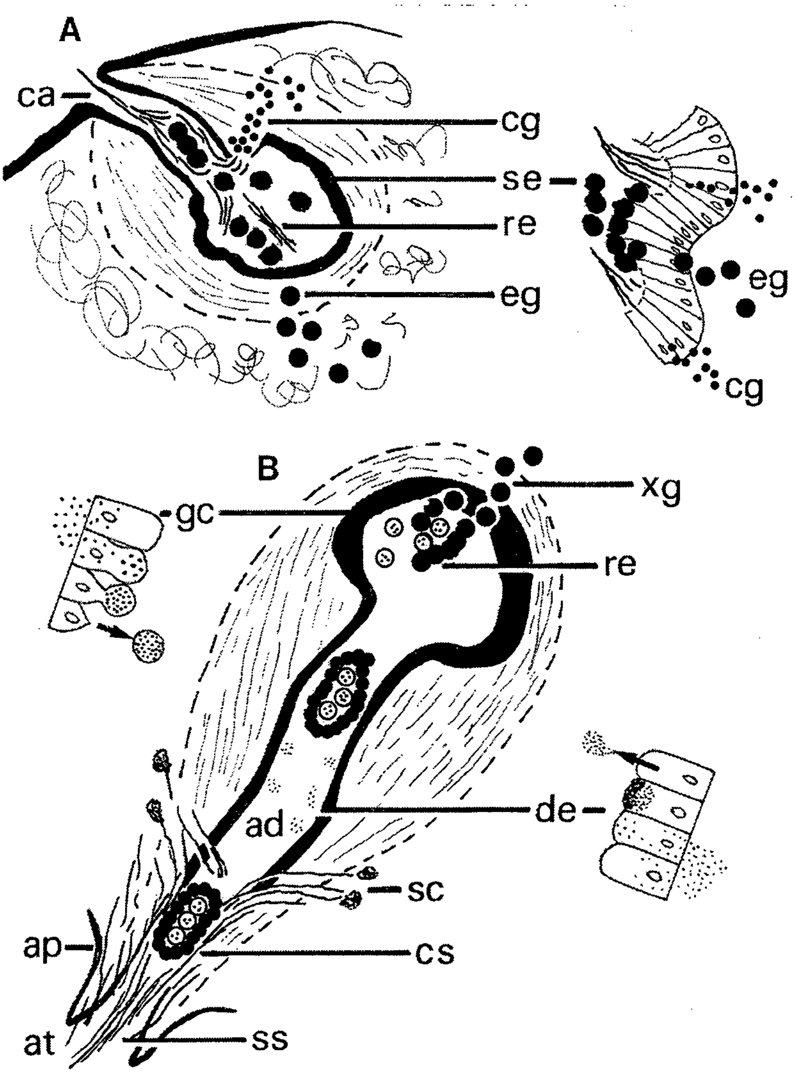


Figure 4.22 A schematic representation of the mechanisms of sclerotin formation in two types of musculoglandular organs.

A. *Artioposthia lucasi.* Type I musculoglandular organ on adenochire. ca - canal, cg - cyanophilic strand cell granules, eg - erythrophilic granules, re - reservoir with sclerotin strands and coarse sclerotin granules, se - secretory epithelium.

B. *Coleocephalus fuscus* Type VI musculoglandular organ (adenodactyl). ad - adenodactyl duct, ap - adenodactyl papilla, at - atrium, cs - cyanophilic sclerotin strands surrounding sclerotin from reservoir, de - duct epithelium, gc - globule cell epithelium, re - adenodactyl reservoir, sc - sclerotin strand cells in surrounding tissue, ss - sclerotin strands, xg - xanthophilic granules from extrabulbar glands.

granules in the reservoir and proximal duct coat the pale cyanophilic globules containing erythrophilic granules. This secretion derives from glands adjacent to the copulatory apparatus and pass through the adenodactyl wall into the reservoir. As the combined mass passes down the adenodactyl duct it is further coated by fine erythrophilic secretions from the duct, and appears laminar in form (Fig.4.22).

The sclerotin mass enters the papillial duct of the adenodactyl and is coated by the sclerotin strands, then passes into the antrum. The globules are intact in the layered sclerotin. At a later stage they disappear leaving small vesicles in the mass, possibly the condensation phase. In contrast to the sclerotin globules in *P. manokwari*, these globules do not become chromophobic.

4.12.3 Sclerotin sourced from adenodactyli vs. sclerotin from the atrial epithelium

The presence of adenodactyli in the copulatory apparatus may also mean a concomitant reduction or absence of cocoon-forming cells and tissues in the antrum, apparent in *Coleocephalus fuscus*, *Artioposthia* species and in *Pasipha ercilla* where the atrial wall is lined by a flat non-glandular epithelium (Froehlich 1978). Comparing adenodactyli in *Coleocephalus fuscus*, *Artioposthia howitti*, and *A. lucasi* it appears that different components and cocoon wall-forming mechanisms may be involved in each species. In *Artioposthia lucasi* adenodactyli on the adenochires (Plate 4.2) and the atrial epithelium adjacent to the female glandular duct both appear to secrete cocoon-wall elements, and formed laminate is present in the lumen of adenodactyli and antrum. In this species sclerotins from both sources (adenodactyli and antrum) combine, as suggested by discontinuities in areas of cocoon wall laminate.

Adenodactyls are apomorphic structures. If they represent essentially a sequestered atrial secretory epithelium, then duplication of function with the residual atrial epithelium is to be expected. The cytology and nature of secretions in each structure will show differences. Detailed histological and histochemical studies of musculoglandular organs may clarify their functions. The configuration and morphology of adenodactyli in particular taxa may be associated with the need for an overall architecture to the cocoon wall laminate - that is, how the laminate is arranged to form a spheroidal cocoon.

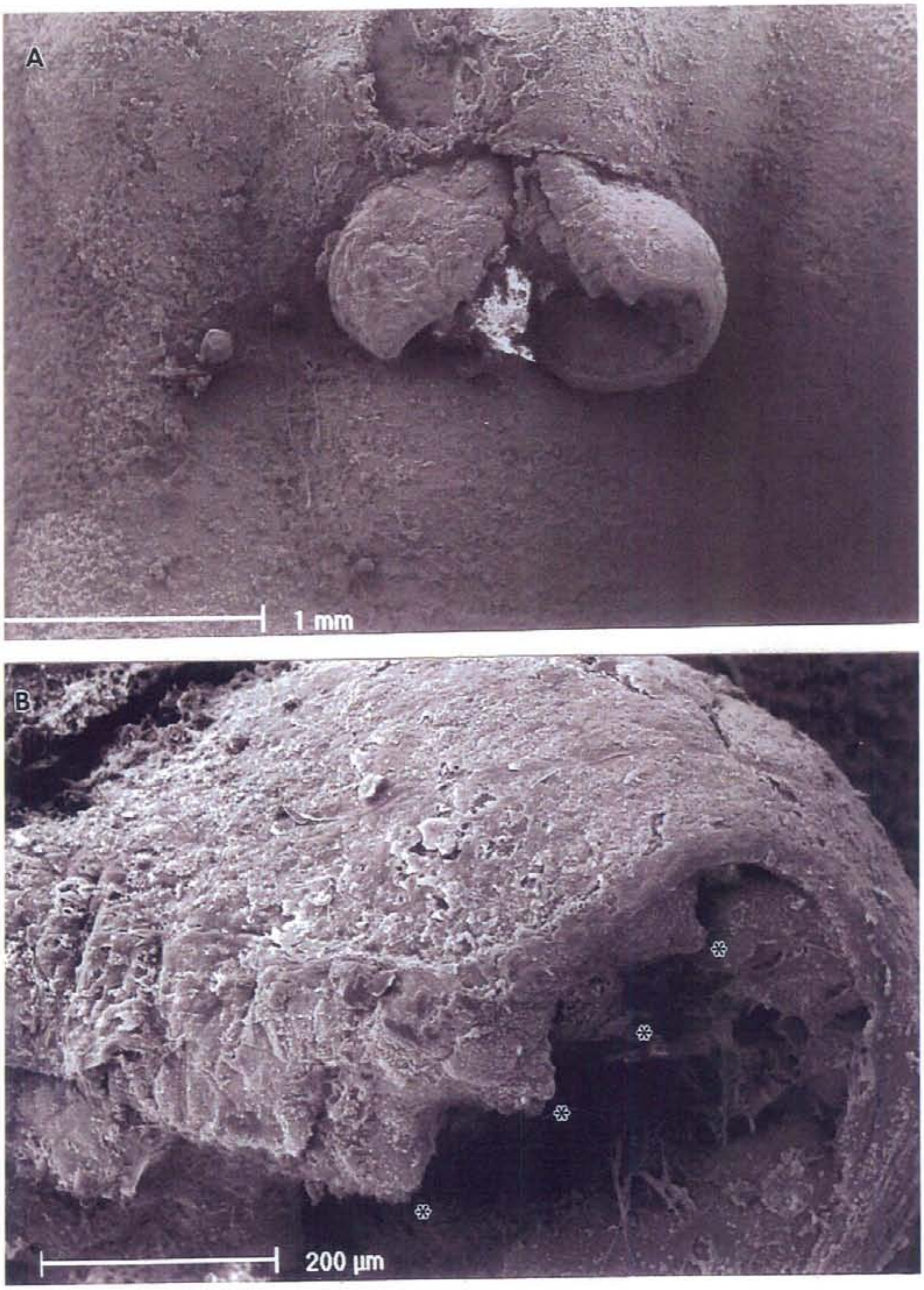


Plate 4.2. *Artioposthia lucasi*, SEM (a) Ventral surface showing the adenochires protruding from the gonopore (b) The left adenochire showing the papillae of the embedded Type I adenodactyli (*).

The site of cocoon formation may vary between taxa, suggested by the presence of cocoon-forming elements in both male or female antra, and both, and the presence of MGOs in either male or female antra, or both.

4.12.4 Stabilisation of the cocoon wall

In *Platydemus manokwari* the cocoon sclerotin continues to stabilise through a quinnone or other tanning mechanism, indicated by colour changes over a few days from a lemon yellow, tan, to dark brown and black. Elements of the phenol-oxidase pathway responsible for the tanning process have been identified in both vitellocytes and in antral cocoon-making secretions (Winsor 1998b). Cocoon colour changes during sclerotin stabilisation have been described in all families of the Terricola. Quinone-tanned cocoons have also been described in Paludicola (Nurse 1950; Jenkins and Brown 1963; Yanagita and Yamamoto 1981) and in Maricola (Sluys 1989b). The involvement of phenol oxidase, di and polyphenols, and proteins in cocoon formation in *Platydemus manokwari* suggests that sclerotin stabilisation is achieved by a quinnone tanning mechanism. Stabilisation of the tryptophane-based cocoon wall proteins might also follow the tryptophane - kynurein - hydroxykynurenin -hydroxyanthranilic acid (sclerotizing precursor) pathway. The cocoon wall gave a positive reaction for diphenols only.

Tryptophane was the only amino acid to be fully identified histochemically in the copulatory apparatus of *Platydemus manokwari* (in Winsor 1990) and is most likely to be a component of the cocoon wall sclerotins. It has the highest hydrophobicity index of those listed by Waite (1983). This property causes components containing tryptophane to aggregate in aqueous environments and facilitates subsequent cross-linking and stabilisation of the cocoon wall. The hydrophobicity of tryptophane may ensure minimal moisture loss from cocoons after deposition onto the substratum. This presence of this amino acid may therefore be an adaptation to a terrestrial life. Support for this view comes from the amino acid analysis of the cocoon walls of *Dendocoelopis* and *Fasciola* (Yanagita and Yamamoto 1981). These taxa live in an aqueous environment and the cocoon is not a problem; tryptophane is absent in their cocoon walls.

4.12.5 Role of vitelline cell products

Within the cocoon vitelline cells are attached to the inside of the membrane as a distinct layer. There is no evidence to suggest their direct participation in the development of the outer cocoon wall as in the Paludicola (Burr 1912; Toedtmann 1913), or obvious change in the inner membrane. However frail thin-walled structures resembling cross sections of micro-cocoons were noted within the cocoon mass. This may be evidence of a relict mechanism of cocoon formation by self-assembly of components derived mainly from vitelline cells. Phenol oxidase present in the vitellocytes may participate in the cocoon tanning process.

4.12.6 Cocoon wall laminate

The outer cocoon wall is a fibrous composite (laminate) of at least two scleroproteins with apparent parallel orientation at right angles to the inner cocoon wall stabilised by tanning (Plate 4.3). The outer cocoon wall exhibits form birefringence under polarised light, and structurally is a Wiener composite body as defined by Neville (1993). The architecture of the outer wall composite over the cocoon inner layer has yet to be ascertained. Examination of the translucent outer cocoon wall of *Artioposthia lucasi* by incident light microscopy reveals areas with coarse corrugations interpreted as adenodactyl marks, and a fine whorled thumbprint-like pattern suggestive of a mono domain helicoid architecture in the wall composite. This contrasts to the smooth cocoon wall of *Caenoplana hoggii,* and slightly rugose cocoon wall of *Platydemus manokwari,* the architectural patterns in which are unclear. Ultrastructural studies of cocoon walls should resolve these uncertainties, and may provide data or characters that will assist in the classification of terricolans.

4.12.7 The Triclad model of cocoon formation

The cocoon formation in *Platydemus manokwari* (in Winsor 1990; 1998c) differs from observations of the process in Paludicola, tacitly assumed in the literature to apply to both Maricola (Sluys 1989a) and the Terricola (Hyman 1951). Both mechanisms share the formation of the pre-cocoon membrane (Stage 1 previously). In the Terricola the cocoon wall is assembled from atrial cell secretions that form a

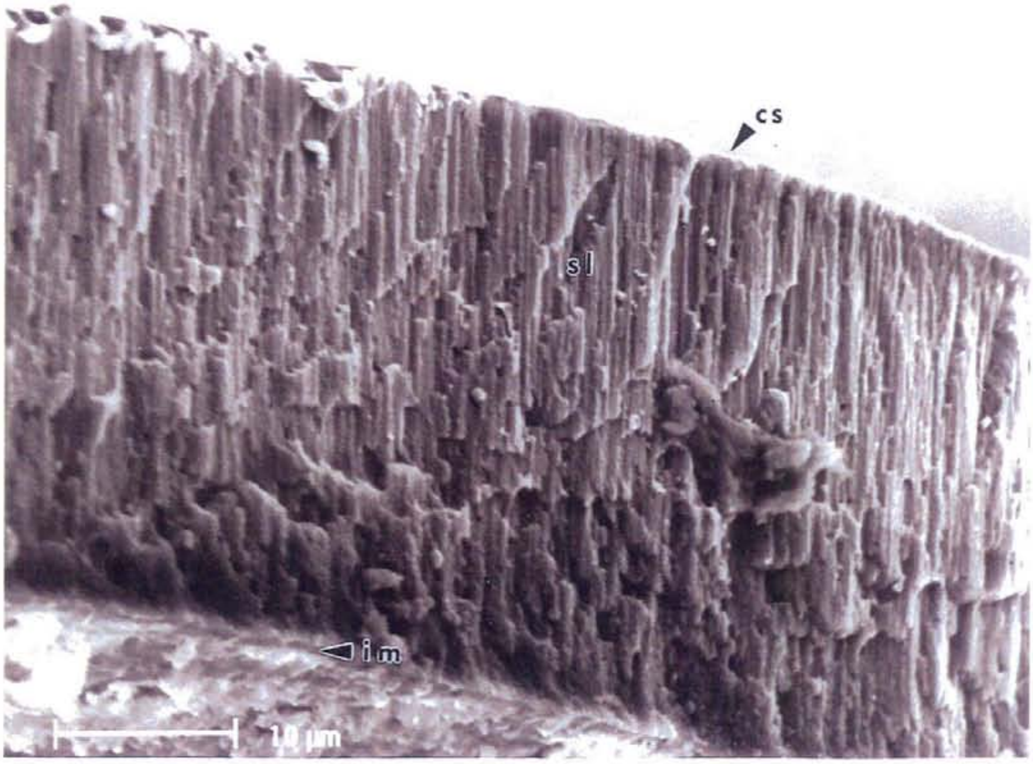


Plate 4.3 *Artioposthia lucasi*, SEM. Cocoon wall showing detail of the sclerotin laminate. cs- cocoon outer surface, sl - sclerotin laminate, im - inner membrane.

sclerotin composite on the *outer* surface of the pre-cocoon capsule. In the Paludicola the cocoon wall is formed from *within* the capsule largely by the shell-globule component of the numerous vitellogenic cells present (Burr 1912; Toedtmannn 1913; Yanagita and Yamamoto 1981). However an alternative interpretation of evidence presented by Yanagita and Yamamoto (1981) in their description of cocoon formation in *Dendrocoelopsis ezensis* (Paludicola) suggests that the mechanism operating in this species in fact follows the Terricola model (Winsor 1998b). At present it is uncertain to what extent mechanisms of cocoon formation in triclads reflect phylogenetic relationships.