THE CANE TOAD: A NEW HOST FOR HELMINTH PARASITES IN AUSTRALIA

Thesis submitted by

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Abstract

The helminth fauna of native Australian amphibians and the introduced toad, *Bufo marinus* was studied. Species composition and ecological relationships of the helminths were considered in detail. In addition, the relationship of one helminth species, *Rhabdias* sp., to the health of the toad was considered.

A total of 27 helminth species (14 Nematoda, 8 Digenea, 2 Cestoda, 2 Acanthocephala, 1 Monogenea) was collected from both the toad and native amphibians in this study. Six helminth species were found to only infect toads in this study: *Dolichosaccus juvenilis*, *Zeylanurotrema spearei*, *Cosmocerca* sp. 2, *Cosmocerca* sp. 3, *Austraplectana* sp., adult acanthocephalans. Two of these species (*D. juvenilis* and adult acanthocephalans) had been reported from native fauna in previous studies. Three species were found to infect only native amphibians in this study: *Parapolystoma* sp., *Seuratascaris numidica*, and Onchocercidae gen. sp.

All of the helminth species collected from *B. marinus* in this study, with the possible exception of *Rhabdias* sp. and *Mesocoelium* sp. for taxonomic reasons, can be determined as having an Australian origin. The majority were acquired by the toad from native amphibians. Some species, however, were thought to have transferred to the toad from native reptiles.

At least 70% of toads and native frogs were infected with at least one helminth species. Maximum number of helminth species for an individual toad was 6, whereas for native frogs it was 4.

Bufo marinus had a more diverse helminth community than native frogs at both a host individual and host population level. The use of diversity indices in helminth community ecology and the concept of core and satellite species, particularly in relation to amphibian helminth communities, is discussed.

Comparison of the helminth fauna of *B. marinus* and a native frog, *Litoria inermis*, was undertaken in detail. Relationships of total helminth intensity and species richness to various factors, including host sex and snout-vent length and month of collection were calculated for both host species. Reasons for the possible disparity between helminth infection levels for *B. marinus* and *Lit. inermis* are discussed.

Only one helminth species, *Rhabdias* sp., was thought to have potential as a biological control agent for the toad in Australia. Detailed studies of the life cycle of *Rhabdias* sp., natural infection levels within a population of *B. marinus* and its relationship with the health of the toad were undertaken.

Rhabdias sp. infected over 80% of toads collected from QDPI, with a mean intensity of 16 nematodes per infected toad. Intensity of infection had a significant relationship with length of toad for subadult toads only. Average length of *Rhabdias* sp. within an infrapopulation had a significant relationship to host length for subadult and middle size class toads.

Distribution of *Rhabdias* sp. within the toad population was aggregated, with degree of aggregation increasing with toad size class.

Sex of toad had a significant relationship with average length of *Rhabdias* sp. only in Class II toads, where male toads had larger nematodes.

Rainfall was an important environmental factor influencing infection of toads with *Rhabdias* sp. The majority of *Rhabdias* sp. recruitment into the toad population occurred during the late wet season, although small amounts of recruitment occurred throughout the year.

Development of *Rhabdias* sp. from embryonated egg to infective third stage larva, in the laboratory, took 4 days at 24°C. Development was only observed via a free-

living sexual cycle, with only one larva produced per free-living female.

Experimental infections were hampered by a high death rate among the metamorph *B. marinus* and *Limnodynastes ornatus* used. Over 50% of metamorphs exposed to infective larvae of *Rhabdias* sp. became infected. Number of larvae penetrating the metamorph was significantly related to the success of infection. Lower infection dosages produced proportionately higher levels of infection.

Haematological data for *B. marinus* in Australia is presented for the first time. Presence of a *Rhabdias* sp. infection significantly decreased levels of red blood cells, packed cell volume and haemoglobin concentration. Level of *Rhabdias* sp. infection also significantly decreased these levels, but not to the same extent as presence of *Rhabdias* sp. alone.

Declaration

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

Diane P. Barton February 1995

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Diane P. Barton February 1995

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Chapter 1: Introduction and Aims

1.1 Literature Review

1.1.1 Introduction of a New Host

Introducing an animal into an ecosystem exposes it to the many selective pressures of that system, including adaptation to a new physical environment and a new complex of species (Weir 1977). The introduced animal also exposes the system to which it is introduced to new selective pressures. Exposure of new and resident host species to their respective parasites is one of these selective pressures (Holmes 1979; Freeland 1983). Differential susceptibility of invading and resident hosts to new parasite species will determine long-term parasitological effects of the introduction.

Freeland (1983) found that there were significant differences between parasites of resident host and invading species in the majority of vertebrate (e.g. rabbit) invasions of Australia. These differences were primarily due to the non-susceptibility of the introduced mammals to the parasite fauna of resident marsupials. Bull (1964) found a similar situation for rabbits in New Zealand.

In most cases, invading host species have susceptibilities overlapping those of the resident hosts (Holmes 1979; Freeland 1983), and can therefore be termed suitable hosts for at least some of the resident parasites (Holmes 1979). This leads to exchange of parasites between hosts, enriching the parasite fauna of each (Holmes & Price 1986; Leong & Holmes 1981; Esch, Shostak, Marcogliese & Goater 1990).

Two possible outcomes of the introduction of a new suitable host are:

1) That the new host introduces new parasites to the

system.

The possibility of introducing parasites to a system with a new host is low if the number of hosts introduced is small (Manter 1967; Freeland 1983). A threshold density of suitable hosts is required for a parasite to be able to establish and maintain its life cycle in a system (Dobson & May 1986). This threshold density is lower for parasites with aggregated distributions (e.g. helminths, the macroparasites of Anderson 1979) than parasites with a more random distribution through a host population (e.g. protozoa, viruses, the microparasites of Anderson 1979). This leads to macroparasites being more common invaders than microparasites, as fewer host individuals are required, theoretically, for the parasites' establishment (Dobson & May 1986).

If parasites were introduced with an invading host population, unfavourable environmental conditions for free-living stages or lack of a suitable intermediate host can impede their transmission to susceptible/ suitable resident host species (Manter 1967; Pence & Windberg 1984; Dobson & May 1986; Freeland *et al.* 1986). Parasites with direct life cycles are more likely to become established than parasites with indirect life cycles, due to the dependence of the latter on intermediate hosts or vectors (Petrushevski 1961; Dobson & May 1986). Some helminths with indirect life cycles can, however, become established if they have a wide specificity to an intermediate host (Kennedy 1993a).

A detailed account of the introduction of the toad *Bufo marinus* to Australia was presented by Easteal (1981). The toad had been distributed throughout the Caribbean and Pacific from South America. One hundred and one toads were brought to Australia from Hawaii in 1935, allowed to breed, and their progeny transplanted within the country (Mungomery 1936; Easteal 1981). This small number of animals, as well as the subsequent release of progeny, rather than adults, are suggested as

reasons why the toad might not have introduced helminths to Australia (Freeland *et al.* 1986; Cribb & Barton 1991). Delvinquier and Freeland (1988) concluded that lack of a suitable vector was responsible for the failure of the toad, *B. marinus*, to introduce any of its native blood protozoa to Australia.

2) The new host acquires parasites from the resident host species.

Taxonomic affinity or ecological similarity between hosts (Leong & Holmes 1981) leads to overlapping susceptibility to resident parasites (Holmes 1979; Freeland 1983). Presence and abundance of resident hosts will determine which parasites an introduced host will acquire (Holmes 1979, 1982; Leong & Holmes 1981; Marcogliese & Cone 1991). The introduction of a suitable host will then lead, theoretically, to an increase in the range of a parasite species (Holmes 1979; Freeland 1983). The new host acts as a population amplifier for the parasite (Holmes 1979) which increases the number of infective stages in the ecosystem and doses received by the individual host (Holmes 1979; Freeland 1983; Dobson & Keymer 1990).

Dependent on which of the two situations above occurs, the effects of the acquired parasite, in either the resident (situation 1) or the introduced (situation 2) host will have either, or both, of the two following outcomes.

Firstly, the new parasite may depress fitness of host individuals as they spread to hosts with which they have not co-adapted (Holmes 1982; Freeland 1983; Dobson & May 1986; Holmes & Price 1986). Thus, the acquired parasite may produce extensive mortality in, and directly exclude, certain host species. A decline in host species richness in the community may result

(Holmes 1979; Minchella & Scott 1991). van Riper *et al.* (1986) found that the introduction of avian malaria (*Plasmodium relictum capistranoae*) to Hawaii in the early 1900's was responsible for the exticntion of many endemic species. Although the vector for the malaria had already been introduced into the islands, it was only after infected birds were introduced that malaria became established. The distribution of endemic Hawaiian birds is now limited to high altitudes where the vector is unable to survive. Sakanari and Moser (1990) reported on pathology associated with *Lacistorhynchus dollfusi* (Cestoda) in introduced bass (*Morone saxatilis*) on the United States west coast. Native west coast fish (presumably of a different species) did not produce a pathological response to this parasite. In introduced fish, however, the parasite produced large external lesions. This response was, however, less marked in *M. saxatilis* from introduced west coast populations compared to naive fish from the source of the east coast populations. A related species, *L. tenuis* occurred in *M. saxatilis* on the east coast, but produced no pathological response (Sakanari & Moser 1990). Direct proof of this relationship, however, is difficult to provide. Anderson (1972) suggested that meningeal worm (*Parelaphostrongylus tenuis*; Nematoda) caused a decline in moose (*Alces alces*) populations where the range of the usual host, the white-tailed deer (*Odocoileus virginianus*), was expanding due to changes in forest management. Although *P. tenuis* produced no disease symptoms in white-tailed deer, it was fatal in moose. Recent studies (see Whitlaw and Lankester 1994a,b) on moose and deer population densities do not support the hypothesis of Anderson (1972) and suggest that other mortality factors may be as important in moose decline.

Secondly, the acquired parasite co-adapts with the

new host species but has no noticeable deleterious effect on its health. Four species of amphibian protozoan parasites have been reported as introduced into Australia by *B. marinus* (Delvinquier & Freeland, 1988). Three of these species (*Trichomitus batrachorum*, *Hyalodaktylethra renacaujo* and *Myxidium immersum*) have subsequently adapted to resident frog species. Delvinquier and Freeland (1988) also found *B. marinus* had acquired 10 protozoan species from Australian frogs. Coho salmon (*Oncorhynchus kisutch*), introduced to the Cold Lake system in Canada, acquired 12 of the 13 species of helminths with which it was infected from resident fish (Leong & Holmes 1981). No pathogenesis was reported to occur in either of the examples cited above.

1.1.2 Indirect effects of introduction of a new host

Alteration of the environment (Pence & Windberg 1984) or competition with resident species for prey items which act as intermediate hosts (Holmes 1979) are some indirect effects of the introduction of a new host. The introduction of phytophagous fish into lakes in Poland changed the parasite fauna of resident fish species due to habitat alteration by the introduced fish (Grabda-Kazubska *et al.* 1987). Changes in aquatic vegetation led to decreased abundance and distribution of certain intermediate hosts which altered the structure of parasite infracommunities in resident fish. Competition for prey items then led to specialisation on particular prey items which altered the parasite infrapopulations within resident hosts (Holmes 1979).

1.1.3 Introduction of *Bufo marinus* to Australia

The family Bufonidae is found throughout the Americas, Africa and Eurasia; no member of this family

occurs naturally in the Australopapuan region (Cogger 1992). Man, however, has assisted the dispersal of this family, and in particular the genus *Bufo*, so it is now distributed worldwide. *Bufo marinus* has been introduced to at least 90 distinct areas throughout the Caribbean and Pacific (Easteal 1981) from its natural range of Central and South America (Zug & Zug 1979).

It was widely suggested that toads would provide a useful biological control for various crop pests throughout the Caribbean and Pacific area (Mungomery 1935, 1936). The pests of concern in Australia were the greyback cane beetle (*Dermolepida albohirtum*) and the Frenchi beetle (*Lepidiota frenchi*) (see van Buerden 1981), beetles similar to the Puerto Rican cane beetle which had been "successfully" controlled by the toad (Mungomery 1935, 1936). Although points were raised against the introduction of the toad (Froggatt 1936) these were not thought sufficient to prevent the introduction taking place (Mungomery 1935, 1936). Kinghorn (1938) suggested that had the introduction of *B. marinus* been researched by herpetologists, instead of entomologists, the introduction might never have taken place. Concern for controlling the beetle in as short a time as possible overrode any consideration for the long-term effects of the toads' introduction.

One hundred and one toads were collected from Hawaii in 1935 (Mungomery 1936; Easteal 1981) for shipment to Australia. These toads were kept at Gordonvale in North Queensland for breeding (Mungomery 1936) and by March, 1937, 62,000 toadlets had been released in the region from Mosman to Bundaberg (Easteal 1981; Easteal & Floyd 1986).

The distribution of the toad now covers the area from Boorooloola (Northern Territory (NT); Freeland, W.J. 1990, pers. comm.) through Cape York (to the level of Heathlands Ranger Station; McLeod, T. 1990, pers. comm.)

along the Queensland coast to northern New South Wales (Lismore; Spencer, H. 1993, pers. comm.) (Figure 2.1). Freeland and Martin (1985) predict that the toad will naturally (i.e. with no assistance from man) reach Darwin (NT) by soon after the turn of the century. It is suggested, moreover, that toads will be able to colonise most of the Australian coast, from Broome (Western Australia) eastward to Adelaide (South Australia) and also in isolated patches in southern WA and Tasmania (van Beurden 1981; Freeland 1985). With the aid of man-assisted dispersals it would be possible for the toads' distribution to cover this area in a shorter amount of time (Freeland & Martin 1985). Wherever the toad has been introduced, in Australia or elsewhere, it has rapidly become the most abundant and visible amphibian in that area (Froggatt 1936; Zug *et al.* 1975). Following this initial post-introduction expansion, however, toad population size usually declined and remained at low densities (Freeland 1986). Reasons for this decline remain unknown, although Freeland *et al.* (1986) postulated that an, as yet unknown, microbe was responsible in Australia.

The primary concerns of the introduction of the toad were its effects on the native fauna as a competitor, predator, prey item (due to its toxicity), and/or the introduction of a disease/parasite (Froggatt 1936; Freeland 1985, 1987; Covacevich & Archer 1975; Easteal & Floyd 1986).

It has been suggested that toads have reduced native frog numbers through competition for space, especially breeding sites, and food (Covacevich & Archer 1975; Freeland 1985). Freeland and Kerin (1988) found that native frogs and toads did not substantially overlap in resource utilisation during the dry season, which is the period of highest competition between frog species. It was postulated that toads, especially in the dry season,

had a narrower, more specialised niche than native frogs (Freeland 1987; Freeland & Kerin 1988). This study, however, was undertaken during the dry season when most amphibians become fairly restricted in movement (Duellman & Trueb 1986). Zug *et al.* (1975) found that *B. marinus* introduced to New Guinea also did not compete with native frogs due to large differences in habits and habitats occupied.

The primary prey items for toads in its native range are terrestrial arthropods, especially ants and beetles (Zug & Zug 1979; Strüssmann *et al.* 1984), and ants and termites in Australia (Freeland *et al.* 1986; Freeland & Kerin 1988). Generally, habitat variation is the factor which determines food availability (Zug *et al.* 1975; Zug & Zug 1979; Easteal & Floyd 1986). Toads associated with humans tend to forage for a shorter amount of time than toads in a natural environment but obtain a higher variety of food, e.g. beetles and cockroaches (Zug & Zug 1979; Freeland *et al.* 1986).

The largest concern regarding the toad is the effect on its predators due to its toxicity (Covacevich & Archer 1975; but see Madsen and Shine 1994). The overall impact on predator populations is difficult to assess (Covacevich & Archer 1975; Freeland 1987). Toad skin and poison glands contain a mixture of toxins including bufogenins (e.g. Bufalin, Marinobufagin), which increase the heart muscle beat of the predator, and serotonin which is a vasoconstrictor (Tyler 1987). The combination of these toxins leads to cardiac arrhythmia (irregular heart beat; West 1988) and pulmonary oedema (fluid on the lungs; West 1988) culminating in death (Licht & Low 1968; Tyler 1987).

One bite or mouthing of a toad can cause death of a predator almost immediately (Covacevich & Archer 1975; Freeland 1987; Tyler 1987). Covacevich and Archer (1975) reported the death of a specimen of *Dasyurus*

geoffroii (Western native cat) in 30 minutes after it mouthed a cane toad. Even in their natural range, toads are preyed upon by few predators (Licht & Low 1968; Zug & Zug 1979), and the list of known Australian predators is short (Covacevich & Archer 1975; Freeland 1987; Ingram & Covacevich 1990). The only native species known to regularly consume *B. marinus* without any obvious ill effects is the keelback snake, *Tropidonotus mairii*, which is a common predator of native frogs (Covacevich & Archer 1975; Ingram & Covacevich 1990). Other animals that have been reported able to consume *B. marinus* in Australia include *Kuhlia rupestris* (Jungle Perch), *Crocodilus porosus* (Estuarine Crocodile) and *Hydromys chrysogaster* (Water rat) (Covacevich & Archer 1975; Freeland 1987).

Despite previous sporadic and occasional records of parasites, no record can be found of tests performed to determine if the toad carried any parasites and/or diseases at the time of introduction. Thus, it is unknown what parasites the toad may have introduced to the Australian fauna. The extent of parasites that may have adapted to the toad from the native fauna is also unknown (Easteal & Floyd 1986). It is unknown whether interaction of toads and native fauna *via* their parasites has affected population distributions of native fauna. The purpose of this thesis is to evaluate this interaction.

1.2 Aims of Thesis

This study will look at aspects of helminth infections of *Bufo marinus* in Australia. It concentrates on the effects of the introduction of *B. marinus* on the helminth communities of native Australian frogs.

Specifically, this study:

1. identifies the helminth fauna of *Bufo marinus* in Australia and attempts to determine its origin(s);
2. describes and compares the structure of the helminth community in native and introduced amphibians in Australia; and
3. examines the effects of one helminth, the nematode *Rhabdias* sp., on the health, specifically the haematology, of *Bufo marinus*.

Chapter 2: General Materials and Methods

2.1 Collection of Hosts

2.1.1 *Bufo marinus*

Specimens of *Bufo marinus* were collected on an opportunistic basis from various locations throughout their range in Australia (Figure 2.1) (see Appendix 1 for locations of study sites). Toads were collected at night by hand and kept in bins or damp hessian bags until dissection within 2 days of capture.

Dissection followed a fatal dose (2-5 drops, dependent on toad size) of `Lethabarb' (Euthanasia injection Pentobarbitone solution) sprinkled onto the dorsal surface. Following snout-vent length (SVL; in mm) and weight (in g) measurements, a ventral incision was made from the pelvic to the pectoral girdle exposing the body cavity. The following organs were removed and placed in separate dishes containing a 0.8% sodium chloride solution (`Toad Ringer'; see Appendix 2) for inspection: lungs, stomach, intestine and rectum, urinary bladder, kidney, liver and gall bladder. Occasionally, the mouth cavity, nostrils, heart and leg musculature were also examined. Organs were examined under a stereo microscope using transmitted light.

Sex of toad was recorded as either subadult (all toads below 60mm SVL) or adult male or adult female, as determined at time of autopsy.

2.1.2 Native frogs

Frogs from various locations (Figure 2.2) were captured at night by hand and kept in moist calico herpetological bags until dissection the following day. Dissection procedure was the same as outlined above for *B. marinus*.

Figure 2.1 Map of collection sites of *Bufo marinus* throughout Queensland and the Northern Territory.

Numbers in parentheses indicate number of *Bufo marinus* collected at site.

Line represents limit of distribution of toads at present time.

MVR = Mountain View Road.

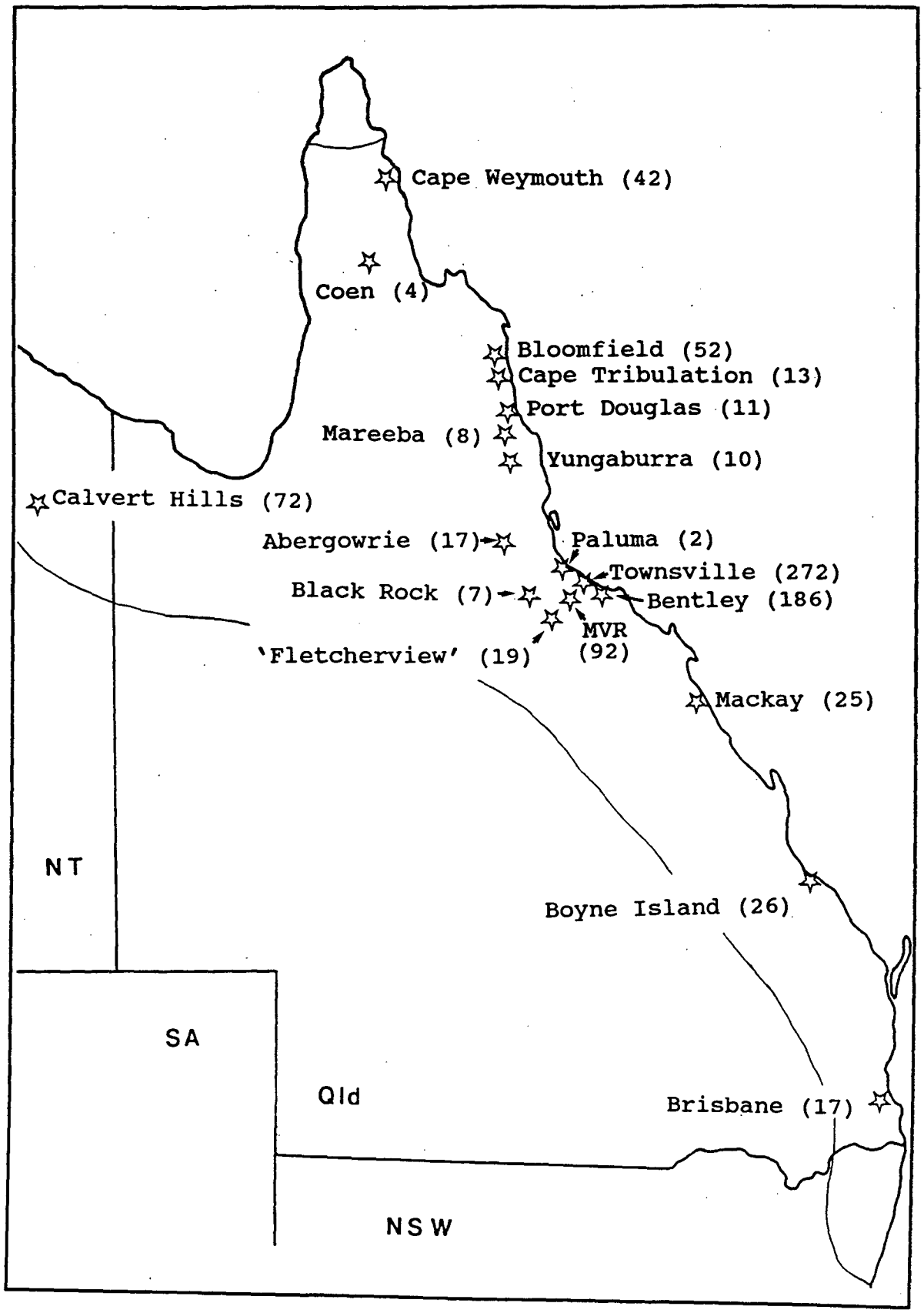
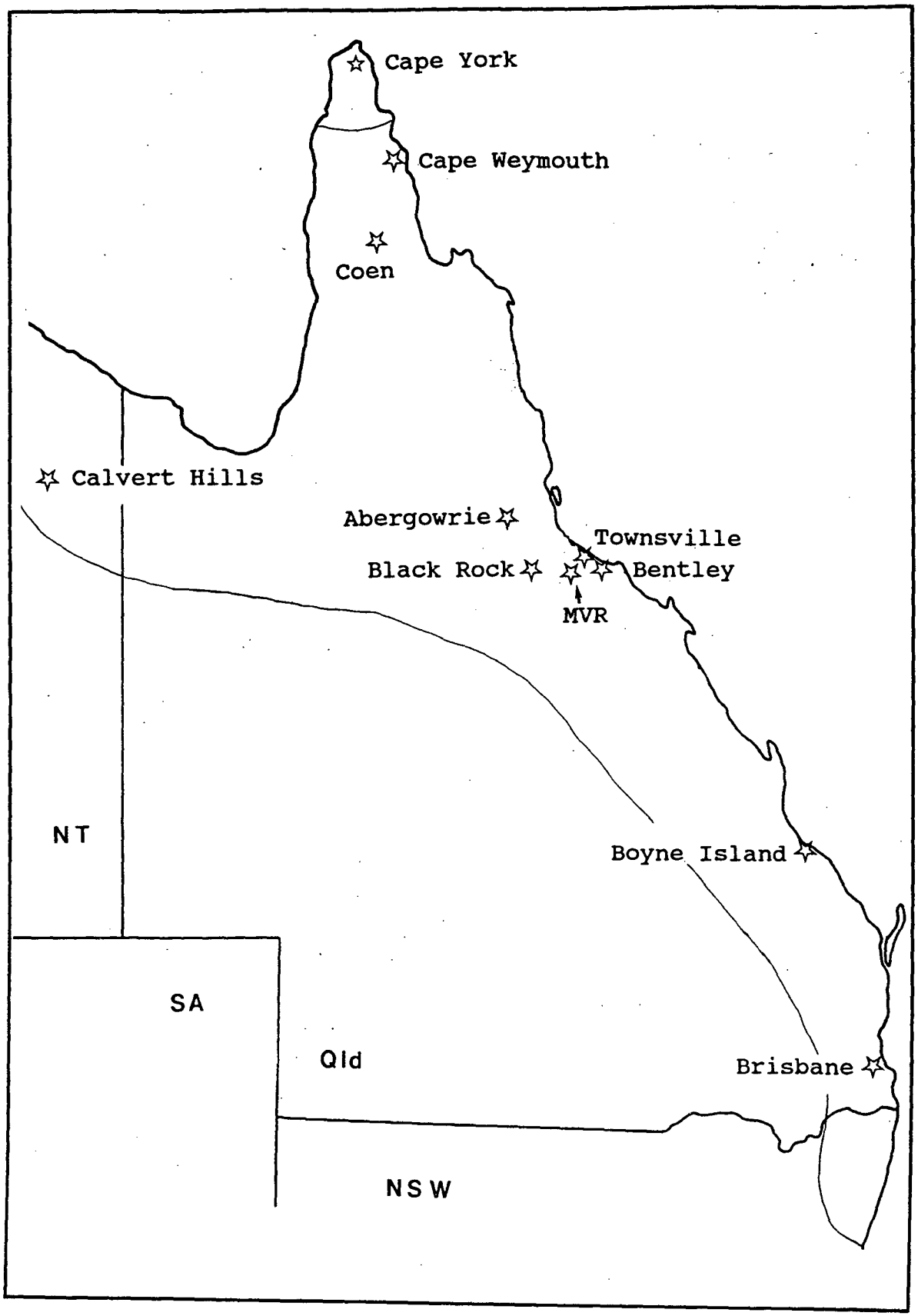

Cape Weymouth (42)
Coen (4)
Bloomfield (52)
Cape Tribulation (13)
Port Douglas (11)
Mareeba (8)
Yungaburra (10)
Calvert Hills (72)
Abergowrie (17)
Paluma (2)
Townsville (272)
Black Rock (7)
Bentley (186)
MVR
(92)
'Fletcherview' (19)
Mackay (25)
NT
Boyne Island (26)
SA
Qld
Brisbane (17)
NSW

Figure 2.2 Map of collection sites of native amphibians in Queensland and the Northern Territory. Refer to Table 3.5 for numbers of each species of native frog from each location.

Line represents limit of distribution of toads at present time.

MVR = Mountain View Road.


Cape York
Cape Weymouth
Coen
Calvert Hills
Abergowrie
Townsville
Black Rock
Bentley
MVR
NT
Boyne Island
SA
Qld
Brisbane
NSW

2.2 Collection of Helminths

Helminths were removed from dissected organs with the aid of fine dissecting forceps and placed in separate cavity blocks filled with `Toad Ringer'. Numbers and location within host for each type of helminth were recorded for each host.

Some living helminths were studied after partial flattening beneath a coverslip, using bright field and differential interference contrast (Nomarski) microscopy.

When the studies of live specimens were completed, helminths were fixed by one of the following methods (for recipes see Appendix 2):

Calcium Acetate buffered Formalin - flattened or unflattened; stored in Formalin

Bouin's fluid - flattened or unflattened; stored in 70% ethanol

Berland's fixative - unflattened; stored in 70% ethanol

Carnoy's fixative - unflattened; stored in 45% acetic acid with 5% glycerine (nematodes only).

Digenean and cestode specimens were stained with dilute Gower's Carmine or Acetocarmine, dehydrated through a graded ethanol series, cleared in xylene and mounted in Canada balsam. Unstained specimens were mounted in the same way. Some Berland's fixed specimens were sectioned at 5µm and stained with haematoxylin and eosin.

Nematode and acanthocephalan specimens were studied as wet preparations after clearing in Lactophenol mountant. Following drawings and measurements, the specimens were returned to 70% ethanol. Nematode specimens in Carnoy's fixative were stained in 1% synthetic orcein in 45% acetic acid following the method

of Ballantyne (1971) (see Appendix 3).

Measurements of helminths are presented in micrometres as means with the range in parentheses. Drawings were made with the aid of a camera lucida.

2.3 Definition of terms

Definitions of ecological terms follow Margolis *et al.* (1982). Prevalence of infection is the percentage of hosts infected in a sample. Mean intensity of infection is the average number of helminth individuals per infected host. Total helminth intensity is the total number of helminth individuals of all helminth species per host individual. Species richness is the number of helminth species per host.

2.4 Statistical analyses

Data was analysed using the Statistix 3.0 Analytical Software package or the AseasyAs statistical package. Graphical presentations were completed with the SigmaPlot 5.0 package.

More detailed descriptions of statistical analyses are given in the appropriate chapters.

Chapter 3: Helminth Parasites of Australian Amphibia

3.1 Introduction

This chapter presents the results of a field survey of the helminth parasites of Australian Amphibia, including the introduced toad, *Bufo marinus*. Amphibians were collected from throughout Queensland and the Northern Territory over a three year period. General infection parameters are detailed for each helminth species. A short description is also provided, but this is not meant to be a detailed taxonomic appraisal. The possibility of the introduction and/or local acquisition of parasites by the toad is also discussed.

3.1.1 Literature Review: Helminths of the Cane Toad, *Bufo marinus*.

Bufo marinus is infected with a wide variety of helminths throughout its geographic range (see Speare 1990). The work of Speare (1990), combined with the checklists of amphibian parasites by Prudhoe and Bray (1982; platyhelminths), Baker (1987; nematodes), and Edmonds (1989; Acanthocephala), record a total of 75 "species" of helminths (total of 85 records) from *B. marinus* (Table 3.1). This total is composed of 36 records of nematodes, 29 digeneans, 6 cestodes, 3 acanthocephalans and 1 monogenean. Of these, 24 records (22 species) are from populations outside the toads' native range and a further 3 records from zoo animals. Many of these records only classify the helminth to genus or, sometimes, to Class (for example, see Freeland *et al.* 1986). There are few genera amongst these that are specific to *B. marinus*. The majority are found in amphibians and reptiles worldwide (see Prudhoe & Bray 1982; Baker 1987).

Since the publication of these checklists, five further records of *B. marinus* as a host for helminths have been published (see Table 3.1). One of these is from a native toad population in Mexico (Etges 1991), whereas the other four are from introduced populations (Cribb & Barton 1991; Jones & Delvinquier 1991; Goldberg & Bursey 1992). This total of 90 helminth records may change with detailed taxonomic work on the helminths of *B. marinus*.

a) South America

In its natural range of Central and South America *B. marinus* has been recorded with 59 species of helminths (28 nematodes, 25 digeneans, 4 cestodes, 1 monogenean, and 1 acanthocephalan) (Prudhoe & Bray 1982; Baker 1987; Speare 1990; Etges 1991) (Table 3.1). The studies of parasites of *B. marinus* in its natural range are generally isolated reports of new species and give no indication of the ecology of the parasite fauna.

b) Australia

The majority of reports of parasites from *B. marinus* in Australia (Table 3.1) are lists of the toad as a new host for previously recorded helminths. There have been six such reports, which are detailed below. Accurate identification of the helminths is assumed, pending further study.

In a study of the genus *Mesocoelium* (Digenea) from Amphibia in Australia, Malaysia and Hawaii, Yuen (1965) recorded *M. mesembrinum* from *B. marinus* in Australia (Brisbane, Qld.). The species had been first described by S.J. Johnston (1912) from *Hyla* (now *Litoria*) *caerulea* in NSW. This parasite must have been acquired by the toad from native amphibians as it was first recorded prior to the toads' arrival in Australia.

Bufo marinus was recorded as a host for

Table 3.1. Published records of helminths from *Bufo marinus*.

Species	Country/Region	Ref
Monogenea:		
Parapseudopolystoma cerrocoloradensis	Venezuela	s
Digenea:		
Catadiscus cohni	S. America	s/pb
Catadiscus sp.	Colombia	pb
Choledocystus elegans	Brazil	s/pb
Choledocystus hepaticus	S. & C. America	s/pb
Choledocystus intermedius	C. America	pb
	Trinidad*	pb
Choledocystus vesicalis	Venezuela	s/pb
Clinostomum attenuatum L	Mexico	e
Creptotrema lynchi	Colombia	s/pb
Glypthelmins linguatula	Brazil	s/pb
Glypthelmins palmipedis	S. America	s/pb
Glypthelmins robustus	Colombia	s/pb
Gorgoderina cryptorchis	Brazil	pb
Gorgoderina diaster	Colombia	s/pb
Gorgoderina megalorchis	C. America	s/pb
	Puerto Rico*	s/pb
Gorgoderina parvicava	S. America	s/pb
Gorgoderina rochalimai	Guadeloupe*	s/pb
Gorgoderina sp.	Colombia	pb
Haematoleochus fuelleborni	Brazil	s/pb
Langeronia macrocirrus	C. America	pb
Langeronia provitellaria	Costa Rica	pb
Mesocoelium danforthi	Jamaica*	s/pb
Mesocoelium incognitum	Brazil	pb
	Hawaii*	s/pb
Mesocoelium mesembrinum	Australia*	s/pb
Mesocoelium monas	S. America	s/pb
	Jamaica*	s/pb
	New Guinea*	pb
	American Samoa*	gb
Mesocoelium sociale	Colombia	s/pb
	Florida Is.*	s/pb
	Pacific Is.*	s/pb
Mesocoelium travassosi	Brazil,Costa Rica	s/pb
Mesocoelium waltoni	Brazil	s/pb
Mesocoelium sp.	Australia*	s/pb
	Colombia	s/pb
Lecithodendriidae	Australia*	s
Trematodes	Australia*	s
Zeylanurotrema spearei	Australia*	cb
Cestodes:		
Cylindrotaenia americana	S. America	s/pb
Proteocephalus bonariensis	S. America	s/pb
Spirometra mansoni L	Australia*	s
Proteocephalid cysts L	Australia*	s
Nematotaenia hylae	Australia*	jd
Nematotaeniidae sp.	Brazil	pb
Taenia filariformis	Argentina	pb

Nematodes:		
Aplectana hoffmani	Costa Rica	b
Aplectana incerta	Mexico	b
Aplectana itzocanensis	Costa Rica	s/b
Aplectana vellardi	Bermuda*	s/b
Cruzia empera	Venezuela	b
Cruzia morleyi	Mexico	b
Foleyella sp.	Brazil	s
Icosiella neglecta	Venezuela	b
Maxvachonia flindersi	Australia*	s/b
Ochoterenella albareti	Guyane	s/b
Ochoterenella caballeroi	C. America	s
Ochoterenella chiapensis	C. America	s
Ochoterenella complicata	Colombia	s
Ochoterenella digiticauda	C. America	s/b
	Jamaica*	s/b
Ochoterenella dufourae	Guyane	s/b
Ochoterenella figueroai	C. America	s
Ochoterenella guyanensis	F. Guyane	s/b
Ochoterenella lamothei	C. America	s
Ochoterenella nanolarvata	C. America	s
Ochoterenella oumari	Guyane	s/b
Ochoterenella royi	Guyane	s/b
Ochoterenella vellardi	Brazil	s/b
Ochoterenella sp.	Colombia	s
Orneoascaris (=*Amplicaecum*)	London Zoo#	s
Oswaldocruzia subauricularis	Brazil	s/b
Oswaldocruzia mazzai	Argentina	b
Oswaldocruzia sp.	London Zoo#	s
Oxyascaris similis	Brazil	s/b
Parathelandros mastgurus	Australia*	b
Parathelandros sp.	Australia*	s/b
Parapharyngodon kartana	American Samoa*	gb
Rhabdias fuelleborni	S. America	s/b
Rhabdias sphaerocephala	S. America	s/b
	Bermuda*	s/b
Rhabdias sp.	London Zoo#	s
Filaria	F. Guiana	s
Microfilaria L	Colombia	s
Nematodes	Australia*	s
Acanthocephala:		
Acanthocephalus correalimai	Brazil	s
Porrorchis hylae L	Australia*	ed
Acanthocephalan cysts L	Australia*	s

*, introduced populations of toads; #, zoo records; b, Baker 1980; C. America, Central America; cb, Cribb & Barton 1991; e, Etges 1991; ed, Edmonds 1989; F. Guiana, French Guiana; gb, Goldberg & Bursey 1992; jd, Jones & Delvinquier 1991; L, larval stage; pb, Prudhoe & Bray 1982; s, Speare 1990; S. America, South America.

Parathelandros mastgurus (Nematoda) by Inglis (1968) in Brisbane (Qld). This species had previously been recorded from *Litoria caerulea* in localities ranging from Townsville (Qld) to Sydney (NSW) and *Lit. gracilenta* in Townsville (Baylis 1930; Inglis 1968). As this species was first described prior to the toads' introduction, it must also have been acquired by the toad post-introduction.

Maxvachonia flindersi (Nematoda) was reported from various amphibian hosts, including *B. marinus*, by Mawson (1972). This species had previously been reported as *Aplectana flindersi* (Johnston & Mawson 1941) and subsequently as *Austracerca flindersi* (Inglis 1968) in frogs from South and West Australia. Although this parasite was recorded after the introduction of the toad, it was originally found well out of the toads' range. Therefore it must have been acquired by the toad from Australian frogs.

Sparganid infections (larval stage of ?*Spirometra mansoni*) occur in a wide variety of animals, from amphibians to mammals, and were reported by Bennett (1978) to occur in a natural infection in *B. marinus*. The life cycle of this helminth is assumed to pass between an amphibian and a mammalian carnivore. Because toads are poisonous, killing their predators (see Chapter 1), it is unlikely that this parasite would have been able to transmit from toads had it been introduced with the toad. It must, therefore, have been acquired since the toads' introduction. Also, its low host specificity would enable *B. marinus* to acquire this helminth.

Freeland *et al.* (1986) studied the parasite fauna within populations of toads over a wide geographical range. Helminths were classified into classes, and occasionally to genera, with a total of 7 `types' of helminths found. From this study Freeland *et al.* (1986)

concluded that the toad was infected with none of its native helminths and thus must have acquired these infections from Australian fauna. Two of the examples cited by Freeland *et al.* (1986) in reaching this conclusion, namely members of the genus *Mesocoelium* and members of the family Lecithodendriidae, are also present in *B. marinus* in its natural range (see Prudhoe & Bray 1982) so this conclusion is invalid without further detailed study of the species involved.

Delvinquier and Freeland (1988) completed a detailed study of the protozoa of *B. marinus*, comparing records of protozoa from *B. marinus* from Australia and South America. They concluded that *B. marinus* had introduced four species of protozoa to Australia, and three of these had adapted to the Australian frog fauna. The remaining ten species infecting *B. marinus* in Australia must have been acquired from the native fauna. Native frogs were postulated as the most likely source of these protozoa.

Bufo marinus was listed as a paratenic host for larval *Porrorchis hylae* (Acanthocephala) by Edmonds (1989). *Porrorchis hylae* was originally recorded by T.H. Johnston (1912) as an Echinorhynch encysted larva from *Litoria aurea* in Sydney, and subsequently (1914) as *Echinorhynchus hylae* from *Lit. caerulea* in Queensland (near Brisbane). This helminth species has been recorded by various Australian workers (see Edmonds 1989) to infect many amphibian hosts (*Litoria* spp., *Limnodynastes* spp.). The toad must have acquired this helminth species after its introduction to Australia.

Adult *Nematotaenia hylae* (Cestoda) were found in only two of over 767 *B. marinus* examined by Jones and Delvinquier (1991; including unpublished data of Assoc. Prof. R. Speare). Jones and Delvinquier (1991) suggested that *N. hylae* is a native parasite that has adopted *B. marinus* as a new host due to the helminth's

wide host and geographical distribution within Australia. Also, the genus *Nematotaenia* has never been recorded from South America.

Zeylanurotrema spearei was recorded from *B. marinus* in North Queensland by Cribb and Barton (1991). The only other member of this genus was reported from Sri Lanka by Crusz and Sanmugasunderam (1974) in an agamid lizard. A similar lizard was postulated as the original host for this parasite in Australia (Cribb & Barton 1991), from which it has since been acquired by the toad. This unusual genus of digenean has never been previously recorded from South America or Australia, so its exact origin is still unknown.

Only after comparison of the helminths found in *B. marinus* in Australia with helminths from its native range and Australian amphibians can specific determination of the origins of these helminths be made. The provisional conclusion from these records, however, would be that all parasites so far acquired by the toad in Australia have a local origin.

c) Other introduced populations of *Bufo marinus*

Other records of parasitic infection in *B. marinus* outside its natural range are primarily from the Caribbean Islands to which it has been introduced, or from zoo specimens.

Six species of digeneans have been recorded in *B. marinus* in the Caribbean, of which three are of the genus *Mesocoelium* (*M. sociale*, see Fischthal & Kuntz 1967; *M. danforthi*, see Mettrick & Dunckley 1968; *M. monas*, see Wong & Bundy 1985). Two other species belong to the genus *Gorgoderina* (*G. rochalimai*, see Jourdane & Theron 1975; *G. megalorchis*, see Prudhoe & Bray 1982) and the last species was *Choledocystus intermedius* (see Everard 1975). Only *M. danforthi* and *G. rochalimai* had not previously been recorded in the toads' natural

range.

Mesocoelium sociale was also recorded by Fischthal and Kuntz (1967) to occur in the Pacific islands of Fiji and British Solomon Islands. *Mesocoelium incognitum* was recorded in Hawaii by Yuen (1965) and *Mesocoelium monas* was reported by Prudhoe and Bray (1982) from *B. marinus* in New Guinea and in American Samoa by Goldberg and Bursey (1992). All these species have been recorded to occur in populations of *B. marinus* in its natural range.

Three nematode species have been reported from the Caribbean islands by Williams (1959; *Aplectana vellardi* and *Rhabdias sphaerocephala*) and Wong and Bundy (1985; *Ochoterenella digiticauda*). Of these species, only two had been previously reported from Central American *B. marinus* (*Rh. sphaerocephala* and *O. digiticauda*). Goldberg and Bursey (1992) recorded *Parapharyngodon kartana* in *B. marinus* in American Samoa. This helminth usually infects skinks (*Emoia nigra* and *E. samoense*) and is considered a pseudoparasitism, possibly acquired after ingestion of an infected skink. A further three nematodes have been reported from zoo specimens in London by Keymer (1974; *Rhabdias* sp., *Oswaldocruzia* sp. and *Amplicaecum* sp.). No indication was given of the origin of these toads, so it is unknown if these parasites were native to the toad or acquired from specimens within the zoo.

d) Helminths of Australian Amphibia

Studies of the helminth fauna of Australian amphibia had been carried out by SJ Johnston (1912) in NSW and Nicoll (1914, 1918) in Queensland prior to the introduction of the toad. Their work concentrated on the description of new trematode species, but Johnston (1912) did report the presence of nematode infestations. Further studies, primarily on nematodes, were carried out by TH Johnston and Mawson in South Australia, an

area out of the range of *B. marinus*, throughout the 1940's (Johnston & Mawson 1941, 1942, 1949). Over 70 species of helminths have been recorded from 63 species of native frogs (see Appendix 4). No study has looked at the present helminth fauna of Australian frogs to determine what, if any, helminths might have been introduced with *B. marinus*.

3.1.2 Aims of Chapter

This Chapter has two main aims:

1. to identify the helminth fauna of *Bufo marinus* throughout its range in Australia.

2. to assess evidence for origins of this fauna through comparison of helminths collected from native fauna and literature records of both Australian fauna and *Bufo marinus* in its native habitat.

3.2 Materials and Methods

3.2.1 Taxonomy of helminths

Classification of helminth to genus followed the system of Prudhoe and Bray (1982) for Monogenea, Digenea and Cestoda, the CIH Keys to the Nematode Parasites of Vertebrates (Hartwich 1974; Chabaud 1975, 1978; Anderson & Bain 1976, 1982; Petter & Quentin 1976; Durette-Desset 1983) and Spencer Jones and Gibson (1987) for the Nematoda, and Amin (1985) for the Acanthocephala. Preliminary taxonomic descriptions were prepared following comparison of collected specimens with museum specimens and literature records. Classification of helminth to species, where possible, followed examination of relevant literature records and/or museum specimens.

Sources from which specimens were borrowed and museums in which specimens have been deposited are indicated in the text as follows: AM, Australian Museum, Sydney, Australia; BM(NH), The Museum of Natural History, London, U.K.; CAS, Czechoslovak Academy of Sciences, České Budějovice, Czechoslovakia; THC, collection of Dr T.H. Cribb, Department of Parasitology, University of Queensland, Brisbane, Australia; MKJ, collection of Dr M.K. Jones, Electron Microscopy Unit, University of Queensland, Brisbane, Australia; JCP, collection of Professor J.C. Pearson, Department of Parasitology, University of Queensland, Brisbane, Australia; QM, Queensland Museum, Brisbane, Australia; RS, collection of Assoc. Prof. R. Speare, Anton Brienl Centre for Tropical Medicine, James Cook University of North Queensland, Townsville, Australia; SP, collection of Dr S. Pichelin, Department of Parasitology, University of Queensland, Brisbane, Australia; MNHN, Museum of Paris, Paris, France; USNMHC,

United States National Museum Helminthological Collection, Beltsville, Maryland, U.S.A.

The following specimens were studied for comparative purposes: USNMHC No. 59630 *Dolichosaccus rastellus subulatus*; BM(NH) No. 1923.6.27.94, 1933.7.19.105, 1933.7.19.120, 1933.7.19.110, 1922.10.24.3, 1933.7.19.119 *Opisthioglyphe rastellus*; BM(NH) No. 1981.7.24.1-5 (2 slides), 1984.10.9.17, 1961.10.3.3-6 (2 slides), unregd (4 slides) *Dolichosaccus rastellus*; BM(NH) No. 1929.4.8.104 *Lecithopyge rastellum subulatum*; USNMHC No. 36505 (2 slides), 51591 *Dolichosaccus trypherus*; AM No. W334a *Dolichosaccus trypherus*; BM(NH) No. 1968.4.29.26 *Dolichosaccus trypherus*; QM GL 11848 *Dolichosaccus trypherus*; AM No. W335 *Dolichosaccus ischyrus*; AM No. W336 *Dolichosaccus diamesus*; USNMHC No. 51422 (2 slides) *Brachysaccus anartius*; AM No. W337a *Brachysaccus anartius*; QM GL11846 *Dolichosaccus anartius*; AM No. W338 *Brachysaccus symmetrus*; QM GL11280 *Dolichosaccus juvenilis*; USNMHC No. 61722 *Opisthioglyphe lygosomae*; USNMHC No. 79790 (vial) *Dolichosaccus* (*Lecithopyge*) *novaezelandiae*; BM(NH) No. 1946.12.31.105, 1982.5.21, 1977.8.2.1-4 *Dolichosaccus novaezelandiae*; USNMHC No. 73025 *Dolichosaccus schmidti*; CAS No. D-232 *Opisthioglyphe cophixali*; CAS No. D-233 *Dolichosaccus grandiacetabularis*; CAS No. D-234 (2 slides) *Dolichosaccus longibursatus*; 39571 *Mesocoelium danforthi*; 31762 (3 slides), 61711 (3 slides) *Mesocoelium sociale*; 45639 (2 slides) *Mesocoelium waltoni*; 56938 (2 slides) *Mesocoelium travassosi*; W341a *Oophoroplanetes* (*Mesocoelium*) *mesembrinum*; W342 *Oophoroplanetes* (*M.*) *oligoon*; W343 *O.* (*M.*) *megaloon*; W332a *Diplodiscus megalochrus*; W333 *Diplodiscus microchrus*.

The following specimens have been deposited in museums: QM GL1273, GL1274-76, GL1211-1272, GL1277-1282 *Zeylanurotrema spearei*; AHC No. 18984a-c, S1779(1-3)

Zeylanurotrema spearei; BM(NH) No. 1990.12.7.3-5 *Zeylanurotrema spearei*; QM QL18322 *Dolichosaccus symmetrus*; BM(NH) No. 1992.12.21.1 *Dolichosaccus symmetrus*; USNMHC No. 82706 *Dolichosaccus symmetrus*; CAS No. D-303 *Dolichosaccus symmetrus*; QM QL18323 *Dolichosaccus juvenilis*; BM(NH) No. 1992.12.21.2 *Dolichosaccus juvenilis*; USNMHC No. 82707 *Dolichosaccus juvenilis*; CAS No. D-304 *Dolichosaccus juvenilis*; QM QL18324 *Dolichosaccus helocirrus*; BM(NH) No. 1992.12.21.3-4 *Dolichosaccus helocirrus*; USNMHC No. 82708 *Dolichosaccus helocirrus*; CAS No. D-305 *Dolichosaccus helocirrus*; MNHN No. 676MDa-b *Johnpearsonia pearsoni*; SAM 23804.1-9 *Johnpearsonia pearsoni*; QM G210154-210158 *Johnpearsonia pearsoni*.

3.2.2 Origins of helminths of *Bufo marinus*

Literature records of helminths recorded from *B. marinus* were studied. Records were divided into natural (South and Central America) and introduced (Caribbean and Pacific) populations. Records of helminths of Australian native frogs were also obtained and divided into pre- and post-toad introduction (1935).

The helminth species found in *B. marinus* in Australia in this study were compared with the above records to determine the likely source of origin.

Helminths were determined as having a definite Australian origin if

a) the genus had never been recorded from any host group from South America, and/or

b) the genus had previously been recorded from any host group from Australia, either

i) before the introduction of the toad (1935), or

ii) in areas well out of the present

distribution of the toad, for example, South Australia, Western Australia or Tasmania.

A helminth was determined as having a possible Australian origin if the genus had never been recorded in South America or Australia but had been recorded, from any host group, from the Australopapuan/Asian area.

Helminths were determined as having a South American origin if the genus had previously been recorded from South America, and especially if from *B. marinus* or a closely related species, for example *B. arenarum*.

Helminths were determined as having an unknown origin if the genus had previously been recorded from South America (especially from *B. marinus*) and Australia either prior to the toads' introduction or in areas well out of its present distribution. Helminths that could not be identified to genus were also determined as having an unknown origin.

3.3 Results

3.3.1 General

a) *Bufo marinus*

In total, 875 toads were collected from 18 locations in Queensland (Qld) and the Northern Territory (NT) (Figure 2.1) from April 1989 to March 1992. Table 3.2 presents sex and SVL data for toads from each location; male toads were more often collected.

Of the 875 toads, 641 (73.26%) were infected with at least one helminth species. No significant difference was found between the sexes ($F_{1,818}$=0.67, p=0.4118) where 75.7% of male toads and 72.1% of female toads were infected with at least one helminth species. Subadult toads for which sex could not be determined, however, were significantly different ($F_{2,872}$=5.80, p=0.0033) with only 50.9% infected with at least one helminth species. Distribution of the number of helminth species per host individual for all toads, independent of geographical location caught, is shown in Figure 3.1. The same data, separated into geographical locations, is presented in Figure 3.2. The most heavily infected toads were collected from Bentley (Figure 3.2), followed by toads from Cape Weymouth and Townsville. Maximum number of helminth species for any toad individual was 6.

Throughout the range of the survey, *B. marinus* was found to be infected with a total of 24 species of helminths (Table 3.3). Six of these species were found to be unique to toads in this study, the remaining 18 species were shared with native frogs. Total prevalence and mean intensity of infection of the helminths found (12 Nematoda, 8 Digenea, 2 Acanthocephala, and 2 Cestoda) over the range of the survey are presented in Table 3.4. *Rhabdias* sp. and *Mesocoelium* sp. dominated the helminth fauna with the two highest recorded

Table 3.2. Numbers of different sexes, range and mean snout-vent length (mm) of toads collected from 18 geographical locations as shown in Figure 2.1.

Location	Sex			Total	Mean length	Range	
	Male	Female	Subadult			Min	Max
Abergowrie	6	8	3	17	94.0	40.0	119.5
Bentley	142	44	0	186	95.7	58.0	117.0
Black Rock	1	6	0	7	126.2	114.5	138.5
Bloomfield	33	14	5	52	86.7	46.5	129.0
Boyne Island	11	14	1	26	92.8	51.0	122.0
Brisbane	11	2	4	17	90.4	44.0	136.5
Calvert Hills	38	25	9	72	88.9	33.5	136.5
Cape Tribulation	5	8	0	13	96.6	73.0	125.0
Cape Weymouth	26	16	0	42	100.5	64.5	176.5
Coen	4	0	0	4	105.1	92.5	115.5
`Fletcherview'	18	1	0	19	103.9	53.0	117.0
Mackay	4	12	9	25	63.1	41.0	93.0
Mareeba	5	3	0	8	94.3	84.5	101.0
MVR	83	9	0	92	95.4	69.0	118.0
Paluma	0	2	0	2	122.3	75.5	169.0
Port Douglas	3	8	0	11	79.9	68.0	95.5
Townsville	173	76	23	272	86.6	46.0	130.5
Yungaburra	6	3	1	10	72.9	58.0	93.5
Total	569	251	55	875	90.88	33.5	176.5

Figure 3.1 Distribution of the number of helminth species per host individual for 24 of the 26 amphibian species collected in this study. *Mixophyes* sp. and *Litoria lesueuri* were not included as both species were represented by a sample of 1 (both infected with 3 helminth species).

Cr., *Crinia*; *Lim.*, *Limnodynastes*; *Lit.*, *Litoria*; *S.*, *Sphenophryne*; *Up.*, *Uperoleia*.

Numbers in parentheses indicate number of host specimens collected.

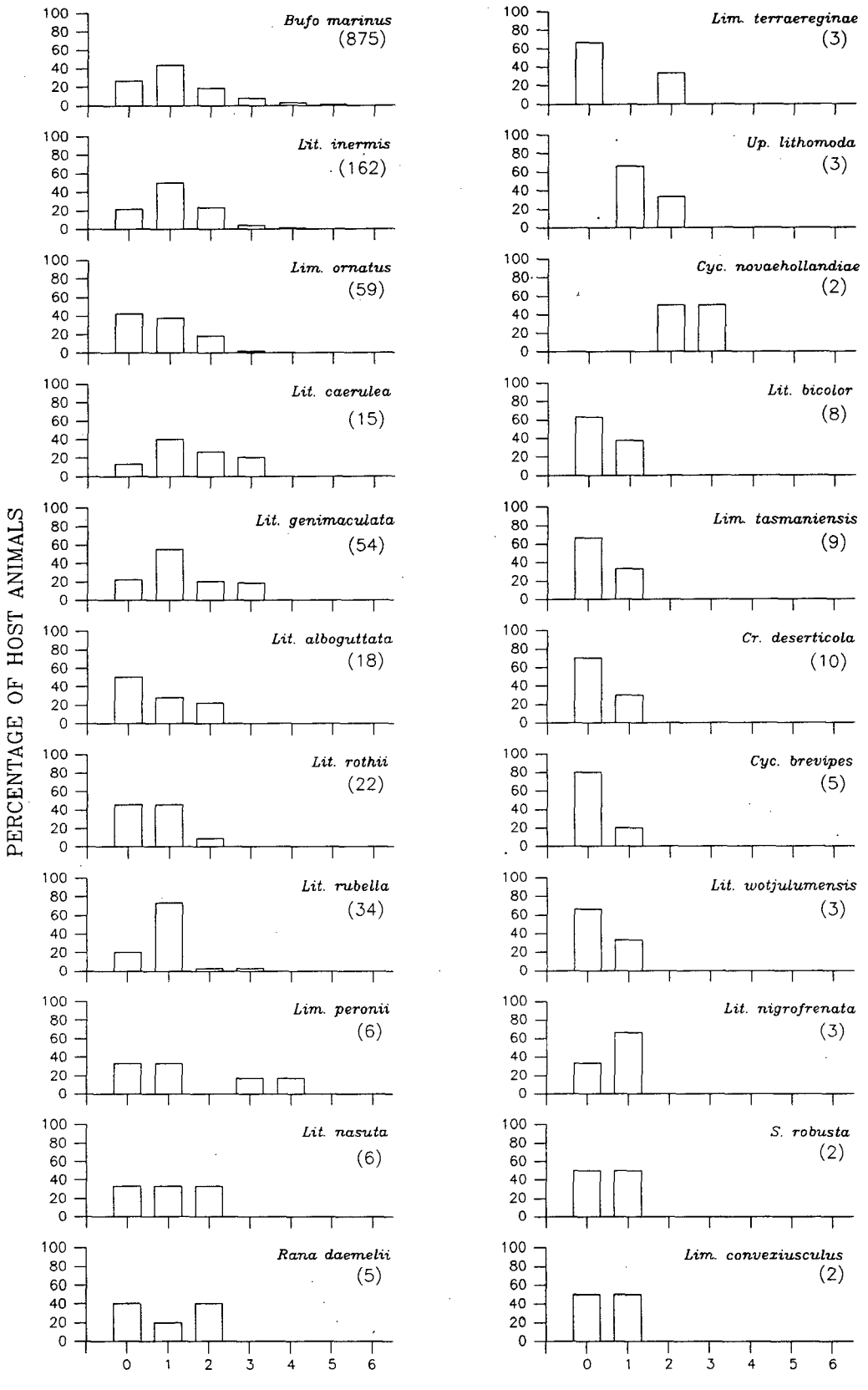
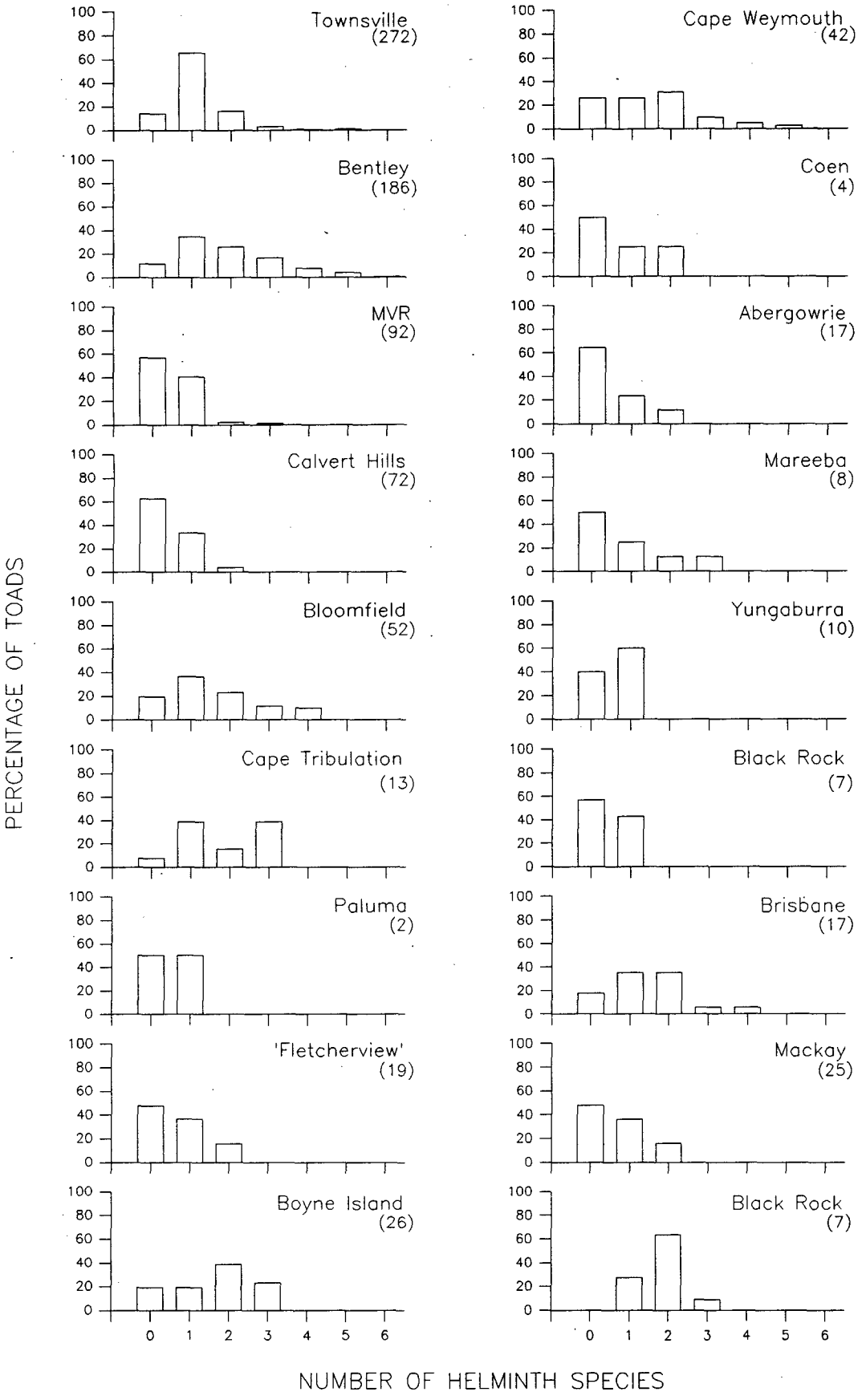

Bufo marinus (875)
Lit. inermis (162)
Lim. ornatus (59)
Lit. caerulea (15)
Lit. genimaculata (54)
Lit. alboguttata (18)
Lit. rothii (22)
Lit. rubella (34)
Lim. peronii (6)
Lit. nasuta (6)
Rana daemelii (5)
Lim. terraereginae (3)
Up. lithomoda (3)
Cyc. novaehollandiae (2)
Lit. bicolor (8)
Lim. tasmaniensis (9)
Cr. deserticola (10)
Cyc. brevipes (5)
Lit. wotjulumensis (3)
Lit. nigrofrenata (3)
S. robusta (2)
Lim. conveziusculus (2)
PERCENTAGE OF HOST ANIMALS
NUMBER OF HELMINTH SPECIES

Figure 3.2 Distribution of the number of helminth species per individual *Bufo marinus* at 18 geographical locations.

Numbers in parentheses indicate number of toads collected.


Townsville (272)
Cape Weymouth (42)
Bentley (186)
Coen (4)
MVR (92)
Abergowrie (17)
Calvert Hills (72)
Mareeba (8)
Bloomfield (52)
Yungaburra (10)
Cape Tribulation (13)
Black Rock (7)
Paluma (2)
Brisbane (17)
'Fletcherview' (19)
Mackay (25)
Boyne Island (26)
Black Rock (7)
PERCENTAGE OF TOADS
NUMBER OF HELMINTH SPECIES

List of helminth species collected from *Bufo marinus* and native frogs in Australia in this study.

Helminth species	*Bufo marinus*	Native frogs
Monogenea		
Parapolystoma sp.	-	+
Digenea		
Diplodiscus sp.	+	+
Dolichosaccus symmetrus	+	+
Dolichosaccus juvenilis	+	*
Dolichosaccus helocirrus	+	+
Mesocoelium sp.	+	+
Pleurogenoides sp.	+	+
Zeylanurotrema spearei	+	-
Digenea larva	+	+
Cestoda		
Nematotaenia hylae	+	+
Diphyllobothrium sp. spargana	+	+
Nematoda		
Rhabdias sp.	+	+
Johnpearsonia pearsoni	+	+
Parathelandros mastigurus	+	+
Cosmocerca sp. 1	+	+
Cosmocerca sp. 2	+	-
Cosmocerca sp. 3	+	-
Austraplectana sp.	+	*
Spinicauda sp.	+	+
Kreisiella sp.	+	+
Maxvachonia sp.	+	+
Seuratascaris numidica	-	+
Onchocercidae gen. sp.	-	+
Undetermined adults	+	+
Nematode larva	+	+
Acanthocephala		
Undetermined adult	+	*
Porrorchis hylae larvae	+	+

+ Presence of helminth in host group in this study
* Presence of helminth in host group in previous studies
- Absence of helminth in host group

Table 3.4. Overall prevalence and mean intensity of infection of helminths collected from *Bufo marinus* in Australia. N, Number of geographical locations (from Figure 2.1) helminth species present in *Bufo marinus*.

Helminth species	Site in Host	N	Prevalence (%)	Mean Intensity	Maximum Intensity
Digenea					
Mesocoelium sp.	Intestine	14	18.4	8.8	127
Dolichosaccus symmetrus	Intestine	5	3.9	7.3	32
Dolichosaccus juvenilis	Intestine	4	5.1	10.2	56
Dolichosaccus helocirrus	Intestine	5	2.5	12.7	73
Pleurogenoides sp.	Intestine	3	1.8	5.7	21
Diplodiscus sp.	Rectum	1	0.1	2.0	2
Zeylanurotrema spearei	Urinary bladder	3	5.9	53.9	231
Digenea larvae	Intestine	3	1.0	1.4	2.0
Cestoda					
Nematotaenia hylae	Intestine	1	0.1	1.0	1
Diphyllobothrium sp.	Muscle	5	1.3	2.6	7

/cont.

Table 3.4 (cont.)

Helminth species	Site in Host	N§	Prevalence (%)	Mean Intensity	Maximum Intensity
Nematoda					
Rhabdias sp.	Lung	8	43.2	18.7	230
Cosmocerca sp. 1	Rectum	7	2.7	5.5	58
Cosmocerca sp. 2	Rectum	1	0.7	12.0	54
Cosmocerca sp. 3	Rectum	1	0.6	3.0	6
Johnpearsonia pearsoni	Intestine	6	8.6	3.7	14
Maxvachonia sp.	Intestine	6	4.9	3.0	15
Parathelandros mastigurus	Rectum	7	2.6	9.6	70
Spinicauda sp.	Intestine	1	2.1	6.0	32
Kreisiella sp.	Intestine	1	0.7	3.0	10
Austraplectana sp.	Rectum	3	3.0	28.0	100
Nematode L	Mesentery	8	6.2	9.9	44
Unknown Nematodes	Intestine	7	0.2	2.1	9
Acanthocephala					
Undetermined Adult	Intestine	1	0.8	1.2	2
Porrorchis hylae cyst	Mesentery	5	0.9	2.5	7

prevalence of infection. *Zeylanurotrema spearei*, *Austraplectana* sp. and *Rhabdias* sp. had the highest mean intensities of infection. All other species were rarely encountered, and were usually present in low intensities.

No helminths were collected from the kidney, liver, gall bladder, heart, nostrils or mouth cavity.

b) Native Amphibia

In total, 424 frogs representing 23 species were collected from 13 geographical locations (Figure 2.2). Cape York was the only location beyond the range of *B. marinus*. Table 3.5 presents sex and SVL data for each species of frog collected from each location; males were more commonly caught than both females and subadults.

Of the 424 native frogs collected, 300 (70.75%) were infected with at least one helminth species. *Litoria inermis* and *Limnodynastes ornatus* were the only native frog species collected in sufficient numbers to allow a test between the sexes. No significant difference was found between the sexes of *Lit. inermis* in the numbers infected with at least one helminth species (♂=76.0%, ♀=80.5%, Subadult=80.0%; $F_{2,159}=0.62$, $p=0.5434$), nor for *Lim. ornatus* (♂=57.1%, ♀=59.3%; $F_{2,56}=0.53$, $p=0.5909$). Figure 3.1 presents the distribution of the total number of helminth species per individual frog for all species (except *Mixophyes* sp. and *Litoria lesueuri*). *Litoria inermis* was the most heavily infected native frog species, followed by *Lim. ornatus*, *Lit. caerulea* and *Lit. genimaculata*. Maximum number of helminth species for any frog individual was 4.

Twenty-one helminth species (adult and larval) were collected overall (see Table 3.3). Only three helminth species were unique to frogs: *Parapolystoma* sp., Onchocercidae gen. sp. and *Seuratascaris numidica*. The helminth fauna was dominated by nematodes (11 species)

Table 3.5. Numbers of each sex and range of snout-vent length (mm) for 23 species of native frogs collected in this study.

Frog species	Geographical Location	Sex			Total	SVL	
		Male	Female	Subadult		Min	Max
Crinia deserticola	Bentley	6	2	0	8	14.0	16.0
	Townsville	2	0	0	2	17.0	17.0
Limnodynastes							
convexiusculus	C. York	1	1	0	2	39.0	62.0
ornatus	Abergowrie	0	0	1	1	32.5	
	Bentley	8	5	0	13	24.0	30.5
	Boyne Is.	0	1	0	1	29.0	
	Brisbane	0	1	0	1	39.5	
	Calvert Hills	0	1	0	1	41.0	
	C. Weymouth	1	0	0	1	36.5	
	C. York	0	3	0	3	34.5	37.5
	Coen	4	1	0	5	38	40.0
	MVR	1	1	0	2	30.5	34.5
	Townsville	17	14	0	31	20.5	36.5
peronii	Boyne Is.	4	2	0	6	20.5	35.0
tasmaniensis	Townsville	3	6	0	9	25.5	28.0
terraereginae	Boyne Is.	1	1	1	3	23.0	38.5
Mixophyes sp.	Mt. Lewis	0	1	0	1	80.0	
Sphenophryne robusta	C. York	1	1	0	2	16.5	22.0
Uperoleia lithomoda	Townsville	3	0	0	3	22.5	24.5
Cyclorana							
brevipes	Black Rock	0	0	1	1	21.0	
	Coen	3	1	0	4	44.0	49.0
novaehollandiae	Coen	1	0	0	1	85.5	
	Townsville	1	0	0	1	80.0	
Rana daemelii	C. Weymouth	1	1	0	2	85.5	86.5
	C. York	3	0	0	3	54.5	57.0

/cont.

Table 3.5. (cont.)

Frog species	Geographical Location	Sex			Total	SVL	
		Male	Female	Subadult		Min	Max
Litoria							
alboguttata	Coen	2	0	0	2	62.0	65.5
	MVR	0	1	0	1	51.0	
	Townsville	9	7	0	15	49.0	68.0
bicolor	Boyne Is.	2	1	0	3	18.0	26.0
	Townsville	4	1	0	5	20.5	27.0
caerulea	Abergowrie	5	1	1	7	57.5	96.0
	Townsville	6	1	1	8	42.5	92.0
genimaculata	Paluma	53	1	0	54	31.0	60.0
inermis	Bentley	64	72	5	141	21.0	36.0
	MVR	7	9	0	16	28.5	38.0
	Townsville	4	1	0	5	29.0	34.0
lesueuri	Brisbane	1	0	0	1	41.0	
nasuta	Bentley	0	2	0	2	32.5	34.0
	C. Weymouth	0	1	0	1	45.5	
	Townsville	3	0	0	3	32.0	42.0
nigrofrenata	C. York	2	1	0	3	39.0	41.0
rothii	Abergowrie	1	0	0	1	51.0	
	Bentley	2	0	0	2	44.5	46.5
	Black Rock	1	0	0	1	44.0	
	Townsville	16	1	1	18	26.0	50.5
rubella	Bentley	0	1	0	1	26.0	
	MVR	4	1	0	5	32.5	50.0
	Townsville	17	11	0	28	23.5	42.0
wotjulumensis	Calvert Hills	1	2	0	3	39.0	52.5

followed by digeneans (6 species), cestodes (2 species), and monogeneans and acanthocephalans (1 species each). *Rhabdias* sp. and *Parathelandros mastgurus* were the two most commonly encountered nematodes, and *Mesocoelium* sp. was the most commonly encountered digenean. Mean intensity for all helminth species, except *Mesocoelium* sp., was low.

c) Checklists

Table 3.6 presents a Parasite-Host checklist which records, for each helminth species, the host species, geographical location, prevalence and mean intensity of infection. Most helminth species infected fewer than five host species. *Rhabdias* sp. infected the most host species (15) followed by *Maxvachonia* sp. and *Mesocoelium* sp. (10 each) and *Parathelandros mastgurus* (9). Table 3.7 presents a Host-Parasite checklist which records, for each host species, the helminth species and location within the host it was recovered from.

3.3.2 Taxonomy of Helminths

In total, 27 types of helminths were found to infect amphibians in Australia in this study (see Table 3.3). Of these, 18 were common to both the introduced toad and native frogs, 6 were found to infect toads only and 3 were found in native frogs only.

Johnpearsonia pearsoni and Onchocercidae gen. sp. are recorded from Australia, from any host group, for the first time. *Johnpearsonia pearsoni* is a new genus and species of nematode parasitic in various Australian amphibians (Durette-Desset, M.-Cl. 1993, pers. comm.). Similarly, Onchocercidae gen. sp. is a new genus (Jones, H. 1993, pers. comm.) collected only from *Lit. genimaculata*.

Table 3.6. Parasite-Host checklist of helminths found in Australian Amphibia in this study. For each helminth species, the host species and geographical location is listed with prevalence (Prev) and mean intensity (Mean Int) of infection. *, Frogs collected by Dr Mal Jones at Wildman River Station, Northern Territory (no prevalence or intensity data available).

1. PHYLUM PLATYHELMINTHES

CLASS MONOGENEA

Order Polyopisthocotylea Odhner 1912

Family Polystomatidae Carus 1863, emended Gamble 1896

Subfamily Polystomatinae Gamble 1896

		Prev (%)	Mean Int
Parapolystoma sp.			
Litoria genimaculata	Paluma	50.0	2.1

CLASS TREMATODA Rudolphi 1808

Order Digenea Van Beneden 1858

Family Paramphistomidae Fischoeder 1901

Diplodiscus sp.			
Cyclorana novaehollandiae	Townsville	100.0	10.0
Litoria alboguttata	Townsville	6.3	4.0
Litoria caerulea	Abergowrie	14.3	2.0
*Litoria dahlii**	Wildman R.		
Bufo marinus	Abergowrie	5.9	2.0

Family Telorchiidae Stunkard 1924

Subfamily Opisthioglyphinae Dollfus 1949

Dolichosaccus symmetrus (Johnston 1912) Yamaguti 1958

Litoria inermis	Bentley	0.7	1.0
Bufo marinus	Townsville	2.9	8.9
	Bentley	9.1	8.4
	Boyne Is.	11.5	2.3
	Brisbane	64.7	3.9
	Mackay	16.0	8.8

Dolichosaccus juvenilis (Nicoll 1918) Travassos

	1930		
Bufo marinus	Townsville	3.3	11.3
	Bentley	14.0	11.0
	MVR	1.1	8.0
	Boyne Is.	34.6	7.1
Dolichosaccus helocirrus	Barton 1994		
Litoria caerulea	Abergowrie	14.3	2.0
Bufo marinus	Townsville	3.3	20.6
	Bentley	11.8	3.9
	MVR	21.7	15.5
	Boyne Is.	11.5	17.3
	Mareeba	12.5	4.0

Family Brachycoeliidae Johnston 1912

Mesocoelium sp.			
Limnodynastes ornatus	Boyne Is.	100.0	1.0
Limnodynastes peronii	Boyne Is.	33.3	1.5
Limnodynastes terraereginae	Boyne Is.	33.3	1.0
Cyclorana novaehollandiae	Coen	100.0	24.0
Litoria bicolor	Boyne Is.	33.3	1.0
Litoria caerulea	Townsville	12.5	1.0
Litoria genimaculata	Paluma	40.7	13.6
Litoria inermis	Bentley	2.1	2.0
Rana daemeli	C. Weymouth	50.0	26.0
Bufo marinus	Townsville	13.6	9.5
	Bentley	28.5	6.3
	Calvert Hills	1.4	1.0
	Bloomfield	11.5	1.7
	C. Tribulation	38.5	2.6
	C. Weymouth	28.6	18.4
	Coen	50.0	4.0
	Abergowrie	5.9	1.0
	Mareeba	25.0	1.5
	Ch. Towers	21.1	9.3
	Boyne Is.	61.5	8.6
	Brisbane	64.7	3.9
	Mackay	16.0	8.5
	Port Douglas	63.6	32.3

Family Lecithodendriidae Odhner 1910

Pleurogenoides sp.			
*Litoria dahlii**	Wildman R.		
Litoria inermis	Bentley	12.1	3.1
*Litoria nasuta**	Wildman R.		
*Litoria rothii**	Wildman R.		
*Litoria tornieri**	Wildman R.		
Bufo marinus	Bentley	7.0	5.1
	Ch. Towers	10.5	12.0
	Boyne Is.	3.9	1.0

Family Brachylaimidae Joyeux & Foley 1930

Zeylanurotrema spearei Cribb & Barton 1991

Bufo marinus	Bloomfield	65.4	73.3
	C. Tribulation	38.5	44.4
	C. Weymouth	31.0	6.7

Digenea Not Further Identified

Digenea larva

Limnodynastes ornatus	Bentley	7.7	1.0
Litoria caerulea	Abergowrie	14.3	20.0
Bufo marinus	Townsville	2.6	1.4
	Bloomfield	5.8	1.3
	Mackay	4.0	2.0

CLASS CESTOIDEA

Order Cyclophyllidea Braun 1900

Family Nematotaeniidae Lühe 1910

Nematotaenia hylae Hickman 1960

Cyclorana novaehollandiae	Townsville	100.0	10.0
Litoria alboguttata	Townsville	6.3	1.0
Litoria inermis	Bentley	1.4	1.0
Bufo marinus	Bentley	0.5	1.0

Order Pseudophyllidea Carus 1863

Family Diphyllobothriidae Lühe 1910

Diphyllobothrium sp. spargana

Limnodynastes peronii	Boyne Is.	33.3	1.5
Litoria caerulea	Abergowrie	14.3	1.0
Litoria inermis	Bentley	5.0	3.4
Bufo marinus	Townsville	0.7	2.0
	Bentley	0.5	1.0
	C. Weymouth	2.4	1.0
	Boyne Is.	23.1	3.7
	Mackay	4.0	1.0

2. PHYLUM NEMATODA

CLASS SECERNENTEA

Order Rhabditida

Superfamily Rhabditoidea

Family Rhabdiasidae Railliet 1916

Rhabdias sp.			
Crinia deserticola	Bentley	25.0	3.0
Limnodynastes ornatus	Townsville	16.1	2.0
	Bentley	61.5	3.8
Limnodynastes peronii	Boyne Is.	33.3	1.0
Limnodynastes tasmaniensis	Townsville	22.2	1.0
Mixophyes sp.	Mt. Lewis	100.0	1.0
Uperoleia lithomoda	Townsville	66.7	1.0
Litoria alboguttata	Townsville	50.0	4.4
	MVR	100.0	1.0
Litoria bicolor	Townsville	25.0	7.0
Litoria caerulea	Townsville	50.0	5.0
Litoria inermis	Townsville	60.0	2.0
	Bentley	68.1	2.3
Litoria lesueuri	Brisbane	100.0	3.0
Litoria nasuta	Townsville	33.3	2.0
	Bentley	100.0	3.0
Litoria rothii	Townsville	5.6	2.0
Litoria rubella	Townsville	3.6	1.0
	MVR	20.0	1.0
Bufo marinus	Townsville	80.5	23.6
	Bentley	73.1	10.3
	MVR	2.2	1.5
	Bloomfield	1.9	1.0
	Abergowrie	5.9	1.0
	Mareeba	25.0	1.0
	Mackay	24.0	7.2
	Port Douglas	100.0	42.4

Order Strongylida

Superfamily Trichostrongyloidea

Family Amphibiophilidae Durette-Desset & Chabaud 1981

Johnpearsonia pearsoni Durette-Desset, Ben Slimane, Cassone, Barton, & Chabdaud 1994

Limnodynastes ornatus	Townsville	6.5	14.0
	Bentley	38.5	1.0
	MVR	50.0	7.0
	Coen	20.0	1.0
Mixophyes sp.	Mt. Lewis	100.0	24.0
Uperoleia lithomoda	Townsville	33.3	3.0
Cyclorana novaehollandiae	Townsville	100.0	4.0
	Coen	100.0	2.0
Litoria alboguttata	Townsville	6.3	6.0
	MVR	100.0	1.0
Litoria inermis	Bentley	0.7	1.0
	MVR	6.3	1.0
Litoria rubella	Townsville	3.6	1.0
Bufo marinus	Townsville	1.1	3.3
	Bentley	16.7	3.4
	MVR	34.8	4.3
	C. Weymouth	4.8	1.0

	Coen	25.0	14.0
	Ch. Towers	31.6	1.0

Order Oxyurida

Superfamily Oxyuroidea

Family Pharyngodonidae Travassos 1919

Parathelandros mastgurus Baylis 1930			
Crinia deserticola	Bentley	12.5	1.0
Limnodynastes tasmaniensis	Townsville	11.1	3.0
Mixophyes sp.	Mt. Lewis	100.0	28.0
Litoria caerulea	Townsville	12.5	8.0
	Abergowrie	85.7	8.0
Litoria genimaculata	Paluma	1.9	2.0
Litoria inermis	Townsville	60.0	7.0
	Bentley	27.0	2.7
	MVR	18.8	4.3
Litoria rothii	Townsville	22.2	8.8
	Bentley	100.0	7.0
	Abergowrie	100.0	5.0
Litoria rubella	Townsville	75.0	5.5
	Bentley	100.0	1.0
	MVR	80.0	23.3
Bufo marinus	Bentley	4.8	13.9
	MVR	3.3	3.0
	Bloomfield	5.8	2.0
	C. Tribulation	23.1	18.3
	Abergowrie	5.9	16.0
	Black Rock	14.3	3.0
	Boyne Is.	11.5	2.3

Order Ascaridida

Superfamily Cosmocercoidea

Family Cosmocercidae (Railliet 1916, subfam.) Travassos 1925

Cosmocerca sp. 1			
Uperoleia lithomoda	Townsville	33.3	1.0
Litoria genimaculata	Paluma	3.7	1.0
Litoria rothii	Townsville	11.1	2.5
Litoria rubella	Townsville	3.6	1.0
Rana daemeli	C. Weymouth	100.0	29.0
	C. York	33.3	4.0
Bufo marinus	Townsville	3.7	7.4
	Bentley	1.6	1.0
	MVR	1.1	1.0
	Bloomfield	5.8	1.0
	C. Tribulation	30.8	8.3
	Abergowrie	5.9	1.0
	Port Douglas	18.2	8.5

Cosmocerca sp. 2			
Bufo marinus	Yungaburra	60.0	12.0
Cosmocerca sp. 3			
Bufo marinus	Brisbane	29.4	3.0
Austraplectana sp.			
Bufo marinus	Bloomfield	36.5	27.4
	C. Tribulation	46.2	34.5
	Brisbane	5.9	1.0
Maxvachonia sp.			
Limnodynastes ornatus	Bentley	7.7	1.0
	Calvert Hills	100.0	18.0
Limnodynastes terraereginae	Boyne Is.	33.3	7.0
Litoria bicolor	Townsville	20.0	1.0
Litoria caerulea	Townsville	12.5	16.0
Litoria inermis	Bentley	0.7	4.0
	MVR	6.3	1.0
Litoria nasuta	Bentley	50.0	2.0
Litoria nigrofrenata	C. York	66.7	1.0
Litoria rothii	Townsville	22.2	2.5
Litoria wotjulumensis	Calvert Hills	33.3	1.0
Bufo marinus	Bentley	16.1	3.4
	Calvert Hills	2.8	1.0
	C. Weymouth	16.7	2.4
	Abergowrie	11.8	1.0
	Mareeba	12.5	1.0
	Boyne Is.	3.9	3.0

Superfamily Ascaridoidea

Family Ascarididae Baird 1853

Seuratascaris numidica (Seurat 1917) Sprent 1985

Litoria nasuta	C. Weymouth	100.0	1.0
Rana daemeli	C. Weymouth	50.0	1.0

Superfamily Heterakoidea

Family Heterakidae Railliet & Henry 1912

Subfamily Spinicaudinae Travassos 1920

Spinicauda sp.			
Limnodynastes convexiusculus	C. York	50.0	3.0
Bufo marinus	C. Weymouth	42.9	6.0

Order Spirurida

Superfamily Physalopteroidea

Family Physalopteridae (Railliet 1893, subfam.) Leiper 1908

Kreisiella sp.			
Sphenophryne robusta	C. York	50.0	1.0
Bufo marinus	C. Weymouth	14.3	3.0

Superfamily Filarioidea

Family Onchocercidae (Leiper 1911)

Onchocercidae gen. sp.			
Litoria genimaculata	Paluma	3.7	3.5

Nematoda Not Further Identified

Nematoda			
Limnodynastes ornatus	Townsville	32.3	14.3
	Bentley	23.1	9.7
	Coen	100.0	5.4
	C. Weymouth	100.0	6.0
Limnodynastes peronii	Boyne Is.	50.0	3.3
Cyclorana brevipes	Coen	25.0	1.0
Litoria caerulea	Townsville	62.5	3.2
Litoria inermis	Bentley	2.1	2.3
Litoria lesueuri	Brisbane	100.0	15.0
Litoria nasuta	Townsville	33.3	1.0
Bufo marinus	Townsville	1.8	1.2
	Bentley	4.8	2.2
	MVR	2.2	1.0
	Bloomfield	1.9	1.0
	C. Tribulation	7.7	1.0
	C. Weymouth	2.4	2.0
	Brisbane	11.8	2.0
Nematode larvae			
Litoria alboguttata	Townsville	6.3	10.0
Litoria caerulea	Townsville	12.5	5.0
Litoria genimaculata	Paluma	1.9	1.0
Litoria inermis	Bentley	2.8	1.3
Litoria lesueuri	Brisbane	100.0	1.0
Bufo marinus	Bentley	8.6	6.3
	Calvert Hills	37.5	10.9
	Bloomfield	7.7	14.0
	C. Weymouth	4.8	10.0
	Abergowrie	5.9	10.0
	Mareeba	12.5	23.0
	Black Rock	28.6	14.0
	Ch. Towers	5.3	1.0

3. PHYLUM ACANTHOCEPHALA

Order Polymorphida Petrochenko 1956

Family Plagiorhynchidae Golvan 1960

Subfamily Porrorchinae Golvan 1956

Porrorchis hylae (Johnston 1914) Schmidt & Kuntz 1967

Limnodynastes ornatus	C. York	33.3	2.0
Bufo marinus	Bentley	0.5	2.0
	Bloomfield	1.9	1.0
	Boyne Is.	3.9	1.0
	Brisbane	23.5	3.5
	Mackay	4.0	2.0

Acanthocephala Not Further Identified

Acanthocephala

Bufo marinus	Bloomfield	13.5	1.2

Table 3.7. Host-Parasite checklist for helminths found in Australian Amphibia in this study. Under each host species is listed the helminth species and its location within the host. Helminths marked with an * represent a new host record for that helminth species; where that helminth is unidentified (e.g. Nematoda) * indicates that the host species has never had that helminth group recorded from it. A, Acanthocephala; C, Cestoda; D, Digenea; M, Monogenea; N, Nematoda.

CLASS AMPHIBIA

Order Anura

Family Myobatrachidae

Crinia deserticola (Liem & Ingram 1977)
N *Rhabdias* sp. (lung)*
Parathelandros mastgurus (rectum)*

Limnodynastes convexiusculus (Macleay 1877)
N *Spinicauda* sp. (rectum)*

Limnodynastes ornatus (Gray 1842)
D *Mesocoelium* sp. (small intestine)*
Digenea larva (mesentery)*
N *Rhabdias* sp. (lung)*
Johnpearsonia pearsoni (intestine)*
Maxvachonia sp. (intestine, rectum)*
Nematoda (lungs, intestine, body cavity)*
A *Porrorchis hylae* cyst (stomach wall)*

Limnodynastes peronii (Duméril & Bibron 1841)
D *Mesocoelium* sp. (small intestine)*
C *Diphyllobothrium* sp. spargana (leg musculature)
N *Rhabdias* sp. (lung)
Nematoda (body cavity)

Limnodynastes tasmaniensis Günther 1858
N *Rhabdias* sp. (lung)
Parathelandros mastgurus (rectum)*

Limnodynastes terraereginae Fry 1915
D *Mesocoelium* sp. (small intestine)*
N *Maxvachonia* sp. (rectum)*

Mixophyes sp.
N *Rhabdias* sp. (lung)*
Johnpearsonia pearsoni (intestine)*
Parathelandros mastgurus (intestine)*

Uperoleia lithomoda Tyler, Davies & Martin 1981
N *Rhabdias* sp. (lung)*

Johnpearsonia pearsoni (intestine)*
Cosmocerca sp. 1 (rectum)*

Family Hylidae

Cyclorana brevipes (Peters 1871)
N Nematoda (body cavity)*

Cyclorana novaehollandiae Steindachner 1867
D *Diplodiscus* sp. (rectum)*
Mesocoelium sp. (small intestine)*
C *Nematotaenia hylae* (intestine)
N *Johnpearsonia pearsoni* (intestine)*

Litoria alboguttata (Günther 1867)
D *Diplodiscus* sp. (rectum)*
C *Nematotaenia hylae* (intestine)*
N *Rhabdias* sp. (lung)*
Johnpearsonia pearsoni (intestine)*
Nematoda larva (mesentery)*

Litoria bicolor (Gray 1842)
D *Mesocoelium* sp. (small intestine)*
N *Rhabdias* sp. (lung)*
Maxvachonia sp. (rectum)*

Litoria caerulea (White 1790)
D *Diplodiscus* sp. (rectum)
Dolichosaccus helocirrus (small intestine)*
Mesocoelium sp. (small intestine)
Digenea larva (stomach wall)*
C *Diphyllobothrium* sp. spargana (leg musculature)
N *Rhabdias* sp. (lung)
Parathelandros mastgurus (rectum)
Maxvachonia sp. (rectum)
Nematoda (intestine)
Nematode larva (mesentery)

Litoria dahlii (Boulenger 1896)
D *Diplodiscus* sp.*
Pleurogenoides sp.*

Litoria genimaculata (Horst 1883)
M *Parapolystoma* sp. (urinary bladder)*
D *Mesocoelium* sp. (small intestine)*
N *Parathelandros mastgurus* (rectum)*
Cosmocerca sp. 1 (rectum)*
Onchocercidae gen. sp. (body cavity)*
Nematode larva (mesentery)*

Litoria inermis (Peters 1867)
D *Dolichosaccus symmetrus* (small intestine)*

Mesocoelium sp. (small intestine)*
Pleurogenoides sp. (small intestine)*
C *Nematotaenia hylae* (intestine)
Diphyllobothrium sp. spargana (leg musculature)*
N *Rhabdias* sp. (lung)*
Johnpearsonia pearsoni (intestine)*
Parathelandros mastgurus (rectum)*
Maxvachonia sp. (rectum)*
Nematoda (intestine)*
Nematode larva (mesentery)*

Litoria lesueuri (Duméril & Bibron 1841)
N *Rhabdias* sp. (lung)
Nematoda (body cavity)
Nematode larva (mesentery)*

Litoria nasuta (Gray 1842)
D *Pleurogenoides* sp.
N *Rhabdias* sp. (lung)
Maxvachonia sp. (rectum)
Seuratascaris numidica (intestine)*
Nematoda (body cavity)

Litoria nigrofrenata (Günther 1867)
N *Maxvachonia* sp. (rectum)*

Litoria rothii (De Vis 1884)
D *Pleurogenoides* sp.*
N *Rhabdias* sp. (lung)*
Parathelandros mastgurus (rectum)*
Cosmocerca sp. 1 (rectum)*
Maxvachonia sp. (intestine)*

Litoria rubella (Gray 1842)
N *Rhabdias* sp. (lung)*
Johnpearsonia pearsoni (intestine)*
Parathelandros mastgurus (rectum)*
Cosmocerca sp. 1 (rectum)*

Litoria tornieri (Nieden 1923)
D *Pleurogenoides* sp.*

Litoria wotjulumensis (Copland 1957)
N *Maxvachonia* sp. (rectum)*

Sphenophryne robusta (Fry 1912)
N *Kreisiella* sp. (intestine)*

Family Ranidae

Rana daemeli (Steindachner 1868)
D *Mesocoelium* sp. (small intestine)*
N *Cosmocerca* sp. 1 (rectum)*

Seuratascaris numidica (intestine)

Family Bufonidae

Bufo marinus (Linneaus 1758)
D *Diplodiscus* sp. (rectum)
Dolichosaccus symmetrus (small intestine)
Dolichosaccus juvenilis (small intestine)*
Dolichosaccus helocirrus (small intestine)*
Mesocoelium sp. (small intestine)
Pleurogenoides sp. (small intestine)*
Zeylanurotrema spearei (urinary bladder)
Digenea larva (intestine, rectum)
C *Diphyllobothrium* sp. spargana (leg musculature, intestinal wall)
N *Rhabdias* sp. (lung)*
Johnpearsonia pearsoni (intestine)*
Parathelandros mastgurus (intestine, rectum)
Cosmocerca sp. 1 (rectum)*
Cosmocerca sp. 2 (rectum)*
Cosmocerca sp. 3 (rectum)*
Austraplectana sp. (rectum)*
Maxvachonia sp. (intestine, rectum)
Spinicauda sp. (rectum)*
Kreisiella sp. (intestine)*
Nematoda (rectum, intestine)
Nematode larva (mesentery, stomach wall)
A Acanthocephala (intestine)
Porrorchis hylae cyst (mesentery)

Zeylanurotrema spearei was recorded as a new species from toads in Australia by Cribb and Barton (1991).

Dolichosaccus helocirrus was recorded as a new species parasitic in *B. marinus* and *Litoria caerulea* by Barton (1994a).

Drawings of all helminths are presented in Figures 3.3-3.17, except for those presently being described by other workers (*Parapolystoma* sp. and Onchocercidae gen. sp.), those previously described with no alteration to that description (*Z. spearei*, *S. numidica*) and all larval and unknown adult helminths. Helminths designated as unknown were usually in too poor a condition to allow identification and drawing. Descriptions and tables of comparative measurements for these helminths are presented in Appendix 5.

3.3.3 Origins of helminths found in *Bufo marinus* in Australia.

Of the helminth species found to infect *B. marinus* in Australia in this study, 14 have a definite Australian origin, 2 have a possible Australian origin, and 8 have an unknown origin. No helminths were determined as of South American origin.

Of the helminths with a definite Australian origin, 5 were identified as species which had previously been described from native fauna: *Dolichosaccus symmetrus*, *Dolichosaccus juvenilis*, *Porrorchis hylae*, *Parathelandros mastgurus*, and *Nematotaenia hylae*. A further six helminths with a definite Australian origin, *Dolichosaccus helocirrus*, *Pleurogenoides* sp., *Diplodiscus* sp., *Maxvachonia* sp., *Kreisiella* sp., and *Austraplectana* sp. fulfil the criteria of Section 3.2.3 in that the genus has only been recorded in Australia. A further three helminths, all larval, were included in

Figure 3.3 *Diplodiscus* sp., wholemount, collected from *Bufo marinus*, ventral view.

Scale bar: 150µm.

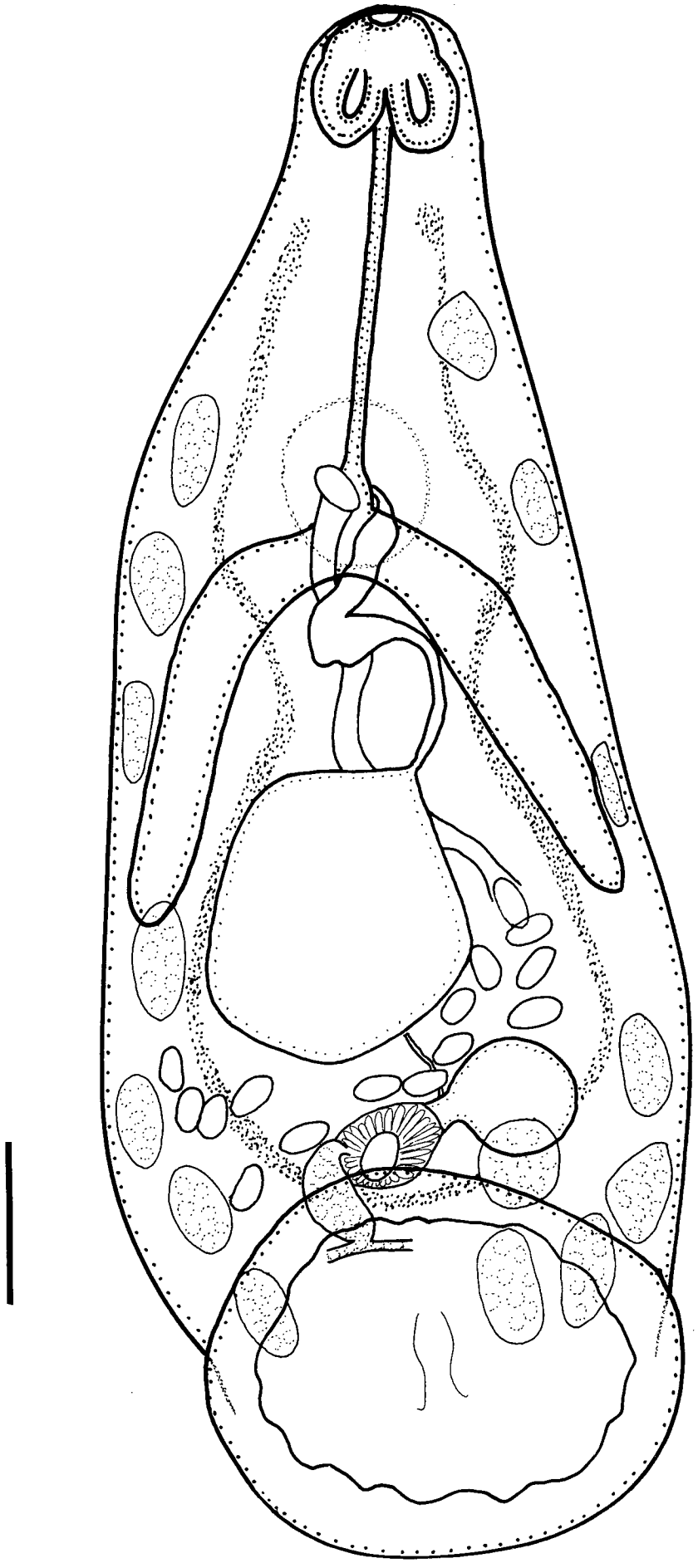


Figure 3.4 *Dolichosaccus symmetrus*, wholemount, collected from *Bufo marinus*, ventral view.

Scale bar: 750µm.

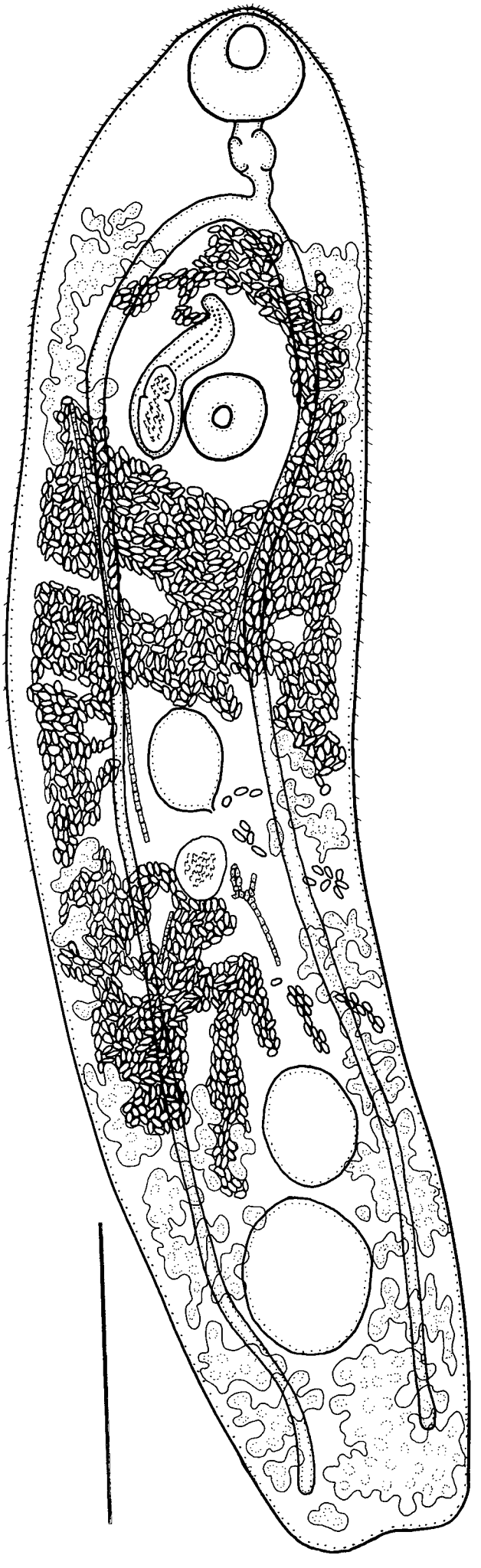


Figure 3.5 *Dolichosaccus juvenilis*, wholemount, collected from *Bufo marinus*, ventral view.

Scale bar: 300µm.

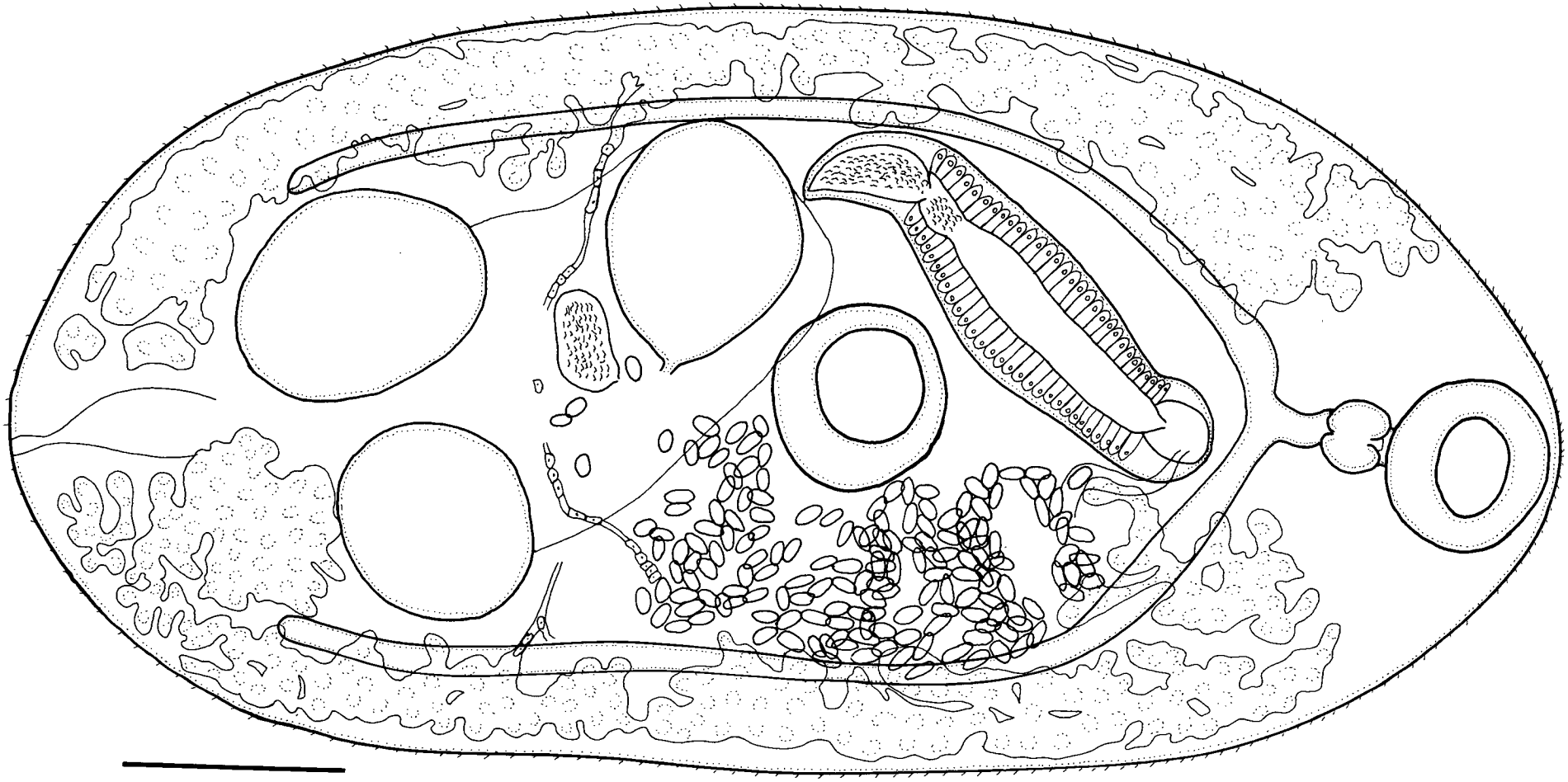


Figure 3.6 *Dolichosaccus helocirrus*, wholemount, collected from *Bufo marinus*, ventral view.

Scale bar: 300µm.

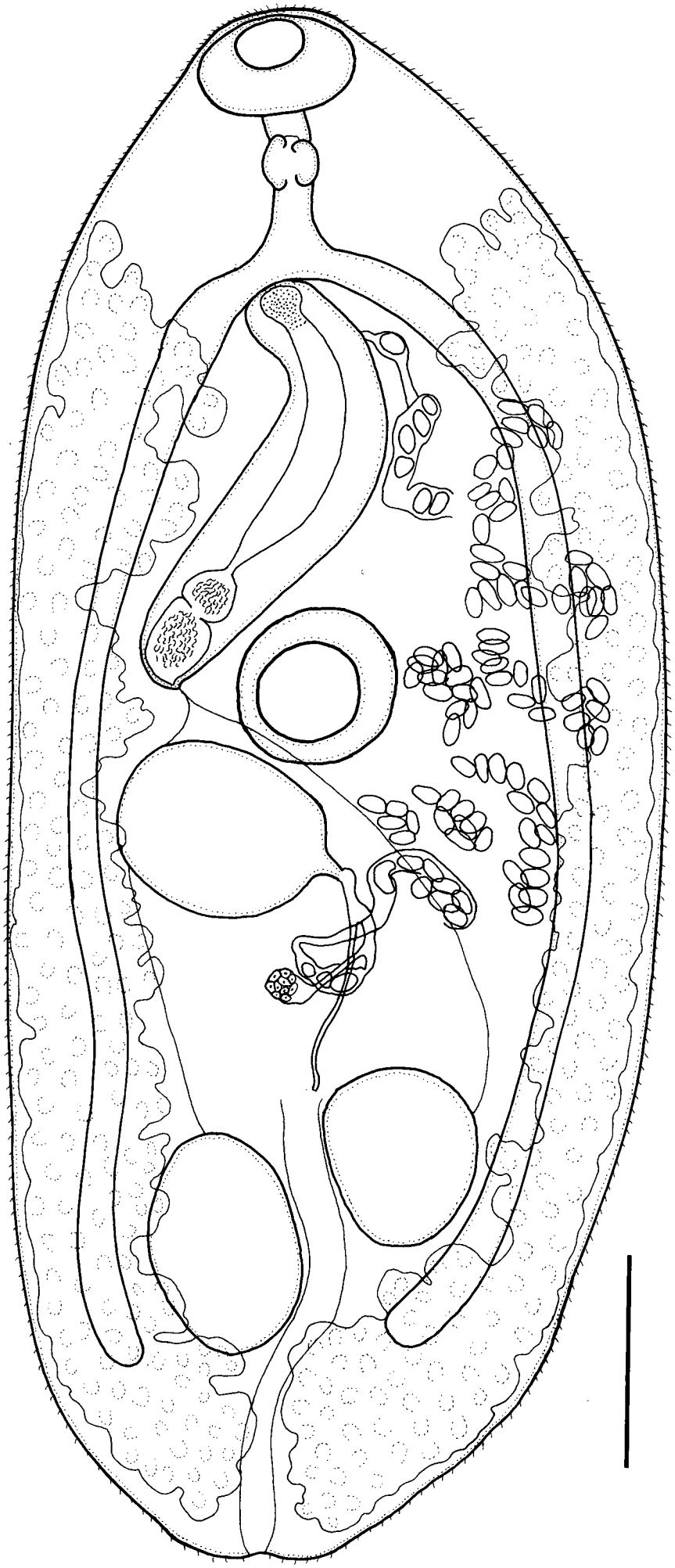


Figure 3.7 *Mesocoelium* sp., wholemount, collected from *Bufo marinus*, ventral view.

Scale bar: 150µm.

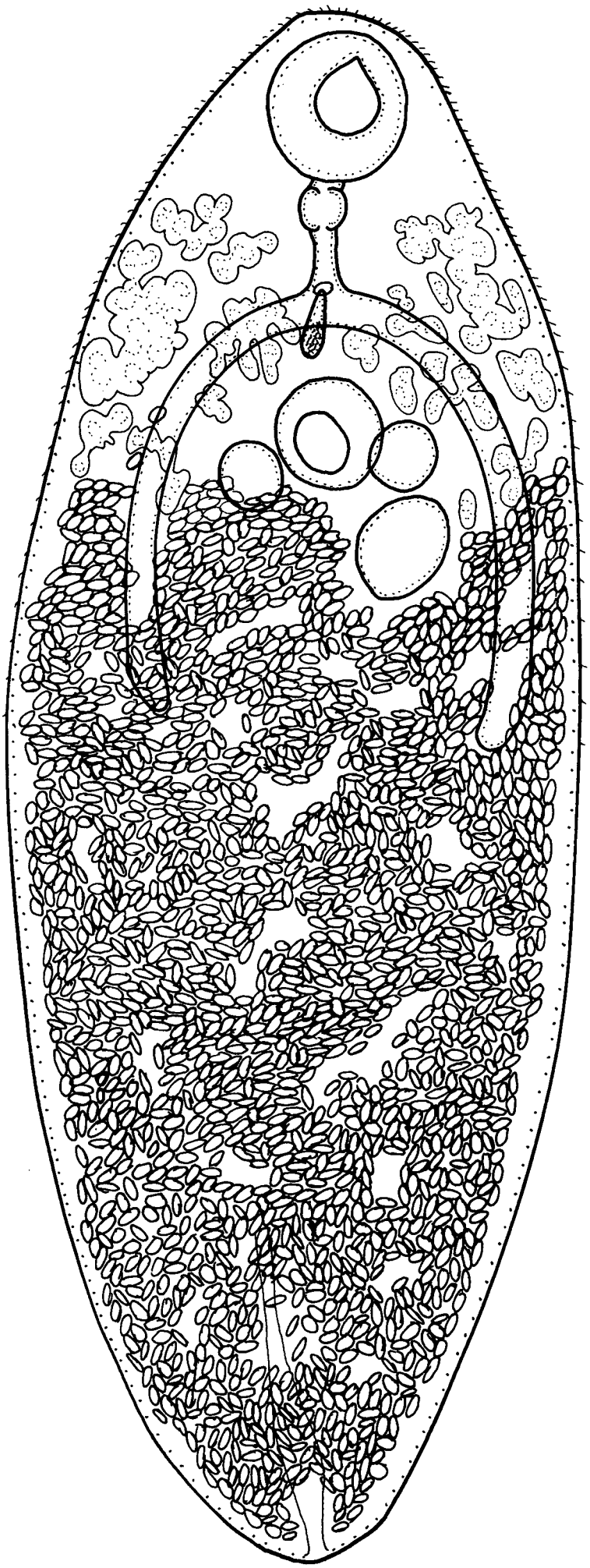


Figure 3.8 *Pleurogenoides* sp., wholemount, collected from *Bufo marinus*, ventral view.

Scale bar: 100µm.

Figure 3.9 *Nematotaenia hylae* scolex, wholemount, collected from *Cyclorana novaehollandiae*, ventral view.

Scale bar: 100µm.

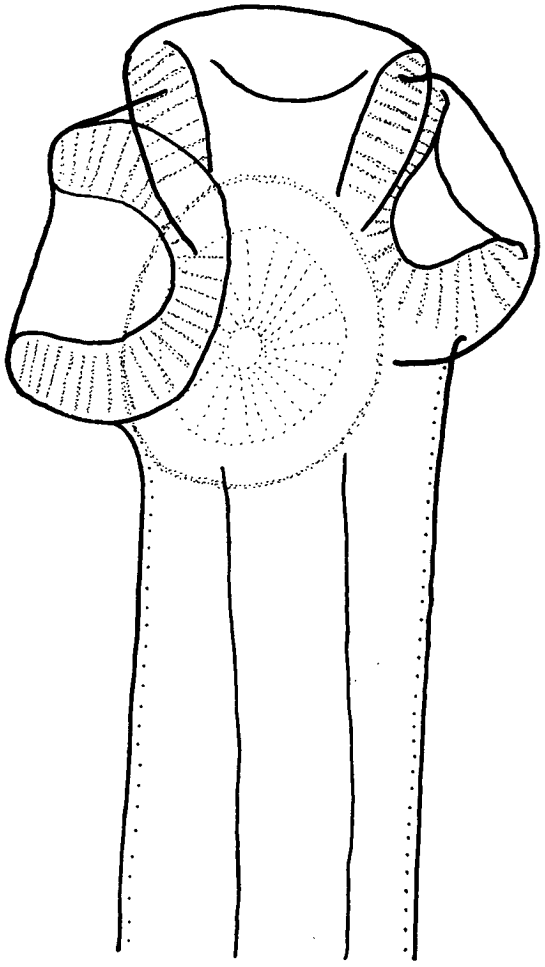


Figure 3.10 Parasitic stage of *Rhabdias* sp., wholemount, collected from *Bufo marinus*.

a, anterior end.

b, posterior end.

Scale bar: 100µm.

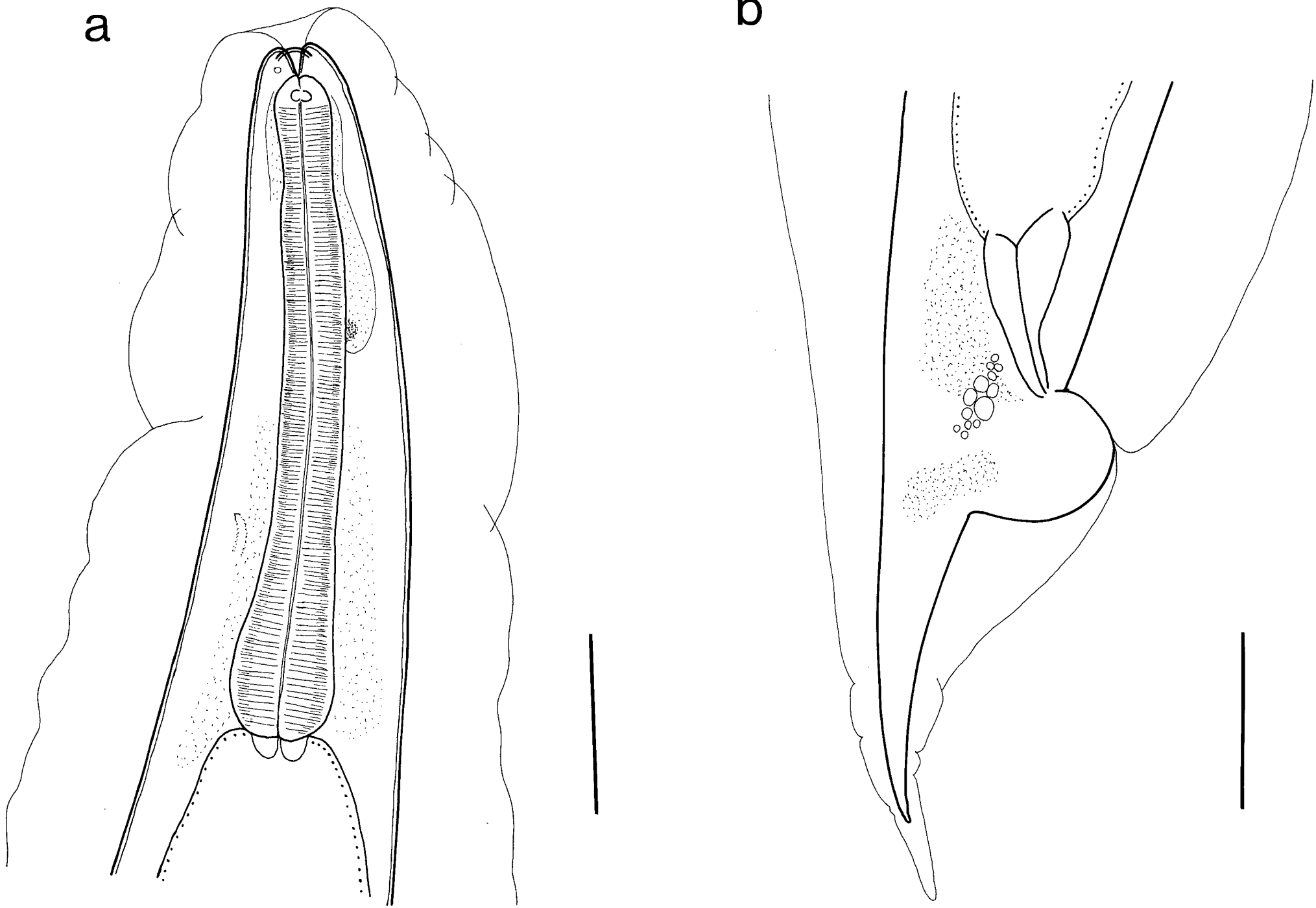

a
b

Figure 3.11 *Johnpearsonia pearsoni*, wet preparation, collected from *Bufo marinus*.

a, anterior end of female, lateral view.

b, tail of female, lateral view.

c, caudal bursa of male, ventral view.

Scale bars: a, 100µm; b, 100µm; c, 150µm.

a, alae; s, spicules; v, vulva.

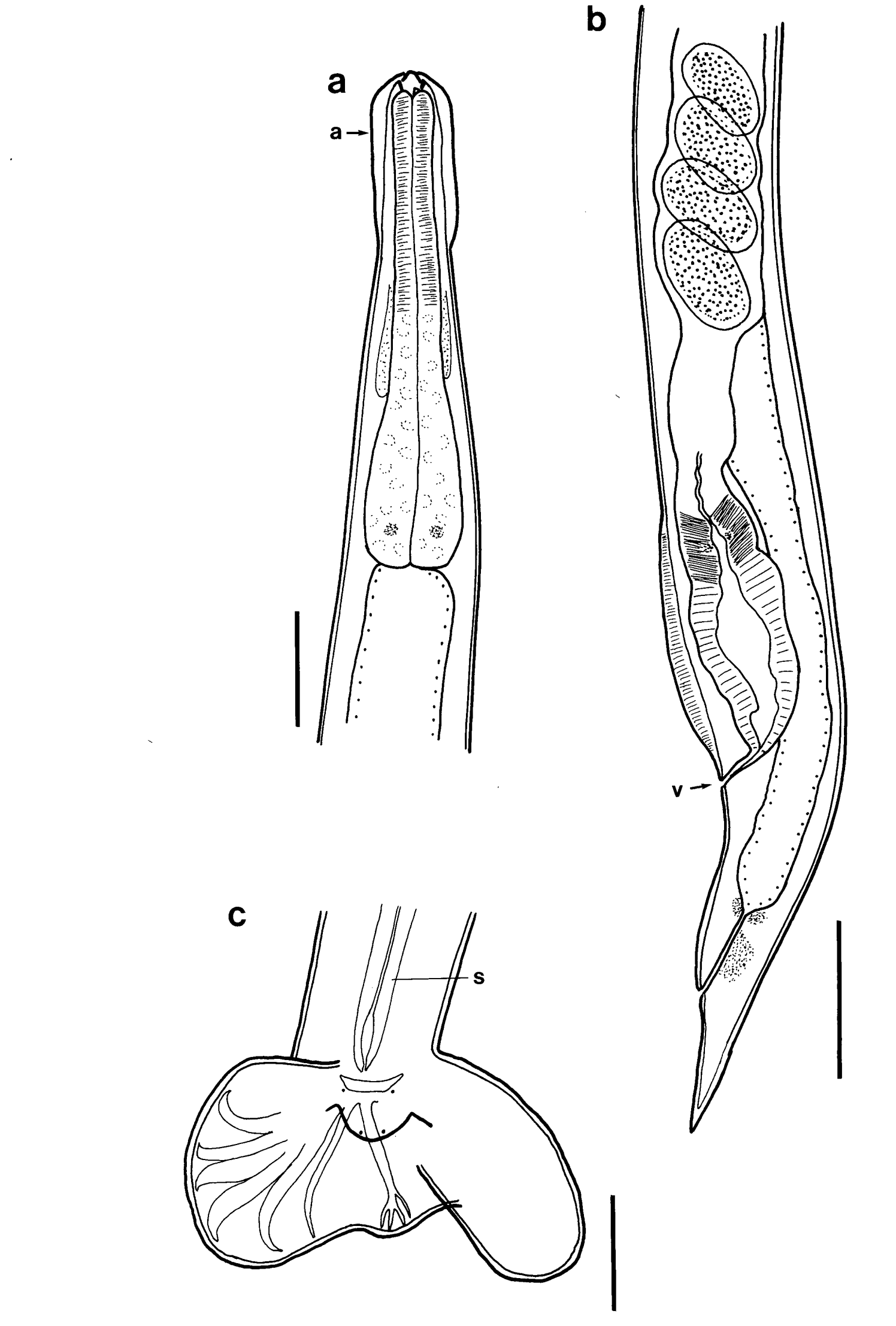

a
a
b
v
c
s

Figure 3.12 *Parathelandros mastgurus*, wet preparation, collected from *Bufo marinus*.

a, anterior end of female, lateral view.
b, tail of female, lateral view.
c, anterior end of male, lateral view.
d, tail of male, lateral view.

Scale bars: a, 100µm; b, 400µm; c, 100µm; d, 100µm.

s, spicules; v, vulva.

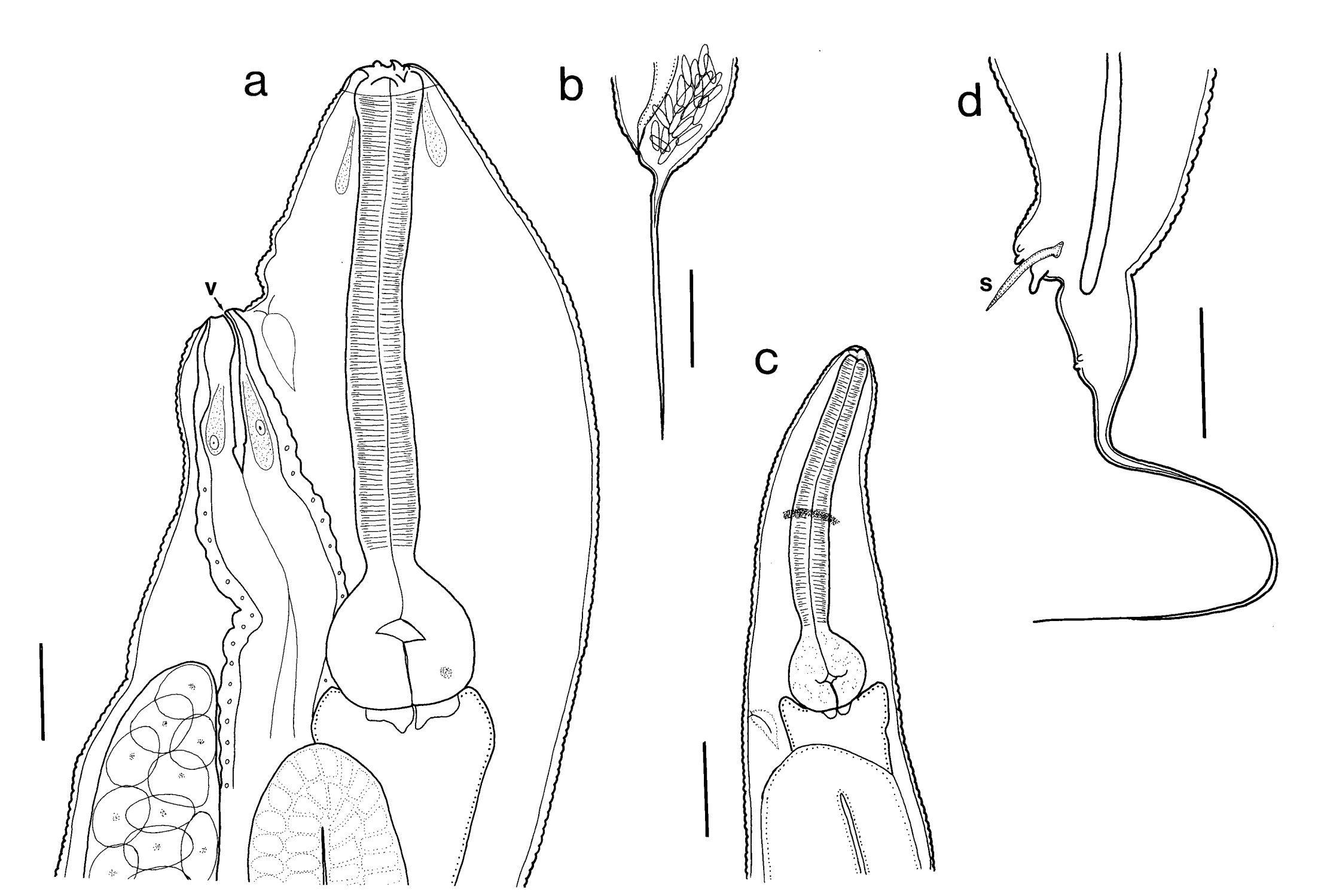

a
b
c
d
v
s

Figure 3.13 *Cosmocerca* spp., wet preparation, collected from *Bufo marinus*.

a, anterior end of female *Cosmocerca* sp. 1, lateral view.

b, anterior end of male *Cosmocerca* sp. 3, lateral view.

c, tail of male *Cosmocerca* sp. 3, lateral view.

Scale bars: a, 100µm; b, 100µm; c, 200µm.

e, excretory pore; p, plectane.

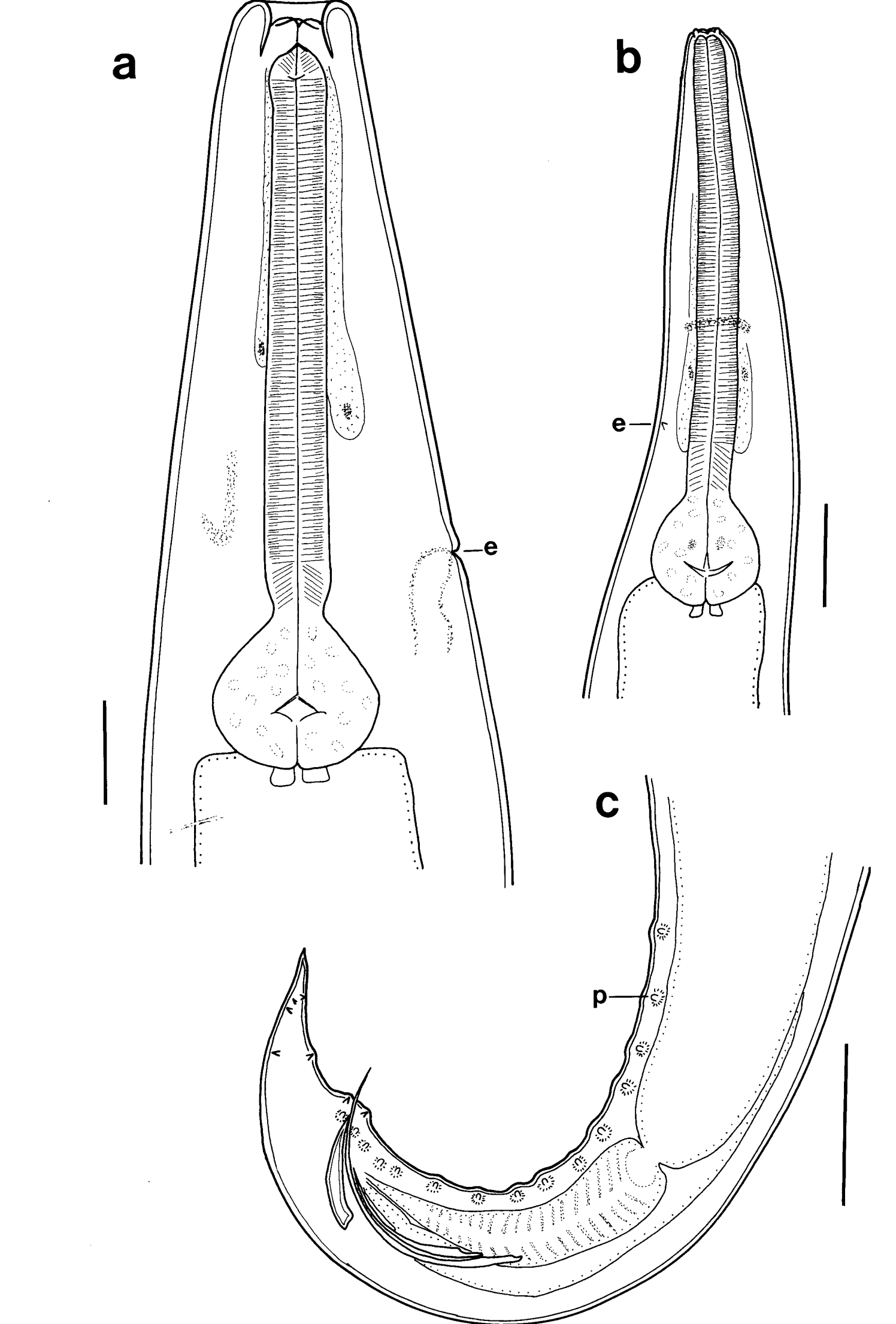

a
e
b
e
c
p

Figure 3.14 *Austraplectana* sp., wet preparation, collected from *Bufo marinus*.

a, whole female specimen, lateral view.

b, anterior end of male, lateral view.

c, tail of male, lateral view.

Scale bars: a, 200µm; b, 50µm; c, 200µm.

s, spicule; v, vulva.

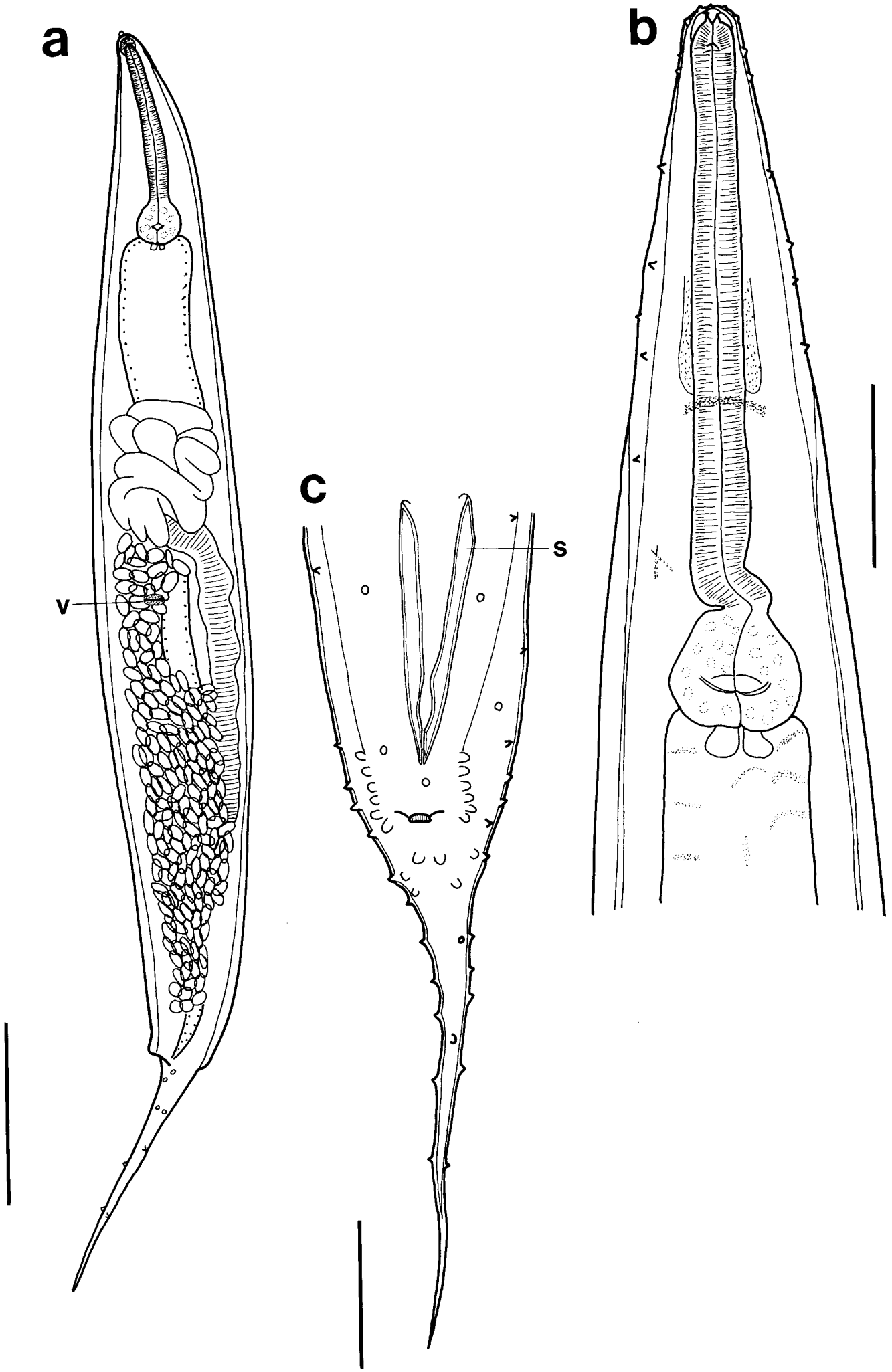
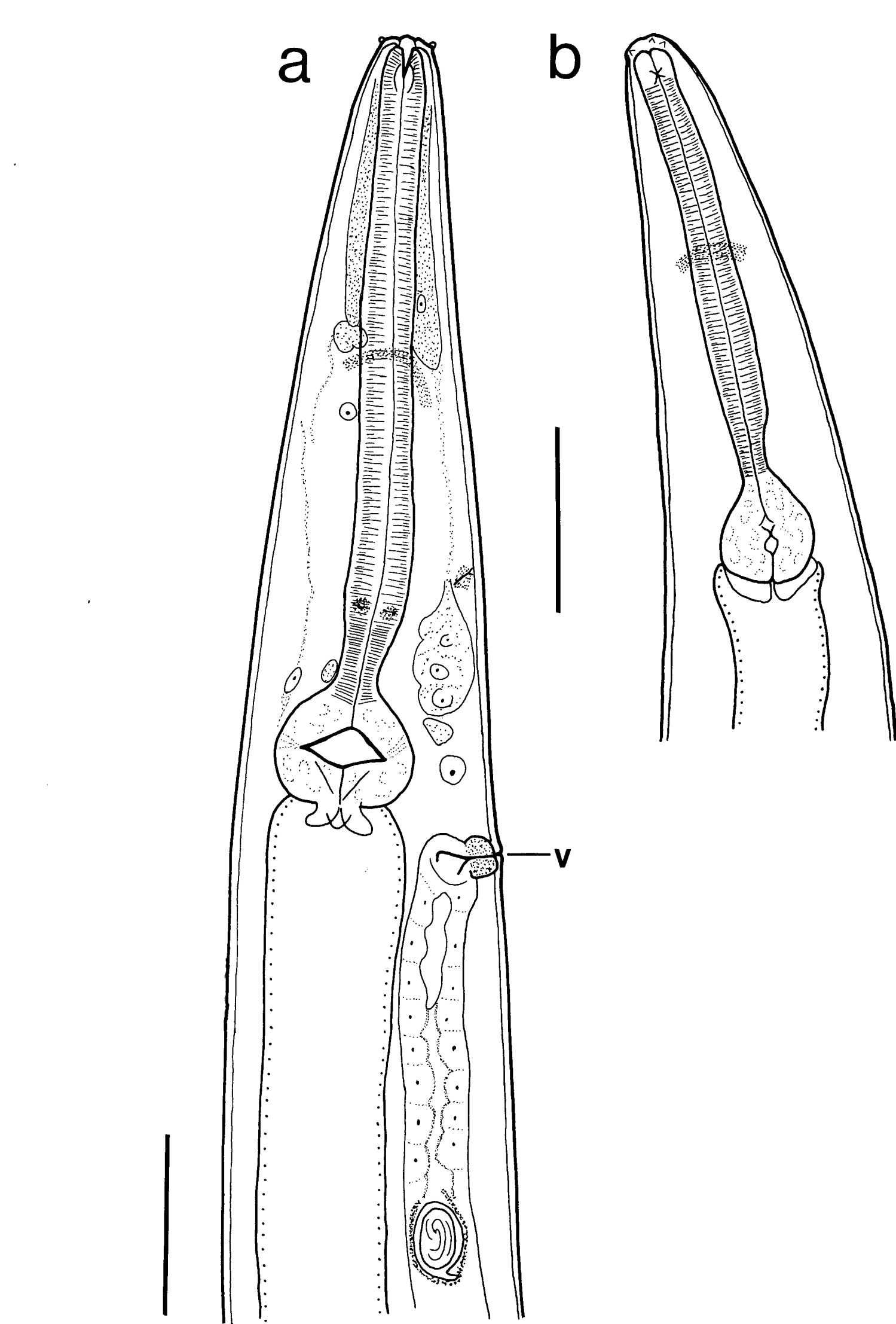

a
v
b
c
s

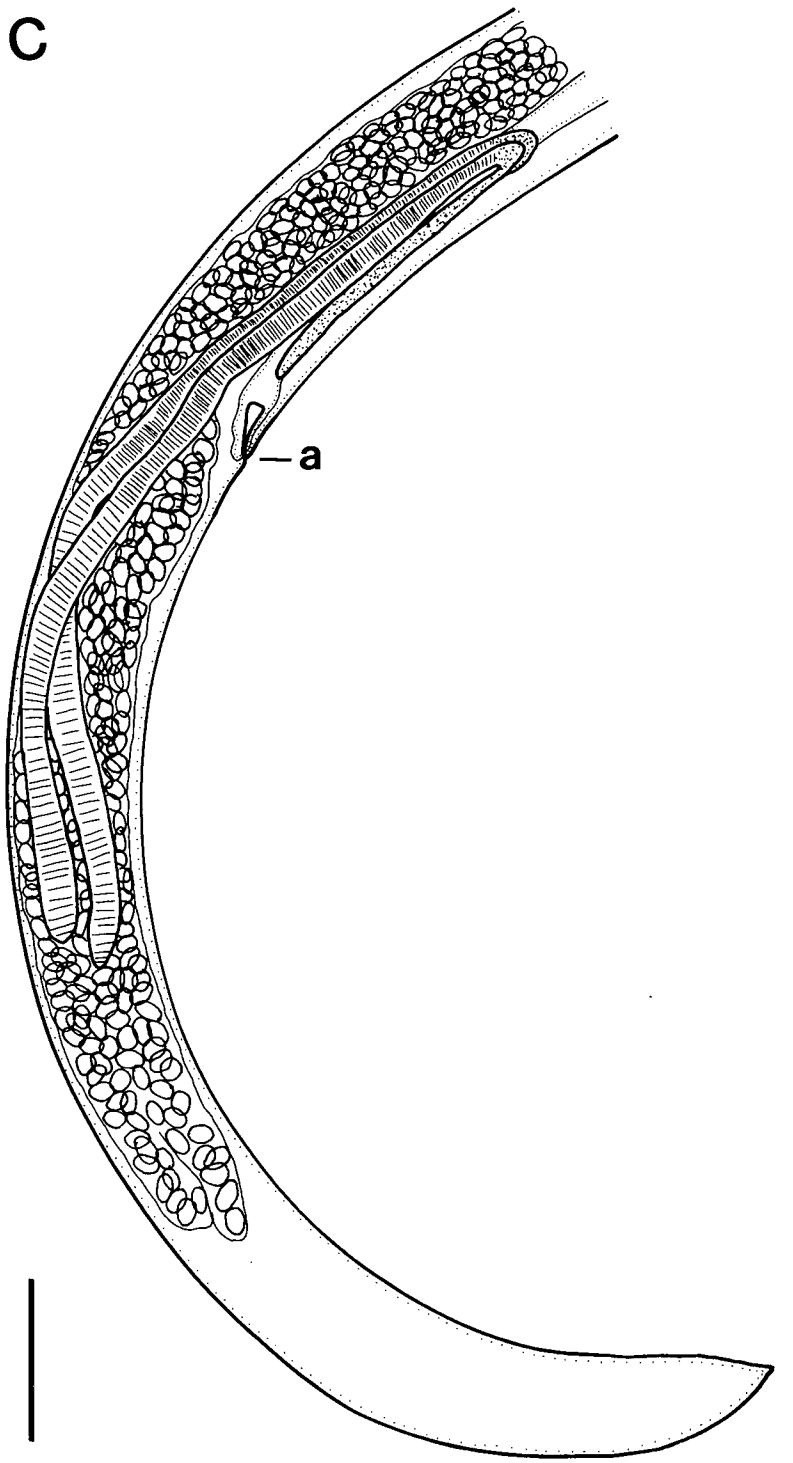
Figure 3.15 *Maxvachonia* sp., wet preparation, collected from *Bufo marinus* (female specimen) and *Litoria rothii* (male specimen).

a, anterior end of female, lateral view.
b, tail of female, lateral view.
c, anterior end of male, lateral view.
d, tail of male, lateral view.

Scale bars: a, 200µm; b, 400µm; c, 200µm; d, 100µm.

a, anus; g, gubernaculum; v, vulva.


a
b
v


c
a

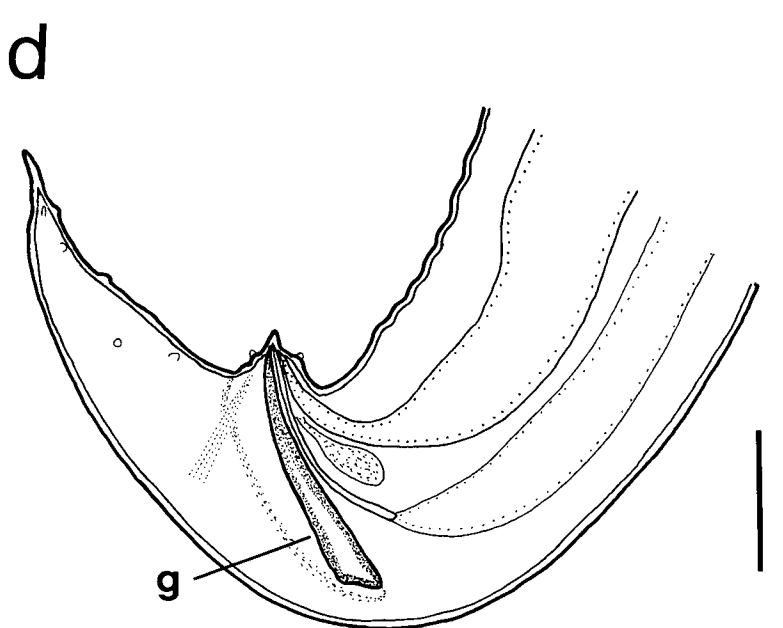

d
g

Figure 3.16 *Spinicauda* sp., wet preparation, collected from *Bufo marinus*.

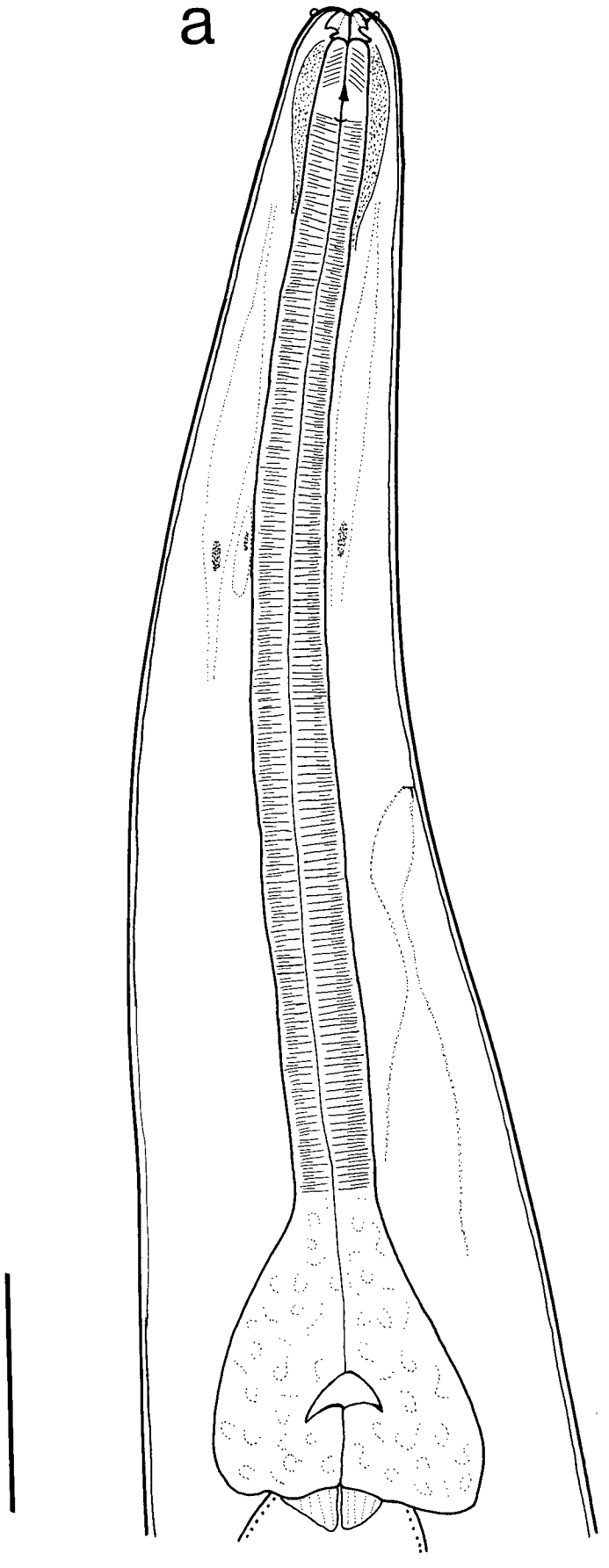
a, anterior end of female, lateral view.

b, tail of male, lateral view.

c, tail of female, lateral view.

Scale bars: a, 200µm; b, 200µm; c, 200µm.

p, preanal sucker.


a

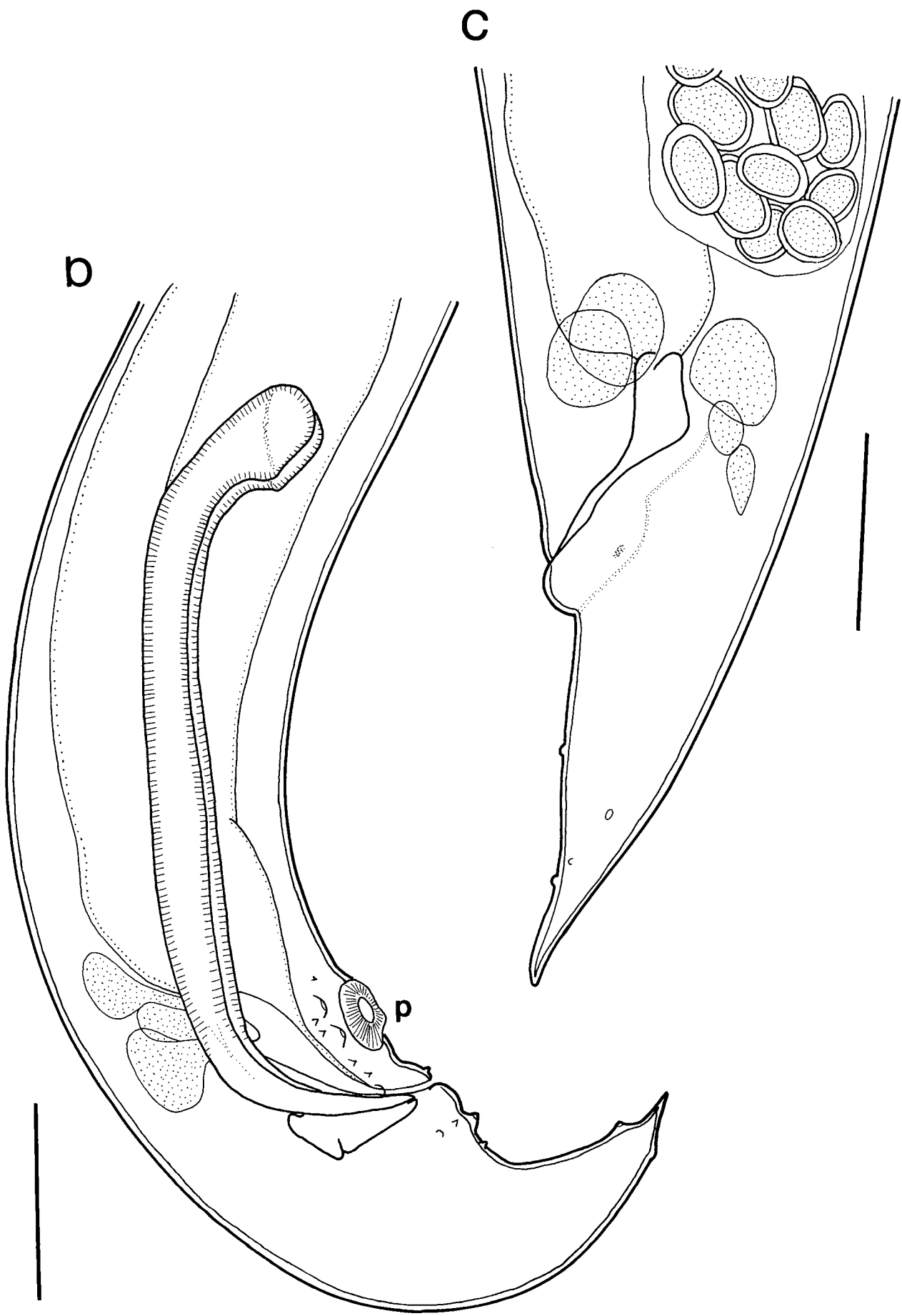
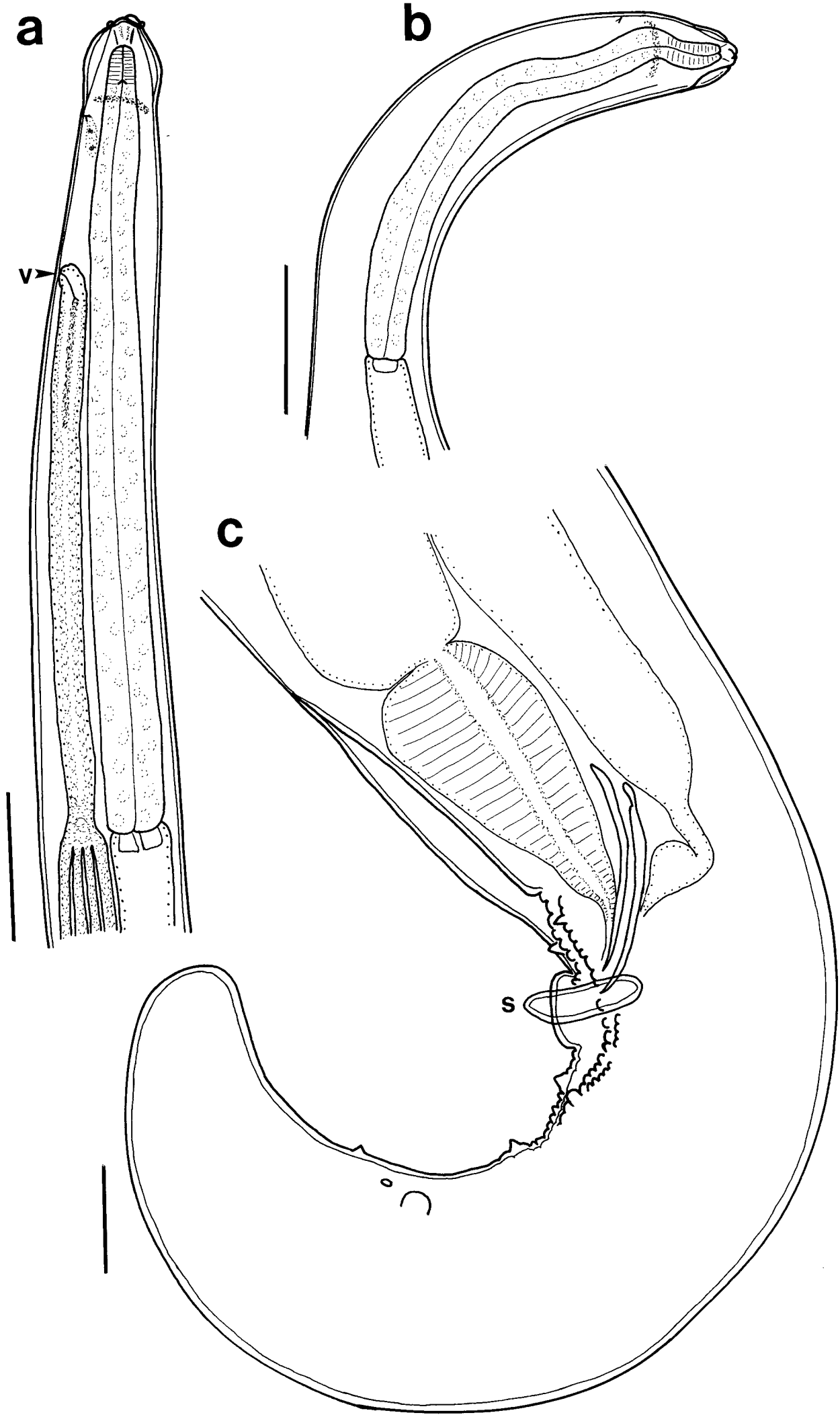

c
b
p

Figure 3.17 *Kreisiella* sp., wet preparation, collected from *Bufo marinus*.

a, anterior end of female, lateral view.
b, anterior end of male, lateral view.
c, tail of male, lateral view.

Scale bars: a, 500µm; b, 500µm; c, 100µm.
s, spicule; v, vulva.


a
v
b
c
s

this group due to their life cycle stage: *Diphyllobothrium* sp. spargana, Digenea larva, Nematoda larva.

Zeylanurotrema spearei and *Johnpearsonia pearsoni* are determined as having a possible Australian origin as they have never been recorded in Australia or South America previously, but related species have been recorded in the Australopapuan/Asian region.

Of the 8 helminths with an unknown origin, 2 were determined as such due to an inability to identify them further than Phylum or Class (Acanthocephala and Nematoda). The genus *Spinicauda* has been recorded to infect reptilian hosts in both South America and Australia (see Jones 1979). The remaining 3 helminth genera (*Rhabdias*, *Mesocoelium*, *Cosmocerca*; 5 species) have been recorded to infect both toads in South America and native fauna in Australia and identification of all these helminths to species is required before a decision can be made as to their origin.

3.4 Discussion

This is the first detailed survey of the helminth parasites of Australian Amphibia, including the introduced toad *Bufo marinus*. Previous workers (Freeland *et al.* 1986; Speare 1990) have surveyed toads for the presence of disease agents, including helminths, but have not identified the components of the helminth fauna or compared it to the helminth fauna of native frogs. Until this project, the helminth fauna of toads in Australia was virtually unknown.

The collection of toads covered as wide a geographical area as possible. Unfortunately, large samples could not be collected from all areas due to the

opportunistic sampling methods. Toads in northern Queensland were, however, well sampled and prevalence and intensity of infection data is probably a better reflection of population characteristics than samples from southern Queensland. Similarly, frog samples could have, ideally, been larger. Smaller size (compare Tables 3.2 and 3.5), better camouflage, and collection restrictions due to government protection, all combined to keep samples small. In addition, lack of public or moral objections to killing toads usually led to an `over-abundance' of toads for dissection, while the dissection of large numbers of frogs was not looked on in the same light.

Male amphibians were encountered, and subsequently collected, more times than female and subadult specimens. The relationship between sex and age of host with development of the helminth fauna of amphibians, in general, is poorly understood. Male, female, and sometimes even subadult, amphibians tend to inhabit different habitats and eat different diets (Richards, S.J. 1993, pers. comm.) and, as a consequence, can develop different helminth faunas (see Lees 1962; Kuc & Sulgostowska 1988a; Goldberg & Bursey 1991c; Muzzall 1991b). Host diet and habitat preference are important factors influencing the transmission dynamics of helminths and their distribution among host individuals (Goater *et al.* 1987). This aspect is discussed in detail in Chapter 4.

Helminths infected at least 70% of toads and native frogs collected in this study. This level is similar to results found for other amphibian species. For example, 51.9% of *Bufo debilis debilis*, 87% of *B. valliceps valliceps*, 75% of *B. woodhousii woodhousii* (McAllister *et al.* 1989), 97% of *B. alvarius*, and 67% of *B. cognatus* (Goldberg & Bursey 1991a) were infected with helminths. Only 38% and 53%, respectively, of the North American

Hylid, *Pseudachris crucifer crucifer*, and Ranid, *Rana sylvatica*, were infected with helminths (Muzzall & Peebles 1991). Adult salamanders were more variable in the number of animals infected with helminths (53% of *Plethodon cinereus cinereus*, Muzzall 1990; 88.9% of *Ambystoma laterale*, Muzzall & Schinderle 1992). Goldberg & Bursey (1991c) found only 23% of female *B. punctatus*, but 75% of males, to be infected with helminths. Reasons for these varying prevalence of infections are discussed in detail in Chapter 4.

Maximum number of helminth species per host individual differed between toads and native frogs; toads had higher intensity of infection. Although other studies have detailed the total number of helminth species collected from a host species, few have listed the maximum number of helminth species from an individual host. Aho (1990) recorded the average number of helminth species per host individual as 3.54 for anurans and 2.76 for salamanders. The results of this study are above average for anurans.

Nematodes were the predominant helminth type found in both toads and frogs in this study. Digenea were quite common, while cestodes, acanthocephalans and monogeneans were rarely encountered. Nematodes dominated the helminth fauna of *B. alvarius*, *B. cognatus* and *Scaphiopus couchii* studied by Goldberg and Bursey (1991a). Cestodes were the next most frequently encountered helminth group. However, *Nematotaenia dispar* (Cestoda) had the highest prevalence of infection in *B. alvarius*, but *Aplectana itzocanensis* (Nematoda) had the highest mean intensity. The three *Bufo* species studied by McAllister *et al.* (1989) were all infected with only 2 helminth species. *Pseudachris c. crucifer* had a helminth fauna dominated by nematodes, but the only trematode, *Glypthelmins pennsylvaniensis*, had the highest prevalence and mean intensity (Muzzall & Peebles

1991). Nematodes were the most commonly encountered helminths in *R. temporaria* (*Rhabdias bufonis*) and *R. arvalis* (*Oswaldocruzia bialata*) (Kuc & Sulgostowska 1988b). *Rana temporaria* studied by Lees (1962) also had nematodes as its dominant helminth type. Frogs were most commonly infected with *Cosmocerca ornata*, while *Rhabdias bufonis* had the highest mean intensity in males and *Aplectana acuminata* in females.

The distribution pattern of the number of helminth species per host individual is basically the same for all species in this study (Figure 3.1) with high numbers of frogs infected with few helminth species and low numbers of frogs infected with many helminths. Differences in the pattern can be explained by small sample sizes, where a true indication of infection patterns could not be determined. This same pattern is found for toads from various sites (Figure 3.2). Parasites are usually distributed unevenly through a host population (Dobson & Keymer 1990). The majority of parasites are often concentrated in a few heavily infected hosts, which leaves the majority of hosts with few or no parasites (Dobson & Keymer 1990). The most heavily infected host species (*B. marinus* from Bentley, Cape Weymouth and Townsville, *Lit. inermis*, *Lim. ornatus*, *Lit. caerulea* and *Lit. genimaculata*) were, generally, the species with the larger sample sizes. The larger the sample size, the greater the possibility to find more helminth species (see Dobson & Pacala 1992). This is not always the case depending on the level of infection in the host individuals collected. For example, *Mixophyes* sp. and *Lit. lesueuri* were both only collected once, but both were infected with three helminth species.

Despite the overall similarity between the helminth faunas of toads and frogs, toads appear to have a richer fauna. The toad helminth fauna is, however, dominated

by two species: *Rhabdias* sp. and *Mesocoelium* sp. All other helminth species were found in less than 10%, and the majority in less than 5%, of hosts investigated. *Mesocoelium* sp., *Rhabdias* sp., and *Parathelandros mastgurus* dominated the helminth fauna of frogs. The mean intensity of the helminth species, with the exception of *Mesocoelium* sp., were low. This dominance by one or two species seems characteristic of amphibian helminth faunas (see Lees 1962; Fransden 1974; Kuc & Sulgostowska 1988b; McAllister *et al.* 1989; Goldberg & Bursey 1991a; Muzzall & Schinderle 1992). Combined with the low maximum number of helminth species and the high number of host individuals infected with one helminth species, it is not surprising to find only one or two helminth species dominant.

Host range of most helminth species (Table 3.6) is low, with only a few helminths infecting over 10 host species. *Rhabdias* sp. has the widest host range which could be attributed to its life cycle (see Chapter 5). The only species of *Rhabdias* so far described in Australia, *Rh. hylae*, infects a wide variety of host species over a vast geographical area (see Appendix 4). Further work is required to determine if these species are the same or, indeed, if a number of *Rhabdias* species exist in Australia.

Of the 6 helminth species that infected only toads, 2 have been described from native amphibians in other studies.

Dolichosaccus juvenilis was first recorded from *Cyclorana brevipes* in Townsville by Nicoll (1918) (see Chapter 3 Literature Review).

Members of the genus *Austraplectana* have been recorded from various amphibian hosts in Australia (Johnston & Mawson 1941; Baker 1981). An undescribed species was also reported by Baker (1981) from amphibians in Queensland. The species recorded in this

study can not be determined as either *A. kartanum* or the undescribed species until comparison with museum specimens is carried out. Measurements of this species (see Table A5.9) do not correspond to those provided for *A. kartanum* (Johnston & Mawson 1941; Baker 1981). The *Austraplectana* sp. from this study is a smaller worm, but the spicules in the male are much larger. Baker (1981) did not provide measurements for the undescribed species.

The genus *Cosmocerca* requires revision (Baker 1987). One species, *C. limnodynastes*, has been described from *Lim. dorsalis* in South Australia (Johnston & Simpson 1942). Two other species described at the same time, *C. australiensis* and *C. propinqua*, were both transferred to the genus *Parathelandros* by Inglis (1968). Baker (1982) reported an unidentified Cosmocercinae sp. from Queensland frogs. Members of the genus *Cosmocerca* have also been reported from various amphibians, but not *B. marinus*, in South America (see Baker 1987). Comparison of measurements (Table A5.8) showed *Cosmocerca* sp. 1 to be a much larger worm than any of the other three species listed. Female specimens only were collected from *Cosmocerca* sp. 2 which were considered sufficiently different by the larger numbers of larvae free *in utero* and an indistinct vulva to be termed a separate species. Collection of male specimens in the future may unite this species with either of the others. *Cosmocerca* sp. 3 was differentiated from *Cosmocerca* sp. 1 due to the presence of 15 pairs of plectanes, and larger body and spicule size in the male. *Cosmocerca* sp. 1 has only 10 pairs of plectanes. Both species were differentiated from *C. limnodynastes* which has 5 pairs of plectanes (Johnston & Simpson 1942) and a smaller body and spicule size.

Only 2 helminth species can be considered truly unique to toads in this study. *Zeylanurotrema spearei*

was suggested by Cribb and Barton (1991) to have originated from an agamid (dragon) lizard, but as yet *B. marinus* is the only recorded host for this species. The adult acanthocephalan could not be identified beyond Class due to the poor condition of the specimens. This species may infect Australian fauna, but further wildlife parasitological surveys need to be carried out to identify other host species.

Three helminth species in this study were unique to frogs. *Parapolystoma* is the only genus of the family Polystomatidae to occur in amphibians in the Australasian area (Prudhoe & Bray 1982). Monogeneans, which have an aquatic free-living infective stage, that live in terrestrial hosts must coincide their sexual activity with that of the host (Prudhoe & Bray 1982; Tinsley 1990), which is also reliant on water for breeding (Duellman & Trueb 1986). Amphibians which are more aquatic in their lifestyle (e.g. *Lit. genimaculata*; Richards, S.J. 1993, pers. comm.) are more heavily parasitised by monogeneans than are terrestrial amphibians (e.g. *B. marinus*) (Fransden 1974; Lluch *et al.* 1987). *Litoria genimaculata* was the only `aquatic' frog collected in this study. Comparison of the helminth faunas of other aquatic frogs, such as *Lit. nannotis* (Richards, S.J. 1993, pers. comm.) would be of interest.

This is the second record of a member of the family Onchocercidae from amphibians of the Australasian region, the first being *Ochoterenella papuensis* from *Cornufer papuensis* (Ranid) in New Guinea (Johnston 1967). This species is considered *incertae sedis* by Esslinger (1986). Preliminary examination has ascertained that this second species does not fall into either of the 2 present subfamilies (Jones, H.I. 1993, pers. comm.). Many members of this family (genus *Ochoterenella*) have been recorded from *B. marinus* in

South America (see Table 3.1). These species are reliant on mosquitoes for transmission (Bain & Prod'hon 1974); mosquitoes have been observed to feed on native frogs (Richards, S.J. 1993, pers. comm.) and toads (Schwarzkopff, L. 1992; pers. comm.) in Australia. Whether this species has been introduced with the toad and transferred to native frogs, or is a native parasite, remains unknown. No specimens have yet been found in *B. marinus* in Australia. More samples, particularly including blood smears (to test for the larval microfilaria stage), need to be taken.

Seuratascaris numidica is restricted in its Australian distribution to Cape York (Sprent 1985). This is the first listing of *Lit. nigrofrenata* as a host for this species, previously only known from *Rana daemeli*. Comparative measurements (Table A5.11) show the female specimens collected in this study to be much larger than previously recorded (Sprent 1985). Morphological features, otherwise, are highly comparable.

There are two possible explanations for the presence of helminth parasites in the toad in Australia. Either they are natural parasites of the toad, introduced to Australia with the toad, or they are Australian parasites acquired in Australia. Many factors stand against the introduction of a helminth into Australia with the toad, but the possibility can not be ruled out. *Mesocoelium incognitum* was recorded to have been introduced with the toad to Hawaii (Yuen 1965). The stop-start history of the introductions of *B. marinus* to various locations, including Hawaii (Easteal 1981), with only small numbers collected for each introduction, could prevent the transmission of helminths (Manter 1967; Freeland 1983). Also, as the toads were maintained in captivity upon introduction to Australia, and only their progeny released (Easteal 1981), it is

unlikely that helminths were introduced and transmitted with the toad.

The introduction of an animal, and possibly its parasites, is of interest in an area such as northern Queensland, due to its unique zoogeographical position. The base of Cape York is recognised as the junction of northern and southern limits of distribution for many species (Zweifel & Tyler 1982). North Queensland is also, basically, parasitologically unexplored (see Jones 1991 for an example). The introduction of a helminth into this area could be masked due to lack of prior knowledge about this area. Careful consideration of all possible origins must be given.

Dolichosaccus symmetrus, *D. juvenilis*, *Parathelandros mastgurus* and *Porrorchis hylae* were all first recorded in Australia prior to the introduction of the toad. *Nematotaenia hylae* was recorded after the introduction of the toad, but in Tasmania, an area well out of the toads' distribution (see Literature Review). All of these species, except *D. symmetrus* and *D. juvenilis* have previously been recorded from *B. marinus* in Australia (see Appendix 4).

Probable Australian origins were determined for genera that had been recorded in the Australian region but never in South America: *Diplodiscus*, *Dolichosaccus*, *Pleurogenoides*, *Zeylanurotrema*, *Austraplectana*, *Maxvachonia*, *Kreisiella*, and *Johnpearsonia pearsoni*

Two species of the genus *Diplodiscus* have previously been recorded in Australian frogs prior to the introduction of the toad (see SJ Johnston 1912). *Diplodiscus*, as a genus, is restricted to the Palearctic, Oriental, Ethiopian and Australasian regions (Prudhoe & Bray 1982). *Diplodiscus megalochrus* was described from *Litoria aurea* and *Limnodynastes peroni*, while *D. microchrus* was described from *Litoria ewingii* and *Lim. tasmaniensis* (SJ Johnston 1912). *Diplodiscus*

megalochrus and *D. microchrus* were synonymised by Bravo-Hollis (1941), stating the characteristics used by Johnston (1912) could possibly be accounted for by age and state of contraction of the parasite. This view is not accepted pending further study of the genus. Comparative measurements of the members of the genus *Diplodiscus* recorded from Australian Amphibia (Table A5.1) shows the species found in this study to be closest to *D. microchrus*. Two members of this subfamily have previously been recorded from *Bufo marinus* in Brazil and Colombia, *Catadiscus cohni* and an undetermined species of *Catadiscus* (Table 3.1). *Catadiscus* is primarily a Neotropical genus (Prudhoe & Bray 1982). There are 2 records of *D. subclavatus* from the Americas (Prudhoe & Bray 1982), one from *Leptodactylus sibilator* in Brazil and the other from *Rana catesbeiana* in North America.

Dolichosaccus helocirrus is considered Australian in origin due to the large number of species of this genus already recorded from the Australasian area, while this genus has never been recorded from South America[1]. Two other species of this genus found in *B. marinus* in this study have been determined with a definite Australian origin (see Barton 1994a).

Members of the genus *Pleurogenoides* have been recorded from frogs and reptiles throughout the Palearctic, Oriental, Ethiopian and Australian regions (Prudhoe & Bray 1982). Two species have been recorded from Australian frogs prior to the introduction of the toad (SJ Johnston 1912). A related genus, *Langeronia*, has been recorded from *B. marinus* in South America (Table 3.1). Comparison of measurements of

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[1]Travassos (1924) described D. amplicava from Eloisa nasus (Amphibia) in Brazil, but later (1930) transferred the species to the genus Opisthioglyphe on the basis of its possessing a single seminal vesicle.

Pleurogenoides sp. with the 2 Australian species (Table A5.5) shows this species to be closest to *P. solus* in body measurements, but close to *P. freycineti* in egg measurements. Further study of this genus is required.

Zeylanurotrema spearei Cribb and Barton 1991, is postulated to have an Australian origin due to the reasons outlined in Section 3.1.1 (see also Cribb & Barton 1991).

As described above, members of the genus *Austraplectana* have been recorded only from amphibians in Australia (Baker 1981).

The genus *Maxvachonia* has been primarily reported from reptiles, especially the Scincidae, Agamidae and Gekkonidae, and has a wide geographical range throughout the world (Mawson 1972). *Maxvachonia flindersi* has been reported from many amphibian host species, including the toad, ranging from Perth to Alice Springs to Queensland (Johnston & Mawson 1941; Mawson 1972). Male specimens of *Maxvachonia* sp. were found only in *Lit. rothii* (Table A5.10) and were larger worms than described for *M. flindersi* (Johnston & Mawson 1942). Female specimens in this study were also larger worms. Comparison of specimens from this study with museum specimens of *M. flindersi* are required to determine if these species are the same.

The genus *Kreisiella* has been previously reported to infect West Australian lizards of the families Gekkonidae and Agamidae (Jones 1985, 1986). Comparison of measurements of species of *Kreisiella* collected in this study with species described by Jones (Table A5.13) showed the species to be different. The much smaller egg measurements of the species collected in this study was due to their not being viable (Jones, H.I. 1993, pers. comm.).

Johnpearsonia pearsoni is closely related to the genus *Batrachonema* which has been described from

amphibians in Malaysia and Peru. The genus *Johnpearsonia*, however, is of probable Australian origin (Durette-Desset, M.-Cl. 1993, pers. comm.).

The origin of the species of *Spinicauda* found in this study remains unknown, as members of the genus have been recorded from both Australian and South American hosts (see Jones 1979). The Australian species are *S. australiensis* from *Tiliqua scincoides* (Scincidae) in Townsville (Baylis 1930) and *S. moretonis* from *Morelia spilotes* (Boidae) in southern Queensland (Jones 1979). Comparative measurements of *Spinicauda* sp. from this study with these 2 species (Table A5.12) showed the former species to be a larger nematode, with the male possessing much larger spicules. Further work on the identity of this species is presently being carried out (Jones, H.I. 1993, pers. comm.).

Origins can not be determined for helminths not identified beyond Class or Phylum. More specimens, in better condition, are required for identification of these helminths.

Without identification to species, the origins of *Mesocoelium* sp. and *Rhabdias* sp. can not be determined. Members of both genera have been recorded in *B. marinus* in South America and in Australian frogs, either prior to the toads' introduction (*Mesocoelium*, see SJ Johnston 1912; Nicoll 1914) or after the introduction but in areas well out of its present distribution (*Rhabdias*, see Johnston & Simpson 1942; Ballantyne 1971). Both of these genera require revision, with confusion over the validity of diagnostic characters and host ranges making the identification of species difficult.

The genus *Mesocoelium* has been revised many times. Cheng (1960) recognised 28 of the 32 described species, while Freitas (1963) recognised only 7. Richard (1965) questioned the synonymies of Freitas due to a lack of evidence and suggested that studies needed to be carried

out on the degree of variation within a species. Nasir and Diaz (1971) recognised only 4 species based on the characteristics of sucker ratio and egg size. This work is not accepted here pending further study of the genus.

The genus *Rhabdias* is in need of a major revision. Two species have been recorded from *B. marinus* in Brazil, *Rh. fuelleborni* (see Travassos 1926) and *Rh. sphaerocephala* (see Kloss 1974). The identity of *Rh. sphaerocephala* from Brazil was questioned by Baker (1987) as it was first recorded from toads in Europe (Goodey 1924), then subsequently from toads in South America. More work needs to be done on the species of *Rhabdias* infecting toads in South America. Only one species has been recorded in Australian amphibians, *Rh. hylae* (see Johnston & Simpson 1942; Ballantyne 1971). This species has a wide host and geographical distribution (see Appendix 4), in comparison to the relatively narrow host and geographical distribution of the South American species. More work is required on the Australian *Rhabdias* species to determine if more than one species occurs. Techniques to determine this will have to extend beyond basic taxonomic morphometrics, due to the similarity in the morphology of the species (Ballantyne 1971). Study of the free-living generation is important (Ballantyne 1971; Kloss 1974) and the use of DNA sequence data, as employed by Luton *et al.* (1992) for the differentiation of *Dolichosaccus* species, will be needed.

Chapter 4: Ecology of Helminth Parasites of Australian Amphibia

4.1 Introduction

This chapter will consider the community ecology of helminths infecting amphibians in Australia. By comparing the structure of the helminth fauna of the introduced toad with the helminth fauna of selected sympatric native amphibians, we can see how successfully the toad has integrated itself into the native fauna from a parasitological viewpoint. From Chapter 3, it is apparent that the cane toad is a successfully exploited host by more species of helminths than any native amphibian thus far studied. But presence of parasite alone does not indicate if this host is fully successful in increasing overall transmission of the parasite. No work has yet compared the helminth faunas of native and introduced amphibians in Australia.

4.1.1 Literature Review: Ecology of parasitic helminths

a) Ecology of parasitic helminths within hosts.

i) General

Ecology is the relationship of an organism to its environment for the essential elements necessary for its life processes (Olsen 1974). For parasites, the environment includes both the habitat within the host (the microenvironment) and the habitat of the host (the macroenvironment) (Dogiel 1961; Noble *et al.* 1963). Parasites can be considered a characteristic of their host, just as are its biochemical and morphological traits (Ernst & Ernst 1980). Thus, parasites should be considered to play an important part in the ecology of

their hosts, a factor which has been ignored by many vertebrate ecologists (see comments by Dobson & Hudson 1986; Holmes & Price 1986; Peterson 1991; Toft 1991). Also, parasite ecology, as a field of study, has been largely ignored by ecologists (Moore & Simberloff 1990). For example, parasites are only mentioned twice in the standard ecology text of Krebs (1985), although they form the focus of the specialised works of Dogiel *et al.* (1961), Kennedy (1975, 1976) and Esch, Bush and Aho (1990).

ii) Community ecology of parasites

The basic premise of parasite community ecology is that each host individual contains a separate and distinct community of parasites allowing for possible replication of communities between hosts of the same species (Holmes 1979; Pence *et al.* 1983; Holmes & Price 1986; Goater *et al.* 1987; Esch, Shostak, Marcogliese & Goater 1990; Moore & Simberloff 1990). Within a single host individual is found parasite infrapopulations (Holmes & Price 1986; Goater *et al.* 1987; Aho 1990), each consisting of all the members of a given parasite species within that host individual. All of the infrapopulations within a host individual make up an infracommunity (Holmes & Price 1986; Goater *et al.* 1987; Aho 1990), which is the unit replicated within a host species.

The infracommunity level determines the abundance and distribution of parasites within a host individual (Holmes & Price 1986; Goater *et al.* 1987). Although this level of study comprises the basic data collected in a parasitological survey (Holmes & Price 1986), few studies of this aspect have been undertaken, especially for amphibians (Aho 1990).

A range of community organisations exists at the infracommunity level, from isolationist to interactive

(Holmes & Price 1986; Goater *et al.* 1987; Stock & Holmes 1987; Esch, Shostak, Marcogliese & Goater 1990; Moore & Simberloff 1990; Cornell & Lawton 1992). Between these two extremes lie a range of communities which exhibit intermediate characteristics (Esch, Shostak, Marcogliese & Goater 1990).

An isolationist community has low parasite species diversity and levels of infection; the community structure is suggested to rely upon minimal responses to the presence of other parasite individuals (Holmes & Price 1986). There appear to be many `vacant potential niches' (Esch, Shostak, Marcogliese & Goater 1990), with a low colonising ability into the system to fill these `niches' (Cornell & Lawton 1992). Isolationist parasite communities were found by Aho (1990) to predominate in amphibians and reptiles.

Interactive communities are at the opposite end of the spectrum, with high species diversity and levels of infection (Holmes & Price 1986; Goater *et al.* 1987; Esch, Shostak, Marcogliese & Goater 1990). Parasite communities of aquatic birds are commonly of this type (for examples see Hair & Holmes 1975; Bush & Holmes 1986b; Stock & Holmes 1987). A comparison of the criteria of isolationist and interactive communities is presented in Table 4.1.

All the infrapopulations sampled from a given host species in an ecosystem make up a metapopulation, whereas all the infracommunities within a given host population make up the component community (Esch, Shostak, Marcogliese & Goater 1990). The component community level determines the parasite species richness of a host population (Holmes & Price 1986).

All the individuals of a given parasite species, regardless of life cycle stage, present in an ecosystem represent the suprapopulation (Holmes & Price 1986; Esch, Shostak, Marcogliese & Goater 1990). The compound

Table 4.1. Characteristics of isolationist versus interactive parasite infracommunities. Explanations of terms in text. Adapted from Sousa, W.P. 1994.

Characteristic	Type of infracommunity structure: Isolationist	Interactive
Rate at which host is colonised by parasite	Low	High
Average density of parasite infrapopulations	Low	High
Frequency of interspecific interactions	Low	High
Species distributions in resource space	Individualistic; insensitive to presence of other species	Evenly distributed; responsive to presence of other species
Species diversity	Low; community unsaturated with species; some niches empty	High; community saturated with species; some niches empty
Equilibrium	Non-equilibrial	Equilibrial
General nature of infracommunity	Stochastic factors important	Evolutionary `mature'
Examples	Amphibians, reptiles, some mammals (e.g. *Apodemus sylvaticus*)	Birds, some fish (e.g. *Gasterosteus aculeatus*)

community level is made up of the suprapopulations of all parasite species in the ecosystem, which is usually a definable habitat unit such as a lake (Leong & Holmes 1981; Holmes & Price 1986; Esch, Shostak, Marcogliese & Goater 1990). Parasite species distribution and abundance within and between habitat units is determined by the study of compound communities (Leong & Holmes 1981; Bush & Holmes 1986b; Holmes & Price 1986). Understandably, these levels of parasite community ecology are rarely studied.

Development of the structure of these communities has been explained by various theories related to the evolutionary relationships between host and parasite (Holmes & Price 1986; Bush *et al.* 1990; Esch, Shostak, Marcogliese & Goater 1990).

Host body or population size, or the extent of the host species' range, is thought to influence the structure of its helminth community (Holmes & Price 1986). This theory, known as the island size hypothesis, suggests that a larger host species should be infected with a greater diversity of parasite species than smaller hosts. Host body weight was the only factor found by Gregory *et al.* (1991) to significantly affect parasite community structure in birds, where larger birds harboured more species of nematodes and trematodes (but not cestodes) than smaller birds. Host size was found to have no effect, however, on the species richness of helminths of amphibians and reptiles (Aho 1990).

Difficulty of invasion for the parasite forms the basis of the island distance hypothesis (Dogiel 1961; Holmes & Price 1986; Esch, Shostak, Marcogliese, & Goater 1990). If the host is difficult to colonise, the parasite community will be isolationist in character (Esch, Shostak, Marcogliese & Goater 1990), due to low species richness. Geographical isolation, combined with

small size of lake, was reported by Dogiel (1961) to be responsible for the small number of parasite species in freshwater fish from lakes in Russia.

Coevolution of parasites and their hosts (the cospeciation hypothesis; Holmes & Price 1986) produces specialisation in both host and parasite, leading to an isolationist infracommunity. Hosts should inherit, however, a common parasite fauna from their common ancestor which means, for example, that fish species in different lakes will have similar parasites due to similar evolutionary patterns between them (Holmes & Price 1986). Cospeciation was found not to have occurred in helminths of amphibians and reptiles (Aho 1990) as these hosts did not posses an unique helminth fauna. Amphibian isolationist helminth infracommunities, therefore, are not due to cospeciation. Few host specialists and dominance of the community by host generalists were found for helminths of these hosts.

Acquisition of parasites over time (the time hypothesis) is thought to lead to an eventually stable parasite community (Holmes & Price 1986). In this situation the parasite species have adapted to each other and the available resources to produce tight species packing and narrow, stable niches (Holmes & Price 1986). This hypothesis, therefore, predicts that phylogenetically older hosts (e.g. fish) will have a greater parasite species diversity than `newer' groups (e.g. mammals). Kennedy, Bush and Aho (1986) and Bush *et al.* (1990) found, however, that the phylogenetic age of a host group did not determine its species richness. Older groups (fish, amphibians and reptiles) had fewer parasite species than mammals and birds (see Table 3 of Sousa 1994) and concluded that habitat of host group (i.e. terrestrial or aquatic) was more important. The time hypothesis may be applicable in situations where a

host has been introduced into a new area without its existing parasites (Holmes & Price 1986), for example the cane toad in Australia.

Transfer of parasite species between host species within an ecosystem unit, for example a lake, may produce an interactive parasite community (Leong & Holmes 1981; Holmes & Price 1986; Esch, Shostak, Marcogliese & Goater 1990). An infective `pool' (Leong & Holmes 1981) is present in the unit allowing transmission of parasites to hosts, dependent on host density and level of contact with the `pool' (Esch, Shostak, Marcogliese & Goater 1990; Davidson *et al.* 1991). The pattern of transfer of parasite species is dependent upon the numerical dominance of the host species, their taxonomic affinity and host specificity of the parasite (see Neraasen & Holmes 1975; Leong & Holmes 1981; Pence *et al.* 1983; Bush & Holmes 1986a; Holmes & Price 1986; Stock & Holmes 1987; Esch, Shostak, Marcogliese & Goater 1990).

This last theory has given rise to the concept of core and satellite parasite species (Hanski 1982). Core species are generally single host species specialists, adapted to each other and widespread and abundant within that host species. Core parasite species found ubiquitously across a host species' geographic range are known as indicator species for that host species (Custer & Pence 1981). Satellite species are acquired by exchange from the ecological associates of the host and are sporadic and less abundant. Within an infracommunity, it is the core species that will interact, usually in a negative association, whereas secondary and satellite species are isolated from other species (Sousa 1994). In exchange, one host's core species becomes another's satellite species (Freeland 1983; Holmes & Price 1986). Bush and Holmes (1986a), however, found that there existed a third group of

species between the core and satellite species, which they termed secondary species. A parasite species with a prevalence of infection over 70% was considered a core species, below 40% was a satellite species, whereas a secondary species had an intermediate prevalence (Bush & Holmes 1986a). Aho (1990) suggested, however, that due to the isolationist characteristic of amphibian parasite communities, that species be considered either common (prevalence over 50%) or rare (below 50%).

Host species from the same community do not carry the same set of parasite species (Freeland 1983). Similar kinds of hosts living in the same geographic area are unlikely to have parasite communities with similar numbers of species due to unpredictable occurrence of parasite mutation, new host defences and interactions among parasites (Freeland 1986). The presence of "screens", such as host habits, habitats and behaviour, determines the species composition of the parasite communities (Holmes 1986). This situation was found by Stock and Holmes (1987) for four species of grebes collected from the same locations. Although parasites were shared among the grebes, the bulk of the breeding population of any particular parasite species was found only in one host species.

A comprehensive study of parasite community ecology was undertaken by Leong and Holmes (1981) on the parasite communities of fish in lake ecosystems in Canada. They found that parasites of the numerically dominant hosts (salmonids: cisco and whitefish) were the numerically dominant parasites, although the number of taxa of parasites was greater in the less abundant hosts (non-salmonids). Exchange of parasites among host species determined part of the parasite communities within hosts. The greatest amount of exchange was found between related fish, particularly the salmonids, showing that taxonomic affinity and population size are

primarily important in determining the amount of parasite exchange (Leong & Holmes 1981; see also Dogiel 1961). Stock and Holmes (1987) found that a lower degree of specificity of parasites allowed exchange between related hosts (grebes), which led to an increase in species richness in the parasite community of individual host species. Taxonomic affinity between species of amphibians and reptiles was not found to be important in determining the richness of their parasite fauna (Aho 1990). This is due to the helminth fauna of amphibians and reptiles consisting, primarily, of host generalists.

Abundance of helminths within a host species is related to both regional and local scales of distribution (Aho 1990). Regional richness is the total number of helminth species found in a host species across the entire geographic range of the host (Aho 1990). Patterns of local richness, the total number of helminth species found in that host species at a particular site (Aho 1990), determine the regional richness. Host species with larger geographic distributions have a higher regional richness due to the larger number of local environments the distribution covers (Aho 1990). Migratory host species also have a higher regional richness due to the wide variety of habitats encountered *en route* (Neraasen & Holmes 1975). Helminth faunas can, however, lose diversity due to the loss of helminths during migration which can not be replaced at the new location (Dogiel 1961; Neraasen & Holmes 1975).

iii) Parasite community ecology within a host population

Parasites are distributed unevenly throughout a host species population (Dobson & Keymer 1990). The majority of parasites (species or individuals) are often

concentrated in a few heavily infected hosts, which leaves the majority of hosts with few or no parasites (Pennycuick 1971b; Dobson & Keymer 1990).

This clumped pattern of distribution for a potentially lethal parasite produces a characteristic pattern in the host population of many infected individuals, some sick, and few dead (Pennycuick 1971b; Holmes & Price 1986). This characteristic has been used as an indirect method for estimating host mortality (Kennedy 1984; Adjei *et al.* 1986). Kennedy (1984) found no evidence of frequency distributions implying parasite induced mortality for fish infected with diplostomatid (Digenea) metacercariae. The use of a peaked host age-parasite abundance curve and decrease in the degree of dispersion of parasites in the older host age classes did not conclusively prove mortality due to parasites (Kennedy 1984).

Adjei *et al.* (1986), however, determined that a negative binomial distribution with a truncated end, that is with the more heavily infected hosts missing from the population, was evidence of parasite-induced mortality. Infection levels over 3-5 larval *Callitetrarhynchus gracilis* (Cestode) per fish (*Saurida* spp.) led to a 50% chance of death for the infected fish (Adjei *et al.* 1986). Pennycuick (1971a, 1971b) found a truncated distribution for *Schistocephalus solidus* (Cestoda) in the stickleback, *Gasterosteus aculeatus*. This could not, however, be attributed solely to the effect of parasite-induced mortality.

Distribution of parasites within a host species is related to the population density of the definitive host (Davidson *et al.* 1991; Neraasen & Holmes 1975; Albert & Curtis 1991), intermediate host(s) (Conneely & McCarthy 1986), food habits of the definitive host (Stock & Holmes 1987), and transmission dynamics of the parasites (Pence & Windberg 1984; Goater *et al.* 1987).

Environmental patchiness and effects of season can generate aggregated helminth distributions (Stock & Holmes 1987) and account for the unpredictability of helminth infections between hosts (Dogiel 1961; Aho 1990).

A broadly similar diet between host individuals or species should lead to similar helminth faunas (Hoberg & Ryan 1989; Stock & Holmes 1987). Within a species, however, environmental patchiness may override the expected similarity (see Hoberg & Ryan 1989). Between host species, specialisation on different dietary items in otherwise broadly overlapping diets, allows for vastly different and diverse parasite communities (Stock & Holmes 1987).

Related to environmental patchiness is the factor of seasonality in determining helminth community richness, due to effects on host behaviour and parasite acquisition. Changes in activity levels and feeding preferences throughout the year affected parasite infestation in eels (Conneely & McCarthy 1986) and other fish (Albert & Curtis 1991). Rising environmental temperatures, especially for cold blooded animals, such as the cane toad, is usually the cue for breeding and many parasites have adjusted their life cycles for this time (see Font 1983; Tocque & Thoney 1991).

b) Helminth Communities in Amphibians and Reptiles.

Amphibians and reptiles (collectively known as herps) have received little attention from parasite ecologists (Lluch *et al.* 1987; Esch, Shostak, Marcogliese & Goater 1990; Aho 1990). The majority of work on helminths of these hosts has concentrated on faunistic surveys or discussions on phylogenetic relationships between host and parasite (see Aho 1990; Muzzall 1991b). The

community ecology of helminths of herps is emerging as an interesting and informative field of research (Aho 1990). Generally, however, the helminth fauna of amphibians and reptiles remains unknown, and studies at the infracommunity level practically non-existent (Aho 1990).

Of the 30 species of helminths reported by Aho (1990) to occur in various herps from different orders (salamanders and anurans), two were found to have an almost cosmopolitan distribution. *Cosmocercoides variabilis* (Nematoda) and *Brachycoelium salamandrae* (Digenea) have been recorded from 3 families of salamanders and 2 families of anurans. Eighteen helminth species were found to be core species in one or more populations, with 5 of these a core species in more than one genus of host. Aho (1990; see also Duellman & Trueb 1986) found that the majority of helminths present in amphibians and reptiles were host generalists, with few specialists present in the community.

The helminth communities of herps have been found by various workers to be depauperate (low in species richness) and isolationist in character. Examples of this type of community have been reported for salamanders (Goater *et al.* 1987; Aho 1990; Muzzall 1990, 1991a), lizards (Goldberg & Bursey 1990, 1991a, 1991b; Dobson *et al.* 1992) and frogs (Muzzall 1991b). However, comparatively rich helminth faunas have been reported from chelonians (Esch *et al.* 1979), marine turtles (Blair, D. 1993, pers. comm.) and also in ranid frogs from Poland (Kuc & Sulgostowska 1988a, 1988b). From his review of herp parasite literature Aho (1990) found that freshwater turtles had the highest level of species richness (4.92 species per host) which declined through anurans (3.54), salamanders (2.76) and lizards (2.06) to snakes (1.88).

The formation of a depauperate, isolationist helminth

community was suggested to be due to various factors of host biology and physiology (Kennedy, Bush & Aho 1986; Goater *et al.* 1987; Aho 1990; Muzzall 1991a, 1991b). These factors were a generalised, opportunistic diet, simple intestinal system, low vagility and an ectothermic metabolism.

Two types of foraging are evident in Amphibia: ambush predation and wide-ranging predation (Toft 1980; Duellman & Trueb 1986; Aho 1990). Ambush predators, e.g. pelobatid toads and leptodactylids, have less complex parasite communities (Aho 1990) due to the low variety of prey encountered, primarily orthopterans and spiders (Toft 1980). The wide-ranging predators, e.g. ranid frogs, have richer communities, which may also be due to development of anti-predator defences, such as poison glands, which decrease the risk of predation and can, thus, increase foraging time (Toft 1980; Aho 1990).

The habit of amphibians and reptiles as opportunistic feeders, selecting for prey size, not type (Duellman & Trueb 1986), leads to a relatively lower exposure to both variety and numbers of parasites (Aho 1990). The factor of diet breadth is of prime importance for parasites dependent upon a food web for transmission (Aho 1990).

Muzzall (1991a) suggested that the broad diet of the newt *N. viridescens* was outweighed by other factors, such as low vagility and ectothermy, to produce a depauperate helminth community. However, Goldberg and Bursey (1990, 1991b) found that the broad, non-selective diet of the alligator lizards, *Gerrhonotus multicarinatus webbi* and *G. coeruleus principis*, contributed to their species poor helminth community.

The helminth community of the salamanders studied by Goater *et al.* (1987) was dominated by nematodes (*Capillaria inequalis* (now known as *Amphibiocapillaria tritonispunctati*) and *Thelandros magnavulvaris*) which

have direct life cycles. The biology of the host is important in this situation, as shown by Muzzall (1991a) in his study of the helminths of newts. As the newts are aquatic in life style, all their helminths have aquatic life cycles; the newt helminth community was dominated by helminths with indirect life cycles (digeneans).

Changes in diet with age of host leads to changes in the parasite fauna (Dogiel 1961; Dronen 1977; Muzzall 1991a, 1991b). Larger hosts are able to consume larger and more prey and have more surface area for parasites to establish (Dogiel 1961; Muzzall 1991a, 1991b). Additionally, parasites may accumulate as the host ages. Increased intensity of infection and species richness in older *Rana catesbeiana*, *R. clamitans* and *Notophthalmus viridescens* were found by Muzzall (1991a, 1991b). Similar results were found by Goater *et al.* (1987) for salamanders and Kuc and Sulgostowska (1988a) for *Rana ridibunda*. Dronen (1977) found that level of infestation of *Haematoleochus* spp. (Digenea) in *Rana pipiens* and *R. catesbeiana* varied with host size. Small frogs were uninfected, most likely due to an inability to catch the intermediate host (odonates). Infection levels peaked in medium sized frogs but declined in larger frogs due to resistance, not a dietary change.

The amphibian intestinal system is morphologically alike along its entire length, with a small valve differentiating the large and small intestines (Duellman & Trueb 1986). Even the inclusion of an appendix/caecum in the intestinal system (as in turtles and lizards) does not guarantee a diverse helminth community (Aho 1990). Turtles have a complex community but lizards have one of the poorest community richness levels. Theoretically, the more differentiated the intestine, the more "niches" there are to occupy which leads to higher diversity (Kennedy, Bush & Aho 1986).

The ability to disperse, or vagility, is limited in amphibians due to their dependence on moisture (Duellman & Trueb 1986; Goater *et al.* 1987; Aho 1990). Amphibians have, however, developed a wide range of behavioural adaptations to allow dispersal from a water source (Duellman & Trueb 1986). These adaptations include nocturnal activity, dense aggregations in burrows and limited activity periods throughout the year (Duellman & Trueb 1986) which restricts exposure to helminths (Aho 1990). *Scaphiopus couchii* (Pelobatidae) is active for only two months per year, and enters the water on 1-3 nights of this time (Tinsley 1983). Thus, this is the only time it is exposed to, and can release, larvae of the monogenean *Pseudodiplorchis americanus* (Tocque & Thoney 1991).

Aquatic amphibians are less restricted within their habitat (Duellman & Trueb 1986). Preferences within this habitat, however, can influence parasite acquisition (Goater *et al.* 1987). The salamander *Leurognathus marmorata* has a depauperate helminth fauna (4 species) as it inhabited strong currents which hindered parasite transmission (Goater *et al.* 1987). Anurans have a more diverse helminth community than salamanders due to their ability to leave the water and feed on a wider variety of organisms (Muzzall 1991b).

This dependence on moisture, and the adaptations of parasites to deal with this host behaviour, has enabled researchers to differentiate hosts, and their environments, by abundances of types of helminths (Fransden 1974; Prokopic & Krivanec 1975; Kuc & Sulgostowska 1988b). Hosts can be divided into one of three groups: aquatic, terrestrial or amphibious, based on differences in prevalence and intensity of different parasites and life cycle characteristics (Frandsen 1974; Lluch *et al.* 1987). Generally, aquatic hosts have richer and larger helminth communities than terrestrial

amphibians (Aho 1990).

Aquatic frogs, e.g. *Rana esculenta*, have a parasite fauna dominated by trematodes (monogeneans and digeneans) (16 out of the 23 species of parasite, Prokopic & Krivanec 1975; 17 out of 21, Kuc & Sulgostowska 1988b) due to the parasites' dependence on an aquatic system to complete their life cycle. Nematodes, on the other hand, characterised a terrestrial amphibian (e.g. *Bufo bufo*, *B. viridis*) as they did not require large amounts of water for completion of their life cycle. Amphibious species (e.g. *R. temporaria*; Kuc & Sulgostowska 1988b) had a mixed helminth fauna of nematodes and trematodes (see also Fransden 1974; Smyth & Smyth 1980). These parasites usually utilise, as a second intermediate host, an aquatic larva of a terrestrial insect (Lluch *et al.* 1987). Even helminth species of wide distribution and very low host specificity, however, have a dominant host to which they have adapted their mode of life (Prokopic & Krivanec 1975).

Even within a broad ecological heading, such as terrestrial, microhabitat preferences among groups of hosts can lead to definable variations in parasite prevalence and intensity (McAllister *et al.* 1989). This was shown for three sympatric species of toads, *Bufo debilis debilis*, *Bufo valliceps valliceps*, and *Bufo woodhousii woodhousii*, all terrestrial in habit. The more arid-adapted toad, *B. d. debilis*, had the narrowest niche, being found primarily in burrows, and had the lowest prevalence of infection (51.9%) with parasites. Moderate parasitism was found in *B. w. woodhousii* (75%) which is restricted to localised stream edges. The toad with the widest variation in habitats, *B. v. valliceps*, had the highest prevalence of infection (87%). Species richness also increase from 4 parasite species in *B. d. debilis* to 6 species in *B. v. valliceps*; intensity of

infection was not recorded. Possible exchange of parasitic stages would occur when the toads spawned, when the three species were found in similar habitats (McAllister *et al.* 1989). Similar results were found by Goldberg and Bursey (1991a) for three different species of toads (*B. alvarius*, *B. cognatus*, *Scaphiopus couchii*), with differences in parasite load, prevalence and intensities of infection between the toad species. Certain *Bufo* species were found by Frandsen (1974) to be rarely parasitised by trematodes, as they were terrestrial in habit, and bred in temporary ponds where the molluscan intermediate hosts for trematodes were not present.

Being ectothermic, amphibians are dependent on environmental temperature for body heat. Thus environmental temperature is a primary restricting factor of amphibian biology, determining activity and feeding rates (Duellman & Trueb 1986; Muzzall 1991a). This subsequently affects exposure to parasites and/or intermediate hosts (Aho 1990; Aho *et al.* 1991) and the population biology of the parasites involved (Tocque & Thoney 1991). Therefore, restricted behaviour due to temperature may enhance the isolationist character of the helminth community (Goater *et al.* 1987; Aho 1990) by increasing the difficulty of colonisation.

Community diversity of helminths within a host may also be affected by habitat stability or predictability (Esch *et al.* 1979; Aho 1990). Helminth community diversity was greatest in *Chrysemys scripta scripta* (Chelonia) collected from a stable environment, when compared to turtles collected from a thermally altered pond. The differences in diversity may also be influenced by the helminth life cycle, where indirect life cycles may easily be broken in an unstable habitat (Esch *et al.* 1979).

4.1.2 Aims of Chapter

This Chapter is divided into two parts which explore the 2 main aims of this section:

1. Part A looks at the population structure of a helminth species, *Rhabdias* sp., within a population of toads. *Rhabdias* sp. is thought to be the best potential biological control agent for the toad in Australia (see Chapter 5) from the helminths so far collected. This part of the study examines toads from metamorphosis and details the development of the *Rhabdias* sp. infrapopulation with changing toad size, sex and time.

2. Part B looks at the structure of helminth infracommunities in two amphibian species. *Litoria inermis* is a native frog species found throughout Queensland and often in association with *Bufo marinus*. Both species were common at Bentley where this part of the study took place. This part of the study details the infracommunity and component community of both amphibian species, compares their structure, and discusses the relevance of diversity indices in the study of amphibian helminth communities.

Part A: Helminth Population Ecology

4.2 Materials and Methods

4.2.1 Description of study site

The Queensland Department of Primary Industries Veterinary Pathology Laboratory (QDPI) is located in the suburb of Oonoonba on the southern limits of Townsville city (Figure 4.1). QDPI is bounded on two sides by the tidal reaches of Ross River; the other two sides are open grassland used for cattle grazing. The site contained a water impoundment closely surrounded by vegetation, and also contained aquatic vegetation.

Coverage of the water impoundment was 100m long x 20m wide x 50cm deep in peak capacity, but was dry in the latter parts of the dry season (August-November). Average size was 20m x 10m x 20cm.

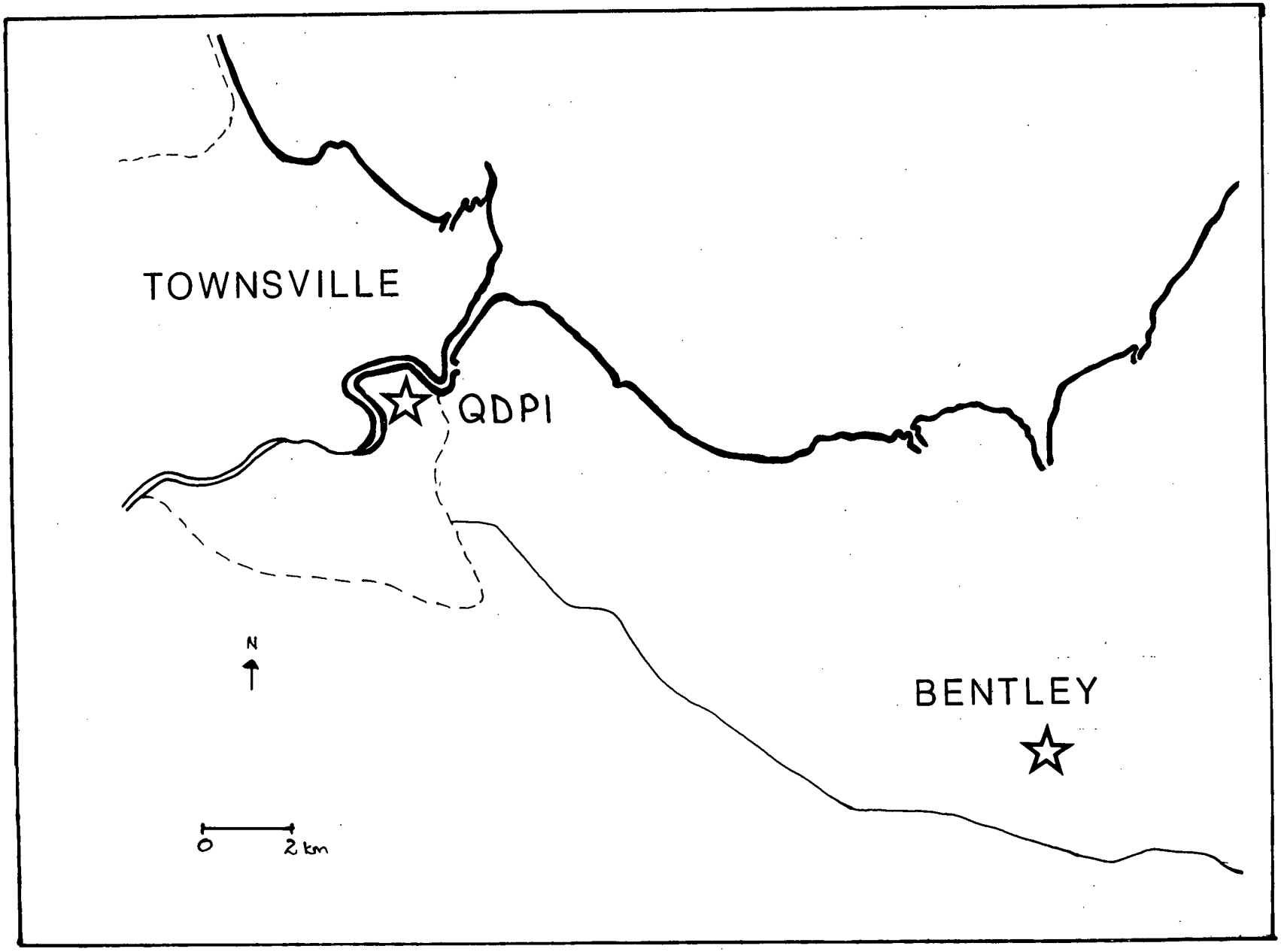
4.2.2 Collection of specimens

Toads were collected from QDPI at four week intervals from August 1990 to March 1992 (with the exception of September & October 1990 and October & November 1991). Two collections were made in February 1992. Efforts were made to collect toads of all sizes. Collection and dissection followed the method outlined in Chapter 2.

Numbers of the nematode Rhabdias sp. (see Chapter 3 for discussion of identity) collected from each lung were recorded. Nematodes were relaxed in Berland's fixative, then stored in 70 % alcohol. Each nematode was measured under a dissector microscope with a calibrated eye piece.

Figure 4.1 Location of the two sampling sites (QDPI and Bentley) involved in the ecological study.

Dashed line indicates the Townsville city limits. Solid line indicates the Bruce Highway.


TOWNSVILLE
QDPI
N
BENTLEY
0
2 km

4.2.3 Statistical Analyses

a) General

Number of nematodes collected per toad were plotted and the relationship between the observed distribution and a predicted Poisson (random) distribution was determined by a Chi-squared (χ^2) goodness-of-fit test. Degree of aggregation of nematodes among toads was determined by calculation of a variance to mean ratio (VMR). The distribution of *Rhabdias* sp. was highly aggregated so all data (intensity of infection and average length of nematodes) were transformed using a $\log_{10}(x+1)$ transformation. Nematode length was also transformed as length and variance were positively correlated (see Zar 1984). Standard parametric statistical tests, such as ANOVA and correlation, were then performed with transformed data (Zar 1984). All other statistical tests, such as t-tests, were performed with untransformed data as the differences between samples were normally distributed (see Zar 1984).

Preference of *Rhabdias* sp. for either left or right lung was determined by a paired t-test.

b) Relationship between infection parameters and toad length.

Relationships between intensity of *Rhabdias* sp. infection and average length of nematodes with toad SVL in each infrapopulation were tested using simple correlation coefficients. Relationship of intensity of infection to average length of nematodes was determined by partial correlation coefficient, adjusted for toad SVL ($r_{il.t}$, where i = intensity of infection, l = length of nematode, t = toad SVL).

Following these analyses, data were divided among three host size/sexual maturity classes. Subadult toads (Class I) were all toads <60mm SVL whose sex could not

be determined. The mid size class (60.5-90mm SVL; Class II) contained toads whose sex could only be determined by dissection. Fully sexually mature toads were toads whose sex could be determined in the field from secondary sexual characteristics (>90.5mm SVL class; Class III). Each size class was examined, as above, for relationships between toad SVL, intensity of *Rhabdias* sp. infection, and average length of nematodes. Size of toad was used instead of age because techniques for ageing toads in Australia have yet to be developed and tested (Schwarzkopf, L. 1992, pers. comm.). The size classes outlined above are thought to cover the following age groups: subadult toads (Class I) are assumed to be first year toads (from metamorphosis to 1 year of age); Class II toads are from year 1 to year 3; Class III toads are over 3 years of age. Longevity of toads in the wild remains unknown.

Frequency distributions of number of nematodes per toad were plotted for each size class. A Chi-squared goodness-of-fit test determined the relationships between the observed and predicted Poisson distributions. A VMR was also calculated for each size class.

A Chi-squared homogeneity test was performed to determine if there was a difference in the proportion of *Rhabdias* sp.-infected toads between toad size classes.

Relationships, as tested above, were determined for adult toads (Classes II and III combined) and compared to the same relationships for Class I toads.

c) Relationship between infection parameters and adult toad sex.

To determine if sex of adult toad is correlated with intensity of *Rhabdias* sp. infection or average length of nematodes one way ANOVAs were performed. Only the two adult toad size classes (II and III) were analysed for

relationships between toad sex and parameters of *Rhabdias* sp. infection.

d) Relationship between infection parameters and month of collection.

Rainfall data, covering the collection period, was provided by QDPI. This data was collected to compare population dynamics of *Rhabdias* sp. infection with a prevalent weather condition.

Monthly variations in intensity of *Rhabdias* sp. infection and the average length of nematodes were determined by one way ANOVAs. These relationships were tested for each of the toad size classes (I, II, and III), adult and subadult toads, and adult male and female toads (combined from Classes II and III).

e) Annual patterns in populations of *Rhabdias* sp.

Data analysed in this section were not $\log_{10}(x+1)$ transformed. The range of lengths recorded for *Rhabdias* sp. were separated into 5 approximately equal size classes (<4.2, 4.3-6.6, 6.7-9.0, 9.1-11.4, >11.5 mm) not based on any biological reason. Number of *Rhabdias* sp. in each class was recorded and converted to a percentage of the total number of *Rhabdias* sp. for that month. Length frequency distributions of *Rhabdias* sp. length were plotted for the three toad size classes.

4.3 Results.

4.3.1 General

Rhabdias sp. was found to infect 82.8% (480 of 580) of toads collected from QDPI, with a mean intensity of 16.1 nematodes per infected toad (see Appendix 7). Intensity of infection ranged from 1 to 230.

A frequency distribution for *Rhabdias* sp. is presented in Figure 4.2; the distribution was not random (χ^2_{14}=8260.9, p<0.001). The calculated VMR (37.04) showed the distribution to be highly aggregated. Number of toads with zero parasites was the highest class (17.2%); 14.1% of toads studied had over 30 nematodes each.

Rhabdias sp. had no preference for either right or left lung (t_{479}=1.86, p=0.0629); mean intensity of infection in the left lung (8.21±0.58) was slightly larger than for the right (7.85±0.55).

4.3.2 Relationship between infection parameters and toad length.

Snout-vent length of toads collected ranged from 22.0 to 117.5mm, with an average length of infected toads of 65.56mm (average length of all toads collected: 62.41mm). Results of statistical analyses of relationships between toad SVL and parameters of *Rhabdias* sp. infection are presented in Table 4.2; only infected toads were included.

Overall (Figure 4.3), positive correlations were found between toad SVL and intensity of *Rhabdias* sp. infection, and toad SVL and average length of nematodes. Intensity of *Rhabdias* sp. infection and average length of nematodes was not found to have a significant relationship.

For subadult toads (<60mm SVL) (Figure 4.4), positive correlations existed for the relationships between SVL and intensity of *Rhabdias* sp. infection, and SVL and average length of nematodes. The partial correlation between intensity of *Rhabdias* sp. infection and average length of nematodes was not significant.

For the 60.5-90mm SVL size class (Figure 4.5), the relationship between SVL and average length of nematodes

Figure 4.2 Frequency distribution of numbers of *Rhabdias* sp. per toad for all toads collected at QDPI for a 20 month period.

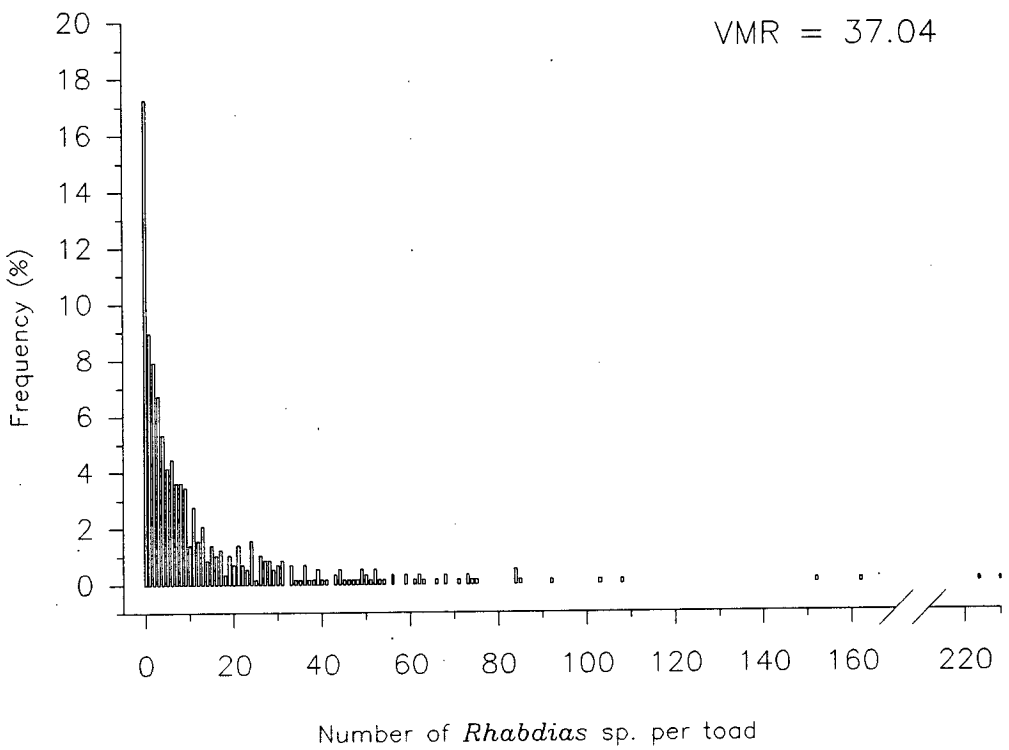

VMR = 37.04
Frequency (%)
20
18
16
14
12
10
8
6
4
2
0
0
20
40
60
80
100
120
140
160
220
Number of *Rhabdias* sp. per toad

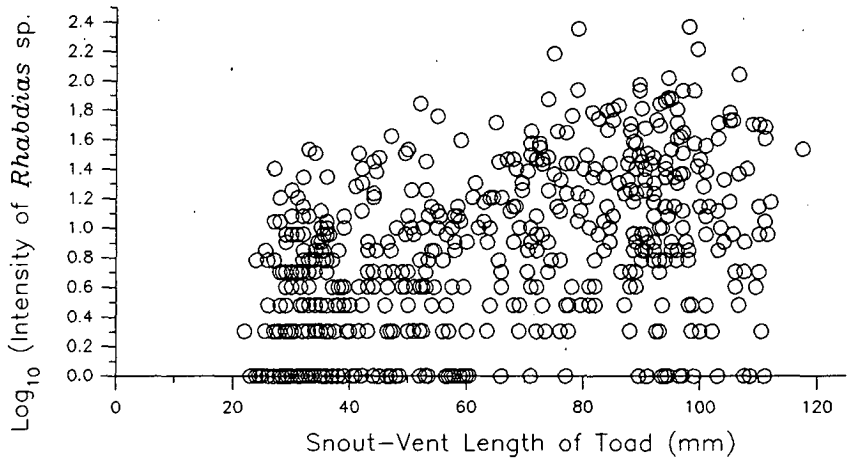
FIG 4.2

Table 4.2. Results of analysis of relationships between toad snout-vent length with intensity of *Rhabdias* sp. infection and average length of nematodes in an infrapopulation by simple correlation, and between the parameters of *Rhabdias* sp. infection by partial correlation, adjusted for toad length. *Rhabdias* sp. infection parameters have been $\log_{10}(x+1)$ transformed. (i = intensity of *Rhabdias* sp. infection, l = length of nematodes, t = toad length, N = number of toads, df = degrees of freedom, PROB = probability, SIG = significant relationship, NS = not significant relationship, *Rh.* = *Rhabdias* sp.).

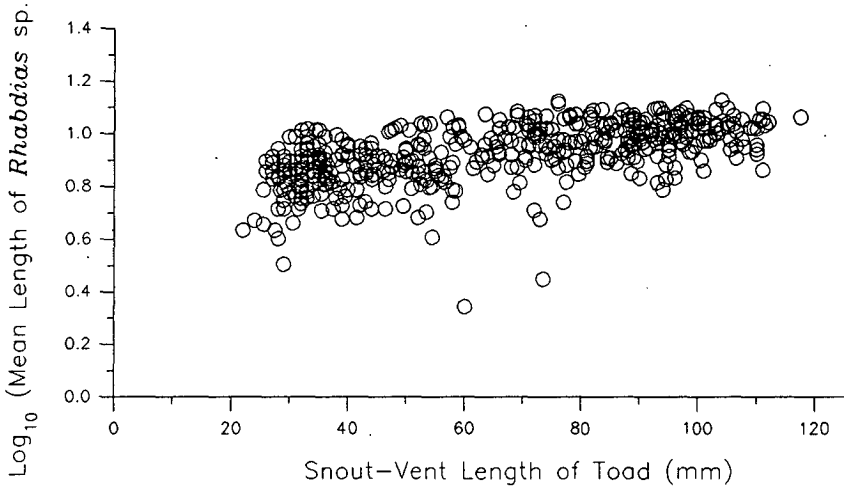
FACTORS	TEST	N	DF	RESULT	PROB	SIG/NS
Overall:						
Toad length v # *Rh.*	Simple Correlation	480	479	$r = 0.44$	$p < 0.0001$	SIG
Toad length v *Rh.* Length	Simple Correlation	480	479	$r = 0.59$	$p < 0.0001$	SIG
Rh. length v # *Rh.*	Partial Correlation	480	479	$r_{il.t} = 0.03$	$p > 0.05$	NS
Toads <60 mm:						
Toad length v # *Rh.*	Simple Correlation	219	218	$r = 0.18$	$0.01>p>0.005$	SIG
Toad length v *Rh.* length	Simple Correlation	219	218	$r = 0.22$	$p < 0.0001$	SIG
Rh. length v # *Rh*	Partial Correlation	219	218	$r_{il.t} = 0.09$	$0.2>p>0.1$	NS
Toads 60.5-90 mm:						
Toad length v # *Rh.*	Simple Correlation	147	146	$r = 0.16$	$0.1>p>0.05$	NS
Toad length v *Rh.* Length	Simple Correlation	147	146	$r = 0.18$	$0.05>p>0.02$	SIG
Rh. length v # *Rh.*	Partial Correlation	147	146	$r_{il.t} = -0.03$	$p > 0.5$	NS
Toads >90.5 mm:						
Toad length v # *Rh.*	Simple Correlation	114	113	$r = -0.04$	$p > 0.5$	NS
Toad length v *Rh.* Length	Simple Correlation	114	113	$r = 0.11$	$0.5>p>0.2$	NS
Rh. length v # *Rh*	Partial Correlation	114	113	$r_{il.t} = -0.11$	$0.5>p>0.2$	NS

Figure 4.3 Relationship between
a) intensity of *Rhabdias* sp. infection and snout-vent length (SVL) of toad,
b) average length of *Rhabdias* sp. and SVL, and
c) average length of *Rhabdias* sp. and intensity of *Rhabdias* sp. infection
for all toads collected from QDPI over a 20 month period.

a


Log10 (Intensity of Rhabdias sp. + 1)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
Snout–Vent Length of Toad (mm)

b


Log10 (Mean Length of Rhabdias sp. + 1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
Snout–Vent Length of Toad (mm)

c

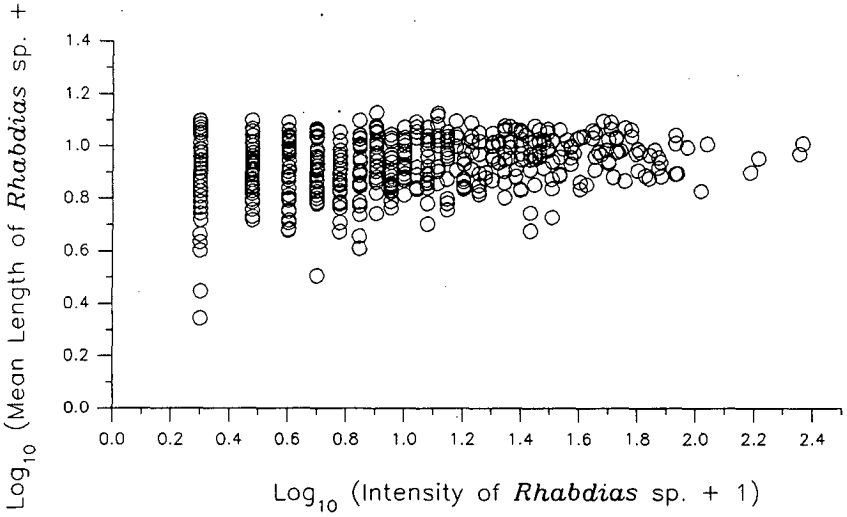

Log10 (Mean Length of Rhabdias sp. + 1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
Log10 (Intensity of Rhabdias sp. + 1)

Figure 4.4 Relationship between
a) intensity of *Rhabdias* sp. infection and snout-vent length (SVL) of toad,
b) average length of *Rhabdias* sp. and SVL, and
c) average length of *Rhabdias* sp. and intensity of *Rhabdias* sp. infection
for subadult toads (<60mm SVL) collected.

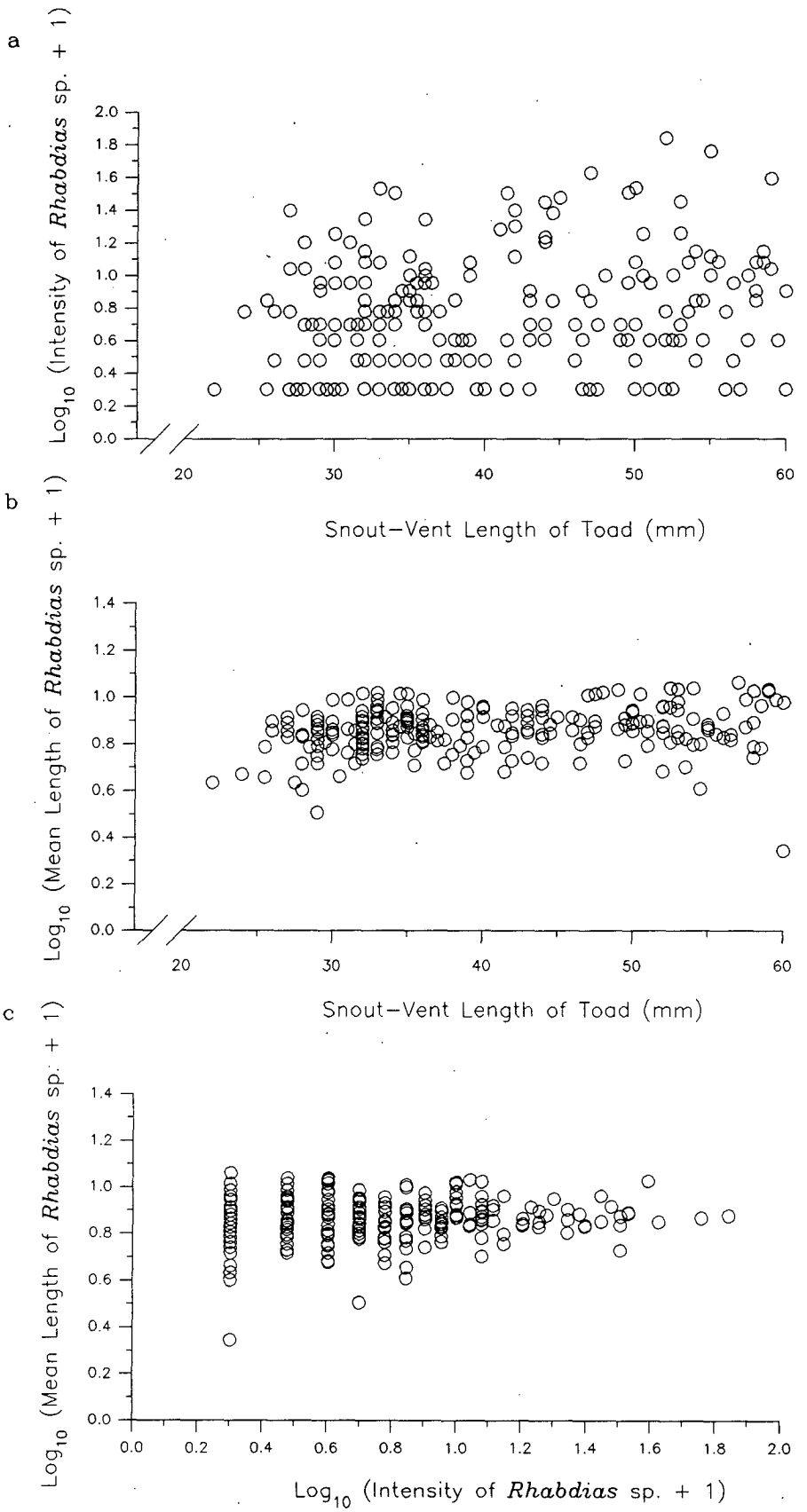
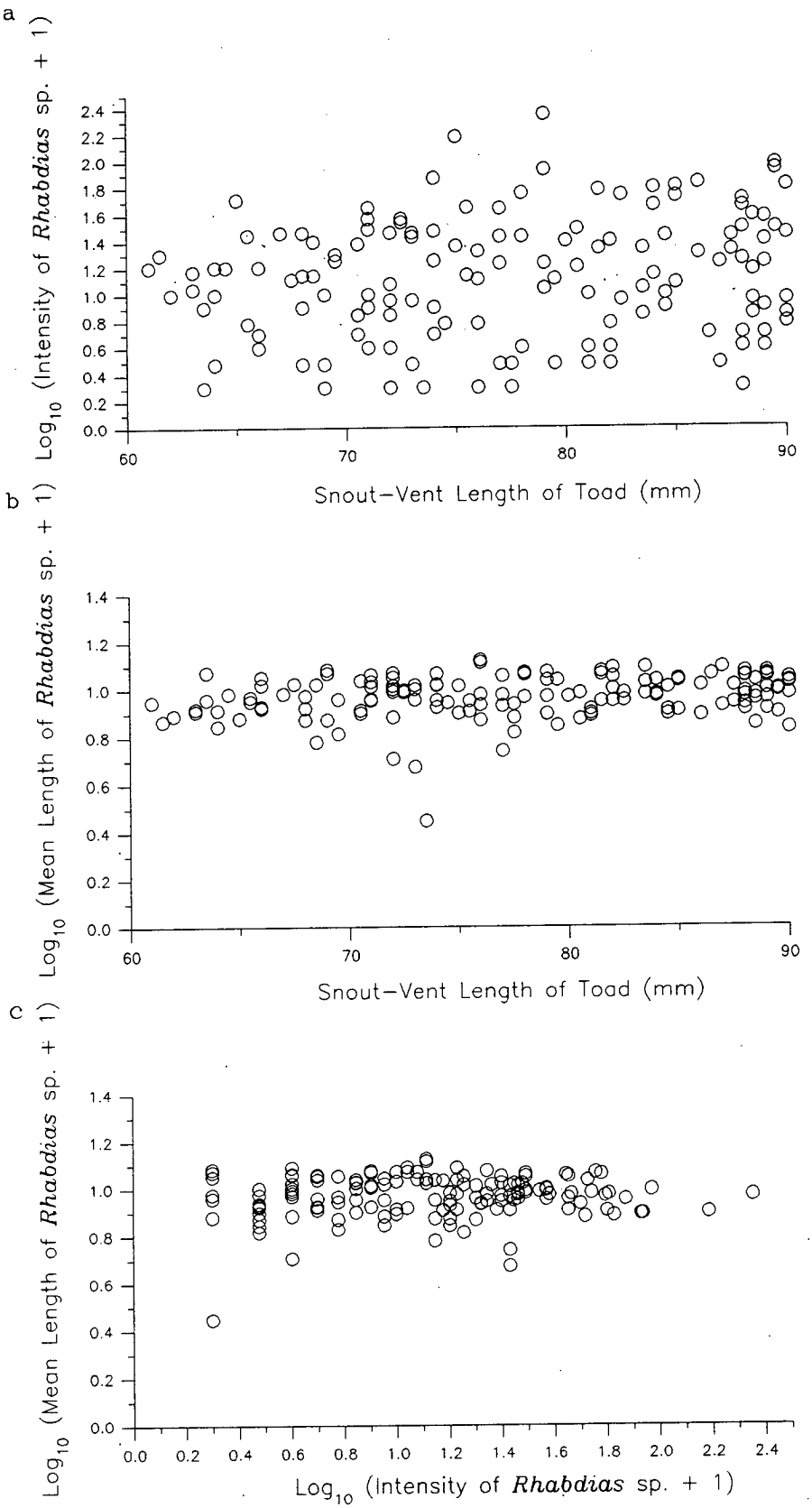

a
Log10 (Intensity of Rhabdias sp. + 1)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
20
30
40
50
60
Snout–Vent Length of Toad (mm)
b
Log10 (Mean Length of Rhabdias sp. + 1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
20
30
40
50
60
Snout–Vent Length of Toad (mm)
c
Log10 (Mean Length of Rhabdias sp. + 1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Log10 (Intensity of Rhabdias sp. + 1)

Figure 4.5 Relationship between
a) intensity of *Rhabdias* sp. infection and snout-vent length (SVL) of toad,
b) average length of *Rhabdias* sp. and SVL, and
c) average length of *Rhabdias* sp. and intensity of *Rhabdias* sp. infection
for mid-size class toads (60.5-90mm SVL) collected.


a
Log10 (Intensity of Rhabdias sp. + 1)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
60
70
80
90
Snout–Vent Length of Toad (mm)
b
Log10 (Mean Length of Rhabdias sp. + 1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
60
70
80
90
Snout–Vent Length of Toad (mm)
c
Log10 (Mean Length of Rhabdias sp. + 1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
Log10 (Intensity of Rhabdias sp. + 1)

was significant. Both the relationships between SVL and intensity of *Rhabdias* sp. infection, and intensity of *Rhabdias* sp. infection and average length of nematodes were not significant.

The largest size class showed all three relationships, as tested above, to be not significant. Negative relationships were found between intensity of *Rhabdias* sp. infection and both toad SVL and average length of nematodes.

Frequency distributions of number of *Rhabdias* sp. per toad in the three toad size classes are shown in Fig 4.6 (a-c). None of the distributions in the 3 toad size classes corresponded to a Poisson (<60mm: χ^2_9=1455.8, p>0.5; 60.5-90mm: χ^2_{11}=399.9, p>0.5; >90.5mm: χ^2_{10}=258.3, p>0.5). All three toad classes showed highly aggregated distributions; calculated VMR became more aggregated as the host size class increased (<60mm: 11.28, 60.5-90mm: 36.45, >90.5mm: 41.13). Toads with zero helminths dominated the distributions of both Class I and III toads (27% and 9.5%, respectively). In Class II, toads with 2 or 3 nematodes dominated (total of 14.2%). Maximum infection levels in the three size classes were 68, 224, and 230, respectively.

Significant difference was found between the proportion of infected toads in the three size classes (χ^2_2=42.7, p<0.001). In Class I the ratio of infected to uninfected toads was 2.7:1, in Class II the ratio was 21:1 while in Class III the ratio was 9.5:1.

Intensity of *Rhabdias* sp. infection differed significantly between adult (Class II and III, combined) and subadult toads ($F_{1,478}$=100.49, p<0.0001); mean intensity of *Rhabdias* sp. in subadult toads was 0.76±0.02, in adult toads 1.15±0.03. Average length of nematodes also differed significantly between the two groups ($F_{1,478}$=196.99, p<0.0001); subadults: 0.86±0.01mm, adults: 0.98±0.01mm.

Figure 4.6 Frequency distribution of numbers of *Rhabdias* sp. per toad for

a) <60mm SVL toads,

b) 60.5-90mm SVL toads, and

c) >90.5mm SVL toads.

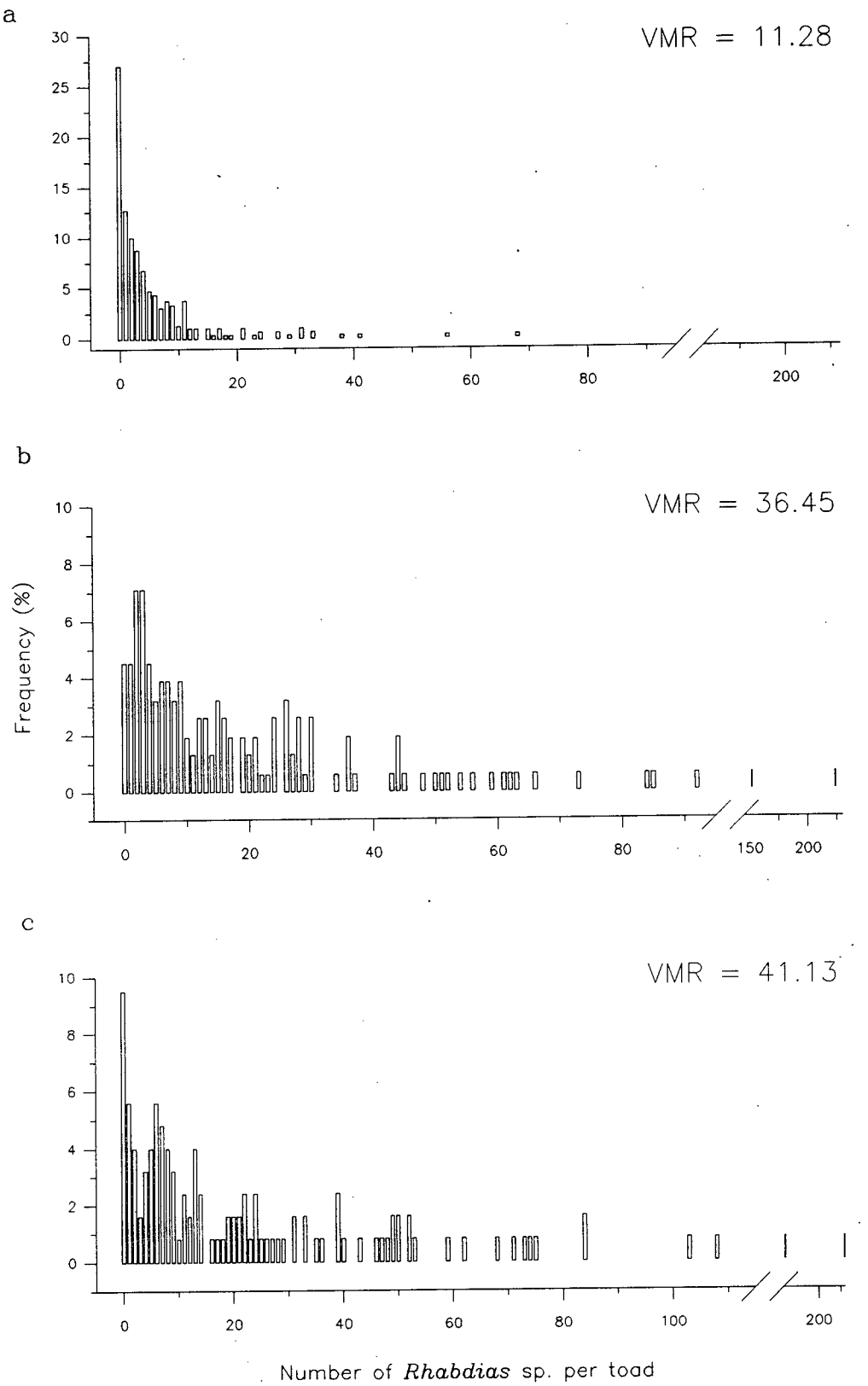

a
VMR = 11.28
30
25
20
15
10
5
0
0
20
40
60
80
200
b
VMR = 36.45
Frequency (%)
10
8
6
4
2
0
0
20
40
60
80
150
200
c
VMR = 41.13
10
8
6
4
2
0
0
20
40
60
80
100
200
Number of Rhabdias sp. per toad

FIG 4.6

4.3.3 Relationship between infection parameters and adult toad sex.

Over the collection period, 125 adult female and 155 adult male toads were collected (Classes II and III combined). Results of the statistical analyses are presented in Table 4.3; only infected toads are included. Overall, no significant difference in intensity of infection between sex was found (average intensity for ♂: 1.19±0.04; ♀: 1.10±0.05). Average length of *Rhabdias* sp. in an infrapopulation showed significant difference between sex (♂: 1.00±0.01mm; ♀: 0.96±0.01mm).

When the two adult size classes (60.5-90mm, >90.5mm) were considered independently, sex of toad was found to have a significant relationship with average length of *Rhabdias* sp. in the 60.5-90mm size class (66 male, 81 female toads collected). In this class, females had smaller nematodes (0.95±0.01mm) than males (0.99±0.01mm). For all other relationships, sex of adult toad was not significant. In Class III, 82 male and 32 female toads were collected.

4.3.4 Relationship between infection parameters and month of collection.

Collection of *B. marinus* from QDPI occurred for 16 of the 20 months sampled. Figure 4.7 presents monthly rainfall data at QDPI for the collection period. The wet season for 1990-1991 occurred from December 1990 to February 1991, with the peak of rainfall occurring in the latter month (>701mm). During this time the water impoundment was converted into a flowing creek which emptied into Ross River. By March 1991 the impoundment had returned to still water and remained with water until August 1991 from which time it was empty. The wet

Table 4.3. Results of one-way analysis of variance (ANOVA) of relationship between sex of adult toad and intensity of *Rhabdias* sp. infection and average length of nematodes in an infrapopulation. Legend as for Table 4.1.

FACTORS	TEST	N	DF	RESULT	PROB	SIG/NS
Overall:						
Sex of toad v # *Rh.*	1 way ANOVA	261	1,259	F = 2.41	p = 0.1219	NS
Sex of toad v *Rh.* Length	1 way ANOVA	261	1,259	F = 15.43	p = 0.0001	SIG
Toads 60.5-90 mm:						
Sex of toad v # *Rh.*	1 way ANOVA	147	1,145	F = 0.20	p = 0.6551	NS
Sex of toad v *Rh.* Length	1 way ANOVA	147	1,145	F = 8.54	p = 0.0040	SIG
Toads >90.5 mm:						
Sex of toad v # *Rh.*	1 way ANOVA	114	1,112	F = 1.80	p = 0.1829	NS
Sex of toad v *Rh.* Length	1 way ANOVA	114	1,112	F = 1.61	p = 0.2065	NS

Figure 4.7 Level of rainfall at QDPI over the collection period from August 1990 to March 1992.

Numbers in parentheses indicate number of toads collected in that monthly sample.

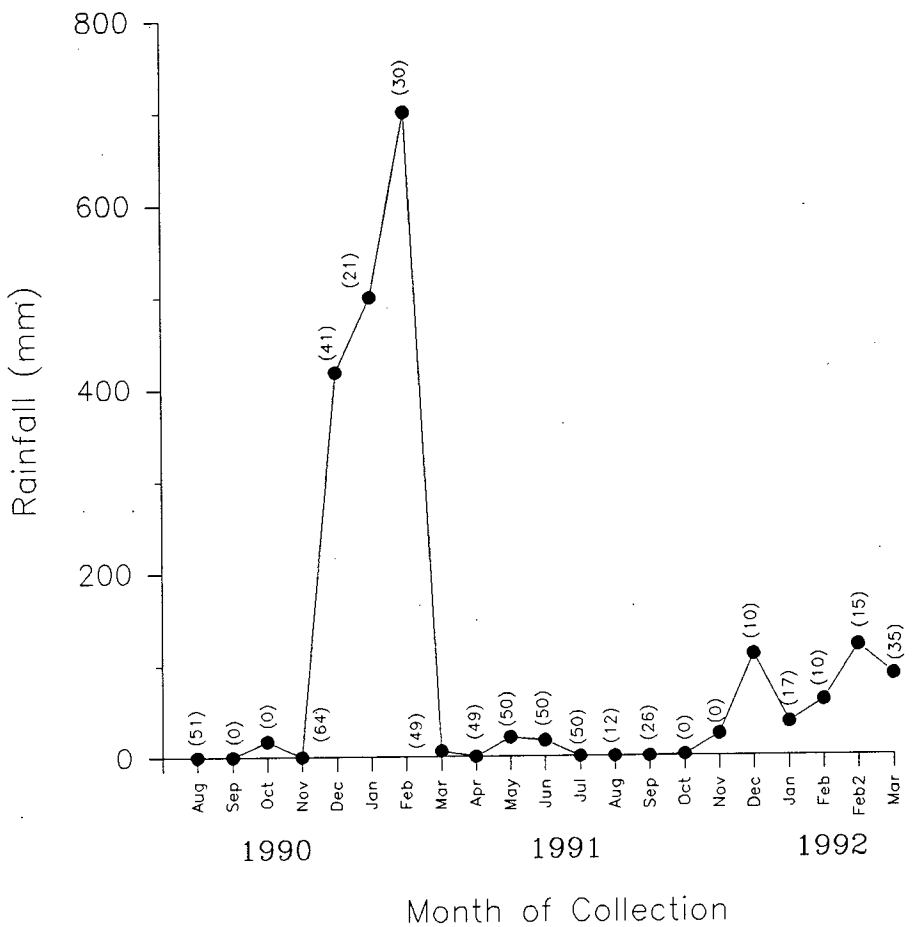

800
600
400
200
0
Rainfall (mm)
(51)
(0)
(0)
(64)
(41)
(21)
(30)
(49)
(49)
(50)
(50)
(50)
(12)
(26)
(0)
(0)
(10)
(17)
(10)
(15)
(35)
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Feb2
Mar
1990
1991
1992
Month of Collection

FIG 4.7

season for 1991-1992 failed, with only scattered showers over this period allowing water to accumulate in the impoundment for short periods of time. Two samples were collected three weeks apart in February 1992 due to heavy rain following the usual February collection.

Results of statistical analyses on relationships between month of collection, toad length, and the parameters of *Rhabdias* sp. infection are presented in Table 4.4.

Overall, significant difference in intensity of infection of *Rhabdias* sp. and average length of nematodes was found between months.

Separation of toads into size classes and analysis of relationship of month of collection showed significant relationships occurred for Class I toads in both parameters of *Rhabdias* sp. infection (Figure 4.8a,b). Subadult toads collected in the first three samples (August to December 1990) represent the Class I cohort for 1990 which became included in the next size class (60.5-90mm) in subsequent samples (see Figure 4.9). Average length of nematodes in the 1990 Class I cohort significantly differed between these 3 months ($F_{2,89}$=15.27, $p<0.0001$); intensity of infection did not differ between months ($F_{2,89}$=0.70, $p=0.5036$).

The group of subadult toads collected from March to December 1991 represent a new cohort from first infection in March with a mean intensity of 0.43±0.04 which rose to 0.96±0.21 by August 1991 (see Figure 4.8a, Table A7.1). This difference was statistically significant ($F_{6,120}$=5.78, $p<0.0001$). Average length of nematodes fluctuated over this same period (see Figure 4.8b, Table A7.1) but the difference was not significant ($F_{6,120}$=1.37, $p=0.2294$). Average length was high in March (0.87±0.02mm), June (0.84±0.02mm) and September (0.88±0.05mm), while subadults collected in May and August, 1991 had the lowest average lengths

Table 4.4. Results of one-way analysis of variance (ANOVA) of relationships between month of collection and intensity of *Rhabdias* sp. infection and average length of nematodes in an infrapopulation in all toads collected and in the three separate toad size classes. Legend as for Table 4.1.

FACTORS	TEST	N	DF	RESULT	PROB	SIG/NS
Overall:						
Month v # *Rh.*	1 way ANOVA	480	16,463	F = 5.86	p < 0.0001	SIG
Month v *Rh.* Length	1 way ANOVA	480	16,463	F = 18.68	p < 0.0001	SIG
Toads <60 mm:						
Month v # *Rh.*	1 way ANOVA	219	9,209	F = 5.78	p < 0.0001	SIG
Month v *Rh.* Length	1 way ANOVA	219	9,209	F = 8.06	p < 0.0001	SIG
Toads 60.5-90 mm:						
Month v # *Rh.*	1 way ANOVA	147	15,131	F = 1.60	p = 0.0819	NS
Month v *Rh.* Length	1 way ANOVA	147	15,131	F = 5.25	p < 0.0001	SIG
Toads >90.5 mm:						
Month v # *Rh.*	1 way ANOVA	114	15,98	F = 0.88	p = 0.5924	NS
Month v *Rh.* Length	1 way ANOVA	114	15,98	F = 3.68	p < 0.0001	SIG

Figure 4.8 Relationship of month of collection with
a) mean intensity of *Rhabdias* sp. infection, and
b) mean length of *Rhabdias* sp. per infrapopulation for toads in the 3 size classes of <60mm, 60.5-90mm, and >90.5mm from QDPI.

Points have been offset to allow for easier interpretation; all samples were collected on the same date for that month.

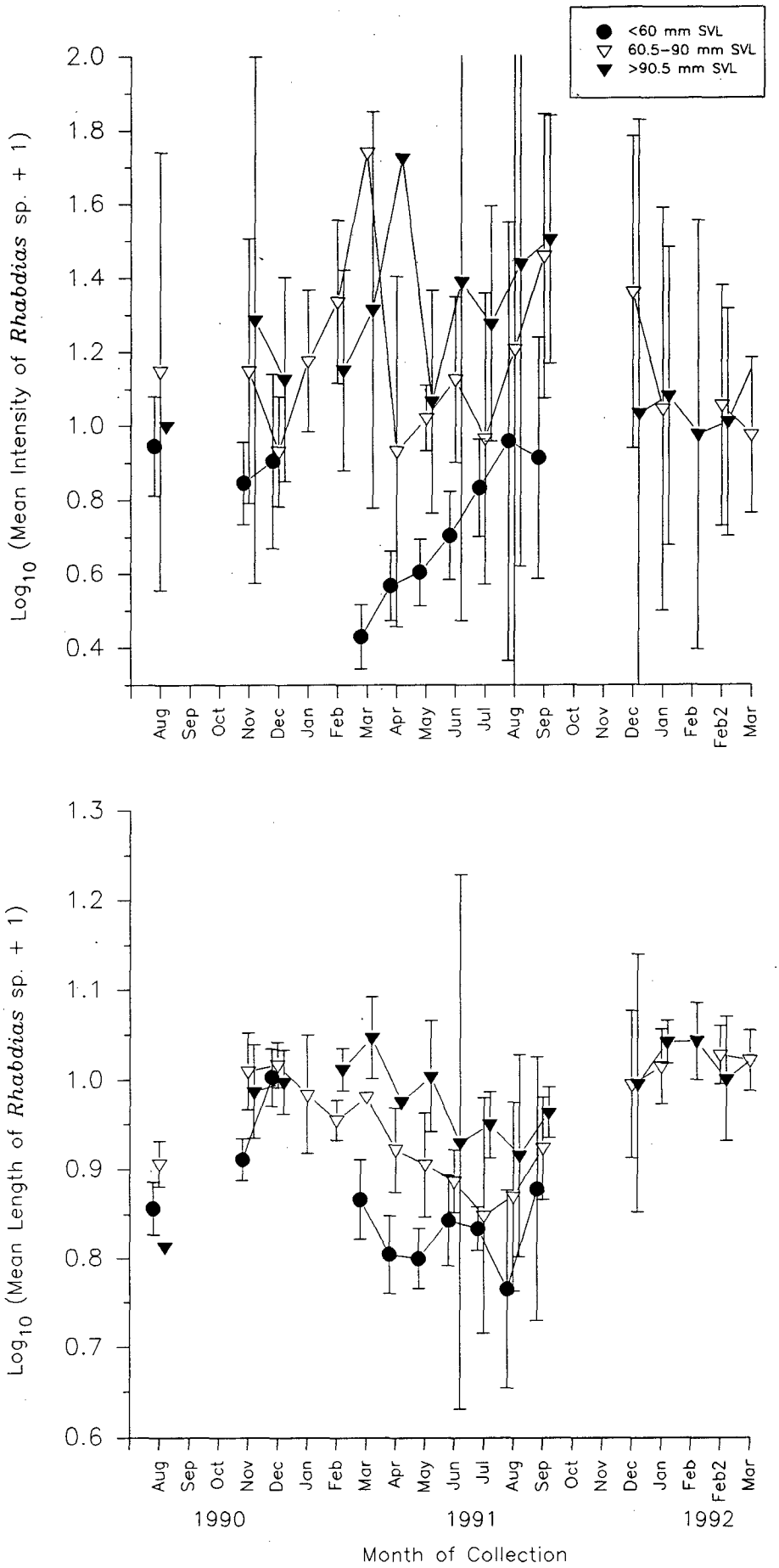
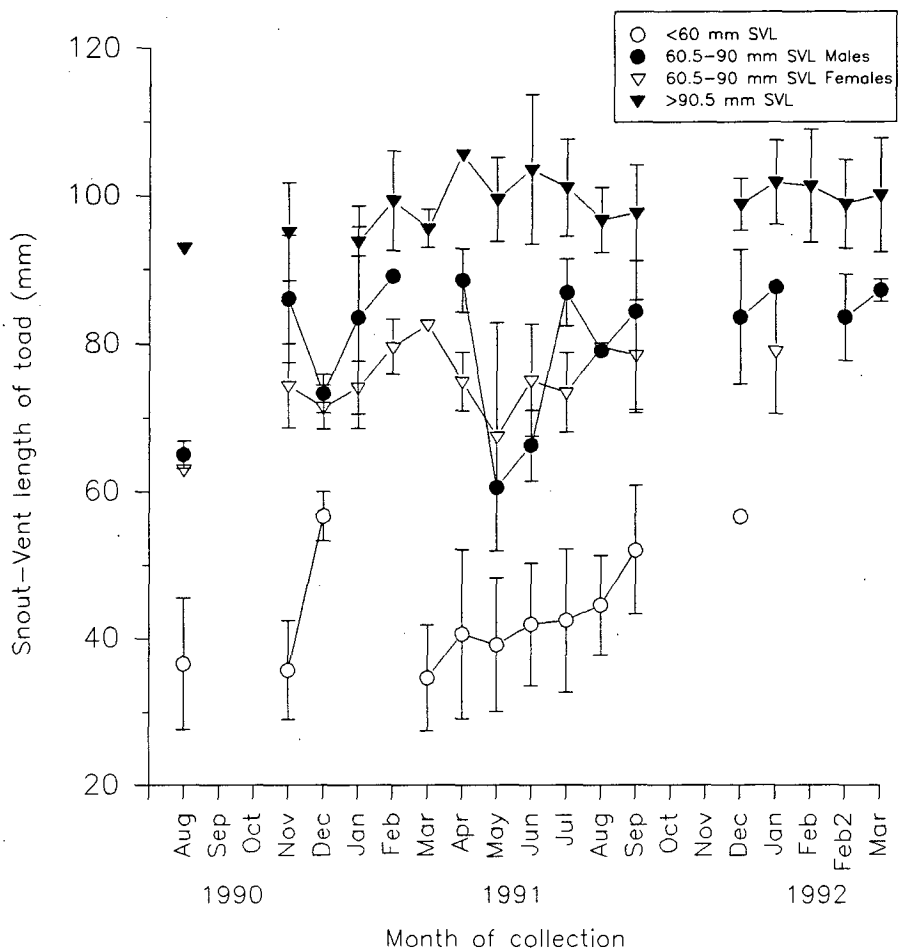

<60 mm SVL
60.5–90 mm SVL
>90.5 mm SVL
Log10 (Mean Intensity of Rhabdias sp. + 1)
Log10 (Mean Length of Rhabdias sp. + 1)
Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Feb2 Mar
1990
1991
1992
Month of Collection

Figure 4.9 Mean snout-vent length of toads in three toad size classes collected from QDPI over a 20 month period.


<60 mm SVL
60.5–90 mm SVL Males
60.5–90 mm SVL Females
>90.5 mm SVL
Snout–Vent length of toad (mm)
120
100
80
60
40
20
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Feb2
Mar
1990
1991
1992
Month of collection

(0.80±0.02mm and 0.80±0.04mm, respectively). Sample sizes of subadult toads declined in the last 3 collections (August, September, December); no subadults were collected in the 1992 samples.

Intensity of *Rhabdias* sp. infection for Class II toads did not significantly differ between months (Figure 4.8a). Average length of nematodes and month of collection did have a significant relationship (Figure 4.8b). Average nematode length declined from 1.01±0.02mm (November 1990) to 0.87±0.02mm (August 1991), then rose sharply over the final months of sampling (see Figure 4.8b).

The largest toad size class (>90.5mm) showed a similar pattern of results to the mid size class. Relationship between month of collection and intensity of *Rhabdias* sp. was not significant (see Figure 4.8a) while month of collection and average length of *Rhabdias* sp. was (Fig 4.8b). Mean intensity of *Rhabdias* sp. infection fluctuated over the collection period. Highest levels of infection were recorded in November 1990 (1.29±0.28), April (1.72) and September 1991 (1.50±0.15), with lowest levels recorded in August 1990 (1.00), May 1991 (1.07±0.13) and February 1992 (1.01±0.14). The peak of infection in March (Class II) and April 1991 (Class III) follows the heavy rainfall (see Figure 4.7) of January and February. The second peak in September 1991 does not follow a peak in rainfall. Decline in average length of nematodes follows the rainfall peak of February 1991, but rises again from August 1991 near the end of the dry season. This pattern is not repeated in 1992. Mean length of *Rhabdias* sp. within an infection also fluctuated but followed the pattern found in the mid size class with a decline from 1.05±0.02mm (March 1991) to 0.92±0.04mm (August 1991). This was followed by a sharp rise to 1.04±0.01mm in January 1992 (see Table A7.1).

As the general pattern of the relationship between intensity of infection and average length of nematodes was similar for Class II and Class III (see Figure 4.8a,b), this data was combined and analysed. Intensity of *Rhabdias* sp. infection differed significantly between months in the combined adult size classes (see Figure 4.10a) ($F_{16,244}$=1.77, p=0.0362). Although fluctuations were apparent, peaks of infection occurred in November 1990 (1.20±0.14), March (1.39±0.18) and September 1991 (1.48±0.11). Level of infection declined, and stayed lower, for the last five collections.

Average length of nematodes in adult toads significantly differed between months ($F_{16,244}$=7.35, p<0.0001). Length of nematodes tended to be highest in the wet season (December-February) in the two years studied (see Figure 4.10b).

Results of analyses of the relationships between adult toad sexes and parameters of *Rhabdias* sp. infection over the collection period are shown in Table 4.5 and Figure 4.11 (see also Table A7.2). Level of *Rhabdias* sp. infestation (Figure 4.11a) and average length of nematodes (Figure 4.11b) varied significantly between months in male toads. Female toads, however, only had a significant relationship with average length of nematodes (Figure 4.11b). Mean intensity of infection fluctuated widely in both sexes over the collection period. Males had a peak of intensity in August 1991 of 1.93±.09 with a sharp decline to 1.40±0.21 by December 1991. Females were less variable over this period, but followed the same pattern of peak (1.28±0.70, September 1991), followed by decline (0.60±0.43, December 1991). The initial peak of infection for both sexes in March (females) and April (males) 1991 followed the heavy rainfall of February (see Figure 4.7). The subsequent, and higher, peak in August (males) and September 1991 (females) did not

Figure 4.10 Relationship of month of collection with
a) mean intensity of *Rhabdias* sp. infection, and
b) mean length of *Rhabdias* sp. per infrapopulation for subadult (Class I) and adult (Classes II and III combined) toads.

Points have been offset to allow for easier interpretation; all samples were collected on the same date for that month.

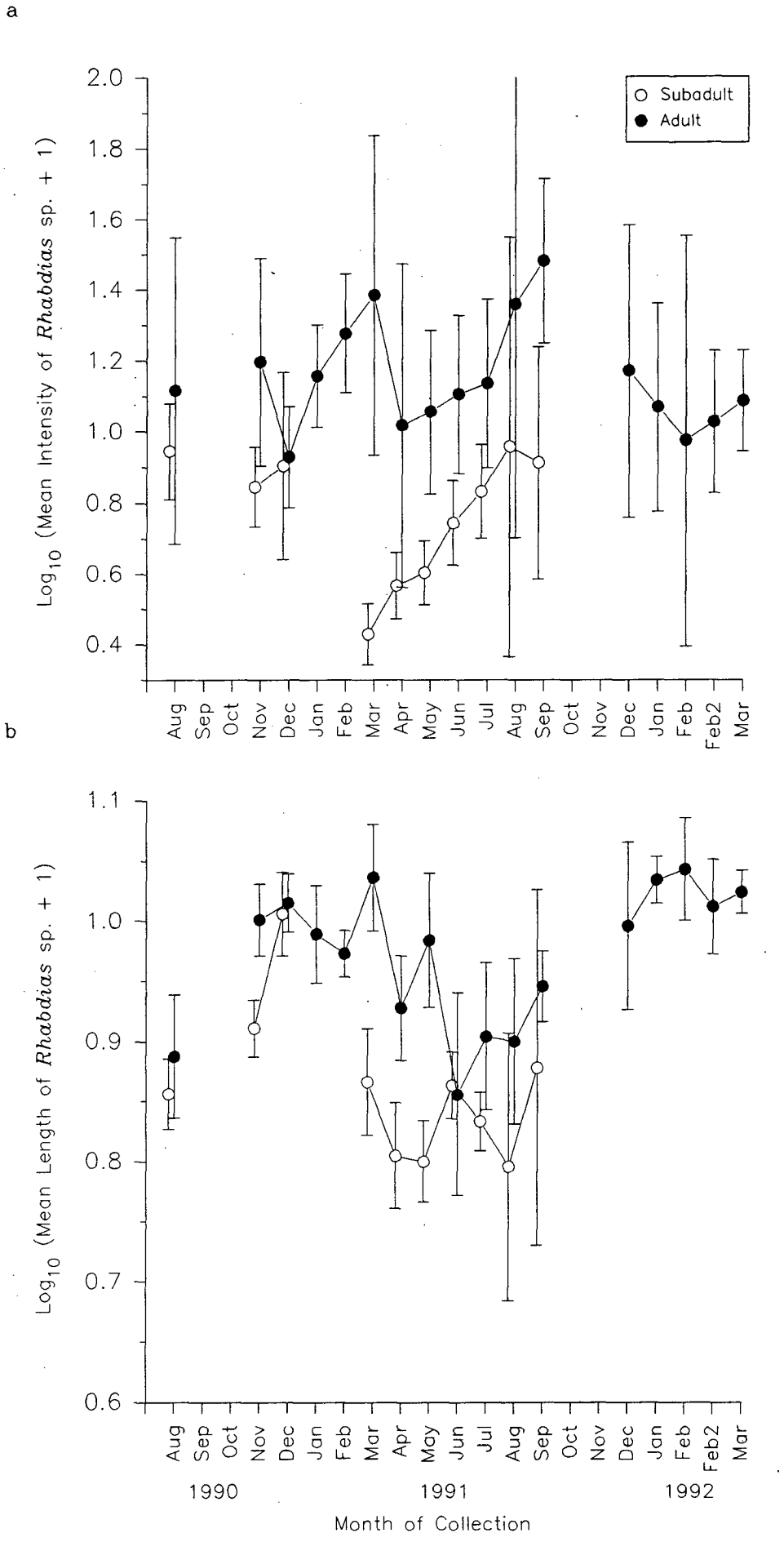

a
b
Subadult
Adult
Log_{10} (Mean Intensity of *Rhabdias* sp. + 1)
Log_{10} (Mean Length of *Rhabdias* sp. + 1)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
1.1
1.0
0.9
0.8
0.7
0.6
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Feb2
Mar
1990
1991
1992
Month of Collection

FIG 4.10

Table 4.5. Results of one-way analysis of variance (ANOVA) of relationships between month of collection and intensity of *Rhabdias* sp. infection and average length of nematodes in an infrapopulation for the two adult toad sexes. Legend as for Table 4.1.

FACTORS	TEST	N	DF	RESULT	PROB	SIG/NS
Male toads:						
Month v # *Rh.*	1 way ANOVA	148	16,131	F = 2.91	p = 0.0004	SIG
Month v *Rh.* Length	1 way ANOVA	148	16,131	F = 6.88	p < 0.0001	SIG
Female toads:						
Month v # *Rh.*	1 way ANOVA	113	15,97	F = 1.97	p = 0.0248	NS
Month v *Rh.* Length	1 way ANOVA	113	15,97	F = 2.92	p = 0.0008	SIG

Figure 4.11 Relationship of month of collection with
a) mean intensity of *Rhabdias* sp. infection, and
b) mean length of *Rhabdias* sp. per infrapopulation for adult male and adult female toads (Classes II and III combined).

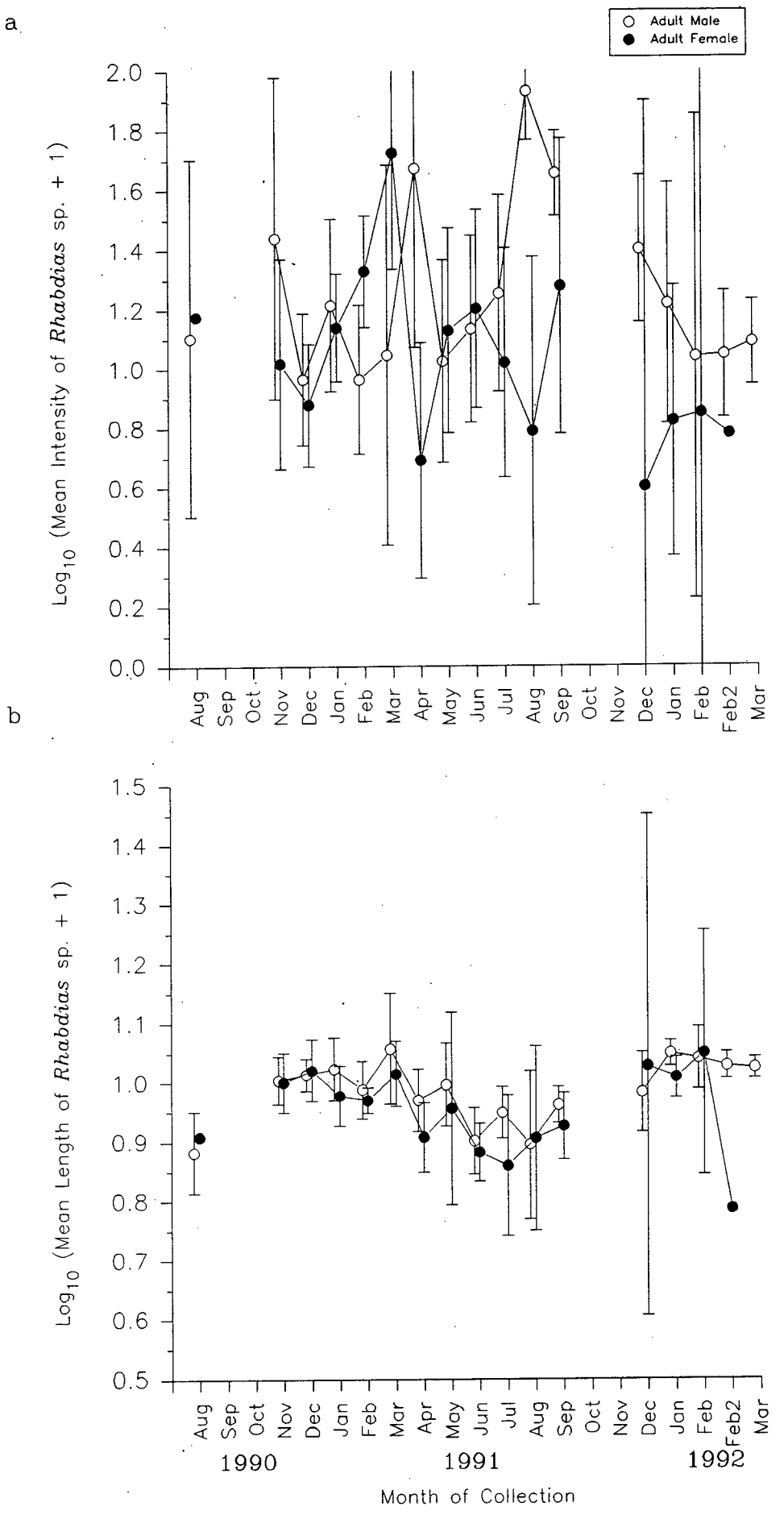

a
Adult Male
Adult Female
Log10 (Mean Intensity of Rhabdias sp. + 1)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Feb2
Mar
b
Log10 (Mean Length of Rhabdias sp. + 1)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
1990
1991
1992
Month of Collection

FIG 4.11

follow heavy rainfall. Levels of infection declined rapidly after this peak.

Mean length of *Rhabdias* sp. followed the same trends as described for the size classes above, declining slowly over the period from December 1990 (♂: 1.01±0.05mm, ♀: 1.02±0.08mm) to August 1991 (♂: 0.89±0.07mm, ♀: 0.91±0.08mm). Mean length then rose rapidly over the next four collection months but started to decline by February 1992. Length of nematodes in female toads declined abruptly in February(2) 1992. No females were collected in March 1992. Decline in mean length followed the heavy rainfalls of February 1991 (see Figure 4.7) with the subsequent rise occurring at the end of the dry season (August-December 1991). Declines in average length of nematodes in 1992 again followed rain.

4.3.5 Annual patterns in populations of *Rhabdias* sp.

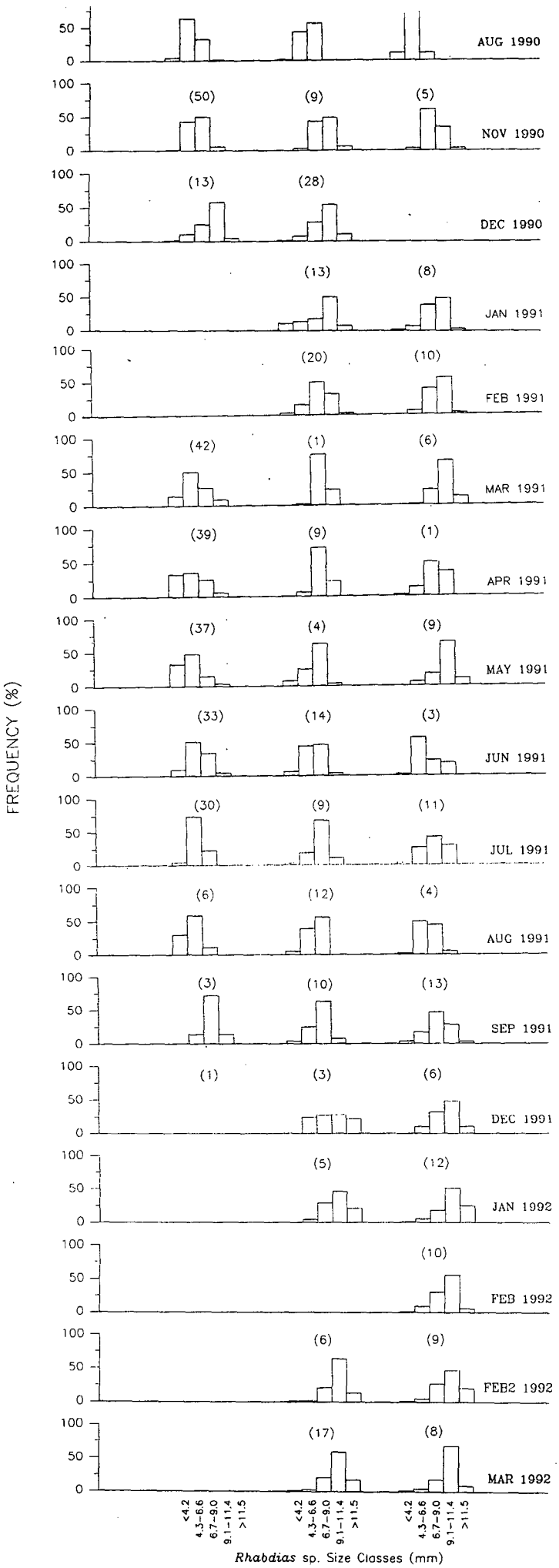
Length of *Rhabdias* sp. (untransformed data) collected ranged from 1.20 to 12.36mm, with an overall average length of 7.65mm. Size frequency distributions of *Rhabdias* sp. in the three toad size classes are shown in Fig 4.12.

Subadults collected from August to December 1990 represented the end of the Class I cohort for 1990 which became included in the next size class over the 1990-91 wet season (see Figure 4.9). Modal size class of *Rhabdias* sp. in this group, at the end of the 1990 dry season, increased from the second smallest to the second largest.

The cohort of subadult toads collected from March to December 1991 showed the development of infection during the first year from initial infection. Growth of nematodes over this period was slow and the large number

Figure 4.12 Frequency distributions of the 5 classes of *Rhabdias* sp. in the three toad size classes of <60mm, 60.5-90mm, and >90.5mm SVL for toads collected from QDPI.

Numbers in brackets indicate the number of toads collected in each sample.


AUG 1990
NOV 1990
DEC 1990
JAN 1991
FEB 1991
MAR 1991
APR 1991
MAY 1991
JUN 1991
JUL 1991
AUG 1991
SEP 1991
DEC 1991
JAN 1992
FEB 1992
FEB2 1992
MAR 1992
FREQUENCY (%)
<4.2
4.3–6.6
6.7–9.0
9.1–11.4
>11.5
Rhabdias sp. Size Classes (mm)
<60 mm 60.5–90 mm >90.5 mm
TOAD SIZE CLASSES

of infective stages, represented by the high numbers of nematodes in the smaller size classes, kept the average length of nematodes low (as seen in Fig 4.8b).

Modal nematode size class in Class II toads tended to remain the same throughout the samples. A high level of *Rhabdias* sp. recruitment was still apparent in this size class (for example, January and February 1991) and the slow growth of these worms led to a lower average length from May to August (see Figure 4.8b).

Class III toads contained primarily the larger worm size classes. This group of toads, however, acquired a few *Rhabdias* sp. infective stages in the periods of highest transmission when the other two host size classes were also receiving high numbers. Usually, the modal size class remained large, but this was occasionally influenced by an influx of smaller nematodes (for example, June to August 1991). Growth of nematodes in Class III toads appeared to be rapid (compared to Class I toads). Number of nematodes in the largest size class (>11.5mm) were more apparent in the late dry season months (September-December).

4.4 Discussion

The population structure of the nematode *Rhabdias* sp. within a *Bufo marinus* population is described for the first time.

A comparison of the infection levels of species of the genus Rhabdias from this study with literature reports is presented in Table 4.6. Generally infections with *Rhabdias* species, as reported in the literature, show low mean intensity. Prevalence of infection, however, varies considerably between host species. Level of infection of *Rh. sphaerocephala* from *B. marinus*

Table 4.6 Levels of infection of *Rhabdias* species as recorded from various amphibians in the literature and the present study.

SPECIES	HOST	N	PREV	MEAN INT.	MAX RANGE	LOCATION	REFERENCE
sphaerocephala	*Bufo marinus*	40	82.5	10	50	Bermuda Is	Williams 1960
ranae	*Rana catesbeiana*						
	(>100 mm)	33	12.1	0.18		Nth Carolina	Brandt 1936
	(<100 mm)	38	15.8	0.237		Nth Carolina	Brandt 1936
	Rana sphenocephala	60	48.3	2.48		Nth Carolina	Brandt 1936
	Bufo fowleri	62	1.61	0.05		Nth Carolina	Brandt 1936
	Scaphiopus holbrooki	60	1.7	0.017		Nth Carolina	Brandt 1936
	Pseudacris brimleyi	55	41.8	0.8		Nth Carolina	Brandt 1936
	Hyla crucifer	60	3.33	0.08		Nth Carolina	Brandt 1936
	Rana sylvatica	300	71-75	7.5	30	Canada	Baker 1979b
bufonis	*Rana esculenta*		3.0	1.2	2	Poland	Kuc&Sul. 1988
	Rana temporaria		73.1	2.9	13	Poland	Kuc&Sul 1988
	Rana arvalis		48.6	15.2	115	Poland	Kuc&Sul 1988
	Rana terrestris	197	91.0	12.0	90	Poland	Plasota 1969
	Bufo bufo	435	60-100	6-14	183	Europe	Goater 1992*
sp.#	*Bufo marinus*	580	82.8	16.1	230	QDPI (Aust.)	This study
		875	43.2	18.72	230	Australia	This study@

Kuc&Sul 1988 = Kuc & Sulgastowska 1988b; *Summarised from three studies; #See Table 3.6 for further examples from Australian native frogs; @Includes data from QDPI.

in the Bermuda Is.[1] (see Williams 1960) is closest to that found in this study for *Rhabdias* sp.

Differences in infection parameters of species of the genus *Rhabdias* (Table 4.5) may be attributed to host habitat preference (Fransden 1974; Kuc & Sulgostowska 1988b). Amphibians which prefer a terrestrial habitat, such as *B. marinus* (see Behler & King 1979), have a helminth fauna dominated by nematodes, particularly nematodes with direct life cycles (Kuc & Sulgostowska 1988b). Members of the genus *Rhabdias* have a direct life cycle (Baker 1979a) and thus could be expected to dominate the helminth fauna of terrestrial amphibians. On the other hand, aquatic amphibians, such as *Rana esculenta* (see Frazer 1989), have a digenean-dominated helminth fauna (Kuc & Sulgostowska 1988b) due to the environmental requirements of the digenean life cycles (Prudhoe & Bray 1982). Consequently, levels of infection with *Rhabdias* species in aquatic amphibians are low (compare *Rh. bufonis* infections in *R. esculenta* and *R. terrestris* in Table 4.5).

Although infections with *Rhabdias* sp. were high in toads collected at QDPI, this phenomenon did not occur in all toads sampled throughout this study (see Table 3.6). Due to the free-living part of the direct life-cycle, environmental conditions (such as humidity, temperature and rainfall) determine the distribution and abundance of *Rhabdias* sp. in Australia (see Chapter 3).

Maximum intensity of infection with species of *Rhabdias* has not always been recorded (see Table 4.5) but the value of 230 in this study is the highest so far. This may be a function of host size where larger hosts have more nematodes due to old age and/or a longer exposure time.

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[1]*Rhabdias sphaerocephala* is a native parasite of *B. marinus* introduced to Bermuda with the toad in 1855 (Easteal 1981) (see Chapter 3).

Frequency distributions of nematode parasites are typically overdispersed (Anderson & Gordon 1982; Toft 1991; Boag *et al.* 1992) which, theoretically, generally conform to a negative binomial distribution (Pennycuick 1971b; Anderson 1982; Anderson & Gordon 1982; Boag *et al.* 1992). The majority of hosts harbour few parasites while a few hosts harbour the major proportion of the total parasite population (Anderson 1982; Toft 1991; Dobson *et al.* 1992). This type of dispersion can be generated in a number of ways such as variability in exposure (host behaviour) or susceptibility/resistance (host genetics and immunity) (Anderson & Gordon 1982) and heterogeneity in the spatial distribution of parasite free-living infective stages (Dobson *et al.* 1992). The presence of an aggregated distribution cannot provide information about processes generating such patterns (Anderson 1982; Gregory *et al.* 1992). A negative binomial distribution assumes that frequencies will increase to some modal (most numerous) value to the left of the mean value before decreasing (Pielou 1977). The distribution for *Rhabdias* sp. in this study did not follow this pattern, but decreased from hosts with zero parasites (modal value) without an initial increase. For these reasons I did not fit the distribution of *Rhabdias* sp. in toads to a negative binomial. The distribution of *Rhabdias* sp. was, however, overdispersed.

Similar patterns of distribution were found for natural *Rh. ranae* infections (Baker 1979b) and experimental *Rh. bufonis* infections (Goater 1992). Neither author indicated if hosts with zero parasites was the modal group or compared their data to a negative binomial distribution.

Host size was used as an approximate indicator of host age in this study. It is known that growth patterns of anurans prevent the use of body size as an accurate and

reliable indicator of age (Halliday & Verrell 1988). Rapid growth occurs for several weeks after metamorphosis then slows down until sexual maturity, after which there are long periods of no, or very slow, growth (Zug & Zug 1979; Halliday & Verrell 1988). Growth rates also vary between season and with food availability (Zug & Zug 1979). Toads in long established populations (for example Townsville) in Australia have a smaller body size to those in frontier populations (for example Cape Weymouth) (Freeland 1986; Table 3.2). Toads in tropical regions can reach adult size within a year but in temperate areas can take two years (Straughan 1966; Easteal & Floyd 1986). All these reasons make the use of body size, as done in this study, inappropriate, but until skeletochronological studies are carried out on tropical amphibians (Halliday & Verrell 1988) this is the best estimator we have. Even then, estimation of age is only assumed accurate for toads below 100 mm SVL (Zug & Zug 1979) which toads in tropical areas may reach within two years (Zug & Zug 1979; Easteal & Floyd 1986).

The size categories used here, however, appear to correspond to data presented by various authors for size-at-sexual-maturity of *B. marinus*. Zug and Zug (1979) found female *B. marinus* were sexually mature at 70-80mm SVL (Papua New Guinea) or 90-100mm SVL (Panama), while males matured at 85-95mm SVL (Panama). A toad of 75mm SVL was assumed to be approximately 2 years of age by Straughan (1966). Although there may be overlap between the size classes used in this study and size-at-sexual-maturity, I believe the size classes reflect the important changes in host behaviour that could influence the dynamics of parasite infection.

Size of host was an important factor in determining level of infection with *Rhabdias* sp. at QDPI. Generally, both intensity of infection and average

length of nematodes per infrapopulation were positively related to host size (Table 4.1). This overall result led to the conclusion that intensity increased with toad length. After analysis of the size classes, however, this relationship remained true for the smallest size class only.

Infection of newly metamorphosed toads occurred rapidly, although at a low level (Figure 4.12). Intensity increased steadily over the following months. Newly metamorphosed *R. terrestris* became rapidly infected with *Rh. bufonis;* however the worm numbers remained fairly constant throughout the frogs' life (Plasota 1969). This was suggested to be due to the direct free-living cycle of the nematode, where changes in host diet with age would not affect *Rh. bufonis* infection levels (Plasota 1969). The effect of changes in host behaviour with age on levels of *Rhabdias* species infections have not previously been considered.

Toad behaviour alters from subadult to adult (Freeland & Kerin 1991). Post-metamorph toads are highly susceptible to dehydration and congregate in high numbers around the waters' edge (Freeland & Kerin 1991). As they grow, the toads move away from the water (Freeland & Kerin 1991), becoming nocturnal in activity, hiding in burrows or under logs during the day (Duellman & Trueb 1986).

Although the relationship of host length and intensity of infection was not significant for the 60.5-90mm SVL size class, intensity does still increase slightly. It is important to note that large toads do not become as easily infected as smaller toads. This statement is supported by evidence from the decline in proportion of large toads infected and the numbers of *Rhabdias* sp. larvae entering the host (see Figure 4.12). Although peak transmission of *Rhabdias* sp. occurs late in the wet season (March-May 1991), the number of

Rhabdias sp. entering large *B. marinus* is small. It would appear that once an old *Rhabdias* sp. infection dies out, it is not replaced, leading to the decline in proportion of infected toads in this size class. The lack of a truncated frequency distribution for *Rhabdias* sp. in the larger toads (see Figure 4.6) also suggests that infections of *Rhabdias* sp. are naturally lost and not due to some parasite-mediated mortality of heavily infected hosts.

Similar declines in proportion of infected toads in large size classes have been noted for *Rh. ranae* in *R. catesbeiana* (Brandt 1936) and *R. sylvatica* (Baker 1979b). Goater (1992) found *Rh. bufonis* in experimental infections to decline in numbers after 6 weeks post infection. Regardless of the number of larvae given to the toad (10 to 160), by 12 weeks post infection the numbers had declined to below 20 nematodes per toad.

Suggested possible reasons for these declines were an increased resistance to re-infection in the natural situation (Baker 1979b) and a density-dependent regulation of the helminth population in the experimental situation (Goater 1992). An immune response may be functioning in *B. marinus* in this study. However, I suggest that some other physiological or behavioural barrier to larval penetration is occurring. Abdominal skin thickness increases in larger toads (pers. obs.) and this may lead to prevention of penetration.

During the wet season (main breeding season), toads of all sizes congregate around water, although males are the most commonly encountered (Hearnden 1991) calling to females from the waters' edge. Females are more dispersed and come to the water primarily to breed (Hearnden, M.N. 1993, pers. comm.). Thus, females are only near the water for a short period, while males stay near the water for weeks at a time. Over the dry

season, toads alter their behaviour again, dispersing from the water (see Behler & King 1979; Freeland & Kerin 1991). Dispersal of large toads away from the water would assist in the preclusion of new recruits. Intensity of infection was found to be affected by sex of frog, with males generally more heavily parasitised than females (Lees 1962; Plasota 1969). In the case of *Rh. bufonis*, the female sex hormone is thought to depress the intensity of infection (Lees 1962). No such relationship was found in this study (Table 4.3).

Behavioural aspects may also explain the dramatic differences in intensity of infection with *Rhabdias* sp. in other amphibians at this site (see Chapter 3). A combination of factors of abdominal skin thickness and host behaviour is most likely to be responsible.

Change in parameters of *Rhabdias* sp. infection can, thus, be attributed to rainfall, its effect on toad behaviour and survival of *Rhabdias* sp. larvae. The increase in intensity of infection in toads from July 1991 (Figure 4.8a) can be related to the rainfall in May and June 1991 (Figure 4.7). This increase was more dramatic in males than females due to their staying close to the dam for longer periods. The peak in *Rhabdias* sp. infection in males in January 1992 (Figure 4.10a) was due to a small amount of rain in December 1991 (Figure 4.7). Although this rain was not sufficient to fill the dam, enough males were attracted to the area to cause an increase. The time lag between rainfall and rise in intensity of infection is due to the period between acquisition of infection and its appearance in the lungs. This effect was also found by Plasota (1969) for *Rh. bufonis*.

Average length of *Rhabdias* sp. appeared to increase with increasing length of toad in the overall infection. Again, upon analysis of the separate toad size classes, the relationship remained for the smallest and middle

size classes only.

Increasing average length with increasing toad size in the smallest toad size class can be related to growth of nematodes of the initial infection. Although the overall average did increase, Figure 4.8b did not show a clear relationship, which could be due to continued acquisition of infections over this period (Figure 4.12). This fact is shown in Figure 4.8a, with high numbers of small nematodes in the <60mm SVL toads. As the intensity of infection is increasing, new worms keep the average length low, although the average length would be increasing enough to make the relationship significant. This relationship remains in the middle size class as the toads are still growing, but are not gaining significant numbers of new infections after they have moved away from the water. Thus the average nematode length increases with toad size. This relationship ceases in the largest size class as the toads' present infection begins to die and is not replaced at an equivalent rate.

Sex of host has been found by many workers to significantly affect factors of parasite infection. In this study, however, only average length of nematode in Class II toads was affected; males had larger worms than females.

Intensity was found not to be related to average length of *Rhabdias* sp. in the infection. No significant crowding effect was evident in this study, although a negative relationship was apparent. This is in contrast to Goater (1992) who found that high numbers of *Rh. bufonis* caused decreased size and fecundity of worms. Baker (1979b) found growth of *Rh. ranae* to be affected by the number of nematodes and, in the smaller frogs, size of frog.

The actual life span of *Rhabdias* sp. is unknown. Baker (1979b) found *Rh. ranae* to only survive for one

season, with one or two generations within this time. Due to the temperate environment, worms overwintered in the lungs of the host, enabling frogs to contain near-gravid worms the following spring. In this study, however, temperature differences between summer and winter were not substantial, with rainfall the most limiting climatic factor for toad behaviour. Transmission of nematodes can occur all year if there is sufficient rainfall to keep toads close to water. I believe *Rhabdias* sp. found in *B. marinus* in this study is able to survive for approximately 2 years. The mean length of nematodes in the three size classes show gradual increase over the collection period. The smallest host size class is predominately infected with small nematodes (Figure 4.8b) which grow over the winter period to be mid-size nematodes. In the middle host size class, the nematodes grow further, reaching close to maximum size. Although the trend in the largest size class is not as apparent due to the small sample sizes, the worms remain large and close to maximum size. Declines in number of *Rhabdias* sp. in the larger nematode size classes (see Figure 4.12) show the loss of these larger worms.

At no stage were immature *Rhabdias* sp. found in the body cavity of *B. marinus*, in contrast to the studies of Baker (1979b) and Goater (1992). They found immature worms in the body cavity of hosts in infections over 10 worms for *Rh. ranae* and *Rh. bufonis*, respectively. Baker (1979b) suggested this phenomenon was due to new recruits developing slightly in the body cavity before entering the lungs. Periodic absence of worms in the body cavity suggested a period of no transmission. Goater (1992) suggested that in the experimental situation, however, there was a space limiting effect within the lung preventing larval penetration.

The patterns observed in this study must be due to

factors of toad activity, behaviour and susceptibility, as well as nematode life cycle, transmission pattern and longevity. Although the host-related factors are generally well known and can be related to parasite acquisition, the importance of the parasite-related factors remain unknown.

Part B: Helminth Community Structure

4.2 Materials and Methods

4.2.1 Description of study site

Bentley is a privately owned property at the `Bentley Estate', approximately 30km south of Townsville (see Figure 4.1). The water impoundment sampled was located 2km from the homestead in open Eucalypt woodland. The area around the impoundment was grazed by cattle but has not been cleared. The impoundment consisted of a natural stream that had been dammed by a 3m stone wall. One side of the impoundment was steep and rocky, with little vegetation, whereas the other side had a more gentle slope and vegetation which extended to the waters' edge. Aquatic vegetation was minimal.

The impoundment area varied considerably over the collection period, ranging from a fast-flowing stream to dry. Average water coverage was 10m x 10m x 1m.

4.2.2 Host species

a) *Bufo marinus*

Bufo marinus were collected from Bentley on ten occasions between January 1990 and March 1992. Collections occurred in January, February, March, April, May and August 1990, April 1991, and January, February and March 1992.

Toads were kept overnight in moist hessian bags and dissected the following day as outlined in Chapter 2.

b) *Litoria inermis*

Litoria inermis were collected from Bentley on six occasions between September 1990 and February 1992.

Months of collection were September and November 1990, April and May 1991, and January and February 1992.

Frogs were kept in moist herpetological bags overnight and dissected the following day as outlined in Chapter 2.

4.2.3 Measures of helminth community structure

Analyses of helminth community structure of both *B. marinus* and *Lit. inermis* were carried out at the infracommunity (host individual) and component community (host species) level.

Relationship between intensity of infection, for all helminth species, and SVL of host was determined by simple correlation coefficient. One way ANOVAs were used to determine the relationship of intensity with both sex of host and month of collection. Relationships of total helminth intensity and species richness to the same parameters were also tested as outlined above.

Relationship of host SVL, irrespective of species, with helminth intensity and species richness was tested by correlation coefficient.

Number of helminth species per host individual were plotted. Degree of aggregation of helminth species (individual and combined) among hosts was determined by calculation of a variance to mean ratio (VMR).

A Chi-squared homogeneity test was performed to determine if there was a difference in the proportion of infected hosts between sexes.

Measures of infracommunity structure used were: mean number of helminth individuals per host individual, mean number of helminth species per host, and mean Brillouin's Index per host (infected hosts only). Measures of component community structure (taken from grouped data) were: total number of helminth species,

number of component species, Simpson's Index, and Shannon-Weiner Index.

Analysis of the similarity of the helminth communities of *B. marinus* and *Lit. inermis* was completed with a Jaccard similarity index following the methods outlined by Magurran (1988).

$$C_j = \frac{j}{a+b-j}$$

where j is the number of shared helminth species, a is the number of helminth species for *B. marinus*, and b is the number of helminth species for *Lit. inermis*.

Comparison of helminth community structure between *B. marinus* and *Lit. inermis* was done by a two sample t-test.

Analyses of community structure follow methods as outlined by Magurran (1988). Bias of an index to either species richness or evenness (Magurran 1988) precludes that a range of indices should be calculated for helminth community studies. Values for indices were calculated for each infracommunity and included all helminths, irrespective of site of infection, using natural logarithms ($\log_e$). The final average value is expressed as a mean ± 1 SE_μ.

The reciprocal of Simpson's diversity index (D) is calculated as 1/D, where

$$D = \sum \frac{n_i(n_i-1)}{N(N-1)}$$

where n_i is the number of individuals of species i, and N is the total number of individuals.

Shannon-Weiner diversity index (H') is calculated as

$$H' = -\sum p_i \ln(p_i)$$

where p_i is the proportion of the collection belonging to species i.

Brillouin's diversity index (HB), appropriate for fully censused communities (Pielou 1977), was calculated as

$$HB = \frac{[\ln N! - \Sigma \ln n_i!]}{N}$$

where N is the total number of specimens collected, n_i is the number of specimens belonging to species i, and the symbol ! represents a factorial. Evenness, the relative abundance of individuals of species, was calculated as J = HB/HB_{max} where

$$HB_{max} = \frac{1}{N} * \ln\left[\frac{N!}{[\frac{N}{S}]!^{S-r} * ([\frac{N}{S}]+1)!^{r}}\right]$$

where S is the total number of helminth species, [N/S] is the integer value of N/S, and r=N-S[N/S].

Helminth species are determined as core species if their prevalence of infection is over 70%, secondary species if 40-70%, or satellite species if less than 40% (Stock & Holmes 1987). Further definitions of helminth species abundance were taken from Aho (1990) for common species (prevalence greater than 50%) and rare species (less than 50%). A component species is defined as having a prevalence of at least 10% (Bush *et al.* 1990).

4.3 Results

4.3.1 *Bufo marinus*

Of the 186 toads collected, 165 (88.7%) were infected with at least 1 helminth; maximum number of helminth species per toad was 6. A total of 12 helminth species were found to infect toads at Bentley: Mesocoelium sp., *Dolichosaccus symmetrus*, *D. juvenilis*, *Dolichosaccus*

helocirrus, *Pleurogenoides* sp., *Rhabdias* sp., *Maxvachonia* sp., *Johnpearsonia pearsoni*, *Parathelandros mastigurus*, *Cosmocerca* sp. 1, Nematode larvae, and Cestode larvae (Spargana) (Table 4.7). Of these 12 species, only *Rhabdias* sp. was considered a core or common species. No secondary species were recorded. The remaining species were considered satellite or rare.

Rhabdias sp. was the commonest helminth encountered, although *Parathelandros mastigurus* was the most numerous. Nematodes dominated the helminth fauna (6 of the 12 species) followed by digeneans (5 species) and cestodes (1 species). Of the digeneans, *Mesocoelium* sp. was the most frequently encountered, although *Dolichosaccus juvenilis* was the most numerous. *Rhabdias* sp. had the highest intensity of infection levels, followed by *P. mastigurus* and *Mesocoelium* sp. *Parathelandros mastigurus* had the most aggregated distribution through the host population, followed by *Rhabdias* sp. and *Mesocoelium* sp.

Average snout-vent length of all toads collected was 95.65mm (58.0-117.0mm); average length of infected toads was 95.48mm (58.0-116.5mm).

Maximum helminth intensity was 114; 49.5% of toads were infected with 10 or fewer helminth individuals. Overall VMR of infection was 27.75 (Figure 4.13). Mean intensity of infection for all helminth species was 16.53±1.61. Helminth intensity had a significant relationship with month of collection (Table 4.8a); helminth intensity declined over the sample period but increased following rainfall in February 1992 (Figure 4.14a). Variance within the samples, however, was high. Helminth intensity also had a significant relationship with sex of toad (Table 4.9a); male toads had higher levels of infection (16.38±2.02) than female toads (8.95±1.36). If *Rhabdias* sp. was removed from the analysis, however, the relationship was no longer

Table 4.7. Helminth species infecting 186 *Bufo marinus* collected from Bentley.

Helminth species	No. infected (% infected)	Mean Intensity	SE_{μ}	Minimum	Maximum	VMR
Mesocoelium sp.	53 (28.5)	6.30	1.43	1	58	21.45
Dolichosaccus symmetrus	17 (9.1)	8.35	2.48	1	32	19.46
Dolichosaccus juvenilis	26 (14.0)	11.00	2.13	1	39	19.89
Dolichosaccus helocirrus	7 (3.8)	3.86	0.74	2	8	4.58
Pleurogenoides sp.	13 (7.0)	5.08	1.11	1	14	7.69
Rhabdias sp.	136 (73.1)	10.37	1.41	1	92	28.70
Maxvachonia sp.	30 (16.1)	3.43	0.60	1	15	5.99
Johnpearsonia pearsoni	31 (16.7)	3.42	0.54	1	14	5.48
Parathelandros mastigurus	9 (4.8)	13.89	8.18	1	70	52.05
Cosmocerca sp.	3 (1.6)	1.00	0.00	1	1	
Nematode larvae	17 (9.1)	6.47	1.53	1	1	11.74
Diphyllobothrium sp.	1 (0.5)	1.00	0.00	1	1	

Figure 4.13 Frequency distribution of total helminth intensity (all helminth species) per toad for all toads collected from Bentley.

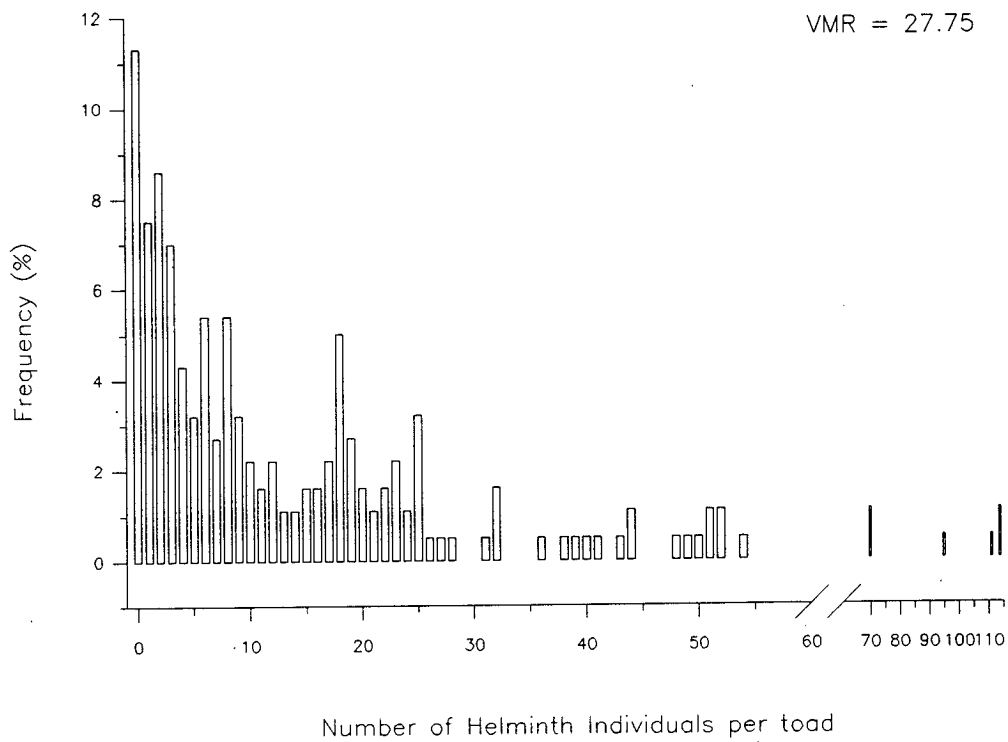

VMR = 27.75
Frequency (%)
12
10
8
6
4
2
0
0
10
20
30
40
50
60
70 80 90 100110
Number of Helminth Individuals per toad

Table 4.8. Relationship between intensity of helminth species infection in a) *Bufo marinus* and b) *Litoria inermis* collected at Bentley with month of collection. Analyses involved only those hosts infected with that particular helminth species.

a) *Bufo marinus*

Helminth sp.	N	df	F	Prob.	SIG/NS
Rhabdias sp.	136	9,126	1.89	0.0586	NS
Mesocoelium sp.	53	8,44	0.63	0.7450	NS
D. symmetrus	17	6,10	0.88	0.5432	NS
D. juvenilis	26	5,20	1.05	0.4177	NS
D. helocirrus	7	3,3	0.34	0.7976	NS
Maxvachonia sp.	30	8,21	0.61	0.7599	NS
J. pearsoni	31	8,22	11.9	<0.0001	SIG
Pa. mastigurus	9	5,3	0.36	0.8480	NS
Pleurogenoides sp.	13	6,6	2.15	0.1864	NS
Nematode larvae	17	5,11	2.72	0.0773	NS
Diphyllobothrium sp.	1				
Cosmocerca sp. 1	3				
Total Helminth Int	165	9,155	2.68	0.0065	SIG
Species Richness	165	9,155	2.98	0.0027	SIG

b) *Litoria inermis*

Helminth sp.	N	df	F	Prob.	SIG/NS
Rhabdias sp.	93	4,88	1.34	0.2619	NS
Mesocoelium sp.	3				
D. symmetrus	2				
Maxvachonia sp.	2				
J. pearsoni	1				
Pa. mastigurus	38	4,33	0.47	0.7588	NS
Pleurogenoides sp.	17	3,13	2.38	0.1172	NS
Nematode larvae	4	2,1	0.25	0.8165	NS
Diphyllobothrium sp.	7	1,5	0.02	0.8871	NS
Unknown nematodes	3				
Nematotaenia hylae	2				
Total Helminth Int	118	5,112	1.08	0.3775	NS
Species Richness	118	5,112	1.92	0.0949	NS

Figure 4.14

a) Relationship of month of collection with mean helminth intensity (all helminth species) of *Bufo marinus* collected from Bentley.

b) Relationship of month of collection with mean species richness of *Bufo marinus* collected from Bentley.

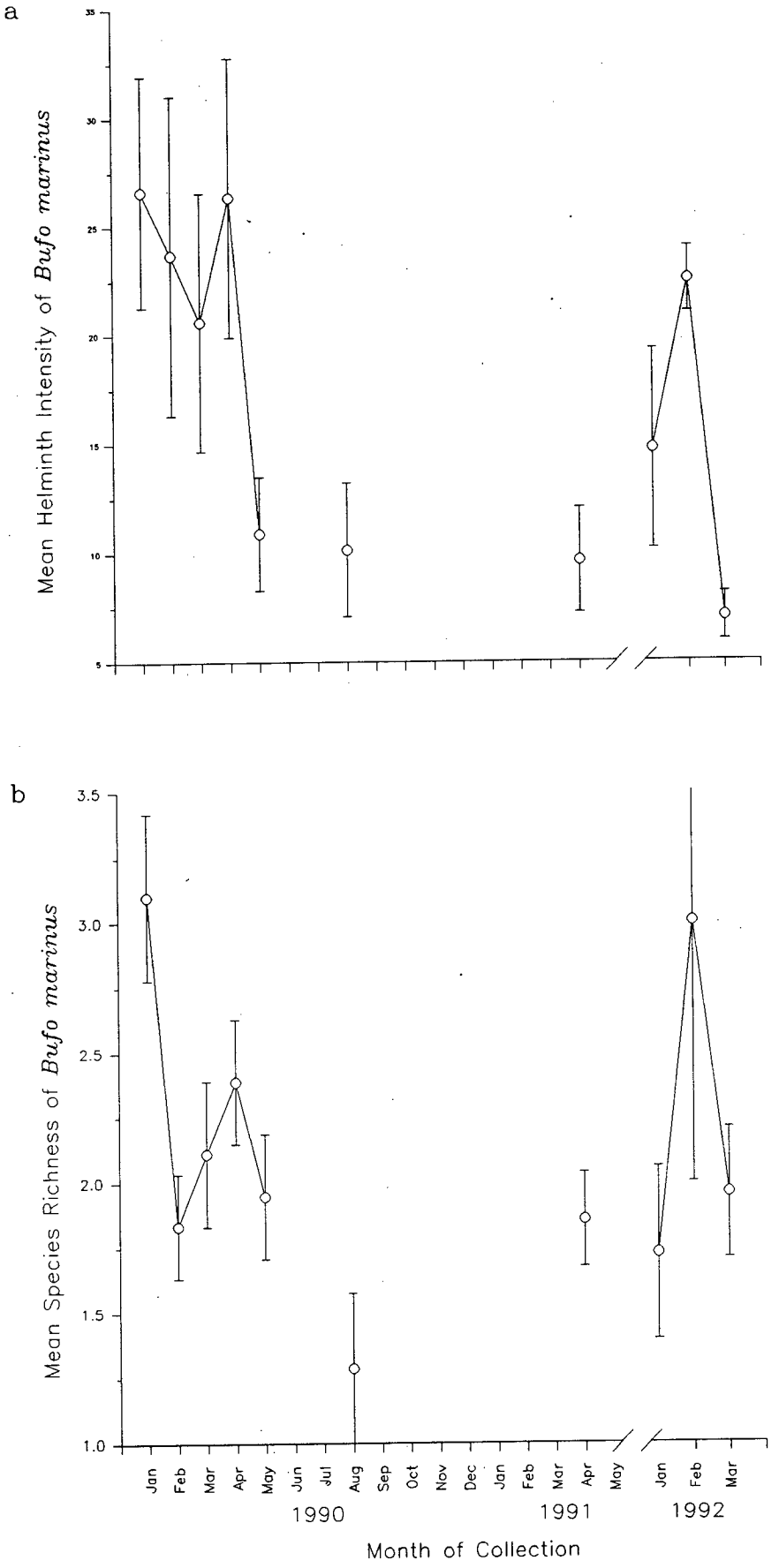

a
Mean Helminth Intensity of Bufo marinus
35
30
25
20
15
10
5
b
Mean Species Richness of Bufo marinus
3.5
3.0
2.5
2.0
1.5
1.0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jan
Feb
Mar
1990
1991
1992
Month of Collection

fig 4.14

Table 4.9. Relationship between intensity of helminth species infection in a) *Bufo marinus* and b) *Litoria inermis* collected at Bentley with sex of host. Analyses involved only those hosts infected with that particular helminth species.

a) *Bufo marinus*

Helminth sp.	N	df	F	Prob.	SIG/NS
Rhabdias sp.	136	1,134	5.15	0.0249	SIG
Mesocoelium sp.	53	1,51	0.33	0.5696	NS
D. symmetrus	17	1,15	0.65	0.4338	NS
D. juvenilis	26	1,24	1.93	0.1771	NS
D. helocirrus	7	1,5	0.49	0.5133	NS
Maxvachonia sp.	30	1,28	0.77	0.3866	NS
J. pearsoni	31	1,29	1.03	0.3183	NS
Pa. mastigurus	9	1,7	0.28	0.6116	NS
Pleurogenoides sp.	13				
Nematode larvae	17	1,15	1.78	0.2016	NS
Diphyllobothrium sp.	1				
Cosmocerca sp. 1	3				
Total Helminth Int	165	1,163	4.83	0.0294	SIG
Species Richness	165	1,163	0.27	0.6018	NS

b) *Litoria inermis*

Helminth sp.	N	df	F	Prob.	SIG/NS
Rhabdias sp.	93	2,90	0.65	0.5278	NS
Mesocoelium sp.	3				
D. symmetrus	2				
Maxvachonia sp.	2				
J. pearsoni	1				
Pa. mastigurus	38	1,36	1.78	0.1909	NS
Pleurogenoides sp.	17	1,15	0.77	0.3931	NS
Nematode larvae	4				
Diphyllobothrium sp.	7	1,5	3.12	0.1377	NS
Unknown nematodes	3	1,1	0.33	0.6667	NS
Nematotaenia hylae	2				
Total Helminth Int	118	2,115	0.68	0.5136	NS
Species Richness	118	2,115	2.86	0.0596	NS

significant ($F_{1,163}$=2.20, p=0.1403). No significant relationship was found between helminth intensity and length of toad (Table 4.10a).

Average species richness was 2.097 (1-6) (infected toads only); 34.9 % of toads had 1 helminth species, only 1 toad was infected with 6 helminth species (Figure 4.15). A significant relationship was found between species richness and month of collection (Table 4.8a). Species richness followed a similar trend as for helminth intensity with an increase following rain (Figure 4.14b). The sample size for February 1992, however, was small (2 toads). No significant relationship was found between species richness and sex (Table 4.9a) or length of toad (Table 4.10a).

Male toads were more commonly collected (143) than female toads (43). No difference was found, however, in the proportion of infected toads for each sex (χ^2_1=0.0064, p=0.9364).

Of the 12 helminth species, only *Johnpearsonia pearsoni* had a significantly different level of infection between months of collection (Table 4.7a). Samples from 1992 had higher levels of infection than previous samples (Figure 4.16).

Only *Rhabdias* sp. had a significant relationship with sex of host (Table 4.9a). Male toads had significantly higher levels of *Rhabdias* sp. infection (12.22±1.83) than females (5.029±0.99).

Intensity of *Johnpearsonia pearsoni* and *Pleurogenoides* sp. infection had a positive significant relationship between intensity of infection and SVL of toad (Table 4.10a; Figure 4.17a,c). *Parathelandros mastigurus* also had a significant relationship to toad SVL (Table 4.10a); the relationship, however, was negative (Figure 4.17b).

Table 4.10. Relationship between intensity of helminth species infection in a) *Bufo marinus* and b) *Litoria inermis* collected at Bentley with snout-vent length (SVL) of host. Analyses involved only those hosts infected with that particular helminth species. In this situation, degrees of freedom (df) can be calculated by N-1.

a) *Bufo marinus*

Helminth sp.	N	μ SVL	r	Prob.	SIG/NS
Rhabdias sp.	136	94.73	0.1128	0.2>p>0.1	NS
Mesocoelium sp.	53	95.52	0.0650	p>0.5	NS
D. symmetrus	17	98.26	-0.143	p>0.5	NS
D. juvenilis	26	94.04	0.2852	0.2>p>0.1	NS
D. helocirrus	7	104.0	0.4136	0.5>p>0.2	NS
Maxvachonia sp.	30	98.88	-0.121	p>0.5	NS
J. pearsoni	31	96.60	0.4005	0.05>p>0.02	SIG
Pa. mastigurus	9	92.78	-0.716	0.02>p>0.01	SIG
Pleurogenoides sp.	13	100.3	0.5709	0.05>p>0.02	SIG
Nematode larvae	17	98.06	0.0767	p>0.5	NS
Diphyllobothrium sp.	1	96.50			
Cosmocerca sp. 1	3	84.17			
Total Helminth Int	165	95.48	0.0798	0.5>p>0.2	NS
Species Richness	165	95.48	0.0856	0.5>p>0.2	NS

b) *Litoria inermis*

Helminth sp.	N	μ SVL	r	Prob.	SIG/NS
Rhabdias sp.	93	27.80	-0.123	0.5>p>0.2	NS
Mesocoelium sp.	3	29.33			
D. symmetrus	2	29.25			
Maxvachonia sp.	2	29.75			
J. pearsoni	1	27.00			
Pa. mastigurus	38	29.03	0.3183	0.05>p>0.02	SIG
Pleurogenoides sp.	17	29.62	-0.112	p>0.5	NS
Nematode larvae	4	28.75	-0.507	0.5>p>0.2	NS
Diphyllobothrium sp.	7	28.79	0.6733	0.05>p>0.02	SIG
Unknown nematodes	3	25.33	-0.945	0.10>p>0.05	NS
Nematotaenia hylae	2	30.00			
Total Helminth Int	118	28.22	0.0650	0.5>p>0.2	NS
Species Richness	118	28.22	0.0975	0.5>p>0.2	NS

Figure 4.15 Distribution of number of helminth species per host individual for *Bufo marinus* and *Litoria inermis* collected from Bentley.

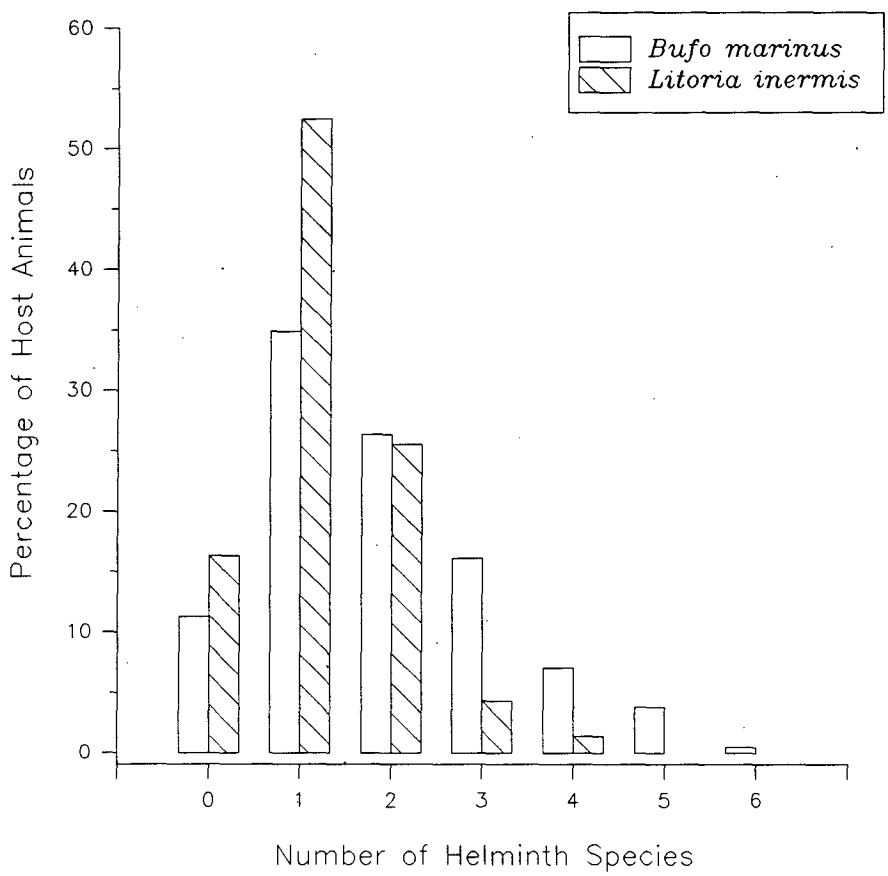

Bufo marinus
Litoria inermis
Percentage of Host Animals
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Number of Helminth Species

Figure 4.16 Relationship of month of collection with mean intensity of *Johnpearsonia pearsoni* in *Bufo marinus* collected at Bentley.

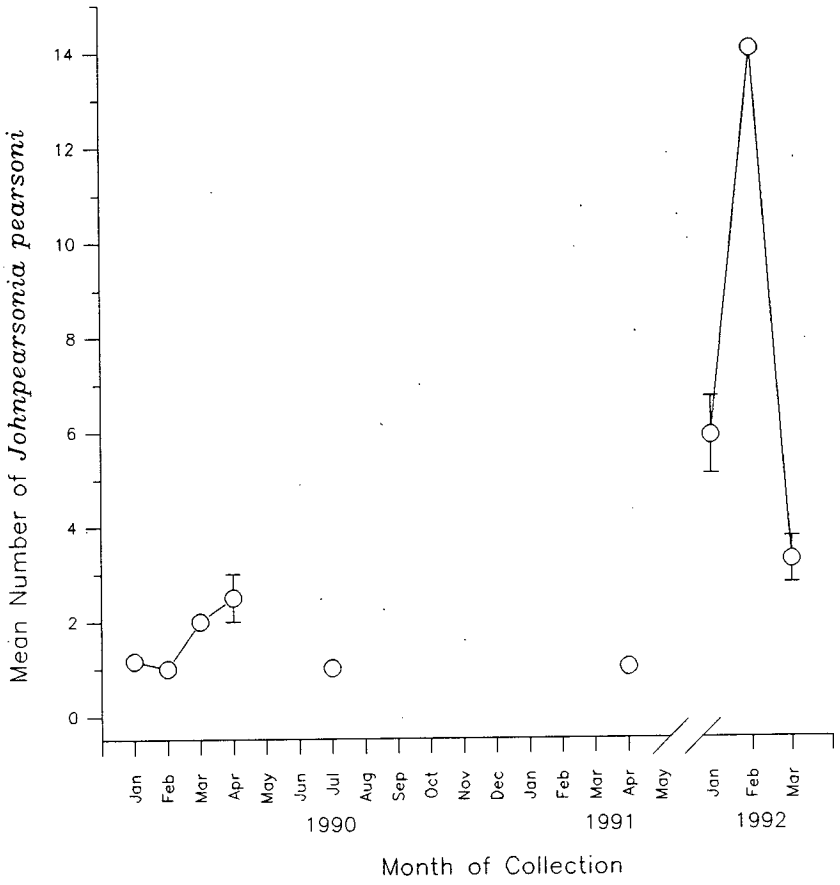

Mean Number of Johnpearsonia pearsoni
0
2
4
6
8
10
12
14
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jan
Feb
Mar
1990
1991
1992
Month of Collection

FIG 4.17

Figure 4.17 Relationship of snout-vent length of *Bufo marinus* with intensity of

a) *Johnpearsonia pearsoni*,

b) *Parathelandros mastigurus*, and

c) *Pleurogenoides* sp. at Bentley.

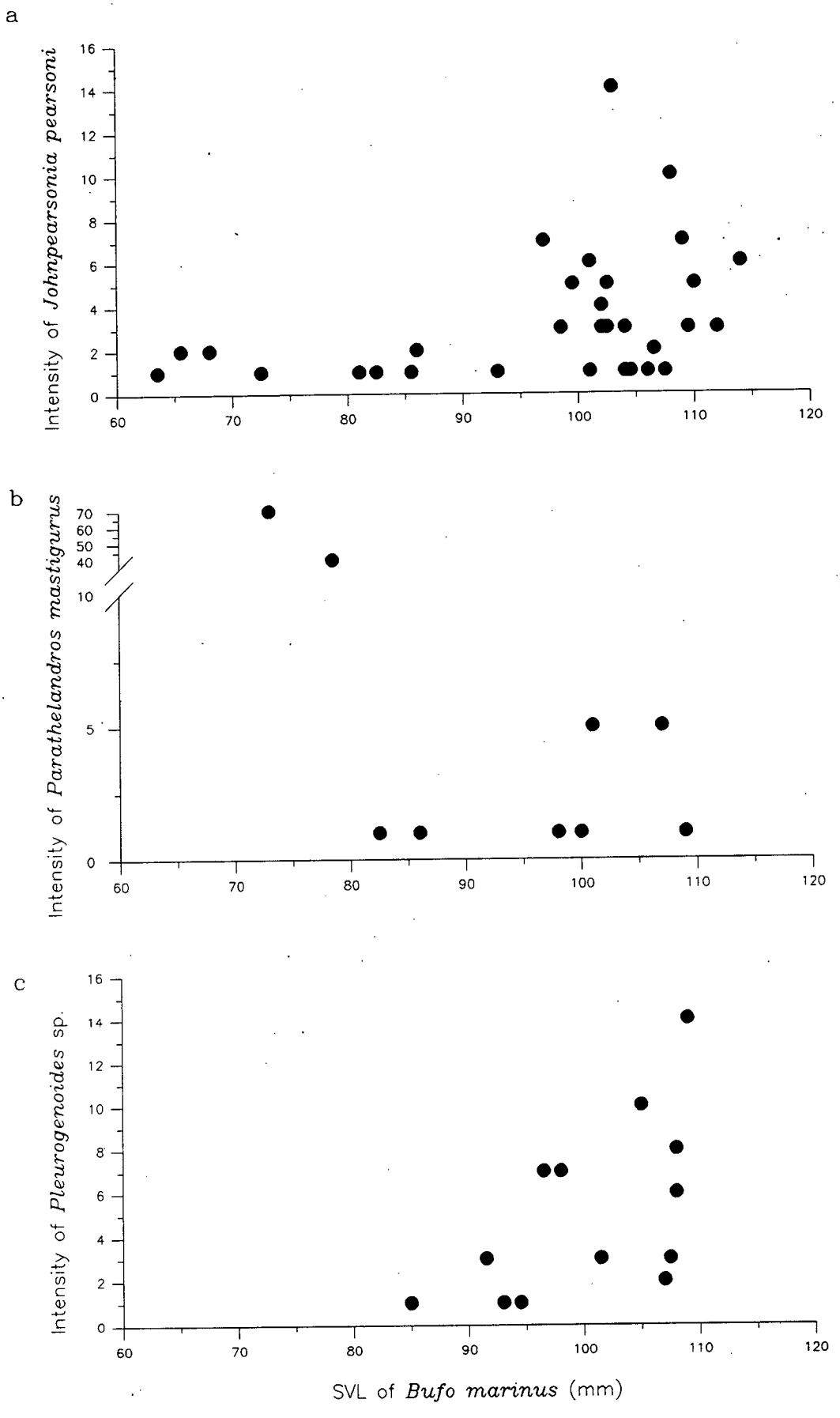

a
Intensity of Johnpearsonia pearsoni
16
14
12
10
8
6
4
2
0
60
70
80
90
100
110
120
b
Intensity of Parathelandros mastigurus
70
60
50
40
10
5
0
60
70
80
90
100
110
120
c
Intensity of Pleurogenoides sp.
16
14
12
10
8
6
4
2
0
60
70
80
90
100
110
120
SVL of Bufo marinus (mm)

FIG 4.16

4.3.2 *Litoria inermis*

A total of 141 *Lit. inermis* was collected from Bentley from September 1990 to February 1992. Of these, 118 (83.69%) were infected with at least one helminth species; maximum number of helminth species per frog was 4. A total of 11 helminth species were found to infect *Lit. inermis* at Bentley: *Mesocoelium* sp., *Dolichosaccus symmetrus*, *Pleurogenoides* sp., *Rhabdias* sp., *Johnpearsonia pearsoni*, *Maxvachonia* sp., *Parathelandros mastigurus*, nematode larvae, unknown adult nematodes, *Nematotaenia hylae*, and cestode larvae (Spargana) (Table 4.11). Of these 11 species, no species were considered core. Only *Rhabdias* sp. was considered a secondary or common species. The remaining species were considered satellite or rare species. Nine of the 11 species were shared with *B. marinus* (compare Tables 4.7 and 4.11).

Rhabdias sp. was the commonest helminth encountered, although cestode larvae had the highest mean intensity. Nematodes dominated the helminth fauna (6 of the 11 species) followed by digeneans (3 species) and cestodes (2 species). Of the digeneans, *Pleurogenoides* sp. was the most numerous and commonly encountered species. *Pleurogenoides* sp. had the highest maximum infection levels and most aggregated distribution, followed by *Rhabdias* sp. and *Parathelandros mastigurus*, respectively.

Average snout-vent length of all *Lit. inermis* collected was 28.19mm (21.0-36.0mm); average SVL of infected frogs were 28.22mm (21.0-36.0mm). A total of 5 juvenile frogs were collected in the samples. Of the adult frogs, 72 (52.9%) were female while 64 (47.1%) were male. No significant difference was found in the proportion of frogs infected for the three sexes (χ^2_2=1.567, p=0.4569) nor for adult sexes only (χ^2_1=1.525, p=0.2168).

Table 4.11. Helminth species infecting 141 *Litoria inermis* collected from Bentley.

Helminth species	No. infected (% infected)	Mean Intensity	SE_{μ}	Minimum	Maximum	VMR
Mesocoelium sp.	3 (2.1)	1.00	0.00	1	1	
Dolichosaccus symmetrus	2 (1.4)	1.00	0.00	1	1	
Pleurogenoides sp.	17 (12.1)	3.06	1.27	1	22	9.28
Rhabdias sp.	93 (66.0)	2.39	0.19	1	10	1.42
Maxvachonia sp.	2 (1.4)	3.00	1.00	2	4	0.67
Johnpearsonia pearsoni	1 (0.7)	1.00	0.00	1	1	
Parathelandros mastigurus	38 (27.0)	2.71	0.33	1	10	1.49
Nematode larvae	4 (2.8)	1.25	0.25	1	2	0.20
Unknown Nematodes	3 (2.1)	2.33	1.33	1	5	2.29
Nematotaenia hylae	2 (1.4)	1.00	0.00	1	1	
Diphyllobothrium sp.	7 (5.0)	3.29	0.71	1	7	1.09

Maximum helminth intensity of infection found was 25; 56.1% of frogs were infected with three or fewer helminth individuals. VMR of infection was 3.44 (Figure 4.18). Mean intensity of infection for all helminth species was 3.64±0.33. No significant relationship was found between helminth intensity and month of collection (Table 4.8b), sex (Table 4.9b) or SVL of host (Table 4.10b). If samples of *B. marinus* and *Lit. inermis* were combined, however, the relationship between helminth intensity and host SVL became highly significant (r_{326}=0.3525, p<0.0001) (Figure 4.19a).

Average species richness was 1.46 (1-4); 52.5% of frogs were infected with 1 helminth species, only 2 frogs had 4 helminth species (Figure 4.15). No significant relationship was found between species richness and month of collection (Table 4.8b), sex (Table 4.9b) or SVL of host (Table 4.10b). If samples of *B. marinus* and *Lit. inermis* were combined, however, the relationship between species richness and host SVL became highly significant (r_{326}=0.2772, p<0.0001) (Figure 4.19b).

No helminth species had a significant relationship between intensity of infection and month of collection (Table 4.8b).

No helminth species had a significant relationship between intensity of infection and sex of host (Table 4.9b).

SVL of *Lit. inermis* had a significant positive relationship with infection levels of *Parathelandros mastigurus* and cestode larvae (Table 4.10b; Figure 4.20). No other helminth species infection levels were correlated to host SVL.

Figure 4.18 Frequency distribution of total helminth intensity (all helminth species) per host for all *Litoria inermis* collected from Bentley.

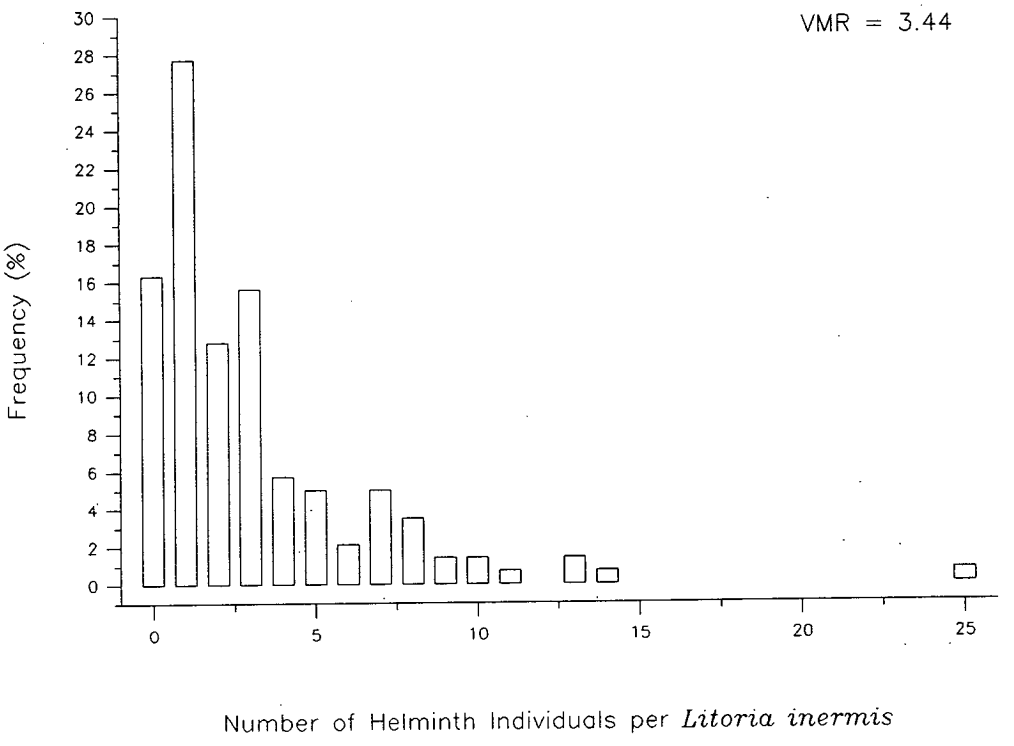

VMR = 3.44
Frequency (%)
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
5
10
15
20
25
Number of Helminth Individuals per *Litoria inermis*

FIG 4.19

Figure 4.19 Relationship of snout-vent length of *Bufo marinus* and *Litoria inermis* (combined) with

a) total helminth intensity, and

b) species richness.

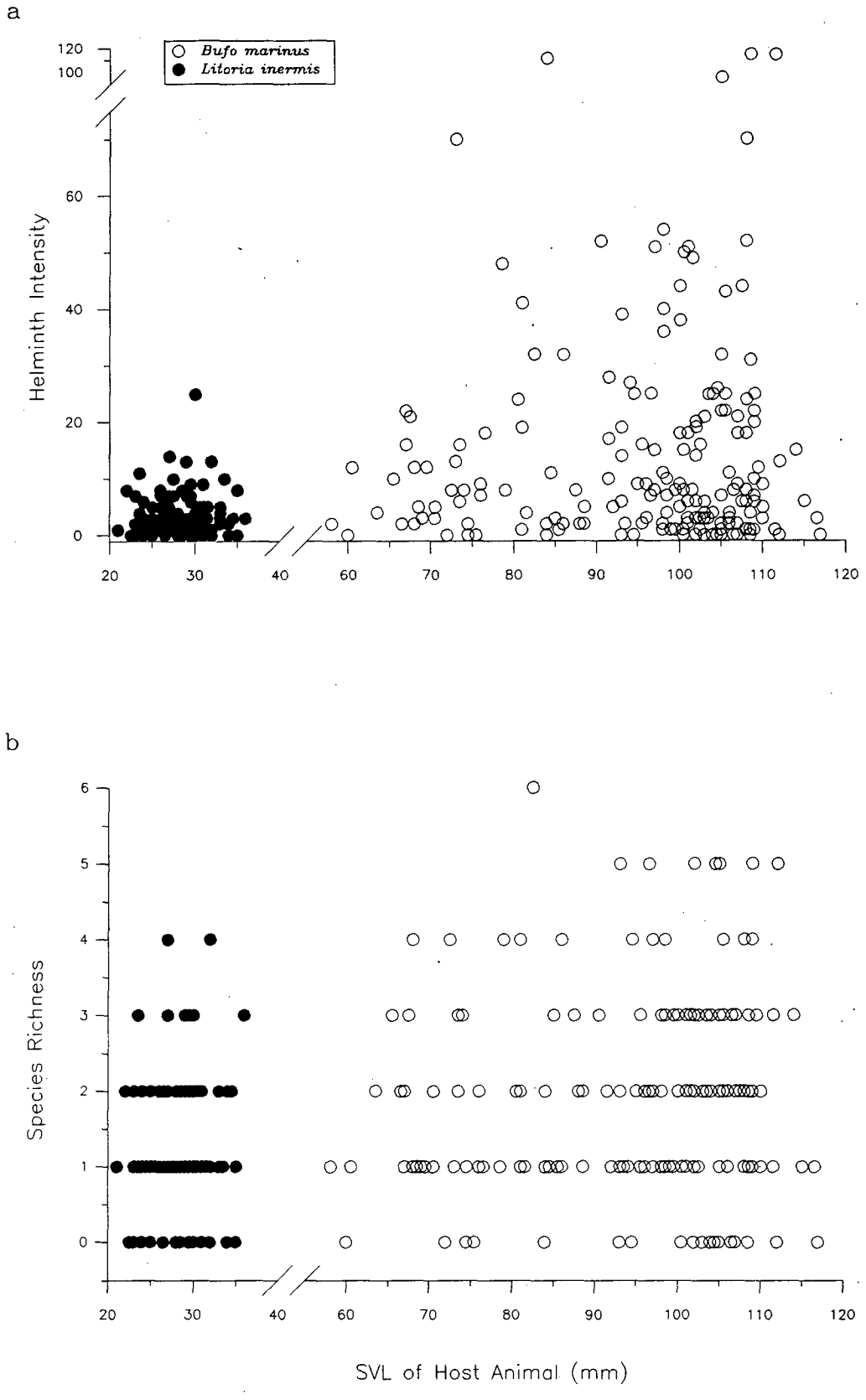
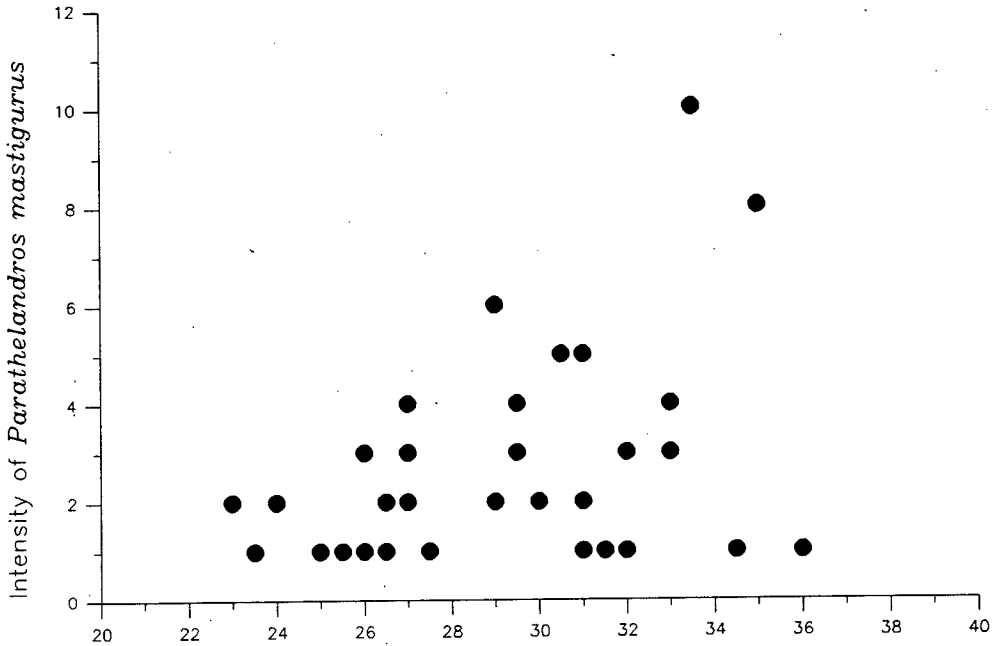
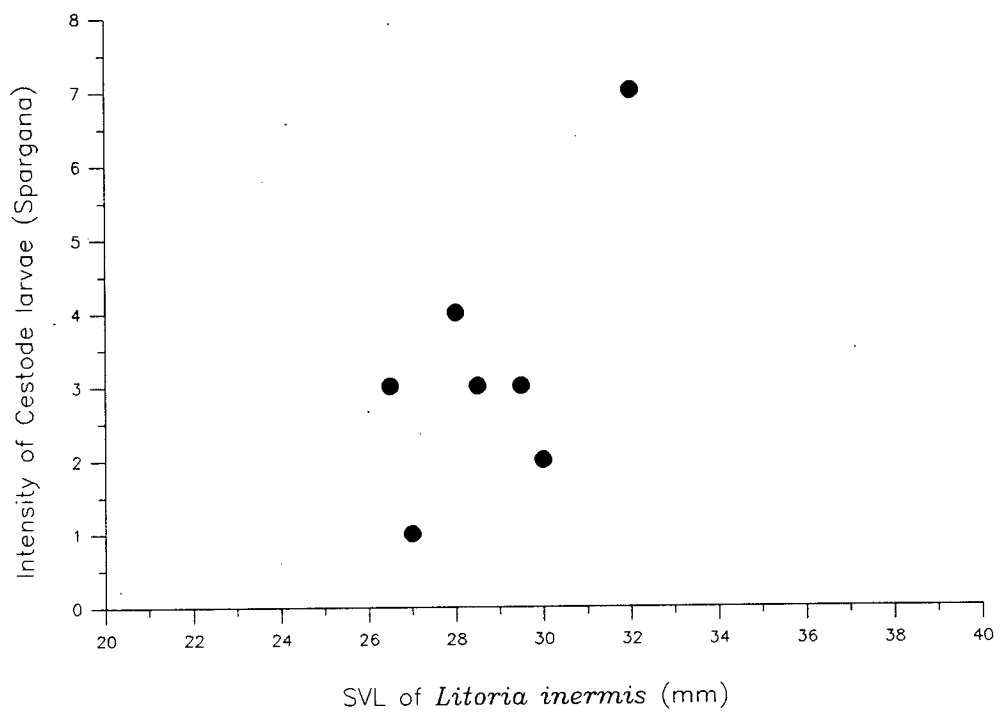

a
Bufo marinus
Litoria inermis
Helminth Intensity
120
100
60
40
20
0
20
30
40
60
70
80
90
100
110
120
b
Species Richness
6
5
4
3
2
1
0
20
30
40
60
70
80
90
100
110
120
SVL of Host Animal (mm)

Figure 4.20 Relationship of snout-vent length of *Litoria inermis* with intensity of *Parathelandros mastigurus* collected from Bentley.

a


Intensity of *Parathelandros mastigurus*
0
2
4
6
8
10
12
20
22
24
26
28
30
32
34
36
38
40

b


Intensity of Cestode larvae (Spargana)
0
1
2
3
4
5
6
7
8
20
22
24
26
28
30
32
34
36
38
40
SVL of *Litoria inermis* (mm)

4.3.3 Comparison of Helminth Communities

Due to the monthly variations in various characteristics and inconsistency of collection of both host species in sufficient numbers, the collection of hosts only in April 1991 (33 *B. marinus*, 53 *Lit. inermis*) were selected for detailed comparison.

In April 1991, *B. marinus* was infected with 9 helminth species and *Lit. inermis* with 8 species; 5 of these helminth species were shared (see Table 4.12). The Jaccard similarity index of the helminth communities of *B. marinus* and *Lit. inermis* was 0.417. *Rhabdias* sp. was the most commonly encountered helminth for both host species. If only the intestinal helminths were considered, however, *Mesocoelium* sp. was the most prevalent helminth infecting *B. marinus*, while *Dolichosaccus juvenilis* had the highest mean intensity. *Pleurogenoides* sp. was the most prevalent helminth infecting *Lit. inermis*. Cestode larvae, although found more frequently than *Pleurogenoides* sp., infect the muscles of the host.

The diversity characteristics of the infracommunities of *B. marinus* and *Lit. inermis* are presented in Table 4.13. *Bufo marinus* was infected with a significantly higher mean number of helminths (t_{31}=2.57, p=0.0151), mean number of helminth species ($t_{44.8}$=2.65, p=0.0112) and mean Brillouin's index ($t_{39.9}$=3.01, p=0.0046).

The diversity characteristics of the component communities of *B. marinus* and *Lit. inermis* are presented in Table 4.14. Only 4 helminth species for *B. marinus*, and 2 for *Lit. inermis*, were considered component species (see Table 4.12). *Rhabdias* sp. was the only component species infecting both host species. *Bufo marinus* had higher values for the Simpson's Index and the Shannon-Weiner Index.

Table 4.12. Helminth infracommunities of 33 *Bufo marinus* and 53 *Litoria inermis* collected from Bentley in April 1991.

Helminth species	*Bufo marinus*					*Litoria inermis*				
	No. Inf. (%)	Mean Int.	SE_{μ}	Min	Max	No. Inf. (%)	Mean Int.	SE_{μ}	Min	Max
Mesocoelium sp.	9 (27.3)	5.11	1.25	2	12	2 (3.8)	1.00	0.00	1	1
Dolichosaccus symmetrus	1 (3.0)	2.00	0.00	2	2	1 (3.0)	1.00	0.00	1	1
Dolichosaccus juvenilis	1 (3.0)	10.0	0.00	10	10					
Dolichosaccus helocirrus	4 (12.1)	4.50	1.19	3	8					
Pleurogenoides sp.	3 (9.1)	6.33	2.33	2	10	4 (7.5)	8.50	4.74	1	22
Rhabdias sp.	25 (75.8)	6.32	2.13	1	49	44 (83.0)	2.00	0.27	1	10
Maxvachonia sp.	6 (18.2)	2.50	1.12	1	8	1 (1.9)	2.00	0.00	2	2
Johnpearsonia pearsoni	3 (9.1)	1.00	0.00	1	1					
Cosmocerca sp. 1	1 (3.0)	1.00	0.00	1	1					
Nematode larvae						1 (1.9)	1.00	0.00	1	1
Nematotaenia hylae						1 (1.9)	1.00	0.00	1	1
Diphyllobothrium sp.						6 (11.3)	3.33	0.84	1	7

Table 4.13. Diversity characteristics of the infracommunities of helminths of *Bufo marinus* and *Litoria inermis* collected from Bentley in April 1991.

Characteristic	*Bufo marinus*	*Litoria inermis*
No. of hosts examined	33	53
Mean No. of helminths	9.64	3.26
SE_{μ}	2.40	0.65
Mean No. of helminth sp.	1.86	1.30
SE_{μ}	0.18	0.01
Mean Brillouin's Index	0.30	0.09
SE_{μ}	0.06	0.03
Mean Evenness (J)	0.48	0.16
SE_{μ}	0.09	0.05

Table 4.14. Diversity characteristics of the component communities of helminths of *Bufo marinus* and *Litoria inermis* collected from Bentley in April 1991.

Characteristic	*Bufo marinus*	*Litoria inermis*
Number of helminth sp.	9	8
No. of component sp.	4	2
Simpson's Index	2.65	2.50
Shannon-Weiner Index	1.37	0.98
Dominant species	*Rhabdias*	*Rhabdias*

4.4 Discussion

The structure of a helminth community of *Bufo marinus* is described for the first time. In addition, the helminth community structure of a native amphibian, *Litoria inermis*, is described for the first time.

The helminth communities of both *B. marinus* and *Lit. inermis* are dominated by nematodes (see Tables 4.7 and 4.11), which is indicative of terrestrial amphibians (see Fransden 1974). The majority of the nematodes in this study have direct life cycles (see Anderson 1992) either through skin penetration (for example *Rhabdias* sp.) or consumption of infective larvae. Kennedy, Laffoley, Bishop, Jones and Taylor (1986) and Aho (1990) suggested that nematodes with direct life cycles would play a major role in the species richness of isolationist helminth communities, as were found here. The proportion of digeneans was high in both *B. marinus* and *Lit. inermis* but does not indicate an aquatic lifestyle for these hosts (compare with results of Kuc & Sulgostowska 1988b for *Rana esculenta*). Goldberg and Bursey (1991a) calculated that members of the family Bufonidae would be infected with an average of 4 nematode species. *Bufo marinus* was found to have a higher number of nematode species in this study. A similar figure for hylid frogs has not been calculated.

Monthly variation in both total helminth intensity and species richness of *B. marinus* is most probably due to variations in rainfall as found for *Rhabdias* sp. at QDPI (see Part A). Although rainfall data was not available for Bentley, the pattern was similar to that for QDPI (Figure 4.7). High levels of rainfall in the wet season of 1990-1991 (December-March) led to a general decline in both infection parameters. At this time the water impoundment was turned into a flowing stream which cleared the impoundment of its aquatic

life. Reductions in helminth numbers and species after this time would be due to the loss of infected intermediate hosts from the area and dispersal of amphibians away from the main water source. Increases in both helminth intensity and species richness over 1991 was due to the generally dry conditions which caused the impoundment to decrease in size and concentrated the hosts of both species around its edge (Alford, R.A. 1993, pers. comm.). The values for February 1992 are misleading in that only 2 toads were collected, both of which were heavily infected with helminths.

Values for helminth intensity and species richness for *Lit. inermis* were not affected by month of collection. The smaller sample sizes of *Lit. inermis* may have prevented these patterns being noted.

Another factor to consider is the recent introduction of *B. marinus* to this helminth community. As the toad and helminths have not had the time to co-evolve to the same extent as has *Lit. inermis*, the helminth fauna of *B. marinus* may be more easily affected by small environmental changes which may produce substantial seasonal changes. *Litoria inermis* and its parasite fauna, on the other hand, have adapted to each other better and are, therefore, more robust to environmental changes.

Johnpearsonia pearsoni infecting *B. marinus* was the only helminth that had significant variation in infection levels over the collection period. This relationship may be artificially amplified by the high levels of infection in February 1992. This sample should be treated cautiously due to the small sample size of 2 toads. Transmission of a related helminth species, *Oswaldocruzia pipiens*, to *Rana sylvatica* occurred during the late summer and fall in Canada (Baker 1978) which would equate, climatically (by

rainfall), with the end of the dry season in this study. At this time of year the size of the water impoundment is decreasing causing a concentration of hosts which could facilitate transmission of the nematode.

Variation between toad sexes in level of helminth intensity was due to the variation of *Rhabdias* sp. Once *Rhabdias* sp. was removed, the relationship was no longer significant. This significant result was in contrast to that found in Part A, where there was no relationship between sex of toad and intensity of *Rhabdias* sp. infection. Microhabitat preferences by male and female toads may be more pronounced at Bentley due to the greater heterogeneity of possible shelter sites (logs, impressions of cattle footprints, crevices in rocks) than at QDPI (logs). Differences in infection levels of *Aplectana itzocanensis* (Nematoda) in *B. alvarius* in Arizona were explained by Goldberg and Bursey (1991a) by a similar method.

Total helminth intensity and species richness were not found to be significantly related to host SVL for either host species. Various helminth species, however, did have a significant relationship.

Johnpearsonia pearsoni had a positive relationship, indicating a continual acquisition of infective stages through the toads' life. Low numbers of infected *Lit. inermis* (1) did not allow for determination of a similar relationship in that host species. *Oswaldocruzia pipiens* did not appear to increase in intensity of infection with increasing size of *R. sylvatica* (Baker 1978). Maximum levels of infection for *O. pipiens* was around 15 (Baker 1978); a similar level was found for *Johnpearsonia pearsoni* in this study.

Parathelandros mastigurus infection levels increased in *Lit. inermis* but decreased in *B. marinus* with increasing host SVL. Numbers of toads infected with *P. mastigurus* were small (9), so this data should be

treated cautiously. A possible explanation may be found in the greater dispersal ability of toads away from a water source which may remove them from the source of infection, preventing replacement of lost infections. *Litoria inermis*, on the other hand, tends to stay close to the water source (Dr R. Alford pers. comm.) which could lead to increasing levels of infection. Dietary changes with age of host is not applicable in this situation as *P. mastigurus* has a direct life cycle (see Anderson 1992).

Pleurogenoides sp. was found only in toads with a SVL greater than 80mm, but was found in *Lit. inermis* with a SVL of less than 40mm. The relationship in toads must be due to a change in some behavioural characteristic that allows toads to come into contact with infected intermediate hosts at that time. This change could be due to either a dietary shift or a change in habitat. The suggestion of *Pleurogenoides* sp. utilising a large intermediate host appears improbable due to the infections in *Lit. inermis*. It may be possible, however, that *Pleurogenoides* is utilising more than one intermediate host, one of which is small and eaten by *Lit. inermis*, and the other large and eaten by *B. marinus*. A related helminth species *Pleurogenes claviger* which infects frogs in Europe, utilises the aquatic larvae of terrestrial insects, such as mayflies, as the intermediate host (Grabda-Kazubska 1971). Frogs become infected by eating the flying insects. The ability to catch these hosts changes with size of host (Duellman & Trueb 1986), as was found for *Haematoleochus* spp. in *Rana* spp. (Dronen 1977).

In general, the characteristics of the helminth fauna of both *B. marinus* and *Lit. inermis* correspond to those outlined by Aho (1990) for anurans. The helminth community is depauperate and dominated by parasites with direct life cycles that are host generalists. The

community is also considered isolationist due to the low number of helminth species and individuals present (see Table 4.1).

Average species richness for both *B. marinus* and *Lit. inermis* were higher than the average of 0.98 calculated by Aho (1990) for anurans in general. Total number of helminth species was at the upper limit described by Aho (1990). Average helminth intensity for *B. marinus* was higher than the anuran average (see Aho 1990), whereas *Lit. inermis* had a lower value. This is of interest considering *B. marinus* is an introduced host that has acquired the majority of its helminths from Australian native frogs (see Chapter 3). The toad, therefore, would appear to have successfully adapted to the Australian parasite fauna, and them to it.

The selection of April 1991 for the detailed comparison of helminth communities in *B. marinus* and *Lit. inermis* was due to the collection of sufficient numbers of each host species to allow statistical analyses.

Bufo marinus was found to have a significantly more diverse helminth community than *Lit. inermis*. Higher values for all parameters were recorded for *B. marinus* at both the infracommunity and component community levels. Although helminth species were shared between the host species, level of similarity was below 0.5. In addition, infection levels were generally higher in *B. marinus*. The only exception to this was *Pleurogenoides* sp., which may be due to the reasons outlined previously. Mean intensity of *Rhabdias* sp. is of interest because, although the two host species have similar prevalence of infection (*Lit. inermis* is actually higher), intensity of infection is different. Reasons for this disparity remain unknown but may be related to factors of host biology and/or physiology (see Chapter 5 for a more detailed discussion).

Possible reasons for *B. marinus* having the more diverse helminth community include host size, diet, habit and habitat differences. In addition, this increased diversity implies that the position of *B. marinus* in the Australian environment lies "across" the habitats of a wide spectrum of native amphibians and reptiles. The helminths of these native hosts, therefore, would appear not to be highly host-specific.

Within each host species, neither helminth intensity nor species richness was related to SVL. When the data for both host species was combined, however, these relationships did become significant. Whether this result is actually due to the effect of host size (only 30% of the relationship is explained by this parameter) or a combination of other host factors, such as diet and habitat, remains unclear.

Bufo marinus is a wide foraging predator (Strüssmann et al. 1984) whereas *Lit. inermis* is an ambush predator (pers. obs.). The diet of the 2 host species at Bentley differed in the size (small for *Lit. inermis* and large for *B. marinus*) and the variety (small flying insects for *Lit. inermis* and large beetles, ants, large flying insects for *B. marinus*) of prey taken. Ambush predators, theoretically, have a less diverse helminth fauna due to the lower variety of prey encountered (Aho 1990). Of the 8 helminth species collected from *Lit. inermis* in April 1991, 6 are reliant on dietary transmission (see Prudhoe & Bray 1982). The helminth fauna of *B. marinus*, however, is dominated by nematodes with direct life cycles (see Anderson 1992), which increases the richness of the helminth fauna (Kennedy, Bush & Aho 1986; Aho 1990).

The use of diversity indices in community ecology is important to define the variety and relative abundance of the species present (Magurran 1988). Unfortunately in helminth ecology, the use of indices is sporadic and

the variety of indices used is often far greater than the actual diversity of the community that they are measuring. To ease the comparison between data sets for helminth communities, parasitologists must reach consensus on which index to use. Helminth communities of amphibians and reptiles are also difficult to analyse due to their low species richness and dominance of the community by host generalists (see Aho 1990).

A range of diversity indices values should be presented for each helminth community due to different biases of either species richness or evenness (Magurran 1988). These values should include the statement of the species richness and helminth intensity (total and mean values), Simpson's Index, Shannon-Weiner Index, mean Brillouin's index and an evenness value (such as J, the evenness of Brillouin's index) (see Kennedy 1993b for example).

The Shannon-Weiner and Brillouin's indices are both better indicators of species richness, whereas Simpson's index is biased toward species dominance (Magurran 1988). Due to its computational simplicity and sensitivity to changes in abundance of the commonest species, many workers prefer Simpson's index over the Shannon-Weiner index (Magurran 1988). High evenness is usually equated with high diversity and determines the equality of the abundance of each species in the community (Magurran 1988).

Diversity values of the Shannon-Weiner and Brillouin's indices are generally between 1.5 and 3.5, although the Brillouin's index will always be a lower value (Magurran 1988). The values found in this study for *B. marinus* and *Lit. inermis* are well below this range. Theoretically, Brillouin's index is the more satisfactory of the 2 indices (Magurran 1988), however its computational difficulty is a major factor against its widespread use.

The concept of core and satellite species works well in highly diverse and species rich helminth communities, such as those found in aquatic birds (see Stock & Holmes 1987). In species-poor communities, such as those found in amphibians and reptiles, however, such terms are not meaningful. Helminth communities of amphibians are generally made up of host generalists which freely exchange between host species. This makes the community, by definition (see Bush & Holmes 1986a), satellite. For such species-poor communities even the classification of species as common or rare (see Aho 1990) does not detail the community enough. Listing the component species (prevalence greater than 10%) would give a better idea of the diversity of the community.

The study of the community ecology of helminths of amphibians is an interesting and informative field. As this field is in its infancy, however, it is necessary to standardise the methods of reporting the community structure. It is hoped that this study has achieved this by detailing the structure of the helminth communities of two amphibian species in Australia.

Chapter 5: Biological Control for *Bufo marinus*?

5.1 Introduction

Control of *B. marinus* in Australia is the primary aim of present toad research. In this context, I have considered the pathological affects of helminths on *B. marinus* in natural infections. Of the 24 helminths found to infect *B. marinus* in Australia (see Chapter 3) only *Rhabdias* sp. appears to have any potential as a control agent (Speare, R. 1991, pers. comm.). The blood-feeding habit of this nematode, coupled with its direct life-cycle, are suggested to allow for a rapid increase in intensity of infection causing a significant pathological reaction. Study into this relationship, between *B. marinus* and *Rhabdias* sp., however, has not yet taken place.

5.1.2 Literature Review: Biological control for *Bufo marinus*?

a) Why control *Bufo marinus*?

Bufo marinus is seen by many Australians as a destructive liability within the Australian ecosystem (Freeland 1985). It is "known" to adversely affect the native fauna through its voracious appetite and toxicity (see Freeland 1985, 1987; Covacevich & Archer 1975; Easteal & Floyd 1986). These claims, however, are usually lacking in substantiated evidence. An example of this is the widespread belief that toads are causing the decline in native frog numbers throughout Queensland. Although the toad may be having some, as yet unknown, effect, frog numbers are declining in areas well out of the present range of the toad. Public perception of the toad, however, remains one of general mistrust.

At present, toad research in Australia is concentrating on the toads' ecology, reproductive and population biology, and effects on native wildlife. Research directed towards finding a biological control agent is occurring primarily in South America. Whatever the control agent may be, once found it will receive far more study (Freeland 1985) on its biology and potential impact on Australian fauna prior to its introduction than was the case for the toad itself.

Certain criteria are required to be filled for the successful implementation of a biological control agent. The agent needs to be easily, and cheaply, cultured and released. Strict host specificity is needed to avoid undesirable infections of native animals. A moderate pathogenicity is best as this depresses the host population, but enables enough animals to be present (the threshold density of Dobson & May 1986) to ensure continued transfer of the agent. Successful control occurs when the pest species is kept below a population density that causes economic damage (in the case of insects; Murdoch 1992). A successful control level for the toad could, possibly, be when their presence in the Australian environment as at a similar level to that for the more common native frogs (for example *Lit. caerulea*).

b) Helminths as possible biological control agents.

Few disease organisms have been found to produce a inflammatory response in *B. marinus* (Speare 1990). Of all the organisms reported by Speare (1990), only the virus *Toddia* sp. appeared to have potential use in biological control of the toad. The effects of helminth parasites of *B. marinus*, however, on the health of the toad have received little attention (Speare 1990).

Helminths are suggested to not directly kill their host, but depress its fitness to a level where the host

will die if placed under stress (Zug & Zug 1979; Speare 1990). Parasites with moderate or low pathogenicity and a direct life cycle, theoretically, will achieve maximum host population regulation (Anderson 1979; see above).

Capillaria hepatica, a nematode with a direct life cycle requiring the death of the host (*Mus domesticus*) for transmission, has been studied for its ability to regulate host populations (Barker *et al.* 1991). Despite promising early results in the laboratory, field enclosure experiments failed to provide conclusive evidence of parasite-mediated host regulation (Barker *et al.* 1991). An unknown regulatory mechanism was, however, responsible for the depression of both control and experimental mice populations and obscured any effect of *C. hepatica* (Barker *et al.* 1991).

Helminths should still be considered as a viable option in the search for a biological control agent for the toad.

Of the helminths recorded from *B. marinus*, in both native and introduced populations, only two species were reported to produce inflammatory responses (Speare 1990). These were the nematode *Rhabdias sphaerocephala* (see Williams 1960) and a larval cestode, recorded as *Spirometra mansoni* (see Bennett 1978).

i) *Spirometra mansoni* has a wide host range, being reported in Australian amphibians, reptiles and mammals (Bennett 1978). Similar pathogenic responses were reported in these hosts (Bennett 1978). This parasite, therefore, could not be used as a biological control agent for the toad due to its low host specificity.

ii) Species of the genus *Rhabdias* generally have a low pathogenicity, but have been reported to produce an inflammatory reaction, associated with pneumonia, in certain snakes (Brannian 1984) and *B. marinus* (Hammerton 1933; zoo specimen).

Williams (1960; Bermuda Is.) recorded the death of a

4cm *Bufo* sp. experimentally infected with 1,000 larvae of *Rh. sphaerocephala*. The validity of the species *Rh. sphaerocephala* is in doubt due to reasons outlined in Chapter 3, and thus the exact species used by Williams (1960) in his study is unknown. Further study of the *Rhabdias* species infecting *B. marinus* in its natural range are needed to clarify this problem.

Rhabdias bufonis, in experimental conditions, seriously affected the growth and survival of juvenile (2-5 months post metamorphosis) *B. bufo* (Goater & Ward 1992). This result was due to a decline in food intake by infected toads. Under natural conditions, mortality induced by *Rh. bufonis* would act in addition to other forms of mortality (Goater & Ward 1992) and may act to depress the fitness to a level where the toad succumbs to other factors (Zug & Zug 1979).

A *Rhabdias* species has been found in *B. marinus* in Australia (see Chapter 3), but whether this species is the Australian *Rh. hylae*, previously recorded from native frogs (see Johnston & Simpson 1942) or an introduced South American species remains unknown.

Members of the genus *Rhabdias* are found world-wide in the lungs of amphibians and reptiles (Ballantyne 1971; Baker 1979a, 1980). The nematodes feed on red blood cells (Ballantyne 1971; Colam 1971) by rupturing the lung tissue and feeding directly on the blood leaking from the capillaries (Colam 1971). Effects of this blood feeding nematode on the haematology of toads have not been described (Speare 1990).

Where known, the life cycle of species of the genus *Rhabdias* involves a parasitic and a free-living phase (Ballantyne 1971; Kloss 1974; Baker 1979a). The parasitic stage is a hermaphroditic adult which releases fully-embryonated eggs (Ballantyne 1971; Baker 1979a). The eggs are carried into the intestine in a mucous mass (Ballantyne 1971) to be voided with the faeces (Williams

1960). Hatching of eggs in the host rectum has been observed by Ballantyne (1971) for *Rh. hylae* but not by Baker (1979a) for *Rh. americanus* or *Rh. ranae*.

The free-living dioecious generation has fully developed males and females within 27hrs (Kloss 1974; Baker 1979a). Free-living *Rh. sphaerocephala* males were more difficult to observe than females due to their smaller size, fewer numbers and shorter life span (Williams 1960).

Infective larvae develop within the body of the female within 5 days (Baker 1979a). Escape of larva(e) occurred after ingestion of internal organs of the parent body and rupturing of the outer cuticle. This process is known as matricidal endotoky (Kloss 1974; Baker 1979a). Number of larvae per female is thought to be under genetic control, but variable, dependent on the climatic conditions (Kloss 1974). *Rhabdias* species from *Bufo ictericus* in humid areas of Brazil always produced 2 larvae, whereas in drier areas 2 or 3 larvae were produced (Kloss 1974).

Infective larvae penetrate the amphibian across the abdominal skin (Kloss 1974; Baker 1979a). Larvae migrate through the body cavity before penetrating the lungs (Williams 1960; Ballantyne 1971; Baker 1979a).

c) Effects of helminths on host haematological values.

Few studies have considered the effects of disease on the haematology of an amphibian host (see Kameswari & Rao 1987; Gruia-Gray & Desser 1992). Although base levels of various haematological values for *B. marinus* in the literature are rare (Table 5.1), many other amphibian species have been studied and can be used for comparison.

Amphibian red blood cells are nucleated and large relative to other vertebrates (Mitruka & Rawnsley 1977).

Table 5.1. Literature records of packed cell volume and haemoglobin concentration for *Bufo marinus*.

Reference	N	Country[a]	Packed cell volume (%)	Haemoglobin concentration (g/dL)
Hall 1966[b]	20	?	37.3 ± 8.4	11.1 ± 2.6
Stuart 1951	72	Guatemala		8.66 ± 0.13
Gil 1975[c]	♂ 22	Venezuela	27.36-33.0	
	♀ 28		30.43-31.88	
Rodulfo & Acuða 1979[c]				
	♂ 41	Venezuela	38.58-47.83	10.3-13.79
	♀ 47		42.5-48.5[d]	11.0-13.31
Tufts *et al.* 1987[b]	10	?	22.3 ± 1.4	

[a] Where locality is unknown, toads were obtained from a commercial supplier.
[b] Data from both sexes combined.
[c] Toads collected from two geographical localities; means from both represented.
[d] Values estimated from graph.

Primary sites for red blood cell production are the bone marrow and/or spleen, dependent on the species under study (Duellman & Trueb 1986; Nikinmaa 1990). Completion of haemoglobin synthesis is undertaken in the circulating immature red blood cells (Nikinmaa 1990).

Research on amphibian haematological parameters has found considerable variation due to sex, activity, season and disease (Harris 1972; Friedmann 1974; Gil 1975; Kameswari & Rao 1987; Tufts *et al.* 1987).

Male amphibians usually have higher haematological values than females (Harris 1972; Gil 1975) but this is not always the case (Gatten & Brooks 1969). No significant differences were found for blood parameters between sex of *B. marinus* (Gil 1975), although males had higher values for packed cell volume.

Packed cell volume and haemoglobin concentration were found to significantly increase in amphibians after activity (Tufts *et al.* 1987; Nikinmaa 1990). Red blood cells were liberated from the spleen due to nervous stimulation (Nikinmaa 1990). Levels of red blood cells returned to normal approximately 1 hour after exercise for *B. marinus* (Tufts *et al.* 1987). The rise was suggested to be due to an increase in the number of circulating red blood cells relative to plasma volume (Tufts *et al.* 1987).

Differences in blood parameters related to season, particularly the spawning period, have been recorded in amphibians by many workers. Generally amphibians have reduced red blood cell counts over the breeding season (Kaplan & Crouse 1956; Harris 1972; Friedmann 1974). Kaplan and Crouse (1956) suggested a decline in haemoglobin in female *Rana pipiens* was due to the diversion of iron compounds to egg production. A similar decline was also reported for male urodeles (*Taricha granulosa*) by Friedmann (1974).

Few researchers have documented the effects of

disease on amphibian haematology (see Kaplan 1951; Kameswari & Rao 1987; Gruia-Gray & Desser 1992). Indeed, few have documented the health status of their animals when reporting base haematological values. Kaplan (1951) compared *R. pipiens* infected with red leg disease (*Pseudomonas hemophilia*) to uninfected animals and found blood parameters, such as red blood cell counts and haemoglobin concentration, to be significantly decreased in infected frogs. The degree of this reduction was related to the severity of disease (Kaplan 1951). Kaplan and Crouse (1956) related seasonal changes in blood parameters to disease resistance for *R. pipiens*.

Naturally infected *R. tigrina* were compared to uninfected frogs by Kameswari and Rao (1987). The frogs had either single or multiple helminth species infections; intensity of infection for each species was not recorded. Both haemoglobin concentration and red blood cell count were significantly decreased in infected animals, the proportion of the decrease dependent upon number of helminths species involved. Intestinal trematodes were found to be correlated with the largest decrease in values. Kameswari and Rao (1987) suggested this may have been due to trematodes somehow impairing the intestinal function. No indication was given of the feeding habits of the helminths concerned.

Red blood cell counts were also found to be significantly decreased in *R. catesbeiana* infected with frog erythrocytic virus (FEV) by Gruia-Gray and Desser (1992). Infection with FEV increased the volume of red blood cells; packed cell volume, therefore, was not significantly decreased by infection. Although haemoglobin concentration was not significantly affected by FEV infection, Gruia-Gray and Desser (1992) suggested the oxygen carrying capacity was probably impaired

(mechanism not explained), indirectly affecting the health of *R. catesbeiana*.

Due to the low number of papers dealing with amphibian haematology and parasitic disease, I will also briefly consider the same system in other cold-blooded, aquatic vertebrates.

A series of papers on the effects of the nematode *Anguillicola crassus* on the general health, including haematological factors, of the eel, *Anguilla anguilla* were presented by Boon and co-workers (Boon, Lokin, Ceusters & Ollevier 1989; Boon, Augustijn, Cannaerts, Lokin, Machiels & Ollevier 1990; Boon, Cannaerts, Augustijn, Machiels, Charleroy & Ollevier 1990). *A. crassus* has a blood-sucking pre-adult stage which occurs in the swim bladder of the eel. This parasite is causing substantial losses in the commercial eel farms in Europe (Boon, Lokin, Ceusters & Ollevier 1989). In a study of wild caught *A. anguilla*, Boon, Lokin, Ceusters & Ollevier (1989) found no relationship between haematocrit values in infected and non-infected eels. Also there was no significant relationship between number of parasites and packed cell volume. The number of nematodes reported per eel, however, was small, with the majority of infected eels having only 1 nematode. A series of experimental infections with *A. crassus* were reported by Boon, Augustijn, Cannaerts, Lokin, Machiels and Ollevier (1990) and Boon, Cannaerts, Augustijn, Machiels, Charleroy and Ollevier (1990). Infection levels of 5, 10 and 20 third-stage larvae (L_3) led to infected eels showing a decrease in body weight; however, the decrease was not proportional to infection level (Boon, Cannaerts, Augustijn, Machiels, Charleroy & Ollevier 1990). The packed cell volume was significantly affected by infection level with the heaviest infected eel having the lowest haematocrit value (Boon, Augustijn, Cannaerts, Lokin, Machiels &

Ollevier 1990). A threshold level of 4 L_3 per week, in a trickle infection, was required to significantly affect the packed cell volume (Boon, Augustijn, Cannaerts, Lokin, Machiels & Ollevier 1990). From these results, Boon, Augustijn, Cannaerts, Lokin, Machiels and Ollevier (1990) concluded that *A. crassus* did affect the health of the eel, *A. anguilla*, in artificial conditions. Sublethal infections, however, did not affect blood parameters in naturally infected eels (Höglund *et al.* 1992).

Health of the charr, *Salvelinus alpinus*, was found to be affected by infection with the cestode *Eubothrium salvelini* by Hoffmann *et al.* (1986). Host condition as well as blood parameters (packed cell volume, red blood cell count and haemoglobin concentration) declined with increasing intensity of infection. No other pathological effects were observed. This decline in health was due to an increased rate of destruction of red blood cells caused by production of toxins by the cestode.

5.1.2 Aims of Chapter

This chapter explores the biology of *Rhabdias* sp. in detail and looks at its potential as a control agent for the introduced cane toad.

The specific aims of this chapter are:

1. to determine the life cycle of *Rhabdias* sp. which infects *Bufo marinus* in Australia.

2. to determine if *Rhabdias* sp. does affect the survival of *B. marinus* metamorphs through infection experiments. Susceptibility of a native amphibian species, *Limnodynastes ornatus*, to larvae of *Rhabdias* sp. will also be tested.

3. to determine the effects of natural infections of *Rhabdias* sp. on various haematological parameters of *B. marinus*. Toads were collected from a *Rhabdias* sp.-free location (`Fletcherview') to provide base values for these parameters.

Part A: Life Cycle of *Rhabdias* sp.

5.2 Materials and Methods

5.2.1 Culture preparation

Rhabdias sp. cultures were prepared following the methods of Ballantyne (1971) and Kloss (1974). Filter paper (Whatman 9.0cm) was placed in the bottom of a plastic petri dish (8.5cm diameter) and moistened with distilled water. A small amount of fresh host faeces was placed in the centre of the filter paper. Gravid parasitic adults recovered from host lungs were lacerated and placed upon the faecal mass. The petri dish was then covered and maintained at 24°C. The culture was lightly moistened every two days with distilled water.

Samples were collected by gently washing the culture with a small amount of distilled water. Washings were collected in a clean petri dish and observed under a binocular dissecting microscope using transmitted light. Collection of *Rhabdias* sp. from the washings was done with a glass pipette with a fine drawn-out tip.

5.2.2 Studies undertaken

a) Life cycle

Samples were taken for 7 days at 12 hour intervals from the start of culture. Specimens of *Rhabdias* sp. were placed onto a clean microscope slide in distilled water for study under a compound microscope. Observations were made on stage of development. On completion of live studies, worms were fixed on the slide with 70% ethanol. Detailed measurements and drawings were then completed.

Unused washings were returned to the culture to continue development.

b) Effect of temperature

Two cultures were maintained at each of 4, 10, 18, 24 and 30°C, in constant temperature rooms, with a 12hr light:12hr dark regime. Regular sample collections occurred as outlined above. Rate of development and number of larvae produced per free-living female were recorded.

5.3 Results

5.3.1 Life Cycle of *Rhabdias* sp.

Development of *Rhabdias* sp. from embryonated egg, dissected from the parasitic stage, to infective third stage larva took 4 days at 24°C. Only development via a free-living sexual cycle was observed.

An embryonated egg (Figure 5.1), dissected from the lacerated adult, was elongate, oval, with membranous shell, 117 (114-122)μm long and 54.8 (52-58)μm wide, containing active larva. Newly hatched larva (Figure 5.2) 430.8 (392-476)μm long and 27.2 (26-28)μm wide, oesophageal length 123.2 (112-132)μm.

Adult free-living females present in culture by 24hrs, body length 786.8 (724-860)μm and width 51.3 (44-60)μm, oesophageal length 150.7 (140-160)μm; uterus didelphic, each arm folded proximally, 320-350μm long, large cells present near vulva (Figure 5.3a). Only one larva developed per female, development commenced by 32hrs (Figure 5.3b-c). By 48hrs, active larva present within uterus (Figure 5.3d, Figure 5.4a,b). By 72hrs, larva free within female body which devoid of internal

Figure 5.1 Eggs of *Rhabdias* sp. with fully formed larva, dissected from parasitic adult collected from lung of *Bufo marinus*.

Scale bar = 50μm.

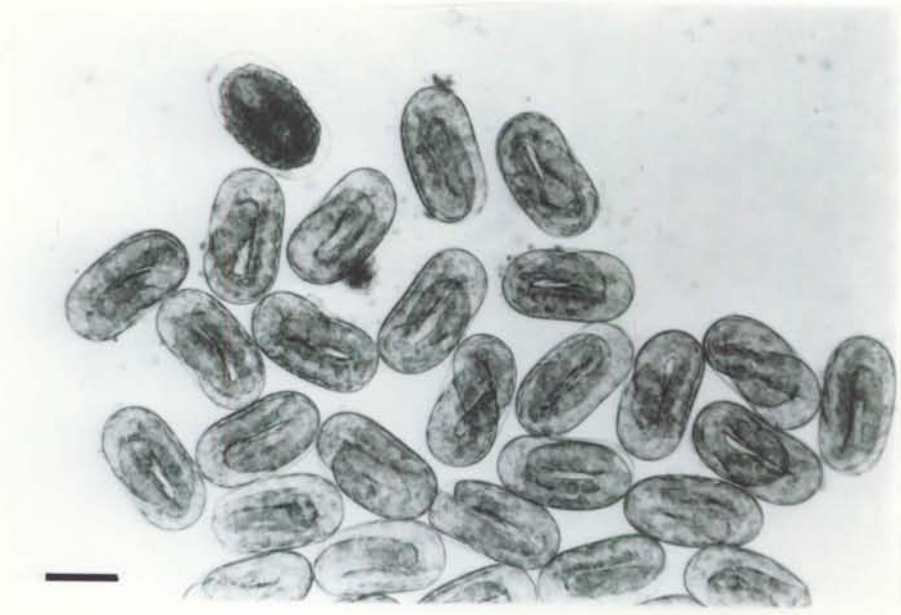


Figure 5.2 Larva of *Rhabdias* sp., freshly released from egg.

Scale bar = 50μm.

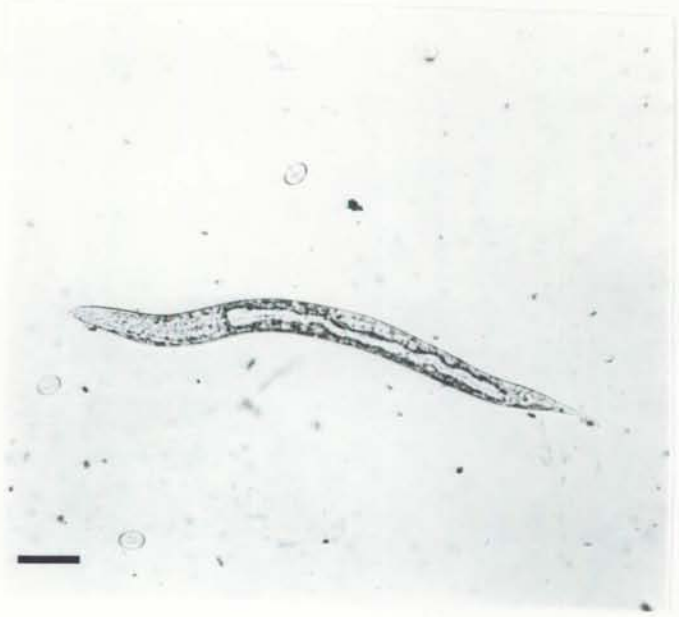


Figure 5.3 Development of larva within adult free-living female *Rhabdias* sp. in culture.

a) Genital system of adult free-living female *Rhabdias* sp.

b) Genital system of adult free-living female *Rhabdias* sp. with developing embryo in one arm of uterus at 32hr in culture.

c) Genital system of free-living female *Rhabdias* sp. with developing embryo at 40hr in culture.

d) Genital system of adult free-living female *Rhabdias* sp. with fully formed larva *in utero* at 48hr.

Arrow indicates position of non-functional vulva.

d, developing embryo; l, larva; u, uterus.

Scale bars: a) 50μm; b) 50μm; c) 10μm; d) 10μm.

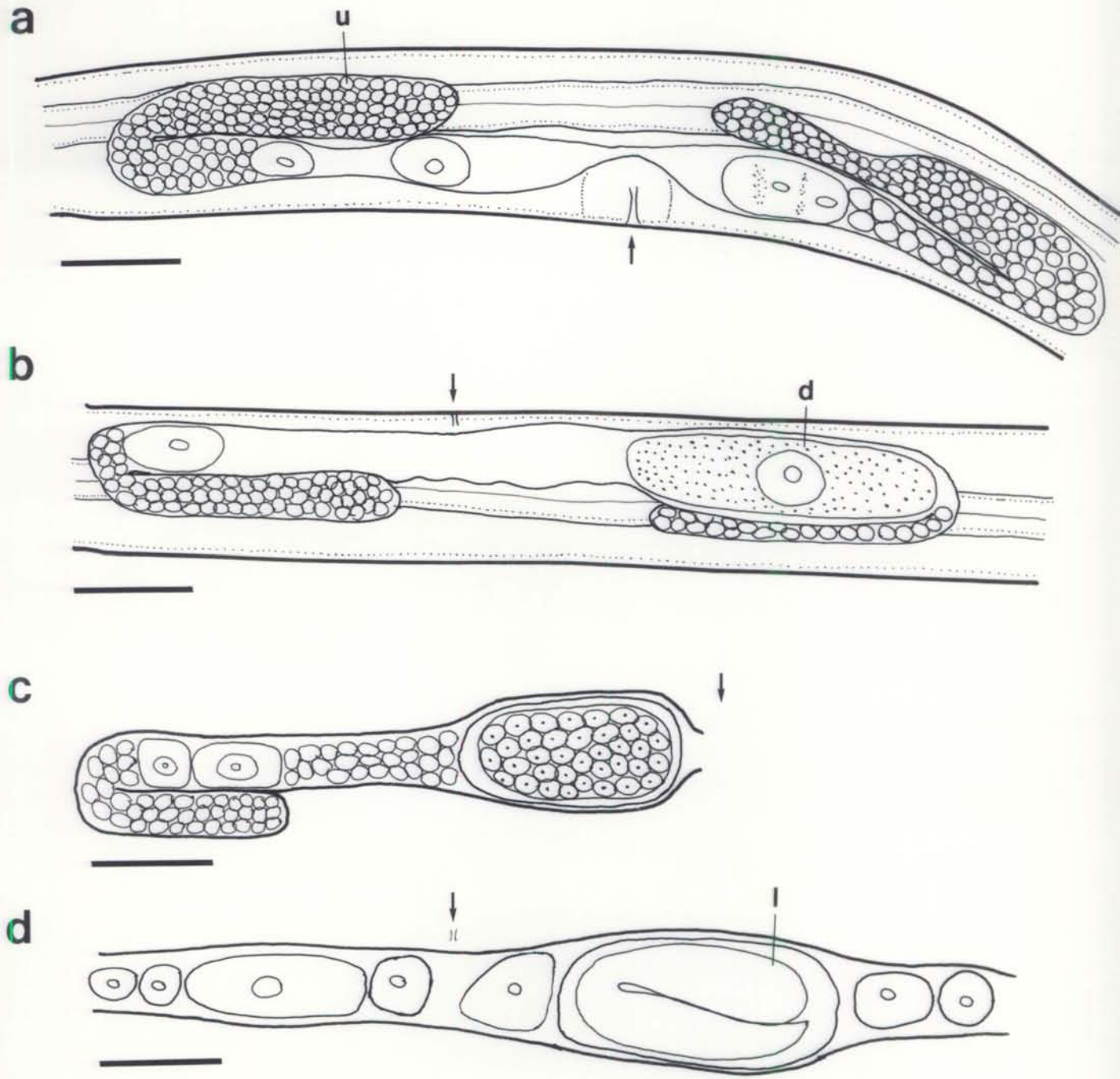

a
u
b
d
c
d
l

Figure 5.4 Development of larva within adult free-living female *Rhabdias* sp. in culture.

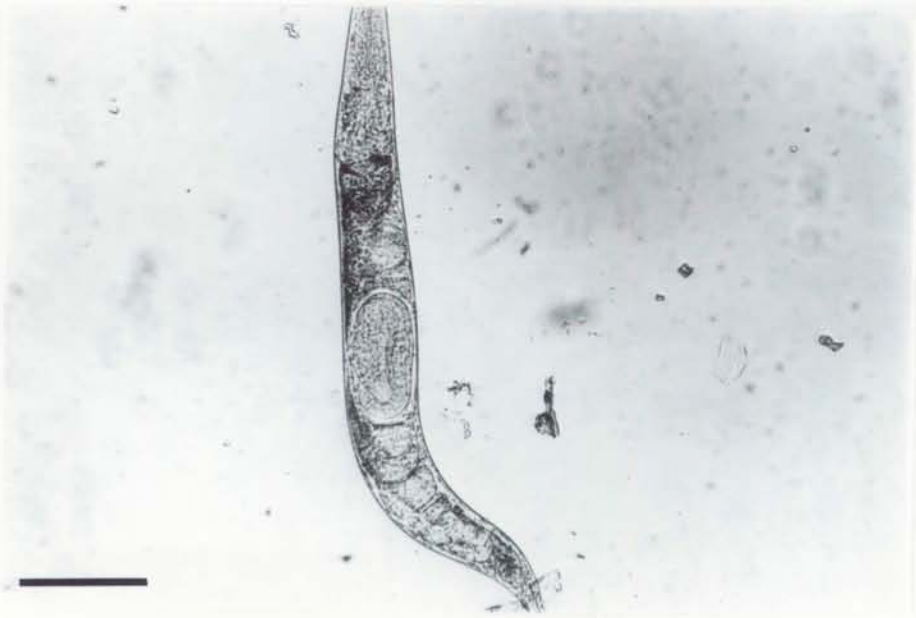
a) Fully formed larva within arm of uterus at 50hr in culture.

b) Close up of larva within uterus of female.

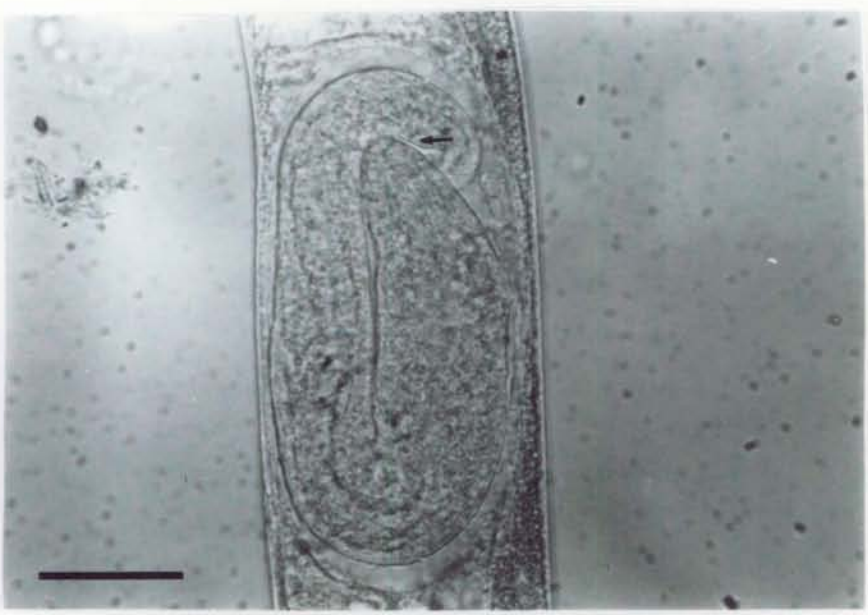
Arrow indicates position of head of larva.

Scale bars: a) 100μm; b) 25μm.

a



b



organs (Figure 5.5); female length 838 (808-896)μm and width 68 (64-80)μm, larval length 738 (704-824)μm and width 30 (24-32)μm.

Adult free-living males present in culture by 24hrs (Figure 5.6a), only present for one day, outnumbered by females approximately 2:1; 527 (522-532)μm long and 31 (28-34)μm wide, oesophageal length 138 (136-140)μm, spicule length 34 (32-36)μm (Figure 5.6b). Six pairs of papillae counted on ventral surface of male (Figure 5.6b).

Larva escapes from shell of female body by 77 hrs to become free in culture. By 96 hrs, larvae migrating away from faecal mass, often with anterior extremity pointed upward.

5.3.2 Effect of Temperature

Results of the effect of temperature on the number of *Rhabdias* sp. larvae produced per free-living female are presented in Table 5.2. At each temperature, at least 10 females per dish were selected for analysis. After 8 days, both the 4 and 10°C cultures had not developed. The 18°C culture required 5 days to complete development; the 24 and 30°C cultures required 4 days. Only one larva was produced per female, regardless of temperature.

5.4 Discussion

Development of *Rhabdias* sp. in this study resembled that found for other species of *Rhabdias* collected from amphibians (see Williams 1960; Ballantyne 1971; Kloss 1974; Baker 1979a). Development to infective larva always involved a free-living sexual phase, similar to that found by Ballantyne (1971) for *Rh. hylae*.

Figure 5.5 Larval *Rhabdias* sp. within confines of body of free-living female stage. Larva in process of `eating-out' female body.

Arrow indicates position of larval head.

Scale bar = 100μm.

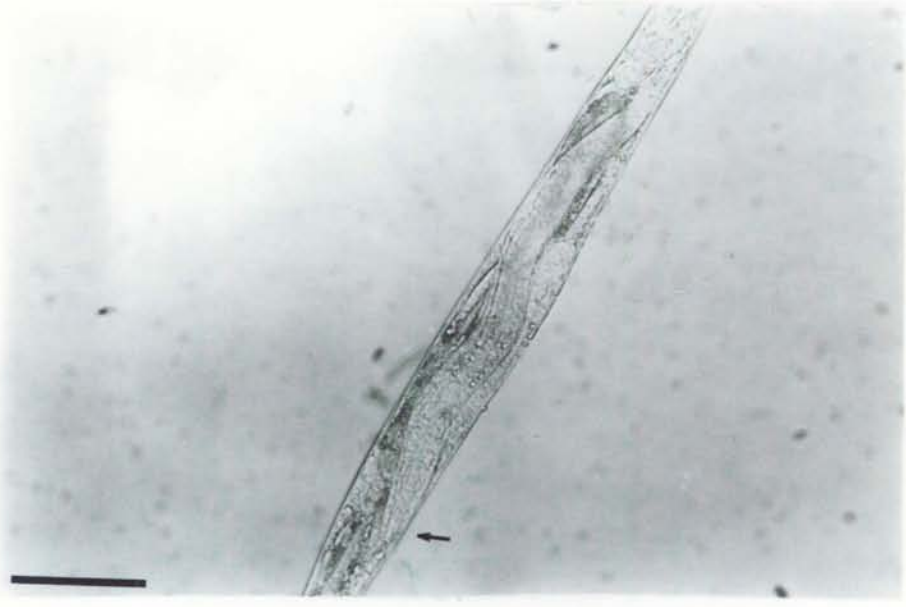


Figure 5.6

a) Free-living adult male *Rhabdias* sp. in culture.

b) Posterior end of free-living adult male *Rhabdias* sp. in culture.

Arrows indicate positions of 6 pairs of papillae.

Scale bars: a) 50μm; b) 35μm.

a

b

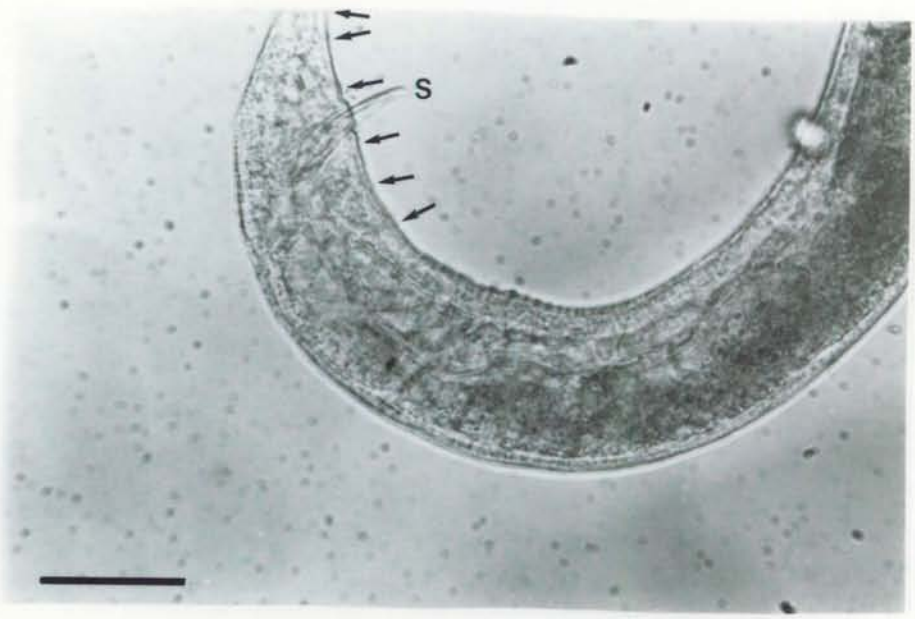


Table 5.2 Time for development and number of larvae of *Rhabdias* sp. produced at various culture temperatures.

Temperature (°C)	No. larvae per ♀	Days till larvae released from ♀
4	0	no development
10	0	no development
18	1	5
24	1	4
30	1	4

Larval nematodes were observed in the rectum of toads during routine dissections. These toads, however, were also infected with cosmocercoid and oxyurid nematodes (see Chapter 3), the larvae of which are similar in appearance to larval rhabdiasoid nematodes. The identity of these larvae was not determined. Ballantyne (1971) observed eggs of *Rh. hylae* to hatch in the rectum of the host. Unless these larvae were voided before the reproductive system began development, they died. This aspect was not studied in detail in this study.

Time of development to adult free-living stages for *Rhabdias* sp. was the same as for *Rh. fuelleborni* (Kloss 1974) and *Rh. americanus* (Baker 1979a). Williams (1960) suggested free-living males of *Rh. sphaerocephala* were difficult to find due to their small size and short life span. Males of *Rhabdias* sp. in this study, in contrast, were relatively easy to find, although their life span was much shorter than for the female.

Measurements of *Rhabdias* sp. free-living stages do not correspond to measurements given for *Rh. hylae* (Ballantyne 1971) or *Rh. fuelleborni* (Kloss 1974) (Table 5.3). No measurements for *Rh. sphaerocephala* were available. A more detailed study of the life cycle stages of all species of *Rhabdias* found in *B. marinus*, in Australia and South America, is needed to help solve the problem of identification of *Rhabdias* sp. in this study.

Temperature did not produce any change in the number of larvae produced per female for *Rhabdias* sp. in this study. Experiments, however, were not carried out on effects of changes in humidity. In northern Australia, rainfall is a more important climatic factor than heat and humidity may prove a more important factor in determining the number of larvae per free-living female.

Table 5.3 Comparison of measurements of free living stages of *Rhabdias hylae* and *Rh. fuelleborni* from the literature and *Rhabdias* sp. from this study. Measurements from this study given as a mean with range in parentheses.

Stage	*Rh. hylae* (Ballantyne 1971)		*Rh. fuelleborni* (Kloss 1974)		*Rhabdias* sp. (This study)	
	Length	Width	Length[a]	Width[a]	Length	Width
1st stage free-living larva	460	25			430.8 (392-468)	27.2 (26-28)
Female	924	37	1100	60	786.8 (724-860)	51.3 (44-60)
Male	735	29	900	50	527 (391-609)	31 (21-33)
Infective L_3[b]	566	20	700	20	738	30

[a] Length and width of life cycle stages estimated from figures.
[b] Only one infective L_3 measured.

Part B: *Rhabdias* sp. Infection experiments

5.2 Materials and Methods

5.2.1 Infection Procedure

Rhabdias sp. infective larvae were collected from 24°C cultures as outlined above. Known numbers of larvae were removed from the washings and placed into clean cavity blocks with a small amount of distilled water. Amphibians to be infected were measured (SVL), toe-clipped for individual identification and placed in the cavity block for a period of 2hrs. A lid was placed on the cavity block to prevent escape of the animal but allow the entry of air.

Control animals were measured, toe-clipped and placed in cavity blocks containing distilled water only. They were subjected to the same conditions and period of confinement as above.

After the infection period, the animals were washed with distilled water and returned to an aquarium where they were held until dissection. The number of larvae remaining in the cavity block was recorded. This number was subtracted from the original number placed in the block to determine the number of larvae which penetrated the animal.

a) *Bufo marinus*

Parasite-free *B. marinus* were obtained for the experiments by collecting late-stage tadpoles (back legs present) from various sites around Townsville. Tadpoles were placed in 10L buckets containing aerated rain water and fed regularly on boiled lettuce. Buckets were cleaned weekly.

Buckets were checked daily for metamorphosed tadpoles (toadlets) which were removed and placed in a bare

aquarium containing a small container of water. Cultured wingless fruit flies were released into the aquarium daily for the toadlets to feed on. Aquaria were cleaned weekly.

All *B. marinus* toadlets used in experiments 1 and 2 were at least 2 weeks post-metamorphosis. Infection dosages of *Rhabdias* sp. were set at 50 L_3 per toadlet. Control animals were randomly selected for each experiment.

Due to the difficulties experienced in rearing and maintaining large numbers of toadlets for the length of the experiment, all further experiments involved wild-caught *B. marinus* toadlets. Toadlets were collected around water sources in the Townsville region; efforts were made to collect smaller toadlets to reduce the risk of natural *Rhabdias* sp. infection being present. To allow for possible natural *Rhabdias* sp. infection, all toadlets collected were kept in aquaria for at least two weeks prior to experiments which enabled natural infections to develop to a recognisable larger size. Results from Chapter 4 (Part A) showed length of *Rhabdias* sp. to increase relatively quickly in these small toads. Toadlets were maintained in similar conditions to those outlined above.

Experiment 3 utilised toadlets with the same infection dosage as for experiments 1 and 2, 50L_3 per toadlet.

The infection dosage of experiment 4 was 2L_3 per toadlet. Toadlets used in this experiment were approximately 3 weeks post-metamorphosis. No control animals were used in this experiment. Two toadlets were dissected at intervals of two days.

Experiments 5 and 6 used approximately 4 week post-metamorphosis toadlets with an infection dosage of 10L_3 each. No control animals were used in these experiments. Toadlets were dissected at 7, 9 and 11

days post infection (p.i.) in experiment 5 and at 6 and 10 days p.i. in experiment 6.

Experiment 7 used 4 week post-metamorphosis toadlets with an infection dosage of $20L_3$ each. No control animals were used in this experiment. Toadlets were dissected at 10 days p.i.

At time of dissection, number and location of *Rhabdias* sp., as well as stage of development, were recorded. Animals found dead during the experiment were dissected to attempt to determine the cause of death.

Success rate of infection was calculated from the number of *Rhabdias* sp. found in infected animals. Division of this number by the number of larvae which originally penetrated the animal produced a success index.

Natural *Rhabdias* sp. infections (natural index) were recognised if the *Rhabdias* sp. found was mature or developed beyond what was possible in the time of the experiment (from information available in Ballantyne 1971; Kloss 1974).

b) *Limnodynastes ornatus*

Limnodynastes ornatus tadpoles were collected and maintained as described above. Frog and toad metamorphs were maintained in separate aquaria. Five metamorphs were used in experiment 1 for infection with $30L_3$ each. One control animal was selected. All animals were dissected 6 days p.i.

Experiment 2 utilised wild-caught *Lim. ornatus* collected and maintained as described above. Infection dosage for this experiment was $10L_3$ per frog. No control animals were used. All animals were dissected at 10 days p.i.

Success rate of infection and presence of natural infection was determined as outlined above.

5.3 Results

5.3.1 *Bufo marinus*

A total of 75 metamorph *B. marinus* were used in 7 experiments.

Experiments 1 and 2 (mean SVL, at beginning of experiment, 10.25 and 13.4 mm, respectively) were not completed as all animals, including controls, were found dead by 7 days p.i. No signs of pathology could be found and cause of death remains unknown. No *Rhabdias* sp. was found in any animal, despite all toadlets acquiring larvae (25-50) at the beginning of the experiment.

Results of experiment 3 are presented in Table 5.4a. Three (75%) toads (mean SVL of experimental toads 38.1mm) became infected with *Rhabdias* sp., the success index of infection ranged from 0.025 to 0.68 (mean 0.36). One control toadlet was naturally infected with *Rhabdias* sp.

Results of experiment 4 are presented in Table 5.4b. Successful infections occurred in four (50%) toadlets (mean SVL 14.65mm); index of success of infection was 0.5 in all cases. No natural infections were found in either experimental or control toadlets.

Results of experiment 5 are presented in Table 5.4c. Seven of 10 toadlets (mean SVL 18.1mm) became infected with *Rhabdias* sp. Index of success ranged from 0.17 to 0.8; no natural *Rhabdias* sp. infections were found. Three toads died during the experiment, two of which harboured no *Rhabdias* sp. infection. In one toadlet, the acquired *Rhabdias* sp. infection was found in the body cavity. This toadlet, however, had been found dead, so determination of this site as a natural location for infection could not be confirmed.

Results of experiment 6 are presented in Table 5.4d.

Table 5.4 Results of infection experiments with *Rhabdias* sp. and metamorph *Bufo marinus*. Dose = number of larvae of *Rhabdias* sp. in cavity block; #*Rh.* pen. = number of *Rhabdias* sp. penetrating host; dpi = days post infection; SI = success index; NI = natural index; fd = found dead; * = *Rhabdias* sp. found in body cavity of host.

a) Experiment 3: Infection dosage 50 L_3.

SVL (mm)	Dose	#*Rh.* pen.	dpi	# *Rhabdias* found	SI	NI
29.0	50	40	10	10	0.4	0
33.5	50	40	10	0	0	0
45.0	50	40	10	1	0.025	0
45.0	50	40	10	27	0.68	0
47.0	0	0	10	0	0	0
50.0	0	0	10	0	0	29

b) Experiment 4: Infection dosage 2 L_3.

SVL (mm)	Dose	#*Rh.* pen.	dpi	# *Rhabdias* found	SI	NI
16.0	2	2	2	0	0	0
12.0	2	2	2	0	0	0
12.5	2	2	6	0	0	0
13.0	2	2	6	1	0.5	0
13.0	2	2	8	0	0	0
13.0	2	2	8	0	0	0
18.0	2	2	10	1	0.5	0
15.5	2	2	10	1	0.5	0
20.0	2	2	12	1	0.5	0
13.5	2	2	12	0	0.0	0

.../Cont.

Table 5.4. (Cont.)
c) Experiment 5: Infection dosage 10 L_3.

SVL (mm)	Dose	#*Rh.* pen.	dpi	# *Rhabdias* found	SI	NI
16.0	10	10	11	0	0	0
16.0	10	10	7	6	0.6	0
18.0	10	10	7	3	0.3	0
19.5	10	10	9	6	0.6	0
18.5	10	10	11	8	0.8	0
19.0	10	6	11	1	0.17	0
17.0	10	8	fd	1*	0.17	0
17.0	10	10	fd	0	0	0
18.0	10	7	fd	0	0	0
22.0	10	10	11	3	0.3	0

d) Experiment 6: Infection dosage 10 L_3.

SVL (mm)	Dose	#*Rh.* pen.	dpi	# *Rhabdias* found	SI	NI
20.5	10	3	6	0	0	0
19.0	10	9	fd	0	0	0
18.0	10	10	10	3	0.3	0
22.0	10	10	10	6	0.6	0
16.5	10	8	fd	1	0.13	1
17.0	10	8	fd	3	0.38	0
18.0	10	9	10	3	0.33	0
16.0	10	7	fd	2	0.29	0
17.0	10	8	10	1*	0.13	0
15.0	10	7	fd	0	0	0

.../Cont.

Table 5.4. (Cont.)
e) Experiment 7: Infection dosage 20 L_3.

SVL (mm)	Dose	#*Rh.* pen.	dpi	# *Rhabdias* found	SI	NI
17.0	20	18	fd	0	0	0
22.5	20	19	10	6	0.32	0
20.0	20	0	10	0	0	2
18.0	20	10	fd	0	0	0
17.0	20	16	10	0	0	0
18.5	20	15	10	8*	0.53	0
19.5	20	0	fd	0	0	0
16.0	20	14	fd	0	0	2
19.0	20	18	10	11	0.61	0

Seven of ten toadlets (mean SVL 17.9mm) became infected. Index of success ranged from 0.13 to 0.38; a natural infection was found in one toad which also harboured an experimental infection. Five of the toads died during the experiment, two of which did not harbour an experimental infection. One toadlet was found to harbour a nematode in the body cavity.

Results of experiment 7 are presented in Table 5.4e. Thirty percent of toads became infected in experiment 7 (mean SVL 18.6mm); index of success ranged from 0.32 to 0.61. Three experimental animals were found dead in this experiment; none were infected with experimental *Rhabdias* sp. infection. Two of the animals were naturally infected with *Rhabdias* sp. infections, neither of which acquired experimental infections. One toadlet was found to harbour a *Rhabdias* sp. in the body cavity.

The results of experiments 3 to 7 were pooled, to give a total of 45 toadlets, of which 2 were control animals. Infection with *Rhabdias* sp. was successful in 55.81% of toadlets exposed to larvae. Average intensity of acquired infection was 4.75 *Rhabdias* sp. per infected toadlet. Range of infection was 1-27. A total of 11 toadlets (24%) died during the experiments, of which 4 (36%) were infected with *Rhabdias* sp.

No correlation existed between proportion of larvae penetrating the toadlet and SVL of toadlet (r_{42}=-0.1418, 0.5>p>0.2), nor between SVL of toadlet and success index (r_{40}=0.1863, 0.5>p>0.2).

No relationship was found between mortality of toadlet and dose of *Rhabdias* sp. ($t_{17.8}$=2.00, p=0.0609).

Rhabdias sp. was found in the lungs of experimental toadlets by 6 days p.i.

5.3.2 *Limnodynastes ornatus*

Results of *Rhabdias* sp. experimental infections with

metamorph *Lim. ornatus* are presented in Table 5.5. Fifteen metamorph *Lim. ornatus* were used in two experiments.

Experiment 1 (mean SVL 10.7mm) had no successful infections. One animal was found dead in this experiment, but did not contain a *Rhabdias* sp. infection.

Experiment 2 (mean SVL 18.95mm) had a 60% success rate. Success index of infection (excluding metamorphs that did not acquire infections) averaged 0.34 (range: 0.1-0.7). Four animals contained natural *Rhabdias* sp. infections, two of which also acquired the experimental infection. Two animals were found dead during the experiment, neither of which had acquired, or possessed, *Rhabdias* sp. infections.

Rhabdias sp. was found in the lungs of *Lim. ornatus* by day 10 p.i. A number of larvae of *Rhabdias* sp. were also found in the body cavity of *Lim. ornatus* (5 of the 6 animals that became infected).

No significant relationships were found between SVL of host and dose of *Rhabdias* sp. (r_9=0.1247, $p>0.5$), SVL and success index (r_9=-0.1998, $p>0.5$), and dose and success index (r_9=-0.3026, $0.5>p>0.2$).

5.4 Discussion

The high death rate of metamorphs severely affected the outcome of the experimental infections. No reason for the death of these animals during the experiment could be found. The high death rates in the first two *B. marinus* experiments led me to believe that the infection dosage was killing the toads, but this did not explain death of control animals. Metamorphs were used in these experiments, in preference to older hosts, due to the, relatively, larger numbers available, suspected

Table 5.5 Results of infection experiments with *Rhabdias* sp. and metamorph *Limnodynastes ornatus*. Legend as for Table 5.3. Bcav., body cavity; Left, left lung; Rght, Right lung. Legend as for Table 5.4.

SVL (mm)	Dose	#*Rh.* pen.	dpi	# *Rhabdias* found				SI	NI
				Left	Rght	Bcav.	Total		
19.0	10	9	fd	0	0	0	0	0	0
27.0	10	9	10	1	0	1	2	0.22	2
23.0	10	10	fd	0	0	0	0	0	0
18.5	10	10	10	0	0	1	1	0.1	0
20.0	10	10	10	0	0	2	2	0.2	0
19.0	10	9	10	0	0	4	4	0.44	0
17.5	10	10	10	0	0	0	0	0	2
15.5	10	10	10	2	5	0	7	0.7	3
16.0	10	5	10	0	0	2	2	0.4	0
14.0	10	10	10	0	0	0	0	0	3

ease of maintenance in the lab, and ease of infection (speculated from data obtained in Chapter 4 Part A).

The period following metamorphosis is the period of highest mortality in an amphibian life cycle (Jameson 1956; Licht 1974). Susceptibility to predation is considered the most important factor in mortality in the wild (Jameson 1956; Licht 1974). Food availability is not considered important; laboratory experiments have shown *Rana* species metamorphs could survive a month without food following metamorphosis (Licht 1974). Goater and Ward (1992) found, however, a decreased intake of food in *B. bufo* metamorphs infected with *Rh. bufonis*, which caused a significant decrease in growth rate and a significant increase in mortality. It would appear that metamorphs are able to survive without food, given no other physiological stress. In the situation encountered here, the natural mortality rate, coupled with the stress of the experiment, probably led to the metamorphs' death. In addition, fruit-fly may be an inadequate source of nutrition for metamorphs (Speare, R. 1993, pers. comm.) which may have contributed to a poor state of health that eventually led to the metamorphs' death. The establishment of a good laboratory metamorph rearing scheme prior to the experiments would have improved the quality of the results.

In future experiments older metamorphs, of a similar age to that used by Goater and Ward (1992; 2-5 months) should be used. Goater and Ward (1992) did not report any losses of control animals during their experiments.

Lower infection dosages did lead to lower death rates among the metamorphs in my experimental infections, and also a relatively high success rate of infection. It would appear that low rates of infection are best to build infection levels in young amphibians. Presence of natural *Rhabdias* sp. infections did not appear to

prevent acquisition of experimental infections.

Older *Lim. ornatus* metamorphs (experiment 2) showed similar results to *B. marinus* with a higher success rate of infection and fewer deaths. The smaller *Lim. ornatus*, however, were infected with higher doses of *Rhabdias* sp. larvae, which combined with the high natural mortality of this life cycle stage, may have led to their death. Many more *Rhabdias* sp. were found in the body cavity of *Lim. ornatus* than for *B. marinus*. Whereas *Rhabdias* sp. may reach the lungs of *B. marinus* by 6 days p.i., the route possibly takes longer in *Lim. ornatus*, and the nematodes may spend an amount of time in the body cavity where they `mature slightly' as postulated by Baker (1979a) for *Rh. ranae*. The results obtained for *Lim. ornatus* were, however, comparable to *B. marinus* in the percentage of metamorphs infected and the range of success indices. These preliminary results show the need for further experimentation on the life cycle of *Rhabdias* sp. in Australia to determine the full host range of *Rhabdias* sp. and susceptibilities of native host species.

Infections with *Rhabdias* sp. in this study were done by contact of the hosts with water containing infective larvae. The possibility, however, of infection of hosts via the mouth or cloaca cannot be ruled out. The importance of skin penetration as a route of infection should be considered experimentally. A physiological barrier may be acting in conjunction with, or instead of, an ecological barrier preventing mass infections of *Rhabdias* sp. in native amphibians (see Chapter 3). The failure of *Rhabdias* sp. to penetrate *B. marinus*, however, shows the problem probably lies in the experimental technique, not with larval penetration.

PART C: Haematology

5.2 Materials and Methods

5.2.1 Collection of samples

Toads were collected from three sites; two were known as *Rhabdias* sp.-infected sites (QDPI and Bentley; see Figure 4.1), the other a known *Rhabdias* sp.-free site (`Fletcherview', the James Cook University of North Queensland Veterinary Research farm; see Figure 2.1).

Toads were heavily anaesthetised with `Lethabarb'. When each toad no longer reacted to external stimuli (touching of eye, pinching of abdominal skin), it was dissected. A ventral incision was made from the pelvic to the pectoral girdle exposing the body cavity. The pericardium was opened by snipping the covering tissue.

A blood sample was collected by heart puncture and placed in an EDTA ([ethylenedinitrilo]tetraacetic acid) vial on ice until analysis. The SVL, sex and total number of *Rhabdias* sp. collected for each toad was recorded.

Before analysis, the EDTA vials were allowed to warm to room temperature while spinning on a Coulter bench top mixer. The following parameters were measured: packed cell volume (PCV), haemoglobin concentration (Hb), and red blood cell count (RBC). Values for the Wintrobe erythrocytic indices were calculated from these measurements as follows:

Mean Corpuscular Volume (MCV) = PCV/RBC x 10

Mean Corpuscular Haemoglobin (MCH) = Hb/RBC x 10

Mean Corpuscular Haemoglobin Concentration (MCHC) = Hb/PCV x 100

Red blood cell counts involved the mixture of whole EDTA blood diluted with 1% (v/v) formalin (40% Formaldehyde) in 3% trisodium citrate to a dilution of

1:200. A subsample was taken to count cells on a haemocytometer (W. Schreck Hofheim/T.S. slide 1/10 mm deep, 1/400 sq. mm). Duplicate counts of 5 squares were taken, and an average count calculated; this number was referred to as N. To calculate the number of red blood cells per μl of blood, N was multiplied by 10,000 (see Jain 1986).

Packed cell volume was determined using microcapillary tubes and centrifugation at 9000rpm for 10min in a bench top microhaematocrit centrifuge (Biofuge A, Hereaus-Christ GmbH, Osterode am Harz, West Germany). Measurement of the haematocrit was made using a sliding scale.

Haemoglobin concentration was measured following the cyanomethaemoglobin method of Jain (1986). A mix of 20μl of whole EDTA blood and 5ml KCN reagent was prepared. Using a commercially available kit (Test combination Haemoglobin, Boeh ringer Mannheim Mbtt, Mannheim, Germany) the cyanomethaemoglobin levels were read spectrophotometrically at 546nm against a distilled water blank and converted to g/dl using a dilution factor of 36.77.

5.2.2 Statistical Analyses

a) Relationship between toad snout-vent length and sex with blood parameters.

To allow for a possible host-dependent relationship between snout-vent length and sex of toad with the three blood parameters, only toads collected from `Fletcherview' (the uninfected site) were used in this analysis. Correlation coefficients were used to determine the relationship between toad length and the blood parameters. A one way ANOVA was used to determine the relationship between sex of toad and the blood parameters.

b) Relationship between month of collection and blood parameters.

One way ANOVAs were used to determine the relationship between month of collection and *Rhabdias* sp. intensity and the blood parameters. Only toads infected with *Rhabdias* sp. were used for the analysis, independent of site.

c) Relationship between intensity of *Rhabdias* sp. infection and blood parameters.

Relationship between intensity of *Rhabdias* sp. infection and blood parameters (RBC, PCV, Hb, MCV, MCH, and MCHC) were determined by correlation coefficients. At first, all toads (infected and uninfected) were included in the analyses; for further analyses, the toads were divided into the following groupings:

* both infected sites (combined QDPI and Bentley)
* QDPI only
* Bentley only
* toads infected with *Rhabdias* sp. only, independent of site

d) Relationship between site of collection and blood parameters.

Relationship between site of collection and the blood parameters was determined by a one way ANOVA for the two *Rhabdias* sp.-infected sites. As no difference was found between these sites, both sites were combined and compared to `Fletcherview'.

e) Relationship between presence of *Rhabdias* sp. infection and blood parameters.

Relationships between presence of *Rhabdias* sp. infection and the blood parameters were determined by a series of one way ANOVAs. Toads were separated into

infected (independent of intensity) and uninfected animals, independent of site. The same analysis was also undertaken for toads within the two infected sites.

5.3 Results

5.3.1 General Results.

A summary of host data, including SVL and intensity of infection of *Rhabdias* sp., for toads collected from QDPI, Bentley and `Fletcherview' is presented in Table 5.6. Intensity of infection of *Rhabdias* sp. was significantly different between the two infected sites (QDPI and Bentley) ($F_{1,207}$=26.4, P<0.0001). Toads collected from QDPI were more heavily infected (average ± S.D.: 20.6±2.6) than Bentley (7.0±1.4). Toads collected from `Fletcherview' were not infected with *Rhabdias* sp.

Average values for the haematological values of RBC, PCV, Hb, MCV, MCH, and MCHC are presented in Table 5.7. Values presented for toads collected from `Fletcherview' are considered base haematological values for *B. marinus* in Australia.

5.3.2 Relationship between toad snout-vent length and sex with blood parameters.

a) Toad snout-vent length

No significant relationships were found between toad SVL and the blood parameters for toads collected from `Fletcherview' (Table 5.8).

b) Toad sex

No significant relationships were found between toad sex and blood parameters for toads collected from

Table 5.6. Summary of host data for toads collected from QDPI, Bentley and `Fletcherview' for blood samples. For QDPI and Bentley samples, only toads infected with *Rhabdias* sp. were used; `Fletcherview' toads are uninfected. N gives the total number of toads collected, independent of presence of *Rhabdias* sp. infection. Values are presented as a mean±SE_{μ}.

Location	Sex	N	Mean SVL of host	Prevalence (%)	Mean Intensity of infection	Range of intensity of infection
QDPI	♂	47	91.4±1.3	97.9	28.0±5.4	1-230
	♀	70	86.5±1.3	92.9	15.3±1.9	1-57
	Total	117	88.5±1.0	94.9	20.6±2.6	1-230
Bentley	♂	67	101.5±1.0	58.2	6.9±1.9	1-59
	♀	25	93.8±2.1	84.0	7.1±1.9	1-34
	Total	92	98.8±1.1	65.2	7.0±1.4	1-59
`Fletcherview'	♂	4	106.8±1.7			
	♀	13	94.6±2.2			
	Total	17	97.5±2.1			

Table 5.7. Values for haematological parameters of *Bufo marinus* infected with *Rhabdias* sp. (QDPI and Bentley) and uninfected (`Fletcherview'). Values recorded for `Fletcherview' toads are considered `base haematological values' for *Bufo marinus* in this study. Values are presented as a mean±SE_{μ}.

Location	Sex	RBC	PCV	Hb	MCV	MCH	MCHC
QDPI	♂	0.67±0.03	30.74±1.14	8.90±0.34	471.1±17.2	151.5±8.9	28.58±0.96
	♀	0.60±0.02	29.04±1.00	8.72±0.29	574.0±82.3	176.8±19.2	29.85±1.04
	Total	0.63±0.02	29.75±0.75	8.79±0.22	529.2±67.2	165.7±11.5	29.30±0.72
Bentley	♂	0.60±0.05	31.66±1.94	9.31±0.50	513.6±50.0	166.7±14.0	29.61±1.03
	♀	0.52±0.05	21.70±1.54	6.83±0.37	482.4±62.4	149.2±15.8	31.59±1.53
	Total	0.57±0.04	27.51±1.47	8.47±0.38	499.3±38.9	154.1±10.8	30.41±0.87
`Fletcherview'	♂	0.78±0.18	36.88±2.54	9.67±0.82	629.5±246.5	163.8±63.2	26.29±1.83
	♀	0.86±0.06	36.65±1.40	8.24±0.45	448.7±40.7	97.7±3.10	22.69±1.09
	Total	0.84±0.06	36.71±1.18	8.58±0.41	491.2±63.2	112.8±15.2	23.54±0.99

Table 5.8 Results of analysis of relationships between snout-vent length and sex of toad on red blood cell count, packed cell volume, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration. All toads were collected from the *Rhabdias* sp.-free site at `Fletcherview'. df = degrees of freedom.

Factors	Test	df	Result	Probability	Sig/NS
Snout-vent length v RBC	Correlation	16	r = 0.0383	p > 0.5	NS
Snout-vent length v PCV	Correlation	16	r = -0.0186	p > 0.5	NS
Snout-vent length v Hb	Correlation	16	r = 0.1664	0.5>p>0.2	NS
Snout-vent length v MCV	Correlation	16	r = 0.0311	p>0.5	NS
Snout-vent length v MCH	Correlation	16	r = 0.1148	p>0.5	NS
Snout-vent length v MCHC	Correlation	16	r = 0.1810	0.5>p>0.2	NS
Sex of toad v RBC	1 way ANOVA	1,15	F = 0.36	p = 0.5590	NS
Sex of toad v PCV	1 way ANOVA	1,15	F = 0.01	p = 0.9398	NS
Sex of toad v Hb	1 way ANOVA	1,15	F = 2.37	p = 0.1447	NS
Sex of toad v MCV	1 way ANOVA	1,15	F = 1.52	p = 0.2367	NS
Sex of toad v MCH	1 way ANOVA	1,15	F = 4.12	p = 0.0604	NS
Sex of toad v MCHC	1 way ANOVA	1,15	F = 2.63	p = 0.1258	NS

`Fletcherview' (Table 5.8). Males had higher values for packed cell volume and haemoglobin concentration but females had higher red blood cell counts (see Table 5.7).

5.3.3 Relationship between month of collection and the blood parameters.

Toads were collected on 5 occasions from QDPI (February, November, December 1990, January, March 1991) and Bentley (November, December 1990, April, May 1991, March 1992) and once at `Fletcherview' (March 1992).

Intensity of *Rhabdias* sp. was found to differ significantly between months of collection (Table 5.9). Mean intensity of infection was significantly lower for April and May 1991 and March 1992 (Figure 5.7).

Month of collection showed significant differences in the three blood parameters of RBC count, PCV and Hb concentration (Table 5.9). Values for all three parameters were significantly higher in March 1992 (Figure 5.8a-c). Although the relationships for MCV, MCH and MCHc were not significant, similar trends were apparent (Figure 5.8d-f).

5.3.4 Relationship between intensity of infection and blood parameters.

Overall (comparison between the three sites), intensity of *Rhabdias* sp. infection showed significant negative correlations with the three blood parameters of red blood cell count, packed cell volume and haemoglobin concentration (Table 5.10). Variation in the measurements of all three parameters were high in toads with low infection levels of *Rhabdias* sp. (Figures 5.9,

Table 5.9 Results of analysis of relationships between month of collection and mean intensity of *Rhabdias* sp. infection (only toads infected with *Rhabdias* sp. included), red blood cell count, packed cell volume, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration. df = degrees of freedom.

Factors	Test	df	Result	Probability	Sig/NS
OVERALL:					
Month v RBC	1 way ANOVA	4,162	F = 11.20	p < 0.0001	Sig
v PCV	1 way ANOVA	7,181	F = 15.25	p < 0.0001	Sig
v Hb	1 way ANOVA	7,214	F = 12.64	p < 0.0001	Sig
v MCV	1 way ANOVA	4,92	F = 0.92	p = 0.4600	NS
v MCH	1 way ANOVA	4,114	F = 0.92	p = 0.4580	NS
v MCHC	1 way ANOVA	7,127	F = 1.47	p = 0.1844	NS
v *Rhabdias* Intensity	1 way ANOVA	7,218	F = 4.33	p = 0.0002	Sig

Figure 5.7 Relationship of mean intensity of *Rhabdias* sp. to month of collection for 8 collection periods.

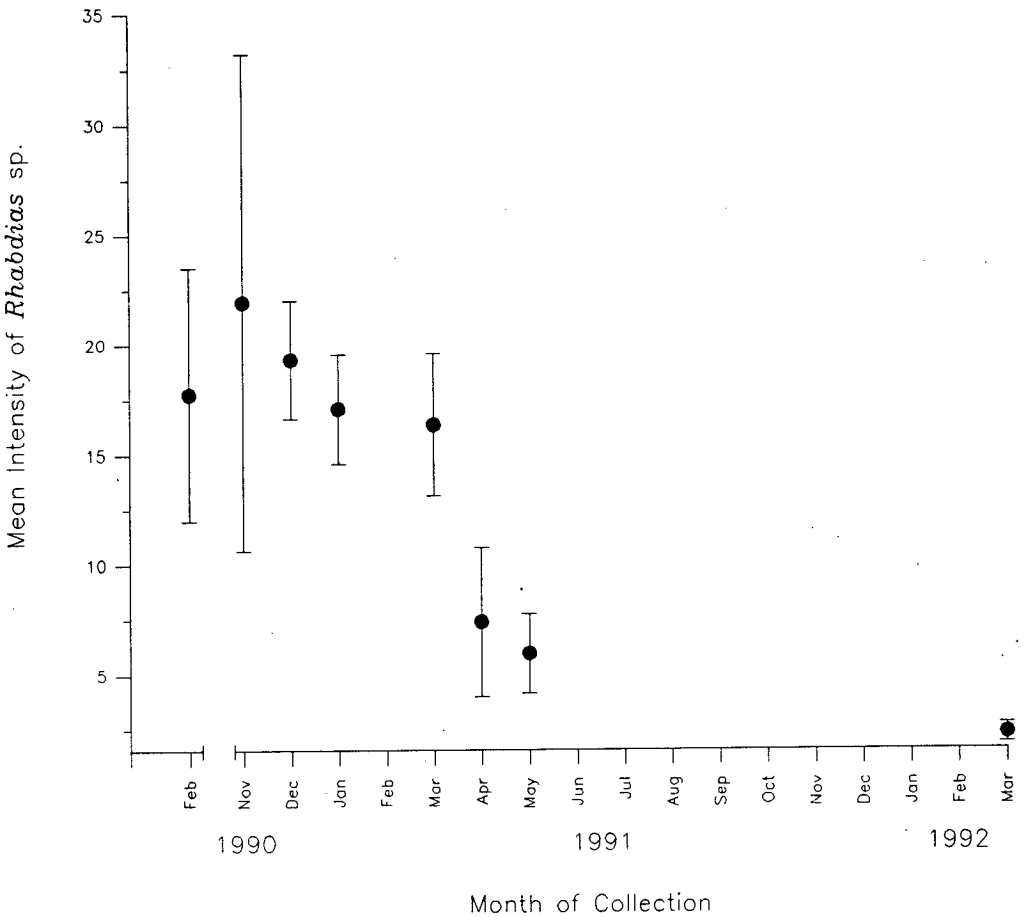

Mean Intensity of *Rhabdias* sp.
35
30
25
20
15
10
5
Feb
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
1990
1991
1992
Month of Collection

FIG 3.8

Figure 5.8 Relationship of month of collection to
a) mean red blood cell count,
b) mean packed cell volume,
c) mean haemoglobin concentration,
d) mean corpuscular volume,
e) mean corpuscular haemoglobin, and
f) mean corpuscular haemoglobin concentration.

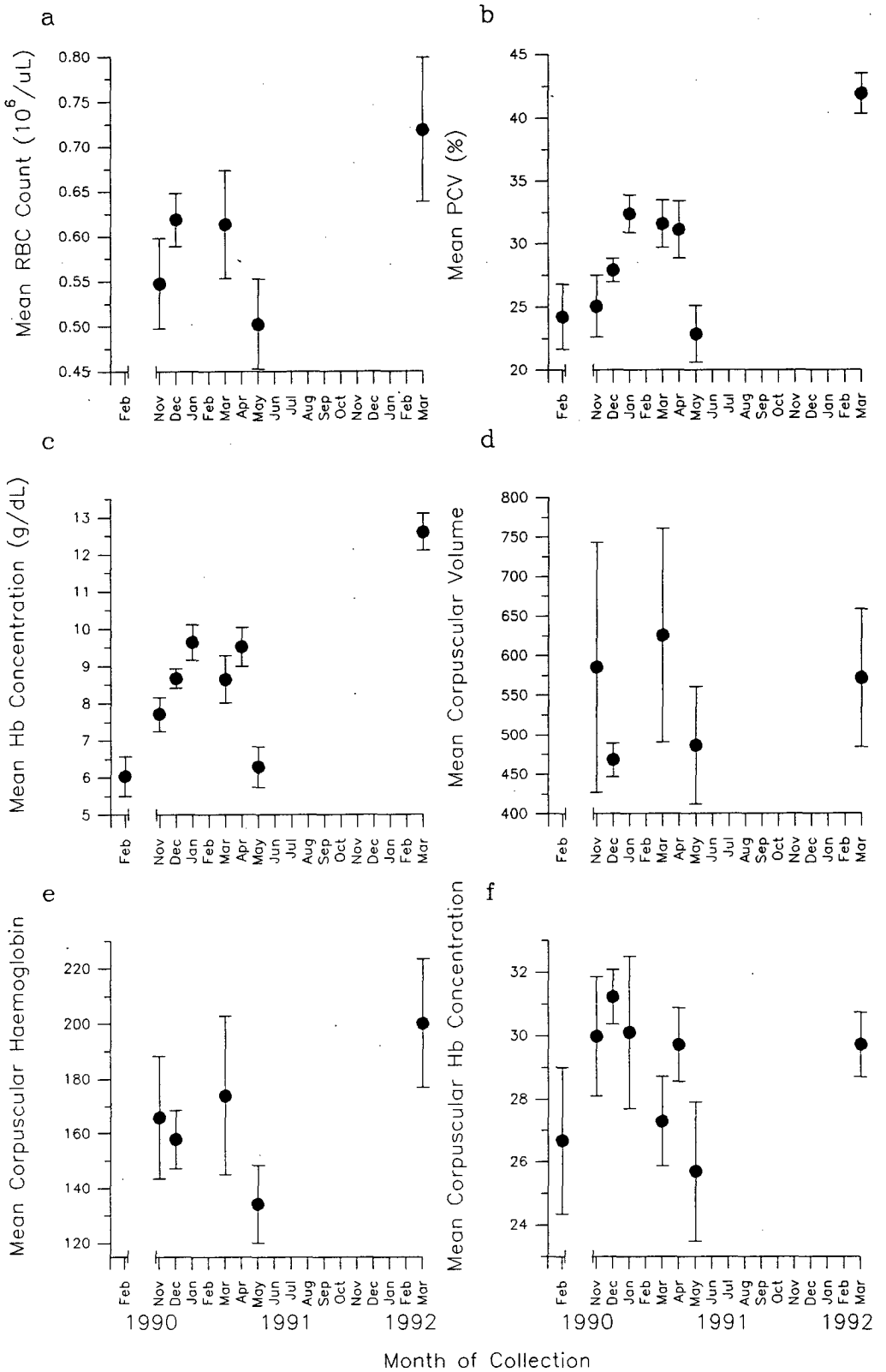

a
Mean RBC Count (10^6/uL)
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
b
Mean PCV (%)
45
40
35
30
25
20
c
Mean Hb Concentration (g/dL)
13
12
11
10
9
8
7
6
5
d
Mean Corpuscular Volume
800
750
700
650
600
550
500
450
400
e
Mean Corpuscular Haemoglobin
220
200
180
160
140
120
f
Mean Corpuscular Hb Concentration
32
30
28
26
24
Feb
Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar
1990
1991
1992
Month of Collection

Table 5.10 Results of analysis of relationships between intensity of *Rhabdias* sp. infection and red blood cell count, packed cell volume, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration. n = number of toads. Sig = significant relationship, NS = not significant relationship. Degrees of freedom can be calculated by n-1.

Factors	Test	n	Result	Probability	Sig/NS
OVERALL:					
Rhabdias Intensity v RBC	Correlation	167	r = -0.1551	0.05>p>0.02	Sig
Rhabdias Intensity v PCV	Correlation	189	r = -0.1939	0.01>p>0.005	Sig
Rhabdias Intensity v Hb	Correlation	222	r = -0.1875	0.005>p>0.002	Sig
Rhabdias Intensity v MCV	Correlation	141	r = 0.0086	p>0.5	NS
Rhabdias Intensity v MCH	Correlation	167	r = -0.0186	p>0.5	NS
Rhabdias Intensity v MCHC	Correlation	185	r = -0.0721	0.05>p>0.02	NS
INFECTED SITES ONLY:					
Rhabdias Intensity v RBC	Correlation	150	r = -0.1226	0.2>p>0.1	NS
Rhabdias Intensity v PCV	Correlation	172	r = -0.1657	0.05>p>0.02	Sig
Rhabdias Intensity v Hb	Correlation	205	r = -0.2015	0.01>p>0.005	Sig
Rhabdias Intensity V MCV	Correlation	160	r = 0.0241	p>0.5	NS
Rhabdias Intensity v MCH	Correlation	150	r = -0.0516	p>0.5	NS
Rhabdias Intensity v MCHC	Correlation	124	r = 0.0049	p>0.5	NS
QDPI SITE ONLY:					
Rhabdias Intensity v RBC	Correlation	80	r = -0.1236	0.5>p>0.2	NS
Rhabdias Intensity v PCV	Correlation	95	r = -0.1690	0.1>p>0.05	NS
Rhabdias Intensity v Hb	Correlation	114	r = -0.1943	0.05>p>0.02	Sig
Rhabdias Intensity v MCV	Correlation	65	r = -0.0076	p>0.5	NS
Rhabdias Intensity v MCH	Correlation	80	r = -0.0768	0.5>p>0.2	NS
Rhabdias Intensity v MCHC	Correlation	92	r = 0.0560	p>0.5	NS

.../Cont.

Table 5.10. (Cont.)

Factors	Test	n	Result	Probability	Sig/NS
BENTLEY SITE ONLY:					
Rhabdias Intensity v RBC	Correlation	70	r = -0.1594	0.2>p>0.1	NS
Rhabdias Intensity v PCV	Correlation	77	r = -0.2537	0.05>p>0.02	Sig
Rhabdias Intensity v Hb	Correlation	91	r = -0.2498	0.02>p>0.01	Sig
Rhabdias Infection v MCV	Correlation	76	r = 0.1197	0.5>p>0.2	NS
Rhabdias Intensity v MCH	Correlation	70	r = -0.0730	p>0.5	NS
Rhabdias Intensity v MCHC	Correlation	59	r = -0.0834	p>0.5	
INFECTED TOADS ONLY:					
Rhabdias Intensity v RBC	Correlation	119	r = -0.0622	0.5>p>0.2	NS
Rhabdias Intensity v PCV	Correlation	139	r = -0.0905	0.5>p>0.2	NS
Rhabdias Intensity v Hb	Correlation	167	r = -0.1381	0.1>p>0.05	NS
Rhabdias Intensity v MCV	Correlation	97	r = -0.0082	p>0.5	NS
Rhabdias Intensity v MCH	Correlation	119	r = -0.0700	0.5>p>0.2	NS
Rhabdias Intensity v MCHC	Correlation	135	r = 0.0400	p>0.5	NS

Figure 5.9 Relationship of red blood cell count to intensity of *Rhabdias* sp. infection for all toads collected.

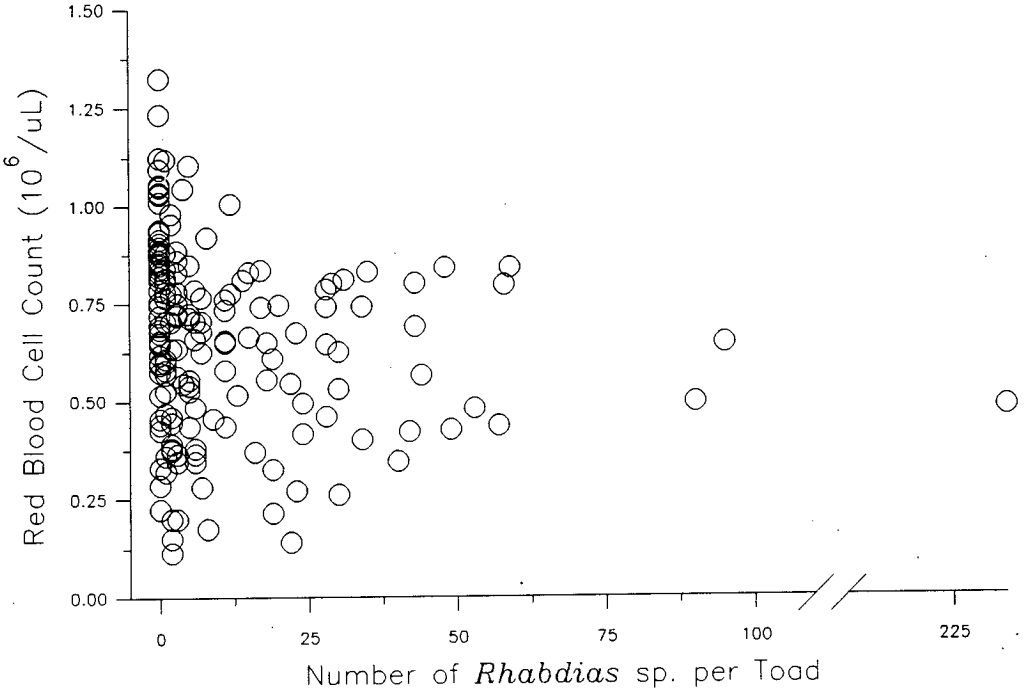

1.50
1.25
1.00
0.75
0.50
0.25
0.00
Red Blood Cell Count (10^6/uL)
0
25
50
75
100
225
Number of *Rhabdias* sp. per Toad

FIG 5.10

5.10a, 5.11a). No relationship was found between intensity of infection and the remaining blood parameters (Table 5.10).

Intensity of infection within the infected sites (QDPI and Bentley combined) showed significant negative relationships still existed for PCV and Hb concentration (Table 5.10). Red blood cell count, however, was no longer significantly related. The strengths of the relationships were less than for the overall data. The pattern of the relationship (Figures 5.10b, 5.11b) was similar to that found overall except the variation of measurement for toads with low infection levels had decreased. No significant relationships were found for the remaining blood parameters (Table 5.10).

QDPI toads, when analysed separately, showed only haemoglobin concentration to be significantly decreased by increasing intensity of *Rhabdias* sp. infection (Table 5.10). Haemoglobin concentration measurements were more closely grouped in these toads than for all toads collected (Figure 5.11c). The remaining parameters were not significantly decreased (Table 5.10).

Significant negative relationships were found between intensity of infection and both PCV and Hb concentration for toads collected from Bentley (Table 5.10). Variations in measurements from toads with low levels of *Rhabdias* sp. infection were, however, high (Figures 5.10c, 5.11d). Maximum levels of *Rhabdias* sp. infection were also much lower than for the QDPI toads (see Table 5.6). No significant relationship was found between intensity and the remaining blood parameters.

When only infected toads, pooled from both infected sites, were analysed there was no significant relationship between intensity of infection and the blood parameters (Table 5.10). The relationships were, however, still negative.

Figure 5.10 Relationship of packed cell volume to intensity of *Rhabdias* sp. infection for

a) all toads collected,
b) toads collected from infected sites only (QDPI and Bentley, combined), and
c) toads collected from Bentley only.

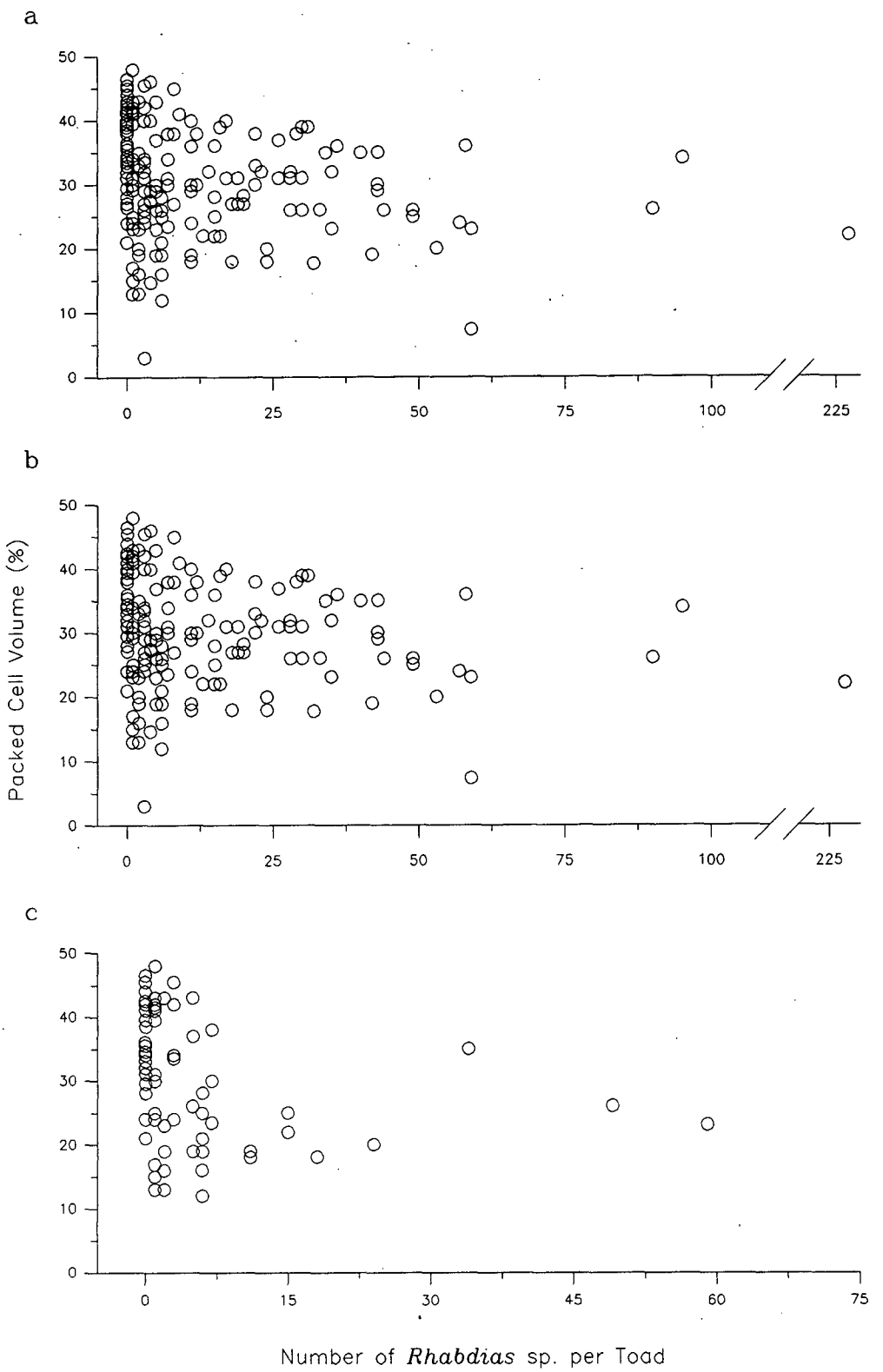
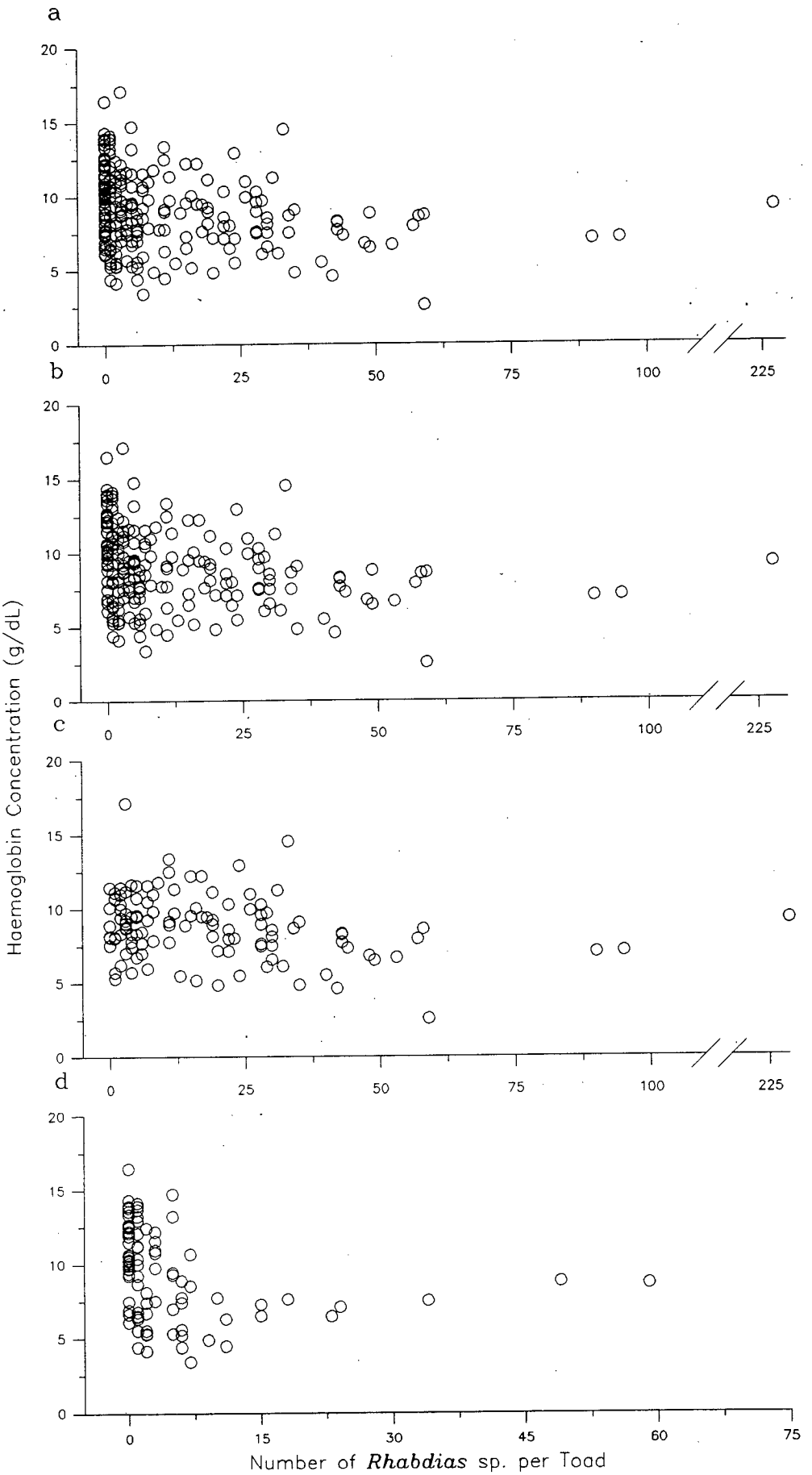

a
b
c
Packed Cell Volume (%)
Number of *Rhabdias* sp. per Toad
0
10
20
30
40
50
0
25
50
75
100
225
0
15
30
45
60
75

Figure 5.11 Relationship of haemoglobin concentration to intensity of *Rhabdias* sp. infection for

a) all toads collected,
b) toads collected from infected sites only (QDPI and Bentley, combined),
c) toads collected from QDPI only, and
d) toads collected from Bentley only.


a
b
c
d
Haemoglobin Concentration (g/dL)
Number of *Rhabdias* sp. per Toad

5.3.5 Relationship between site of collection and blood parameters.

Despite the significant difference in intensity of *Rhabdias* sp. infection between sites (see above), none of the blood parameters had a significant difference between the two infected sites (Table 5.11). The two sites were, therefore, combined for comparison against `Fletcherview'.

Significant differences were found for RBC counts, PCV, MCH and MCHC between the infected and uninfected sites (Table 5.11). Measurements for RBC counts and PCV were significantly higher at `Fletcherview' than for the infected sites (Figure 5.12a,b), whereas MCH and MCHC were significantly lower (Figure 5.12e,f). Haemoglobin concentration and mean corpuscular volume did not vary significantly between the sites (Table 5.11; Figure 5.12c,d).

5.3.6 Relationship between presence of *Rhabdias* sp. infection and blood parameters.

Comparison of infected and uninfected toads, independent of site, showed significant differences between the two groups for RBC count, PCV and Hb concentration (Table 5.12). For these three parameters, uninfected toads had significantly higher values than infected toads (Figure 5.13a-c). No significant difference was found for the remaining blood parameters (MCV, MCH and MCHC) (Table 5.12); uninfected toads had lower values for these parameters than infected toads (Figure 5.13d-f).

Within an infected site, comparison of the same data produced two completely different results. QDPI toads showed no significant differences between the two groups

Table 5.11 Results of analysis of relationships between site of collection and infection, red blood cell count, packed cell volume, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration. df = degrees of freedom.

Factors	Test	df	Result	Probability	Sig/NS
INFECTED SITES ONLY:					
Site v RBC	1 way ANOVA	1,148	F = 0.47	p = 0.4950	NS
Site v PCV	1 way ANOVA	1,170	F = 0.37	p = 0.5460	NS
Site v Hb	1 way ANOVA	1,203	F = 2.51	p = 0.1146	NS
Site v MCV	1 way ANOVA	1,166	F = 1.85	p = 0.1756	NS
Site v MCH	1 way ANOVA	1,148	F = 0.75	p = 0.3882	NS
Site v MCV	1 way ANOVA	1,122	F = 0.44	p = 0.5088	NS
INFECTED SITES v UNINFECTED SITE:					
Site v RBC	1 way ANOVA	1,165	F = 12.19	p = 0.0006	Sig
Site v PCV	1 way ANOVA	1,187	F = 9.27	p = 0.0027	Sig
Site v Hb	1 way ANOVA	1,220	F = 0.56	p = 0.4543	NS
Site v MCV	1 way ANOVA	1,139	F = 0.07	p = 0.7918	NS
Site v MCH	1 way ANOVA	1,165	F = 4.88	p = 0.0286	Sig
Site v MCHC	1 way ANOVA	1,183	f = 17.02	p = 0.0001	Sig

Figure 5.12 Relationship of site of collection to
a) mean red blood cell count,
b) mean packed cell volume,
c) mean haemoglobin concentration,
d) mean corpuscular volume,
e) mean corpuscular haemoglobin, and
f) mean corpuscular haemoglobin concentration.

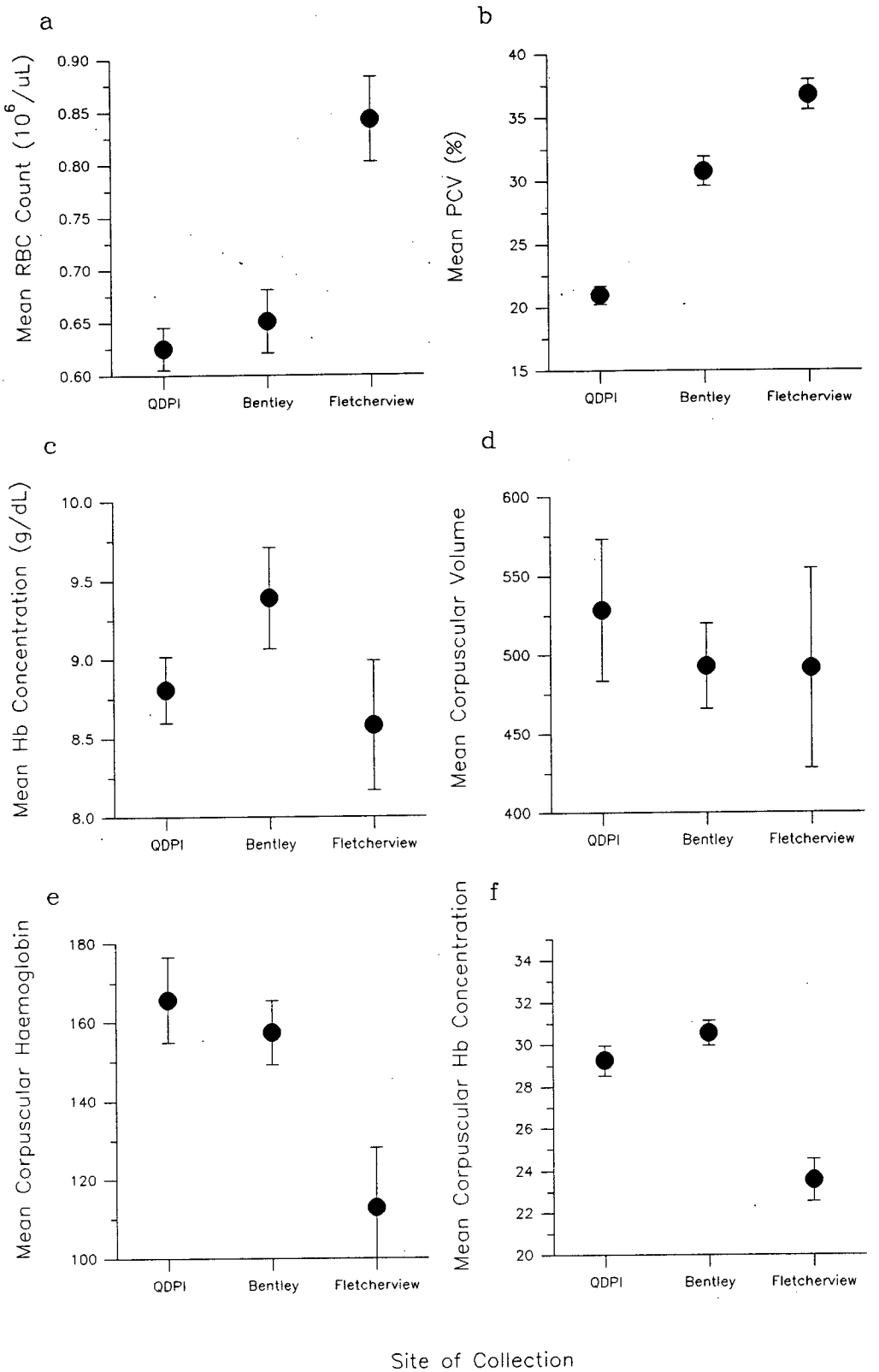
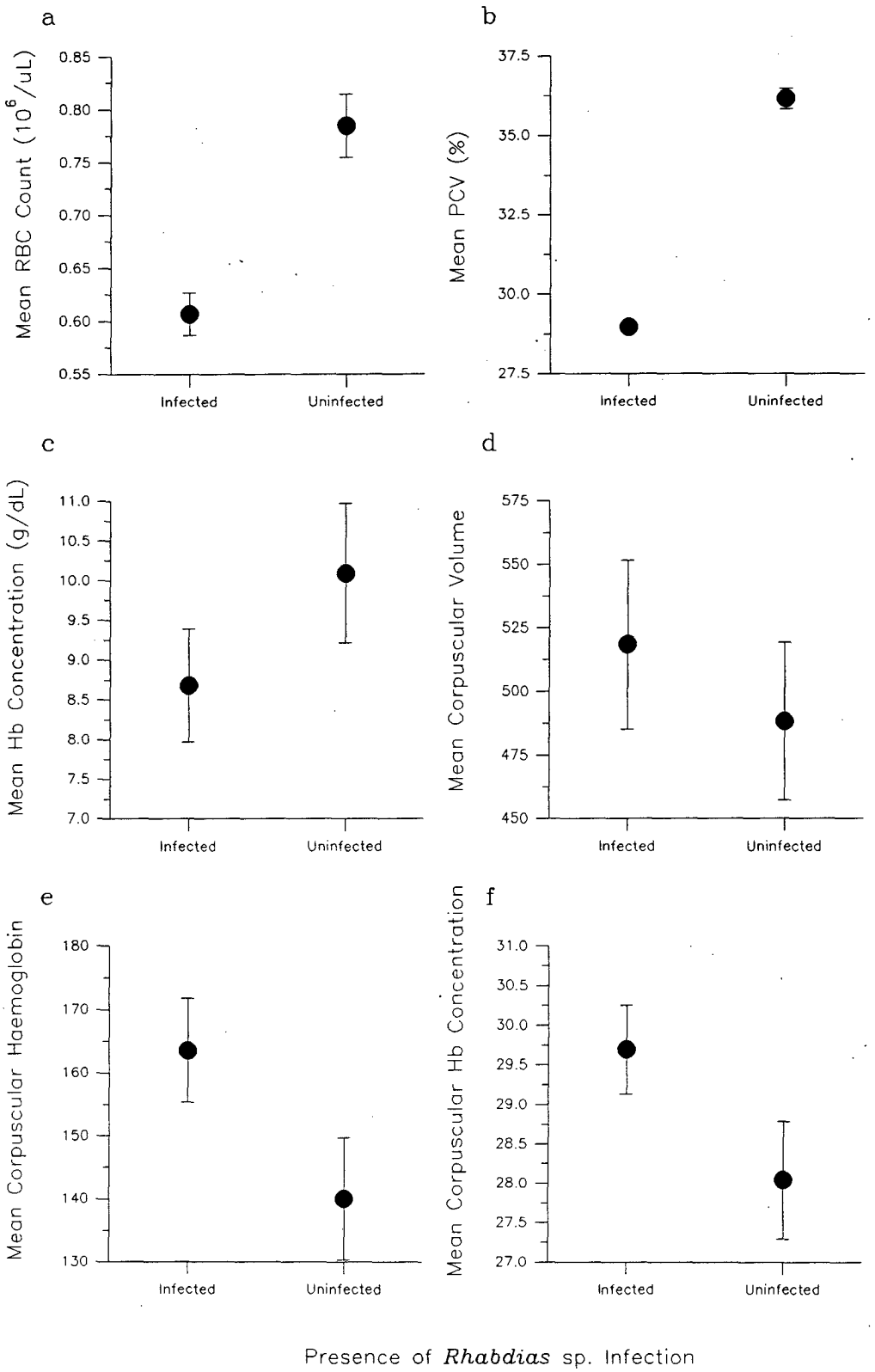

a
Mean RBC Count (10^6/uL)
0.90
0.85
0.80
0.75
0.70
0.65
0.60
QDPI
Bentley
Fletcherview
b
Mean PCV (%)
40
35
30
25
20
15
QDPI
Bentley
Fletcherview
c
Mean Hb Concentration (g/dL)
10.0
9.5
9.0
8.5
8.0
QDPI
Bentley
Fletcherview
d
Mean Corpuscular Volume
600
550
500
450
400
QDPI
Bentley
Fletcherview
e
Mean Corpuscular Haemoglobin
180
160
140
120
100
QDPI
Bentley
Fletcherview
f
Mean Corpuscular Hb Concentration
34
32
30
28
26
24
22
20
QDPI
Bentley
Fletcherview
Site of Collection

Table 5.12 Results of analysis of relationships between presence of *Rhabdias* sp. infection and red blood cell count, packed cell volume, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration. df = Degrees of freedom.

Factors	Test	df	Result	Probability	Sig/NS
OVERALL: (Present v Absent)					
v RBC	1 way ANOVA	1,165	F = 21.80	p < 0.0001	Sig
v PCV	1 way ANOVA	1,187	F = 30.51	p < 0.0001	Sig
v Hb	1 way ANOVA	1,220	F = 13.26	p = 0.0003	Sig
v MCV	1 way ANOVA	1,139	F = 0.32	p = 0.5733	NS
v MCH	1 way ANOVA	1,165	F = 2.71	p = 0.1013	NS
v MCHC	1 way ANOVA	1,183	F = 2.56	p = 0.1116	NS
QDPI ONLY: (Present v Absent)					
v RBC	1 way ANOVA	1,78	F = 0.17	p = 0.6784	NS
v PCV	1 way ANOVA	1,93	F = 1.54	p = 0.2179	NS
v Hb	1 way ANOVA	1,112	F = 0.08	p = 0.7810	NS
v MCV	1 way ANOVA	1,63	F = 0.01	p = 0.9385	NS
v MCH	1 way ANOVA	1,78	F = 0.00	p = 0.9938	NS
v MCHC	1 way ANOVA	1,90	F = 0.17	p = 0.6781	NS
BENTLEY ONLY: (Present v Absent)					
v RBC	1 way ANOVA	1,68	F = 13.90	p = 0.0004	Sig
v PCV	1 way ANOVA	1,75	F = 15.84	p = 0.0002	Sig
v Hb	1 way ANOVA	1,89	F = 18.53	p < 0.0001	Sig
v MCV	1 way ANOVA	1,57	F = 0.09	p = 0.7703	NS
v MCH	1 way ANOVA	1,68	F = 0.18	p = 0.6730	NS
v MCHC	1 way ANOVA	1,74	F = 0.06	p = 0.8105	NS

Figure 5.13 Relationship of presence of *Rhabdias* sp. infection to

a) mean red blood cell count,
b) mean packed cell volume,
c) mean haemoglobin concentration,
d) mean corpuscular volume,
e) mean corpuscular haemoglobin, and
f) mean corpuscular haemoglobin concentration for all toads collected, independent of site.


a
Mean RBC Count (10^6/uL)
0.85
0.80
0.75
0.70
0.65
0.60
0.55
Infected
Uninfected
b
Mean PCV (%)
37.5
35.0
32.5
30.0
27.5
Infected
Uninfected
c
Mean Hb Concentration (g/dL)
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
Infected
Uninfected
d
Mean Corpuscular Volume
575
550
525
500
475
450
Infected
Uninfected
e
Mean Corpuscular Haemoglobin
180
170
160
150
140
130
Infected
Uninfected
f
Mean Corpuscular Hb Concentration
31.0
30.5
30.0
29.5
29.0
28.5
28.0
27.5
27.0
Infected
Uninfected
Presence of *Rhabdias* sp. Infection

for any blood parameter (Table 5.12). It should be noted, however, that there were only 6 (5%) uninfected toads collected from QDPI. Comparison of the means for QDPI toads for each parameter showed uninfected toads had higher packed cell volume and haemoglobin concentration but lower RBC count, MCV, MCH and MCHC (Figure 5.14). Bentley toads, alternatively, showed highly significant differences between the two groups for RBC counts, PCV and Hb concentration (Table 5.12). For these three parameters, uninfected toads had significantly higher values (Figure 5.14a-c). The remaining parameters were not significantly different (Table 5.12), with the average values approximately equal for both groups of toads (Figure 5.14d-f).

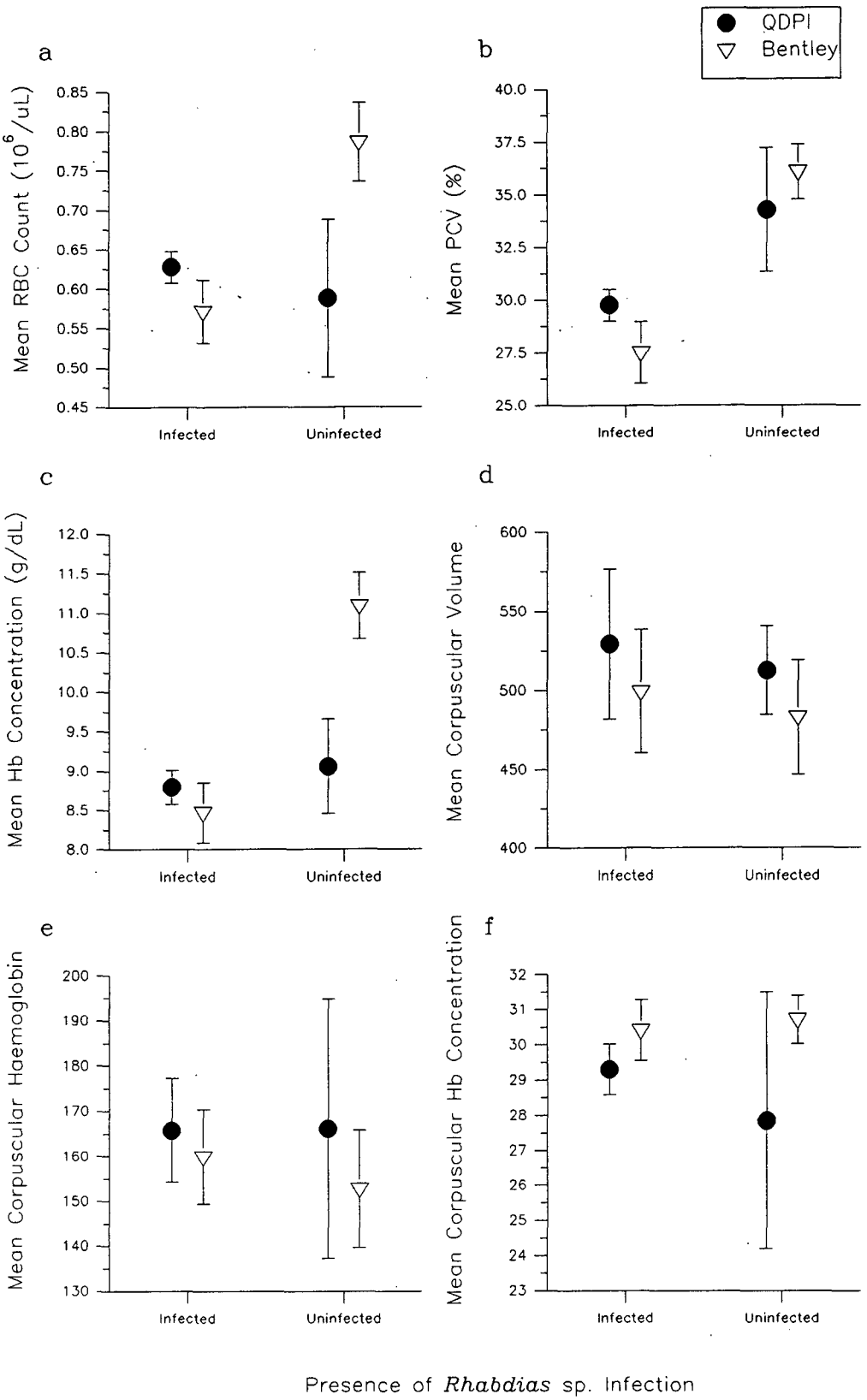
5.4 Discussion

This is the first record of haematological values for *B. marinus* in Australia. Base values for this data was collected from `Fletcherview' toads to rule out possible effects from *Rhabdias* sp. infection on the data. Season (March, 1992: autumn) may have slightly affected the values as it is just after the summer breeding period, which is a period of anaemia for amphibians (see Kaplan & Crouse 1956; Harris 1972; Friedmann 1974). However, the 1991-1992 breeding season in northern Australia was affected by the El Nino climatic phenomenon which prevented the customary summer monsoonal rain. This rain triggers mass spawning in most Australian amphibians (Hearnden 1991). At the time of collection from `Fletcherview', few tadpoles or small metamorphs were present at the site, indicating little breeding had occurred (pers. obs.). Thus, the summer anaemia effect may have been reduced this season.

Values found for the haematological parameters of *B.*

Figure 5.14 Relationship of presence of *Rhabdias* sp. infection to

a) mean red blood cell count,
b) mean packed cell volume,
c) mean haemoglobin concentration,
d) mean corpuscular volume,
e) mean corpuscular haemoglobin, and
f) mean corpuscular haemoglobin concentration for toads collected from QDPI and Bentley.


a
b
c
d
e
f
QDPI
Bentley
Mean RBC Count (10^6/uL)
Mean PCV (%)
Mean Hb Concentration (g/dL)
Mean Corpuscular Volume
Mean Corpuscular Haemoglobin
Mean Corpuscular Hb Concentration
Infected
Uninfected
Presence of *Rhabdias* sp. Infection

marinus in this study (Table 5.7) resemble values recorded in the literature (Table 5.1). Packed cell volumes from Australian toads were within the range of literature records while haemoglobin concentration was lower. It is difficult to draw many conclusions from this, however, as the disease status of the toads in Table 5.1 are unknown.

Uninfected toads collected from `Fletcherview', showed no significant relationship between length or sex of toad and the blood parameters. Thus, if this result is extrapolated into the infected sites, any alteration in the blood parameters should be due to *Rhabdias* sp. infection, not host-related factors. Certain host-related factors, such as nutrition, cannot be ruled out as habitats at the infected sites and `Fletcherview' were not identical. While the differences found in this study were not significant, male toads did have higher values for packed cell volume and haemoglobin concentration. Gil (1975) found similar results for *B. marinus* in Brazil, with no significant difference between the sexes for packed cell volume, although males did have higher values.

It was unfortunate that collections of toads from the three locations could not occur more regularly or coincide with each other. Difficulties in access to properties, particularly in the wet season, and capture of sufficient toads to use for analysis made the collection of coincident samples impossible. Future studies should consider regular sampling of blood over a longer period of time to assess the true seasonality of the blood parameters.

In addition, determination of the true effect of infection with *Rhabdias* sp. in an experimental situation should be considered. Toads kept in captivity usually suffer from an increase in infection levels of *Rhabdias* sp. due to its direct life cycle (see Chapter 5 Part A)

which allows it to transmit within an enclosure (Speare, R. 1993, pers. comm.). Collection of blood from toads over a period of time by live heart puncture would give an indication of the effect of increasing levels of infection. This solution however leads to the problem of identification, and quantification, of *Rhabdias* sp. infection from faecal samples (see Chapter 5 Part A).

The pattern of variation over time that was evident in this study, however, did show the importance of *Rhabdias* sp. infection to the blood parameters of toads. Mean level of infection of *Rhabdias* sp. decreased over the sampling period (Figure 5.8). Levels of red blood cells, packed cell volume and haemoglobin all increased over the same time (Figure 5.9). Decrease in values for blood parameters related to an increase in level of disease has been reported by Kaplan (1951) for *Rana pipiens* with red leg disease.

Intensity of *Rhabdias* sp. infection was only significantly related to levels of red blood cells, haemoglobin concentration and packed cell volume when large numbers of uninfected toads were present in the data. Presence or absence of infection had a more significant relationship to levels of these three parameters.

While intensity of infection was significant in the overall data, when the uninfected toads were removed from the analysis this significance was lost. Thus, increasing infection levels had no more a significant relationship with levels of the blood parameters than did the presence of a single *Rhabdias* sp. This is in contrast to the situation reported by Kameswari and Rao (1987) where decreases in levels of red blood cells and haemoglobin concentration were proportional to increases in the number of helminth species infecting *R. tigrina*.

The result of no significant difference between infected and uninfected toads at QDPI should be treated

cautiously due to the low number of uninfected animals collected (6 of 117). The result from Bentley is a more accurate representation of the importance of the presence of *Rhabdias* sp. infection on the blood parameters. This is because the distribution between infected and uninfected toads (60 and 32, respectively) was more even than at QDPI.

Another factor supporting the importance of presence of *Rhabdias* sp. was found in the analysis of site of collection. Although there was a significant difference in intensity of *Rhabdias* sp. infection between the two infected sites, there was no significant difference between levels of the three blood parameters.

This study has shown the presence of *Rhabdias* sp. to be related to decreases in various host blood parameters. Although toads are capable of supporting quite high levels of *Rhabdias* sp. infection (see Chapter 4 Part A), it is possible that *Rhabdias* sp. could be having a measurable effect on toad health. The significance of this relationship, however, remains unknown. Collection of more baseline blood data over a longer collection period to allow for seasonal variation, coupled with coincident samples from *Rhabdias* sp. infected and *Rhabdias* sp.-free sites is required. This will provide the answer to the importance of *Rhabdias* sp. in its relationship to toad health.

Chapter 6: General Discussion

The helminth fauna of Australian amphibians, including that of the introduced toad *Bufo marinus*, has been surveyed in detail for the first time. Prior to this study, knowledge of this fauna, particularly in northern Australia, was practically non-existent with regards to both species composition and ecological relationships.

A total of 27 helminth species was collected from both the toad and native amphibians in this study. No helminths were collected from the kidney, liver, gall bladder, heart, mouth cavity, or nostrils of any amphibian. Of the 27 records, four were determined to be new species: *Zeylanurotrema spearei* (see Cribb & Barton 1991), *Dolichosaccus helocirrus* (see Barton 1994a), Onchocercidae gen sp. (description in preparation), and *Johnpearsonia pearsoni* (see Durette-Desset *et al.* 1994). Many helminth taxonomic problems were highlighted by this work, particularly for the genera *Rhabdias*, *Mesocoelium* and *Cosmocerca*. Clarification of the taxonomic problems within these genera are required and will have to involve more sophisticated techniques than morphological analyses. The use of DNA sequence data, despite its own inherent difficulties, will enhance the information gathered in studies similar to this one by allowing the accurate identification of species and inference of possible phylogenetic relationships.

The range of host species for many helminths has been expanded and many of the amphibian species studied here are recorded as a host for the first time. In addition, certain helminth genera which had only previously been recorded from reptiles, for example *Kreisiella* and *Spinicauda*, were found in amphibians. The majority of the helminth genera collected are regarded as host generalists and members of these genera have been recorded from various amphibian taxa around the world (see Prudhoe & Bray 1982; Baker 1987).

The toad helminth fauna was found to consist primarily of species which had been previously recorded in native frogs and had, thus, transferred to the toad after its introduction. Some helminth species, for example *Zeylanurotrema spearei*, however, may have been acquired from native reptiles; a study of the helminth fauna of native reptiles within this region needs to be undertaken.

Of the 27 helminth species collected, three were recorded only from native frogs. All three species, *Parapolystoma* sp., *Seuratascaris numidica* and Onchocercidae gen. sp., occurred in areas where toads were present either in small numbers (Paluma) or were new arrivals (Cape Weymouth). In addition, the life cycles of these helminths are relatively specialised and these helminths could be regarded as host specialists. Further collections of toads from these areas are needed to determine if *B. marinus* is capable of `breaking into' such a specialised helminth life cycle in Australia.

No helminths infecting toads in Australia were determined as having a South American origin. *Rhabdias* sp. and *Mesocoelium* sp., due to the reasons outlined above, however, cannot be ruled out as possible introductions with the toad without further detailed taxonomic analysis.

The technique of collecting host specimens from around water sources led to higher numbers of male hosts in the samples. Differences in helminth infection occur between sex and age of hosts due to different behaviour patterns. This fact should be recognised, and acknowledged, when describing patterns of helminth acquisition for host species.

Over half the amphibians collected were infected with at least one helminth species. Intensity of infection, however, was relatively low for the majority of helminths. These low levels of infection are probably due to a combination of the low number of hosts collected

and the depauperate nature of amphibian helminth communities. These infection levels, however, were comparable with records from temperate amphibian species (see Aho 1990). Collection of amphibian species from the wet tropics would assist in determining whether a depauperate helminth community is typical of amphibians in general.

Nematodes dominated the helminth faunas of the amphibians collected. This was due to the generally terrestrial habits of the hosts studied. The only `aquatic' species sampled was *Litoria genimaculata* which was found to be infected with a helminth fauna dominated by helminths requiring water for transmission, for example *Parapolystoma* sp. (see Prudhoe & Bray 1982). Many of the truly `aquatic' frogs, for example *Litoria nannotis*, have never been sampled for helminths.

Bufo marinus was found to have a more diverse helminth community compared to native frogs, and in particular *Lit. inermis*. Both at a host individual and host population level, the toad was infected with a larger number of helminth individuals and species. Even when helminth species were shared between toads and *Lit. inermis* at similar prevalence, for example *Rhabdias* sp., the intensity of infection was significantly different. Whether this relationship is due to some difference in susceptibility, exposure and/or diffrential mortality remains unknown.

Factors such as host size may be influencing the levels of helminth infection in the amphibians studied, and more research, particularly on frogs of a similar size to *B. marinus*, for example *Cyclorana novaehollandiae*, are needed. Again, however, these frogs are primarily terrestrial in habit. Comparison of the helminth fauna of *B. marinus* with other amphibian species in South America would clarify whether *B. marinus* is naturally infected with a diverse range of helminths.

Diversity indices in helminth ecology, in general, if used incorrectly, can create more difficulties than they are worth. A consensus must be reached on which indices to use to ease future comparison between data sets. Use of a range of indices that are biased to different aspects of community structure are best to give an overall picture (as in Kennedy 1993b). Stating the indices used for each level of community structure studied should become a requirement of all future community ecology reports.

The concept of core and satellite species, and their involvement in an interactive versus an isolationist community, needs to be reviewed for amphibian helminth communities (see Sousa 1994). The depauperate nature of amphibian helminth communities, in comparison to those of birds and fish as studied by Holmes and co-workers in formulating this concept, makes this system of classification inadequate. As the helminth community of amphibians is isolationist in character, species should be determined as either a component (prevalence greater than 10%) or rare species. Listing all helminth species found, with their relevant prevalence and mean intensity of infection, is highly recommended for all studies, including standard parasite taxonomic papers. Valuable information is otherwise lost with the exclusion of such `base' data.

The introduction of *B. marinus* to Australia has been reported to affect Australia's native fauna to varying degrees. The relationship of *B. marinus* to native fauna *via* its parasites, however, has not previously been considered. It has been shown in this study that *B. marinus* has acquired the majority, if not all, of its Australian helminths from native fauna. In addition, the toad in Australia is infected at similar, if not greater, levels of infection. Unfortunately, no quantitative study of the helminth fauna of native amphibians was

undertaken prior to the introduction of *B. marinus*.

The role of *B. marinus* as a dispersal agent for infective stages of these acquired helminths has not been considered. Theoretically, the high number of toads present in an amphibian community, infected with such levels of helminths, should create a continuous output of infective stages. This theory is dependent on the fecundity of the helminths being similar to that of the same helminth species in its original host. I have no reason to consider that this is not the case as all helminth species found appeared to have similar reproductive status, independent of the host species. Future studies on the parasite fauna of toads may consider methods of quantifying this aspect.

Although the toad may be increasing the number of infective helminth stages in the environment, there appears to be no obvious deleterious relationship within the native amphibian community. Observed declines in levels of certain amphibian species, however, should not be forgotten. The toad may not be directly influencing native amphibian population structure, but the effect of increased levels of helminth infections caused by the presence of the toad may push a declining native amphibian species to the edge.

Of the 27 helminth species recorded in this study, only *Rhabdias* sp. was suggested as a possible biological control agent for the toad. Members of the genus *Rhabdias* sp. are blood-feeding with a direct life cycle and have been reported to cause minor pathological damage in captive hosts. The population structure of *Rhabdias* sp. within wild toads was detailed in this study. Experimental studies of the life cycle of *Rhabdias* sp. and the effects of *Rhabdias* sp. on the health of the toad were also examined.

Infection of newly metamorphosed wild toads with *Rhabdias* sp. occurred rapidly, although initially at a

low level. Intensity of infection increased steadily over the following months as the toads grew. After the first year, however, due to the continual acquisition of *Rhabdias* sp. when the toads returned to the water impoundment, patterns in *Rhabdias* sp. acquisition and growth became harder to discern. The larger toads appeared to lose their *Rhabdias* sp. infection, which was probably due more to changes in their behaviour, and physiology (thicker abdominal skin), than parasite-induced mortality. Lack of a truncated frequency distribution for *Rhabdias* sp. in the toad population supported this theory. Future studies of the relationship between *Rhabdias* sp. and *B. marinus*, as well as other amphibian species, in Australia should consider these aspects in detail.

Rainfall was found to be a major climatic influence on the acquisition of *Rhabdias* sp. Again, behaviour of the host related to rainfall was the primary reason for these patterns, where congregations of toads, usually in response to rainfall, would lead to an increase in intensity of *Rhabdias* sp.

Use of host size as an indicator of age for amphibians is not recommended for various reasons. The lack of skeletochronological data for toads in Australia, however, precludes the use of this technique. The size classes that were used in this study did appear to correspond well with host behavioural changes.

Development of *Rhabdias* sp. from embryonated egg to infective third-stage larva via a free-living generation took 4d at 24°C. *Rhabdias* sp. failed to complete its life cycle at temperatures below 18°C. A free-living sexual phase was always included in the life cycle. Only one larva per free-living female was produced in culture, regardless of the temperature at which it was held. Use of the free-living part of the life cycle to identify species of the genus *Rhabdias* has been recommended by

various workers (see Ballantyne 1971; Kloss 1974). Variation in the number of larvae produced per free-living female (see Kloss 1974), however, makes this technique of dubious systematic value. Coupled with the DNA sequence technique, however, this method could prove a powerful taxonomic tool. Further experiments to look at the effects of humidity on the number of larvae produced, however, are required.

Infection experiments with *Rhabdias* sp. were hampered by the high death rate of metamorph toads and *Limnodynastes ornatus*. Before any further experiments in this area, a laboratory procedure for the raising, and maintaining, of large numbers of metamorphs needs to be finalised. Once this system is established, studies into lethal level of infection and development of the infection within the host can be completed.

When the experimental infections were successful, however, low infection dosages with *Rhabdias* sp. produced the best results for success rate of infection. Reasons for this remain unknown, although Goater and Ward (1992) found that after a period of time, infection declined to a similar level, independent of the infection dosage. Unfortunately, experiments in this study were unable to continue for a sufficient time to allow for this decline to be detected. An unknown physiological factor maybe determining infection levels of *Rhabdias* sp. within the metamorphs. While similar numbers of *B. marinus* and *Lim. ornatus* metamorphs were experimentally infected (55.8% and 60%, respectively), mean intensity and range of infection was much lower in *Lim. ornatus*. Further experiments are required to determine what this factor could be. The role of skin thickness, immunological and ecological factors need to be determined.

Haematological values recorded for *B. marinus* in Australia (from `Fletcherview') resemble records for *B. marinus* in South America. This is the first record of

base haematological values for *B. marinus* in Australia. These values need to be treated cautiously, however, as only a small number of toads were sampled. The possibility of a physiological effect related to the time of collection (breeding season) can not be ruled out. A longer term study, looking at the seasonality of levels of the blood parameters of toads in Australia needs to be undertaken.

The presence of *Rhabdias* sp. infection significantly affected levels of red blood cells, packed cell volume and haemoglobin concentration. Intensity of infection, however, was not such a significant factor. That is, the presence of a single *Rhabdias* sp. did not alter the levels of blood parameters more than did the presence of many *Rhabdias* sp. individuals. Reasons for this remain unknown, although future studies could look at rate of red blood cell production to determine if *Rhabdias* sp.-infected toads are somehow compensating for the red blood cells lost to *Rhabdias* sp. Size or weight of the spleen, a site of red blood cell production, may be used to give an indication of this.

No relationship was found between the blood parameters and season, toad sex or toad length. The collection of toads for this part of the study was, however, particularly haphazard, so these results should be treated cautiously. Future studies need to collect toads on a regular basis to determine seasonal patterns, particularly in relation to seasonal patterns of helminth infections. In addition, collections of large numbers of toads will give a more accurate representation of the relationships between toad sex and length with the blood parameters.

Results of this study do not support the use of *Rhabdias* sp. as a biological control agent for *B. marinus* in Australia. Without further taxonomic evidence, the presence of *Rhabdias* sp. in such a variety of amphibian

species, and possibly also in reptiles, shows *Rhabdias* sp. to have too low a host specificity. In addition, there may be more than one species of *Rhabdias* in Australia, one of which may have been introduced with the toad. Until this question is resolved *Rhabdias* sp. can not be considered as a biological control agent.

In addition, the lack of a parasite-mediated host mortality by *Rhabdias* sp. prevents support for *Rhabdias* sp. as a control agent. The indirect effects of *Rhabdias* sp. on the health of the toad, however, must be considered. *Rhabdias* sp. may enhance a natural state of anaemia, particularly during the breeding season, which may make the toad susceptible to secondary infections. On the other hand, *Rhabdias* sp. could potentially act in conjunction with a viral control agent to produce overall control of the toad. The direct life cycle of *Rhabdias* sp. could be used to advantage for ease of transmission of such a virus to toads. In this case, however, the virus would have to be highly host specific due to the possible low specificity of the vector. The blood-feeding habit of *Rhabdias* sp. could also be used to advantage when combined with a virus that lysed red blood cells. This combination could cause a sufficient anaemia to cause death of the host.

The question of a biological control agent for the toad in Australia still remains unanswered. This study has shown, however, that due to the `generalist' nature of the parasite community of both native amphibians and reptiles in Australia, whatever the control agent may be, it must be highly host specific.

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Appendix 1: Location of study sites

Location	State	Longitude	Latitude
Abergowrie	Qld	146°00'E	18°27'S
Bentley	Qld	146°57'E	19°22'S
Black Rock	Qld	144°07'E	19°05'S
Bloomfield	Qld	145°21'E	15°56'S
Boyne Island	Qld	151°21'E	23°57'S
Brisbane	Qld	153°01'E	27°30'S
Calvert Hills	NT	137°25'E	17°13'S
Cape Tribulation	Qld	145°29'E	16°05'S
Cape Weymouth	Qld	143°23'E	12°55'S
Charters Towers	Qld	146°11'E	19°53'S
Coen	Qld	143°12'E	13°57'S
Mackay	Qld	149°11'E	21°09'S
Mareeba	Qld	145°25'E	17°00'S
MVR*	Qld	146°57'E	19°30'S
Paluma	Qld	146°12'E	19°01'S
Port Douglas	Qld	145°28'E	16°29'S
Townsville	Qld	146°49'E	19°15'S
Yungaburra	Qld	145°35'E	17°17'S

* Mountain View Road

Appendix 2: Recipes

Acidified Carmine

10 g	Carmine C.I. 75470
100 ml	45 % Acetic Acid

Berland's Fixative

95 ml	Glacial Acetic Acid
5 ml	40 % Formaldehyde

Bouin's Fluid

75 ml	Saturated Picric acid in distilled water
5 ml	Glacial Acetic Acid
25 ml	40 % Formaldehyde

Calcium Acetate Buffered Formalin

10 ml	40 % Formaldehyde
1 g	Calcium Chloride (Anhydrous)
5 ml	Glacial Acetic Acid
85 ml	Tap water

Carnoy's Fixative

60 ml	100 % Ethanol
30 ml	Chloroform
10 ml	Glacial Acetic Acid

Gower's Carmine

1 g	Acidified Carmine
10 g	Potassium Alum
200 ml	Distilled water

Lactophenol

50 ml	Phenol (liquid)
50 ml	Lactic Acid
100 ml	Glycerol
50 ml	Distilled Water

`Toad Ringer'

80 ml	filtered Sea Water
360 ml	Distilled Water

Appendix 3: Fixation and mounting of nematodes using Carnoy's fixative

Method taken from Ballantyne 1971:26.

1. Some water was removed from the cavity block.
2. The cavity block and contents were heated gently until the nematodes stopped moving.
3. The heating source was removed and the nematodes left for 5 minutes to ensure death.
4. As much water as possible was removed and Carnoy's fixative added.
5. Nematodes were left in fixative for half an hour or more (maximum time 24 hours).
6. 90% alcohol.
7. 70% alcohol containing 5% glycerine.
8. Nematodes were stored in this mixture, but for best results they had to be stained within a week.
9. 70% alcohol.
10. Distilled water.
11. 45% acetic acid. Nematodes were left in 45% acetic acid for at least one hour (or longer if they had been stored for a long period).
12. Stain in 1% synthetic orcein in 45% acetic acid. The time necessary to stain material varied from 6 to 24 hours. Warming hastened and improved staining but care was needed as the acetic acid evaporated.
13. After staining, specimens placed in 45% acetic acid containing 5% glycerine (a small amount of stain was added if worms were lightly stained).
14. Specimens were examined and stored in the above mixture.

Other staining schedules follow those as outlined in Pritchard and Kruse (1982).

Appendix 4

This Appendix contains a copy of a Parasite-Host and Host-Parasite checklist as published in *The Records of the South Australian Museum*.

A CHECKLIST OF HELMINTH PARASITES OF AUSTRALIAN AMPHIBIA

DIANE P. BARTON

BARTON, D. P. 1994. A checklist of helminth parasites of Australian Amphibia. *Rec. S. Aust. Mus.* 27(1): 13–30.

This checklist includes all original references, and any other references which do more than repeat original work, of helminths occurring in Australian amphibians published up to 1992. Museum listings are also included, where available. Most records pertain to free-ranging animals; where they do not, they have been annotated appropriately.

Helminths are arranged as follows: Monogenea, Digenea, Cestoda, Nematoda, Acanthocephala, in both the parasite-host and host-parasite checklists.

Hosts are presented by family with consideration given to recent taxonomic changes.

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Manuscript received 20 August 1992.

Introduction

In 1939 May Young produced a checklist of helminth parasites recorded from Australian hosts. Thirteen amphibian hosts, infected with a total of 30 helminth species, were included. There has been no further compilation of solely Australian records from amphibians since then. The aim of this work is to produce an updated checklist of amphibian helminth parasites in Australia.

Included in this checklist are all original references, and any other references which do more than repeat original work, published up to 1992. Museum collections of amphibian parasites are also included, where available. Most records pertain to free ranging animals; where they do not, they have been annotated appropriately (e.g. experimental).

Comments on host taxonomy

Host names used in this checklist follow Cogger (1992), with the following exceptions:

i) *Kyarranus* Moore, 1958 is accepted as a valid genus (see Frost 1985).

ii) *Litoria pearsoniana* (Copland, 1961) is accepted as a valid species (see Frost 1985).

In the parasite-host checklist, host names are given as they were listed in the original publications. In the host-parasite checklist, the names have been updated to those used by Cogger (1992). The original names are also given with reference to the new name when there is an element of confusion.

All species formerly referred to the family Leptodactylidae are now placed in the family Myobatrachidae (see Cogger 1992).

All *Hyla* species are now referred to the genus *Litoria* (see Cogger 1992).

Limnodynastes dorsalis and *L. d. dumerilii* recorded from South Australia, Queensland and New South Wales are referred to *L. dumerilii*. *Limnodynastes dorsalis* is only present in Western Australia (see Cogger 1992).

Litoria aurea and *L. a. raniformis* recorded from South Australia, Victoria and Tasmania are referred to *L. raniformis* (see Cogger 1992).

Litoria aurea recorded from Western Australia is referred to *Litoria* spp., as the range of *L. aurea* does not extend to Western Australia (see Cogger 1992).

Crinia sp. recorded from the Flinders Ranges, South Australia, in the Australian Helminthological Collection (AHC) list are most likely *C. riparia*. A group of these frogs was collected from Warren Gorge, which is within the range of *C. riparia* (Dr Margaret Davies, pers. comm.). A more precise geographical location is, however, required to differentiate *C. riparia* from *C. signifera*, so they must remain *Crinia* sp.

Litoria jervisiensis recorded from South Australia is referred to *L. ewingii* (see Cogger 1992).

Mixophyes sp. collected from the Bunya Mts, Queensland (AHC 6172), could be either *M. fasciolatus* or *M. iteratus*. Examination of the host specimen would be needed to determine the exact species.

Uperoleia marmorata collected from New England National Park, New South Wales (AHC 8055), could be either *U. rugosa* or *U. laevigata*. Davies & Littlejohn (1986) showed both species to be present in this region, while *U. marmorata* was restricted to the north-west of Western Australia. All *U. marmorata* in this checklist are from eastern Australia and helminths from these host specimens are referred to *Uperoleia* spp. Again, examination of the host specimen would be needed to determine which species is correct.

Bufo marinus was introduced to Australia in 1935

from South America (via Hawaii) (Easteal 1981). It is 'naturally' found in Queensland, northern New South Wales and eastern Northern Territory. Any locations recorded out of this range are from laboratory animals acquired from a commercial supplier.

Records of helminths from frogs in New Guinea are included only if that frog species is also found in Australia.

Comments on helminth taxonomy

Helminth nomenclature follows Prudhoe & Bray (1982) for Monogenea, Digenea and Cestoda, the CIH Keys to the Nematode Parasites of Vertebrates (Hartwich 1974; Chabaud 1975*a*, 1975*b*, 1978; Anderson & Bain 1976, 1982; Petter & Quentin 1976; Durette-Desset 1983) and Spencer Jones & Gibson (1987) for the Nematoda, and Amin (1985) for the Acanthocephala.

The taxonomic status of many genera and species infecting amphibians is in need of revision. Generally, original records of helminths are treated as correct, unless it is known to the author that appropriate revision has taken place.

All helminths are recorded in the parasite-host checklist under the current name with any synonyms also listed.

All previous records of lung nematodes from Australian frogs have been referred to *Rhabdias hylae* Johnston & Simpson, 1942, by Ballantyne (1971), a view accepted in this checklist.

Nasir & Diaz (1971) synonymised the Australian representatives of the genus *Mesocoelium* as *M. megaloon* Johnston, 1912, and *M. monas* (Rudolphi, 1819), Teixeira de Freitas, 1958 (*M. microon* Nicoll, 1914, *M. mesembrinum* Johnston, 1912, *M. oligoon* Johnston, 1912). This is not accepted here, pending further work on the genus.

Frogs often serve as an intermediate host for cestodes, being infected with plerocercoids/spargana in the musculature. The identification of this life cycle stage is impossible without knowledge of the definitive host. In other groups (Acanthocephala, Digenea), larval stages are often identifiable.

Explanation of Format

This checklist has been compiled from all published records up to 1992 known to the author, and from lists of museum holdings.

References to Prudhoe & Bray (1982) on microfiche are shown as 'mf' following the page number.

The lists are arranged as follows:

1. Parasite species are arranged systematically. The amphibian hosts are listed for each helminth followed by the state or territory of origin (? denotes the state or territory was not referred to), literature references, and museum collection numbers, where available. The hosts are arranged with the type host first and all others listed alphabetically after.
2. Host species are arranged alphabetically within each family. The helminths from each host species are listed below the host with the phase of development and site of infection recorded, where known.
3. References.

Authors whose names appear frequently are referred to, where appropriate, by initials, as follows:

LMA L. Madeline Angel
MRY May R. Young
PMM Patricia M. Mawson
SJJ Stephen J. Johnston
THJ T. Harvey Johnston

The major helminth parasite groups are referred to by their initials:

M Monogenea
D Digenea
C Cestoda
N Nematoda
A Acanthocephala

Museums and other sources which are referred to as having amphibian parasites in their collection are abbreviated as follows:

AHC Australian Helminthological Collection, (in the South Australian Museum) Adelaide, SA
AM Australian Museum, Sydney, NSW
BM(NH) Natural History Museum, London, England
CAS Institute of Parasitology, Czechoslovak Academy of Sciences, Ceské Budejovice, Czechoslovakia
QM Queensland Museum, Brisbane, Qld
SAM South Australian Museum, Adelaide, SA
SP Personal collection of Ms Sylvie Pichelin, Parasitology Department, University of Queensland, Brisbane, Qld
TM Tasmanian Museum and Art Gallery, Hobart, Tas

State names are abbreviated as follows:

NSW New South Wales
NT Northern Territory
Qld Queensland
SA South Australia, including Kangaroo I. & Pearson I.

Tas	Tasmania, including Bass Strait Islands (King & Flinders)
Vic	Victoria
WA	Western Australia

ORDER AND ARRANGEMENT OF PARASITES AS PRESENTED UNDER EACH HOST

1. Phylum Platyhelminthes

Class Monogenea Carus, 1863
Order Polyopisthocotylea Odhner, 1912

Family POLYSTOMATIDAE Carus, 1863, emended Gamble, 1896
Subfamily Polystomatinae Gamble, 1896

Class Trematoda Rudolphi, 1808
Order Digenea Van Beneden, 1858
Suborder Prosostomata Odhner, 1905

Family PARAMPHISTOMATIDAE Fischoeder, 1901
Subfamily Diplodiscinae Cohn, 1904

Family GORGODERIDAE Looss, 1901

Family ALLOCREADIIDAE Stossich, 1903

Family PLAGIORCHIIDAE Lühe, 1901, emended Ward, 1917
Subfamily Haematoloechinae Teixeira de Freitas & Lent, 1939, emended Yamaguti, 1958

Family TELORCHIIDAE Stunkard, 1924
Subfamily Opisthioglyphinae Dollfus, 1949

Family BRACHYCOELIIDAE Johnston, 1912

Family LECITHODENDRIIDAE Odhner, 1910

Family BRACHYLAIMIDAE Joyeux & Foley, 1930

Family DIPLOSTOMIDAE Poirier, 1886

Family DOLICHOPERIDAE Yamaguti, 1971

Not further identified

Class Cestoidea Rudolphi, 1808
Order Pseudophyllidea Carus, 1863

Family DIPHYLLOBOTHRIIDAE Lühe, 1910

Order Proteocephalidea Mola, 1928

Family PROTEOCEPHALIDAE La Rue, 1911

Order Cyclophyllidea Braun, 1900

Family NEMATOTAENIIDAE Lühe, 1910

Not further identified

2. Phylum Nematoda

Class Secernentea
Order Rhabditida

Superfamily Rhabditoidea
Family RHABDIASIDAE Railliet, 1916

Order Strongylida

Superfamily Trichostrongyloidea
Family MOLINEIDAE (Skrjabin & Schulz, 1937) Durette-Desset & Chabaud, 1977

Order Oxyurida

Superfamily Oxyuroidea
Family PHARYNGODONIDAE Travassos, 1919

Order Ascaridida

Superfamily Cosmocercoidea
Family COSMOCERCIDAE (Railliet, 1916, subfam.) Travassos, 1925

Superfamily Ascaridoidea
Family ASCARIDIDAE Baird, 1853

Order Spirurida

Superfamily Physalopteroidea
Family PHYSALOPTERIDAE (Railliet, 1893, subfam.) Leiper, 1908

Superfamily Habronematoidea
Family HEDRURIDAE Railliet, 1916

Superfamily Filarioidea

Not further identified

3. Phylum Acanthocephala

Class Palaeacanthocephala Meyer, 1931
Order Echinorhynchida Southwell & MacFie, 1925

Family ECHINORHYNCHIDAE Cobbold, 1876

Order Polymorphida Petrochenko, 1956

Family PLAGIORHYNCHIDAE Golvan, 1960

Not further identified

ORDER AND ARRANGEMENT OF HOSTS AS PRESENTED IN HOST-PARASITE CHECKLIST

Class Amphibia
Order Anura

Family MYOBATRACHIDAE
Adelotus
Arerophryne

Assa
Crinia
Geocrinia
Heleioporus
Hyperoleia
Kyarranus
Leptodactylid
Limnodynastes
Metacrinia
Mixophyes
Myobatrachid
Neobatrachus
Paracrinia
Philoria
Pseudophryne
Ranidella
Rheobatrachus
Taudactylus
Uperoleia

Family HYLIDAE
Chiroleptes
Cyclorana
Hyla
Litoria

Family RANIDAE
Rana

Family BUFONIDAE
Bufo

Unidentified Anura

PARASITE-HOST CHECKLIST

1. Phylum Platyhelminthes

Class Monogenea Carus, 1863
Order Polyopisthocotylea Odhner, 1912

Family POLYSTOMATIDAE Carus, 1863, emended Gamble, 1896
Subfamily Polystomatinae Gamble, 1896

Parapolystoma bulliense (Johnston, 1912), Ozaki, 1935
syn. *Polystomum bulliense* Johnston, 1912
Hyla phyllochroa, NSW, SJJ 1912: 297, AM W.346, QM GL 12109, GL 12160, AHC 2200 (wholemount), 2217–2219 (sections)
Hyla lesueurii, NSW, SJJ 1912: 297
Litoria citropa, NSW, AHC 5167
Litoria pearsoniana, Qld, SP
Parapolystoma sp.
Litoria nyakalensis, Qld, SP

Class Trematoda Rudolphi, 1808
Order Digenea Van Beneden, 1858
Suborder Prosostomata Odhner, 1905

Family PARAMPHISTOMATIDAE Fischoeder, 1901
Subfamily Diplodiscinae Cohn, 1904

Diplodiscus megalochrus Johnston, 1912
Hyla aurea, NSW, SJJ 1912: 302, AM W.332, QM GL 11851
Frog, NSW, AHC 3310
Hyla caerulea, Qld, THJ 1916*b*: 60
Limnodynastes peronii, NSW, SJJ 1912: 302
Litoria caerulea, Qld, Prudhoe & Bray 1982: 199 mf
Diplodiscus microchrus Johnston, 1912
Hyla ewingii, NSW, SJJ 1912: 307, AM W.333
Limnodynastes tasmaniensis, NSW, SJJ 1912: 307
Diplodiscus **sp.**
Bufo marinus, Qld, AHC 14, 2978, 3028, 3553, 3563, 3576, 3875
Hyla aurea, NSW, AHC 12683
Hyla caerulea, Qld, QM GL 12350
Amphistome
Bufo marinus, Qld, AHC 4944
Distoma **sp.**
Hyla aurea, ?, MRY 1939: 74

Family GORGODERIDAE Looss, 1901

Gorgodera australiensis Johnston, 1912
Hyla aurea, NSW, SJJ 1912: 326, AM W.340a, AM W. 395, AM W.19850, QM GL 11860, GL 12161
Limnodynastes dorsalis, SA, AHC 3511
Limnodynastes peronii, NSW, SJJ 1912: 326, AM W.340 (this number is given for *H. aurea* in SJJ 1912: 326, but in AM records is for *L. peronii*)
Gorgodera **sp.**
Hyla aurea, NSW, AHC 12680; Vic, AHC 4539; SA, AHC 3529, 3532
Limnodynastes dorsalis, SA, AHC 3498, 3502, 12698
Limnodynastes tasmaniensis, SA, AHC 3489

Family ALLOCREADIIDAE Stossich, 1903

Allocreadiidae sp.
Cyclorana cultripes, Qld, QM GL 11285

Family PLAGIORCHIIDAE Lühe, 1901, emended Ward, 1917
Subfamily Haematoleochinae Teixeira de Freitas & Lent, 1939, emended Yamaguti, 1958

Haematoleochus australis (S.J. Johnston, 1912), Inglis, 1932 syn. *Pneumonoeces australis* S.J. Johnston, 1912
Hyla aurea, NSW, SJJ 1912: 321, AM W.339, W.339a, W.396, W.19849; ?, QM GL 11868, GL 1197

Limnodynastes peronii, NSW, SJJ 1912: 321
Litoria aurea, Tas, AHC 5404
Litoria moorei, WA, Prudhoe & Bray 1982: 83
mf, BM(NH) 1967.10.23.7–9

Family TELORCHIIDAE Stunkard, 1924
Subfamily Opisthioglyphinae Dollfus, 1949

Dolichosaccus anartius (S.J. Johnston, 1912) Yamaguti, 1958
syn. *Brachysaccus anartius* S.J. Johnston; 1912
Hyla aurea, NSW, SJJ 1912: 317, AM W.337, W.398, QM GL 11846, AHC 12685, 12686; ?, QM GL 11868, GL 11997
Limnodynastes peronii, NSW, SJJ 1912:317

Dolichosaccus diamesus S.J. Johnston, 1912
Hyla freycineti, NSW, SJJ 1912: 315, AM W.336, W.19848

Dolichosaccus ischyrus S.J. Johnston, 1912
Limnodynastes dorsalis, NSW, SJJ 1912: 314, AM W.335
Hyla caerulea, NSW, SJJ 1912: 314; Qld, THJ 1916: 60

Dolichosaccus juvenilis (Nicoll, 1918), Travassos, 1930
syn. *Brachysaccus juvenilis* Nicoll, 1918
Chiroleptes brevipalmatus, Qld, Nicoll 1918: 368
Cyclorana cultripes, Qld, QM GL 11280

Dolichosaccus symmetrus (S.J. Johnston, 1912), Yamaguti, 1958
syn. *Brachysaccus symmetrus* Johnston, 1912
Hyla caerulea, NSW, SJJ 1912: 319, AM W.338
Bufo marinus, Qld, AHC 13

Dolichosaccus trypherus S.J. Johnston, 1912
Limnodynastes peronii, NSW, SJJ 1912: 310, AM W.334, QM GL 11850
Hyla aurea, NSW, SJJ 1912: 310, QM GL 11850; SA, AHC 12704
Limnodynastes dorsalis, SA, AHC 12699
Limnodynastes tasmaniensis, SA, AHC 3485, 3487, 3488
Litoria moorei, WA, BM(NH) 1968.4.19.16

***Dolichosaccus* sp.**
syn. *Brachysaccus* sp.
Bufo marinus, Qld, AHC 18, 2973, 2975, 3559, 3874, 4952, 4953, 5192
Hyla aurea, NSW, AHC 3527, 12682
Hyla caerulea, Qld, AHC 12690
Hyla sp., ?, MRY 1939: 75
Limnodynastes dorsalis, SA, AHC 3512, 3513, 12677
Limnodynastes fletcheri, SA, AHC 4583
Limnodynastes tasmaniensis, SA, AHC 3485, 3487, 3488

Family BRACHYCOELIIDAE Johnston, 1912

Mesocoelium megaloon S.J. Johnston, 1912
Hyla ewingii, NSW, SJJ 1912: 335, AM W.343
Litoria caerulea, ?, Freitas 1963: 179 (noted that this specimen should be *M. mesembrinum*)
Litoria ewingii, ?, Prudhoe & Bray 1982:117
mf

Mesocoelium mesembrinum S.J. Johnston, 1912
Hyla caerulea, NSW, SJJ 1912: 330, AM W.341, W.341b, W.393, W.394, AHC 4538
Bufo marinus, Qld, Yuen 1965: 271
Litoria aurea, ?, Prudhoe & Bray 1982: 117
Litoria caerulea, Qld, THJ 1916*b*: 60; NSW, QM GL 11861

Mesocoelium microon Nicoll, 1914
Litoria caerulea, Qld, Nicoll 1914: 339, QM GL 11131
Cyclorana cultripes, Qld, QM GL 11278
Litoria gracilenta, Qld, Nicoll 1914: 339, QM GL 11169

Mesocoelium oligoon S.J. Johnston, 1912
Hyla citropus, NSW, SJJ 1912: 336 AM W.342

***Mesocoelium* sp.**
Bufo marinus, Qld, Freeland et al. 1986: 496, AHC 16, 17, 2967, 2973, 2975, 3138, 3876, 4949, 4951, 4955; SA, AHC 4547 (*Mesocoelium* sp. 2 of LMA)
Hyla caerulea, Qld, AHC 3517–3521, 3523, 3524

Family LECITHODENDRIIDAE Odhner, 1910

Pleurogenoides freycineti (S.J. Johnston, 1912), Travassos, 1930
syn. *Pleurogenes freycineti* Johnston, 1912
Hyla freycineti, NSW, SJJ 1912: 342, AM W.344

Pleurogenoides solus (S.J. Johnston, 1912), Travassos, 1930
syn. *Pleurogenes solus* Johnston, 1912
Hyla aurea, NSW, SJJ 1912: 345, AM W.345, W.19851, W.19852

***Pleurogenes* spp.**
Hyla spp., ?, MRY 1939: 75

Lecithodendriid sp.
Bufo marinus, Qld, Freeland et al. 1986: 496

Family BRACHYLAIMIDAE Joyeux & Foley, 1930

Zeylanurotrema spearei Cribb & Barton, 1991
Bufo marinus, Qld, Cribb & Barton 1991: 207, QM GL 1273, 1274–76, AHC 18984, BM(NH) 1990.12.7.3–5

Family DIPLOSTOMIDAE Poirier, 1886

Fibricola intermedius (Pearson, 1959),

Sudarikov, 1961
syn. *Neodiplostomum intermedium* Pearson, 1959
Hyla pearsoni, ?, diplostomula, Pearson 1961: 135
Hyla caerulea, paratenic host, ?, Pearson 1961: 136
Hyla latopalmata tadpole, ?, Pearson 1961: 135
Leptodactylid sp., ?, Pearson 1961: 135
Mixophyes fasciolatus tadpole, ?, Pearson 1961: 135

Family DOLICHOPERIDAE Yamaguti, 1971

Dolichoperoides macalpini (Nicoll, 1918), Johnston & Angel, 1940
syn. *Dolichopera macalpini* Nicoll, 1918
Limnodynastes sp. tadpole, SA, metacercaria, THJ & Angel 1940: 381, AHC 201320
Hyla aurea raniformis, SA, metacercaria, THJ & Angel 1940: 382
Limnodynastes dorsalis (*dumerili*), SA, metacercaria, THJ & Angel 1940: 382
Limnodynastes tasmaniensis (*platycephalus*), SA, metacercaria, THJ & Angel 1940: 382
Tadpole, SA, metacercaria, AHC 2725

Digenea Not Further Identified

Cercaria ameriannae T.H. Johnston & Beckwith, 1947
Limnodynastes sp., SA, diplostomula, (experimental), THJ & Beckwith 1947: 578, AHC 20219
Tadpole, SA, diplostomula, (experimental), AHC 2272
Cercaria angelae T.H. Johnston & Simpson, 1944
Limnodynastes tasmaniensis tadpole, SA, cysts, AHC 2825; experimental infection of *L. tasmaniensis* tadpoles produced *Tetracotyle* cysts (THJ & Simpson 1944: 131)
Tadpole, SA, metacercaria, AHC 2829, cysts, AHC 2831, 2833
Cercaria ellisi T.H. Johnston & Simpson, 1944
Crinia signifera tadpole, SA, metacercaria, (experimental), THJ & Simpson 1944: 89
Tadpole, SA, cyst, AHC 20206
Cercaria lethargica T.H. Johnston & Muirhead, 1949
Tadpole, SA, AHC 2821
Cercaria natans T.H. Johnston & Muirhead, 1949
Limnodynastes tasmaniensis tadpole, SA, (experimental), THJ & Muirhead, 1949: 104 (belongs to *Echinostomum* group); AHC 12402
***Cercaria* sp.**
Tadpole, SA, (K.I. stylet: experimental), AHC 20260 (Echinostome J: experimental), AHC 20261
(Stylet J.W.: experimental), AHC 20262
Diplostomula
Hyla aurea, SA, AHC 12390
Hyla peronii, SA, AHC 12838
Limnodynastes sp., SA, (experimental), AHC 12398
Limnodynastes tasmaniensis, SA, AHC 4125, 4134, 12702
Echinostome cysts
Frog, SA, AHC 12712
Hyla aurea, SA, AHC 12713
Tadpole, SA, AHC 12387; (experimental), AHC 12722
***Halipegus* sp.**
Litoria caerulea, NT, AHC 5405
Plagiorchid cysts
Hyla aurea, SA, AHC 12388
Strigeid cysts
Hyla aurea, SA, AHC 12384, 12386, 12394
Limnodynastes tasmaniensis, SA, AHC 12380
***Tetracotyle* cysts**
Hyla aurea, SA, AHC 12382
Digenea cysts
Bufo marinus, Qld, cysts, Freeland et al. 1986: 494
Frog, NSW, cysts, AHC 12393
Hyla aurea, NSW, cysts, AHC 12372, 12373, 12390, 12392, 12718–12721
Hyla peroni, SA, cysts, AHC 12401
Limnodynastes dorsalis, SA, cysts, AHC 12369, 12385, 12400, 12406, 12407
Limnodynastes tasmaniensis, SA, cysts, AHC 12370, 12371, 12389, 12395; 12397, 12399, 12406, 12407
Tadpole, SA, cysts, AHC 12375–12377, 12403; (experimental), AHC 12379
Digenea
Bufo marinus, Qld, Freeland et al. 1986: 496; Qld, AHC 15, 19, 2004, 2969, 2971, 2977, 3145, 3157, 3309, 3313, 3535–3552, 3555–3558, 3561, 3562, 3564–3575, 3577–3580, 3880, 3947, 4077, 4078, 4099, 4101, 4215, 4351, 4889, 5020, 5021
Hyla aurea, NSW, AHC 12687, 12681, 4546, 4537, 4536, 4535; SA, AHC 3520, 4083, 4341, 4579, 12688
Hyla peroni, SA, AHC 12396
Limnodynastes dorsalis, SA, AHC 3494–3497, 3499–3501, 3504–3510, 4545, 4548–4550, 12676, 12700
Limnodynastes fletcheri, SA, AHC 12678
Limnodynastes sp., SA, AHC 3478–3480, 3482, 3483
Limnodynastes tasmaniensis, SA, AHC 1877, 3484
Litoria caerulea, Qld, AHC 3522, 3525, 3526, 12691; NT, AHC 4544

Litoria dahlii, NT, AHC 6809, 6993
Litoria moorei, WA, AHC 8545
Litoria rothii, Qld, AHC 7181
Rheobatrachus silus, Qld, AHC 6232
Taudactylus diurnus, Qld, AHC 8237

Class Cestoidea Rudolphi, 1808
Order Pseudophyllidea Carus, 1863

Family DIPHYLLOBOTHRIIDAE Lühe, 1910

***?Ligula* sp.**
Hyla aurea, NSW, larval stage, Haswell 1890: 661 (recorded as having possible affinities with *Ligula*)
Hyla caerulea, Qld, AHC 2350–2352
Spirometra erinacei Rudolphi, 1819
Litoria rubella, NT, AHC 17857
Diphyllobothriidae spargana
(*?Diphyllobothrium* (=*Spirometra*) *erinacei* (Rudolphi, 1819))
Bufo marinus, Qld, AHC 4100
Hyla aurea, NSW, WA, THJ 1912: 70
Hyla caerulea, Qld, NSW, THJ 1912: 70
Hyla latopalmata, ?, (experimental), Sandars 1953: 67
Hyla latopalmata tadpole, ?, (experimental), Sandars 1953: 67
? *Spirometra mansoni* (Cobbold, 1882), Stiles & Taylor, 1902 *Bufo marinus*, spargana, Bennett 1978: 756

Order Proteocephalidea Mola, 1928

Family PROTEOCEPHALIDAE La Rue, 1911

***Ophiotaenia* sp.**
Hyla aurea, ?, SJJ 1914: 44; SA, AHC 2825
Proteocephalus hylae (S.J. Johnston, 1912), Prudhoe & Bray, 1982
syn. *Ophiotaenia hylae* S.J. Johnston, 1912
Hyla aurea, NSW, THJ 1912: 63
Litoria aurea, NSW, QM G 423
Litoria moorei, WA, BM(NH) 1968.4.19.1–5; AHC 8178
Proteocephalid plerocercoids
Bufo marinus, Qld, Freeland et al. 1986: 496
Crinia laevis, Tas, Hickman 1960: 20
Crinia signifera, Tas, Hickman 1960: 20
Hyla aurea, Vic, AHC 2327; SA, AHC 8696
Limnodynastes peronii, Tas, Hickman 1960: 20

Order Cyclophyllidea Braun, 1900

Family NEMATOTAENIIDAE Lühe, 1910

Cylindrotaenia criniae (Hickman, 1960), Jones, 1987
syn. *Baerietta criniae criniae* Hickman, 1960
Crinia tasmaniensis, Tas, Hickman 1960: 18, TM K710–712
Ranidella tasmaniensis, Tas, Jones 1987: 207
Cylindrotaenia minor (Hickman, 1960), Jones, 1987
syn. *Barietta criniae minor* Hickman, 1960
Crinia tasmaniensis, Tas, Hickman 1960: 18
Crinia laevis, Tas, Hickman 1969: 18
Crinia signifera, Tas, Hickman 1960: 18; TM K716–717
Ranidella tasmaniensis, Tas, Jones 1987: 211
Assa darlingtoni, NSW, Jones 1987: 212, QM GL 4887; Qld, Jones & Delvinquier 1991: 492
Geocrinia laevis, Tas, Jones 1987: 211
Philoria loveridgei, Qld, Jones & Delvinquier 1991: 492
Ranidella signifera, Tas, Jones 1987: 211
Nematotaenia hylae Hickman, 1960
Hyla ewingii, Tas, Hickman 1960: 8, TM K705, K707–709
Litoria ewingii, Tas, Jones 1987: 184, 185
Bufo marinus, Qld, Jones & Delvinquier 1991: 492
Crinia signifera, Tas, Hickman 1960: 8, TM K706
Cyclorana novaehollandiae, Qld, Jones & Delvinquier 1991: 492
Limnodynastes ornatus, Qld, Jones & Delvinquier 1991: 492
Litoria fallax, Qld, Jones 1987: 185
Litoria inermis, Qld, Jones 1987: 185
Litoria latopalmata, Qld, Jones 1987: 185, QM GL 4886
Litoria pallida, Qld, Jones & Delvinquier 1991: 492
Litoria peronii, Qld, Jones & Delvinquier 1991: 492
Ranidella parinsignifera, Qld, Jones 1987: 185, QM GL 4887
Ranidella signifera, Tas, Jones 1987: 184, 185
Ranidella riparia, SA, Jones & Delvinquier 1991: 492
Uperoleia rugosa, Qld, Jones & Delvinquier 1991: 492
***Nematotaenia* sp.**
Hyla caerulea, ?, MRY 1939: 74; NSW, THJ 1916*a*: 195, Prudhoe & Bray 1982:12 mf
Hyla freycineti, ?, MRY 1939: 75; NSW, THJ 1916*a*: 194, Prudhoe & Bray 1982:12 mf
Hyperoleia marmorata, ?, MRY 1939: 75; NSW, THJ 1916*a*: 194, Prudhoe & Bray 1982: 12 mf
Triplotaenia mirabilis Boas, 1902
Hyla aurea, ?, MRY 1939: 74 (usually a cestode of marsupials; see Prudhoe & Bray 1982:3 mf for discussion)

Cestoda Not Further Identified

Bufo marinus, Qld, AHC 10, 46, 4892
Crinia signifera, SA, AHC 4419, 4424, 20687
Crinia sp., SA, AHC 4234
Hyla aurea, NSW, SJJ 1912: 291; Vic, AHC 2326; SA, larva, AHC 4584
Hyla caerulea, NSW, SJJ 1912: 290; Qld, AHC 1223
Hyla ewingi, NSW, AHC 4082; SA, AHC 4304, 4369
Hyla ewingi alpina, NSW, AHC 4079–4081
Hyla freycineti, NSW, SJJ 1912: 291
***Hyla* sp.**, SA, AHC 40
Hyperoleia marmorata, NSW, SJJ 1912: 290
***Limnodynastes* sp.**, Qld, AHC 2376; SA, AHC 2378
Metacrinia nichollsi, WA, AHC 48
Rheobatrachus silus, Qld, AHC 8913
Frog, SA, AHC 20678

2. Phylum Nematoda

Class Secernentea
Order Rhabditida

Superfamily Rhabditoidea
Family RHABDIASIDAE Railliet, 1916

Rhabdias australiensis Moravec & Sey, 1990
Rana daemeli, Qld, Moravec & Sey 1990: 283, CAS N–450
Rhabdias hylae Johnston & Simpson, 1942
Hyla aurea, NSW, THJ & Simpson 1942: 176, SJJ 1912: 291 (lung nematode); VIC, THJ & Simpson 1942: 176; SA, Ballantyne 1971: 51
Adelotus brevis, Qld, Ballantyne 1971: 51
Crinia georgiana, WA, Ballantyne 1971: 51
Crinia glauerti, WA, Ballantyne 1971: 51
Crinia insignifera, WA, Ballantyne 1971: 51
Crinia leai, WA, Ballantyne 1971: 51
Crinia signifera, NSW, SA, Ballantyne 1971: 50
Crinia subinsignifera, WA, Ballantyne 1971: 51
Crinia victoriana, Vic, Ballantyne 1971: 50
Hyla aurea raniformis, Vic, Ballantyne 1971: 50
Hyla caerulea, QLD, THJ & Simpson 1942: 176
Hyla latopalmata, Qld, Ballantyne 1971: 51
Hyla lesueuri, Qld, Ballantyne 1971: 51
Hyla peroni, NSW, SJJ 1912: 290 (lung nematode); THJ & Simpson 1942: 178
Limnodynastes dorsalis, NSW, THJ & Simpson 1942: 179
Limnodynastes fletcheri, Qld, Ballantyne 1971: 51
Limnodynastes peroni, NSW, SJJ 1912: 290 (lung nematode); THJ & Simpson 1942: 179; Qld, Ballantyne 1971: 51; SA, Ballantyne 1971: 51
Limnodynastes tasmaniensis, NSW, SJJ 1912: 290 (lung nematode), THJ & Simpson 1942: 176; SA, THJ & Simpson 1942: 176, Ballantyne 1971: 50; Vic, Ballantyne 1971: 50
Mixophyes fasciolatus, Qld, Ballantyne 1971: 51
Pseudophryne bibronii, NSW, Ballantyne 1971: 50
Pseudophryne guentheri, WA, Ballantyne 1971: 51
Pseudophryne occidentalis, WA, Ballantyne 1971: 51
Pseudophryne sp., SA, Ballantyne 1971: 51
Rhabdias nigrovenosum (Goeze, 1800)
syn. *Rhabdonema nigrovenosum* Goeze, 1800; listed as a synonym of *Rhabdias bufonis* (Schrank, 1788) in Yamaguti 1961: 84
Hyla aurea, ?, AM W.19853–6
***Rhabdias* sp.**
Hyla aurea, NSW, VIC, THJ & Simpson 1942: 178 (referring to THJ 1938: 151); WA, BM(NH) 1989.1987–1988
Hyla moorei, WA, BM(NH) 1980.263–282
***Rhabdonema* sp.**
Hyla aurea, NSW, Vic, THJ & Simpson 1942: 178 (referring to Haswell 1891)
Hyla caerulea, QLD, THJ 1916*b*: 60

Order Strongylida

Superfamily Trichostrongyloidea
Family MOLINEIDAE (Skrjabin & Schulz, 1937) Durette-Desset & Chabaud, 1977

Oswaldocruzia* (*O.*) *limnodynastes T.H. Johnston & Simpson, 1942
Limnodynastes dorsalis, SA, THJ & Simpson 1942: 172; THJ & PMM 1949: 65
Hyla aurea, NSW, Vic, THJ & Simpson 1942: 172
Hyla peroni, SA, THJ & PMM 1949: 65

Order Oxyurida

Superfamily Oxyuroidea
Family PHARYNGODONIDAE Travassos, 1919

Parathelandros australiensis (Johnston & Simpson, 1942), Inglis, 1968
syn. *Cosmocerca australiensis* Johnston & Simpson, 1942
Limnodynastes dorsalis, SA, THJ & Simpson 1942: 176
Limnodynastes fletcheri, SA, Inglis 1968: 173
Parathelandros carinae Inglis, 1968

Heleioporus albopunctatus, WA, Inglis 1968: 176.
Heleioporus australiacus, WA, Inglis 1968: 176
Heleioporus eyrei, WA, Inglis 1968: 176
Heleioporus psammophilus, WA, Inglis 1968: 176
Neobatrachus pelobatoides, WA, Inglis 1968: 176

Parathelandros johnstoni Inglis, 1968
Heleioporus eyrei, WA, Inglis 1968: 175
Limnodynastes dorsalis, WA, Inglis 1968: 175
Neobatrachus centralis, WA, Inglis 1968: 175 (specimens in poor condition, may be *P. maini* or *P. limnodynastes*)
Neobatrachus pelobatoides, WA, Inglis 1968: 175

Parathelandros limnodynastes (Johnston & Mawson, 1942), Inglis, 1968
syn. *Pharyngodon limnodynastes* Johnston & Mawson, 1942
Limnodynastes dorsalis, SA, THJ & PMM 1942: 94; Inglis 1968: 175
Limnodynastes dorsalis dumerili, SA, THJ & PMM 1942: 94

Parathelandros maini Inglis, 1968
Hyla moorei, WA, Inglis 1968: 176
Hyla adelaidensis, WA, Inglis 1968: 176
Hyla cyclorhyncha, WA, Inglis 1968: 176

Parathelandros mastigurus Baylis, 1930
Hyla caerulea, Qld, Baylis 1930: 359, Inglis 1968: 173; NSW, Inglis 1968: 173
Bufo marinus, Qld, Inglis 1968: 173
Hyla gracilenta, Qld, Baylis 1930: 359
Hyla gracilis, Qld, Inglis 1968: 173 (refers to *Hyla gracilenta* recorded by Baylis 1930)

Parathelandros propinqua (Johnston & Simpson, 1942), Inglis, 1968
syn. *Cosmocerca propinqua* Johnston & Simpson, 1942
Limnodynastes dorsalis, SA, THJ & Simpson 1942: 176

***Parathelandros* spp.**
Bufo marinus, Qld, Freeland et al. 1986: 496
Hyla aurea, WA, (female only), BM(NH) 1980.283–292
Hyla rubella, WA, (female only), BM(NH) 1980.318–317
Oxyurids Not Further Identified
Bufo marinus, Qld, AHC 2276, 4950; Vic, AHC 9048, 9059
Cyclorana sp., NT, AHC 4450
Hyla aurea, Vic, AHC 2311
Hyla caerulea, Qld, AHC 2343; NT, AHC 4947
Limnodynastes dorsalis, SA, AHC 2306, 3176
Limnodynastes tasmaniensis, SA, AHC 1417, 5030
Litoria rothii, Qld, AHC 7156
Litoria rubella, Qld, AHC 7180
Mixophyes sp., Qld, AHC 6172

Order Ascaridida

Superfamily Cosmocercoidea
Family COSMOCERCIDAE (Railliet, 1916 subfam.) Travassos, 1925

Cosmocerca limnodynastes Johnston & Simpson, 1942
Limnodynastes dorsalis, SA, THJ & Simpson 1942: 174

Cosmocercinae gen. sp. 1
Rana daemeli, Qld, Moravec & Sey 1990: 273

Austraplectana kartanum (Johnston & Mawson, 1941), Baker, 1981
syn. *Rallietnema kartanum* Johnston & Mawson, 1941
Hyla jervisiensis, SA, THJ & PMM 1941: 146
Heleioporus eyrei, WA, Inglis 1968: 166
Hyla moorei, WA, Inglis 1968: 166, BM(NH) 1967. 1158–1159
Litoria nasuta, Qld, Baker 1981: 111
Austraplectana sp.
Frog, Qld, Baker 1981: 116

Maxvachonia adamsoni Moravec & Sey, 1990
Litoria infrafrenata, New Guinea, Moravec & Sey 1990: 276, CAS N–449

Maxvachonia ewersi Mawson, 1972
Litoria nasuta, New Guinea, PMM 1972: 105

Maxvachonia flindersi (Johnston & Mawson, 1941), Mawson, 1972
syn. *Aplectana flindersi* Johnston & Mawson, 1941; *Austracerca flindersi* (Johnston & Mawson, 1941) Inglis 1968
Hyla jervisiensis, SA, THJ & PMM 1941: 148
Bufo marinus, Qld, PMM 1972: 104, AHC 5170
Heleioporus australiacus, WA, Inglis 1968: 165
Heleioporus barycragus, WA, PMM 1972: 104
Heleioporus inornatus, WA, PMM 1972: 104, AHC 5180
Heleioporus psammophilis, WA, Inglis 1968: 165
Hyla cyclorhyncha, WA, Inglis 1968: 165
Limnodynastes dorsalis, SA, PMM 1972: 104, AHC 5183
Litoria adelaidensis, WA, PMM 1972: 104, AHC 5172
Litoria caerulea, NT, PMM 1972: 104, AHC 5182
Litoria moorei, WA, PMM 1972: 104, AHC 5175

Falcaustra hylae (Johnston & Simpson, 1942), Chabaud & Golvan, 1957

syn. *Spironoura hylae* Johnston & Simpson, 1942
Hyla aurea, NSW, THJ & Simpson 1942: 173
Cosmocercoid
Bufo marinus, Qld, AHC 5009

Superfamily Ascaridoidea
Family ASCARIDIDAE Baird, 1853

Ophidascaris pyrrhus Johnston & Mawson, 1942
Tadpole, Qld, (experimental infection), QM GL 9107
Frog, Qld, QM GZ 15
Raillietascaris varani (Baylis & Daubney, 1922), Sprent, 1985
Tadpole, ?, QM GL 5674
Seuratascaris numidica (Seurat, 1917), Sprent, 1985
Rana daemeli, Qld, Sprent 1985: 241

Order Spirurida

Superfamily Physalopteroidea
Family PHYSALOPTERIDAE (Railliet, 1893 subfam.) Leiper, 1908

Pseudorictularia disparilis (Irwin-Smith, 1922), Dollfus & Desportes, 1945
syn. *Rictularia disparilis* Irwin-Smith, 1922
Litoria inermis, Qld, Owen & Moorhouse 1980: 1014
Litoria nigrofrenata, Qld, Owen & Moorhouse 1980: 1014
Rana daemeli, Qld, Owen & Moorhouse 1980: 1013
Physaloptera confusa T.H. Johnston & Mawson, 1942
Limnodynastes tasmaniensis, NSW, encysted larva, THJ & Simpson 1942: 178; SA, encysted larva, THJ & PMM 1949:69
Hyla aurea, NSW, encysted larva, THJ & PMM 1942: 91; THJ & Simpson 1942: 178
Hyla caerulea, Qld, encysted larva, THJ & Simpson 1942: 178
Hyla peroni, SA, encysted larva, THJ & PMM 1942: 91; THJ & PMM 1949: 69; THJ & Simpson 1942: 178
Limnodynastes dorsalis, SA, encysted larva, THJ & PMM 1942: 91; NSW, encysted larva, THJ & Simpson 1942: 178
Limnodynastes dorsalis dumerilii, SA, encysted larva, THJ & PMM 1942: 91; THJ & Simpson 1942: 178
***Physaloptera* sp.**
Cyclorana australis, WA, larva AHC 6399
Heleioporus eyrei, WA, AHC 3012
Hyla aurea, SA, AHC 12386
Limnodynastes dorsalis dumerilii, SA, cysts, AHC 2356 (frog taken from intestine of tiger snake, *Notechis scutatus*), 2375

Superfamily Habronematoidea
Family HEDRURIDAE Railliet, 1916

Hedruris hylae Johnston & Mawson, 1941
Hyla jervisiensis, SA, THJ & PMM 1941: 148
***Hedruris* sp.**
Crinia signifera, SA, AHC 28

Superfamily Filarioidea
Filarioidea ?gen. ?sp.

Filaria cochleata Railliet, 1916
syn. *Filaria spiralis* Oerley, 1882
Heleioporus albopunctatus, ?, Oerley 1882: 312

Nematoda Not Further Identified

***Agamonema* sp.**
Hyla caerulea, Qld, encysted larva, THJ 1914: 82
Dorylaimid
Frog, SA, AHC 6417
Nematode larvae
Bufo marinus, Qld, cysts, Freeland et al. 1986: 496
Hyla moorei, WA, BM(NH) 1980.298–307
Arenophryne rotunda, WA, cysts, AHC 6808
Hyla caerulea, Qld, cysts, AHC 2341
Nematodes
Bufo marinus, Qld, Freeland et al. 1986: 496, AHC 8,9, 2974, 3258
Crinia georgiana, WA, AHC 8081, 8079
Crinia glauerti, WA, AHC 8119, 8113
Crinia haswelli, Vic, AHC 8084
Crinia leai, WA, AHC 8115, 8082, 8078
Crinia pseudinsignifera, WA, AHC 8118, 8114
Crinia riparia, SA, AHC 8077
Crinia rosea, WA, AHC 8076
Crinia signifera, NSW, SJJ 1912:290; SA, AHC 20, 22–24, 3617, 6799, 8102, 8105; Vic, AHC 1083, 1098; NSW, AHC 8066
Crinia sp., Vic, AHC 21; SA, AHC 4210, 4211, 4214, 4217, 4219, 4231–4233
Crinia subinsignifera, WA, AHC 8080, 8075
Crinia victoriana, Vic, AHC 8122, 8069, 8070, 8088, 8096, 8099
Cyclorana australis, WA, AHC 12880
Heleioporus eryei, WA, AHC 8120
Hyla adelaidensis, NSW, AHC 1760
Hyla aurea, NSW, SJJ 1912: 291, AHC 3528, 2306, 2308, 2309, 2314–2316, 2318–2321, 2323, 2324; SA, AHC 3520
Hyla aurea raniformis, Vic, AHC 8094
Hyla caerulea, NSW, SJJ 1912: 290, AHC 2339, 2337, 2336, 2333, 2360; NT, AHC 2331; Qld, AHC 2349, 2346, 2344, 2342, 2340, 2338, 2335, 2235
Hyla dentata, NSW, SJJ 1912: 291

Hyla ewingii, NSW, SJJ 1912: 291; SA, AHC 8236
Hyla jervisiensis, SA, AHC 1759, 3615
Hyla lesueurii, NSW, SJJ 1912: 291; Qld, AHC 8238
Hyla peronii, NSW, SJJ 1912: 290; SA, AHC 12396
Hyla phyllochroa, NSW, SJJ 1912: 290
Kyarranus sphagnicolus, NSW, AHC 8247
Limnodynastes dorsalis, NSW, SJJ 1912: 290, AHC 2365, 3362, 2361, 2360; Vic, AHC 8068; Qld, AHC 2367; SA, AHC 2368, 3010, 3176, 8108, 8235
Limnodynastes fletcheri, NSW, AHC 8059
Limnodynastes peronii, NSW, SJJ 1912: 290, AHC 1728, 3477; SA, AHC 8103
Limnodynastes sp., Qld, AHC 2605
Limnodynastes tasmaniensis, NSW, SJJ 1912: 290, AHC 8064; Vic, AHC 36, 8087, 8100; SA, AHC 25, 26, 39, 1877, 1882, 3320, 3619, 3622, 5031, 8101, 8107, 8110, 12389
Litoria aurea, SA, AHC 8073
Litoria booroolongensis, NSW, AHC 8063
Litoria caerulea, Qld, AHC 8061, 8060
Litoria dahlii, NT, AHC 6809, 6993
Litoria ewingii, Vic, AHC 8071, 8072, 8095, 8097
Litoria nigrofrenata, Qld, AHC 6145
Litoria rothii, Qld, AHC 7181
Litoria verreauxii, NSW, AHC 8085
Mixophyes fasciolatus, Qld, AHC 8093, 8056
Neobatrachus pelobatoides, WA, AHC 8121, 8116
Neobatrachus pictus, SA, AHC 8104
Pseudophryne bibronii, Vic, AHC 8090; NSW, AHC 8062; SA, AHC 4213, 4218, 4220–4227, 8089, 8106, 8111
Pseudophryne guentheri, WA, AHC 8117, 8074
Pseudophryne occidentalis, WA, AHC 8112
Pseudophryne semimarmorata, SA, AHC 8109
Uperoleia marmorata, NSW, AHC 8055

3. Phylum Acanthocephala

Class Palaeacanthocephala Meyer, 1931
Order Echinorhynchida Southwell & MacFie, 1925

Family ECHINORHYNCHIDAE Cobbold, 1876

Acanthocephalus criniae Snow, 1971
Crinia tasmaniensis, Tas, Snow 1971: 147, TM K228–230, AHC 18165
Crinia laevis, Tas, Snow 1971: 147
Crinia signifera, Tas, Snow 1971: 147
Pseudoacanthocephalus perthensis Edmonds, 1971
Litoria moorei, WA, Edmonds 1971: 55; AHC 5048, 5051
Limnodynastes dorsalis, WA, Edmonds 1971: 55

Order Polymorphida

Family PLAGIORHYNCHIDAE Golvan, 1960

Porrorchis hylae (Johnston, 1914), Schmidt & Kuntz, 1967
syn. *Echinorhynchus* sp. Johnston, 1912; *Echinorhynchus hylae* Johnston, 1914; *Echinorhynchus bulbocaudatus* Southwell & MacFie, 1925; *Gordiorhynchus hylae* (Johnston, 1914), Johnston & Edmonds, 1948; *Pseudoporrorchis hylae* (Johnston, 1914), Edmonds, 1957
Limnodynastes dorsalis, SA, encysted larva, THJ & Edmonds 1948: 69
Bufo marinus, Qld, encysted larva, Freeland et al. 1986: 496 (identified by Edmonds 1989: 130)
Hyla aurea, NSW, encysted larva, THJ 1912: 84, THJ 1914: 83; SA, NSW, THJ & Edmonds 1948: 69
Hyla caerulea, Qld, encysted larva, THJ 1914: 83, THJ & Edmonds 1948:69

Acanthocephala Not Further Identified

***Acanthocephala* sp.**
Hyla caerulea, NSW, QM GL 12287
Hyla peronii, Qld, QM GL 12346
Acanthocephala
Limnodynastes sp., SA, AHC 3409; larva, AHC 3481

Host - parasite checklist

Order Anura

Family MYOBATRACHIDAE

Adelotus brevis (Günther, 1863)
N *Rhabdias hylae*, (lung)
Arerophryne rotunda Tyler, 1976
N Nematode larva, cysts
Assa darlingtoni (Loveridge, 1933)
C *Cylindrotaenia minor*, (intestine)
Crinia georgiana Tschudi, 1838
N *Rhabdias hylae*, (lung)
N Nematodes, (duodenum, rectum)
Crinia glauerti Loveridge, 1933
N *Rhabdias hylae*, (lung)
N Nematodes, (buccal cavity, rectum, ileum)
Crinia haswelli Fletcher, 1894
see *Paracrinia haswelli*

Crinia insignifera Moore, 1954
N *Rhabdias hylae*, (lung)

Crinia laevis Günther, 1864
see *Geocrinia laevis*

Crinia leai Fletcher, 1898
see *Geocrinia leai*

Crinia parinsignifera Main, 1957
C *Nematotaenia hylae*, (intestine)

Crinia pseudinsignifera Main, 1957
N Nematodes, (ileum)

Crinia riparia Littlejohn & Martin, 1965
C *Nematotaenia hylae*, (intestine)
N Nematodes, (rectum)

Crinia rosea Harrison, 1927
see *Geocrinia rosea*

Crinia signifera (Girard, 1853)
C proteocephalid plerocercoids, (mesentery & under skin)
C *Cylindrotaenia minor*, (duodenum, ileum)
C *Nematotaenia hylae*, (duodenum)
C Cestodes, (small intestine)
N *Rhabdias hylae*, (lung)
N *Hedruris* sp., (stomach)
N Nematodes, (stomach, intestine, buccal cavity, rectum, lung, abdominal cavity)
A *Acanthocephalus criniae*, (duodenum, ileum)

Crinia signifera (Girard, 1853) tadpole
D *Cercaria ellisi*, metacercaria, (kidney, mesenteries, heart lung), (experimental)

Crinia subinsignifera Littlejohn, 1957
N *Rhabdias hylae*, (lung)
N Nematodes, (rectum)

Crinia tasmaniensis (Günther, 1864)
C *Cylindrotaenia criniae*, (duodenum, ileum)
C *Cylindrotaenia minor*, (duodenum, ileum)
A *Acanthocephalus criniae*, (duodenum, ileum)

Crinia victoriana Boulenger, 1888
see *Geocrinia victoriana*

***Crinia* sp.**
C Cestodes, (intestine)
N Nematodes, (intestine, stomach, rectum)

Geocrinia laevis (Günther, 1864)
C proteocephalid plerocercoids, (mesentery)
C *Cylindrotaenia minor*, (duodenum, ileum)
A *Acanthocephalus criniae*, (duodenum, ileum)

Geocrinia leai (Fletcher, 1898)
N *Rhabdias hylae*, (lung)
N Nematodes, (abdominal cavity, duodenum)

Geocrinia rosea (Harrison, 1927)
N Nematodes, (rectum)

Geocrinia victoriana (Boulenger, 1888)
N *Rhabdias hylae*, (lung)
N Nematodes, (duodenum, rectum)

Heleioporus albopunctatus Gray, 1841
N *Parathelandros carinae*, (rectum)
N *Filaria cochleata*, (encapsulated between serous and muscular layers of stomach)

Heleioporus australiacus (Shaw & Nodder, 1795)
N *Parathelandros carinae*, (rectum)
N *Maxvachonia flindersi*, (rectum)

Heleioporus barycragus Lee, 1967
N *Maxvachonia flindersi*

Heleioporus eyrei (Gray, 1845)
N *Parathelandros carinae*, (rectum)
N *Parathelandros johnstoni*, (rectum)
N *Austraplectana kartanum*, (rectum)
N *Physaloptera* sp., (stomach)
Nematodes, (stomach)

Heleioporus inornatus (Lee & Main, 1954)
N *Maxvachonia flindersi*, (rectum)

Heleioporus psammophilus (Lee & Main, 1954)
N *Parathelandros carinae*, (rectum)
N *Maxvachonia flindersi*, (rectum)

Hyperolia marmorata (Gray, 1841)
see *Uperoleia* spp.

Kyarranus loveridgei (Parker, 1940)
C *Cylindrotaenia minor*, (intestine)

Kyarranus sphagnicolus Moore, 1958
N Nematodes, (rectum)

Leptodactylid sp.
see Myobatrachid sp.

Limnodynastes dorsalis (Gray, 1841)
for *Limnodynastes dorsalis* from any state, except WA, see *Limnodynastes dumerilii*
N *Parathelandros johnstoni*, (rectum)
A *Pseudoacanthocephalus perthensis*, (intestine)

Limnodynastes dorsalis dumerilii
see *Limnodynastes dumerilii*

Limnodynastes dumerilii Peters, 1863
D *Gorgodera australiensis*
D *Gorgodera* sp.
D *Dolichosaccus ischyrus*, (intestine)
D *Dolichosaccus trypherus*
D *Dolichosaccus* sp.
D *Dolichoperoides macalpini*, metacercaria, (tissues)
D Digenea cysts
D Digenea, (intestine, stomach)
N *Rhabdias hylae*, (lung)
N *Oswaldocruzia limnodynastes*, (intestine)
N *Parathelandros australiensis*, (rectum, intestine)
N *Parathelandros limnodynastes*
N *Parathelandros propinqua*, (rectum, intestine)
N Oxyurid
N *Cosmocerca limnodynastes*
N *Maxvachonia flindersi*, (rectum)
N *Physaloptera confusa*, encysted larva, (mesentery, stomach, peritoneum)
N *Physloptera* sp., cysts

N Nematodes, (stomach, intestine, rectum)
A *Porrorchis hylae*, encysted larva, (mesenteries)

Limnodynastes fletcheri Boulenger, 1888
D *Dolichosaccus* sp.
D Digenea
N *Rhabdias hylae*, (lung)
N *Parathelandros australiensis*, (rectum)
N Nematodes, (duodenum, rectum)

Limnodynastes ornatus (Gray, 1842)
C *Nematotaenia hylae*, (intestine)

Limnodynastes peronii (Duméril & Bibron, 1841)
D *Diplodiscus megalochrus*, (rectum)
D *Gorgodera australiensis*, (bladder)
D *Dolichosaccus anartius*, (intestine, rectum)
D *Dolichosaccus trypherus*, (duodenum)
D *Haematoleochus australis*, (lungs)
C proteocephalid plerocercoids, (mesentery)
N *Rhabdias hylae*, (lungs)
N Nematodes, (lungs, intestine, rectum, stomach)

Limnodynastes tasmaniensis Günther, 1858
D *Diplodiscus microchrus*, (rectum)
D *Gorgodera* sp.
D *Dolichosaccus trypherus*, (intestine)
D *Dolichosaccus* sp.
D *Dolichoperoides macalpini*, metacercaria (tissues)
D Diplostomula, (buccal cavity)
D Strigeid, cysts
D Digenea cysts, (muscles, subcutaneous)
D Digenea, (gut)
N *Rhabdias hylae*, (lung)
N Oxyurids, (abdominal cavity)
N *Physaloptera confusa*, encysted larva, (stomach, peritoneum)
N Nematodes, (lungs, stomach, intestine, rectum)

Limnodynastes tasmaniensis Günther, 1858 tadpole
D *Cercaria angelae*, cysts, (wall of thorax and rectum, pericardium, tail tissue, base of forleg), (experimental)
D *Cercaria natans*, (kidney tissue, kidney peritoneum), (experimental)

Limnodynastes tasmaniensis (platycephalus) Günther, 1867
see *Limnodynastes tasmaniensis*

***Limnodynastes* sp.**
D Diplostomula, (eye), (experimental)
D Digenea, (stomach, intestine, rectum)
C Cestodes, (coelom)
N Nematodes, (stomach)
A Acanthocephala, (mesentery)
A Acanthocephala, larva, (rectum)

***Limnodynastes* sp. tadpole**
D *Cercaria amerianna*, diplostomula, (tissues), (experimental)
D *Dolichoperoides macalpini*, metacercaria, (tissues)

Metacrinia nichollsi (Harrison, 1927)
C Cestodes

Mixophyes fasciolatus Günther, 1864
N *Rhabdias hylae*, (lung)
N Nematodes, (rectum)

Mixophyes fasciolatus Günther, 1864 tadpole
D *Fibricola intermedius*, metacercaria, (muscles)

***Mixophyes* sp.**
N Oxyurid

Myobatrachid sp.
D *Fibricola intermedius*, metacercaria, (muscle)

Neobatrachus centralis (Parker, 1940)
N *Parathelandros johnstoni*, (rectum)

Neobatrachus pelobatoides (Werner, 1914)
N *Parathelandros carinae*, (rectum)
N *Parathelandros johnstoni*, (rectum)
N Nematodes, (rectum)

Neobatrachus pictus Peters, 1863
N Nematodes, (rectum)

Paracrinia haswelli (Fletcher, 1894)
N Nematodes, (duodenum, rectum)

Philoria loveridgei Parker, 1940
see *Kyarranus loveridgei*

Pseudophryne bibronii Günther, 1858
N *Rhabdias hylae*, (lung)
N Nematodes, (duodenum, rectum, stomach)

Pseudophryne guentheri Boulenger, 1964
N *Rhabdias hylae*, (lung)
N Nematodes, (rectum)

Pseudophryne occidentalis Parker, 1940
N *Rhabdias hylae*, (lung)
N Nematodes, (rectum, stomach)

Pseudophryne semimarmorata Lucas, 1892
N Nematodes, (rectum)

***Pseudophryne* sp.**
N *Rhabdias hylae*, (lung)

***Ranidella* spp.**
for all *Ranidella* species, see the *Crinia* equivalent

Rheobatrachus silus Liem, 1973
D Digenea, (rectum)
C Cestodes

Taudactylus diurnus Straughan & Lee, 1966
D Digenea, (rectum)

Uperoleia marmorata Gray, 1841
for *Uperoleia marmorata* from all states, except WA, see *Uperoleia* spp.

Uperoleia rugosa (Andersson, 1916)
C *Nematotaenia hylae*, (intestine)

***Uperoleia* spp.**
C *Nematotaenia* sp.
C Cestodes, (small intestine)
N Nematodes, (rectum)

Family HYLIDAE

Chiroleptes brevipalmatus Peters, 1871
see *Cyclorana brevipes*

Cyclorana australis (Gray, 1842)
N *Physaloptera* sp., larva, (buccal cavity)
N Nematodes

Cyclorana brevipes (Peters, 1871)
D *Dolichosaccus juvenilis*, (intestine)

Cyclorana cultripes Parker, 1940
D Allocreadiidae sp.
D *Dolichosaccus juvenilis*
D *Mesocoelium microon*

Cyclorana novaehollandiae Steindachner, 1867
N *Nematotaenia hylae*, (intestine)

***Cyclorana* sp.**
N Oxyurids, (rectum)

***Hyla* spp.**
for all *Hyla* species, see the *Litoria* equivalent, with the following exceptions:

i) *Hyla aurea* Lesson, 1829
for *Hyla aurea* from NSW (coastal area), see *Litoria aurea*
for *Hyla aurea* from SA, Tas, Vic, NSW (exclusive of coastal area), see *Litoria raniformis*
for *Hyla aurea* from WA, see *Litoria* spp.

ii) *Hyla ewingi alpina* Fry, 1915
see *Litoria verreauxii*

iii) *Hyla jervisiensis* Duméril & Bibron, 1841
for *Hyla jervisiensis* from all states, except SA, see *Litoria jervisiensis*
for *Hyla jervisiensis* from SA see *Litoria ewingii*

Litoria adelaidensis (Gray, 1841)
for *Litoria adelaidensis* from all states, except WA, see *Litoria* spp.
N *Parathelandros maini*, (rectum)
N *Maxvachonia flindersi*, (intestine)

Litoria aurea (Lesson, 1829)
for *Litoria aurea* from Vic, Tas, SA, NSW (exclusive of coastal area), see *Litoria raniformis*
for *Litoria aurea* from WA, see *Litoria* spp.
D *Diplodiscus megalochrus*, (rectum)
D *Diplodiscus* sp., (rectum)
D *Distoma* sp.
D *Gorgodera australiensis*, (bladder)
D *Gorgodera* sp., (bladder)
D *Haematoleochus australis*, (lungs)
D *Dolichosaccus anartius*, (intestine, rectum)
D *Dolichosaccus trypherus*, (duodenum)
D *Dolichosaccus* sp.
D *Mesocoelium mesembrinum*
D *Pleurogenoides solus*, (intestine)
D Digenea cysts, (nerves, muscles, subcutaneous)
D Digenea, (lung, intestine, rectum)
C ?*Ligula* sp., (muscles, peritoneal cavity, subdermal lymph sinuses)
C Diphyllobothriidae spargana, (thigh muscles)
C *Ophiotaenia* sp., (intestine)
C *Proteocephalus hylae*
C *Triplotaenia mirabilis*
C Cestodes, (intestine, muscle)
N *Rhabdias hylae*, (lung)
N *Rhabdias nigrovenosum*, (lung)
N *Rhabdias* sp., (lung)
N *Rhabdonema* sp.
N *Oswaldocruzia limnodynastes*, (intestine)
N *Falcaustra hylae*, (intestine)
N *Physaloptera confusa*, encysted larva, (mesentery)
N Nematodes, (lung, intestine, rectum, peritoneu abdominal cavity, stomach)
A *Porrorchis hylae*, encysted larva, (mesenteries

Litoria booroolongensis (Moore, 1961)
N Nematodes, (rectum, mesentery)

Litoria caerulea (White, 1790)
D *Diplodiscus megalochrus*
D *Diplodiscus* sp.
D *Dolichosaccus ischyrus*, (intestine)
D *Dolichosaccus symmetrus*, (rectum)
D *Dolichosaccus* sp.
D *Mesocoelium megaloon*, (intestine)
D *Mesocoelium mesembrinum*, (intestine, duodenum)
D *Mesocoelium microon*
D *Mesocoelium* sp.
D *Fibricola intermedius*, metacercaria, (muscles) paratenic host
D *Halipegus* sp.
D Digenea, (intestine)
C ?*Ligula* sp.
C Diphyllobothriidae spargana, (thigh muscle)
C *Nematotaenia* sp.
C Cestodes, (rectum)
N *Rhabdias hylae*, (lung)
N *Rhabdonema* sp., (lungs)
N *Parathelandros mastigurus*, (small intestine, rectum)
N Oxyurid, (intestine)
N *Maxvachonia flindersi*
N *Physaloptera confusa*, encysted larva, (stomach peritoneum)
N *Agamonema* sp., encysted larva, (stomach wall)
N Nematode larva, cysts, (intestine)
N Nematodes, (stomach, intestine, rectum, lung, buccal cavity, abdominal cavity, muscle)
A *Porrorchis hylae*, encysted larva, (liver)
A *Acanthocephala* sp.

Litoria citropa (Duméril & Bibron, 1841)
M *Parapolystoma bulliense*
D *Mesocoelium oligoon*, (duodenum)

Litoria cyclorhyncha (Boulenger, 1882)

N *Parathelandros maini*, (rectum)
N *Maxvachonia flindersi*, (rectum)

Litoria dahlii (Boulenger, 1896)
D Digenea
N Nematodes

Litoria dentata (Keferstein, 1868)
N Nematodes, (intestine)

Litoria ewingii (Duméril & Bibron, 1841)
D *Diplodiscus microchrus*, (rectum)
D *Mesocoelium megaloon*, (intestine)
C *Nematotaenia hylae*, (duodenum)
C Cestodes, (small intestine)
N *Austraplectana kartanum*
N *Maxvachonia flindersi*
N *Hedruris hylae*
N Nematodes, (intestine, rectum, duodenum, mesentery)

Litoria fallax (Peters, 1880)
C *Nematotaenia hylae*, (intestine)

Litoria freycineti Tschudi, 1838
D *Dolichosaccus diamesus*, (stomach)
D *Pleurogenoides freycineti*, (duodenum)
C *Nematotaenia* sp.
C Cestodes, (duodenum)

Litoria gracilenta (Peters, 1869)
D *Mesocoelium microon*
N *Parathelandros mastigurus*, (rectum)

Litoria inermis (Peters, 1867)
C *Nematotaenia hylae*, (intestine)
N *Pseudorictularia disparilis*, (stomach)

Litoria infrafrenata (Günther, 1867)
N *Maxvachonia adamsoni*, (intestine)

Litoria latopalmata Günther, 1867
C Diphyllobothriidae spargana, (muscles), (experimental)
C *Nematotaenia hylae*, (intestine)
N *Rhabdias hylae*, (lung)

Litoria latopalmata Günther, 1867 tadpole
D *Fibricola intermedius*, metacercaria, (muscles)
C Diphyllobothriidae spargana, (experimental)

Litoria lesueurii (Duméril & Bibron, 1841)
M *Parapolystomum bulliense*, (bladder)
N *Rhabdias hylae*, (lung)
Nematodes, (rectum)

Litoria moorei (Copland, 1957)
D *Haematoleochus australis*, (lungs)
D *Dolicosaccus trypherus*, (intestine)
D Digenea, (abdominal cavity)
C *Proteocephalus hylae*, (intestine)
N *Rhabdias* sp.
N *Parathelandros maini*, (rectum)
N *Austraplectana kartanum*, (rectum)
N *Maxvachonia flindersi*, (rectum)
N Nematode larvae
A *Pseudoacanthocephalus perthensis*, (rectum, intestine)

Litoria nasuta (Gray, 1842)
N *Austraplectana kartanum*
N *Maxvachonia ewersi*

Litoria nigrofrenata (Günther, 1867)
N *Pseudorictularia disparilis*, (stomach)
N Nematodes

Litoria nyakalensis Liem, 1974
M *Parapolystoma* sp., (urinary bladder)

Litoria pallida Davies, Martin & Watson, 1983
C *Nematotaenia hylae*, (intestine)

Litoria pearsoniana Copland, 1961
M *Parapolystoma bulliense*, (bladder)
D *Fibricola intermedius*, metacercaria, (muscles) (natural & experimental)

Litoria peronii (Tschudi, 1838)
D Diplostomula
D Digenea cysts, (rectum)
D Digenea
C *Nematotaenia hylae*, (intestine)
N *Rhabdias hylae*, (lung)
N *Oswaldocruzia limnodynastes*
N *Physaloptera confusa*, encysted larva, (mesentery)
N Nematodes, (lungs, rectum)
A *Acanthocephala* sp.

Litoria phyllochroa (Günther, 1863)
M *Parapolystoma bulliense*, (bladder)
N Nematodes, (rectum)

Litoria raniformis (Keferstein, 1867)
D *Gorgodera* sp., (bladder)
D *Haematoleochus australis*
D *Dolichosaccus trypherus*, (intestine)
D *Dolichoperoides macalpini*, metacercaria, (intestine)
D Diplostomula
D Echinostome cysts, (stomach)
D Plagiorchid cysts
D Strigeid cysts, (body wall)
D *Tetracotyle* cysts
D Digenea, (intestine)
C *Ophiotaenia* sp., (intestine)
C proteocephalid plerocercoids
C Cestodes
C Cestode larva, (abdominal cavity)
N *Rhabdias hylae*, (lung)
N *Rhabdias* sp., (lung)
N *Rhabdonema* sp.
N *Oswaldocruzia limnodynastes*, (intestine)
N Oxyurids, (lung, rectum)
N *Physaloptera* sp.
N Nematodes, (mesentery, intestine, stomach, rectum)
A *Porrorchis hylae*, encysted larva, (mesentery)

Litoria rothii (De Vis, 1884)
D Digenea, (small intestine)

N Oxyurid
N Nematodes, (small intestine)

Litoria rubella (Gray, 1842)
C *Spirometra erinacei*
N *Parathelandros* spp., (rectum)
N Oxyurid

Litoria verreauxii (Duméril, 1853)
C Cestodes, (small intestine)
N Nematodes, (rectum)

***Litoria* sp.**
D *Dolichosaccus* spp.
D *Pleurogenes* spp.
C Cestodes

***Litoria* spp.**
identified as *Litoria adelaidensis* from NSW
N Nematodes

***Litoria* spp.**
identified as *Litoria aurea* from WA
C Diphyllobothriidae spargana, (thigh muscle)
N *Parathelandros* spp.

Family RANIDAE

Rana daemeli (Steindachner, 1868)
N *Rhabdias australiensis*, (lung)
N Cosmocercinae gen. sp. 1
N *Seuratascaris numidica*, (stomach, intestine)
N *Pseudorictularia disparilis*

Family BUFONIDAE

Bufo marinus (Linnaeus, 1758)
D *Diplodiscus* sp.
D Amphistome
D *Dolichosaccus symmetrus*, (intestine)
D *Dolichosaccus* sp.
D *Mesocoelium mesembrinum*, (small intestine)
D *Mesocoelium* sp., (intestine, abdominal cavity)
D Lecithodendriid sp., (intestine)
D *Zeylanurotrema spearei*, (urinary bladder)
D Digenea cysts
D Digenea, (intestine, stomach, rectum, abdominal cavity, lung, buccal cavity)
C Diphyllobothriidae spargana
C ?*Spirometra mansoni*, spargana, (muscles)
C Proteocephalid plerocercoids
C *Nematotaenia hylae*, (intestine)
C Cestodes, (intestine, stomach)
N *Parathelandros mastigurus*
N *Parathelandros* spp., (intestine)
N Oxyurid
N *Maxvachonia flindersi*, (rectum)
N Cosmocercoid
N Nematode cysts
N Nematodes, (intestine, rectum, abdominal cavity, stomach wall)
A *Pororchis hylae*, encysted larva

Unidentified Anura

Frog
D *Diplodiscus megalochrus*, (bladder)
D Echinostome cysts, (stomach)
D Digenea cysts
C Cestodes, (buccal cavity)
N *Austraplectana* sp.
N *Ophidascaris phyrrhus*
N Dorylaimid, (intestine)

Tadpole
D *Dolichoperoides macalpini*, metacercaria
D *Cercaria ameriannae*, diplostoma
D *Cercaria angelae*, cysts, metacercaria
D *Cercaria ellisi*, cysts
D *Cercaria lethargica*
D K.I. Stylet cercaria, (experimental)
D J.W. Stylet metacercaria
D Echinostome J cercaria, (experimental)
D Echinostome cysts, (experimental)
D Digenea cysts
D Digenea cysts, (experimental)
N *Ophidascaris pyrrhus*, (experimental)
N *Rallietascaris varani*

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Appendix 5: Taxonomic Descriptions of Helminths

This appendix contains taxonomic descriptions of the helminths found in this study but not determined to species. Only adult helminths are detailed. With the following exceptions, each species is described and a table of measurements presented: *Parapolystoma* sp., *Zeylanurotrema spearei* and Onchocercidae gen. sp. *Parapolystoma* sp. is presently being described by Dr Sylvie Pichelin (Parasitology Department, University of Queensland). *Zeylanurotrema spearei* was described by Cribb and Barton (1991), a copy of which is included in Appendix 6. The two Onchocercidae gen. sp. specimens collected are presently being described by Dr Hugh Jones (Zoology Department, University of Western Australia).

Of the specimens identified to level of species, tables of comparative measurements are provided but not the taxonomic descriptions. The following references can be consulted for taxonomic descriptions of *Dolichosaccus symmetrus* (Johnston 1912; Barton 1994), *D. juvenilis* (Nicoll 1918; Barton 1994), *D. helocirrus* (Barton 1994), *Johnpearsonia pearsoni* (Durette-Desset *et al.* 1994), *Parathelandros mastigurus* (Baylis 1930), and *Seuratascaris numidica* (Sprent 1985). No table of measurements is provided for *Nematotaenia hylae* (see Jones 1987).

Each table of measurements is presented with the species under investigation and various other species in the same, or closely related, genera for comparison. All data is presented in micrometers, unless otherwise stated. Data collected in this study is presented as a mean with the range in parentheses. Data from other studies is presented as given in the relevant text.

The helminths are presented in the order that they appear in the Parasite-Host checklist (Table 3.6).

Abbreviations used:

Intro/Nat, Introduced or natural host populations; *Lim.*, *Limnodynastes*; *Lit.*, *Litoria*.

Measurement details:

Nerve ring, excretory pore and vulva are all measured as distance from anterior end of body.

Oesophagus width is the maximum width of the oesophagus.

Diplodiscus sp.

Body club-shaped, posterior end bluntly rounded, anterior end tapered, widest just anterior to posterior sucker. Tegument unspined. Oral sucker terminal with thick, muscular walls surrounding deep cavity; oral diverticula emerged postero-laterally from oral sucker, with thick muscular walls similar to those of oral sucker. Posterior sucker terminal, with an outer muscular rim and central muscular `plug'. Oesophagus narrow, emerged ventrally between oral diverticula, runs posterad, deflected dorsally past oral diverticula, enlarged into oesophageal bulb anterior to intestinal bifurcation. Intestinal caeca wide, simple, lateral in body, terminate at, or just behind, equator. Gonads in posterior half of body. Common genital pore muscular, ventral, at level of intestinal bifurcation.

Testis single, oval to rounded, intercaecal, generally post-equatorial. Sperm duct single, arises from anterior edge of testis, runs anteriorly and enlarges to form seminal vesicle. Seminal vesicle composed of a few coils, runs anteriorly in midline of body before enters genital pore dorsally.

Ovary much smaller than testis, pear-shaped to rounded, posterior to testis, generally left of midline, posterior to termination of caeca. Oviduct arises from medial side of ovary, dilated in midline as Mehlis' gland, duct from Laurer's canal enters dorsally. Uterus with few coils posterior to testis before runs anteriorly along dorsal edge of testis then ventrally to fill space between testis and bifurcation; enters genital pore ventrally. Eggs large, average 30 (10 - 68) in number. Laurer's canal straight, opens dorsally just anterior to Mehlis' gland. Vitelline follicles large, extracaecal, in lateral fields between oral diverticula and posterior of body, confluent dorsally and ventrally posterior to

caeca. Transverse vitelline ducts not seen; vitelline reservoir in midline of body posterior to Mehlis' gland; duct from reservoir enters gland ventrally near Laurer's canal.

Excretory pore or vessel not observed. Two large ducts observed, run parallel along body and occasionally looped towards the mid body region; may be excretory or lymphatic vessels. Finer ducts apparent in live specimen studied.

Table A5.1. Comparative measurements of members of the genus *Diplodiscus* recorded from amphibian hosts in Australia.

Characteristic	*megalochrus*	*microchrus*	*Diplodiscus* sp.
Body length	3720(3000-4000)	1200	1646(1280-1920)
width	1370(1250-1500)	850	774(696-944)
Oral sucker length			172(144-304)
width	313		230(176-336)
Diverticula length	330		128(80-208)
width			120(80-208)
Oesophagous length	520		336(240-432)
Posterior sucker diameter	1085		700(560-832)
Testis length			335(256-400)
width			304(192-368)
Ovary length			145(100-160)
width			135(96-176)
Egg length	132	125	116(100-136)
width	66	67	82.4(60-112)
Number of eggs	156*	32*	30(10-68)
Hosts	*Lit.aurea* *Lim.peronii*	*Lit.ewingii* *Lim.tasman.*	*Lit.caerulea* *Lit.alboguttata* *Lit.dahlii* *Bufo marinus*
Reference	Johnston 1912	Johnston 1912	This study

*Counted from museum specimens.

Table A5.2. Measurements of *Dolichosaccus symmetrus* and *Dolichosaccus anartius* as recorded in Johnston (1912) and this study from amphibians in Australia.

Characteristic	*anartius*	*symmetrus*	*symmetrus*
Body length	3300	1470	3744
width	1200	570	942
Oral sucker length			260
width	203	256	298
Ventral sucker length			216
width	212	215	218
Left Testis length	147	238	
width	175	105	284
Right Testis length		292	
width		288	
Cirrus sac length			493
Ovary length		147	259
width	142	101	218
Egg length	34	34	47
width	19	19	24
Hosts	*Lit.aurea* *Lim.peronii*	*Lit.caerulea*	*Bufo marinus*
Reference	Johnston, 1912	Johnston, 1912	This study

Table A5.3. Measurements of *Dolichosaccus juvenilis* and *Dolichosaccus grandiacetabularis* as recorded in Nicoll (1918), Moravec and Sey (1989) and this study from amphibians in Australia and Papua New Guinea.

Characteristic	*grandiacetabularis*	*juvenilis*	*juvenilis*
Body length	1410	1350-1650	1740
width	789	550-650	850
Oral sucker length	177		180
width	219	130-170	200
Ventral sucker length	279		200
width	285	190-210	210
Left Testis length	258		230
width	174	150	200
Right Testis length	315		210
width	165		180
Cirrus sac length	369		580
Ovary length	123	150	220
width	174	210	260
Egg length	45-48	42-48	47
width	21-24	27	24
Hosts	*Rana grisea*	*Cyclorana brevipes*	*B.marinus*
Location	Papua New Guinea	Queensland (Australia)	Queensland (Australia)
Reference	Moravec & Sey 1989	Nicoll 1918	This study

Table A5.4. Comparative measurements of *Dolichosaccus longibursatus* as recorded in Moravec and Sey (1989) and *Dolichosaccus* sp. from this study.

Characteristic	*longibursatus*	*Dolichosaccus* sp.
Body length	1030	1774
width	449	852
Oral sucker length	136	168
width	163	214
Ventral sucker length	177	214
width	190	226
Left Testis length	109-122	262
width	109-122	191
Right Testis length		265
width		195
Cirrus sac length	340	544
Ovary length	95	248
width	177	286
Egg length	45-51	49
width	21	27
Host	*Rana grunniens*	*Bufo marinus*
Location	Papua New Guinea	Queensland (Australia)
Reference	Moravec & Sey 1989	This study

Mesocoelium sp.

Body elongate, bluntly rounded at both ends. Tegument spinose, dense anterior, terminate at mid body level. Oral sucker subterminal, opening directed ventrally, larger than ventral sucker. Ventral sucker in anterior third of body. Prepharynx short; pharynx immediately posterior to ventral sucker; oesophagus long. Intestinal caeca simple, unbranched, lateral, terminate before equator of body. Gonads in anterior third of body, lateral or posterior to ventral sucker, intra-caecal. Common genital pore at level of intestinal bifurcation in mid line of body.

Testes round, symmetrically placed, either lateral or posterior to ventral sucker. Cirrus sac runs directly posterior from genital pore; contains seminal vesicle in posterior half.

Ovary round to oval, posterior to testes and ventral sucker, always to one side of midline, but side not consistent. No other part of female system observed due to extent of uterus. Uterus fills entire posterior two-thirds of body before runs anteriorly to genital pore. Eggs small, unembryonated. Vitelline follicles from level of pharynx posterior to end of intestinal caecae, lateral to, though sometimes covering, intestinal caeca.

Excretory opening terminal. Excretory bladder I-shape, runs anteriorly through mid line of body.

Table A5.5. Comparative measurements of members of the genus *Mesocoelium* recorded in Australia.

Characteristic	*megaloon*	*oligoon*	*microon*	*mesembrinum*	*mesembrinum*	*Mesocoelium* sp.
Body length	1880	1530	1940	2300-3140	1530-1860	1570(975-2964)
width	358	590	770	644-1190	540-650	669.5(390-1092)
Oral sucker diameter	193.5	215	250	293.4	220	232.3(144-336)
Pharynx diameter		65	80	110	66-76	
Ventral sucker diameter	64.5	129	180	195.6	160-180	154.8(96-208)
Testis length	181	165	210	195	120-150	177.8(112-256)
width	129	99	140		100-140	
Ovary length	112	130	150	163	170-200	232.3(144-336)
width		90			160-180	
Cirrus sac length						159.6(64-256)
Egg length	47	39-52	38	40	34-41	34.72(32-36)
Egg width	29	28	26	25	25-30	
Host	*Lit. ewingii*	*Lit. citropa*	*Lit. caerulea gracilenta Tiliqua scincoides*	*Lit. caerulea*	*Bufo marinus*	*Bufo marinus*
Reference	Johnston 1912	Johnston 1912	Nicoll 1914	Johnston 1912	Yuen 1965	This study

Table A5.6. Comparative measurements of members of the genus *Mesocoelium* recorded from *Bufo marinus* (natural and introduced populations).

Characteristic	*sociale*	*danforthi*	*incognitum*	*mesembrinum*	*Mesocoelium* sp.
Body length	741-2396	680-2430	1151-1249	1530-1860	1570(975-2964)
width	330-1080	360-810	500-900	540-650	669.5(390-1092)
Oral sucker diameter	145-290	120-280	140-250	220	232.3(144-336)
Pharynx diameter	58-126x 63-145	60-120x 60-140	60-100	66-76	
Ventral sucker diameter	102-255	80-210	160-300	160-180	154.8(96-208)
Testis length	85-230	70-180	62-180	120-150	177.8(112-256)
width	75-230	50-140	53-101	100-140	
Ovary length	92-211	70-190	82-200	170-200	232.3(144-336)
width	104-211	60-160	49-147	160-180	
Cirrus sac length					159.6(64-256)
Egg length	29-40	30-35	37-41	34-41	34.72(32-36)
Egg width	20-25	18-25	21-25	25-30	
Location	Florida, Br. Solomon I. Fiji	Jamaica	Brazil	Queensland (Australia)	Queensland (Australia)
Intro/Nat	Introduced	Introduced	Natural	Introduced	Introduced
Reference	Fischthal & Kuntz 1967	Mettrick & Dunkley 1968	Cheng 1960	Yuen 1965	This study

Pleurogenoides sp.

Body rounded or oval. Tegument spinose, spines small anteriorly, larger posteriorly. Oral sucker subterminal, with opening directed ventrally, approximately same size as ventral sucker. Ventral sucker at equator. Prepharynx and oesophagous short. Intestinal caeca simple, unbranched, run lateral, end anterior to ventral sucker, in anterior third of body. Gonads in middle third of body, lateral or anterior to ventral sucker. Common genital pore on lateral edge, at level of oral sucker.

Testes oval or rounded, posterior to caeca, symmetrically placed lateral or just anterior to ventral sucker. Sperm duct runs dorsal to ventral sucker; ducts to testes not seen. Cirrus sac large, anterior to ventral sucker, dorsal to caeca, distal end an elongated S-shape; contains seminal vesicle in basal part of cirrus pouch, prostatic gland large.

Ovary round, intra-caecal, anterior to testes and ventral sucker, on opposite side of body to cirrus sac. Uterus runs lateral from ovary to opposite side of body before returns to original side, turns posterior, loops anteriorly, then again runs lateral, turns posteriorly, then runs anteriorly to level of genital pore, dorsal to caeca. Eggs unembryonated. Mehlis' gland posterior to ovary and lateral to ventral sucker; seminal receptacle large, lateral to ventral sucker; Laurer's canal not observed. Vitelline follicles large, few in number, anterior to intestinal caecae, concentrated on side of body opposite to cirrus sac. Vitelline reservoir posterior to ovary.

Excretory opening terminal. Excretory bladder V-shaped, large primary branches obscured by eggs just posterior to ventral sucker.

A sample of immature *Pleurogenoides* sp. specimens were available from a *Litoria dahlii* collected by MKJ at Wildman R. Station, NT. Description as above, except ovary not present in many specimens, vitelline follicles not present in any specimens, no eggs present in any specimens.

Body 37.44 (25.6-41.6) long and 23.2 (19.2-26.4) wide. Oral sucker larger than ventral sucker, 7.72 (6-8.6) long and 8.28 (6.4-9.8) wide. Ventral sucker not fully developed, 7.52 (6.8-88) long and 6.84 (5.2-7.8) wide. Pharynx 3.44 (2.4-4.0) wide.

Testes 5.36 (2.8-6.8) long and 4.1 (2-5.2) wide. Cirrus sac 17.12 (12.4-22) long.

Ovary only present in larger specimens, 3.9 (3.2-4.2) long and 3.7 (3.2-4.0) wide.

Excretory arms extend to posterior edge of ventral sucker.

Table A5.7. Comparative measurements of members of the genus *Pleurogenoides* recorded from amphibians in Australia.

Characteristic	*freycineti*	*solus*	*Pleurogenoides* sp.
Body length	1450	815	707.2(400-960)
width	890	490	490.4(304-640)
Oral sucker length			117.4(88-140)
width	202	111	133.6(92-170)
Pharynx diameter	64		57.8(48-64)
Ventral sucker length			119.0(80-156)
width	176	121	117.6(96-148)
Testes length	430		136.4(64-180)
width	193	98	109.1(48-152)
Cirrus sac length		245	347.6(220-404)
Ovary length		98	111.3(68-160)
width		49	94.0(56-128)
Egg length	23.6	20	24.6(24-28)
width	11.7	10	12.0
Hosts	*L. freycineti*	*L. aurea*	*Bufo marinus* *L. tornieri* *L. dahlii* *L. rothii* *L. nasuta*
Reference	Johnston 1912	Johnston 1912	This study

Rhabdias sp.

Moderate to large worms. Cephalic extremity rounded, cuticle smooth, inflated along entire body length. Tail bluntly pointed. Oesophagus elongate, slightly expanded at posterior end at junction with intestine; oesophageal valves projecting into intestine. Nerve ring not observed. Excretory pore posterior to mid length of oesophagus. Intestine dark brown in colour throughout entire length, constricted near posterior end into rectum. Anus near posterior extremity, situated on anterior edge of body projection.

Vulva about mid body length, prominent lips. Vagina divergent, one uterus runs anteriorly, other posteriorly, elongate, turn back on themselves to form ovaries. Middle two-thirds of body filled with eggs. Eggs thin shelled, rounded, developed larva near vulva.

Male reproductive system not observed.

Table A5.8. Comparative measurements of parasitic stage of members of the genus *Rhabdias* recorded in Australia and from *Bufo marinus* in natural populations.

Characteristic	*hylae*	*fuelleborni*	*sphaerocephala*	*Rhabdias* sp.
Total length	6500-7800	10000-12000	6704-11600	10820(6960-14460)
Body width max	340-370	470-480	416-480	435(380-480)
O/I*			75-93	206(180-240)
Oesophagous length	380-460	450-500	432-448	464.5(400-512)
Oesophagous width		200	75-82	70.4(64-80)
Nerve ring	170-180			196(112-256)
Excretory pore				285.3(200-400)
Anus-tail	340-400	370-420	330-432	357.8(304-432)
Vulva	3250-3850	4630-5880†	3680-6240	5321(3456-6800)
Egg length	55		94-112	102.9(88-116)
Egg width	10		56-60	54.2(44-64)
Host	*Litoria aurea*	*Bufo marinus*	*Bufo marinus*	*Bufo marinus*
Location	NSW	Brazil	Costa Rica	Queensland
Intro/Nat	Natural	Natural	Natural	Introduced
Reference	Johnston & Simpson 1942	Travassos 1924	Kloss 1974	This study

*Width of body at junction of oesophagous and intestine. †Calculated from information given.

Table A5.9. Comparative measurements of *Oswaldocruzia limnodynastes* and *Johnpearsonia pearsoni* recorded in Australia, and *Batrachonema bonai* from toads in South America.

Characersitic	Sex	*O.limnodynastes*	*J. pearsoni*	*Batrachonema bonai*
Total length	♂	3800	7380(5760-8600)	3400
	♀	6700-9500	9227(5920-11920)	5230
Body width	♂	100	144(120-184)	100
	♀	120-150	170.4(128-216)	250
Oesophagous	♂		394.5(372-440)	450
length	♀	430	440.4(372-520)	586
Oesophagous	♂		57.3(44-80)	
width	♀		68.4(50-92)	
Nerve ring	♂			240
	♀	180		301
Excretory pore	♂			405
	♀	270		567
Anus-tail	♂			
	♀	200	88(68-104)	
Vulva	♀	180	188(144-220)	1670
Cervical alae	♂	100	124(84-146)	95
length	♀		143.4(124-160)	105
Cervical alae	♂		56(48-70)	52
width	♀		61.6(56-70)	85
Spicule length	♂	95	513.2(446-612)	440
Accessory piece length	♂	45	97.6(92-124)	90
Egg length	♀	36	72.7(60-84)	80
width		25	43.6(40-48)	55
Host		*Limnodynastes dorsalis*	*Bufo marinus*	*Bufo typhonius*
Reference		Johnston & Simpson 1942	This study	Durette-Desset *et al.* 1984

Table A5.10. Comparative measurements of members of the genus *Parathelandros* recorded from various amphibians in Australia.

Characteristic	Sex	*mastigurus*	*mastigurus*	*australiensis*	*propinqua*
Total length	♂	1300-1450	2027(1952-2080)		
	♀	3240-4720	4880(4280-5200)	7500-9000	5000
Body width	♂	190	195(168-232)		
	♀	380	515(420-640)	380-480	369
Oesophagous	♂	240-250	374(360-384)		
length	♀	440-500	712(648-864)	353	480
Oesophageal bulb	♂	60-70	83(80-84)		
length	♀	100-110	164(152-168)	126	126
Nerve ring	♂	100			
	♀	125-187		150	126
Excretory pore	♂		392		
	♀	<160-220	268(256-272)		
Tail length	♂	270-320			
	♀	900-1100	1157(1080-1310)	1000	
Spicule length	♂	75	72		
Vulva	♀	160-220	268(256-272)	573	290
Egg length		127-150	161(156-164)	137	
width		37-47	49(38-60)	36	
Host		*Litoria caerulea*	*Bufo marinus* *Lit. caerulea* *Lit. genimaculata* *Lit. inermis* *Lit. rothii* *Lit. rubella* *Lim. tasmaniensis* *Mixophyes sp.* *Crinia deserticola*	*Lim. dorsalis*	*Lm dorsalis*
Reference		Baylis 1930	This study	Johnston & Mawson 1942	

Cosmocerca spp.

Small worms. Mouth with three insignificant lips; opens into small buccal cavity. Oesophagous with long body, narrow isthmus and posterior bulb, with valvular apparatus in centre of bulb. Nerve ring about mid length of oesophagus. Excretory pore just anterior to oesophageal bulb.

Male. Tail curved ventrally, ending in terminal spine; numerous papillae and plectanes: 19 pairs of plectanes in *Cosmocerca* sp. 1, 15 pairs in *Cosmocerca* sp. 3. Plectane dimensions decreasing towards posterior, those around cloaca very small, but still bearing rosette consisting of ring of small teeth at extremity. Papillae scattered over post-cloacal area in *Cosmocerca* sp. 1, much fewer in number in *Cosmocerca* sp. 3. Gubernaculum large, strongly chitinized, wide proximal end, often protruding from cloacal opening. Spicules sub-equal, slender, resembling simple curved rods.

Female. Vulva at mid body. Ovaries arising in anterior part of body, passing forward to just posterior to oesophageal bulb before turning posterior. Coiled larvae present posterior to vulva, many free of egg, but still *in utero*.

Table A5.11. Comparative measurements of members of the genus *Cosmocerca* recorded in Australia.

Characteristic	Sex	*limnodynastes*	*Cosmocerca* sp.1	*Cosmocerca* sp.2	*Cosmocerca* sp.3
Total length	♂	1600	2520		3640(3320-3960)
	♀	4250	6840(6800-6880)	4400(4200-4600)	4360
Body width	♂	185	280		330(300-360)
	♀	485	610(600-620)	360(300-400)	300
Oesophagous length	♂	310	472		604(536-672)
	♀	430	706(664-728)	648(608-716)	600
Oesophageal bulb width	♂	59	176		120(112-128)
	♀		180(176-184)	160(152-168)	128
Nerve ring	♂	149			
	♀	162			
Excretory pore	♂	306			408(384-432)
	♀	431	456(368-544)	386.7(368-416)	432
Anus-tail	♂		208		170(160-180)
	♀		576(560-592)	450.7(432-480)	192
Vulva	♀		3616(3568-3664)		
Spicule length	♂	50-75	156		294(280-308)
Gubernaculum length	♂	110	180		121(120-122)
Egg length	♀	144	130(128-132)	155(150-160)	
Egg width		94	90(84-96)	89(80-98)	
Larva length			400	560	
Host		*Lim. dorsalis*	*Bufo marinus*	*Bufo marinus*	*Bufo marinus*
Reference		Johnston & Simpson 1942	This study	This study	This study

Austraplectana sp.

Short worms, thick body with long thin tail of bumpy appearance. Mouth bordered by three large lips. Oesophagus divided into short pharyngeal part, a long body, short and narrow isthmus, and a large bulb; oesophageal valves project into intestine. Nerve ring mid length of oesophagous body. Excretory pore posterior third of oesophagus. Cuticle of body smooth, with narrow alae.

Male. Tail elongate, ending in sharp point, covered with small phasmids and papillae. Caudal alae projecting and thick, extend from preanal region to tail, supported on each side by 8 papillae. Spicules equal, distal ends meet in midline. Cloaca on slight projection.

Female. Vulva mid body; ovaries and oviducts anterior to vulva; uteri posterior to vulva. Eggs oval, fill posterior half of body. Tail conical and pointed; phasmids present.

Table A5.12. Comparative measurements of members of the genus *Austraplectana* recorded in Australia.

Characteristic	Sex	*kartanum**	*kartanum*§	*Austraplectana* sp.
Total length	♂	3000-3300	3800	1814.4(1312-2592)
	♀	4000	3500	2088(1912-2384)
Body width	♂			102(80-112)
	♀			237.3(208-272)
Oesophagous length	♂	480	512	196.7(162-237.3)
	♀		618	219(212-226)
Nerve ring	♂		246	
	♀		279	
Excretory pore	♂		394	168
	♀		509	141(138-144)
Tail length	♂	140	170	
	♀	300	272	
Vulva	♀		1900	580(540-620)
Spicule length	♂	130	224	347(240-454)
			285	323(232-414)
Egg length	♀			22(20-24)
Egg width				16
Host		*Litoria jervisiensis*	*L. moorei* *L. nasuta*	*Bufo marinus*
Reference		Johnston & Mawson 1941	Baker 1981	This study

*Described as *Raillietnema kartanum* by Johnston and Mawson (1941).
§Type species of genus.

Maxvachonia sp.

Female worms large, more commonly encounteres than smaller males. Mouth bordered by three lips. Oesophagus with long body, narrow isthmus and posterior bulb; oesophageal bulbs project into intestine. Two prominent nuclei present anterior to isthmus. Nerve ring mid length of oesophagus. Excretory pore posterior to nerve ring, close to posterior bulb.

Male. Lateral alae extend for most of body length. Spicules equal in size, weakly developed, blunt-tipped. Gubernaculum large, well chitinised, about same length as spicules, pair of lateral processes near proximal end. Cloacal opening on an elevation of the body wall. Caudal papillae present. Sperm duct undifferentiated posteriorly.

Female. Lateral alae extend to about mid body. Anus distant from posterior end of body. Tail long, anterior half filled with eggs, posterior half clear. Vulva a transverse slit, at posterior end of oesophageal bulb. Vagina runs posterior to vulva, splits into two uterine branches which run parallel towards posterior end of body, posterior to anus, turn and run anterior to ovaries, anterior to anus. Eggs large, prominent spine, coiled larva present in distal end of uterus, surrounded by 'envelope' of tissue material.

Table A5.13. Comparative measurements of members of the genus *Maxvachonia* recorded in Australia.

Characteristic	Sex	*flindersi**	*Maxvachonia*	*Maxvachonia*	*Maxvachonia*
Total length (mm)	♂	2.1			3.952
	♀	8.8-11.1	13.2(1.88-15.84)	10.12(9.88-10.5)	10.12(9.06-10.99)
Body width	♂				320
	♀		398.7(280-460)	333.3(320-340)	318(288-336)
Oesophagous length	♂	340			644
	♀	700-900	851.7(624-1000)	733.3(660-816)	924(832-976)
Oesophageal bulb width	♂	80			86
	♀		149.2(114-174)	132.7(116-146)	194(184-200)
Nerve ring	♂	130			272
	♀	200-290	294(248-344)	248	352
Excretory pore	♂	270			432
	♀	400-510	642(424-752)	493.3(444-560)	730(704-800)
Anus-tail	♂	170			320
	♀	1600-2300	3301(1648-4528)	2189(1848-2728)	3776(2456-5048)
Vulva	♀	530-630	827.3(660-996)	650.7(564-768)	1038(1008-1065)
Spicule length	♂	110			144, 172
Gubernaculum length	♂				156
width					32
Egg length	♀		66.4(60-78)	77.3(70-92)	74.7(56-108)
Egg width			48.3(40-60)	53(50-56)	
Host		*Bufo marinus*	*Bufo marinus*	*Litoria inermis*	*Litoria rothii*
Reference		Johnston & Mawson 1941; Mawson 1972	This study	This study	This study

*Measurements for male from Johnston and Mawson 1941; female measurements from Mawson 1972.

Table A5.14. Measurements of *Seuratascaris numidica* (Seurat 1917) Sprent 1985 recorded from *Rana daemelii* (Ranidae) in Australia.

Characteristic	Sex	*numidica*	*numidica*
Total length	♂	7900-21500	
	♀	14400-37200	75400
Body width max	♂	190-440	
	♀	320-580	540
O/I*	♂	190-350	
	♀	290	360
Oesophagous	♂	1300-2500	
length	♀	2500-3000	4672
Caecum length	♂	650-140	
	♀	1700	2688
Nerve ring	♂	280	
	♀		
Excretory pore	♂	310-460	
	♀	420-450	
Vulva	♀	4400-9200	1520
Spicule length	♂	110-200	
Egg length	♀		960
width			800
Reference		Sprent 1985	This study

*Width of body at junction of oesohagous and intestine.

Spinicauda sp.

Medium sized worms. Head conforms to general Heterakidae pattern as outlined by Inglis (1957); mouth with three lips, each with a pharyngeal tooth and anterior cuticular flange. Oesophagus narrow, distinct pear-shaped oesophageal bulb. Nerve ring encircles narrow part of oesophagus, anterior to excretory pore. Excretory pore mid length of oesophagus. Intestine swollen at junction of oesophagus and intestine.

Male. Tail curved ventrally, ends in fine, sharply pointed terminal spike. Narrow caudal alae supported by pairs of papillae; most pairs lie at level of precloacal sucker, remaining pair lateral to cloacal opening. Preanal sucker with heavily cuticularised rim. Paired spicules equal length, elongate, ventrally curved proximal and distal ends; appear striated along length almost to distal ends. Gubernacular mass present at distal end of spicules, approximately 80 μm in transverse diameter.

Female. Vulva opens on ventral surface, about midbody, with prominent anterior lip. Vagina immediately runs posterior, becomes a wider common uterine trunk and splits into 2 parallel uteri. Eggs thin shelled, oval.

Table A5.15. Comparative measurements of members of the genus *Spinicauda* recorded from various host groups in Australia.

Characteristic	Sex	*australiensis*	*moretonis*	*Spinicauda* sp.
Total length	♂	3890-3990	4120-4500	6536.7(5400-7220)
	♀	4400-4800	6300-6600	7452.0(5180-9040)
Body width	♂	360-380	170	333.3(240-380)
	♀	480	260-280	390.0(200-500)
Oesophagous length	♂	700-750	420-470	1261.3(1056-1544)
	♀	800	750-770	1348.8(1184-1460)
Oesophageal bulb length	♂		90-110	189.3(176-208)
	♀		150-160	223.8(176-256)
Nerve ring	♂	260-300		400.0
	♀	260-300	280-300	470.0(448-492)
Excretory pore	♂	280-350	390	626.7(576-672)
	♀	280-350	500-560	675.0(600-732)
Anus-tail	♂	210-270	240-280	277.3(232-312)
	♀	450-550	380-420	408.8(320-464)
Spicule length	♂	600-650	270-310	876.0(768-956)
Preanal sucker W	♂	70-80	60	67.2(64-72)
Vulva	♀	2100	2270-3150	2859.4(2160-3360)
Egg length	♀	75-80	95-106	87.1(80-94)
Egg width		53-60	62	54.4(52-72)
Host		*Tiliqua scincoides*	*Morelia spilotes*	*Bufo marinus*
Host group		Skinkidae	Boidae	Bufonidae
Reference		Baylis 1930	Jones 1979	This study

Kreisiella sp.

Cylindrical worms of large size. Mouth bordered by 2 lips; enters small buccal capsule. Muscular oesophagus short, followed by wider, and much longer, glandular portion. Nerve ring surrounds posterior portion of muscular oesophagus. Excretory pore posterior to nerve ring at level of junction of muscular and glandular portions of oesophagus. Anterior cuticle slightly inflated posterior to mouth, never extends beyond nerve ring.

Male. Bursa extending to tip of tail, covered with small tile-like tuberculations, arrangement of papillae difficult to ascertain. Cloacal rim appears crenulated and is free of tuberculations. Spicules unequal; left spicule straight, distal end a fine tip; right spicule thicker than left, about a third of size of left, protruding from cloaca in most cases. Male duct terminates in bulbous muscular mass.

Female. Tail conical, bluntly pointed. Anus slightly distant from end of tail, rectum chitinised, many gland cells surround rectum. Vulva lies anterior to mid length of oesophagus; muscular vagina runs posteriorly, branches into 4 uteri, all entirely posterior to vulva. Eggs small, thin shelled.

Table A5.16. Comparative measurements of members of the genus *Kreisiella* recorded from various host groups in Australia.

Characteristic	Sex	*Kreisiella* sp.	*chrysocampa*	*lesueurii*
Total length (mm)	♂	13.8(11.3-15.3)	67.43	16.2
	♀	16.1(14.4-18.5)	15.13	25.8
Body width	♂	424(380-480)	140	170
	♀	393.3(360-440)	360	740
Oesophagous length	♂	1436.8(800-1984)	1260	2260
	♀	2709.3(2320-2960)	2370	2980
Oesophagous width	♂	130.7(112-144)	100	150
	♀	160(128-176)	160	220
Nerve ring	♂	184	120	240
	♀	216	150	240
Excretory pore	♂	368(344-392)	240	390
	♀	318(284-352)	240	400
Tail length	♂	916(880-1056)	460	920
	♀		400	110
Left spicule length	♂	274.4(240-292)	178	320
width		29.6(24-44)		
Right spicule length	♂	97.3(64-132	97	132
width		28(24-36)		
Vulva	♀	840(736-952)	560	800
Egg length	♀	28	53	66
Egg width		20	25	32
Host		*Bufo marinus*	*Egernia inornata*	*Pogona minor minor*
Host group		Bufonidae	Gekkonidae	Agamidae
Reference		This study	Jones 1985	Jones 1986

§Measurements of holotype male and allotype female presented.

Appendix 6: Taxonomic Papers Published

Included in this Appendix are descriptive papers of helminths found in this study that have been published at the time of submission of the thesis. The papers are presented in chronological order.

The papers are:

Cribb T.H. & Barton D.P. (1991) *Zeylanurotrema spearei* sp.n. (Digenea: Brachylaimidae) from the cane toad, *Bufo marinus*, in Australia. *Zoologica Scripta*. **20:** 207-213.

Durette-Desset M.-Cl., Ben Slimane B., Cassone J., Barton D.P. & Chabaud A.G. (1994) *Johnpearsonia* gen. nov. and Johnpearsoniinae subf. nov. (Molineoidea, Nematoda) from *Bufo marinus*, with comments on the primitive trichostrongyle parasites of amphibians and reptiles. *Parasite*. **1:** 153-160.

Barton D.P. (1994) Three new species of the genus *Dolichosaccus* Johnston, 1912 (Digenea: Telorchiidae) from the introduced toad *Bufo marinus* (Amphibia: Bufonidae) in Australia, with the erection of *Meditypus* n. subg. *Systematic Parasitology*. **29:** 121-131.

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Zeylanurotrema spearei sp.n. (Digenea: Brachylaimidae) from the cane toad, *Bufo marinus*, in Australia

THOMAS H. CRIBB and DIANE P. BARTON

Accepted 18 *December* 1990

Cribb, T. H. & Barton, D. P. 1991. *Zeylanurotrema spearei* sp.n. (Digenea: Brachylaimidae) from the cane toad, *Bufo marinus*, in Australia.—*Zool. Scr. 20:* 207–213.

Zeylanurotrema spearei sp.n. is described from the urinary bladder of the introduced cane toad, *Bufo marinus*, from North Queensland, Australia. The other species in *Zeylanurotrema*, *Z. lyriocephali*, has been recorded only from an agamid lizard from Sri Lanka. The new species has differently arranged gut caeca, vitelline follicles and ovary as compared with *Z. lyriocephali*. *Z. spearei* has a Laurer's canal that does not open to the exterior but which forms a glandular Juel's organ. We suggest that *Z. spearei* is an Australian species that has been acquired by the cane toad from a native host. In juvenile specimens of *Z. spearei* a persistent microcercous tail is present. From this and the anatomy of the adult we conclude that *Zeylanurotrema* is a brachylaimid rather than a urotrematid as originally proposed. A new subfamily, the Zeylanurotrematinae, is proposed to accommodate *Zeylanurotrema*.

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Introduction

During a survey of the helminths of the introduced cane toad (*Bufo marinus*) in Australia an unusual trematode was found in the urinary bladder. Trematodes have not previously been recorded from this site in cane toads in Australia. However, a similar trematode has been described from the urinary bladder of the hump-nosed lizard, *Lyriocephalus scutatus* (Agamidae), in Sri Lanka by Crusz & Sanmugasunderam (1974). Crusz and Sanmugasunderam erected a new genus and species, *Zeylanurotrema lyriocephali*, within the family Urotrematidae. The species in the cane toad is distinct from *Z. lyriocephali* and is here described as a new species. The familial relationships of the genus are reconsidered.

Material and methods

Toads were collected by hand and were dissected within a week of capture. The toads were killed with a dose of 'Lethobarb' (Pentobarbitone sodium solution) sprinkled onto the skin. The bladder was removed and placed in 0.85% saline, opened and any trematodes removed. Trematodes were studied, live and partially flattened beneath a coverslip, using bright field and interference microscopy. Eggs were collected from live specimens, placed in saline in covered cavity blocks and maintained at 24°C. Trematodes were fixed in Berland's fluid (Gibson 1979) or nearly boiling 5% formalin and stored in 70% ethanol; some specimens were fixed partially flattened beneath a coverslip with either Bouin's fluid made up with distilled water or calcium acetate buffered formalin. Whole-mounts were stained with Gower's carmine or Mayer's haematoxylin, dehydrated, cleared with xylene or methyl salicylate, and mounted in Canada balsam. Unstained whole-mounts were also prepared in this manner. Sections (7 μm) were stained with haematoxylin and eosin and mounted in thin Canada balsam. Measurements are given in micrometres as ranges with the mean in parentheses. The system of measurements used is based on that recommended for brachylaimids by Mas-Coma *et al.* (1984); the oral sucker, ventral sucker, testes and ovary are thus given as maximum diameter followed by maximum perpendicular diameter. Drawings were made with the aid of a camera lucida. Museum abbreviations used are as follows: AHC, Australian Helminthological Collection at the South Australian Museum, Adelaide; BM(NH), British Museum (Natural History), London; QM, Queensland Museum, Brisbane.

Family Brachylaimidae Stiles & Hassall, 1989
Subfamily Zeylanurotrematinae subfam. n.

Diagnosis

Brachylaimidae. Body robust, with tegumental spines. Oral and ventral suckers of similar size; ventral sucker in anterior half of body. Prepharynx short; pharynx present; oesophagus absent; caeca simple, extending to near posterior end of body. Testes opposite, at posterior end of body. *Vas deferens* developed as external seminal vesicle, leading to non-sperm-storing bursa and cirrus pouch which contains only ejaculatory duct. Common genital pore at posterior end of body. Ovary formed from several deeply separated lobes. Vitellarium consisting of follicles extending laterally from near ventral sucker to testes; vitelline ducts arising from posterior end of vitelline fields. Laurer's canal present, not opening to exterior, forming large, glandular Juel's organ. Uterine seminal receptacle present. Uterus passing anteriorly from ovary in coils to ventral sucker then returning to posterior end of body and opening with cirrus pouch at common genital pore. Eggs small, tanned, operculate. Excretory bladder small, Y-shaped, with sphincters delimiting bladder from ducts, opening dorso-subterminally. Known from the urinary bladders of reptiles and amphibians in Sri Lanka and Australia respectively.

Zeylanurotrema spearei sp.n. (Figs 1–7)

Type material. Holotype and 9 paratypes ex-urinary bladder *Bufo marinus* (L.) (Bufonidae), Bloomfield, North Queensland, Australia

(15°57'S, 145°20'E), October 1989, Coll. D. Smyth. Holotype QM No. GL 1273. Paratypes 3, QM Nos 1274–76, 3, AHC Nos 18984a–c, 3, BM(NH) Nos 1990. 12. 7. 3–5.

Further material. All ex-urinary bladder *B. marinus*: Bloomfield, Coll. D. Blair, May 1989, Coll. D. Smyth, October 1989; Cape Tribulation (16°05'S, 145°29'E), Coll. H. Spencer, January 1990, May 1990; Cape Weymouth (12°37'S, 143°26'E), Coll. D. Barton, November 1989; Garner's Beach (17°49'S, 146°06'E), Coll. R. Speare, July 1988; Moresby (17°38'S, 146°02'E), Coll. P. Rowles, November 1989. 3 AHC S1779 (1–3) no data. QM Nos 1211–1272, 1277–1282.

Etymology. This species is named in honour of our colleague Dr Rick Speare who first drew attention to this species and who has performed important work on Australian helminths.

Description.(Based on holotype and 9 paratypes from one infected toad.) Body elongate, with bluntly rounded posterior and anterior ends, widest approximately at mid-body level, 2070–2269 mm (2193) long and 544–621 μm (570) wide. Body length/width ratio 3.62–4.07 (3.85). Tegument spinose. Oral sucker slightly larger than ventral sucker, 295–318 μm (308) by 263–315 μm (280). Ventral sucker immediately posterior to gut bifurcation, 257–282 μm (269) by 225–270 μm (244). Ratio area oral sucker to area ventral sucker 1:0.68–0.83 (0.76); ratio oral sucker maximum diameter to that of ventral sucker 1:0.84–0.91 (0.88). Prepharynx short; pharynx immediately posterior to oral sucker, 112–135 μm (123) long by 116–135 μm (124) wide; oesophagus absent. Gut caeca simple, unbranched, lateral in body for much of length, turning medial anterior to testes and terminating near mid-line. Forebody 398–456 μm (428), occupying 18.3–20.6 (19.5)% body length. Gonads in posterior quarter of body. Common genital pore at posterior end of body.

Testes opposite, posterior to or slightly overlapping ends of gut caeca, irregularly oval in ventral view; left testis 270–372 μm (338) by 193–250 μm (221); right testis 276–372 μm (323) by 199–228 μm (219). Sperm ducts arise from antero-median face of testes, pass medially and unite to form large, tubular, median *vas deferens* anterior to testes, which forms external seminal vesicle; seminal vesicle passes anteriorly initially then turns posteriorly, contracts to form elongate, median, unspecialized duct which passes posteriorly and joins 'non-sperm-storing bursa' (terminology of Mas-Coma & Montoliu 1986); bursa thick-walled, muscular, surrounded by gland cells, connects with proximal end of cirrus pouch (Fig. 2). Cirrus pouch posterior to testes; containing only short, eversible, slightly convoluted ejaculatory duct; external wall muscular, composed of diffuse fibres; externally surrounded by gland cells (Fig. 2).

Ovary pre-testicular, median, anterior to ends of gut caeca, composed of 5 deeply separated lobes, 270–321 μm (290) by 170–257 μm (222). Oviduct arises at junction of ovarian lobes, unites almost immediately with Laurer's canal and with duct from vitelline reservoir, then enters Mehlis' gland to form oötype (Fig. 3). Mehlis' gland dorsal to ovary, radiating from point almost directly over origin of oviduct. Vitelline follicles lateral, extending from ventral sucker to level of anterior edge of ovary, external to caeca; vitelline ducts run from posterior end of vitellarium, turning medially posterior to ovary to form transverse vitelline ducts and uniting in mid-line to form vitelline reservoir. Laurer's canal not opening to exterior, passing posteriorly from egg-forming complex and immediately expanding to form large receptacle, invariably (in specimens examined) filled with vitelline remnants and sperm, which occupies antero-dorsal space between testes (Fig. 3), lined postero-ventrally with glandular material forming 'Juel's Organ' (Fig. 4) (see Gibson & Bray 1979). Uterus issues from oötype and typically passes posteriorly for short distance, frequently containing sperm, then passes anteriorly in numerous coils to 0–96 μm (39) anterior to posterior margin of ventral sucker; descending limb of uterus passes to level of ovary in tight

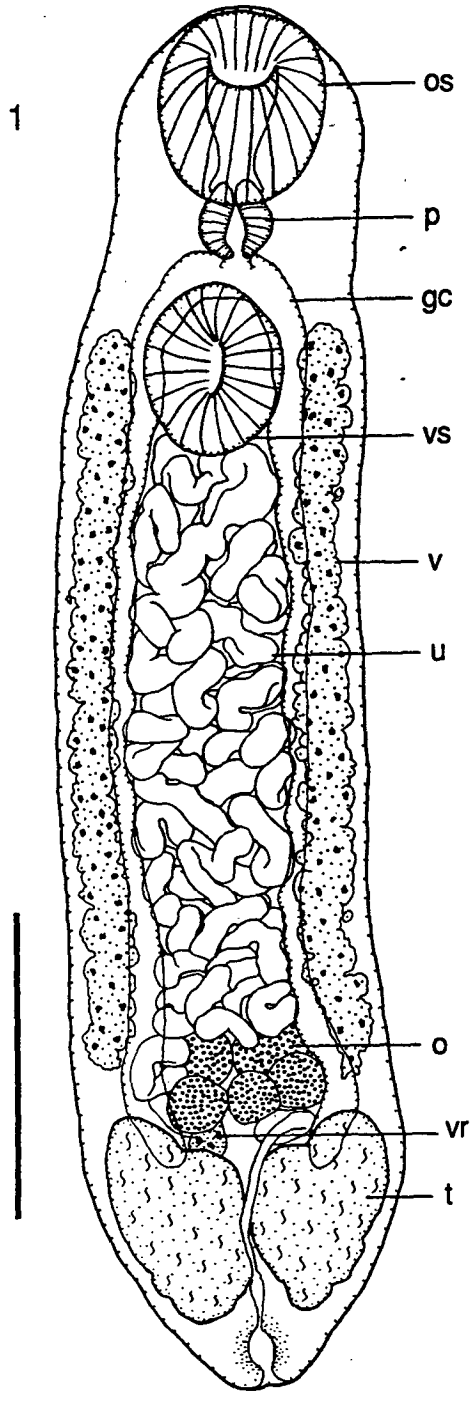
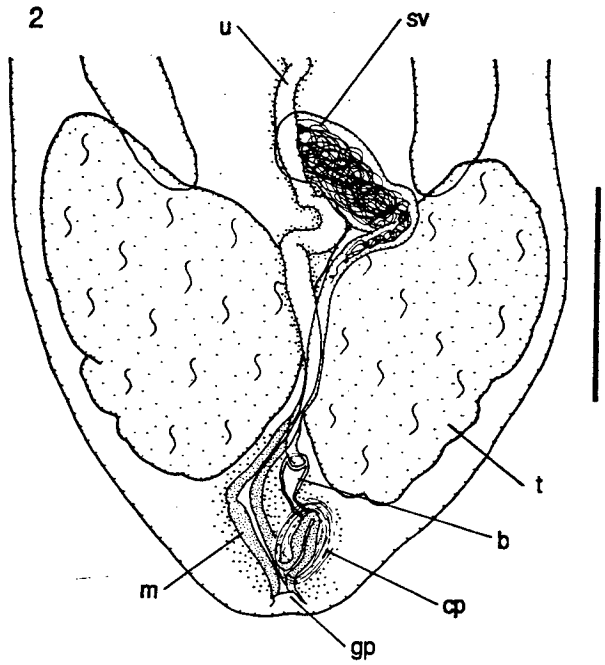
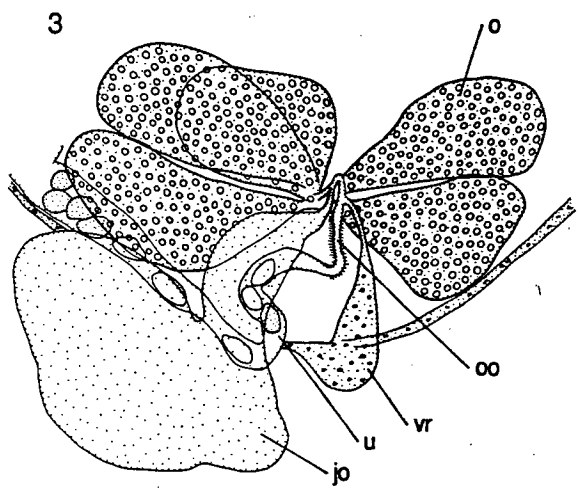
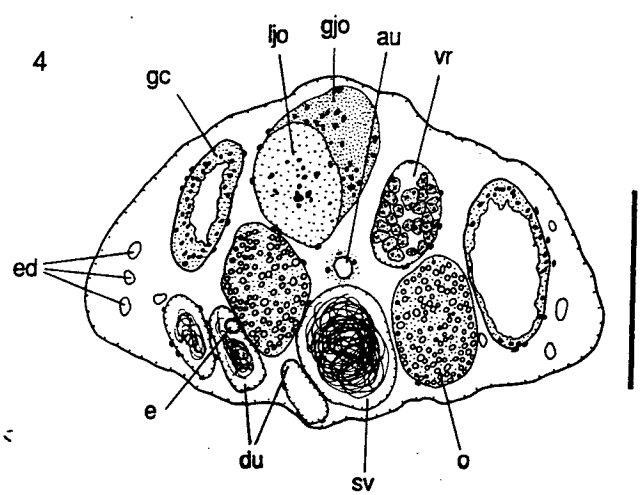


Fig. 1. Zeylanurotrema spearei sp.n. Holotype, ventral view. *gc* gut caecum; *o* ovary; *os* oral sucker; *p* pharynx; *t* testis; *u* uterus; *vr* vitelline reservoir; *vs* ventral sucker.







Figs 2–4. Zeylanurotrema spearei sp.n.—*2*. Paratype, terminal genitalia, ventral.—*3*. Egg-forming complex, dorsal, flattened. Mehlis' glands omitted.—*4*. Transverse section through posterior end; note Juel's organ and sperm in proximal coils of uterus. *au* ascending uterus; *b* bursa; *cp* cirrus pouch; *du* descending uterus; *e* egg; *ed* excretory ducts; *gc* gut caecum; *gjo* glandular portion of Juel's organ; *gp* genital pore; *jo* Juel's organ; *ljo* lumen of Juel's organ; *m* metraterm; *o* ovary; *oo* oötype; *sv* seminal vesicle; *t* testis; *u* uterus; *vr* vitelline reservoir. Scale bars: *2* = 500 μm; *3–4* = 200 μm.

coils, then constricts to form a narrow duct sheathed in gland cells, passes ventral to ovary and posterior to testes; posterior to testes uterus expands to form a large, muscular cavity which is often filled with eggs, then constricts again to form short highly muscular metraterm which opens posteriorly at genital pore. Eggs unembryonated in terminal portion of uterus, numerous, ovoid, tanned, operculate, 41–54 μm (47) long and 24–29 μm (26) wide (n=13).

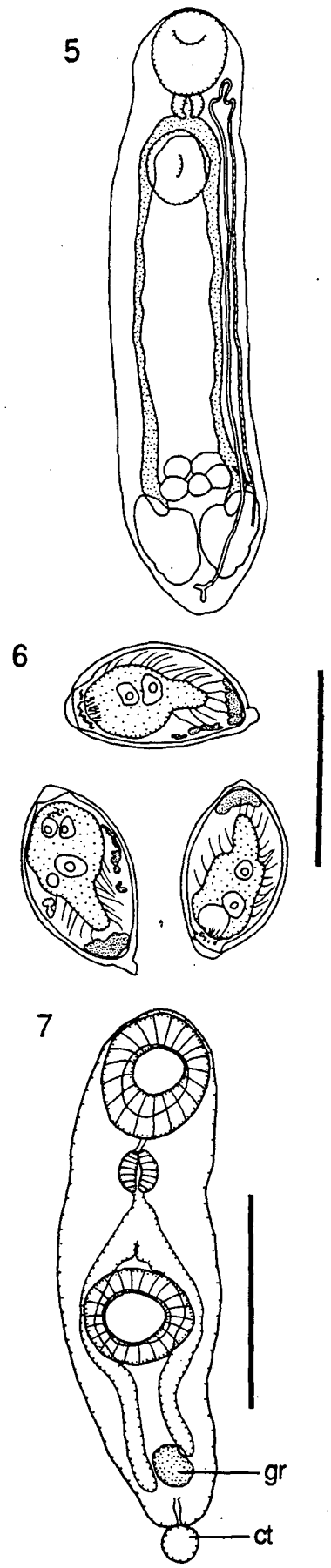
Excretory bladder small, Y-shaped, with sphincters delimiting bladder from ducts, opening dorso-subterminally (Fig. 5) Primary collecting ducts run anteriorly, lateral to intestine, to level of pharynx where they become ciliated and turn and run posteriorly; near testes primary ducts divide to form secondary ducts (Fig. 5).

Variation and allometry. Little variation in the arrangement of organs was observed. Although specimens in the type-series only measured up to 2.3 mm long, specimens up to about 3.5 mm long were common. One unusually large specimen (slightly flattened) measured about 6.6 mm and another (strongly flattened) measured about 8.7 mm. Development of the body is allometric so that in the smallest specimens the forebody occupies about 50% of the body length whereas it occupies only 12% in the largest specimen examined. The earliest egg production observed was in specimens about 1 mm long.

Egg development (Fig. 6). After 8 days at 24°C, eggs laid by specimens of *Z. spearei* sp.n. contained a developed miracidium. The miracidium was ciliated with an apical papilla. No movement was observed within the egg and the miracidia were never observed to hatch from the egg. When the egg was flattened under pressure it split, although not along the opercular line.

Juveniles (Fig. 7). A large range of juvenile specimens was examined. In the smallest seen (458 μm long) the suckers and digestive system were fully formed but the reproductive system consisted of a single primordium at the ends of the gut caeca. On a few specimens (Fig. 7) a small spherical projection was present on the posterior end of the body. This tiny structure was entirely featureless but, because of its position, is here interpreted as being a cercarial tail.

Biology. Infected toads were collected from a variety of habitats ranging from eucalypt forest, to vine forest to rain forest at sites in North Queensland as shown in Fig. 8. Prevalences of infection were as follows: 31% at Cape Weymouth, 65% at Bloomfield, 38% at Cape Tribulation, 29% at Garner's Beach and 100% (1 animal) at Moresby. Intensity of infection ranged from 1 to 231 with a mean of 54 in 52 infections. Infected animals ranged in snout-vent length from 57 to 176.5 mm. There was no significant correlation between size of toad and intensity of infection (r = 0.04, $p > 0.05$).



Figs 5–7.—5. Principal ducts of excretory system, schematic.—*6.* Eggs containing fully developed miracidia.—*7.* Juvenile; note persistent cercarial tail. *ct* cercarial tail; *gr* genital rudiment. Scale bars: *6* = 50 μm; *7* = 200 μm.

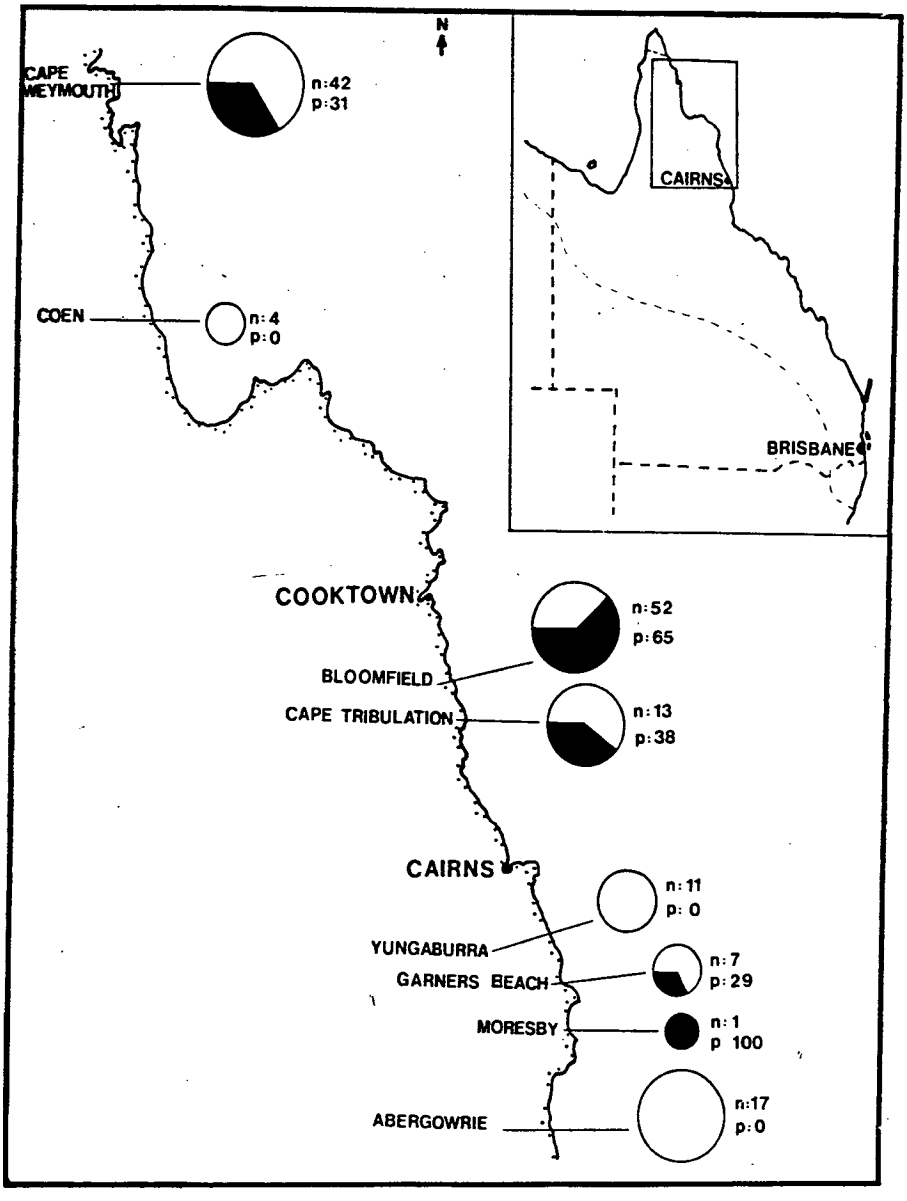


Fig. 8. Sites of infection of *Bufo marinus* with *Zeylanurotrema spearei* in north Queensland. *n* = number of toads examined, *p* = % prevalence of *Z. spearei*. Inset shows boundary of present toad distribution in Australia.

Discussion

The present species clearly belongs to *Zeylanurotrema* Crusz & Sanmugasunderam, 1974 as it has the same general disposition of the suckers, gonads, uterus and vitelline follicles as the type and only other species, *Z. lyriocephali*. However, the present form differs from *Z. lyriocephali* in a number of respects which require the erection of a new species.

Zeylanurotrema lyriocephali was described from 19 specimens from a single host. These specimens were flattened and were all over 7 mm long. Only one of our specimens (also flattened) was in this size range. It has not been possible to compare *Z. spearei* sp.n. with specimens of *Z. lyriocephali* because several efforts to borrow type material of the latter were unsuccessful. However, two characters appear to differ consistently between the two forms. The gut caeca of *Z. lyriocephali* enclose and pass posterior to the testes whereas they terminate near the anterior margin of the testes in *Z. spearei*. The vitelline follicles of *Z. lyriocephali* terminate well posterior to the posterior margin of the ventral sucker but extend well anterior to it in *Z. spearei*. In addition the ovary of *Z. lyriocephali* is relatively much smaller than that of *Z. spearei* and appears, from the original figure, to be far less regular than that of *Z. spearei*. The eggs of *Z. lyriocephali* are 29–41 by 12–20 whereas those of *Z. spearei* are 41–54 (47) by 24–29 (26).

There appear to be two possible explanations for the presence of *Zeylanurotrema spearei* in the cane toad in Australia. Either it is a natural parasite of the cane toad that was introduced to Australia with its host, or it is an Australian species that the cane toad has acquired in Australia. As the cane toads were maintained in captivity in Australia and only their progeny released (Easteal, 1981), we believe that the parasite is unlikely to have been introduced with the toad. In addition, the zoogeographical affinities between Australia and Sri Lanka are stronger than those between Australia and central America. In particular the Agamidae are found from Africa,

through southern Asia, and into Australia but not in any part of the Americas (Webb 1978). We therefore think that *Z. spearei* is probably an endemic Australian species. The natural host or hosts remain unknown. Because the only other member of the genus parasitises an agamid lizard, any of a number of agamids present in north Queensland (Cogger 1986) might be the host in Australia. However, the evident success of this species in an introduced amphibian may point to a native amphibian being the natural host.

Crusz & Sanmugasunderam (1974) proposed the genus *Zeylanurotrema* within the Urotrematidae but pointed out a number of significant differences between *Zeylanurotrema* and the two existing urotrematid genera. In this study we conclude that the genus belongs in the Brachylaimidae. The form of the digestive tract, the excretory system (stenostomate) and the reproductive system are all consistent with the Brachylaimidae. The reproductive system, with a terminal genital pore and the ovary anterior to the testes is not immediately recognizable as typical of the Brachylaimidae (e.g. *Brachylaima*) but in the subfamily Panopistinae the genital pore is characteristically terminal, or nearly so, so that this feature is also consistent with the Brachylaimidae. The form of the male terminal genitalia (i.e. enlarged *vas deferens* acting as external seminal vesicle, 'non-sperm-storing bursa', gland cells surrounding the duct outside the cirrus pouch, and cirrus pouch containing only ejaculatory duct) is strongly characteristic of the Brachylaimidae.

The discovery of what we have interpreted as a retained microcercous cercarial tail on juvenile specimens provides further strong evidence for the placement of the genus in the Brachylaimidae. Microcercous cercariae are known in the families Opecoelidae, Dicrocoeliidae, Nanophyetidae, Paragonimidae, Lissorchiidae, Troglotrematidae, Hasstilesiidae and Brachylaimidae. Of these, the morphology of the adult is consistent with the Brachylaimidae only. In addition, what could be determined of the morphology of the miracidium was consistent with what has been described previously for brachylaimids (e.g. Ulmer 1951*a*).

The most recent critical consideration of the classification of the Brachylaimidae (Mas-Coma & Gallego 1975) recognized three subfamilies, the Brachylaeminae (*sic*), Ityogoniminae and Panopistinae. Yamaguti (1971) recognized these three subfamilies as well as the Leucochloridiomorphinae and Scaphiostominae. Regardless of which classification is accepted, *Zeylanurotrema* shows most affinity to the Panopistinae in having a robust body, a post-testicular cirrus pouch, and the vitelline follicles extending neither into the forebody nor to the posterior end of the body. However, *Zeylanurotrema* differs from these and all other brachylaimids in having opposite testes and a deeply lobed ovary. The elements of the male system of *Zeylanurotrema* are largely similar to those of other brachylaimids, but the blindly ending Laurer's canal, forming a Juel's organ, is unprecedented within the Brachylaimidae. Juel's organ, defined and discussed by Gibson & Bray (1970), is thought to provide a mechanism for the resorption of excess egg-forming material and spermatozoa instead of this material being passed to the exterior and wasted.

We believe the combination of opposite testes, deeply lobed ovary and Laurer's canal forming Juel's organ to set *Zeylanurotrema* apart from other brachylaimids sufficiently to require a new subfamily. We note in this regard that Gibson & Bray (1979) considered the Juel's organ to be generally of subfamily level importance within the Hemiuroidea. Crusz & Sanmugasunderam (1974) suggested that, because of the morphology of *Zeylanurotrema* and its location within the host, a new subfamily, the Zeylanurotrematinae, might have to be erected for it within the Urotrematidae. Under the terms of the International Code of Zoological Nomenclature this does not constitute a formal proposal so the name Zeylanurotrematinae subfam.n. is proposed here formally.

Prudhoe & Bray (1982) reviewed the literature regarding trematode parasites of amphibians and gave only a single record of a brachylaimid, an immature *Brachylaemus* sp., from an Indian toad by Pande (1938). Prudhoe & Bray suggested that this was probably an accidental infection, a view that seems credible. We know of no other records of brachylaimids from reptiles or amphibians. The demonstration from this work and that of Crusz & Sanmugasunderam (1974) that brachylaimids may be parasites of reptiles and, under some circumstances, amphibians, adds a new dimension to knowledge of the biology of the Brachylaimidae. The use of the urinary bladder as site of infection is also novel for the Brachylaimidae which are mainly intestinal parasites. It is noteworthy, however, that the Panopistinae, to which *Zeylanurotrema* may be most closely related, tend to occupy extra-intestinal sites also. In considering the position of *Zeylanurotrema* we attempted to ignore the host and site in the host as taxonomic characters. The difference seen in these features, however, appears to provide good corroborative evidence that *Zeylanurotrema* is distinct from the remainder of the Brachylaimidae.

The typical brachylaimid life cycle is characterized by a microcercous cercaria produced in a terrestrial snail, the cercaria emerging and infecting another snail in which the metacercaria develops, and the definitive host becoming infected by eating the second snail. There are a number of reports (e.g. Zug *et al.* 1975; Matsumoto *et al.* 1984) that indicate that *Bufo marinus* may eat terrestrial snails so this life cycle is feasible. We are unaware of any reports of juvenile brachylaimids in the definitive host still bearing a cercarial tail. It is conceivable that this unusual finding is an indication of a two host life cycle in which cercariae within the first intermediate (snail) host are infective to the definitive host directly. However, the smallest juvenile examined (458 μm long) was still substantially larger than brachylaimid cercariae reported in the literature (e.g. 210–335 μm (255) for *Brachylaima ruminae* by Mas-Coma & Montoliu (1986); 147–246 μm (198) for *Postharmostomum helicis* by Ulmer (1951*b*); 198–360 μm (259) for *Dollfusinus frontalis* recorded by Mas-Coma & Montoliu (1987); 260 for *Pseudoleucochloridium soricis* by Jourdane (1976)). Because of this we think it most likely that the life-cycle is of the typical three host type.

Acknowledgements

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JOHNPEARSONIA GEN. NOV. AND JOHNPEARSONIINAE SUBF. NOV. (MOLINEOIDEA, NEMATODA) FROM *BUFO MARINUS*, WITH COMMENTS ON THE PRIMITIVE TRICHOSTRONGYLE PARASITES OF AMPHIBIANS AND REPTILES.

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Summary :
A new genus is proposed in the family Amphibiophilidae based on specimens parasitic in *Bufo marinus* from Australia : *Johnpersonia*. The species *Amphibiophilus egerniae* Johnston and Mawson, 1947, parasite of Australian lizard, is classified in this genus. The only genus that it is close to is *Batrachonema* Yuen, 1965, parasitic in malayian Ranidae and peruvian Bufonidae. The two genera were classified in a new subfamily : the Johnpearsoniinae mainly characterised with a bursa of the 1-3-1 type. *Johnpearsonia* is differentiated from *Batrachonema* in that the female is monodelphic and the dorsal lobe of the caudal bursa is not reduced. An analysis of the relationships between the other primitive genera of Trichostrongylina parasitic in poikilothermic animals is done.

KEY WORDS : Nematoda, primitive Trichostrongylina, Molineoidea, Johnpearsoniinae subf.nov. *Johnpearsonia* gen.nov. poikilothermic vertebrates, Australia.

MOTS CLES : *Nematodes, Trichostrongles primitifs, Molineoidea, Johnpearsoniinae nov.sub.fam.* Johnpearsonia *nov.gen. Vertébrés poikilothermes, Australie.*

Résumé : *JOHNPEARSONIA* NOV.GEN. ET *JOHNPEARSONIINAE* NOV.SUBF. (MOLINEOIDEA, NEMATODA) PARASITE DE *BUFO MARINUS*; REMARQUES SUR LES TRICHOSTRONGLES PRIMITIFS PARASITES D'AMPHIBIENS ET DE REPTILES.
Description d'un nouveau genre, Johnpearsonia, *parasite de* Bufo marinus *et d'Amphibiens endémiques en Australie.* Amphibiophilus egerniae *Johnston et Mawson, 1947, parasite d'un lézard australien, est classée dans ce genre. Le seul genre proche,* Batrachonema *Yuen, 1965 est parasite de Ranidae en Malaisie et de Bufonidae au Pérou. La nouvelle sous-famille des Johnpearsoniinae apppartenant à la famille des Amphibiophilidae est créée pour ranger ces deux genres. Cette sous-famille est principalement caractérisée par une bourse caudale de type 1-3-1.* Johnpearsonia *se différencie de* Batrachonema *par une femelle monodelphe et un mâle dont le lobe dorsal de la bourse caudale n'est pas réduit.*
Les relations existant entre les autres genres primitifs de Trichostrongylina parasites de Vertébrés poïkilothermes sont analysées.

INTRODUCTION

A new Molineoid nematode species (Trichostrongylina) was found in north-eastern Australia, in endemic amphibians and in the introduced *Bufo marinus*. The new species has similarities with *Amphibiophilus egerniae*, Johnston and Mawson, 1947, a parasite of Australian lizards.Together these two species differ from the other primitive genera known to occur in poikilothermic animals and a new genus is created for them. An analysis of the relationships between the different genera known at the present time permits a new classification which reflects the phylogeny of the group.

MATERIAL AND METHODS

Toads and native amphibians were collected from various locations throughout Queensland, Australia, and dissected within 2 days of capture. Dissection followed a fatal dose of "Lethabarb" (Euthanasia injection Pentobarbitone solution) sprinkled onto the skin. A ventral incision was made from the pelvic to the pectoral girdle exposing the body cavity . The stomach, intestine and rectum were removed and placed in 0.85% NaCl solution, opened, and any nematodes removed. Worms were fixed in Berland's fluid (Gibson, 1979) and transferred to 70% ethanol for storage.
Morphological terms to describe the bursal rays follow Durette-Desset and Chabaud, 1981 and Durette-Desset, 1985 for the synlophe. Measurements are in micrometers.
Type specimens have been deposited in the collections of the South Australian Museum, Adelaide, Australia (SAM). Voucher specimens have been deposited in the collections of the South Australian Museum (SAM), the Museum of Paris, France (M.N.H.N) and the Queensland Museum , Brisbane, Australia (QM).

RESULTS

A – DESCRIPTIONS

JOHNPEARSONIA PEARSONI, SP.NOV.

Type material : holotype male, allotype female, MNHN 676 MDa, paratypes : 1 male, 1 female, 3 L4 males, 2 L4 females, MNHN 676 MDb from small

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intestine of *Bufo marinus* (Bufonidae), Coen, Queensland. Australia.

Voucher material : from small intestine of the following hosts : 26 *Bufo marinus* (Bufonidae) from Cape Weymouth, Townsville, Bentley, and Mountain View Road near Townsville (Nov. 89 to May 91) and 7 native amphibian species : 2 *Limnodynastes ornatus* (Myobatrachidae) from Coen and Bentley (Nov. and Dec 89), 2 *Cyclorana novaehollandiae* (Hylidae) from Coen and Townsville (Nov. 89 and Feb.90), 1 *Litoria alboguttata* (Hylidae) from Townsville (Dec 89), 1 *Uperoleia lithomoda* (Myobatrachidae) from Townsville (Dec 89), 1 *Notaden melanoscapus* (Leptodactylidae) from Townsville (Feb 92). Queensland. Australia.

ADULTS

Small straight nematodes or with anterior end completely coiled. Excretory pore variable, always posterior to oesophago-intestinal junction; minute triangular-shaped deirids at same level as excretory pore (figs.1,5). Excretory glands well developed.

Head (figs. 2,3,4) : Cephalic vesicle long (110 in male, 130 in female), buccal capsule well developed; dorsal oesophagal tooth present, generally smaller in male (10-13) than in female (10-15). Absence of ring at base of buccal capsule. In apical view, 6 lips separated by 6 interlabia, 6 internal labial papillae, 6 external labial papillae of which lateral labial papillae have shared peduncle with 2 amphids and 4 cephalic papillae. Mouth hexagonal in shape.

Synlophe (studied in 6 males and 5 females) (figs.6 to 9, 11 to 15) : In both sexes, longitudinal cuticular ridges present, non-interrupted, with rounded ends; slight reinforcement of ridges visible in middle part of body in some female specimens.
Ridges appear at base of cephalic vesicle and disappear, in male, at anterior ends of spicules, and in female between level of sphincter and *vagina vera*. In vulvar region, 2 mammilli form ventral alae present; 9 high in transverse section. Left ala (130 long), begins at level of vulva; right ala (150 long) begins about 10µm posterior to it. Ridges orientated perpendicular to body surface, regularly spaced; more widely spaced opposite to median fields.

Male holotype (figs. 16 to 22) : 5300 long and 100 wide in mid-body; cephalic vesicle 110 long by 50 wide; buccal capsule 11 long. Nerve ring, excretory pore and cervical papillae 190, 470, and 470 respectively from anterior end. Œsophagus 325 long.
Bursa of the type 1-3-1, very slightly asymmetric, extended transversely. Rays 5 robust, rays 6 small. Rays 8, narrow, beginning at root of dorsal ray. Dorsal ray relatively long, divided into pairs of small terminal pointed branches. Spicules narrow, 400 long; alae start 250 from proximal end; each spicule terminates in 3 points enclosed within membrane : externolateral branch, ventral branch 70 long which appears on externolateral branch and dorsal branch. Gubernaculum 90 long by 18 wide. Genital cone rounded, 30 long by 40 wide proximally, papillae zero and papillae 7 elongated; papillae 7 bifid at tip.

Female allotype (fig. 10) : 8700 long by 120 wide in mid-body. Cephalic vesicle 130 long by 50 wide. Buccal capsule 12 long. Nerve ring, excretory pore and cervical papillae 220, 590 and 590 respectively from anterior end. Œsophagus 380 long.
Monodelphic, with vestigial posterior genital branch, reduced to a cellular mass, 80 long. Vulva 240 from posterior end. *Vagina vera* 55 long, oblique. Anterior genital branch with ovejector consisting of vestibule 195 long, sphincter 40 long and infundibulum 85 long. Uterus 2240 long containing 68 eggs in morula stage, 65 long by 35 wide. Tail 80 long by 30 wide at level of anus, with caudal spine 20 long.

4 TH LARVAL STAGE

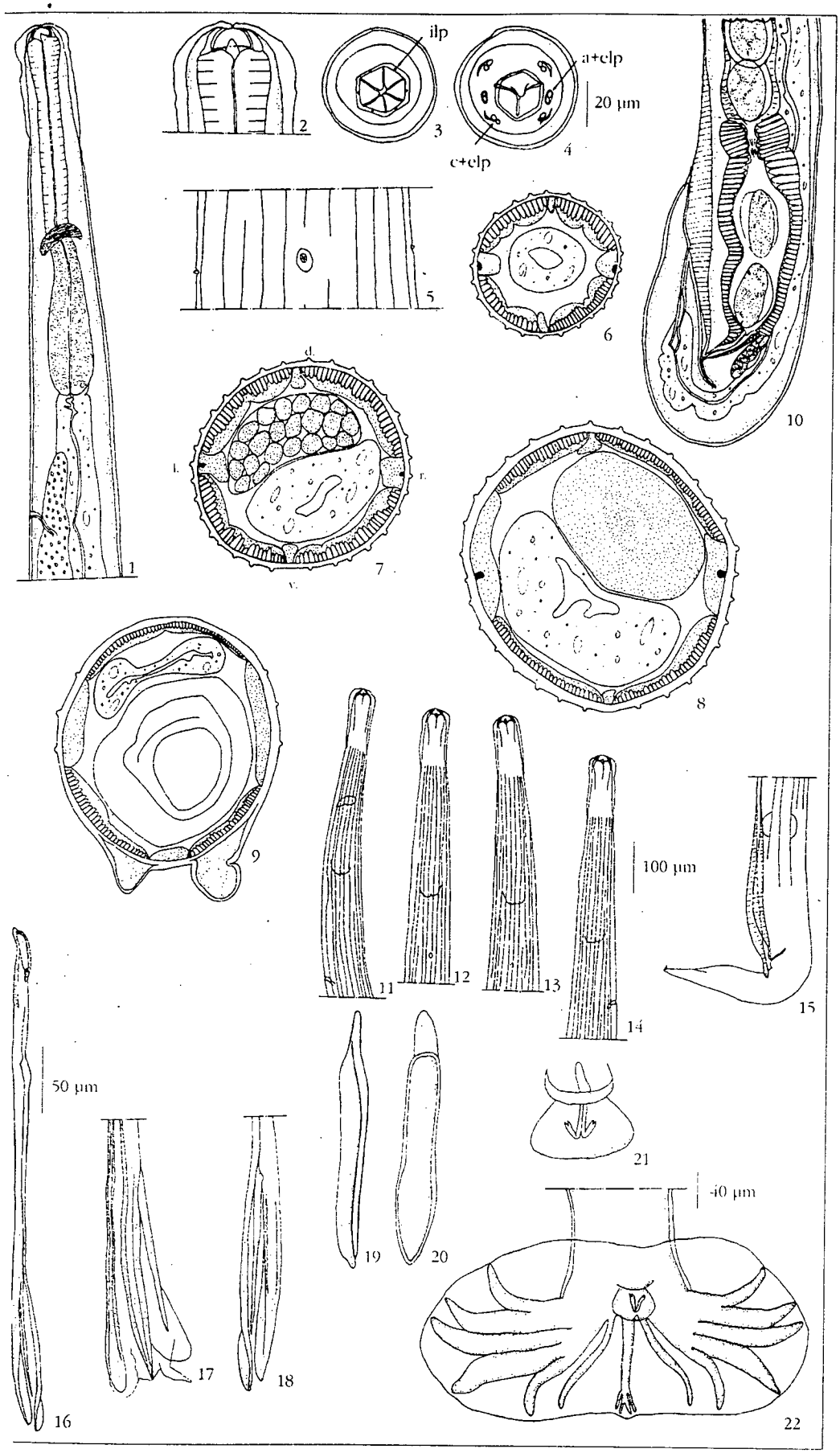
Head (figs. 23, 24, 25) : Absence of cephalic vesicle; small buccal capsule present. In apical view, 6 lips, 6 external labial papillae, 4 cephalic papillae and 2 amphids. Mouth hexagonal; oesophagal tooth absent.

Synlophe (fig. 26) : 2 small lateral ridges with an internal support, orientated perpendicular to body surface.

Male (figs. 27, 28) : Length 2170 width 50 nerve ring, excretory pore and deirids 120, 245 and 245 respectively from anterior end; parts of the genital system well differentiated : testis 460, seminal vesicle 270, deferent canal 270.
Female (fig.29) : Length 2720, width 50 nerve ring, excretory pore and deirids 145, 330 and 330 respectively from anterior end. Vulva 190 from caudal extremity. Anterior genital branch 3 times larger than posterior branch. Latter does not develop but remains visible in adult in vestigial form. Tail, 90 long.

REMARKS

The species does not belong to any of the genera already known in amphibians and reptiles. We regard it as the type species of the new genus *Johnpearsonia pearsoni* named in honour of our colleague Prof. J. C. Pearson from the University of Queensland.

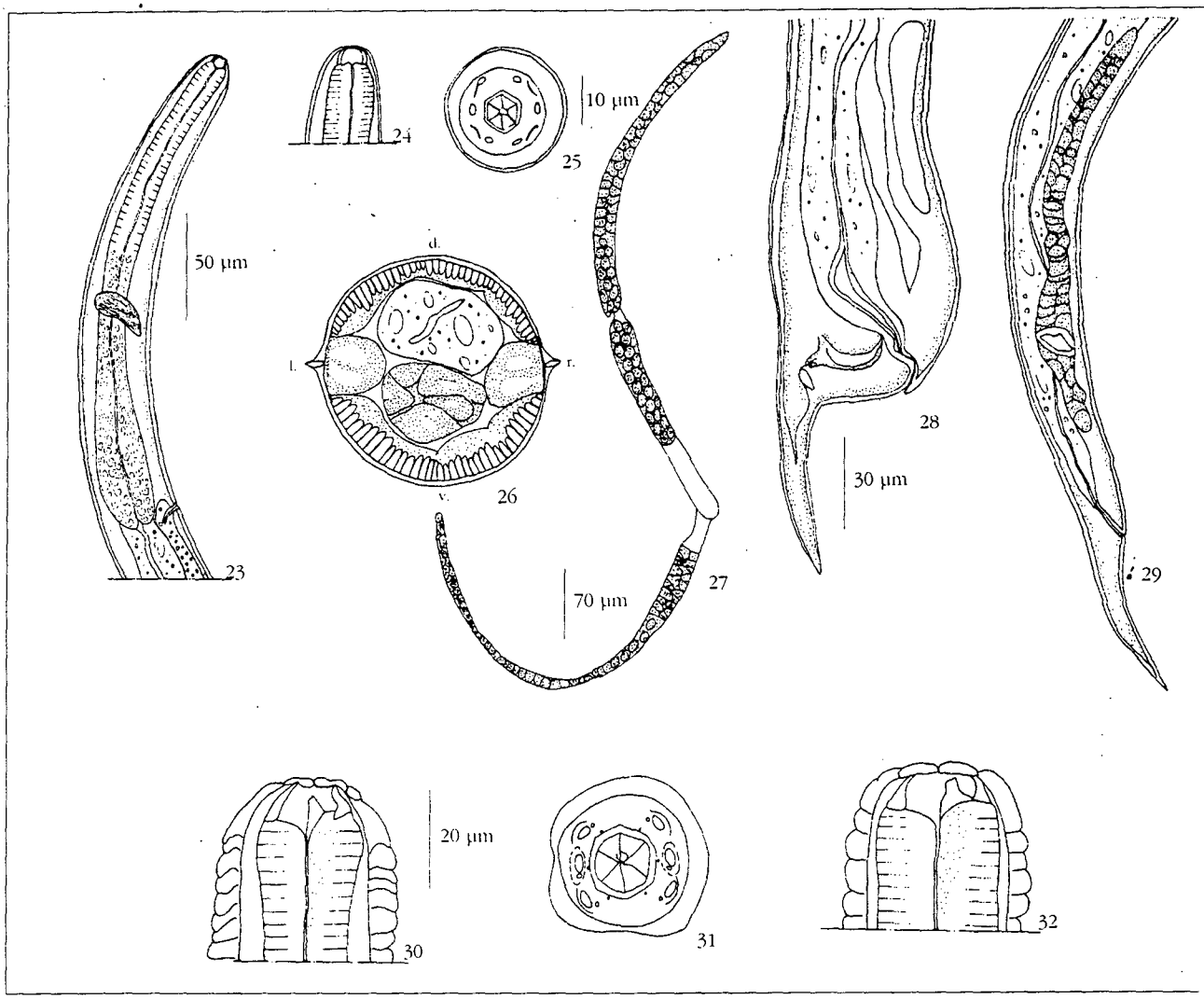


Figs. 1-22. – *Johnpearsonia pearsoni* n.gen., n.sp. from *Bufo marinus*. Adults. 1, anterior part of female, left lateral view; 2, head of female, dorsal view; 3, 4, head of female, apical view, showing the 6 lips and the interno labial papillae then the oesophagal tooth and the other papillae; 5, detail of excretory pore and deirids of female, ventral view; 6-9, synlophe. 6, male, at the oesophago intestinal junction; 7 male, at the middle of the body; 8, female, at the middle of the body ; 9, female, at level of the ovejector; 10, ovejector and tail of female, left lateral view; 11-14, anterior part of female showing the appeareance of cuticular ridges. 11, left lateral view, 12, ventral view, 13, dorsal view, 14, right lateral view; 15, tail of female showing the vulvar lateral alae; 16-18, right spicule, ventral, externo-lateral and dorsal views, 19,20, gubernaculum, right lateral and ventral view; 21, genital cone, ventral view; 22, caudal bursa of the male, ventral view.

Cross sections of the body are orientated as figure 7

a= amphid, ilp= interno-labial papilla, elp= externo-labial papilla, c= cephalic papilla, d = dorsal, v = ventral, r = right, l = left

Scale lines : 1-10, 17 -21 : 20µm; 11-15 : 100µm; 16 : 50µm; 22 : 40µm.



Figs.23-29. – *Johnpearsonia pearsoni* n.gen., n.sp. from *Bufo marinus*. Fourth larval stages. 23, anterior part of female, right lateral view 24, head of female, lateral view ; 25, head of male, apical view ; 26, synlophe of male in midbody ; 27, dissected genital apparat of male showing the testicule, the seminal vesicle and the deferent canal; 28, tail of male ; 29, tail and genital apparat of female, showing the developed anterior genital branch and the atrophied posterior genital branch, right lateral view.

Figs. 30-32. – *Wanaristrongylus cteroti* from *Ctenotus grandis*. 30, 31, head of male, left lateral view, showing the dorsal oesophagal tooth and apical view, showing the 6 lips, the 6 interno labial papillae, the 6 externo labial papillae, the 2 amphids and the 4 cephalic papillae 32, head of female, left lateral view.

Scale lines : 23, 29 : 50µm ; 24,28 : 30µm ; 25, 26 : 10µm ; 27 : 70µm ; 30-32 : 20µm.

JOHNPEARSONIA EGERNIAE
(JOHNSTON & MAWSON, 1947), COMB. NOV.

Synonymy : *Amphibiophilus egerniae* Johnston & Mawson, 1947, p. 23, figs. 1-3

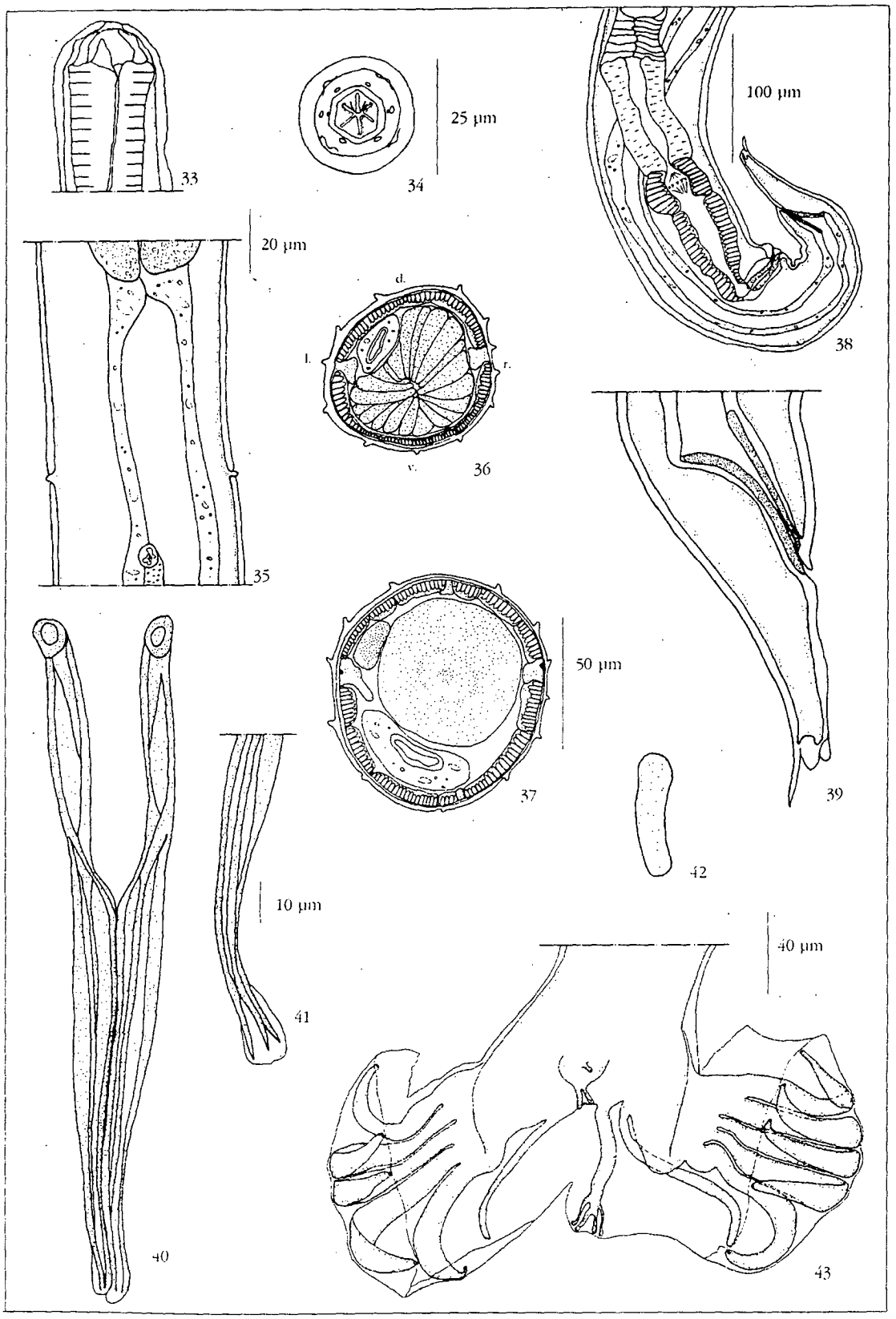
Material examined : 2 males, 2 females, (SAM n° 8667) from the small intestine of *Egernia striata* (Scincidae) ; 5 females (SAM n° 8666) from the small intestine of *Egernia kintorei* (Scincidae), Itavi Rock, Musgrave Range, South Australia.

ADULTS

Small nematodes, loosely coiled. Excretory pore and cervical papillae posterior to oesophago-intestinal junction (fig. 35).

Head (figs. 33, 34) : Cephalic vesicle elongate (80 i male, 82-90 in female), buccal capsule well develc ped, dorsal œsophageal tooth present, smaller i male (9) than in female (10-12). Absence of a ring : base of buccal capsule. In apical view, 6 lips separ: ted by 6 interlabia ; internal labial papillae not obse ved ; 6 external labial papillae, 2 amphids and cephalic papillae. Mouth hexagonal in shape.

Synlophe (studied in one male and one female) (fig 36, 37) : In both sexes, uninterrupted longitudin cuticular ridges not with rounded apices. Ridg appear at base of cephalic vesicle and disappe about 400 anterior to bursa in male, and anterior anus in female. In both sexes, 12 ridges in mid-bod



Figs.33-43. – *Johnpearsonia egerniae* (Johnston and Mawson, 1947) n.comb. from *Egernia striata*. 33, 34, head of male, right lateral view then apical view; 35, detail of excretory pore and deirids in male, ventral view; 36, 37, synlophe at mid body of male then female; 38, ovejector and tail of female, right lateral view; 39, tail of female showing the two ventral tubercles, right lateral view; 40, dissected spicula, ventral view; 41, tip of the right spicule, dorsal view; 42, gubernaculum, ventral view; 43, caudal bursa, ventral view.

Cross sections of the body are orientated as figure 36
d = dorsal, v = ventral, r = right, l = left
Scale lines : 33,35,39 : 20µm; 34 : 25µm; 36, 37 : 50µm; 38 : 100µm; 40-42 : 10µm; 43 : 40µm.

4 dorsal, 6 ventral, 1 right lateral, 1 left lateral. Ridge situated opposite each lateral cord and adjacent ventral crest orientated perpendicular to body surface; other crests orientated slightly from right ventral direction towards left dorsal, axis of orientation inclined 35°-40° from frontal axis. Ridges unequal in size and irregularly spaced. Vulvar alae absent in female.

Male (figs. 40, 41, 42, 43) : Length 4200, 65 wide in mid-body; nerve ring, excretory pore and cervical papillae 175, 360 and 345 respectively from anterior end. Oesophagus 300 long.

Bursa of the 1-3-1 type, very slightly asymmetrical, spread transversely. Rays 5 and 6 robust. Rays 8 narrow, beginning at root of dorsal ray. Dorsal ray relatively long, divides into 2 small branches at distal extremity. Spicules narrow, alate, 160 long, divided into 2 branches at distal extremity, one ventral, one dorsal : dorsal branch slightly curved in internal view, bifurcated in dorsal view. Gubernaculum 30 long, 7 wide. Genital cone triangular in shape, bearing on ventral lip elongated papilla zero and on ventral lip, 2 finger-shaped papillae 7.

Female (figs. 38, 39) : Length 5900, 80 wide in mid-body; nerve ring, excretory pore and cervical papillae 185, 390 and 380 situated from anterior end respectively. Oesophagus 345 long.

Monodelphic; vestigial posterior branch not observed; vulva 135 from posterior end; *vagina vera*, well developed, 32 long; ovejector with vestibule, sphincter and infundibulum 85, 35 and 90 long, respectively. Uterus 1000 long, containing 28 embryonated eggs, 65 long by 35 wide. Tail 53 long with caudal spine 15 and two ventral tubercules 5 in height.

REMARKS

The species differs from *Amphibiophilus* in having a bursa of 1-3-1 type as opposed to 2-3 type, a reduced non-hypertrophied dorsal lobe and a monodelphic rather than didelphic female.

In all these characteristics it resembles the preceding species . The two species can be differentiated by the synlophe, the size of the rays 6 in relation to rays 5; the shape of the spicules and of the gubernaculum and the presence or absence of vulvar alae. We therefore regard the specimens from *Egernia* as a second species in the genus *Johnpearsonia*.

B – DEFINITIONS

JOHNPEARSONINAE SUBF. NOV.

Amphibiophilidae. Bilaterally symmetrical synlophe in the larva but not always in adult; bursa of the 1-3-1 type; female didelphic or monodelphic with caudal spine . Parasitic in Gondwanian amphibians. Type-genus : *Johnpearsonia* gen. nov. Other genus *Batrachonema* Yuen, 1965.

JOHNPEARSONIA GEN. NOV.

Head with 6 lips, well developed buccal capsule and a strong dorsal œsophageal tooth. Absence of a ring at the base of the buccal capsule. Bilaterally symmetrical larval synlophe. Adult synlophe bilaterally symmetrical or with an oblique axis. Bursa of the 1-3-1 type with dorsal lobe neither hypertrophied nor reduced. Rays 8 of bursa beginning at the root of the dorsal ray. Spicules with two or three points. Gubernaculum present. Female monodelphic with caudal spine.

Type species : *J. pearsoni*, sp.nov., parasitic in Australian amphibians. Other species : *J. egerniae* (Johnston and Mawson, 1947), comb. nov. parasitic in Australian lizards.

DISCUSSION

Among Trichostrongylina which parasitize amphibians or reptiles, a group of species can be readily identified as relicts. The presence of six lips, the well developed buccal capsule and the large œsophageal tooth indicate a transitional state from the Strongylina. This exclusively Gondwanian group presents therefore a certain homogeneity, but it is important to analyse it in greater detail since it lies close to the major division within the Trichostrongylina which occurs between the Molineoidea and Heligmosomoidea (cf. Durette Desset and Chabaud, 1993).

Seven genera occur in this group : *Herpetostrongylus*, *Vaucherus*, *Wanaristrongylus*, *Amphibiophilus*, *Batrachostrongylus*, *Batrachonema* and *Johnpearsonia*.

Herpetostrongylus Baylis, 1931 is characterised by the presence of three teeth, a bursa of the 1-3-1 type with rays 2 and 3 more strongly developed than 5 and 6, didelphic female and a synlophe of the heligmosomoidean type (a species with 2 left ventral humps, species with an oblique axis running from a right ventral direction to a left dorsal one). The genus consists of two species from Queensland, *H. python* Baylis, 1931 from *Python spilotes* and *H. varani* Baylis, 1931 from *Varanus gouldii*. As well *H. pythonis* occurs in Oceania (Schmidt and Kuntz, 1972).

Vaucherus Durette-Desset, 1980 is close to the preceding species because of the hypertrophy of rays and of the conservation of the didelphic female, but the bursa has become the 2-2-1 type in place of the 2-3. The genus comprises three species, *V. vauchei* Durette-Desset, 1980 parasitic in *Varanus rudicollis*

Malaysia, *V. indicus* (Deshmukh, 1969), and *V. leiperi* (Sharief, 1957) parasitic in *Varanus indicus* in India. Both *Herpetostrongylus* and *Vaucherus* have an asymmetric synlophe similar to that of the Heligmosomoidea and are therefore distinct from the two species described above.

Wanaristrongylus Jones, 1987 possesses a single tooth in the buccal cavity (figs.30, 31, 32), a synlophe with ridges orientated perpendicularly to the body surface and the female is monodelphic. The bursa is of the 2-3 type in two species and has equally spaced rays in the third. The genus was placed in the Herpetostrongylidae by Jones (1987), but in this family the synlophe is never bilaterally symmetrical. Therefore the synlophe as well as the bursa, indicate that the genus is close to *Amphibiophilus* in the Molineoidea. The genus comprises three species which parasitize Australian lizards, *W. pogonae* Jones, 1987, parasitic in *Pogona minor*; *W. ctenoti* Jones, 1987, parasitic in *Ctenotus grandis* and *W. papangawurpae* Jones, 1987, parasitic in *Nephrurus laevissimus*.

Amphibiophilus Skrjabin, 1916 has a bilaterally symmetrical synlophe with ridges perpendicular to the body surface. The bursa is of the 2-3 type with a very hypertrophied dorsal lobe. The female is didelphic. The genus comprises four species which parasitize Ranidae in eastern and southern Africa, *A. acanthocirratus* Skrjabin, 1916, *A. natalensis* (Walton, 1935), *A. chabaudi* Puylaert, 1967 and *A. versterae* Baker, 1981.

Batrachostrongylus Yuen, 1963 appears to lack a buccal capsule and has a single, robust œsophageal tooth. It has been placed close to *Amphibiophilus* since the buccal capsule is comparable. The female is didelphic. The genus is monotypic with *B. longispiculus* Yuen, 1963, redescribed by Baker (1983), parasitic in *Megophrys nasuta* in Malaysia.

Batrachonema Yuen, 1965 belongs to the Molineoidea since the larval synlophe is bilaterally symmetrical. In the type-species, the bilateral symmetry is preserved in the adult. However in the adult of the second species, the axis of orientation of the ridges runs from right to left while the fourth stage larva has one ridge opposite each lateral field, orientated perpendicular to the body surface, confirming that *Batrachonema* belongs within the Molineoidea. The bursa is of the 1-3-1 type, with a marked reduction of the dorsal lobe. Rays 8 begin on the dorsal ray. The female is didelphic and a ring is present at the base of the buccal capsule. The genus comprises two species, *B. synaptospicula* Yuen, 1965, redescribed by Baker (1983), parasite of Ranidae in Malaysia and *B. bonai* Durette-Desset, Baker and Vaucher 1984, parasitic in *Bufo typhonius* in Peru *.

Johnpearsonia gen. nov. has a bilaterally symmetrical larval synlophe whereas the adult synlophe may be bilaterally symmetrical or may have an oblique axis of orientation with oblique ridges. The bursa is of the 1-3-1 type. The only genus that it is close to therefore is *Batrachonema*. The two species studied above cannot, however, be classified in this genus since the dorsal lobe of the caudal bursa is not reduced, rays 8 begin at the base of the dorsal ray and the female is monodelphic.

We have therefore created a new genus as defined.

Relationships between the genera

The species belonging to the genera named above have in common a primitive cephalic structure with 6 lips, a hexagonal mouth and a buccal capsule but in reality they belong to very different groups :

Herpetostrongylus and *Vaucherus* (Herpetostrongylidae, Herpetostrongylinae) have a synlophe that is never bilaterally symmetrical either in larval or in the adult stage. They therefore belong to the Heligmosomoidea and are distinct from the other genera in the remarkable development of the ventral rays of the bursa. They are known from reptiles (varanids and more rarely snakes) in the Oriental and Australian biogeographic regions and appear to be the origin of the Nicollinidae, a family parasitic in monotremes but which has subsequently invaded Australian marsupials.

Amphibiophilus, *Batrachostrongylus* and *Wanaristrongylus* (Amphibiophilidae, Amphibiophilinae) have a bilaterally symmetrical synlophe. They therefore belong to the Molineoidea. The bursa is of the 2-3 type. The first two genera have a bursa with a very well developed dorsal lobe and have didelphic females; the third one, *Wanaristrongylus*, has a smaller dorsal lobe, and a monodelphic female. *Batrachostrongylus* has an aberrant buccal structure and ovejector.The genus *Amphibiophilus* appears the most primitive morphologically.

Batrachonema and *Johnpearsonia* (Amphibiophilidae, Johnpearsoniinae) have a bilaterally symmetrical synlophe in the larval stage that, sometimes, lose its bilateral symmetry in the adult. They therefore belong to the Molineoidea. In contrast to all the other members of this superfamily, the bursa is of the 1-3-1

* Hasegawa, 1987, reports the occurrence of *B. synaptospicula* in 3 species of Ranidae in Japan. But the axis of orientation is directed from right to left as in *B. typhonius*. According to Baker, 1983, the crests are orientated prependicularly to body in *B. synaptospicula*. The Japan specimens cannot therefore be identified to *B. synaptospicula*.

type, a type only known up until now in the superfamilies Trichostrongyloidea and Heligmosomoidea. Due to the differences in the synlophe and the type of caudal bursa, the two genera appear to be original and need to be separated from the Amphibiophilinae. These characteristics indicate a particularly archaic group. Its geographic distribution supports this view since the species are widespread, occur in amphibians only, and almost all in the Gondwanian region.

The data given above lead to the creation of a new subfamily for which we have proposed the name Johnpearsoniinae nov. subfamily as defined above.

The seven genera with primitive cephalic structures, parasitic in amphibians and reptiles are classified in the following manner :
Molineoidea : Amphibiophilidae-Amphibiophilinae : *Amphibiophilus, Batrachostrongylus* and *Wanaristrongylus*; Amphibiophilidae-Johnpearsoniinae nov. subfam. *Johnpearsonia* and *Batrachonema*. Heligmosomoidea : Herpetostrongylidae-Herpetostrongylinae : *Herpetostrongylus* and *Vaucherius*.

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Three species of the genus *Dolichosaccus* Johnston, 1912 (Digenea: Telorchiidae) from the introduced toad *Bufo marinus* (Amphibia: Bufonidae) in Australia, with the erection of *Meditypus* n. subg.

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Abstract

Members of the genus *Dolichosaccus* are recorded from the introduced cane toad in Australia for the first time. Redescriptions of the species *D. symmetrus* (Johnston, 1912) Yamaguti, 1958 and *D. juvenilis* (Johnston, 1912) Travassos, 1930 are provided and a new species, *D. helocirrus* n. sp., is described. *Litoria caerulea* is also recorded as a host for *D. helocirrus* n. sp. A new subgenus, *Meditypus*, is erected to accommodate *D. juvenilis*, *D. grandiacetabularis* Moravec & Sey, 1989, *D. longibursatus* Moravec & Sey, 1989 and *D. helocirrus* n. sp. *D. schmidti* Fischthal & Kuntz, 1975 is transferred from the subgenus *Dolichosaccus* to the subgenus *Lecithopyge* on the basis of the extent of the uterus. A key to the subgenera is provided.

Introduction

During a survey of the helminths of the introduced cane toad *Bufo marinus* in Australia, three species of the digenean genus *Dolichosaccus* Johnston, 1912 were found. This genus has not previously been recorded from toads in Australia. Prior to this study, six species of *Dolichosaccus* had been recorded from Australian amphibians. Only one of these, *D. juvenilis* (Nicoll, 1918) Travassos, 1930, had previously been recorded in Queensland.

Materials and methods

Toads were collected from various locations and dissected within 2 days of capture. Dissection followed a fatal dose of 'Lethabarb' (Euthanasia injection Pentobarbitone solution) sprinkled onto the skin. A ventral incision was made from the pelvic to the pectoral girdle exposing the body cavity. The stomach, intestine and rectum were removed and placed in 0.85% NaCl solution, opened and any trematodes removed.

Digeneans were studied, live and partly flattened beneath a coverslip, using bright field and differential interference contrast microscopy. Worms were fixed in Berland's fluid (Gibson, 1979), Bouin's fluid made up with distilled water, or calcium acetate buffered formalin. Some specimens were partly flattened beneath a coverslip when fixed. Whole-mounts were stained with Gower's carmine or acetocarmine, dehydrated, cleared with xylene and mounted in Canada balsam. Unstained whole-mounts were also prepared in this manner. Measurements are given in micrometres as means with the range in parentheses. Drawings were made with the aid of a camera lucida. The classification of the helminths followed the system of Prudhoe & Bray (1982).

Sources from which specimens were borrowed and museums in which specimens have been deposited are indicated as follows: AM, Australian

Museum, Sydney, Australia; BM(NH), The Natural History Museum, London, UK; CAS, Czechoslovak Academy of Sciences, České Budějovice, Czechoslovakia; QM, Queensland Museum, Brisbane, Australia; THC, personal collection of Dr Tom Cribb, Department of Parasitology, University of Queensland, Brisbane, Australia; USNMHC, United States National Museum Helminthological Collection, Beltsville, Maryland, USA.

The following specimens were studied for comparative purposes: USNMHC No. 59630 *Dolichosaccus rastellus subulatus*; BM(NH) No. 1923.6.27.94, 1933.7.19.105, 1933.7.19.120, 1933.7.19.110, 1922.10.24.3, 1933.7.19.119 *Opisthioglyphe rastellus*; BM(NH) No. 1981.7.24.1–5 (2 slides), 1984.10.9.17, 1961.10.3.3–6 (2 slides), unregd (4 slides) *D. rastellus*; BM(NH) No. 1929.4.8.104 *Lecithopyge rastellum subulatum*; USNMHC No. 36505 (2 slides), 51591 *D. trypherus*; AM No. W334a *Dolichosaccus trypherus*; BM(NH) No. 1968.4.29.26 *Dolichosaccus trypherus*; QM GL11848 *D. trypherus*; AM No. W335 *D. ischyrus*; AM No. W336 *D. diamesus*; USNMHC No. 51422 (2 slides) *Brachysaccus anartius*; AM No. W337a *B. anartius*; QM GL11846 *D. anartius*; AM No. W338 *B. symmetrus*; QM GL11280 *D. juvenilis*; USNMHC No. 61722 *O. lygosomae*; USNMHC No. 79790 (vial) *D. (Lecithopyge) novaezelandiae*; BM(NH) No. 1946.12.31.105, 1982.5.21, 1977.8.2.1–4 *D. novaezelandiae*; USNMHC No. 73025 *D. schmidti*; CAS No. D-232 *O. cophixali*; CAS No. D-233 *D. grandiacetabularis*; CAS No. D-234 (2 slides) *D. longibursatus*.

Family Telorchiidae Stunkard, 1924
Subfamily Opisthioglyphinae Dollfus, 1949
Genus *Dolichosaccus* Johnston, 1912
Subgenus *Brachysaccus* Johnston, 1912

***Dolichosaccus* (*Brachysaccus*) *symmetrus* (Johnston, 1912) Yamaguti, 1958** (Fig. 1)

Host: *Bufo marinus* Linnaeus, 1758 (Bufonidae).
Locality: Queensland, Australia.
Site: Intestine.
Material: Voucher specimens deposited as BM(NH) No. 1992.12.21.1, *ex* intestine of *Bufo marinus* (Bufonidae), Townsville, North Queensland, Australia (19°15′ S, 146°49′ E), April 1989, Coll. D. Barton; QM No. QL18322, *ex* intestine of *B. marinus*, Townsville, April 1989, Coll. D. Barton; USNMHC No. 82706, *ex* intestine of *B. marinus*, Brisbane, South Queensland, Australia (27°30′ S, 153°01′ E), October 1989, Coll. D. Barton; CAS No. D-303, *ex* intestine of *B. marinus*, Bentley, North Queensland, Australia (19°05′ S, 146°57′ E), April 1990, Coll. D. Barton.

Description

(Measurements based upon 15 mounted specimens) Body long, lancet-shaped, with ends tapered, 3,744 (3,020–4,520) long and 942 (600–1,200) wide. Tegument spinose; spines dense anteriorly but sparse posteriorly. Oral sucker larger than ventral sucker, 260 (208–296) long and 298 (240–344) wide. Ventral sucker in anterior third of body, posterior to intestinal bifurcation, 216 (184–264) long and 218 (168–256) wide. Prepharynx short; pharynx 130 (120–140) in diameter; oesophagus short. Gut-caeca simple, unbranched, lateral in body for entire length, terminate at posterior extremity of body. Gonads in posterior half of body. Common genital pore distinctly posterior to intestinal bifurcation.

Testes round to cuboid, one obliquely behind other in mid-line, in posterior third of body. Anterior testis 238 (184–272) long and 284 (224–312) wide; posterior testis 292 (240–352) long and 288 (256–320) wide. Sperm duct from each testis arises on anterior face, passes anteriorly almost directly to base of cirrus-sac. Cirrus-sac 493 (336–624) long, extends to posterior edge of ventral sucker, contains bipartite seminal vesicle in proximal quarter, then prostatic region, undifferentiated glandular region, and cirrus.

Ovary longitudinally oval, median, in posterior half of body, distant from ventral sucker, 259 (160–448) long and 218 (136–248) wide. Oviduct arises from posterior face of ovary, initially traverses small muscular chamber then receives ducts

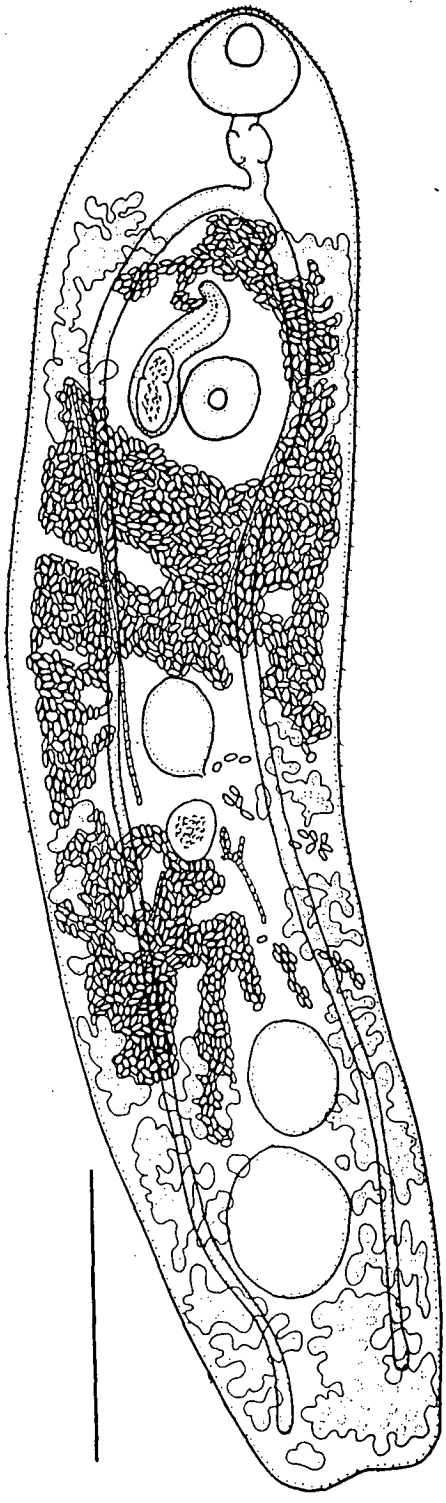

Fig. 1. Dolichosaccus (*Brachysaccus*) *symmetrus*, whole-mount, ventral view. *Scale-bar*: 750 μm.

from seminal receptacle, Laurer's canal and then vitelline reservoir before entering uterus. Mehlis' gland not observed. Uterus highly coiled, overlaps intestinal caeca in mid-body region, extends from level of testes forward to genital pore. Eggs *in utero* yellow, unembryonated, oval, 47 (44–50) long and 24 (20–28) wide. Laurer's canal opens dorsally posterior to ovary. Seminal receptacle posterior to ovary, variable in size, opens in common with Laurer's canal into oviduct. Vitelline follicles large, in 4 separate fields; anterior fields lateral, extend from level of intestinal bifurcation to posterior edge of ventral sucker; posterior fields extend from level of ovary to posterior end of body, fill much of post-testicular region. Vitelline reservoir posterior to seminal receptacle.

Excretory opening near posterior extremity. Excretory bladder not observed.

Molecular sequence of ribosomal internal transcribed spacer regions is reported in Luton *et al.* (1992).

Biology

Infected toads were collected from 5 sites ranging from urban (Townsville, Boyne Is., Brisbane) to open eucalypt farmland (Townsville, Bentley, Mackay). Prevalence of infection was as follows: 2.9% at Townsville; 9.1% at Bentley; 16% at Mackay (21°09′ S, 149°11′ E); 11.5% at Boyne Is. (23°57′ S, 151°21′ E); and 64.7% at Brisbane. Intensity of infection was 1–32 with mean of 7.29 in 34 infections. Infected animals ranged in snout-vent length (SVL) from 51–112.5 mm. There was no significant correlation between SVL and intensity of infection ($r = -0.0680$). Specimens of *D. symmetrus* were also collected from toads in Brisbane and Broken Wheel Waterhole (19°07′ S, 140°31′ E) by Dr Tom Cribb in 1982 and 1983. No data were available on prevalence or intensity of infection.

Differential diagnosis

Dolichosaccus symmetrus Johnston, 1912 is most closely related to *D. anartius* Johnston, 1912 from *Litoria aurea* and *Limnodynastes peronii* from

New South Wales, Australia. The two species were originally differentiated by body size, sucker size and vitelline distribution: *D. symmetrus* was held to be much smaller than *D. anartius*, the oral sucker larger than the ventral sucker (ventral larger than oral for *D. anartius*), and the vitellarium separated into anterior and posterior lateral fields.

The specimens collected from *B. marinus* were identified as *D. symmetrus* due to the interrupted lateral vitelline fields, the testes sitting obliquely one behind the other, and the uterus filling the body space between the testes and the genital pore. Their size, however, provoked the question as to whether *D. symmetrus* should be synonymised with *D. anartius*, which has a similar morphology but is larger. The only morphological difference between the two species was the interrupted lateral vitelline fields. As all specimens collected in this study, regardless of size, exhibited this feature, it was decided not to synonymise the two species.

***Meditypus* n. subg.**

Diagnosis

Telorchiidae. *Dolichosaccus*. Small, elongate worms with rounded ends. Tegument spiny. Oral and ventral suckers well developed, of approximately equal size; ventral sucker in anterior half of body. Prepharynx, pharynx and oesophagus present. Intestinal caeca end blindly close to posterior end of body. Excretory bladder with long stem. Genital pore in mid-line, at or just posterior to intestinal bifurcation, anterior to ventral sucker. Testes large, rounded, opposite or slightly oblique, in posterior half of body. Cirrus-sac banana-shaped, running from genital pore to posterior margin of ventral sucker; seminal vesicle bipartite; cirrus may or may not be armed with spines or knobs. Laurer's canal present. Vitelline glands well developed, lateral along entire body length. Uterus relatively short, lying only between ovary and genital pore.

Type-species: *Dolichosaccus* (*Meditypus*) *juvenilis* (Nicoll, 1918) Travassos, 1930 from *Cyclorana brevipes*, *Bufo marinus*, Australia.

Other species: *D.* (*M.*) *grandiacetabularis* Moravec & Sey, 1989 from *Rana grisea*, Papua New Guinea; *D.* (*M.*) *longibursatus* Moravec & Sey, 1989 from *R. grunniens*, Papua New Guinea; *D.* (*M.*) *helocirrus* n. sp. from *B. marinus*, *Litoria caerulea*, Australia.

Etymology: Named for characteristics intermediate between the subgenera *Dolichosaccus* and *Brachysaccus* (*medius*, Latin for middle; *typus*, Latin for shape, example).

***Dolichosaccus* (*Meditypus*) *juvenilis* (Johnston, 1912) Travassos, 1930** (Fig. 2)

Host: *Bufo marinus* Linnaeus, 1758 (Bufonidae).
Locality: Queensland, Australia.
Site: Small intestine.
Material: Voucher specimens deposited as BM(NH) No. 1992.12.21.2, *ex* intestine of *Bufo marinus* (Bufonidae), Townsville, North Queensland, Australia (19°15′ S, 146°49′ E), April 1989, Coll. D. Barton; QM No. QL18323, *ex* intestine of *B. marinus*, Townsville, March 1990, Coll. D. Barton; USNMHC No. 82707, *ex* intestine *B. marinus*, Boyne Is., Central Queensland, Australia (23°57′ S, 151°21′ E), October 1989, Coll. D. Barton; CAS No. D-304, *ex* intestine of *B. marinus*, Bentley, North Queensland, Australia (19°05′ S, 146°57′ E), April 1990, Coll. D. Barton.

Description

(Measurements based upon 10 mounted specimens). Body elongate oval, with bluntly rounded ends, 1,740 (1,220–2,380) long and 850 (590–1,140) wide. Tegument spinose; spines dense anteriorly but sparse posteriorly. Oral sucker terminal, with aperture directed ventrally, smaller than ventral sucker, 180 (130–260) long and 200 (140–270) wide. Ventral sucker anterior to median line, 200 (140–270) long and 210 (150–280) wide. Prepharynx short; pharynx 70.4 (64–76) in diameter; oesophagus short. Gut caeca simple, unbranched,

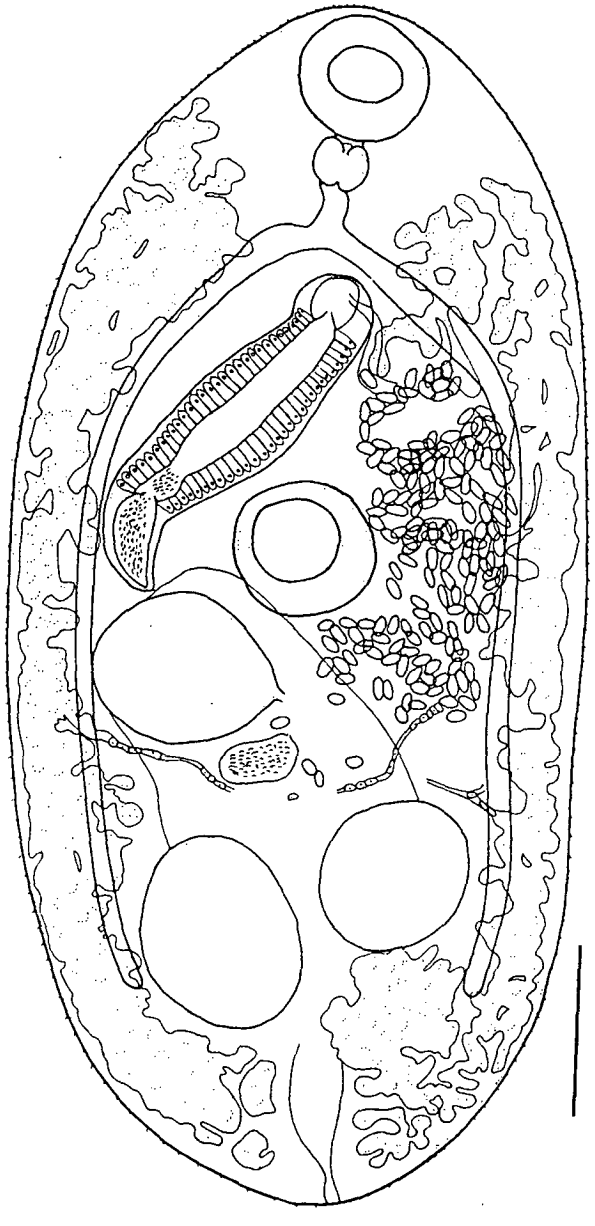

Fig. 2. Dolichosaccus (*Meditypus*) *juvenilis* whole-mount, ventral view. *Scale-bar:* 300 μm.

lateral in body for much of length, terminate near posterior extremity of body. Gonads in posterior half of body. Common genital pore at level of, or just posterior to, intestinal bifurcation.

Testes spherical, in posterior quarter of body, tandem, oblique, or almost opposite, with anterior testis generally to left side. Left testis 230 (160–400) long and 200 (140–300) wide; right testis 210 (160–320) long and 180 (140–280) wide. Sperm duct from each testis arises from anterior face, passes anteriorly almost directly to base of cirrus-sac. Cirrus-sac banana-shaped, 580 (420–760) long, extends to lateral edge of ventral sucker, occasionally posterior to it, contains bipartite seminal vesicle in basal third of cirrus-sac, long prostatic region surrounded by gland-cells and cirrus.

Ovary oval, transversely elongate, immediately posterior to ventral sucker, generally on right side of median line, 220 (150–320) long and 260 (170–370) wide. Oviduct arises medially, initially traverses small muscular chamber then receives in turn duct from seminal receptacle, Laurer's canal and duct from vitelline reservoir before uniting with uterus. Mehlis' gland not observed. Uterus looped, on left side of body, extends from level of ovary to genital pore, does not coil in post-ovarian region. Eggs in uterus yellow, unembryonated, oval, 47 (42–56) long and 24 (20–32) wide. Pore of Laurer's canal not observed. Seminal receptacle posterior to ovary, variable in size. Vitelline follicles large, lateral, extend from anterior to intestinal bifurcation to posterior end of body, overlap caeca dorsally and ventrally for much of length; lateral fields not confluent posterior to testes. Vitelline reservoir lateral to seminal receptacle.

Excretory pore close to posterior extremity. Excretory bladder, long, dorsal to testes, branches not observed.

Biology

Infected toads were collected from 4 geographical locations, all open eucalypt forest. Prevalence of infection as follows: 3.3% at Townsville; 14.0% at Bentley; 1.1% at Mountain View Road (19°30′ S,

146°57′ E); and 34.6% at Boyne Island. Intensity of infection was 1–56 with a mean of 10.22 in 45 infections. Infected animals ranged in snout-vent length from 60.5–122 mm. There was no significant correlation between size of toad and intensity of infection ($r = 0.1465$). Mixed infections of *D. juvenilis* and *D. symmetrus* occurred in 10 animals.

Differential diagnosis

Dolichosaccus juvenilis Nicoll, 1918 most closely resembles *D. grandiacetabularis* Moravec & Sey, 1989 from *Rana grisea* in Papua New Guinea. Differences between the two species were outlined by Moravec & Sey (1989) as: absence of tegumentary spines in *D. juvenilis*; lack of oesophagus in *D. grandiacetabularis*; shape of body; and length of cirrus-sac. Tegumentary spines were found to be present on *D. juvenilis* in this study. The immaturity of the specimens described by Nicoll (1918) may explain the apparent absence of spines. Also, certain methods of fixation may lead to loss of spines from the tegument (Dr D. Blair, pers. comm.). Presence or absence of oesophagus is not considered a good diagnostic character, as it is dependent on the state of contraction of the specimen at the time of fixation. Both *D. grandiacetabularis* specimens were collected from preserved frogs and were contracted, which could cause the oesophagus to shorten to an extent where it appeared absent. Within the specimens of *D. juvenilis* collected in this study, 'presence' of oesophagus was variable. Body shape is also dependent on fixation and can be highly variable. The small number of *D. grandiacetabularis* specimens collected would not show the true extent of body variation. Cirrus-sac measurements for *D. juvenilis* collected in this study reinforce the suggestion of Moravec & Sey (1989) that the two species should remain separate. *D. juvenilis* has a larger cirrus-sac than *D. grandiacetabularis*, even in the immature specimens collected by Nicoll (1918).

I consider that *D. juvenilis* can be differentiated from *D. grandiacetabularis* by differences in oral and ventral sucker sizes, the larger gonad and egg measurements of *D. juvenilis*, and geographical distance. The ventral sucker is larger than the oral sucker in both species, but the difference is not as large in *D. juvenilis* as it is in *D. grandiacetabularis*.

***Dolichosaccus* (*Meditypus*) *helocirrus* n. sp.** (Figs 3, 4)

Hosts: *Bufo marinus* Linnaeus, 1758 (Bufonidae), *Litoria caerulea* White, 1790 (Hylidae).
Locality: Queensland, Australia.
Site: Small intestine.
Type-material: Holotype and three paratypes *ex* intestine of *Bufo marinus* (Bufonidae), Townsville, North Queensland, Australia (19°15′ S, 146°49′ E), April 1990, Coll. D. Barton. BM(NH) No. 1992.12.21.3. Paratypes 2, *ex* intestine of *Litoria caerulea* (Hylidae), Abergowrie, North Queensland, Australia (18°27′ S, 146°0′ E), April 1989, Coll. D. Barton, BM(NH) No. 1992.12.21.4; 5, *ex* intestine of *B. marinus*, Townsville, April 1990, Coll. D. Barton, QM No. QL18324; 6, *ex* intestine of *B. marinus*, Townsville, April 1990, Coll. D. Barton, USNMHC No. 82708; 1, *ex* intestine of *B. marinus*, Townsville, July 1988, Coll. R. Speare, CAS No. D-305.
Etymology: This species is named after its armed cirrus which is covered in small knobs (*helos*, Greek for wart).

Description

(Measurements based upon 10 mounted specimens). Body rounded or oval, with bluntly rounded ends, 1,774 (1,176–2,448) long and 852 (688–1,040) wide. Tegument spinose; spines dense anteriorly but sparse posteriorly. Oral sucker terminal, with opening directed ventrally, slightly smaller than ventral sucker, 168 (130–210) long and 214 (150–270) wide. Ventral sucker preequatorial, 214 (170–250) long and 226 (170–280) wide. Prepharynx short; pharynx 64.6 (56–76) in diameter; oesophagus short. Gut caeca simple, long, unbranched, lateral, end blindly close to posterior extremity, turn slightly medially post-

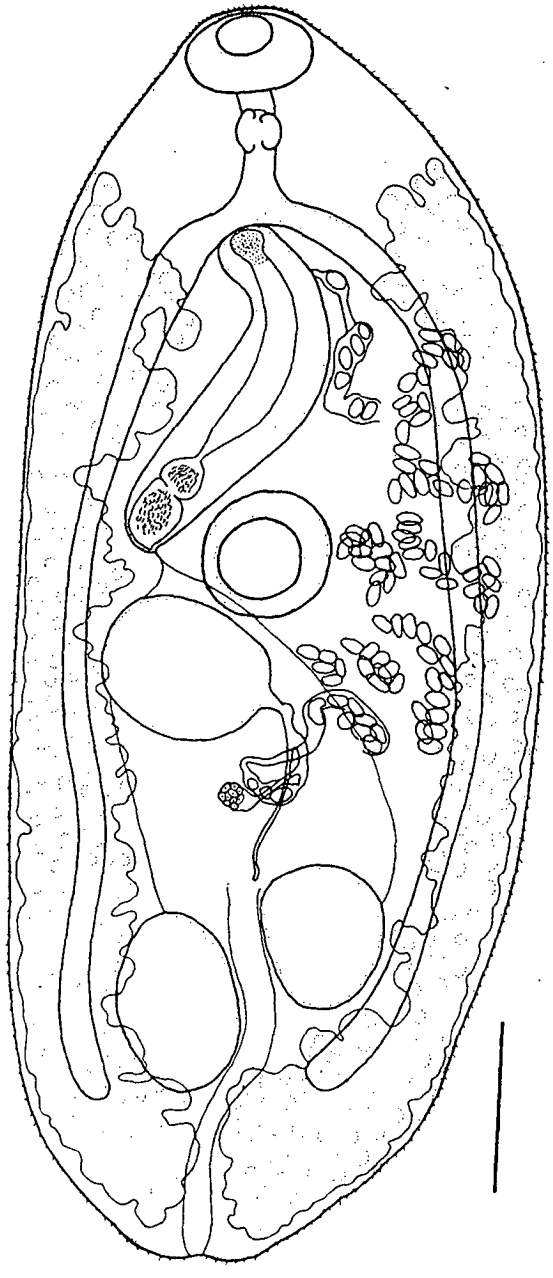

Fig. 3. Dolichosaccus (*Meditypus*) *helocirrus* n. sp. Holotype, whole-mount, ventral view. *Scale-bar*: 300 μm.

erior to testes. Gonads in posterior half of body. Common genital pore at level of, or just posterior to, intestinal bifurcation.

Testes spherical, in last quarter of body, tandem, oblique or almost opposite, with anterior testis generally to left side. Left testis 262 (176–336) long and 191 (136–240) wide; right testis 265 (208–304) long and 195 (152–248) wide. Sperm duct from each testis arises from anterior face, passes anteriorly almost directly to base of cirrus-sac. Cirrus-sac banana-shaped, 544 (384–720) long, extends to lateral edge of ventral sucker (Fig. 4A), contains bipartite seminal vesicle in basal third, followed by prostatic region and cirrus. Two groups of gland-cells present within cirrus-sac; one surrounds seminal vesicle; other surrounds prostatic region. Cirrus covered with small knobs.

Ovary oval, transversely elongate, immediately posterior to ventral sucker, generally on right side of median line, 248 (208–288) long and 286 (240–336) wide (Fig. 4B). Oviduct arises medially, initially traverses small muscular chamber then receives duct from seminal receptacle and Laurer's canal together, followed by duct from vitelline reservoir before uniting with uterus. Mehlis' gland not observed. Uterus looped, overlaps left intestinal caecum, extends from level slightly posterior to ovary to genital pore, does not fill space posterior to ovary. Eggs in uterus yellow, unembryonated, oval, 49 (44–56) long and 27 (22–30) wide. Laurer's canal runs posteriorly, sometimes to level of testes, opens dorsally. Seminal receptacle posterior to ovary, variable in size. Vitelline follicles large; fields lateral, extending from anterior to genital pore to posterior end of body, overlap caeca dorsally and ventrally for much of length, not confluent posterior to testes. Vitelline reservoir lateral to seminal receptacle.

Excretory pore close to posterior extremity. Excretory bladder long, dorsal to testes, passes between testes, then branches.

Molecular sequence of ribosomal internal transcribed spacer region is reported in Luton *et al.* (1992).

Biology

Dolichosaccus (*M.*) *helocirrus* n. sp. infected *B. marinus* in 5 geographical locations, all open eucalypt forest. Prevalence of infection as follows: 12.5% at Mareeba (17°00′ S, 145°25′ E); 3.3% at Townsville; 11.8% at Bentley (19°22′ S,

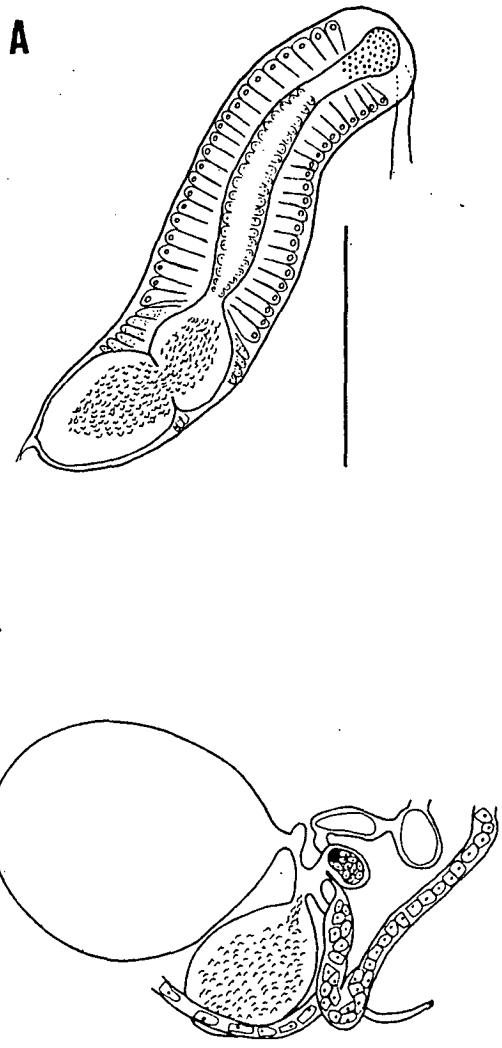

Fig. 4. Dolichosaccus (Medityphis) helocirrus n. sp. A. Male copulatory organ; B. Female reproductive system. *Scale-bars*: 250 μm.

146°57′ E); 21.7% at Mountain View Road (19°30′ S, 146°57′ E); and 11.5% at Boyne Island (23°57′ S, 151°21′ E). Intensity of infection was 1–73 with mean of 12.68 in 22 infections. Infected animals ranged in snout-vent length from 81.5–115 mm. There was no significant correlation between size of toad and intensity of infection (r = 0.1609). Infections were also found in 14.3% (1 of 7) *Litoria caerulea* collected from Abergowrie, with mean intensity of 2.0. Mixed infections of *D. helocirrus* and *D. juvenilis* were only found on 3 occasions, while mixed infections of *D. helocirrus* and *D. symmetrus* occurred 4 times. Two specimens of *D. helocirrus* were collected from a single toad in Brisbane by Dr Tom Cribb in 1983 and further 3 specimens were collected by Dr Rick Speare from toads in Townsville in 1988. No data were available on prevalence or intensity of infection.

Differential diagnosis

Dolichosaccus (*M.*) *helocirrus* n. sp. most closely resembles *D. longibursatus* Moravec & Sey, 1989 in *Rana grunniens* from Papua New Guinea, as both species have cirrus armature. *D. helocirrus* can be differentiated from the latter by the type of cirrus armature, vitelline distribution, body size and geographical location. Vitelline fields are confluent posterior to the testes in *D. longibursatus*, while they remain lateral in *D. helocirrus*. *D. helocirrus* is also larger than *D. longibursatus*. The disjunctive distribution between the two species, with no area of host species overlap, is another reason for separation of the two species.

Discussion

Members of the genus *Dolichosaccus* are reported here, for the first time, from the introduced cane toad *Bufo marinus* in Australia. There are two possible explanations for the presence of members of the genus *Dolichosaccus* in the introduced cane toad in Australia. Either they are natural parasites of the cane toad that were introduced to Australia with their host, or they are Australian species that the cane toad has acquired in Australia. For *D. juvenilis* and *D. symmetrus*, there is no doubt of their origin, as both species were described in Australia prior to the introduction of the toad. The origin of the new species, *D. helocirrus*, however, is more difficult to determine. No species of *Dolichosaccus* have been recorded from amphibians or reptiles in South America (the cane toads' native range; Zug & Zug, 1979) or Hawaii (source

of introduction to Australia; Easteal, 1981)[1]. As the introduced toads were maintained in captivity in Australia and only their progeny released (Easteal, 1981), I believe this parasite was unlikely to have been introduced with the toad. No study was carried out at the time of introduction of the toad on its parasite fauna, thus it is unknown what, if any, parasites the toad introduced to Australia. It is most probable that *D. helocirrus* is an endemic Australian species. It is recorded from only one other frog species, *Litoria caerulea*, at Abergowrie, north of Townsville.

D. helocirrus has a cirrus covered with small knobs while, *D. longibursatus* has small, flat, triangular spines. *D. (Lecithopyge) rastellus subulatus* (Perkins, 1928) Travassos, 1930 from *B. regularis* in Africa is the only other species with an armed cirrus in this genus (Manter & Pritchard, 1964). On examination of museum specimens of this species, the spines were found to be only obvious on a cirrus protruding from a tear in the body. This is unlike both *D. helocirrus* and *D. longibursatus*, where the knobs or spines are quite obvious on an invaginated cirrus.

The body plans of *D. helocirrus* and *D. juvenilis* are almost identical, except for the armed cirrus of *D. helocirrus*. The use of molecular approaches (as in Luton *et al.*, 1992) may be a more reliable method of differentiating between such closely related species.

D. juvenilis has been redescribed from a new host species, *Bufo marinus*, in Queensland. The major difference from Nicoll's description is the presence of tegumental spines on specimens found in this study. Spination of the tegument may become more apparent with maturation. Juvenile specimens of *D. symmetrus* collected in this study showed different spination patterns to adults. Spination was obvious in the anterior part of the juvenile body but missing from the posterior two-thirds, while adult specimens were covered with spines. The worms found in this study are also larger than the specimens described by Nicoll (1918), which were immature. Thus, differences in body and gonad size can be accounted for by maturation of the specimens, but may be due to host differences. Interestingly, the egg size of *D. juvenilis* is similar in both descriptions.

D. symmetrus has been redescribed from a new host species, *B. marinus*, in Queensland. The specimens described here differ from the original description by having larger body, gonad and egg measurements; the sucker dimensions are almost identical. However, larger body measurements may be attributed to differences in microhabitat, i.e. different host species. *D. symmetrus* was originally described by Johnston (1912) from three specimens from the rectum of *Litoria caerulea*. The rectum is an unusual site of infection for members of this genus, which are usually found in the small intestine. Being found in the rectum might suggest that *L. caerulea* was an unsatisfactory host and conditions were not favourable, leading to a stunted growth of the individuals.

Interrupted lateral vitelline fields is a major taxonomic character for the identification of *D. symmetrus*. Other species that possess interrupted lateral vitelline fields include *D. leiolopismae* Allison & Blair, 1987 and *D. rastellus subulatus* (Perkins, 1928) Travassos, 1930, as described by Manter & Pritchard (1964). Variability in the vitelline distribution could be due to differences in fixation (Allison & Blair, 1987); however, in all the *D. symmetrus* specimens collected in this study, this feature was consistent. Further work is required on these species.

Allison & Blair (1987) suggested that *D. schmidti* Fischthal & Kuntz, 1975 belonged to the subgenus *Dolichosaccus*, not to *Lecithopyge*, as the vitelline fields were not confluent anteriorly. However, within the species *D. leiolopismae* from Otago skinks, specimens with non-confluent vitelline fields were also observed. Therefore, this characteristic is not reliable enough to place *D. schmidti* in the subgenus *Dolichosaccus*. Instead, *D. schmidti* should be placed in the subgenus *Lecithopyge* due to the fact that the uterus occupies the space between testes and genital pore.

Meditypus n. subg. is erected to accommodate

[1] Travassos (1924) described *Dolichosaccus amplicava* from *Eloisa nasus* (Amphibia) in Brazil, but later (Travassos, 1930) transferred the species to the genus *Opisthioglyphe* on the basis of an unconstricted seminal vesicle.

D. juvenilis (Nicoll, 1918) Travassos, 1930, *D. grandiacetabularis* Moravec & Sey, 1989, *D. longibursatus* Moravec & Sey, 1989 and *D. helocirrus* n. sp. Characteristics of these species were intermediate between the subgenera *Dolichosaccus* and *Brachysaccus*. *Brachysaccus*-type characteristics were the relative size of the suckers, position of testes and distribution of the vitelline fields. *Dolichosaccus*-type characteristics were the disposition of the uterus and size of the cirrus-sac. All species included in this subgenus are found in the Australo-Papuan region and inhabit amphibians from three families (Ranidae, Bufonidae and Hylidae).

The following is a key to the subgenera of *Dolichosaccus*, incorporating these characteristics.

Key to subgenera

1. Uterus occupies area between ovary and testes 2
– Uterus pre-ovarian 3
2. Ovary distant from ventral sucker ..... *Brachysaccus*
– Ovary directly posterior to ventral sucker ..... *Lecithopyge*
3. Ovary distant from ventral sucker ..... *Dolichosaccus*
– Ovary directly posterior to ventral sucker ..... *Meditypus*

The species within these subgenera are as follows:

D. (*Dolichosaccus*)

trypherus Johnston, 1912, from *Limnodynastes peronii* and *Litoria aurea* in New South Wales, Australia.

ischyrus Johnston, 1912, from *Limnodynastes dorsalis* and *Litoria caerulea* in NSW and Queensland, Australia.

diamesus Johnston, 1912, from *Litoria freycineti* in NSW, Australia.

cheloniae Wang, 1983, from *Chelonia mydas* in Fujian Province, China.

D. (*Lecithopyge*)

rastellus (Olsson, 1876), from various anurans in Europe and Africa.

lygosomae Fischthal & Kuntz, 1967, from *Lygosoma noctua*, New Hebrides.

novazealandiae Prudhoe, 1970, from *Leiopelma archeyi*, *L. hamiltoni* and *L. hochstetteri*, New Zealand.

schmidti Fischthal & Kuntz, 1975, from *Ocadia sinensis* in Taiwan.

ranae Wang, 1980, from *Rana spinosa* and *Staurais wuyiensis* in Fujian Province, China.

leiolopismae Allison & Blair, 1987, from *Leiolopisma nigriplantare*, *L. infrapunctatum*, *L. lineoocellatum* and *Sphenodon punctatus*, New Zealand.

D. (*Brachysaccus*)

anartius (Johnston, 1912) Yamaguti, 1958, from *Litoria aurea* and *Limnodynastes peronii* in NSW, Australia.

symmetrus (Johnston, 1912) Yamaguti, 1958, from *Litoria aurea* in NSW and *Bufo marinus* in Queensland, Australia.

D. (*Meditypus*)

juvenilis (Nicoll, 1918) Travassos, 1930, from *Cyclorana brevipes* and *Bufo marinus* in Queensland, Australia.

grandiacetabularis Moravec & Sey, 1989, from *Rana grisea* in Okasa, Papua New Guinea.

longibursatus Moravec & Sey, 1989, from *Rana grunniens* in Ambunti, Papua New Guinea.

helocirrus n. sp., from *Bufo marinus* and *Litoria caerulea* in Queensland, Australia.

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Appendix 7: Monthly Variations in *Rhabdias* sp. Populations at QDPI.

Monthly variations in the number of toads collected (N), prevalence (P) and mean intensity of infection (I) of *Rhabdias* sp. infection, and average length of nematodes (L) within an infrapopulation for all toads collected from QDPI over a twenty month period. Data presented here is not log transformed. Variations within the three size classes are shown in Appendix 4.1A; variations between the adult sexes (Class II and III combined) are shown in Appendix 4.1B. Values for I and L are for *Rhabdias* sp.-infected toads only.

Table A7.1 Variations within the three toad size classes.

		CLASS I <60 mm SVL				CLASS II 60.5-90 mm SVL				CLASS III >90.5 mm SVL				OVERALL			
Mth	Yr	N	P	I	L	N	P	I	L	N	P	I	L	N	P	I	L
Aug	1990	46	78.3	12.3	6.31	4	100	19.5	7.06	1	100	9.00	5.50	51	80.4	12.9	6.36
Nov	1990	50	90.0	9.36	7.27	9	100	20.3	9.29	5	100	54.0	8.75	64	92.2	14.8	7.70
Dec	1990	13	84.6	9.91	9.13	28	96.4	11.3	9.49					41	92.7	10.9	9.39
Jan	1991					13	100	17.7	8.88	8	100	16.0	9.00	21	100	17.1	8.92
Feb	1991					20	100	35.4	8.07	10	90.0	18.3	9.30	30	96.7	30.1	8.45
Mar	1991	42	33.3	1.86	6.46	1	100	54.0	8.60	6	83.3	30.8	10.2	49	40.8	11.7	7.50
Apr	1991	39	66.7	3.27	5.58	9	88.9	19.1	7.42	1	100	52.0	8.46	49	71.4	8.29	6.08
May	1991	37	75.7	3.64	5.44	4	50.0	9.50	7.05	9	88.9	13.8	9.21	50	76.0	6.08	6.32
Jun	1991	33	75.8	5.64	6.18	14	85.7	16.7	6.76	3	66.7	26.5	7.62	50	78.0	10.1	6.43
Jul	1991	30	90.0	7.82	5.88	9	100	16.6	6.49	11	100	27.6	7.98	50	94.0	14.1	6.49
Aug	1991	6	66.7	12.0	5.33	2	100	43.5	6.42	4	100	47.0	7.34	12	83.3	32.3	6.35
Sep	1991	3	100	7.67	6.64	10	100	48.1	7.52	13	92.3	49.8	8.26	26	96.2	44.1	7.77
Dec	1991	1	0			3	100	24.0	8.92	6	66.7	17.3	9.13	10	70.0	20.1	9.04
Jan	1992					5	100	14.6	9.39	12	91.7	23.9	10.1	17	94.1	21.0	9.84
Feb	1992									10	60.0	17.8	10.1	10	60.0	17.8	10.1
Feb2	1992					6	100	12.8	9.69	9	100	14.2	9.21	15	100	13.7	9.40
Mar	1992					17	94.1	13.5	9.62	18	100	23.2	9.65	35	97.1	18.6	9.63
OVERALL		300	73.0	7.35	6.38	154	95.5	21.0	8.44	126	90.5	26.4	9.1	580	82.8	16.1	7.65

Table A7.2 Variations within the adult toad sexes (CLasses II and III combined).

		Female				Male				OVERALL			
Mth	Yr	N	P	I	L	N	P	I	L	N	P	I	L
Aug	1990	1	100	14.0	7.10	4	100	18.3	6.66	5	100	17.4	6.75
Nov	1990	8	100	14.5	9.08	6	100	56.2	9.13	14	100	32.4	9.10
Dec	1990	12	91.7	8.18	9.64	16	100	13.4	9.39	28	96.4	11.3	9.49
Jan	1991	16	100	17.0	8.71	5	100	17.2	9.60	21	100	17.1	8.92
Feb	1991	26	96.2	33.5	8.40	4	100	8.75	8.75	30	96.7	30.1	8.45
Mar	1991	3	100	56.3	9.37	4	75.0	13.0	10.5	7	85.7	34.7	9.92
Apr	1991	7	85.7	6.83	7.14	3	100	54.7	8.34	10	90.0	22.8	7.54
May	1991	5	60.0	13.3	8.17	8	87.5	12.7	9.04	13	76.9	12.9	8.78
Jun	1991	9	66.7	20.2	6.69	8	100	16.5	7.05	17	82.4	18.1	6.88
Jul	1991	10	100	18.3	6.65	10	100	26.9	7.97	20	100	22.6	7.33
Aug	1991	3	100	6.33	7.15	3	100	85.3	6.91	6	100	45.8	7.03
Sep	1991	10	100	48.2	7.56	13	92.3	49.7	8.22	23	95.7	49.0	7.92
Dec	1991	4	50.0	4.00	9.93	5	100	26.6	8.68	7	100	20.1	9.04
Jan	1992	6	100	9.00	9.22	11	90.9	28.2	10.2	17	94.1	21.0	9.84
Feb	1992	4	50.0	12.5	10.3	6	66.7	20.5	10.0	10	60.0	17.8	10.1
Feb2	1992	1	100	5.00	5.10	14	100	14.3	9.71	15	100	13.7	9.40
Mar	1992					35	97.1	18.6	9.63	35	97.1	18.6	9.63
OVERALL		125	90.4	21.9	8.28	155	98.1	24.5	9.05	280	93.2	23.4	8.71