Studies on the systematics of the cestodes infecting the emu, *Dromaius novaehollandiae* (Latham, 1790)

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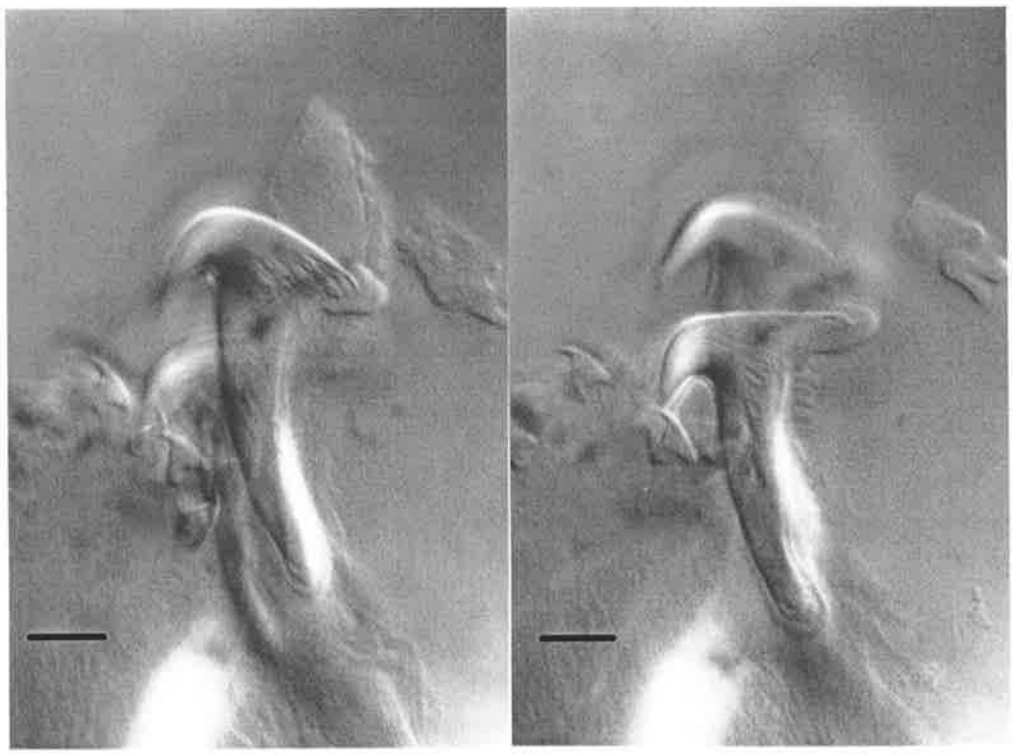

Frontispiece. "Hammer shaped" rostellar hooks of *Raillietina dromaius*. Scale bars = 10 μm.

DEDICATION

For mum

and

for all of the proficient scientists whose regard I value.

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ABSTRACT

Four new species of cestode are described from the emu, *Dromaius novaehollandiae* Latham, 1790and one from the southern cassowary, *Casuarius casuarius* Linnaeus, 1758. All are assigned to the genus *Raillietina* Fuhrmann, 1920 (*sensu lato* Jones and Bray, 1994) on the basis of the possession of two rows of numerous hammer-shaped rostellar hooks, unilateral genital pores, a small cirrus sac which does not reach or just crosses the osmoregulatory canals and egg capsules containing several eggs. In addition, *R. australis* (Krabbe, 1869) from the emu and *R. casuarii* (Kotlan, 1923) from the cassowary are redescribed from Australian specimens. These specimens are compared to and distinguished from all known congeners in the Struthioniformes. The principle diagnostic characters are identified and tabled. This study incorporates a range of analyses to establish the identity of the cestodes infecting *Dromaius novaehollandiae* in Australia.

Large numbers of cestodes were recovered from farmed and wild emus with a maximum intensity of 4795 in a wild bird. *Raillietina beveridgei* was the most predominant species encountered.

The distribution of the cestode species infecting emus indicated that each species occupied a preferred and predictable portion of the intestinal tract that is believed to reinforce reproductive advantage.

Cysticercoids of the five species of *Raillietina, R. australis, R. beveridgei, R. chiltoni, R. dromaius* and *R. michelli*, were recovered from ants belonging to the genus *Pheidole* and are described. Each species was identified on the basis of the number and size of rostellar hooks which corresponds to that of adult worms. There was a trend towards an inverse relationship between size of the cysticercoid and the parasite burden in the intermediate host.

Ultrastructural studies showed that the microtriches present on scoleces do not differ from those reported from other davaineids. Examination of the fine structure of the egg capsule revealed a thin and greatly-folded embryophore. The mechanism of egg hatching appears to begin with mechanical release of the embryo from the capsule followed by expansion of the folded embryophore to encircle the oncosphere.

DNA sequencing techniques were applied to confirm the morphological distinction of adult worms. 18S, ITS2 and CO1 gene sequence analysis provided additional characters to enable species separation.

DECLARATION

This thesis contains no material that has been previously submitted for an award at any university or other tertiary institution and to the best of my knowledge and intention contains no material previously published or written by other persons except where due reference has been made in the text.

I consent that it be made available for loan and/or photocopy.

Michael O'Callaghan

Dated 7/05/2004

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Chapter 1

INTRODUCTION

A vast literature has accumulated through the years, describing such aspects as morphology, taxonomy, lifecycles, physiology, pathogenesis, and host relationships of tapeworms. Even so, much remains unknown (Schmidt, 1986).

1.1 General introduction

Systematics comprises the disciplines of taxonomy, nomenclature and phylogenetics (Mayr, 1969). If systematics is the scientific study of the kinds and diversity of organisms and of any and all of the relationships among them (Simpson, 1961), then any consequent orderly classification (Richardson *et al.*, 1986) is an elementary part of parasitology because it provides a means to recognise organisms. Systematics is therefore dependent on descriptive taxonomy for without accurate identifications, credible parasitological work would not be possible (see Andrews and Chilton, 1999).

Schmidt (1986, p. 1) states "tapeworms have long excited in man a sense of bewilderment and sometimes fear… many of which have never been examined". In particular, very little serious collecting of helminths from birds has taken place in Australia and most helminths have been found incidental to bird collection (Mawson *et al.*, 1986).

Amongst the helminths in Australian birds, the identity of the cestodes infecting the emu, *Dromaius novaehollandiae* (Latham, 1790), has not been established. In this study, the primary objective is to describe and classify the tapeworms infecting the emu using comparative morphology. The acquisition of these data is fundamental, however the consideration of several sources of evidence beyond comparative morphology provides more substantial support for taxonomic decisions (Mariaux, 1996; Sandeman, 2001).

The present work attempts a detailed study of cestode species within a host, gathering comparative data with the objective of increasing the accuracy at which the cestode species can be recognised. Ultimately, it is hoped that this study fills a gap in the knowledge of parasites and host-parasite systems and contributes to the state of knowledge of the cestode fauna of Australian birds (Beveridge and Jones, 2002).

1.2 The host, *Dromaius novaehollandiae* (Latham, 1790)

Large flightless birds or ratites belong to the order Struthioniformes that includes the family Dromaiidae of which the emu is the only representative. The emu, *Dromaius novaehollandiae*, inhabits most of mainland Australia excluding rainforest, settled and very arid areas. Adult emus stand 1.6-1.9 m tall and may weigh up to 45 kg. Birds reach sexual maturity at about two years of age. Wild birds feed mainly on green herbage but also on seeds and insects (Little, 1992).

A closely related family, the Casuariidae is represented in Australia by one species, the southern cassowary, *Casuarius casuarius* (Linnaeus, 1758), which inhabits rainforest (Pollock, 1992). Other members of the Casuariidae include the dwarf cassowary, *C. bennetti* Gould, 1858 and the northern cassowary, *C. unappendiculatus* Blyth, 1860 which together with the southern cassowary, inhabit New Guinea. The emu is distantly related to other ratites including the Rheas, *Rhea americana* (Linnaeus, 1758) and *Pterocnemia pennata* (Orbigny, 1834), the Ostriches *Struthio camelus* Linnaeus, 1758 and *S. molybdophanes* Riechenow, 1883 and the Kiwis, *Apteryx* species Shaw, 1813 (Bruning and Dolensek, 1986; Sibley and Monroe, 1990; Peterson, 2001).

In recent times emu farming, particularly for oil, leather and eggs, has developed as an industry in Australia. In 1996 there were 145 licensed emu farms in South Australia

(SA) (Anon 1998) and 650 nationally with a population of 71,000 emus producing 78,000 chicks annually (Mannion *et al.*, 1995).

1.3 Cestode nomenclature

The tapeworms described from emus belong to the cestode order Cyclophyllidea van Beneden in Braun, 1900. This is the largest cestode order and contains fifteen families as recognised by Jones *et al.* (1994).

The family Davaineidae Braun, 1900 was historically divided into three subfamilies, namely Davaineinae Braun, 1900, Ophryocotylinae Fuhrmann, 1907 and Idiogeninae Fuhrmann, 1907. The Idiogeninae have a paruterine organ that is absent in the Ophryocotylinae and Davaineinae. Jones and Bray (1994) departed from this traditional recognition of three subfamilies by not recognizing the Davaineinae and Ophryocotylinae as distinct subfamilies thus supporting the earlier proposal of Spasskii and Spasskaya (1976). They believed that the nature and origin of the egg capsule is not sufficiently understood to separate these two subfamilies. The Ophryocotylinae were identified by the presence of a persistent sac-like uterus rather than a uterus replaced by egg capsules as occurs in the Davaineinae. Consequently two subfamilies, the Davaineinae and Idiogeninae are recognised today.

Phylum - Platyhelminthes

Class - Cestoda

Order - Cyclophyllidea

Family - Davaineidae

Subfamily - Davaineinae

1.3.1 Characteristics of the family Davaineidae

According to Jones and Bray (1994), the Davaineidae have small, numerous hammer-shaped rostellar hooks - the only autapomorphy of the family. Thirty-one genera occur in the subfamily Davaineinae.

Jones and Bray (1994) regard the significant taxonomic characters at the generic level to be:

- the presence or absence of a rostellum;
- shape and continuity or discontinuity of the rostellar crown;
- hook shape and size, number of rows of rostellar hooks; and
- the presence (or rarely absence) and shape of the suckers.

Of lesser importance are:

- the position of the ovary when distinctly poral is generically significant, as is
- the alternation or unilaterality of genital pores;
- the number of proglottides and osmoregulatory canals, single or paired genitalia and modifications of the genital atrium and terminal genital ducts;
- the number of testes is significant only when low (two) and their distribution, when distinctly and consistently poral, is generally used in conjunction with other characters;
- the number of eggs in each capsule is of generic significance and the number of capsules per proglottis is only generically significant if consistently low.

At the species level, the taxonomic characters of importance are:

- the overall size of the cestode and scolex;
- the size of the rostellum and the size and number of rostellar hooks;

- the size and number of sucker hooks;
- the position of the genital pore;
- the length of the cirrus sac;
- the testis number and distribution;
- the number of eggs per capsule; and
- the presence of accessory rostellar spines.

Cestodes described from emus belong to two genera in the subfamily Davaineinae, namely *Raillietina* and *Cotugnia*.

1.3.2 *Raillietina* Fuhrmann, 1909

Traditionally, *Raillietina* comprised four subgenera, *Paroniella, Skrjabinia, Raillietina* and *Fuhrmannetta*. Hughes and Schultz (1942) listed 225 species and Yamaguti (1959) listed 248 species in the genus. Sawada (1964, 1965) abstracted the morphological features of each species and listed 226 belonging to the four subgenera recognised at that time. Schmidt (1986) recognised a total of 282 species belonging to the four subgenera (Table 1).

Meanwhile, Movsesyan (1966) had elevated the subgenera to generic rank by recognising the significance of the number of eggs per capsule plus the location of genital pores as unilateral or irregularly alternating. Jones and Bray (1994) followed Movsesyan and recognised the generic status of the four traditionally-recognised subgenera.

Table 1. The number of species belonging to the subgenera recorded by Sawada (1964) and Schmidt (1986) and the number of species currently recognised in each genus (Appendix A, Table 45).

Subgenus	Sawada (1964)	Schmidt (1986)	The present*
Fuhrmannetta	17	18	19
Paroniella	47	56	61
Raillietina	129	174	199
Skrjabinia	33	34	37
Unknown generic status	-	14	14

*as genera after Movsesyan (1966)

According to Jones and Bray (1994), the diagnostic features of *Raillietina* are the presence of rostellar hooks in a circular row and armed, partly-armed or unarmed suckers. The proglottides are craspedote. Reproductive organs are single, genital pores are unilateral, testes are numerous. The ovary is situated medially. There are two to eight eggs per capsule (Table 2).

The genus is cosmopolitan and occurs in both birds and mammals.

Table 2. The diagnostic features of *Raillietina, Fuhrmannetta, Paroniella* and *Skrjabinia.*

	Raillietina	*Fuhrmannetta*	*Paroniella*	*Skrjabinia*
Genital pores	Unilateral	Alternate irregularly	Unilateral	Alternate irregularly
Eggs per capsule	2 to ? *	2 to 8	One	One

*The type species of *Raillietina, R. tetragona* (Molin, 1858) is described by some authors as having six to 12 eggs per capsule (cf. Reid, 1962; Sawada, 1965; Soulsby, 1982). Sawada (1965) records the number of eggs per capsule varying in individual *Raillietina* species (*sensu stricto*) from one to 26, but Jones and Bray (1994) suggest two to eight.

1.3.3 *Cotugnia* Diamare, 1893

Cotugnia is one of six nominal genera in the Davaineinae with consistently-paired genitalia. Jones and Bray (1994) recognise only five of these as valid genera, namely *Cotugnia, Abuladzugnia, Rostelugnia, Pavugnia* and *Multicotugnia*. A new genus, *Erschovitugnia*, was erected by Spasskii (1973) to which he allocated *C. collini* Fuhrmann, 1909 because of the large size of the rostellar hooks (70 - 87 μm long). Although the size of the hooks is large for *Cotugnia* species, Jones and Bray (1994) agreed with Schmidt (1986) and Movsesyan (1987) that it is of specific rather than generic significance and *Erschovitugnia* is regarded as a synonym of *Cotugnia*. Schmidt (1986) listed 49 species of *Cotugnia*.

Cotugnia, according to Jones and Bray (1994), is characterised by a broad rostellum armed with a double row of small (rarely large) hammer-shaped hooks. Suckers are rarely armed. The numerous proglottides are craspedote. A dorsal pair of osmoregulatory canals is present or absent but the ventral pair is always present. Genital pores are bilateral. The diagnostic feature is the consistent presence of two genital organs per proglottis. The cirrus sac is small and extravascular. Testes are numerous in one or two fields. Ovaries are lateral and lobed. Vitelline glands are post-ovarian. Egg capsules are numerous and contain a single egg. The cestodes are cosmopolitan and occur in birds.

Cestodes belonging to *Cotugnia* can be separated from *Raillietina* by the presence of egg capsules containing only one egg and consistently-paired genital organs in each proglottis.

1.4 Cestodes of emus

Cestodes were first recorded in emus when Krabbe (1869) published a description of *Taenia australis* from the intestine of a captive emu that died in October 1867 in the Copenhagen (Kjaerboelling's Zoological Garden) Zoo. The emu had arrived at the zoo 1.5 years earlier and was reared in Australia. The synonymies of this tapeworm species is as follows:

Taenia australis Krabbe, 1869, *Davainea australis*: Blanchard, 1891, *Ransomia australis*: Fuhrmann, 1920, *Kotlania australis*: Lopez-Neyra, 1931, *Raillietina australis*: Fuhrmann, 1924

Later, Fuhrmann (1909) described another species, *Cotugnia collini*, from an emu specimen in the Museum for Natural Sciences in Berlin. The synonymies of this is as follows:

Cotugnia collini Fuhrmann, 1909 (syn: *Ershovitugnia collini*: Spasskii, 1973)

1.5 Cestodes from other ratites

Baer (1928) redescribed *Houttuynia struthionis* (Houttuyn, 1773) from Ostriches, *Struthio* species, in Africa, and described a novel variety, *H. s. var. neogaeae,* from the Rhea (*Rhea americanus*) from South America. In Hungary, Kotlan (1923) described *Raillietina casuarii* and *R. infrequens* collected between 1897 and 1899 from a cassowary, *Casuarius bennetti picticollis* Sclater, 1874, in New Guinea.

The principal characters distinguishing the cestodes in ratites appear in Table 3.

Moniezia rhea (Fuhrmann, 1904) (Cestoda: Anoplocephalidae) also occurs in *Rhea americanus*. Specimens were re-described from types and a limited amount of additional material (Beveridge, 1978) collected in Brazil. Cestodes belonging to the genus *Moniezia* are characterised by a scolex without a rostellum, unarmed suckers and paired genitalia. *Moniezia rhea* re-described by Beveridge (1978) is 115-228 mm long and 3-6 mm wide with a scolex 1.2-1.3 mm in diameter and suckers 0.700-0.750 x 0.050 mm.

Table 3. Morphological characters, particularly the size of the strobila and size of rostellar hooks, distinguishing davaineid cestodes of ratites

Host	Ostrich Rhea	Emu	Emu	Cassowary	Cassowary
Cestode species	*Houttuynia struthionis* [1]	*Raillietina australis* [2]	*Cotugnia collini*[3]	*Raillietina casuarii* [4]	*Raillietina infrequens* [4]
Size (cm)	30-60	40	5-7	34	8
Width (mm)	9	1.2	4-5	3	< 1.2
Scolex diameter (mm)	1 – 2	?	1.2-1.34	1 - 1.2	0.5
Rostellum (mm)	0.45-0.60	?	0.85	0.5	0.25
Rostellar hooks (μm)	Large 77 Small 63	12 - 14	87 68-70	48-54 40-46	27-34 21-25
Number of hooks	160-166	340-360	200	250	260
Sucker diameter (mm)	0.4-0.5	?*	0.3-0.38	0.4	0.13
Sucker hooks	Un-armed	5 - 11 μm	Un-armed	10-13 μm	10-15 μm
Eggs/capsule	15 -25	?	1	2-4	unknown

References: [1]. Baer J-G. (1928) [2]. Krabbe H. (1869) [3]. Fuhrmann O. (1909) [4]. Kotlan A. (1923)

*Sawada, (1965) records the sucker diameter incorrectly as five – 11 μm whereas the size of the sucker hooks are five - 11μm.

1.6 Records of cestodes from emus in Australia

Johnston (1910) identified *Davainea australis* that he collected in 1909 from an emu in New South Wales and from an emu collected by J. B. Cleland in the Strelley River district of northwest Western Australia. In a list of parasites recorded from Australian birds, Johnston (1912) identified these two records and that of Krabbe (1869). In the first volume of the Medical Journal of Australia, Johnson (1914) recorded *D. australis* from the emu as a closely-related form of the tapeworms found in the ostrich and rhea. Cleland (1922) summarised previous records and his own findings. He listed *D. australis* and *C. collini* as parasites recorded in Australian birds whilst stating that "there is still very much work to be done in the parasitology of Australian birds" (Cleland, 1922, p. 86).

In a checklist of helminth parasites of Australia (Young, 1939), three cestodes are listed as occurring in the emu, namely *C. collini, R.* (*R.*) *australis* and a *Taenia* species. In this checklist, the author refers to the published descriptions of *C. collini* by Fuhrmann (1909) and *R. (R). australis* by Krabbe (1869). The reference to the *Taenia* sp. (Johnston, 1909a) was later confirmed by that author to be *D. australis* when he subsequently recorded its occurrence in the intestine of an emu in NSW (Johnston, 1910).

Mawson *et al.* (1986) updated the checklist of helminths from Australian birds. *Cotugnia collini, Cotugnia* sp., *R. (R). australis* and *Raillietina* sp. are listed. *Davainea australis* and *T. australis* appear as synonyms of *R. (R). australis.* The authors explain that "some of the material listed (from Australian birds) was not fully identified, particularly the cestodes, as less work has been done in Australia on these parasites than on other helminths" (Mawson *et al.*, 1986, p. 220).

There is thus confusion regarding the cestode fauna of emus. My study examines the status of these helminths.

Chapter 2

GENERAL MATERIALS AND METHODS

A flash of inspiration which creates the idea or the image is not, of course, sufficient in itself, for the idea must then be converted into reality. This surely is the role of technique (Smyth, 1967).

2.1 Cestodes

The cestodes examined were obtained from a variety of sources including preserved material archived in Museums and laboratory collections, as well as fresh specimens from farmed emus slaughtered in abattoirs or fortuitously collected from wild emus following accidental death or, in one case, as a result of culling.

2.2 Location of emu farms

Studies were conducted on emu farms at Keith, SA (36° 06' S, 140° 19' E), Glossop, SA (34° 16' S, 140° 32' E) and Avenue, SA (36° 57' S, 140° 14' E) (Fig. 1).

2.3 Collection of wild emus

Three wild emus were collected, one at Kiki, SA (35° 41' S, 139° 51' E) on 29.vi.1999, one at Meningie, SA (35° 54' S, 136° 27' E) on 11.xi.1999 and one at Ucolta, SA (32° 57' E, 138° 57' S) on 27.viii.2002 (Fig. 1). Material was collected from wild birds with permits from the SA Department of National Parks and Wildlife (# E24326 1, 2 & 3).

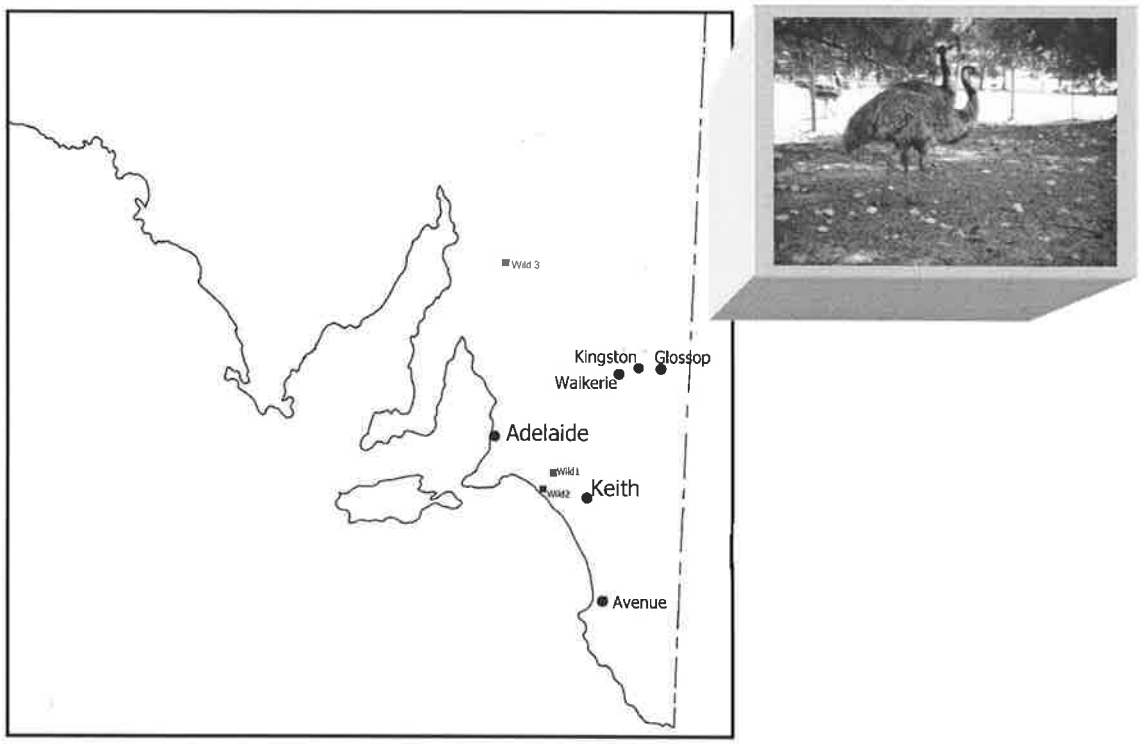


Fig. 1. Map of South Australia depicting sites of emu collection referred to in this study. Emu farm at Keith (inset).

2.4 Location of abattoirs

Portions of, or whole, gastro-intestinal tracts (GITs), blood and liver samples were collected from emus slaughtered at Gateway Meats, Waikerie, SA and Dalriada Meats, Keith, SA (Fig. 1).

2.5 Details of abattoir collections

Between November 1996 and April 1997, a segment of small intestine approximately 5-8 cm long was randomly dissected from the GIT by Mr P. Heap, a meat inspector at the Waikerie abattoirs and sent to the South Australian Research and Development Institute, (SARDI) Parasitology Laboratory. Whole GIT's were collected from abattoirs at Keith, SA and Waikerie, SA between 1998 and 2000.

2.6 Drawings and measurements

Measurements were made with the aid of an eyepiece graticule calibrated with an Olympus objective micrometer. In the text, all measurements appear in millimetres (mm), as the range followed, in parentheses, by the mean and the number of observations.

Drawings were made with the aid of a drawing tube attached to an Olympus BH microscope. Rostellar hooks were measured *en face*, sucker hooks and accessory spines were measured on side.

2.7 Effects of mounting medium

No difference was detected between hooks measured *en face* or on side in either lactophenol or De Fauré's mounting medium (Appendix B, Table 46). De Faure's medium resulted in a permanent mount and was the medium of choice for examining cleared scoleces and cysticercoids and for obtaining measurements of rostellar hooks, sucker hooks and accessory spines.

2.8 Terminology

Apart from the descriptions of cestode species (Chapter 3), cestodes referred to elsewhere in this study were identified using combinations of the following characters: the size of rostellar hooks; the number of rostellar hooks; the size of the scolex; and the size and shape of the cirrus sac.

2.9 Statistical analyses

Statistical analyses were performed using Statistix for Windows. More specific analyses applied to the significance of rostellar hook length and the intestinal distribution of cestode species (Chapter 4) were conducted with the assistance of J. Jones, BiometricsSA. Statistical probabilities were calculated using the two-sample *t*-test adjusted for equal or unequal variance, analysis of variance, the least significant difference (LSD) and the Tukey HSD comparison of means methods and the Kruskal-Wallis test (Morrison, 2002). Analyses have been performed on some data sets even though there is a lack of true replication, i.e., data have been analysed from a subsample of the experimental unit, the emu.

Chapter 3

TAXONOMY OF THE CESTODES INFECTING STRUTHIONIFORMES IN AUSTRALIA

In practice, most cestode species are defined for convenience from morphological criteria, though some difficulties arise in the selection of specific criteria. Undoubtedly, taxonomists obtain by intuitive means an appreciation of variation within taxa that they study and apply this knowledge to the classification they construct (Beveridge, 1974).

3.1 Introduction

The description of the morphology of the cestodes infecting emus is undertaken to determine the number of species infecting emus in Australia and to establish the validity of the cestodes previously described in emus. In addition, it is proposed to compare the morphology of the cestodes with those reported in closely-related hosts.

Wardle (1932a) suggested that examination of a wide range of uniform material, exposed to various techniques is required for specific differentiation of Cestoda. Accepting also that certain characters may alter during relaxation and fixation and be influenced by artificial media (Wardle, 1932b), attempts were made to establish the limits of morphometric characters in a large sample of tapeworms, particularly to assess their use as distinguishing characters.

3.2 Material examined

3.2.1 Australian Helminth Collection (AHC)

Cestodes from emus were obtained from the AHC, at the South Australian Museum, Adelaide (SAMA). The identity of specimens examined is detailed in Table 4. In some cases, only slide material was available for examination. The remainder of

Table 4. Details of the cestodes from emus held in the Australian Helminth Collection (AHC SAMA).

SAMA AHC Number	Bottle (b) or Slide (s)	Locality	Collector	Date
227	(b)	Shelley River, Qld	No data	29.ix.1907
542	(b)	No data	No data	No data
1187	(b)	Mundulla, SA	Dinning	February, 1933
3329	(b)	Parachilna, SA	No data	Oct, 1959
8125	(b)	Vic.	K. Harrigan	No data
9179	(b)	Condobolin, NSW	Ryan	27.i.1971
10005	(b)	Kinchega, NSW	I. Beveridge	31.iii.1974
10006	(b)	Kinchega, NSW	I. Beveridge	31.iii.1974
10511	(b)	Pine Plains, Vic	I. Beveridge	14.v.1971
11008	(b)	Menindee, NSW	I. Beveridge	10.viii.1977
10102	(b)	La Trobe, Vic	I Beveridge	24.vi.1975
11181	(b)	Yunta, SA	G. Ford	1.ix.1981
18391	(b)	Werribee, Vic	K. Harrigan	April, 1988
22926	(b)	Healesville, Vic	I Beveridge	3.vi.1989
26698	(b)	Werribee, Vic	I. Beveridge	23.vi.1995
	(b)	Badgingarra, WA	I. Beveridge	13.xii.2001
20429	(s)	No data	No data	No data
20430	(s)	NSW	TH Johnston &TL Baldock	1914
20431	(s)	North West WA	TH Johnston	No data
20432	(s)	North West WA	JB Cleland	No data
20433	(s)	As for 20430		
20837	(s)	La Trobe, Vic	I. Beveridge	24.vi.1972
21347	(s)	As for 11181		
26205	(s)	Vic	D. Turner	1994
27716	(s)	Wagga, NSW	No data	7.xii.1994
27717	(s)	Bairnsdale, Vic	I. Beveridge	5.xii.1994
27718	(s)	As for 27717		
31574	(b)	Badgingara, WA	I. Beveridge	13.xii.2001

the material examined consisted of preserved cestodes in bottles. Museum numbers may designate single specimens or several cestodes. Collection details are given where available.

3.2.2 Parasitology Laboratory Collection, South Australian Research and Development Institute (SARDI)

The cestodes from emus held in this collection are detailed in Table 5. Material consisted of preserved cestodes in bottles and one sample of stained cestode proglottides on a slide.

Table 5. Details of cestodes from emus held in the SARDI Collection

Identification Number	Bottle (b)	Locality	Collector	Date
4852	(b)	Adelaide Zoo, SA	M. O'Callaghan	22.vi.1987
-	(b)	Kersbrook, SA	I. Beveridge	April, 1989
7496	(b)	Yorktown, SA	M. O'Callaghan	14.viii.1992
11224	(b)	Kyneton, SA	M. O'Callaghan	24.xi.1992
1724	(b)	Port Lincoln, SA	E. Moore	25.ii.1992
10742	(b)	Gawler, SA	E. Moore	28.ix.1994
9070	(b)	Gawler, SA	E. Moore	11.x.1994
9204	(b)	Port Lincoln, SA	E. Moore	17.x.1994
6023	(b)	Kadina, SA	M. O'Callaghan	11.vii.1995
7137	(b)	Avenue Range, SA	M. O'Callaghan	20.ix.1995
1712	(b)	Lock, SA	M. O'Callaghan	9.iii.1996
6259	(b)	Kadina, SA	E. Moore	19.ix.1996
-	(b)	Unknown	P. Heap	14.x.1996
-	(b)	Maitland, SA	M. O'Callaghan	13.iii.1997

3.2.3 Material collected at abattoirs from farmed emus

Between 15.xi.1996 and 30.iv.1997, cestodes were recovered from a segment of GIT collected by Mr P. Heap and sent to the laboratory. Between 27.x.1998 and 20.x.2000, cestodes were recovered from entire GITs collected at abattoirs and transported to the laboratory (Table 6).

3.3 Preparation of cestodes

3.3.1 Staining of cestodes

Cestodes were washed and then relaxed in tap water for up to 15 h. After fixation in 10% buffered formalin, cestodes were stained with Heidenhain's haematoxylin, Celestine blue and Semichon's acetocarmine. The benefit of using three stains was that each stain highlighted different features of internal structure. Once each character had been recognised and described, Celestine blue became the stain routinely used for diagnostic purposes.

The following methods were adopted:

Heidenhain's haematoxylin

1. Cestodes washed in distilled water
2. Stained in Heidenhain's haematoxylin for up to 5 h
3. De-colourised in acid/alcohol (1% hydrochloric acid in 70% ethanol) for up to 2 h
4. De-hydrated through graded ethanol including absolute ethanol
5. Cleared in clove oil for 12-48 h
6. Mounted in Canada balsam

Celestine blue

1. Cestodes washed in distilled water
2. Stained in Celestine blue for one to 2 h
3. Washed in distilled water

4-6. As above.

Semichon's acetocarmine

1. Cestodes placed directly into stain

2. Stained one to 2 h

3. Rinsed in 70% ethanol

4-6. As above

Table 6. Details of material collected at abattoirs. Number of emus examined (in parentheses).

Date	Locality of emu farm and number of emus sampled
15.xi.1996	Waikerie, SA (1); Minlaton, SA (2); Mount Gambier, SA (2)
22.xi.1996	Mount Gambier, SA (2); York Peninsula, SA (2)
5.xii.1996	Glossop, SA (1); Waikerie, SA (2); Ceduna, SA (1)
11.xii.1996	Parilla, SA (2)
14.xii.1996	Tailem Bend, SA (1); Mount gambier, SA (1)
24.i.1997	Waikerie, SA (1); Lameroo, SA (1)
7.ii.1997	Apsley, Vic (1); Berri, SA (1)
19.ii.1997	Millicent, SA (2)
7.iii.1997	Mannum, SA (1); Waikerie, SA (1); Yumali, SA (1)
19.iii.1997	Taplan, SA (1); Mount Gambier, SA (1); Truro, SA (1)
30.iv.1997	Moorook, SA (2)
10.viii.1998	Kingston on Murray, SA (7)
27.x.1998, 8.xii.1998, 30.iii.1999, 25.v.1999 & 29.vii.1999	Keith, SA (25)
20.x.2000	Glossop, SA (5)

3.3.2 Measurements

Measurements of the internal structure of adult cestodes were made on relaxed cestodes that were stained, cleared and mounted in Canada balsam. The period of relaxation in tap water varied from 4-15 h at room temperature. Relaxed cestodes were fixed in 10% buffered formalin and stored in 70% ethanol with 5% glycerol. Scoleces were sliced from whole worms under a dissecting microscope, mounted and cleared in De Faurés medium at 60°C. In order to examine hook shape, scoleces were crushed using gentle pressure to force hooks, hooklets and accessory spines onto their sides.

3.3.3 Terminology

The format of species descriptions and detail of morphological characters is drawn from several authors, mainly the work of Buscher (1975), Deardorff *et al.* (1976), Beveridge (1981), Sato *et al.* (1988) and Jones and Anderson (1996) with the objective of standardising style and format and assisting in accurate identification.

3.4 Results

3.4.1 Species descriptions

3.4.1.1 ***Raillietina australis*** (Krabbe, 1869) Fuhrmann, 1924

FIGS 2-13

Synonyms: *Taenia australis* Krabbe, 1869. K. Danske Vidensk Selsk. Skr. Naturv. Og Math. Afd. 8, 249-363. Figs 296-298; *Davainea australis* Blanchard, 1891; *Ransomia australis* Fuhrmann, 1920; *Kotlania australis* Lopez-Neyra, 1931; *Raillietina australis* Fuhrmann, 1924.

Holotype: In Zoologisk Museum, Copenhagen. Denmark.

Material examined: Kadina, South Australia (SA) (33° 58'S, 137° 48'E), Collector (coll.). M. O'Callaghan, 11.vii.1995, SAMA AHC 31376, British Museum Natural History (BMNH) 2000.5.17.1-10,Werribee, Victoria (Vic.), coll. K. E. Harrigan, iv.1988, SAMA AHC 18391; Shelley River, Queensland (Qld), 29.ix.1907, SAMA AHC 227; Kinchega, New South Wales (NSW), coll. I. Beveridge, 31.iii.1974, SAMA AHC 10006; Yunta, SA, coll. G. E. Ford, 1.ix.1981, SAMA AHC 11181; north-west Western Australia (WA), coll. T. H. Johnston, SAMA AHC S20431.

Description

Cestodes of moderate size, up to 50 long in unrelaxed specimens and up to 110 in relaxed specimens. Maximum width 1.2. Strobila containing approximately 1150 proglottides. Scolex 0.416-0.568 (0.498, n=20) in diameter, usually with retracted rostellum (Figs 2, 9) in fixed specimens. Occasionally with everted rostellum (Fig. 3). Rostellum 0.200-0.288 (0.249, n=10) in diameter armed with 280-362 (326) hammer-shaped hooks arranged in two rows. Larger rostellar hooks 0.021-0.030 (0.025, n=250) in length, smaller rostellar hooks 0.016-0.023 (0.02, n=250) (Figs 4, 10). Base of rostellum armed with 16-20 rows small, rose-thorn-shaped accessory spines 0.002-0.004 in length. Suckers 0.136-0.168 (0.149, n=30) in diameter armed with eight diagonally-arranged rows of hooks 0.005-0.011 in length (Fig. 5).

Proglottides craspedote. Mature proglottides wider than long, 0.160-0.184 (0.171) x 0.800-0.848 (0.822, n=10) (Figs 6, 11). Genital pores single, unilateral, 0.016 in diameter; genital ducts passing between longitudinal osmoregulatory canals. Dorsal osmoregulatory canal 0.048 in maximum diameter, lying internal to smaller ventral osmoregulatory canal, 0.02 in diameter. Transverse osmoregulatory canals connecting left and right ventral canals at posterior margin of each proglottis.

Genital atrium small, situated in anterior half of lateral proglottis margin and surrounded by an accumulation of cells. Cirrus sac elongate 0.152-0.164 (0.158) x 0.016-0.024 (0.020, n=10) (Figs 7, 12) extending to but not beyond dorsal osmoregulatory canal. Distal region of cirrus of greater internal diameter than mid region, armature not seen; proximal region forming small, spherical, internal seminal vesicle, 0.016 in diameter. Cirrus sac in holotype 0.149 x 0.023, also with internal seminal vesicle 0.016 in diameter. Coiled vas deferens passing towards centre of proglottis where it becomes convoluted, occasionally overlying seminal receptacle before passing posteriorly towards ovary. Testes

in poral and aporal fields, numbers 43-8 (5) poral and 11-13 (10, n=40) aporal, bounded by lateral osmoregulatory canal. Testes 0.044-0.052 (0.048, n=10) in diameter.

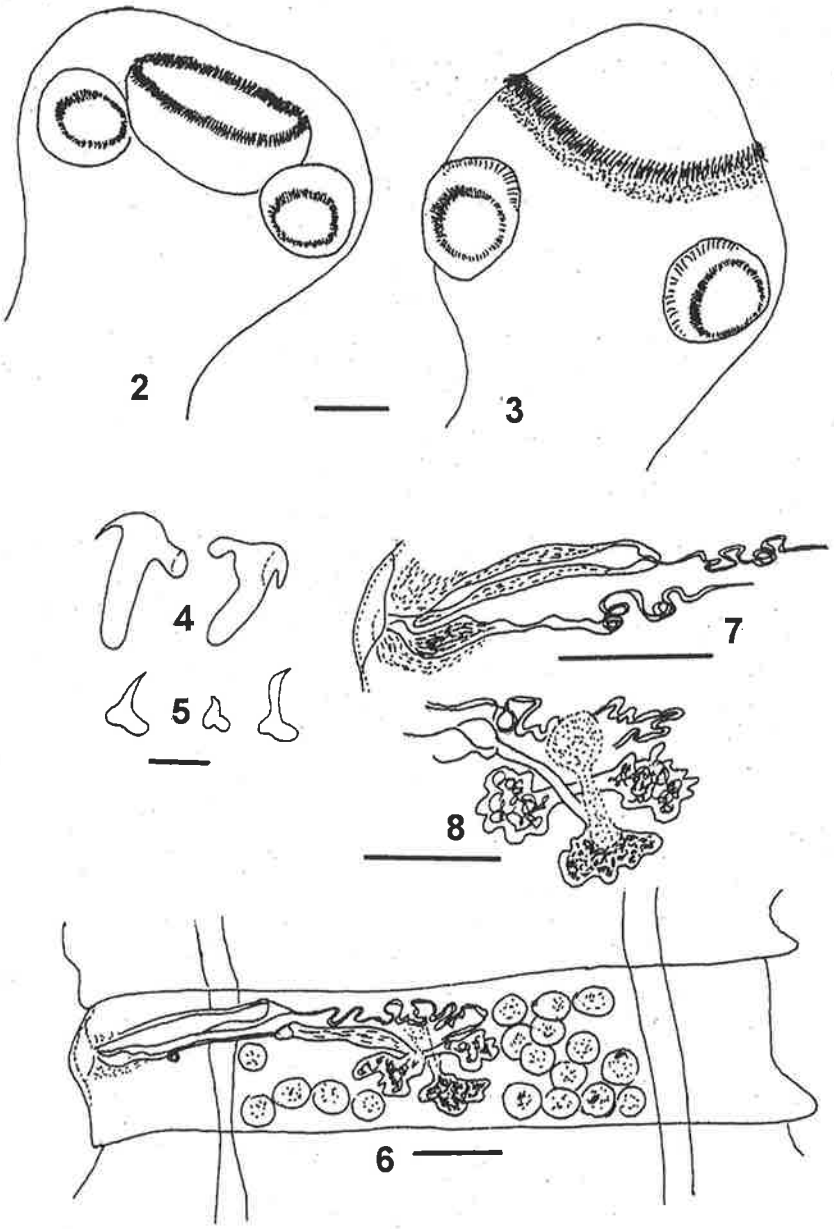
Vagina opening to genital atrium posterior to cirrus sac. Distal region slightly enlarged 0.024-0.032 (0.029) x 0.010-0.016 (0.015, n=10) (Fig. 7). Mid region, narrow, coiled 0.005 in diameter, leading to a seminal receptacle medially posterior to vas deferens, 0.084-0.128 (0.122) x 0.024-0.032 (0.026, n=10) and lying anterior and dorsal to poral lobe of ovary. Ovary distinctly bilobed, situated in mid line of proglottis (Fig. 8). Poral lobe 0.040-0.072 (0.049) x 0.040-0.060 (0.048, n=10), aporal lobe 0.044-0.072 (0.060) x 0.032-0.072 (0.043, n=10) with 3-5 lobules in each lobe. Vitellarium irregularly lobulate, post ovarian, slightly aporal, occasionally dorsal to aporal lobe of ovary, 0.06-0.08 (0.068) x 0.036-0.048 (0.042, n=10). Uterine duct passing anteriorly to developing uterus. Gravid proglottides extending transversely 0.969-1.515 (1.284)x 0.242-0.949 (0.548, n=20) with large osmoregulatory canal up to 0.120 in diameter. Egg capsules irregularly ovoid 0.108-0.132 x 0.080-0.104. Egg capsules 76-110 (88, n=10) per gravid proglottis containing 10-14 (11, n=40) eggs. Terminal proglottides extending transversely, as wide as long 0.58- 0.8 x 0.6- 0.88. Oncosphere 0.012 in diameter, oncospheral hooks 0.005-0.007 long.

Host

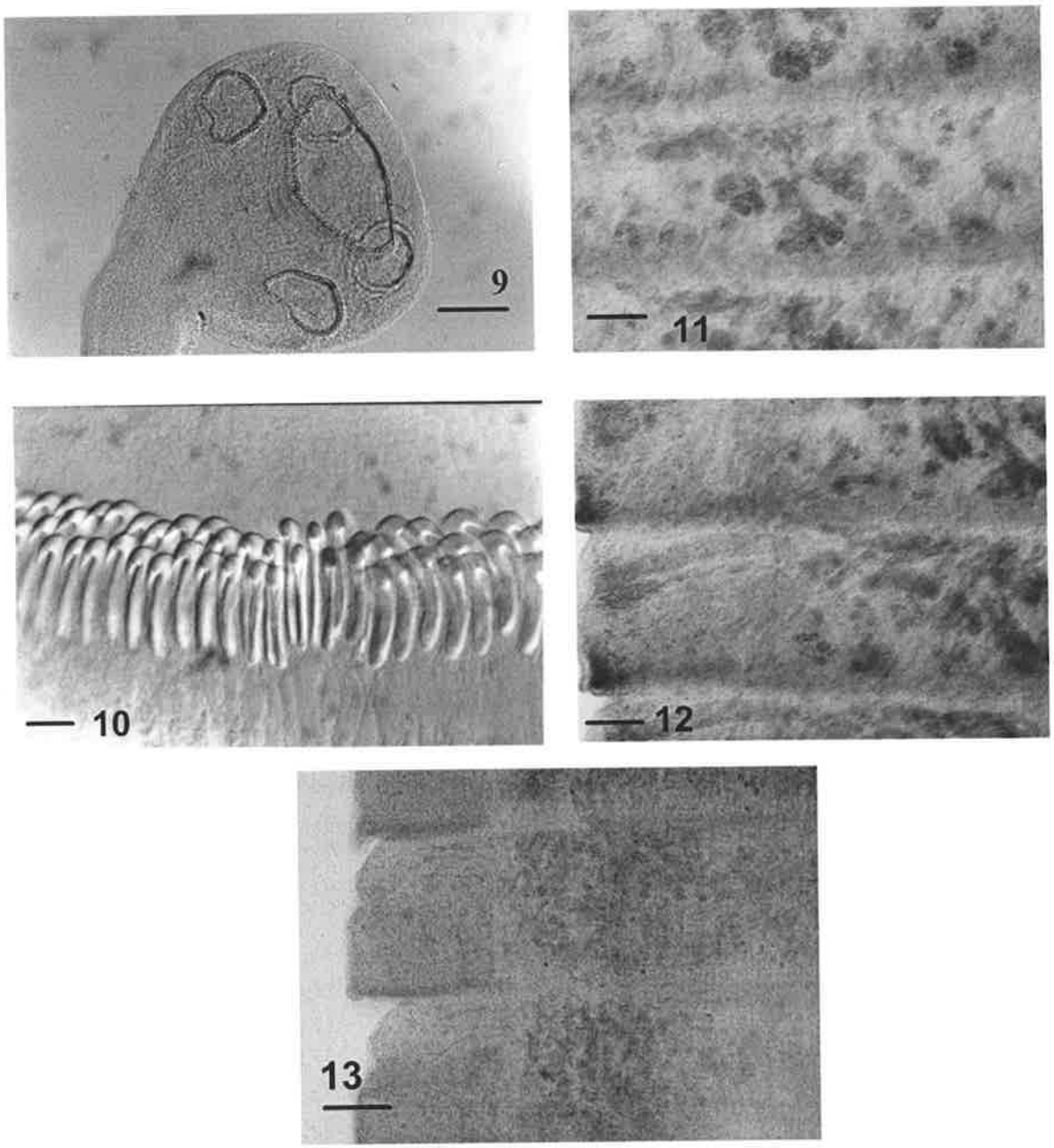
Dromaius novaehollandiae (Latham, 1790) (Struthioniformes: Dromaiidae)

Location in host

Small intestine.



Figs 2-8. *Raillietina australis.* 2. Scolex with retracted rostellum. 3. Scolex with everted rostellum. 4. Rostellar hooks. 5. Sucker hooks. 6. Single mature proglottis. 7. Cirrus and distal vagina. 8. Female genitalia. Scale bars = 0.1 mm, 2, 3, 6-8; 0.01 mm, 4, 5.



Figs 9-13. *Raillietina australis*. 9. Scolex. 10. Rostellar hooks. 11. Mature proglottides. 12. Cirrus and distal vagina. 13. Cirrus and distal vagina of holotype specimen. Scale bars = 0.1 mm, 9; 0.05 mm, 11-13; 0.01 mm, 10.

3.4.1.2 ***Raillietina beveridgei*** O'Callaghan, Davies and Andrews, 2000.

FIGS 14-24

Holotype: Keith, SA (36° 06'S, 140° 19'E), coll. M. O'Callaghan, 30.iii.1999, SAMA AHC S28300.

Paratypes: Lock, SA (33° 34'S, 135° 45'E), coll. M. O'Callaghan, 9.iii.1996, SAMA AHC S28301, 31377, BMNH 2000.5.17.11-30.

Other material examined: Yunta, S A, coll. G. E. Ford, 1.ix.1981, SAMA AHC 11181, S21347; Werribee, Vic., coll. I. Beveridge, 23.vi.1995, SAMA AHC 26698; Mundulla, SA, coll. Dinning, February 1933, SAMA AHC 1187; Bairnsdale, Vic., coll. I Beveridge, 5.xii.1994, SAMA AHC S27717, S27718; Condobolin, NSW, coll. Ryan, 27.i.1971, SAMA AHC 9179; Vic., coll. D.Turner, 1994, SAMA AHC S26205; NSW, coll. T. H. Johnston/T. L. Bancroft, 1914, SAMA AHC S20430, S 20433; La Trobe, Vic., coll. I. Beveridge, 24.vi.1972, SAMA AHC S20837; Bairnsdale, Vic. Coll. I. Beveridge, Scolex only, 5.vii.1994, SAMA AHC S27717, S27718.

Description

Large cestode, up to 160 long in unrelaxed specimens and up to 600 in relaxed specimens; gravid strobila containing approximately 750 segments. Strobila with maximum width 3.8 in relaxed specimens. Scolex 0.480-0.736 (0.609, n=25) wide at suckers (Figs 14, 21). Retracted rostellum 0.192-0.258 (0.234, n=10) diameter with 304-412 (370, n=10) hammer-shaped hooks in two rows. Larger rostellar hooks 0.016-0.021 (0.019, n=250) long; smaller rostellar hooks 0.014-0.019 (0.016, n=250) long (Figs 15, 22). Very small accessory rostellar spines approximately 0.001-0.002 in length on rostellar sac, only visible under high magnification. Suckers circular 0.136-0.168 (0.150, n=10) in

diameter armed with 12-18 rows hooks 0.004-0.011 long (Fig. 16). Neck variable, up to 0.250 in length. Calcareous corpuscles present in posterior half of scolex.

Proglottides craspedote. Mature proglottides wider than long, 1.554-1.932 (1.730) x 0.273-0.399 (0.326, n=20) (Figs 17, 23). Genital pores single, unilateral. Large, ventral, longitudinal osmoregulatory canal 0.108 maximum diameter joined by transverse canal connecting left and right lateral canals in posterior margin of each proglottis. Dorsal canals not seen. Genital anlage appear in approximately segment 150. Male and female genitalia mature in proglottides 200 and 300 respectively, and first eggs appear in 480.

Genital atrium small, situated in anterior half of lateral proglottis margin. Cirrus sac 0.256-0.328 (0.298, n=10) x 0.08 extending to ventral osmoregulatory canal (Figs 18, 24). Distal region of cirrus lined with spines, of greater internal diameter than sinuous mid region; proximal region forms spherical internal seminal vesicle 0.06-0.092 (0.079) x 0.052-0.06 (0.056, n=10), not detectable in proglottides of every cestode examined. Vas deferens greatly coiled, extending anteriorly across midline of each proglottis then returning posteriorly towards ovary. Testes distributed in poral and aporal fields within area defined by ventral osmoregulatory canals, number 5-9 (7, n=30) poral and 12-18 (15, n=30) aporal. Testes sub-circular, 0.08-0.1 (0.088) x 0.08-0.088 (0.083, n=10) not overlying ovary or vitellarium.

Vagina opening to genital atrium posterior to cirrus sac. Distal region with thickened muscular wall 0.088-0.12 (0.106, n=10). Mid region of vagina narrow, coiled, leading medially, posterior to vas deferens to seminal receptacle varying in length from 0.088-0.240 (0.100, n=20), lying anterior to testes and poral lobe of ovary. Sperm duct passing posteriorly from seminal receptacle. Ovary bilobed, 0.084-0.132 (0.11) x 0.08-0.088 (0.082, n=10) with 4-6 lobules in each lobe (Fig. 19). Vitellarium ovoid, 0.112-0.136 (0.12) x 0.08-0.1 (0.087, n=10) situated posterior to ovary; uterine duct passing anteriorly

to developing uterus. Gravid proglottides wider than long, 2.5-2.7 x 0.4-0.5. Terminal proglottides longer than wide, 1.0 x 0.9 (Fig. 14). Gravid proglottides containing 30-40 (35, n=10) egg capsules, 0.168-0.2 (0.184) x 0.144-0.196 (0.161), n=10) each containing 10-12 eggs , 0.04 in diameter. Oncosphere 0.014-0.016 (0.016) x 0.011-0.016 (0.013, n=10). Oncospheral hooks 0.004-0.006 (0.005, n =10).

Host

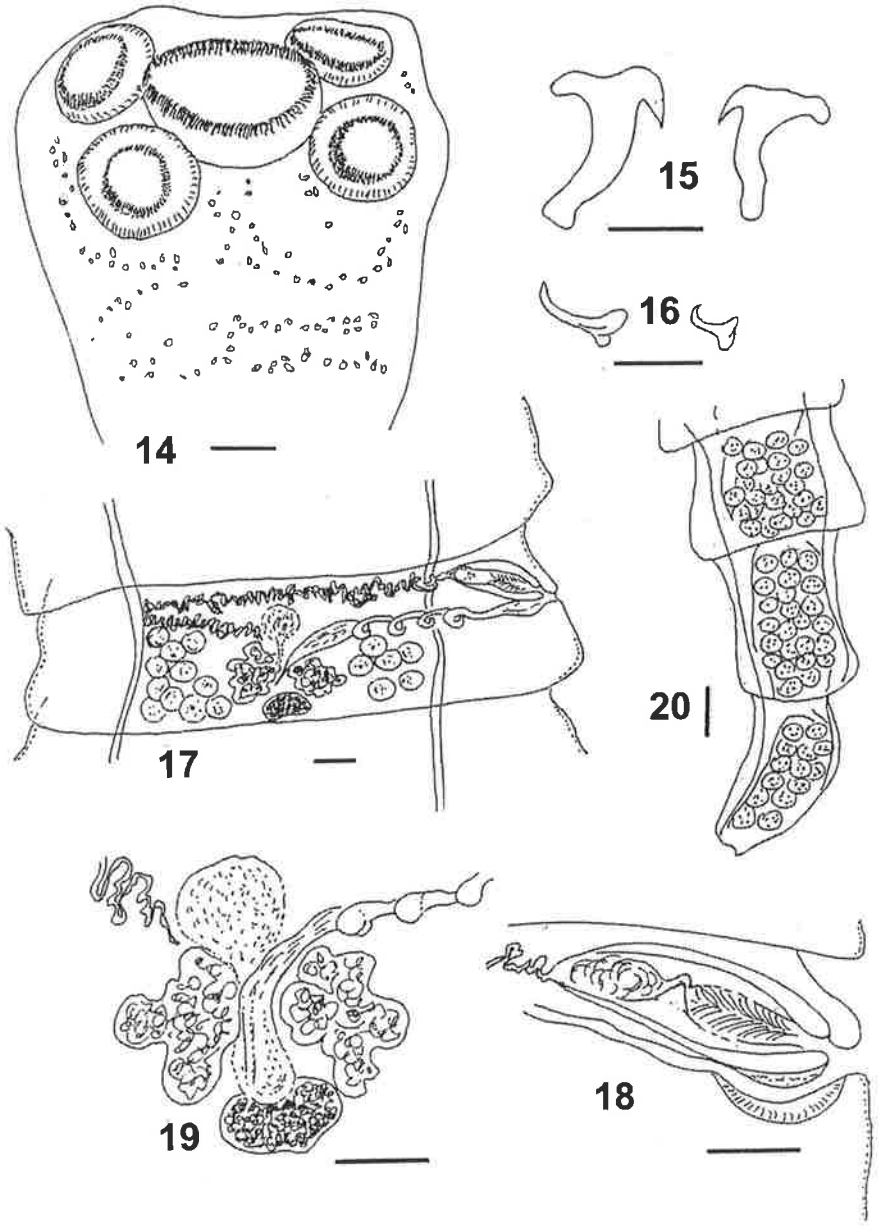
Dromaius novaehollandiae (Latham, 1790) (Struthioniformes: Dromaiidae).

Location in host

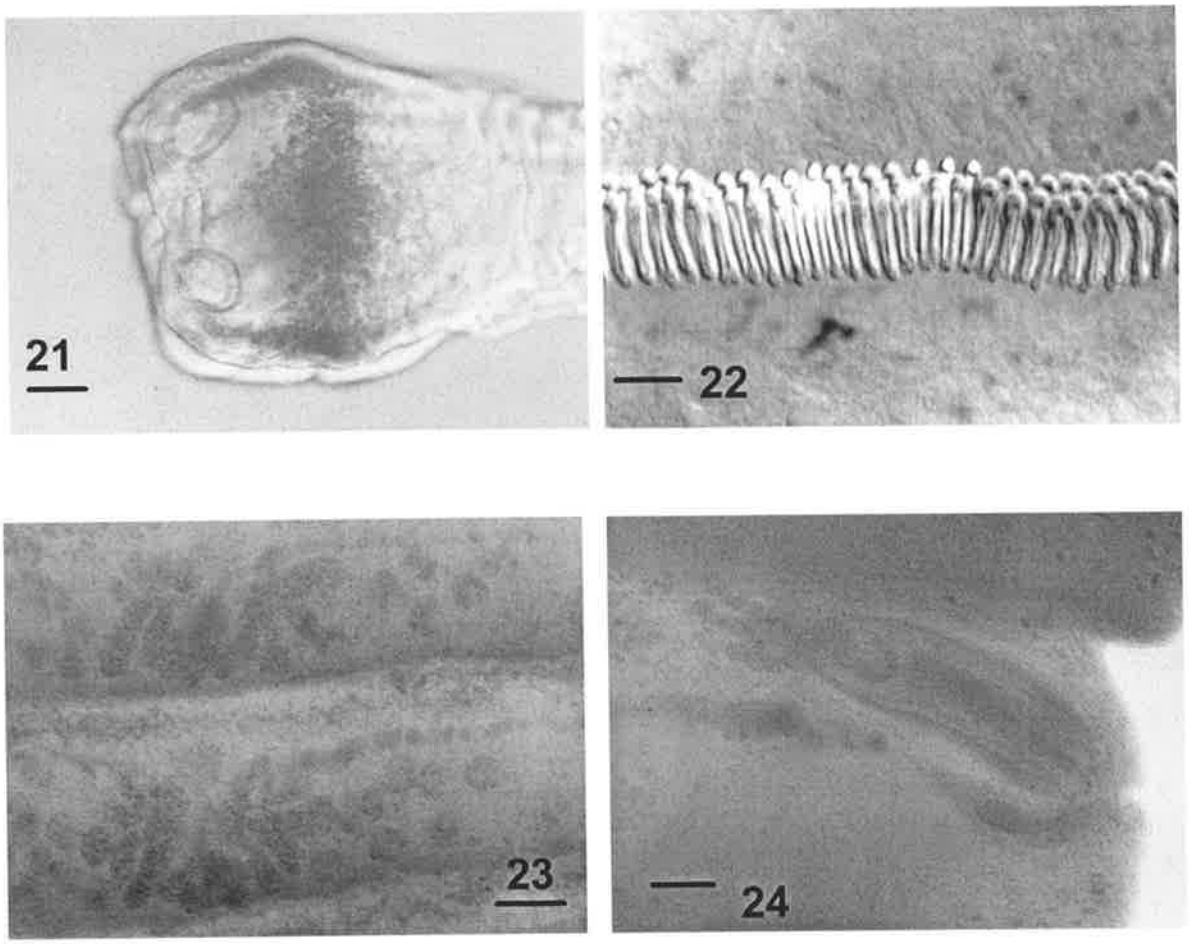
Small intestine.

Etymology

This species is named for Dr I. Beveridge in recognition of his outstanding contribution to our knowledge of the parasites of the Australian endemic fauna and his guidance to me.



Figs 14-20. *Raillietina beveridgei*. 14. Scolex. 15. Rostellar hooks. 16. Sucker hooks. 17. Single mature proglottis. 18. Cirrus and distal vagina. 19. Female genitalia. 20. Terminal gravid proglottides. Scale bars = 0.1 mm, 14, 17-20; 0.01 mm, 15, 16.



Figs 21-24. *Raillietina beveridgei*. 21. Scolex. 22. Rostellar hooks. 23. Mature proglottides. 24. Cirrus and distal vagina. Scale bars = 0.1 mm, 21, 23; 0.05 mm, 24; 0.01 mm, 22.

3.4.1.3 ***Raillietina chiltoni*** O'Callaghan, Davies and Andrews, 2000.

FIGS 25-36

Holotype: Keith, SA (36° 06' S, 140° 19' E), SAMA AHC S28302.

Paratypes: Kersbrook, SA (34° 47'S, 138° 51'E), coll. I. Beveridge, 1.iv.1989, SAMA AHC 31378, BMNH 2000.5.17.31-40.

Description

Cestodes up to 90 long in relaxed specimens, maximum width 1.4. Strobila contain approximately 360 proglottides. Scolex 0.545-0.832 (0.643, n=20) in diameter with eversible rostellum, 0.336-0.480 (0.383, n=10) in diameter, retracted in majority of specimens (Figs 25, 26, 33). Rostellum armed with 250-350 (272, n=10) hammer-shaped hooks in two rows. Larger rostellar hooks 0.026-0.039 (0.032, n=250) in length; smaller rostellar hooks, 0.022-0.034 (0.027, n=250) in length (Figs 27, 34). Rostellar sac at base of rostellum armed with rose-thorn-shaped accessory spines 0.003 in length, visible under high magnification only and in specimens with fully everted rostellum. Suckers 0.136-0.2 (0.171, n=30) in diameter, armed with 8-14 rows of hooks 0.005-0.013 long (Fig. 28). Neck variable in length, 0.4-0.8 in relaxed specimens.

Proglottides craspedote. Mature proglottides 0.890-1.400 x 0.072-0.140 (Figs 29, 35). Genital pores single, unilateral; genital ducts passing between osmoregulatory canals. Dorsal osmoregulatory canal extremely narrow, diameter 0.002, lying internal to ventral osmoregulatory canal, 0.012 maximum diameter. Transverse osmoregulatory canals connecting right and left ventral canals at posterior margin of each proglottis. Dorsal commissures not seen. Genital anlage appearing in proglottis 40 approximately; first mature proglottis 160; first gravid proglottis 280.

Genital atrium small, situated in anterior half of lateral proglottis margin. Cirrus sac 0.104-0.112 (0.108) x 0.036-0.040 (0.038, n=10), not reaching longitudinal osmoregulatory canals (Figs 30, 36). Cirrus unarmed, distal region of greater internal diameter than mid region; leading uncoiled to internal seminal vesicle 0.015 (0.012-0.016) x 0.012 (0.012-0.014). Vas deferens greatly coiled, passing towards centre of proglottis. Testes distributed in two lateral groups, 3-5 (4, n=20) poral and 7-11 (9, n=20) aporal. Testes 0.056-0.068 (0.062, n=20) in diameter; not overlying female genital organs.

Vagina opening to genital atrium posterior to cirrus sac; distal region surrounded by cells, 0.032-0.036 (0.035) x 0.012-0.020 (0.015, n=10). Mid region coiled, often dilated with sperm, leading medially posterior to vas deferens, greatly dilated and saccular anterior to poral lobe of ovary (Fig. 31). Ovary bi-lobed, situated in proglottis midline, enlarging in consecutive mature proglottides, maximum size 0.220 x 0.080 in posterior mature proglottides. Vitellarium similarly enlarging, maximum dimensions 0.184 x 0.080, situated posterior and distal to aporal lobe of ovary. Sperm duct passing posteriorly between lobes of ovary, uterine duct passing anteriorly to developing uterus. Gravid proglottides 1.2-1.7 x 0.200-0.440 (Fig. 32) containing 32-50 (38, n=10) spherical egg capsules, 0.136-0.184 x 0.136-0.192, with 14-17 (15, n=10) eggs per capsule. Oncosphere circular, 0.016-0.020 in diameter, oncospheral hooks 0.006-0.008.

Host

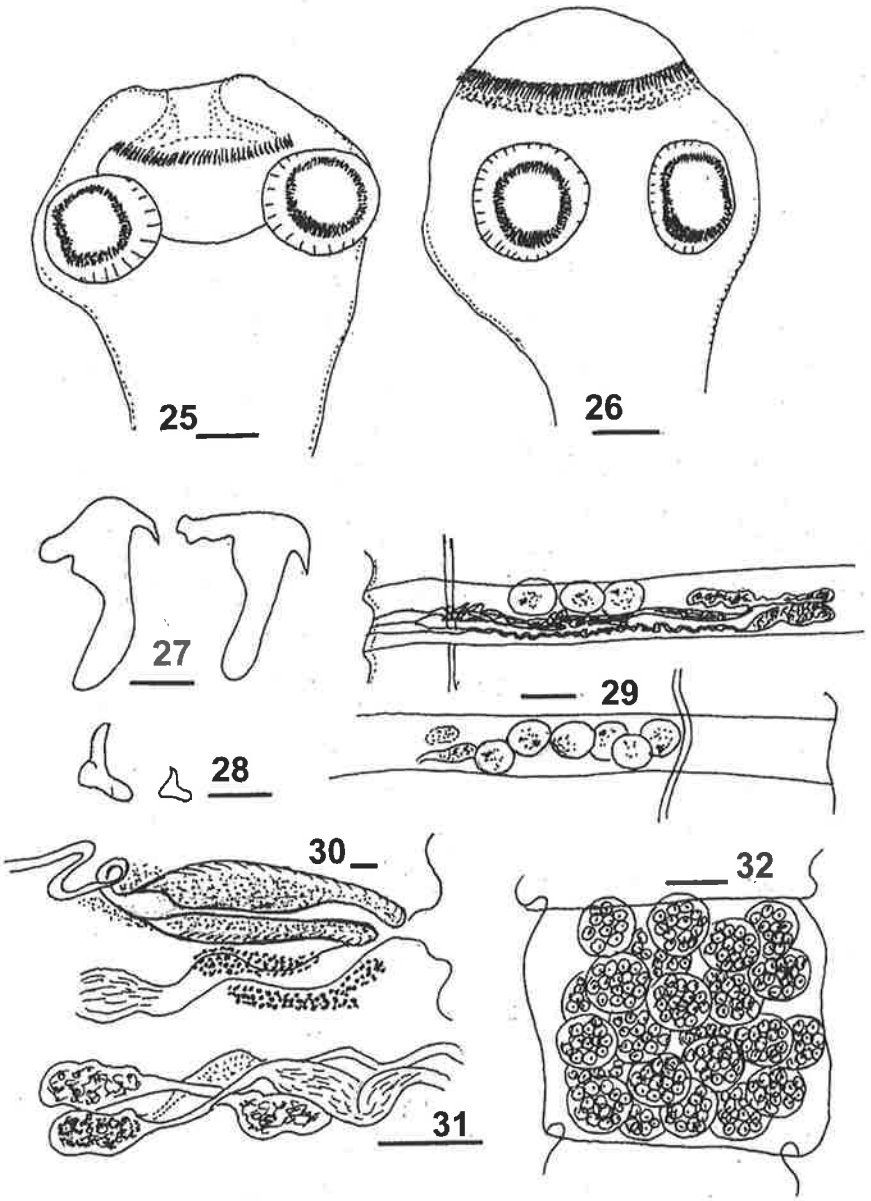
Dromaius novaehollandiae (Latham, 1790) (Struthioniformes: Dromaiidae).

Location in host

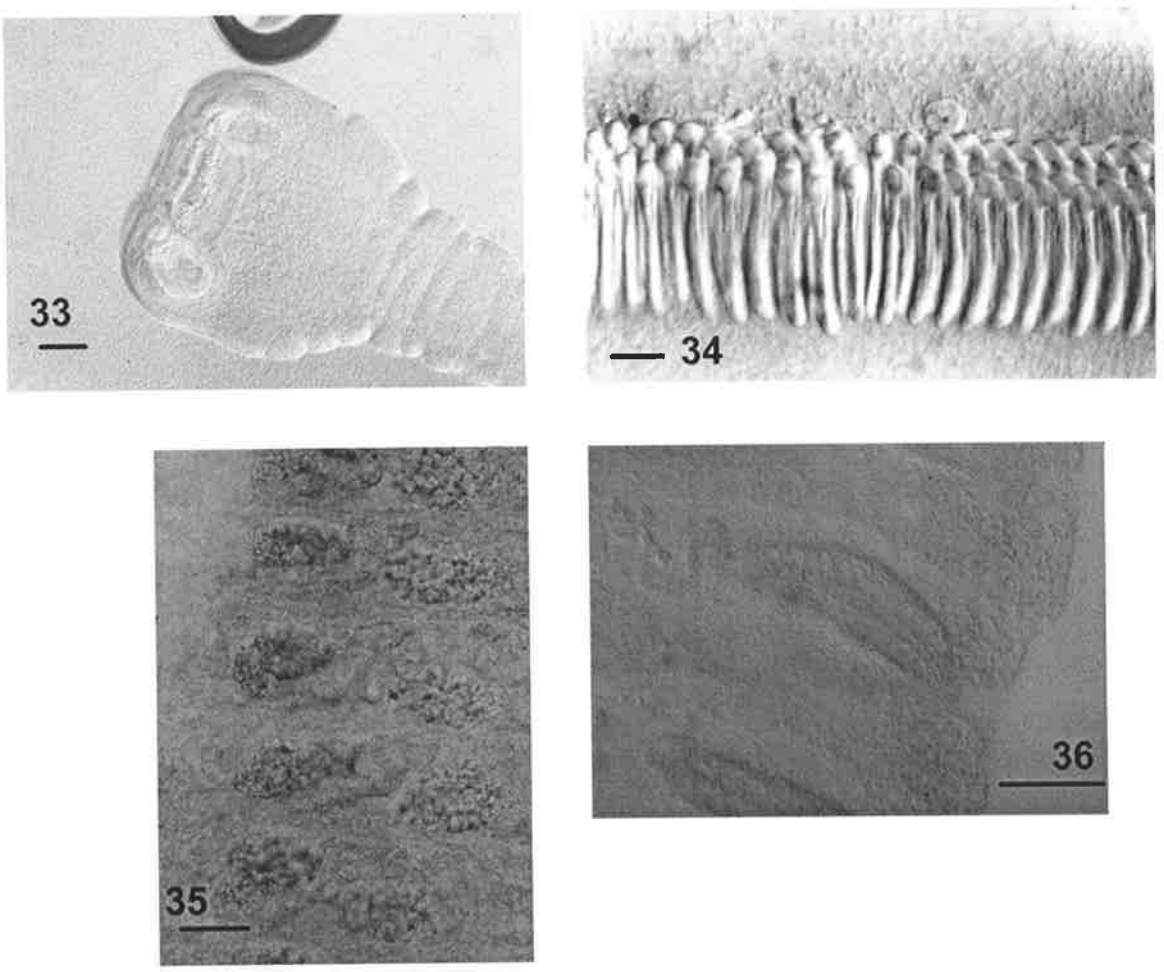
Small intestine.

Etymology

This species is named for Dr N. Chilton, formerly of the University of Melbourne, now University of Saskatchewan, Canada for his contribution to parasitology in Australia.



Figs 25-32. *Raillietina chiltoni*. 25. Scolex with retracted rostellum. 26. Scolex with everted rostellum. 27. Rostellar hooks. 28. Sucker hooks. 29. Single mature proglottis. 30. Cirrus and distal vagina. 31. Female genitalia. 32. Gravid proglottis. Scale bars = 0.1 mm, 25, 26, 29, 31, 32; 0.01 mm 27, 28, 30.



Figs 33-36. *Raillietina chiltoni.* 33. Scolex. 34. Rostellar hooks. 35. Female genitalia. 36. Cirrus and distal vagina. Scale bars = 0.1mm, 33, 35; 0.05 mm, 36; 0.01 mm, 34.

3.4.1.4 ***Raillietina dromaius*** O'Callaghan, Davies and Andrews, 2000.

FIGS 37-48

Holotype: Keith, SA SAMA AHCS28303.

Paratypes: Kingston, S A (34°14'S, 140°21'E), coll. M O'Callaghan, 10.viii.1998, SAMA AHC S28304, S28305, 31379, BMNH 2000.5.17.41-60.

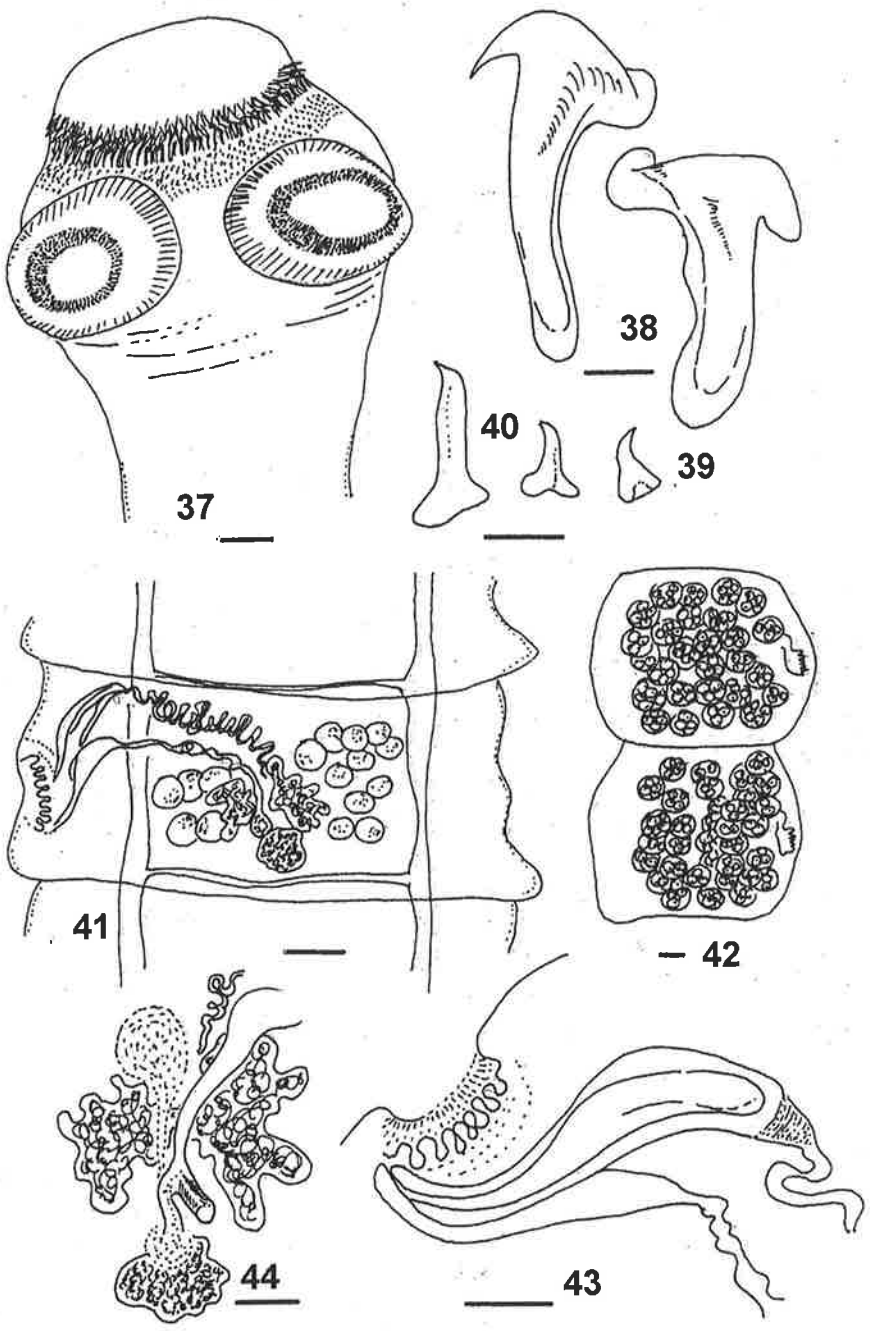
Other material examined: Wagga, NSW, 7.xii.1994, SAMA AHC 27716; Kinchega, NSW, coll. I. Beveridge, 31.iii.1974, SAMA AHC 10005; Menindee, NSW, coll. I. Beveridge, 10.viii.1977, SAMA AHC 11008; Pine Plains, Vic., coll. I. Beveridge, 14.v.1971, SAMA AHC 10511; Condobolin, NSW, 27.i.1971, SAMA AHC 9179; Wagga, NSW, coll. I. Beveridge, 7.xii.1994, Scolex only, SAMA AHC S27716.

Description:

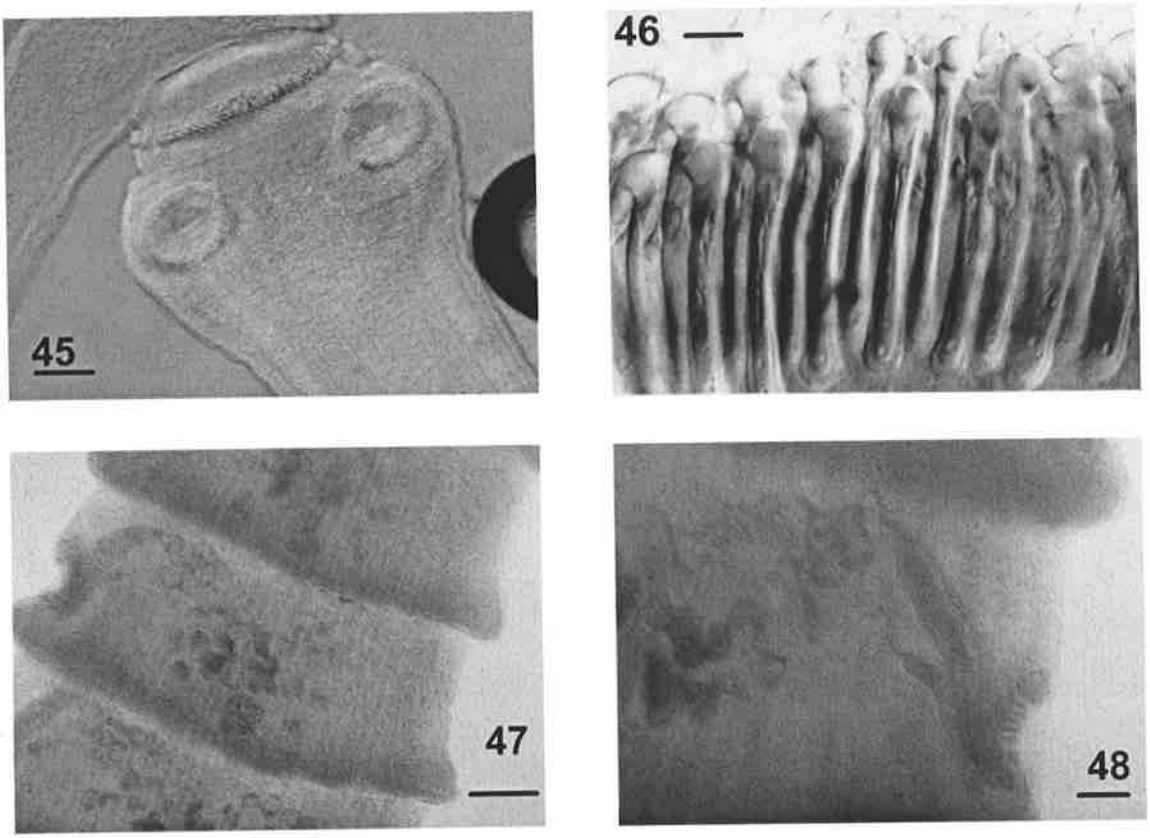
Cestode up to 45 long in unrelaxed specimens and up to 200 in relaxed specimens. Gravid strobila contain 940 proglottides. In relaxed specimens, strobila with a maximum width of 2.12. Scolex 0.480-0.752 mm (0.594, n=20) wide at suckers. Rostellum everted, 0.336-0.448 (0.397, n=20) in diameter (Figs 37, 45), with 124-156 (142, n=10) hammer-shaped hooks in two rows. Larger, inner rostellar hooks 0.05-0.063 (0.056, n=110) long, smaller outer hooks 0.043-0.054 (0.048, n=110) (Figs 38, 46). Scolex surface, posterior to rostellar hooks armed with 15-19 (17, n=25) diagonal rows of rose-thorn-shaped accessory spines 0.008-0.010 (0.009, n=20) long (Fig. 39). Suckers sub-circular 0.192-0.280 (0.234, n=20) x 0.168-0.260 (0.231, n=20) armed with 8-12 rows of hooks varying in length from 0.008-0.02 (Fig. 40). Neck 0.160-0.400 long. Calcareous corpuscles present in neck and less frequently in posterior half of scolex.

Proglottides craspedote. Mature proglottides wider than long, 0.722-1.05 (0.893, n=10) wide x 0.205-0.370 (0.301, n=10) long (Figs 41, 47). Gravid proglottides 0.920-0.980 x 0.740-0.790, 8-10 terminal, urn-shaped proglottides 0.500-0.730 (0.556) x 0.430-0.600 (0.472, n=10) (Fig. 42). Genital pores unilateral, opening into a muscular, plicate genital atrium 0.114-0.135 (0.123, n=10) wide x 0.041-0.082 (0.052, n=10) (Figs 43, 48), extending from the mid-point into posterior half of lateral proglottis margin. Lateral dorsal osmoregulatory canals 0.024-0.032 in diameter joined by transverse commissures in posterior region of proglottides. Ventral osmoregulatory canals not seen. Elongate cirrus sac, 0.246-0.271 (0.257) x 0.041-0.053 (0.044, n=10), extending anteriorly and towards but not reaching lateral osmoregulatory canal. Distal region of cirrus narrow, remainder wide, un-coiled. Vas deferens coiled, voluminous, extending transversely in anterior margin of proglottides. Testes 10-18, in poral and aporal groups, 2-6 (4, n=15) poral and 8-12 (10, n=15) aporal, 0.041-0.057 (0.048, n=15) x 0.040-0.050 (0.040, n=15), lying within lateral osmoregulatory canals.

Vagina opening to genital atrium posterior to male genital pore. Distal region of vagina enlarged, 0.040-0.050 (0.048, n=5) x 0.020-0.024 (0.022, n=5). Mid region sinuous, leading anteriorly and medially, occasionally overlying testes, into a large seminal receptacle, 0.088-0.120 x 0.028-0.040, lying anterior to poral lobe of ovary; sperm duct passes posteriorly, lined with bristles. Ovary bipartite, each lobe of approximately equal size 0.090-0.130 (0.106, n=10) x 0.041-0.061 (0.050, n=10) (Fig. 44). Vitellarium medial, post ovarian, sub-circular 0.074-0.090 (0.082) x 0.066-0.094 (0.08, n=10). Uterine duct passing anteriorly to developing uterus. Egg capsules, spheroidal, 0.136-0.190 (0.156) x 0.099-0.140 (0.124, n=20), 12-18 (15, n=20) in each proglottis; containing 15-22 (17, n=10) eggs, 0.045-0.051 (0.049) x 0.036-0.041 (0.038, n=5). Oncosphere oval 0.017-0.018(0.018, n=5) x 0.014-0.016 (0.015, n=5), embryonic hooks 0.005-0.007 long.



Figs 37-44. *Raillietina dromaius*. 37. Scolex. 38. Rostellar hooks. 39. Accessory rostellar spine. 40. Sucker hooks. 41. Single mature proglottis. 42. Gravid proglottides. 43. Cirrus and distal vagina. 44. Female genitalia. Scale bars = 0.1 mm, 37, 41, 42; 0.05 mm, 43, 44; 0.01 mm, 38-39, 40.



Figs 45-48. *Raillietina dromaius*. 45. Scolex. 46. Rostellar hooks. 47. Mature proglottis. 49. Cirrus and distal vagina. Scale bars = 0.1 mm, 45, 47; 0.05 mm, 48; 0.01 mm, 46.

Host

Dromaius novaehollandiae (Latham, 1790) (Struthioniformes: Dromaiidae)

Location in host

Small intestine.

Etymology

This species is named after the host, *Dromaius novaehollandiae*.

3.4.1.5 ***Raillietina mitchelli*** O'Callaghan, Davies and Andrews, 2000.

FIGS 49-60

Holotype: Keith, SA (36° 06' S, 140° 19' E), SAMA AHC S28306.

Paratypes: Keith, SA SAMA AHC S28307, 31380, BMNH 2000.5.17.61-65.

Other Material examined: Yunta, SA, coll. G. E. Ford, 1.ix.1981, SAMA AHC 11181.

Description

Cestodes up to 120 long in relaxed specimens. Strobila containing approximately 1120 proglottides. Scolex small, 0.224-0.340 (0.298, n=45) in diameter (Figs 49, 50, 57), usually with retracted rostellum, 0.108-0.154 (0.138, n=40) in diameter. Rostellum armed with 296-380 (316, n=20) hammer-shaped hooks in two rows. Larger, inner rostellar hooks 0.009-0.012 (0.011, n=70) long; outer, smaller rostellar hooks 0.008-0.01 (0.009, n=70) long (Figs 51, 58). Surface of rostellar sac when rostellum everted covered by minute accessory spines/microtriches, 0.001-0.002 long, visible under high magnification only. Suckers 0.055-0.088 (0.072, n=40) in diameter, armed with 4-6 rows of hooks 0.004-0.010 long (Fig. 34). Neck absent.

Proglottides craspedote. Mature proglottides wider than long, 0.600-0.900 (0.822) x 0.180-0.220 (0.204, n=10) (Figs 53, 59). Genital pores, single, unilateral. Genital ducts passing between osmoregulatory canals, larger ventral osmoregulatory canal, 0.020 in maximum diameter, lying internal to dorsal canal, 0.012 in maximum diameter. Ventral canal joined by tranverse osmoregulatory canal in posterior margin of proglottides. Transverse dorsal canal not seen.

Genital anlage first appearing in proglottides 400-520. Male and female genitalia mature in proglottides 640-750. First gravid proglottides 1000 with 100-120 gravid proglottides terminating with 10-20 compact proglottides becoming progessively longer than wide (Fig 56).

Genital atrium small, situated in anterior half of lateral proglottis margin. Cirrus sac 0.152-0.176 (0.161) x 0.032-0.044 (0.038, n=10) (Figs 54, 60) not reaching ventral osmoregulatory canal. Distal region of cirrus lined with spines, of greater internal diameter than sinuous mid region; proximal region forms spherical internal seminal vesicle 0.028-0.052 (0.040) x 0.020-0.032 (0.026, n= 10). Vas deferens slightly coiled at midline of proglottis. Testes 0.048-0.060 (0.053, n=10) in diameter, dorsal to and overlying female gonads. Testes 5-6 (5, n=20) per proglottis, one frequently overlying vitellarium with additional testes, one poral and 3-4 aporal.

Vagina opening to genital atrium posterior to cirrus sac. Distal region, dilated, 0.082 x 0.024-0.032, mid region, narrow, straight, leads medially posterior to vas deferens, terminating in fusiform seminal receptacle 0.124-0.152 (0.143) x 0.024-0.036 (0.028, n=10). Ovary bilobed (Fig. 55). Poral lobe 0.064-0.096 x 0.104-0.112, consisting of 1-3 transversely elongate lobules. Aporal lobe, 0.112-0.160 x 128-0.16 consisting of 3-4 lobules. Vitellarium irregularly ovoid, 0.056-0.076 (0.070) x 0.040-0.056 (0.049, n =10). Mehlis gland spherical, anterior to vitellarium 0.024-0.032 (0.028, n=10) in diameter. Uterine duct passing anterior to vitellarium, terminating dorsal to ovary. Uterus absent. Gravid proglottides wider than long 0.084-1.120 (0.964) x 0.272-0.360 (0.316, n=10) containing 9-15 egg capsules 0.140-0.170 (0.150) x 0.100-0.15 0(0.130, n=10) each with 12-18 (15, n=10) eggs 0.041-0.049 (0.045) x 0.035-0.045 (0.040, n=10). Terminal segments shrivelled (Fig. 56). Oncosphere 0.015-0.018 (0.017) x 0.014-0.017 (0.016, n=10); oncospheral hooks 0.004-0.006 long.

Host:

Dromaius novaehollandiae (Latham, 1790) (Struthioniformes: Dromaiidae).

Location in host

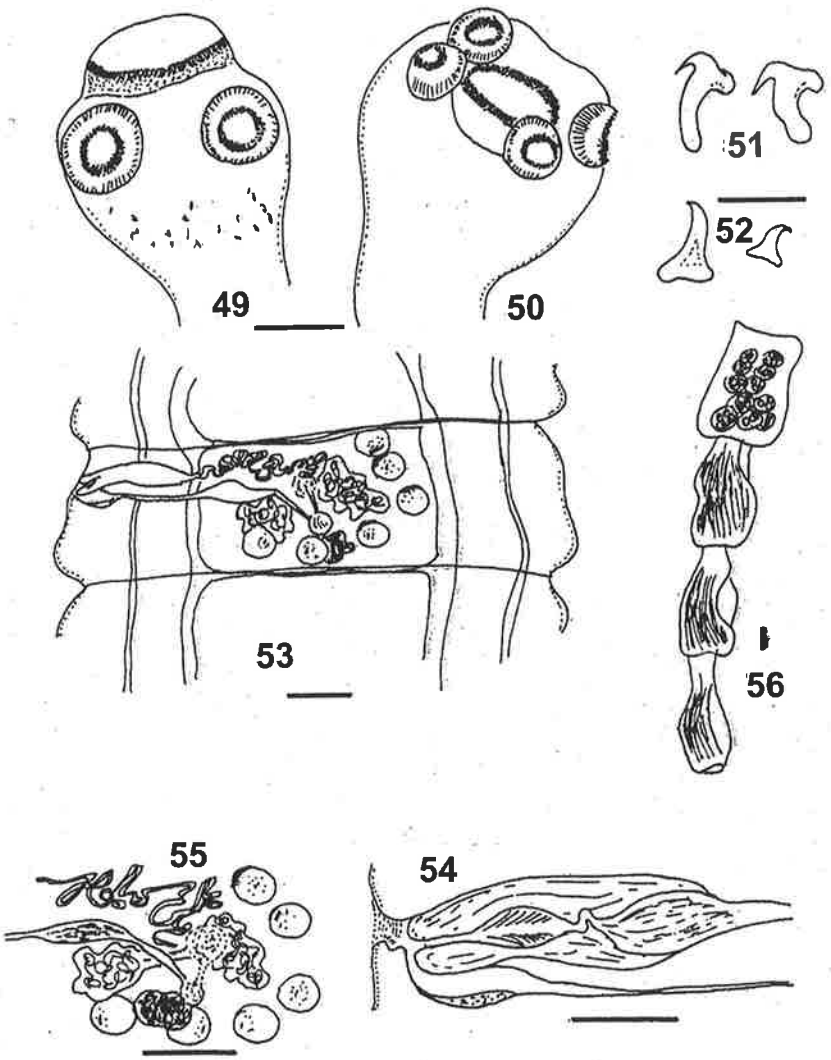
Small intestine.

Etymology

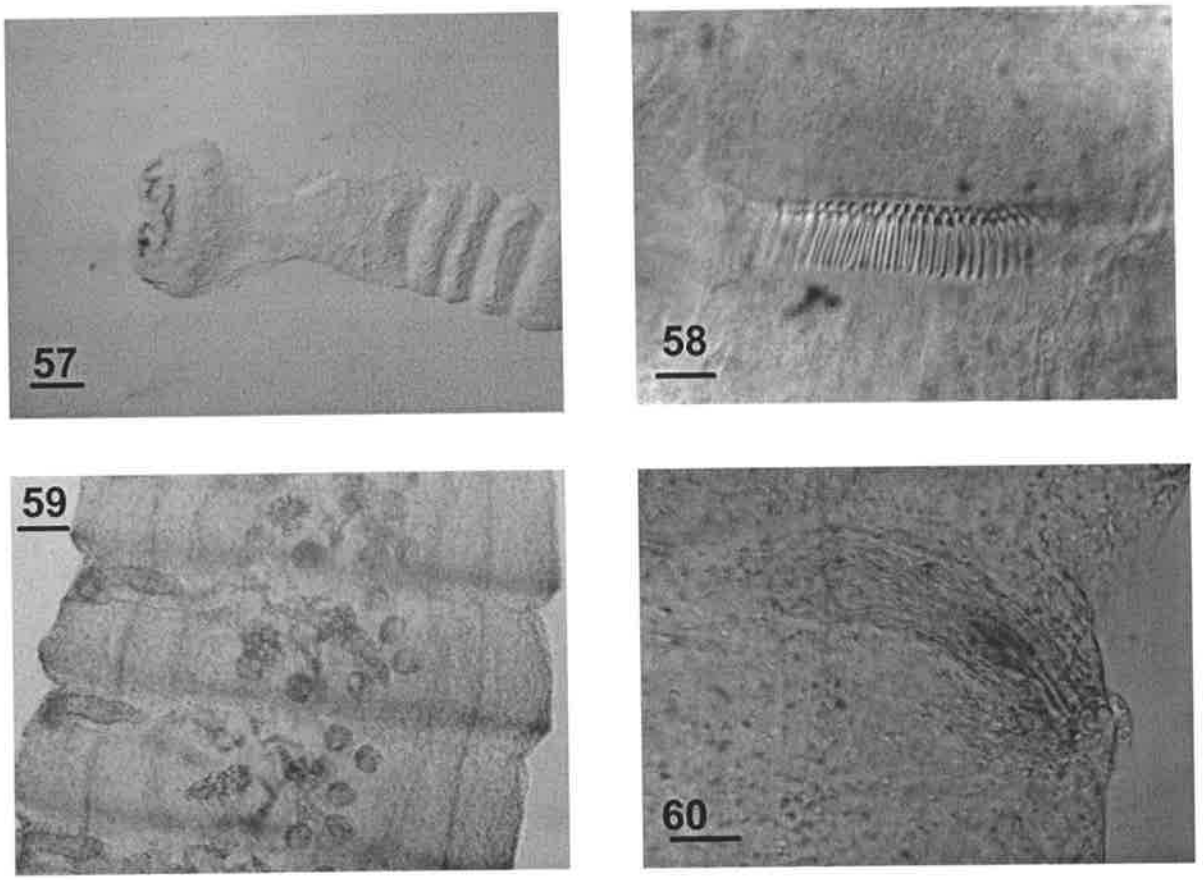
This species is named for the late Sir Mark Mitchell in acknowledgment of support of this project through the Sir Mark Mitchell Foundation.

3.5 Comparison with other species

Of the species of *Raillietina* with hosts in the Struthioniformes, *R. dromaius* resembles *R. casuarii* found in the New Guinean cassowary, *C. picticollis* in the size of the rostellar hooks (Kotlán, 1923). However, *R. dromaius* is smaller than *R. casuarii*, has fewer rostellar hooks (142 v 250), fewer and smaller testes and there are fewer eggs per capsule. *Paroniella appendiculata* Fuhrmann, 1909 described from an unknown host in New Guinea is similar in size to *R. dromaius* with 130 rostellar hooks 0.036-0.043 in length. However, *P. appendiculata* has only one egg per capsule.



Figs 49-56. *Raillietina mitchelli*. 49. Scolex with everted rostellum. 50. Scolex with retracted rostellum. 51. Rostellar hooks. 52. Sucker hooks. 53. Mature proglottis. 54. Cirrus and distal vagina. 55.Female genitalia. 56. Terminal gravid proglottides. Scale bars = 0.1 mm, 49, 50, 53, 55, 56; 0.05 mm, 54; 0.01 mm, 51, 52.



Figs 57-60. *Raillietina mitchelli*. 57. Scolex. 58. Rostellar hooks. 59. Mature proglottides. 60. Cirrus and distal vagina. Scale bars = 0.1 mm, 57, 59; 0.05 mm, 60; 0.01 mm, 58.

(diagnostic for the genus *Paroniella*), a smaller cirrus sac and more testes than *R. dromaius*.

Raillietina chiltoni resembles *R. infrequens* (Kotlan, 1923) in the size of the strobila, scolex and rostellar hooks, the number of rostellar hooks and testes. However, *R. chiltoni* differs from *R. infrequens* in the size of the cirrus sac (0.108 x 0.038) compared with (0.180-0.200 x 0.060) in *R. infrequens*. In addition, the cirrus of *R. chiltoni* has no armature and the internal seminal vesicle is smaller (0.015 x 0.012 v 0.054 long). *Raillietina chiltoni* has a larger rostellum (0.383) than *R. infrequens* (0.250) and has testes in distinctly aporal and poral groups that are never in the midline.

The species of *Raillietina* described here can be distinguished from all congeners in the Struthioniformes by the size and number of the rostellar hooks, size of the scolex and size of the cirrus sac (Table 7).

Table 7. Key features of *Raillietina* species in emus.

	R. australis		*R. beveridgei*		*R. chiltoni*		*R. dromaius*		*R. mitchelli*	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Size of large rostellar hooks	0.025	0.021-0.030	0.019	0.016-0.021	0.032	0.026-0.039	0.056	0.050-0.063	0.011	0.009-0.012
Size of small rostellar hooks	0.020	0.016-0.023	0.016	0.014-0.019	0.027	0.022-0.034	0.048	0.043-0.054	0.009	0.009-0.010
Dimension of cirrus sac										
Length	0.158	0.152-0.168	0298	0.256-0.328	0.108	0.104-0.112	0.257	0.246-0.271	0.161	0.152-0.176
Width	0.020	0.016-0.024	0.80		0.038	0.036-0.040	0.044	0.041-0.053	0.038	0.032-0.044
Number of rostellar hooks	280-362		304-412		302-378		124-156		296-380	
Dimensions of scolex	0.416-0.568		0.480-0.736		0.545-0.832		0.480-0.752		0.224-0.340	

3.6 Closely related species in the Casuariidae

3.6.1 Species descriptions

3.6.1.1 ***Raillietina geraldschmidti*** O'Callaghan, Andrews, Davies and Spratt, 2001

FIGS 61-66, 82,83

Holotype: Scolex on slide, 2 specimens on slides, 3 specimens, Mission Beach, Qld (17° 52' S, 146° 06' E), coll. D. M. Spratt, 3.ix.1999, SAMA AHC 28397, 31475; *Paratypes*: 1 slide, 2 specimens, Mission Beach, Qld (17° 52' S, 146° 06' E), coll. D. M. Spratt, 3.ix.1999, SAMA AHC 28398, 31476; 1 specimen, El Arish, Qld (17° 49' S, 146° 00' E), coll. D. M. Spratt, 28.xi.1999, SAMA AHC 31477; cestode fragments, Etty Bay, Qld (17° 34' S, 146° 05' E), coll. D. M. Spratt, 4.i.1998, SAMA AHC 31478; Mature proglottides on slide, Mission beach, coll. F. Crome & D. M Spratt, 7.vi.1987, SAMA AHC 28399.

Other material: CSIRO wildlife helminth collection, W/L HC C941, W/L HC C939.

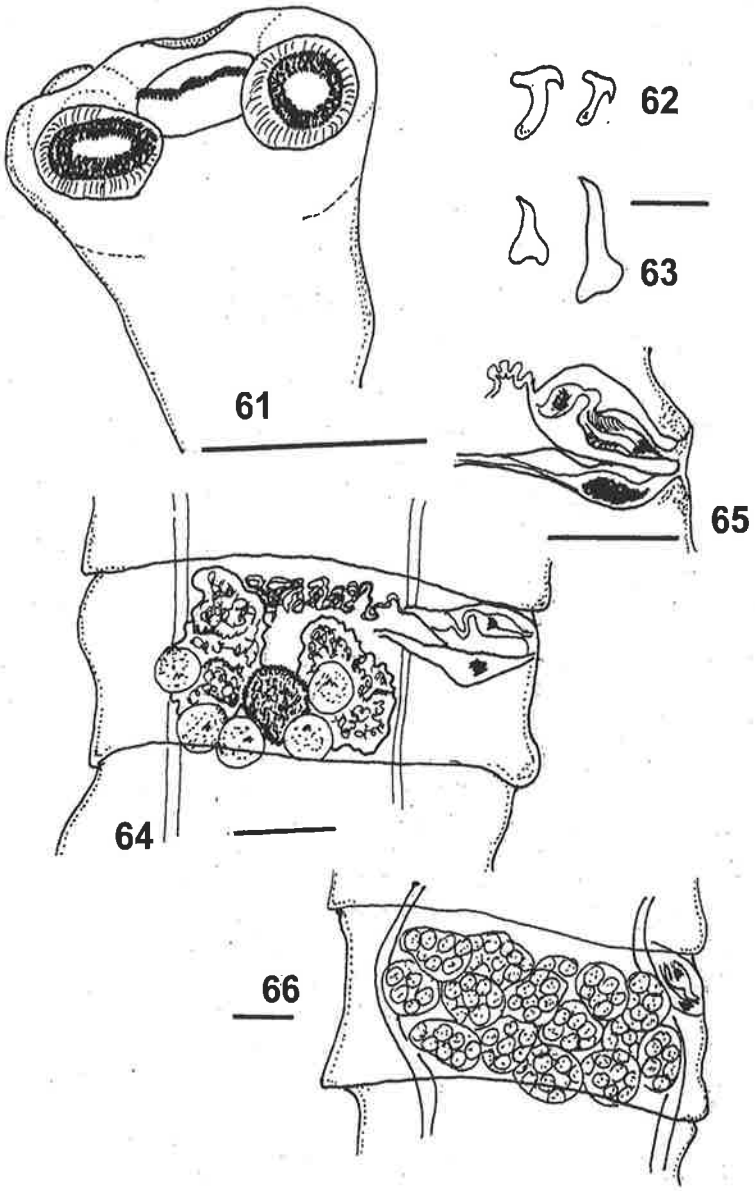
Description

Description based on mounted specimens of three strobilae, cestode fragments consisting of mature proglottides and two scoleces. Small cestode, maximum length 40 in relaxed specimens, maximum width 0.760. Strobilae contain approximately 450 proglottides. Scolex 0.155-0.180 (0.166, n=3) in diameter with retracted rostellum 0.064-0.074 (0.069, n=2) in diameter (Figs 61, 82). Rostellum armed with 218-238 (228, n=2) hammer-shaped hooks in two circular rows. Larger, anterior rostellar hooks 0.008-0.009 (0.008, n=20) in length; smaller, posterior rostellar hooks 0.007-0.008 (0.007, n=20) in length (Figs 62,83). Rostellum armed with minute accessory spines/microtriches 0.001 in

length visible under high magnification only. Suckers 0.052-0.072 (0.059, n=8) in diameter armed with hooks 0.005-0.014 in length (Fig. 63).

Proglottides acraspedote. Immature proglottides longer than wide, 0.112-0.152 (0.130) x 0.036-0.072 (0.060, n=10). Mature proglottides wider than long 0.080-0.144 (0.104) x 0.312-0.560 (0.426, n=10) (Fig. 64). Genital pores single, unilateral. Lateral dorsal osmoregulatory canals 0.028-0.036 in diameter joined by transverse commissures, 0.008 in diameter, in posterior region of proglottides. Ventral osmoregulatory canal not seen. Cirrus sac 0.108-0.124 (0.116) x 0.048- 0.052 (0.049, n=10) (Fig. 65) extending anteromedially to but not crossing lateral osmoregulatory canal. Distal region of cirrus narrow, mid region enlarged, lined with spines, proximal region forms spherical internal seminal vesicle 0.018-0.030 (0.023, n=10) in diameter. External seminal vesicle absent. Vas deferens narrow, greatly coiled, passing medially towards centre of proglottis. Testes 5-7 in number, lying within area bounded by lateral osmoregulatory canals, usually overlying ovary and vitellarium; testes 0.036-0.044 (0.039, n=10) in diameter in poral and aporal groups, 2 poral and 3-4, occasionally 5, aporal.

Vagina and cirrus opening into common genital atrium, vagina opening posterior to cirrus. Distal region of vagina enlarged, 0.040-0.048 x 0.018-0.024, with a seminal receptacle 0.014-0.020 (0.016, n=10) usually containing sperm. Mid-region narrow, leading medially posterior to vas deferens. Ovary bilobed, each lobe circular, lobes approximately equal in size, 0.052-0.120 (0.088) x 0.040-0.108 (0.080, n=20). Vitellarium median, post ovarian, circular 0.048-0.076 (0.062) x 0.040-0.072 (0.053, n=10). Gravid proglottides (Fig. 66) wider than long, 0.240-0.320 (0.251) x 0.480-0.736 (0.650, n=10). Egg capsules 0.072-0.080 (0.075) x 0.064-0.080 (0.066, n=5), spheroidal, 16-20 in each proglottis, containing 11-13 circular eggs 0.020-0.032 (0.026, n=10) in diameter. Oncosphere circular 0.012-0.016 (0.015, n=10) in diameter, embryonic hooks 0.006 long.



Figs 61-66. *Raillietina geraldschmidti*. 61. Scolex. 62. Rostellar hooks. 63. Sucker hooks. 64. Mature proglottis. 65. Cirrus and distal vagina. 66. Gravid proglottis. Scale bars = 0.1 mm, 61, 64-66; 0.01mm, 62, 63.

Host

Casuarius casuarius Linnaeus, 1758 (Struthioniformes: Casuariidae).

Location in host

Intestine.

Etymology

Named for the late Dr G. Schmidt in recognition of his outstanding contribution to our knowledge of cestodes.

3.6.1.2 ***Raillietina casuarii*** (Kotlan, 1923)

FIGS 67-76, 84, 85

Synonyms: *Davainea casuarii* Kotlan, 1923. Ann. Trop. Med. Parasitol. 17, 45-57. Figs 1-5; *Raillietina* (*Ransomia*) *casuarii* Fuhrmann, 1920; *Kotlania casuarii* Lopez-Neyra, 1931; *Kotlanotaurus casuarii* Spasskii, 1973; *Raillietina casuarii* Fuhrmann, 1924.

Material examined: 4 specimens, El Arish, Qld (17° 49' S, 146° 00' E), coll. D. M. Spratt, 28.xi.1999, SAMA AHC 31481; 12 specimens, Mission Beach, Qld, coll. D. M. Spratt, 3.ix.1999 SAMA AHC 31479, 31480; 1 specimen on slide, Queensland University, no collection data, SAMA AHC 28400; 2 strobilae on slides, 6 specimens, Amau, New Guinea (10° 02' S, 148° 40' E), coll. W. B. Hitchcock, 4.ix.1969 SAMA AHC 12878, 22349.

Other material: W/L HC C940, W/L HC 942

Revised description

Description based on mounted specimens of four strobilae and five cleared scoleces. Large cestode, up to 200 in unrelaxed specimens, maximum width 3.4. Strobila contains approximately 700 proglottides. Scolex 0.800-1.048 (0.962, n=5) in diameter with eversible rostellum 0.304-0.360 (0.323, n=5) in diameter (Figs 67, 74, 84). Rostellum armed with 172-212 (190, n=9) hammer-shaped hooks in two circular rows. Larger, anterior rostellar hooks 0.038-0.053 (0.045, n= 50) in length; smaller, posterior rostellar hooks 0.032-0.046 (0.039, n=50) in length (Figs. 68,75,85). Rostellum armed with accessory spines 0.002-0.003 in length visible under high magnification only. Suckers, circular, 0.320-0.368 (0.347, n=9) in diameter, armed with 10-13 rows of hooks 0.005-0.021 in length (Figs 69, 76).

Proglottides craspedote. Mature proglottides wider than long 1.777-1.898 (1.836) x 0.343-0.505 (0.428, n=10) (Fig. 70). Genital pores single, unilateral. Dorsal osmoregulatory canal narrow, 0.010 in diameter, ventral osmoregulatory canal 0.040-0.064 in diameter. Narrow transverse osmoregulatory canals connect right and left dorsal and ventral canals at posterior margin of each proglottis. Large cirrus sac 0.232-0.336 (0.286) x 0.128-0.208 (0.169, n=20) extending anteriorly, not reaching lateral osmoregulatory canals. Distal region of cirrus of greater internal diameter than proximal region, armature not seen, mid-region expanding to form large internal seminal vesicle folded dorsally, 0.096-0.128 (0.102, n=10) maximum diameter (Fig. 71). Vas deferens greatly coiled passing medially towards centre of proglottis. Testes 0.048-0.056 in diameter, number 43-51 per proglottis, always more testes on aporal field; 12-14 (13) in poral field, 31-37 (35) aporal.

Vagina opening to genital atrium posterior to male genital pore, distal region with thickened muscular wall 0.028-0.036 (0.033, n=10) wide. Mid region with thickened wall extends, uncoiled, medially and posterior to vas deferens, region internal to osmoregulatory canals dilated and filled with sperm, proximal region coiled. Ovary bilobed, poral lobe 0.200-0.240 (0.214) x 0.112-0.120 (0.115, n=5), aporal lobe 0.240-0.280 (0.269) x 0.112-0.136 (0.122, n=5) with 3-4 lobules in each lobe. Vitellarium median, post ovarian, sub-circular 0.128-0.152 (0.144) x 0.096-0.136 (0.110, n=10). Uterine duct passing anteriorly to developing uterus (Fig. 72). Gravid proglottides 1.000-2.121 (1.860) x 0.606-1.080 (0.731, n=10) (Fig. 73) filled with egg capsules. Egg capsules sub-spherical to ovoid, containing 1-4 eggs, mostly 1-2, seldom 3 or 4. Capsules containing one egg 0.052-0.072 (0.062) x 0.048-0.064 (0.056, n=10), containing two eggs 0.076-0.104 (0.091) x 0.052-0.072 (0.060, n=10). Approximately 250-300 egg capsules in each proglottis. Eggs spherical 0.040-0.052 (0.045) x 0.032-0.044 (0.039, n=10) containing spherical oncosphere 0.020-0.024 (0.023) x 0.020-0.024 (0.021, n=10), embryonic hooks 0.006-0.008 long.

Host

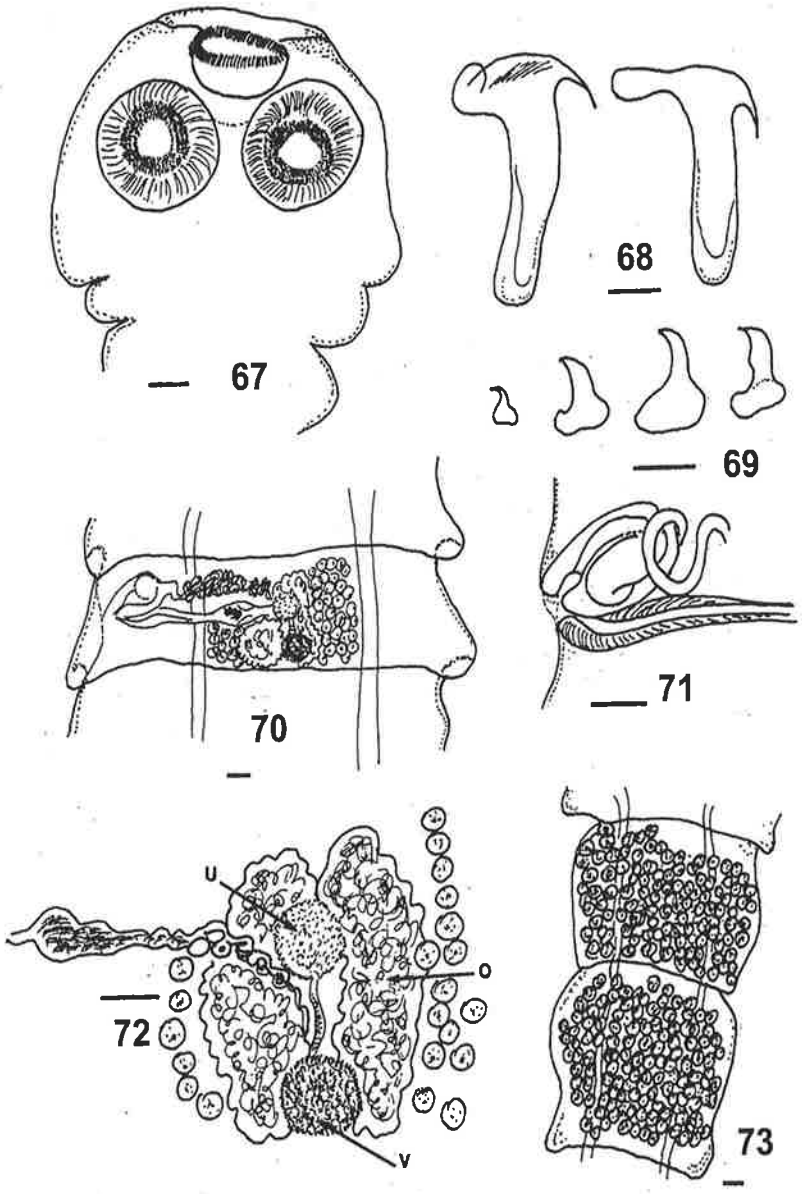
Casuarius casuarius Linnaeus, 1758 (Struthioniformes: Casuariidae).

Location in host

Intestine.

Remarks

These specimens of *R. casuarii* are smaller than those reported previously (140 v. 340) (Table 8). However, Kotlan (1923), in describing the largest cestodes from one locality, observed more contracted and shorter cestodes than those described.



Figs 67-73. *Raillietina casuarii* from Australia. 67. Scolex. 68. Rostellar hooks. 69. Sucker hooks. 70. Mature proglottis. 71. Cirrus and distal vagina. 72. Female genitalia. 73. Gravid proglottides. Scale bars = 0.1 mm, 67, 70-73; 0.01 mm, 68, 69. Legend: o, ovary; u, developing uterus; v, vitellarium.

3.6.1.3 ***Raillietina infrequens*** (Kotlan, 1923)

FIGS 77-81, 86, 87

Synonyms: *Davainea infrequens* Kotlan, 1923. Ann. Trop. Med. Parasitol. 17, 45-57; *Raillietina infrequens* Fuhrmann, 1932.

Material examined: 1 strobila on slide, 2 specimens, Amau, New Guinea , coll. W. B. Hitchcock, 4.ix.1969 SAMA AHC 12878, 22349.

Revised description

Description based on one entire mounted specimen, segments of mature and gravid proglottides and one scolex. Strobilae are 50 long and contain 500 segments with characters that conform to those reported by Kotlan (1923). The scolex (Figs 77, 86) is 0.456 in diameter with a retracted rostellum 0.200 in diameter armed with two rows of hammer-shaped hooks that have become dislodged and some appear to be missing. Larger, anterior rostellar hooks 0.022-0.024 (0.023, n=10) in length; smaller, posterior rostellar hooks 0.017-0.019 (0.018, n=10) in length (Figs 78, 87). Circular suckers 0.116-0.140 (0.128, n=10) in diameter are armed with hooks 0.005-0.014 in length (Fig. 79). In mature segments genital pores are unilateral, with a cirrus sac and vagina which conform with the description and dimensions reported by Kotlan (1923). Cirrus sac 0.160-0.192 (0.174) x 0.048-0.060 (0.056, n=10) (Fig. 80). Gravid segments are wider than long (Fig. 81); up to six terminal segments 0.488-0.056 (0.537) x 0.336-0.520 (0.425) containing 25-32 (28, n=6) egg capsules each with 7-10 (9, n=10) eggs. Egg capsules circular 0.080-0.100 (0.090) x 0.072-0.088 (0.078, n=10).

Host

Casuarius casuarius Linnaeus, 1758 (Struthioniformes: Casuariidae).

Location in host

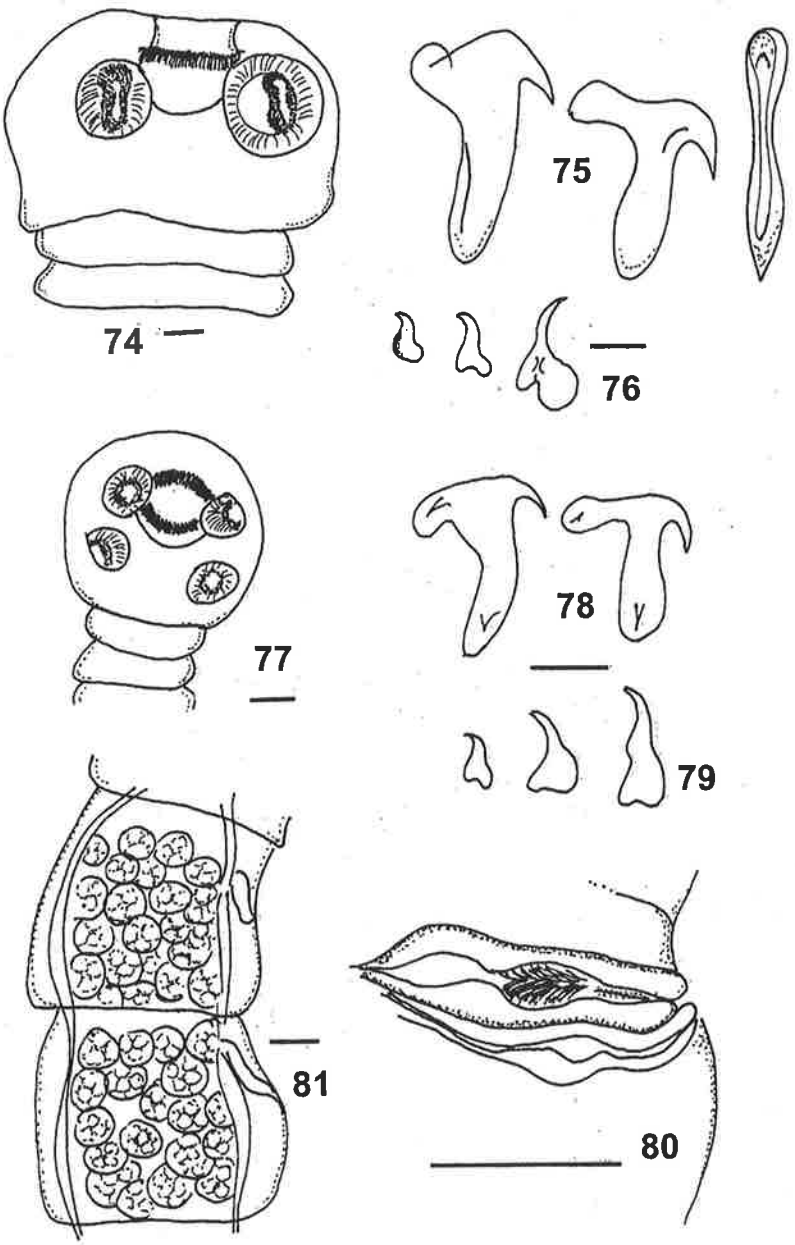
Intestine.

Remarks

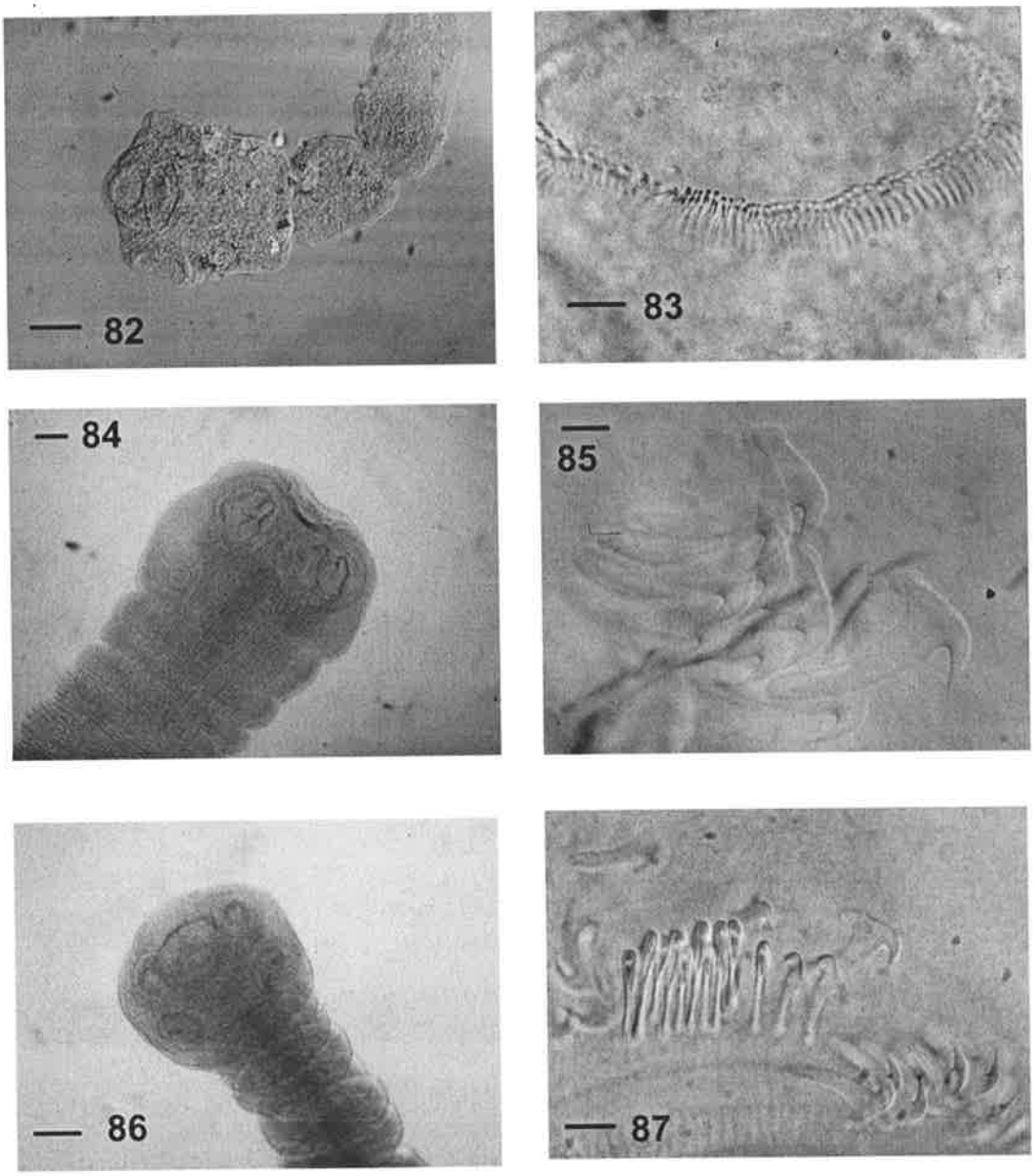
Gravid proglottides were unavailable in the material examined by Kotlan (1923) and consequently he was unable to complete the description of *R. infrequens*. Therefore, a description of gravid segments, although from a limited number of specimens, is presented here. Kotlan (1923) also estimated the size of *R. infrequens* from two fragments that apparently belonged together. The two mounted specimens of *R. infrequens* examined here are in semi-contracted form.

Table 8. Measurements of the principle features of *Raillietina* species in *Casuarius casuarius.*

	R. casuarii Kotlan (1923)	*R. casuarii* Amau, N. G.	*R. casuarii* El Arish, Qld.	*R. infrequens* Kotlan (1923)	*R. infrequens* Amau, N. G.	*R. geraldschmidti*
Size (mm)	340 x 3	140 x 1.5	200 x 3.4	80 x 1.2	50 x 0.920	40 x 0.760
Dimensions of scolex	1.0- 1.2	0.910	0.962	0.500	0.456	0.166
Size of large rostellar hooks	0.048-0.054	0.046-0.053	0.038-0.042	0.027-0.034	0.022-0.024	0.008-0.009
Size of small rostellar hooks	0.040-0.046	0.038-0.046	0.032-0.037	0.021-0.025	0.017-0.019	0.007-0.008
Number of rostellar hooks	250	176-212	172-192	260	-	218-238
Diameter of suckers	0.400	0.344	0.349	0.130	0.128	0.059
Dimensions of cirrus sac	0.250 x 0.160	0.316 x 0.192	0.256 x 0.146	0.180-0.200 x 0.060	0.174 x 0.056	0.116 x 0.049



Figs 74-76. *Raillietina casuarii* from New Guinea. 74. Scolex. 75. Rostellar hooks. 76. Sucker hooks.77-81. *R. infrequens* from New Guinea. 77. Scolex. 78. Rostellar hooks. 79. Sucker hooks. 80. Cirrus and distal vagina. 81. Gravid proglottides. Scale bars = 0.1 mm 74, 77, 80, 81; 0.01 mm 75, 76, 78, 79.



Figs 82-83. *Raillietina geraldschmidti*. 82. Scolex. 83. Rostellar hooks. **Figs 84-85.** *R. casuarii*. 84. Scolex. 85. Rostellar hooks. **Figs 86-87.** *R. infrequens*. 86. Scolex. 87. Rostellar hooks. Scale bars = 0.1 mm, 84, 86; 0.05 mm 82; 0.01mm, 83, 85, 87.

3.7 Cestodes in Struthionidae

The material described below was taken from an ostrich, *Struthio camelus*. The morphological features are detailed for direct comparison with those of *R. australis* from the emu.

3.7.1 Species description

3.7.1.1 ***Raillietina australis*** (Krabbe, 1869)

FIGS 88-96

Synonyms: *Taenia australis* Krabbe, 1869. K. Danske Vidensk Selsk. Skr. Naturv. Og Math. Afd. 8, 249-363. Figs 296-298; *Davainea australis* Blanchard, 1891; *Ransomia australis* Fuhrmann, 1920; *Kotlania australis* Lopez-Neyra, 1931; *Raillietina australis* Fuhrmann, 1924.

Material examined:

Strobila on slide, 4 scoleces on slide and cestode fragments, Monarto, SA (35° 07' S, 139° 08' E), coll. A. Spanner, 23.viii.1995, SAMA AHC 28436, 32855.

Description

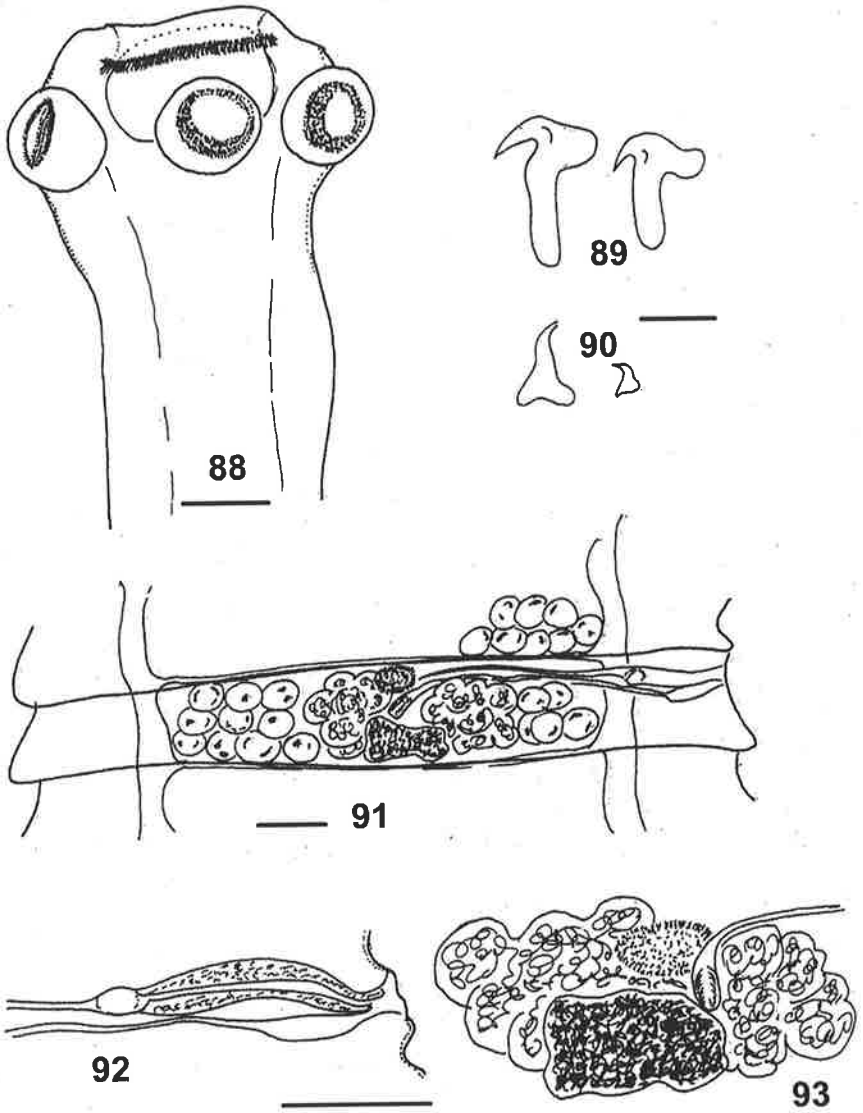
Description based on one complete specimen and four scoleces only. Cestode 130 in length. Gravid strobila contains approximately 1800 segments. Strobila 1.200 in maximum width. Scolex 0.420 in diameter with retracted rostellum 0.200-0.256 in diameter (Figs 88, 94). Rostellum armed with 338-354 (344, n=4) hammer shaped hooks in two circular rows. Larger rostellar hooks 0.018-0.020 (0.018, n=20) in length; smaller rostellar hooks 0.014-0.016 (0.015, n=20) in length (Figs 89, 95). Suckers circular, 0.104-0.120 in diameter, armed with 8-12 rows of hooks 0.004-0.010 in length Fig. 90).

Calcareous corpuscles present in base of scolex, irregularly shaped 0.10-0.12 in diameter. Neck 0.400 in length.

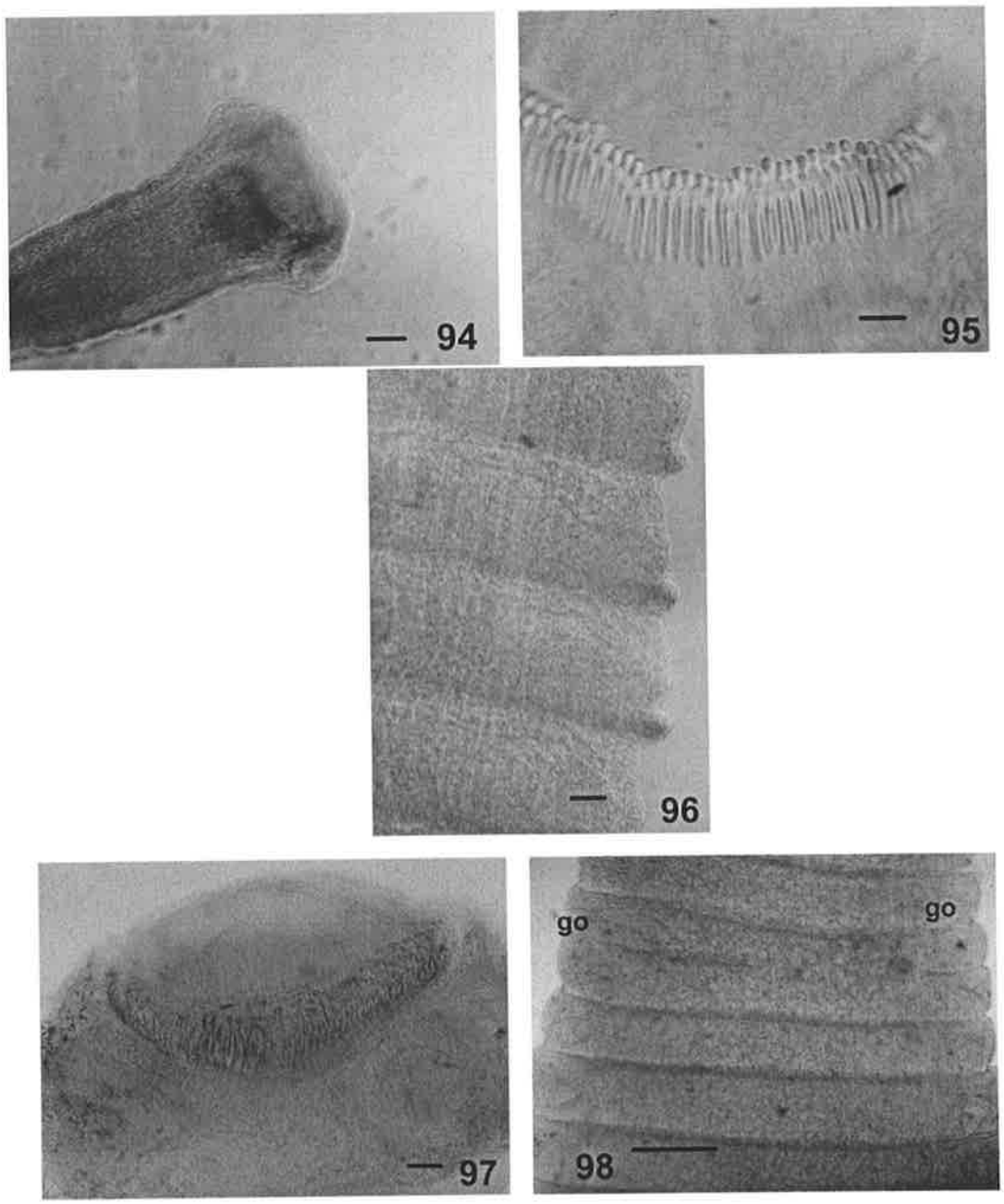
Proglottides craspedote. Mature proglottides wider than long, 0.120-0.140 x 1.040-1.180 (Figs 91, 96). Genital pores single, unilateral. Dorsal osmoregulatory canal with a maximum diameter of 0.060 in gravid segments. Ventral canal not seen. Transverse osmoregulatory canals connecting right and left ventral canals at posterior margin of each proglottis. Genital anlarge appears in proglottis 575 approximately; male and female genitalia mature in proglottides 865 and 1200 respectively; first gravid proglottis number 1730.

Genital atrium small, situated in anterior half of lateral proglottis margin. Cirrus sac 0.172-0.192 (0.183, n=20) x 0.012-0.020, extending to and occasionally just crossing ventral osmoregulatory canal (Fig. 92). Cirrus unarmed, narrow, internal seminal vesicle present 0.020-0.024 in diameter. Vas deferens extending across anterior margin to centre of proglottis. Testes distributed in two lateral groups, 7-9 (9, n=10) poral and 10-14 (12, n=10) aporal. Testes 0.048-0.056 (0.052, n=10) in diameter overlie female genital organs.

Vagina opening to genital atrium posterior to cirrus sac. Distal region dilated, 0.052-0.060 x 0.020-0.028, mid region narrow, uncoiled, leads medially posterior to vas deferens. Ovary bilobed, poral lobe 0.092-0.128 (0.106) x 0.092-0.112 (0.096, n=5), aporal lobe 0.120-0.168 (0.139) x 0.100-0.104 (0.102, n=5) in size with 3-4 spherical lobules in each lobe. Vitellarium, oval or bean shaped 0.120-0.140 x 0.064-0.100 in size, posterior and dorsal to ovary (Fig. 93). Developing uterus present. Gravid proglottides extending transversely, 0.747-1.212 (0.983) x 0.161-0.202 (0.180, n=20). Egg capsules number approximately 40-72 per proglottis, circular, 0.038-0.040 x 0.020-0.022 in size containing 14-18 (16, n=10) eggs per capsule. Oncosphere 0.010-0.012 in diameter.



Figs 88-93. *Raillietina australis* in an ostrich. 88. Scolex. 89. Rostellar hooks. 90. Sucker hooks. 91. Mature proglottis. 92. Cirrus and distal vagina. 93. Female genitalia. Scale bars = 0.1 mm, 88, 91-93; 0.01 mm, 89, 90.



Figs 94-96. *Raillietina australis* in Ostrich. 94. Scolex. 95. Rostellar hooks. 96. Cirrus and distal vagina. **Figs 97-98.** *Cotugnia collini* holotype. 97. Scolex. 98. Mature proglottides. Scale bars = 1.0 mm, 98; 0.1 mm, 94, 97; 0.05 mm, 96; 0.01 mm, 95. Legend: go, genital organs.

Host

Struthio camelus.

Location in host

Small intestine.

3.8 Calcareous corpuscles

The role of calcareous corpuscles is not clear (von Brand, 1979; Smyth and McManus, 1989) although they are observed in most cestodes (Nieland and von Brand, 1969; Chowdhury and De Rycke, 1976). The presence of these corpuscles was highly variable between cestode species and also within individual cestodes of the same species. Calcareous corpuscles were observed in all cestode species examined in this study, however, they have only been recorded when present in the specimens examined for species descriptions.

3.9 Identification of archived material

Cestodes held in the AHC SAMA were identified and their identity appears in Table 9. Material listed as SAMA AHC 20432 could not be identified because of the absence of a scolex and severely contracted proglottides. Material held in the SARDI collection and that collected at abattoirs was also identified (Tables 10 & 11).

3.10 Summary of cestode identifications and prevalence of species

Material examined from the AHC consisted of specimens from five states (Qld 1, NSW 6, Vic. 9, SA 3, WA 3, no locality data 2). *Raillietina beveridgei* was the most commonly identified cestode, occurring in 16 samples, followed by *R. dromaius* in eight, *R. australis* in seven and *R. mitchelli* in two. *Raillietina australis* was identified in each state sampled,

R. beveridgei and *R. dromaius* were present in samples from SA, NSW, WA and Victoria. *Raillietina mitchelli* was identified in only two samples, one from SA and another collected recently in WA. *R. chiltoni* was not encountered.

All of the material with collection data, held in the SARDI laboratory, was from SA. *Raillietina beveridgei* occurred in 11 samples, *R. australis* and *R. dromaius* in two. *R. chiltoni* and *R. mitchelli* were absent in this collection.

Material examined from small portions of intestine collected at abattoirs prior to the commencement of this study, consisted of cestodes from 37 emus collected at 16 localities in SA and one in Victoria. Cestodes identified as *R. beveridgei* occurred at 13 localities in SA, *R. australis* at one, *R. chiltoni* at three and *R. dromaius* at three. *Raillietina dromaius* was identified in the sample from Apsley in Victoria.

In all of the archived samples examined, *R. beveridgei* occurred in 65.6%, *R. australis* in 16.4%, *R. chiltoni* in 4.9%, *R. dromaius* in 23% and *R. mitchelli* in 3.3%. More than one species occurred in 11.5% of samples. One sample contained three species and four species occurred only once (1.6%).

Table 9. Identification of the cestodes of *Raillietina* from emus held in the Australian Helminth Collection (AHC SAMA).

SAMA AHC Number	Identification	Bottle (b) or Slide (s)	Locality	Collector	Date
227	*R. australis*	(b)	Shelley River, Qld	No data	29.ix.1907
542	*R. beveridgei*	(b)	No data	No data	No data
1187	*R. beveridgei*	(b)	Mundulla, SA	Dinning	Feb., 1933
3329	*R. beveridgei*	(b)	Parachilna, SA	No data	Oct, 1959
8125	*R. dromaius*	(b)	Vic.	K. Harrigan	No data
9179	*R. beveridgei* & *R. dromaius*	(b)	Condobolin, NSW	Ryan	27.i.1971
10005	*R. dromaius*	(b)	Kinchega, NSW	I. Beveridge	31.iii.1974
10006	*R. australis*	(b)	Kinchega, NSW	I. Beveridge	31.iii.1974
10511	*R. dromaius*	(b)	Pine Plains, Vic	I. Beveridge	14.v.1971
11008	*R. dromaius*	(b)	Menindee, NSW	I. Beveridge	10.viii.1977
10102	*R. beveridgei*	(b)	La Trobe, Vic	I. Beveridge	24.vi.1975
11181	*R. beveridgei*, *R. australis* & *R. mitchelli*	(b)	Yunta, SA	G. Ford	1.ix.1981
18391	*R. australis*	(b)	Werribee, Vic	K. Harrigan	April, 1988
22926	*R. australis*	(b)	Healesville, Vic	I. Beveridge	3.vi.1989
26698	*R. beveridgei*	(b)	Werribee, Vic	I. Beveridge	23.vi.1995
31574	*R. beveridgei*, *R. australis*, *R. mitchelli* & *R. dromaius*	(b)	Badgingarra, WA	I. Beverdige	13.xii.2001
20429	*R. beveridgei*	(s)	No data	No data	No data
20430	*R. beveridgei*	(s)	NSW	TH Johnston & TLBaldock	1914
20431	*R. australis*	(s)	North West WA	TH Johnston	No data
20432	No Id.	(s)	North West WA	JB Cleland	No data
20433	*R. beveridgei* & *R. dromaius*	(s)	As for 20430		
20837	*R. beveridgei*	(s)	La Trobe, Vic	I. Beveridge	24.vi.1972
21347	*R. beveridgei*	(s)	As for 11181		
26205	*R. beveridgei*	(s)	Vic	D. Turner	1994
27716	*R. dromaius*	(s)	Wagga, NSW	No data	7.xii.1994
27717	*R. beveridgei*	(s)	Bairnsdale, Vic	I. Beveridge	5.xii.1994
27718	*R. beveridgei*	(s)	As for 27717		

Table 10. Identification of cestodes of *Raillietina* from emus held in the SARDI collection.

Identification Number	Identification	Bottle (b)	Locality	Collector	Date
4852	*R. beveridgei*	(b)	Adelaide Zoo, SA	M. O'Callaghan	22.vi.1987
-	*R. beveridgei* & *R. dromaius*	(b)	Kersbrook, SA	I. Beveridge	April, 1989
7496	*R. dromaius*	(b)	Yorktown, SA	M. O'Callaghan	14.viii.1992
11224	*R. beveridgei*	(b)	Kyneton, SA	M. O'Callaghan	24.xi.1992
1724	*R. beveridgei*	(b)	Port Lincoln, SA	E. Moore	25.ii.1992
10742	*R. beveridgei*	(b)	Gawler, SA	E. Moore	28.ix.1994
9070	*R. beveridgei*	(b)	Gawler, SA	E. Moore	11.x.1994
9204	*R. beveridgei*	(b)	Port Lincoln, SA	E. Moore	17.x.1994
6023	*R. australis*	(b)	Kadina, SA	M. O'Callaghan	11.vii.1995
7137	*R. beveridgei*	(b)	Avenue Range, SA	M. O'Callaghan	20.ix.1995
1712	*R. beveridgei*	(b)	Lock, SA	M. O'Callaghan	9.iii.1996
6259	*R. australis*	(b)	Kadina, SA	E. Moore	19.ix.1996
-	*R. beveridgei*	(b)	unknown	P. Heap	14.x.1996
-	*R. beveridgei*	(b)	Maitland, SA	M. O'Callaghan	13.iii.1997

3.11 Discussion

This study of the cestodes collected from farmed and wild emus has resulted in the recognition of five species assigned to *Raillietina* Fuhrmann, 1920 (*sensu* Jones and Bray, 1994) on the basis of the possession of two rows of numerous hammer-shaped rostellar hooks, uni-lateral genital pores, a small cirrus sac which does not reach or just crosses the osmoregulatory canals, and egg capsules containing several eggs. One species was identified as *R. australis* whilst the other four are described as new species.

Krabbe (1869) omitted the dimensions of the scolex, rostellum and suckers in his description of *R. australis* and the strobila was inadequately described. The material described above, based on the examination of 50 cestode specimens, indicates that Krabbe's (1869) measurements of the rostellar hooks (12-14 mm) are consistent with hook width rather than hook length although this cannot be confirmed because of the absence of a scolex in the type material examined. Mature proglottides present in the type material obtained, however, do not differ from specimens examined in this study providing the opportunity to redescribe *R. australis*.

In members of the Struthioniformes, *Cotugnia collini* can be distinguished from *Raillietina* species by the presence of two sets of bilateral genital organs. Examination of holotype material (Museum für Naturkunde, Invalidensstr., Berlin, Gemany), confirmed the presence of bilateral genital organs (Figs 97, 98). *Cotugnia collini* was not found in this study although the number of specimens examined from eastern Australia was limited to specimens held in the AHC which were collected from five localities in NSW, six in Victoria. and one in Qld. I regard *Cotugnia collini* as a *species inquirenda* until new material is collected for a complete reassessment.

Table 11. Identification of cestodes of *Raillietina* collected at abattoirs.

Date	Locality of emu farm and number of emus sampled
15.xi.1996	Waikerie, SA (1); Minlaton, SA (2); Mount Gambier, SA (2) *R. beveridgei; R. beveridgei; R. beveridgei & R. chiltoni*
22.xi.1996	Mount Gambier, SA (2); York Peninsula, SA (2) *R. beveridgei & R. chiltoni ; R.beveridgei*
5.xii.1996	Glossop, SA (1); Waikerie, SA (2); Ceduna, SA (1) *R. australis; R. beveridgei; R. beveridgei*
11.xii.1996	Parilla, SA (2) *R. beveridgei & R. chiltoni*
14.xii.1996	Tailem Bend, SA (1); Mount gambier, SA (1) *R. beveridgei; R. beveridgei*
24.i.1997	Waikerie, SA (1); Lameroo, SA (1) *R. beveridgei; R. beveridgei*
7.ii.1997	Apsley, Vic (1); Berri, SA (1) *R. dromaius; R. dromaius*
19.ii.1997	Millicent, SA (2) *R. beveridgei & R. dromaius*
7.iii.1997	Mannum, SA (1); Waikerie, SA (1); Yumali, SA (1) *R. beveridgei; R. beveridgei; R. dromaius*
19.iii.1997	Taplan, SA (1); Mount Gambier, SA (1); Truro, SA (1) *R. beveridgei; R. beveridgei; R. chiltoni*
30.iv.1997	Moorook, SA (2) *R. beveridgei*
10.viii.1998	Kingston on Murray, SA (7) *R. australis, R. beveridgei & R. dromaius*
27.x.1998, 8.xii.1998, 30.iii.1999, 25.v.1999 & 29.vii.1999	Keith, SA (25) *R. australis, R. beveridgei, R. chiltoni, R. dromaius & R. mitchelli*
20.x.2000	Glossop, SA (5) *R. australis, R. beveridgei, R. chiltoni & R. dromaius*

It should be noted that the specimen recovered from an ostrich and identified as *R. australis* has characters that vary in size from the original description (Krabbe, 1869), and from those described and reported by O'Callaghan *et al.* (2000) of specimens from emus (Table 12). There appear to be no characteristics except size, size of rostellar hooks, number of testes and number of egg capsules separating these specimens. The morphology and dimensions of the cirrus sac, however, are similar and the cirrus sac of *R. australis* (Krabbe, 1869, O'Callaghan *et al.*, 2000) is easily distinguished from that of congeners in

members of the Struthioniformes. In addition, although there is a variation in the size of the rostellar hooks, the shape is the same. Although this character is considered important in separating species in some genera *e.g. Taenia* and *Hymenolepis,* it has been largely neglected in the genus *Raillietina* (see Chandler & Pradatsundarasar, 1956).

The specimen described was collected from an ostrich sharing a zoological enclosure with emus and consequently it is regarded as an accidental infection with a cestode species normally parasitic in emus. Hosts in new or unusual environments often acquire cestodes that are new to them (Kotecki, 1970; Freeman, 1973) and it is common for sympatric host species belonging to the same taxon to share parasites which suggests either a common ancestor or more often an exchange or acquisition of parasites (Goater *et al.,* 1987; Stock and Holmes, 1987; Poulin, 1998). Furthermore, in bird parasites the traits that lead to a high prevalence and intensity of infection, such as exposure to a greater variety of helminths and a greater variety of gastrointestinal habitat, simultaneously increase the probability of colonising new hosts (Poulin, 1999).

Railietina geraldschmidti can be distinguished from congeners in the Casuariidae by the total length and the size of the rostellar hooks, scolex and suckers (Table 13).

Of the species of *Raillietina* described in members of the Struthioniformes, *R. geraldschmidti* most closely resembles *R. mitchelli* described recently by O'Callaghan *et al.* (2000) (Table 13, p. 71). *Raillietina geraldschmidti* differs from *R. mitchelli* in the size of the scolex (0.155-0.180 v. 0.224-0.340), rostellar hooks (0.007-0.009 v. 0.008-0.012) and cirrus sac (0.108-0.124 x 0.048-0.052 v. 0.152-0.176 x 0.032-0.044). In addition, *R. geraldschmidti* is smaller than *R. mitchelli* and has fewer rostellar hooks (228 v. 316). Considering these differences, there is sufficient evidence to suggest that *R. geraldschmidti* represents a new species, however, pending further study, it is possible that this species may become a

Table 12. Key features of *Raillietina australis* in ostrich and emu.

		Raillietina australis from Ostrich	*Raillietina australis* from Emu
Total length		13	50
Maximum width		1.2	1.2
Scolex diameter		0.420	0.416-0.568 (0.498)
Rostellum		0.200-0.256	0.200-0.288 (0.249)
No. of Rostellar hooks		338-354 (344)	280-362 (326)
Rostellar hooks	large	18-20 (18)	21-30 (25)
	small	14-16 (15)	16-23 (20)
Diameter of suckers		0.104-0.120	0.136-0.168
Length of sucker hooks		0.004-0.010	0.005-0.011
Cirrus sac		0.172-0.192 (0.183) x 0.012-0.020	0.152-0.164 (0.158) x 0.016-0.024
No. of testes	poral	7-9	4-7
	aporal	10-14	11-13
No of egg capsules		40-72	76-110

Table 13. A comparison of measurements of the principle features of *Raillietina geraldschmidti* with *R. infrequens* and *R. mitchelli*.

	Raillietina geraldschmidti	*R. infrequens* This study	*R. infrequens* Kotlan (1923)
Total length	40	50	80
Maximum width	0.760	0.920	1.200
Scolex diameter	0.155-0.180 (0.166)	0.456	0.500
Rostellum	0.064-0.074 (0.069)	0.200	0.250
No. of rostellar hooks	218-238 (228)	-	≅ 260
Length of rostellar hooks			
large	0.008-0.009 (0.008)	0.022-0.024 (0.023)	0.027-0.034
small	0.007-0.008 (0.007)	0.017-0.019 (0.018)	0.021-0.025
Sucker diameter	0.052-0.072 (0.059)	0.116-0.140 (0.128)	0.130
Sucker hooks	0.005-0.014	0.005-0.014	0.010-0.015
Cirrus sac	0.108-0.124 (0.116) x 0.048-0.052 (0.049)	0.160-0.192 (0.174) x 0.048-0.060 (0.056)	0.180-0.200 x 0.060
No. testes	5-7	-	9-12
No. egg capsules	16-20	-	25-32

	R. geraldschmidti	*R. mitchelli*
Total length	40	120
Maximum width	0.760	0.960
Scolex diameter	0.155-0.180 (0.166)	0.224-0.340 (0.298)
Rostellum	0.064-0.074 (0.069)	0.108-0.154 (0.138)
No. of rostellar hooks	218-238 (228)	296-380 (316)
Length of rostellar hooks		
large	0.008-0.009 (0.008)	0.009-0.012 (0.011)
small	0.007-0.008 (0.007)	0.008-0.010 (0.009)
Sucker diameter	0.052-0.072 (0.059)	0.055-0.088 (0.072)
Sucker hooks	0.005-0.014	0.004-0.010
Cirrus sac	0.108-0.124 (0.116) x 0.048-0.052 (0.049)	0.152-0.176 (0.161) x 0.032-0.044 (0.038)
No. testes	5-7	5-6
No. egg capsules	16-20	9-15

synonym of *R. mitchelli*. As Beveridge (1974, p. 2) states "the more intensively a species is studied and further information gathered on it, so a need arises to re-structure early classifications in the light of new knowledge".

Similarly *R. infrequens* resembles *R. australis* in the number of rostellar hooks (280-362 v. 260), size of rostellar hooks and size of scolex. The only definitive characters are the dimensions of the cirrus sac, the number of testes and number of egg capsules.

Host-specificity is a characteristic of tapeworms, more pronounced than previously thought and confirmed where detailed studies have been conducted (Bona, 1975; Mariaux, 1996; Caira and Zahner, 2001; Beveridge and Jones, 2002). Whilst discussing the taxonomy of *Raillietina* in humans, rodents and monkeys, Chandler and Pradatsundarasar (1956) considered that in this genus, as in other genera, there appeared to be a considerable degree of host-specificity. The same authors suggested that "lumping" of mammalian species of *Raillietina* should be avoided and it is likely to cause more confusion than simplification.

Bray (1991) emphasised the importance of allopatry as a major factor for speciation. Emus and cassowaries occur in sympatry in far North-eastern Australia although emus do not inhabit rainforest where the solitary cassowary hides in thickets during the day (Pollock, 1992). The occurrence of the two morphologically similar cestode species, *R. mitchelli* and *R. geraldschmidti*, in closely related hosts might be explained by host switching, where a species of cestode is found in two different hosts, only one of which is the natural host, the other a colonised host (Poulin, 1998). Their similarity, however, should not be misinterpreted as having a common ancestor but may be a result of the selection pressure of becoming adapted to similar intestinal environments (Kunz, 2002). Alternatively, the similarity of the morphological characters may indicate the existence of a species-complex in *Dromaius* and *Casuarius* species.

Wardle (1932a) discussed the limitations of morphometric characters in the differentiation of the Cestoda and concluded that non-adaptive characters, that is, the internal morphometric characters, are more likely to provide a stable basis for specific identification. This supports the separation of *R. mitchelli* and *R. geraldschmidti*. However, if the morphology of the rostellar hooks is accepted as a critical character and hook shape is, as previously discussed, as significant as size, then, in the cestode genus *Rallietina* there appears to be considerable cause to assess the validity of morphological, particularly morphometric characters, in determining species. To this end, studies of additional material in Casuariformes are required before the distribution of species and the evolutionary relationships can be further explored.

The number of eggs per capsule observed in the present study exceeds the ranges hitherto reported in taxonomic keys for *Raillietina* (see Jones and Bray, 1994). Individual species described here have ten to 22 eggs per capsule and it is considered therefore, that the genus should be regarded, as Schmidt (1986) simply suggests, as having several eggs per capsule.

Where morphological characters do not define species, the use of molecular definition becomes essential and the only way to provide a sound basis for future analysis (Mariaux, 1996). *Raillietina* (*sensu lato*) is, according to Mariaux (1996), exceedingly complex because of a prolonged history of poor descriptions, extensive synonomies and lack of workable characters differentiated in an ambiguous manner. Therefore the consideration of evidence in addition to comparative morphology provides support for taxonomic decisions. As Stunkard (1957, p.17) states "I know of no more futile effort than fishing for specific characters in the cestode and trematode gene pool. When life-cycles have been worked out, when larval stages and sexual generations are known, when the

ranges of possible intermediate hosts have been assessed, we can begin to define morphological species with real assurance".

The morphological study undertaken has drawn attention to two areas for further study. First, the need to examine molecular characters and other biological criteria to further define the species of *Raillietina* infecting emus and related birds (Chapter 7). Second, the prevalence of species in archived material and that identified from 5-8 cm sections of intestine indicates either an uneven geographical distribution of parasites within Australia or an uneven distribution of parasites within the intestine of the host. The latter seems evident because a greater number of species was collected when whole gastrointestinal tracts were examined. Consequently, it was deemed necessary to determine the number and distribution of cestode species along the length of the gastrointestinal tract (see Chapter 4).

3.12 Summary

A detailed description of the morphology of five cestode species of *Raillietina* infecting the emu in Australia is given. *Raillietina australis* (Krabbe, 1869) is redescribed and four new species identified. Cestodes infecting related hosts in Australia have also been re-described and compared with those infecting the emu. The species of *Raillietina* infecting emus can be distinguished from all congeners in the Struthioniformes by the size and number of rostellar hooks, size of the scolex and dimensions of the cirrus sac.

Chapter 4.

THE HOST-PARASITE RELATIONSHIP BETWEEN *RAILLIETINA* SPECIES AND THE EMU.

Accurate knowledge of the distribution of the helminths in the alimentary tract should lead to a better understanding of the factors affecting population density, nutrition, growth, reproduction and other aspects of their biology (Crompton, 1973).

4.1 Introduction

There is no published information on the intensity of cestode infections in emus. Determining the number of cestodes infecting a species may provide valuable information on the potential for the parasite to cause disease, particularly if the pathogenicity of the species is known (Goater and Holmes, 1997). In fowl, for example, species of *Raillietina* such as *R. echinobothrida* and *R. tetragona* are regarded as pathogenic in heavy infections because of the intestinal reaction associated with the deeply-embedded scoleces (Reid, 1962; Dunn, 1969; Urquhart *et al.,* 1987).

Parasite intensity is regulated by a number of mechanisms, such as exploitation competition, interference competition, host-mediated restriction and parasite-induced host mortality (Poulin, 1998). Fewer parasites become established when parasite burdens are high and attachment space in the gut of the definitive host becomes the limiting resource (Uznanski and Nickol, 1982; Brown, 1986; Hudson and Dobson, 1997; Poulin, 1998).

The ecological niche of an individual parasite is recognised by Hutchinson (1957) as the multi-dimensional volume occupied by parasites, which in turn is defined by several physical and biotic variables. For example, parasites can occupy a defined habitat

determined in response to competition for resources or to facilitate mating, as well as other factors (Rohde, 1994). Stock and Holmes (1988) found that helminth species were usually restricted to a predictable portion of the intestine and that various species were arranged along the entire length of intestine, when they examined the intestinal distribution of helminths in four species of grebes. In species flocks, that is, several related species of parasite occurring in the same host individual at the same time, site segregation is, as Inglis (1971) points out, common. Parasite attachment sites can be enumerated along a linear axis, such as the gastrointestinal tract (GIT), and the niche can be taken as the mean or median position of individual parasite species (Poulin, 1998).

Crompton (1973) and Mettrick (1980) suggested that the intestine may be regarded as a complex linear gradient and the linear distribution of a parasite is probably best expressed in terms of a percentage distance along the alimentary tract. Crompton (1973) further suggested that records of linear distribution of parasites should include the location of an intestinal landmark such as the *diverticulum caecum vitelli* or Meckle's diverticulum which is a point of delineation between the jejunum and ileum and is important in establishing site preference of intestinal parasites such as coccidia (Pellerdy, 1974).

The "crowding effect" (Read, 1951; Read and Simmons, 1963) has been well documented for many species of tapeworm (Roberts, 1961,1966; Roberts & Mong, 1968). Crowding, a consequence of heavy infections, has adverse effects on size and fecundity in cestodes (Smyth and McManus, 1989) but rarely is the impact of parasites on bird populations studied, principally because tapeworms are seen to be benign symbionts that have little impact on the host population (Hudson and Dobson, 1997).

Cestodes absorb their food through the tegument and may be in close contact with the mucosa or extend from a securely attached scolex into the intestinal lumen. If the cestode infections are influenced by the host's nutrition, then the need to note the nutritional

state of the host is self-evident. Poorly nourished hosts are more vulnerable because nutrients are diverted by parasites and because they are less able to launch a strong immunological response (Hudson and Dobson, 1997). Care must be taken to determine whether infection is the cause rather than the effect of nutritional deficiency (Symons, 1989). However, it is conceivable that the bigger, stronger hosts are more capable of supporting high parasite burdens particularly if the parasites are of low pathogenicity or are non-pathogenic.

Endoparasite infection can inhibit skeletal growth (Symons, 1989) and the quality of bone is dependent on mineral content, particularly calcium and phosphorous. Dietary mineral deficiencies in manganese, zinc and selenium are known to cause a variety of problems such as shortened bones, bone deformities and low egg production in poultry (Angel, 1993) and leg weakness is known to occur in emus (Costa *et al.,* 1993). Nadakal and Vijayakumaran Nair (1982) found higher levels of calcium and phosphorous in *R. tetragona* than in the tissues and serum of the chicken host. Cestodes are known to accumulate calcium, magnesium, lead, manganese and phosphorous in soft tissues and in calcareous corpuscles (Desser, 1963; von Brand and Weinbach, 1965, 1975, Kegley *et al.*, 1970), thought to be connected with nutrition levels in the host (von Brand *et al.*, 1969). Biochemical analysis will determine the mineral and trace nutrient levels, particularly calcium, phosphorous and manganese, in *Raillietina* species and detect any associated depletion of nutrients in the host.

There are few records of the length of the GIT of emus. Herd and Dawson (1984) recorded a total length of 445 cm with the small intestine (SI) occupying 315 cm, whilst Fowler (1991) reported the length of intestine and rectum as 287 cm. Histologically the emu intestine, caeca and rectum, are uniform (Herd and Dawson, 1984).

4.2 Materials and Methods.

4.2.1 Enumeration and distribution of cestode species.

Whole GITs from emus originating from three farms (Fig. 1) were collected at abattoirs and GITs from wild emus were dissected from road kills. GITs were transported to the laboratory where the oesophagus and stomach were removed. The remaining intestine was straightened and the total length measured. Beginning anteriorly, the SI and rectum were then divided into 30 cm lengths, each opened longitudinally. A 30 cm section was assumed to represent approximately 10% of the total length of an emu SI and rectum. Cestodes were removed by scraping the entire intestinal mucosa with the edge of a glass slide. The scrapings were mixed with tap water. Cestodes were allowed to relax from four h to overnight before fixing in 10% buffered formalin or 70% ethanol and storing at room temperature prior to examination using a stereomicroscope. Cestode scoleces were dissected from strobila, washed in 70% ethanol, mounted in De Faure's mounting medium, cleared and dried at 60°C, counted and identified to species using the morphological features of the scolex. The length of rostellar hooks was used as the principal criterion for specific determination (Appendix C, Table 47, Fig. 177 and Appendix D, Figs 178, 179, 180).

Each intestinal segment was examined individually and cestodes were recorded in the segment in which the scolex was embedded. Intensity and distribution of other endoparasites encountered (the nematodes, *Dromaeostrongylus bicuspis* Lubimov, 1933 and *Trichostrongylus tenuis* (Mehlis, 1846) Railliet and Henry, 1909 and the trematode, *Brachylaima cribbi* Butcher and Grove, 2001) are recorded in wild birds only and results are found in Appendix E.

Terminology follows that of Bush *et al.* (1997).

4.2.2 Distribution of cestode species in relation to *diverticulum caecum vitell,* ('Meckel's diverticulum')

GITs from two emus at one farm were divided once at Meckels diverticulum. This point was determined by the presence of the small rudimentary dome-like appendage, the remnant of the yolk duct and yolk sac on the external aspect of the SI (McLelland, 1991). Cestodes were counted and identified as above.

4.2.3 Histological examination.

Fresh sections of SI were fixed in buffered formalin and processed routinely (dehydrated, cleared and impregnated with paraffin wax) in an automated tissue processor (Leica TP1050), embedded in paraffin wax, sectioned at approx. 5 μm thickness and stained with haematoxylin and eosin using an automated staining machine (Shandon Varistain 24-4).

4.2.4 The crowding effect

The wet weight (patted dry to remove excess liquid) of *Raillietina beveridgei* was determined by randomly selecting individual adult worms from a pool of relaxed specimens, collected from emu ilia, with infections of varying intensities. Cestodes were then fixed in 10% buffered formalin. Preserved specimens were gently dried on blotting paper to remove surface moisture and weighed as pools of 5, 10, 15, 20, 25 and 30 individuals. The mean wet weight of individual cestodes was calculated from each pool. *Raillietina beveridgei* was the predominant parasite in the ileum and was easily identified macroscopically from samples selected in that intestinal region.

Cestode scoleces, mounted in De Fauré's medium, were measured from infections of varying intensities. The number of capsules in each proglottis and the number of eggs per capsule were estimated by recovering worms from the contents of a SI segment with varying intensities. Gravid proglottides were dissected from the strobila, stained in Celestin blue, cleared in clove oil, mounted in Canada balsam, allowed to dry and examined microscopically.

4.2.5 Biochemical analysis

Plasma and liver samples were collected at abattoirs from slaughtered emus and frozen at -20°C. Cestodes were recovered from corresponding birds, washed in three changes of distilled water and frozen at -20°C until assayed. Dried liver and cestode samples were digested by heating with 0.3 ml concentrated nitric acid (Aristar®) to dryness at 150 °C. The process was repeated before a further 0.3 ml nitric acid was added and the sample heated to dryness overnight at 100 °C. The residue was dissolved in 0.3 ml nitric acid at 100 °C, and made to 5 ml with distilled water. Selenium assays were performed using the fluorimetric procedure of Koh and Benson (1983). Plasma inorganic phosphorous, calcium and magnesium concentrations were assayed using commercially available kits (Trace Scientific Ltd, Melbourne) and an automated, random access biochemical analyser (Cobas Mira, F Hoffman La Roche & Co). Plasma copper levels were determined by atomic absorption spectrophotometry (GBC 906 Flame Atomic Absorption Spectrophotometer). Liver and cestode copper, iron, zinc, and manganese assays were performed using atomic absorption spectrophotometry. Liver and cestode cadmium, lead, sodium and potassium assays were conducted by the Northern Territory University (NTU) using inductive coupled plasma mass spectrophotometry (ICPMS). NTU also performed liver and cestode calcium, magnesium and phosphorous assays using

inductive coupled plasma atomic emission spectroscopy (ICPAES). Liver and plasma were assayed for vitamin B12 using a radioisotope assay kit (Solid Phase No Boil, Diagnostic Products Corporation, USA). All liver and cestode assays (excluding vitamin B12) were conducted on a dry matter basis. Quality control samples were included in each batch of assays. Liver vitamin B12, selenium, copper, manganese and zinc levels reflect reserves and are regarded as better indicators of animal status (Puls, 1994a, b).

4.2.6 Statistical analysis

Statistical analysis was performed to determine if hook length differs significantly between cestode species. A linear mixed model analysis was performed with parameters estimated using residual maximum likelihood (REML) for both fixed and random effects of variance. The main effects, between species and size of hooks, and the interaction effects were analysed using a Wald test to determine which species contributed to the significance using LSD. Similarly, REML was conducted to analyse the interaction between cestode species and intestinal segment. A logarithmic transformation was conducted and the transformed response did not show departure from the assumptions of normality.

4.3 Results

4.3.1 Length of intestine

The SI from 30 emus was measured in this study. The mean length was 266.4 cm (± 55.4 S.D.; range 148-398 cm).

4.3.2 Intensity of cestodes

Cestodes were present in all of the birds examined. The maximum number recovered in a farmed bird was 1,794. The heaviest infection occurred in a wild bird (Table 14). There was no macroscopic evidence of damage to intestinal epithelium. In heavy infections, cestodes were visible in the distal section of the SI (ileum) before it was opened. When present, mature cestodes were visible in each section of opened intestine. A large amount of peritoneal fat was associated with the intestine of farmed birds but noticeably absent in wild emus.

Table 14. The intensity of cestodes recovered from the gastro-intestinal tracts of farmed and wild emus.

	Farmed		Wild		
Bird	Keith, SA	Glossop, SA	Kiki, SA	Meningie, SA	Ucolta, SA
1	636	89	3472	1063	4695
2	306	398			
3	893	509			
4	1004	1794			
5	292	287			

4.3.3 Distribution of cestode species in the small intestine of emus

The highest number of cestode scoleces recovered from each intestinal segment is shown in Table 15.

Table 15. The maximum intensity of cestodes present in an intestinal segment. 1 = anterior, 9 = posterior.

Intestinal section	Highest number of cestodes	Host
1	502	Wild
2	638	Wild
3	573	Wild
4	254	Wild
5	1712	Wild
6	1007	Wild
7	249	Farmed
8	22	Farmed
9	64	Farmed

Table 16. Intensity and distribution of *Raillietina* species in 30 cm segments of SI from farmed emus at Keith, SA; collected 8.xii.1998 and 30.iii.1999.

Intestinal section	*R. beveridgei*					*R. australis*					*R. chiltoni*					*R. dromaius*					*R. mitchelli*				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	1	0	1	0	0	6	7	0	0	0	0	0	2	0	1	28	6	0	34	2	19	6	7	10	5
2	0	2	0	0	0	4	1	0	3	0	2	1	0	26	2	105	103	108	72	10	0	0	0	3	0
3	5	0	30	1	0	0	7	4	1	0	22	38	51	39	26	0	19	0	1	0	0	0	0	0	0
4	0	0	18	37	4	1	4	2	2	2	5	11	13	11	17	2	1	1	0	0	0	0	0	3	0
5	1	48	125	20	154	0	0	0	0	1	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0
6	111	21	379	443	66	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	249	31	149	84	0	0	0	0	0	0	0	0	0	3	0	0	0	2	0	0	0	0	0	1	0
8	10	0	1	12	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	10	0
9	64	0	0	12	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	441	102	703	782	226	11	19	6	6	3	29	50	66	80	46	136	129	111	109	12	19	6	7	27	5

Table 17. Intensity and distribution of *Raillietina* species in 30 cm segments of SI from farmed emus at Glossop, SA; collected 20.x.2000.

Intestinal section	*R. beveridgei*					*R. australis*					*R. chiltoni*					*R. dromaius*					*R. mitchelli*				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	0	0	0	0	0	1	33	21	95	7	0	0	0	0	0	0	14	0	27	1	0	0	0	0	0
2	0	0	1	0	0	2	57	104	444	64	0	1	0	0	2	2	15	2	23	18	0	0	0	0	0
3	0	4	1	1	0	19	72	154	211	74	2	0	0	11	2	2	0	0	0	1	0	0	0	0	0
4	4	149	17	169	20	2	1	24	4	11	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
5	33	52	158	541	81	0	0	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
6	21	1	11	218	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	2	34	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	5	6	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	3	2	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	58	205	198	972	103	25	163	308	761	156	2	1	1	11	8	4	29	2	50	20	0	0	0	0	0

Table 18. Intensity and distribution of *Raillietina* species in 30 cm segments of SI from 3 wild emus; #1 collected at Kiki, SA, (35° 41' E, 139° 51 ' S), 29.vi.1999; #2 collected at Meningie, SA, (35° 54' E, 139° 27' S), 11.xi.1999; # 3 collected at Ucolta, SA, (32° 57' E, 138° 57' S).

Intestinal section	*R. beveridgei*			*R. australis*			*R. chiltoni*			*R. dromaius*			*R. mitchelli*		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	0	1	0	17	197	96	0	0	0	267	20	145	0	5	261
2	1	3	0	19	287	56	4	3	163	443	41	398	0	0	21
3	0	1	0	20	60	45	164	101	416	49	3	23	1	0	89
4	29	131	38	47	13	64	146	9	104	6	1	0	0	5	48
5	954	157	1569	23	1	21	49	1	21	2	1	1	0	0	100
6	970	7	837	10	1	0	27	0	1	0	0	1	0	0	15
7	203	1	147	6	0	0	14	0	0	1	0	0	0	0	0
8	0	3	14	0	0	0	0	3	0	0	0	0	0	0	0
9	0	1	1	0	1	0	0	3	0	0	2	0	0	0	0
Total	2157	305	2606	142	560	282	404	120	705	768	68	568	1	10	534

Table 19. Mean intensity of cestode species in 30 cm segments of all gastro-intestinal tracts of the emus examined (n = 13; 10 farmed birds, 3 wild birds).

Intestinal section	*R. beveridgei*			*R. australis*			*R.chiltoni*			*R. dromaius*			*R. mitchelli*		
	Keith	Glossop	Wild	Keith	Glossop	Wild	Keith	Glossop	Wild	Keith	Glossop	Wild	Keith	Glossop	Wild
1	0.4	0	0.3	2.6	31.4	103.3	0.6	0	0	14.0	8.4	144.0	9.4	0	88.7
2	0.4	0.2	1.3	1.6	134.2	120.7	6.2	0.6	56.7	79.6	12.4	294.0	0.6	0	7.0
3	7.2	1.2	0.3	1.6	106.0	41.7	35.2	3.0	227.0	4.0	0.6	25.0	0	0	30.0
4	11.8	71.8	66.0	2.2	8.4	41.3	11.4	0.8	86.3	0.8	0	2.3	0.6	0	17.7
5	69.6	173.0	893.3	0.2	0.6	15.0	0.2	0.2	23.7	0.4	0	1.7	0	0	33.3
6	204.0	50.6	604.7	0	0.4	3.7	0	0	9.3	0	0	0.3	0	0	5.0
7	102.6	7.2	117.0	0	0.4	2.0	0.6	0	4.7	0.4	0	0.3	0.2	0	0
8	5.0	2.2	5.7	0	0.2	0	0	0	1.0	0.2	0	0	2.0	0	0
9	15.6	1.0	0.7	0	1.0	0.3	0	0	1.0	0	0	0.7	0	0	0
$\bar{x}$ Total	449.8	307.2	1689.3	7.0	282.6	328.0	54.2	4.6	409.7	99.4	21.0	468.0	12.8	0	181.7
N=	5	5	3	5	5	2	5	5	3	5	5	3	5	5	3

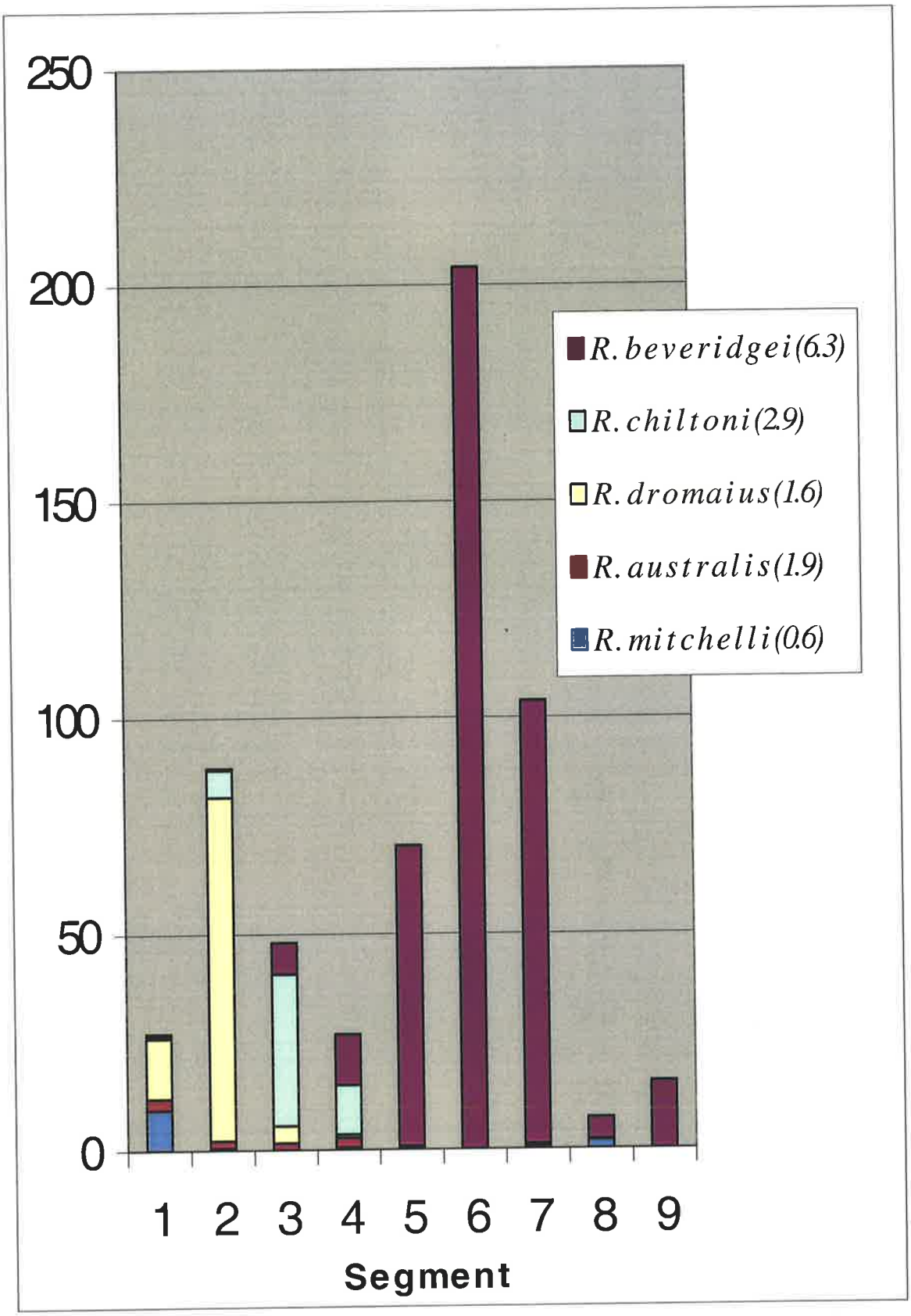


Fig. 99. Mean intensity of *Raillietina* species in 30 cm segments of SI, Keith SA (n=5). Segmental position of the median of each species is shown in parentheses in the legend.

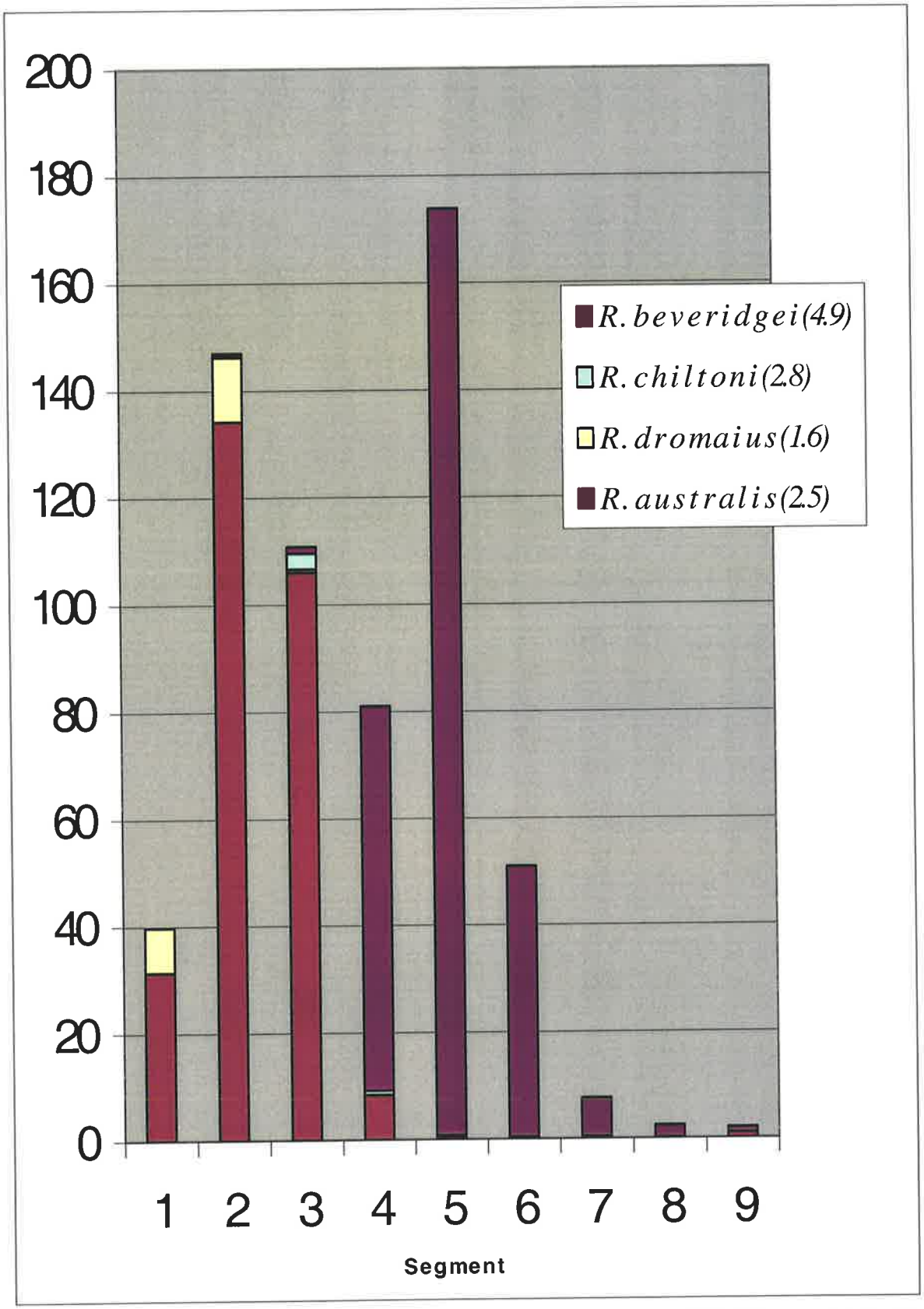


Fig. 100. Mean intensity of *Raillietina* species in 30 cm segments of SI, Glossop SA (n=5). Segmental position of the median of each species is shown in parentheses in the legend.

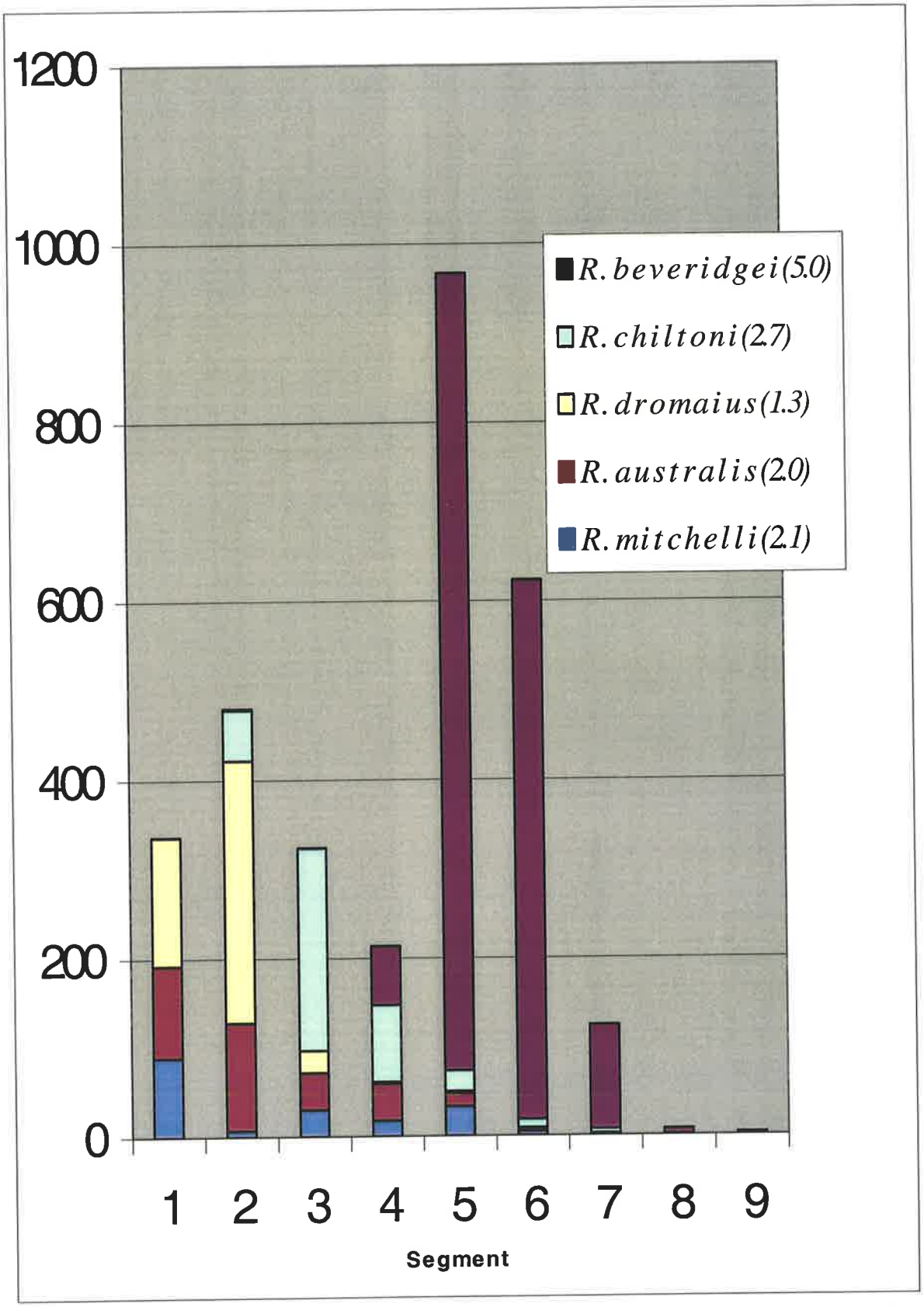


Fig. 101. Mean intensity of *Raillietina* species in 30 cm segments of SI, Wild birds (n=3). Segmental position of the median of each species is shown in parentheses in the legend.

Five species of *Raillietina* were present in GITs collected from farmed emus at Keith and three wild emus. Only four species were recovered from emus farmed at Glossop. The intensity of each cestode species and their intestinal distribution is recorded in Tables 16, 17 and 18. Although the positions in which cestode species were attached varied, separate species appeared to predominate in a different portion of the SI in both farmed and wild emus. The mean intensity of cestode species in intestinal segments of all emus examined appears in Table 19. No cestode scoleces were recovered from the rectum.

Raillietina mitchelli was the least commonly detected parasite and was not recovered from farmed emus examined at Glossop (Table 20).

Table 20. Percentage of *Raillietina* species in farmed and wild emus.

	R. beveridgei	*R. australis*	*R. chiltoni*	*R. dromaius*	*R. mitchelli*
Keith					
1	69.3	1.7	4.6	21.4	3.0
2	32.2	6.3	16.6	42.9	2.0
3	78.7	0.7	7.4	12.4	0.8
4	78.0	0.6	8.0	10.9	2.7
5	77.4	1.0	15.8	4.1	1.7
Glossop					
1	65.2	28.1	2.3	4.5	0
2	51.5	41.0	0.3	7.3	0
3	39.0	60.6	0.2	0.4	0
4	54.2	42.4	0.6	2.8	0
5	35.9	54.4	2.8	7.0	0
Wild					
1	62.1	4.1	11.6	22.1	0.03
2	28.7	52.7	11.3	6.4	0.9
3	55.5	6.0	15.0	12.1	8.9

Raillietina beveridgei was the most predominant cestode species comprising up to 78.7 % of a cestode population. The maximum intensity of an individual species recovered from an intestinal segment is shown in Table 21.

At Keith, where *R.australis* occurred in low numbers, the species was more evenly distributed in the anterior sections of SI and occupied the same region of intestine as *R. chiltoni, R. dromaius* and *R. mitchelli,* although each of these species appeared to accumulate in a more localised region (Table 16, Fig. 99). At Glossop, (Table 17, Fig. 100) however, a similar pattern occurred when *R. chiltoni* was present in low numbers, sharing the same anterior S.I. range as *R. australis* and *R. dromaius*. On this farm, *R. australis* occurred in higher numbers and appeared to accumulate in a more localised region of the S.I. Similar patterns also occur in the wild birds.

Table 21. The intestinal segment in which the maximum intensity of each *Raillietina* species occurs. Proportion of each species and total number of cestodes in the segment from farmed and wild birds.

Cestode species	Intensity	Total cestodes in section	Proportion	Section of intestine
R. mitchelli	261	502	52.0%	1
R. dromaius	443	467	94.9%	2
R. chiltoni	416	573	72.6%	3
R. australis	444	467	95.1%	2
R. beveridgei	1569	1712	91.7%	5

Raillietina beveridgei occupied a more distal region of SI and did not appear to co-occur with any of the other species. Posterior sections of SI were frequently unoccupied by cestodes. It is evident that four cestode species inhabit the anterior portion of intestine whilst *R. beveridgei* inhabits the posterior portion. There appears to be no displacement of species from anterior portions of intestine to posterior portions when large populations of a co-habitating species occurred. There is, rather, an expansion in distribution, adjacent to the preferred site. *Raillietina mitchelli* apparently prefers the anterior section of intestine, particularly the first segment, *R. australis* the first and second segment, *R.chiltoni* the third and fourth segment and *R. dromaius* the first and second segment.

One measure of the location or niche of a species is the median position of individual parasites in the host intestine and the mean of these positions is the measure of the usual location of that species (Stock and Holmes, 1988). The median position, here measured as the location within an intestinal segment, is consistent with the distribution indicated by the mean numbers of cestode species present (Table 19, Figs 99,100,101).

To confirm the distribution, the proportion of each cestode species in intestinal segments was calculated and appears in Figs 102,103,104. These figures have been adjusted by excluding destrobilised cestodes (see section 4.3.6). The distribution of cestode species in two wild emus reflected that of birds farmed at Keith, whilst the other was similar to the birds farmed at Glossop. In each case, the distribution was dependent on the number of *R. australis* and/or *R. dromaius* present in the population. *Raillietina australis* and *R. dromaius* occupied the same intestinal region but did not co-occur together in high intensities. There is little evidence that the distribution of *R. chiltoni* and *R. dromaius* overlapped with increased collective population size.

4.3.4 Statistical analysis

The mixed model analysis showed that the interaction between species and size of rostellar hook was significant ($p < 0.001$) and that the length of hooks is significantly different between species (Appendix D, Figs 184, 185). A graphical representation of the back transformed estimated means is presented in Appendix D, Fig. 186.

The is also a significant interaction between the cestode species and the intestinal segment at the 5% level ($p < 0.001$).This indicates statistically that the number of cestode species within each segment differs depending on species. A back-transformed estimated means analysis for segment by species interaction effect of tapeworms ($p < 0.001$) also appears in Appendix D, Fig. 187. This analysis confirms the earlier observations regarding

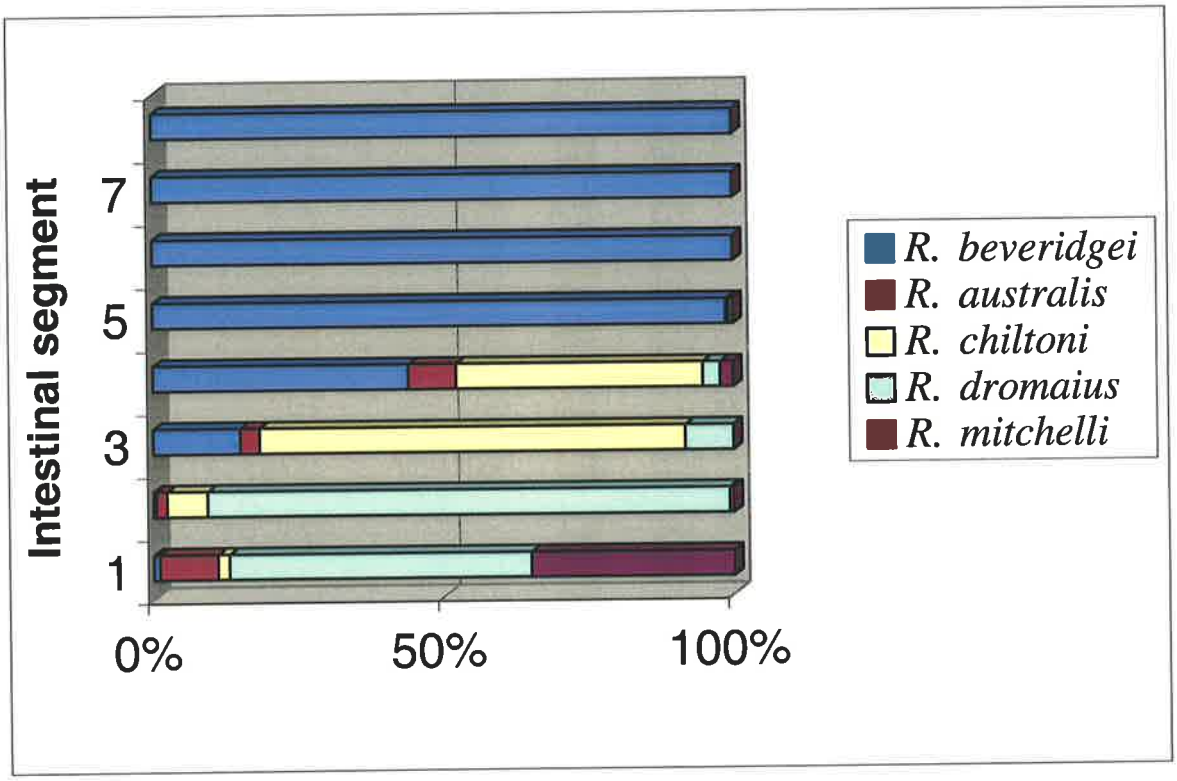


Fig. 102. Proportion of *Raillietina* species in 30 cm segments of intestine of farmed emus collected at Keith, SA; (Mean, n=5).

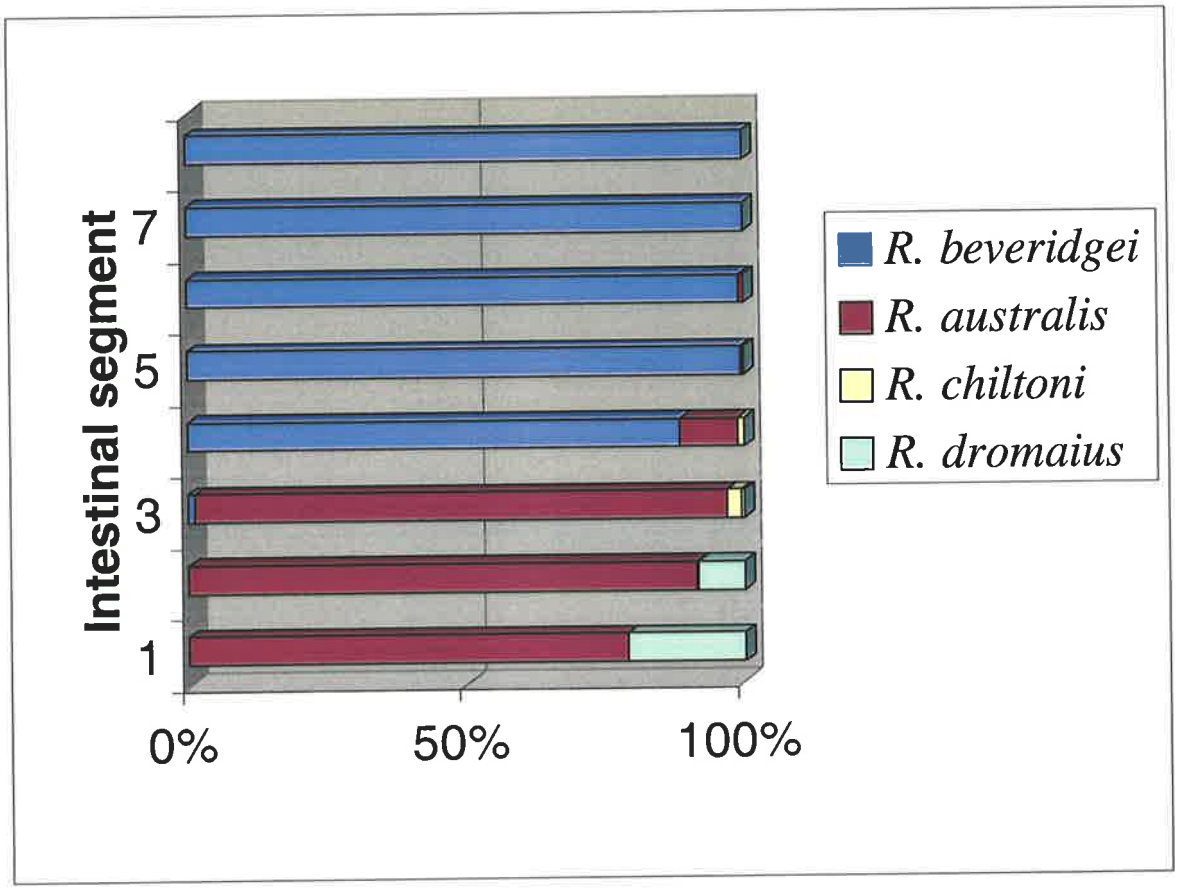


Fig. 103. Proportion of *Raillietina* species in 30 cm segments of intestine of farmed emus collected at Glossop, SA; (Mean, n=5).

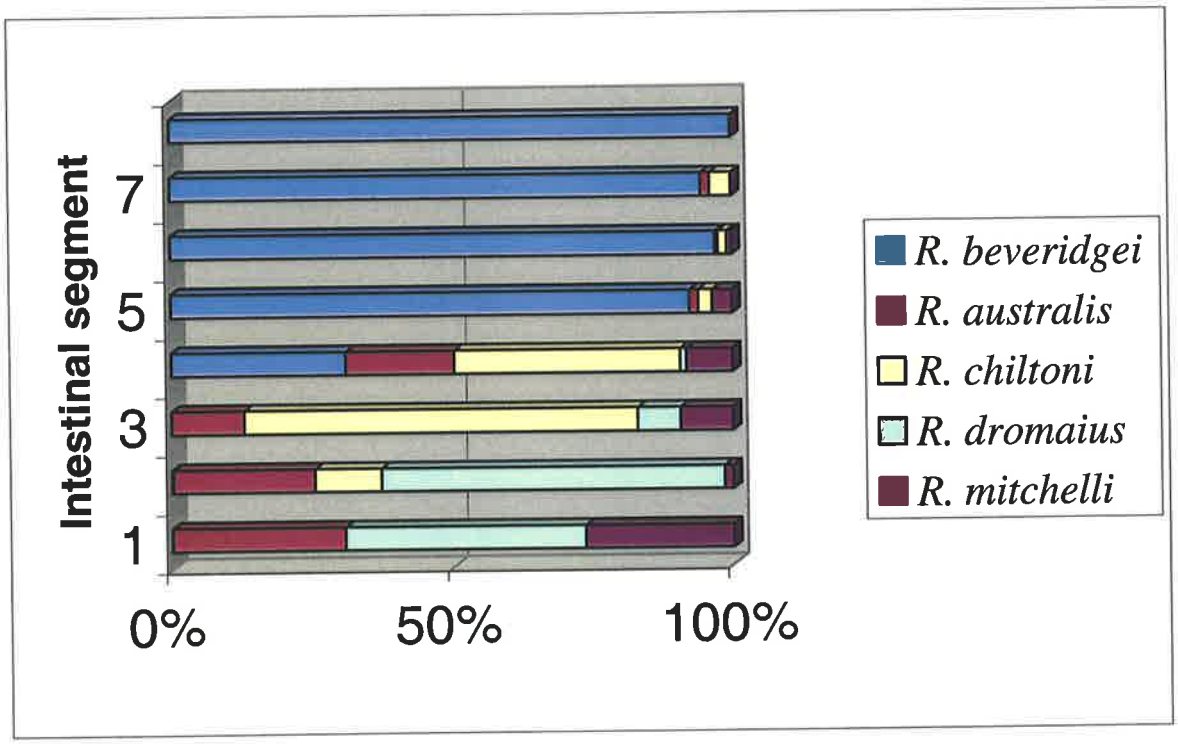


Fig. 104. Proportion of *Raillietina* species in 30 cm segments of intestine from wild emus; (Mean, n=3)

the preferred site of attachment for each cestode species but indicates further that *R. australis* can occupy the first four segments and appears to have a less restricted distribution than the other species inhabiting the anterior intestine.

4.3.5 Distribution of cestodes in relation to Meckel's diverticulum

In two intestines divided at Meckel's diverticulum, the duodenum and jejunum were measured as 40-43% of intestinal length. Four cestode species occupied this region whilst *R. beveridgei* preferred to attach below (i.e. posterior to) Meckel's diverticulum, in the ileum (Table 22)

Table 22. Intensity and distribution of *Raillietina* species in relation to Meckel's diverticulum. Percentage of species in parentheses.

Cestode species / Intestine	Above, anterior to Meckel's Diverticulum		Below, posterior to Meckel's Diverticulum	
	1	2	1	2
R. australis	2 (100%)	4 (100%)	0	0
R. chiltoni	54 (94.7%)	20 (76.9%)	3 (5.3%)	6 (23.1%)
R. dromaius	28 (100%)	84 (88.4%)	0	11 (11.6%)
R. mitchelli.	5 (100%)	3 (100%)	0	0
R. beveridgei	1 (0.3%)	0	371 (99.7%)	292 (100%)

4.3.6 De-strobilised cestodes

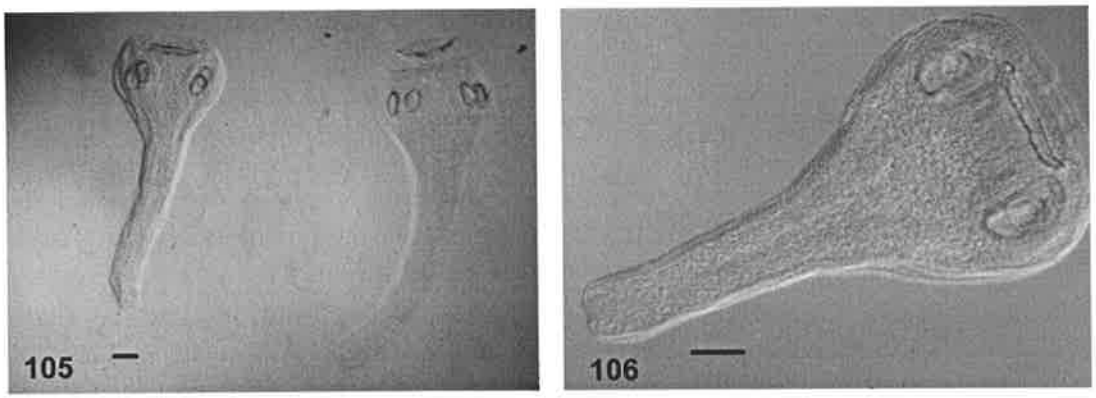
Intact strobila were rarely recovered from the last two segments of SI. When scoleces were recovered in segments posterior to the observed ranges, an estimated 78.6%, 64.0% and 24.2% of *R. beveridgei, R. australis* and *R. chiltoni* occurred as destrobilised, (i.e. scolex and neck only Figs 105, 106).

The scolex and rostellar diameter of de-strobilised scoleces of *R. beveridgei* was significantly smaller than in established cestodes (Table 23).

Table 23. Scolex and rostellar diameter (μm) from established and de-strobilised *Raillietina beveridgei*.

		Diameter	Range	S. D.	N=
Established cestodes	Scolex*	619.8	505.0-727.2	52.7	25
	Rostellum**	280.3	232.0-336.0	30.1	25
De-strobilised cestodes	Scolex	494.5	444.4-545.5	27.4	25
	Rostellum	259.2	240.0-288.0	15.5	25

Significant *P < .001, ** P < .01



Figs 105, 106. Destrobilised *Raillietina beveridgei* from the posterior segments of bird #1 from Keith. Scale bars = 0.1 mm.

4.3.7 Histopathology of the small intestine

There was marked, diffuse lymphoplasmacytic and eosinophil infiltration of the lamina propria with some collapse and condensation of stroma and crypts ahead of the invading parasite and, occasionally, collections of high-protein oedema fluid and compact fibrin around the cestode. Scoleces were found extending into the sub-mucosa with occasional breaks of muscle layers at the edge of the muscularis mucosa. The pathology indicates a potential for intestinal irritation and hyperactivity that may result in high parasite numbers causing decreased weight gain or a loss of condition (Figs 107-110).

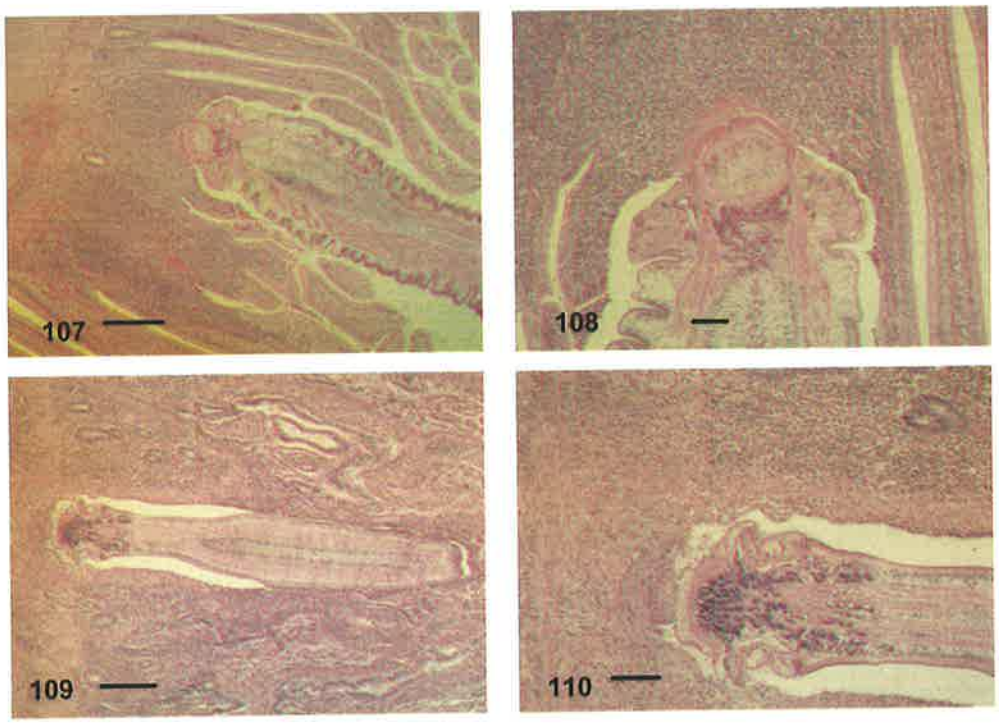
4.3.8 Effect of crowding

4.3.8.1 Size of cestodes measured by wet weight

Differences were detected in the wet weight of *Raillietina beveridgei* taken from regions of intestine containing varying intensities (Table 24). Linear regression analysis indicates that there is no trend towards an inverse relationship.

Table 24. Wet weight of adult *Raillietina beveridgei* from infections of varying intensity.

Number of cestodes	Mean Weight (g) N=30	Range (g)
N= 48	0.2606	0.2192-0.3132
N= 154	0.2238	0.2168-0.2640
N= 249	0.3078	0.2510-0.3376
N= 379	0.1549	0.1344-0.1786
N= 443	0.2410	0.1736-0.3022
N= 541	0.3818	0.3510-0.3960



Figs 107-110. 107, 108. Light micrograph of histological sections (HE) of *Raillietina chiltoni in situ.* 109, 110. Light micrograph of histological sections (HE) of *R. dromaius in situ.* Scale bars = 0.5 mm 107, 109; 0.1 mm 108, 110.

4.3.8.2 Size of cestodes measured by scolex diameter

Differences in the size of scoleces were detected in cestodes taken from the same regions of intestine with varying intensities (Table 25).

Table 25. Mean diameter of scoleces from infections of varying intensity. N= number of *Raillietina* species followed in parentheses by the total number of cestodes in the segment.

R. australis	Segment	Mean diameter of scolex (mm)	S.D.	Range (mm)	Number measured
N= 57(73)	2	0.533	45.6	0.464-0.626	20
N= 197 (223)	1	0.572	34.7	0.505-0.626	20
N= 444 (467)*	2	0.538	47.8	0.464-0.626	20
N= 19 (467)	2	0.441	48.5	0.343-0.505	16
N= 19 (24)**	3	0.516	79.3	0.404-0.707	19
R. beveridgei					
N= 52 (52)	5	0.750	46.6	0.646-0.828	50
N= 541 (541)*	5	0.642	54.6	0.525-0.767	50
R. dromaius					
N=41 (334)**	2	0.670	77.7	0.505-0.889	45
N=108 (108)*	2	0.672	63.3	0.525-0.848	45
N=267 (284)*	1	0.680	63.3	0.566-0.808	45
N=398 (638)	2	0.736	44.4	0.646-0.808	45
N=23 (467)**	2	0.676	95.5	0.504-0.856	18

Significant * $p < .001$ ** $p < .01$

4.3.8.3 Number of egg capsules per proglottis

The mean number of egg capsules per proglottis decreased with the intensity of infection (Table 26).

Table 26. Mean number of egg capsules in proglottides of *Raillietina* from segments containing varying infections.

R. beveridgei	Mean number	S.D.	Range	Number counted
N= 6	44.3	4.5	39-53	12
N= 967	31.5	3.5	27-39	12
N= 21	49.4	2.9	46-53	10
N= 443	38.8	7.0	29-47	10
R. australis				
N= 2	80.0	3.7	74-86	10
N= 444*	51.9	8.0	39-60	10

Significant* $p < .001$

4.3.8.4 Number of eggs per capsule

The number of eggs per capsule did not vary with intensity of infection (Table 27).

Table 27. Mean number of eggs per capsule from *Raillietina* in varying intensities.

R. beveridgei	Mean number	S.D.	Range	Number counted
N= 7	13.0	1.8	10-17	40
N= 970	13.2	1.7	10-17	40
N= 21	13.1	1.4	11-17	20
N= 443	13.3	1.9	10-17	20
R. australis				
N= 2	10.5	0.9	9-12	20
N= 444	10.5	1.4	9-13	20

not significant

4.3.9 Biochemical analysis

Biochemical analysis of cestodes appears in Table 28. Plasma and liver analyses are shown in Appendix F, Table 51 and Appendix G, Table 52. Levels of manganese, lead, phosphorous, magnesium and calcium were higher in cestodes than in the liver of the infected emu.

4.4 Discussion

In this study, the distribution of *Raillietina australis* and *R. chiltoni* was less restricted when they were detected at low intensities. When species were detected at higher intensity, the site of attachment became more restricted (Tables 16, 17 and 18).

Raillietina australis preferred the same intestinal region as *R. dromaius,* however it appears that *R. australis* cannot successfully select the region of preference when *R. dromaius* occurrs in high intensities as shown in Tables 16 and 18. There is evidence here (Table 17, Fig. 100) to suggest that these two cestode species do not co-occur in high intensities in the same host. This may be a result of competition for attachment sites or competitive exclusion (Holmes, 1973).

Table 28. Biochemical analysis of *Raillietina* from infected emus.

Locality	Cestodes Date Collected	Cestode sp.	Selenium umol/Kg	Copper mmol/Kg	Iron mmol/Kg	Zinc mmol/Kg	Manganese mmol/Kg	Cadmium mg/Kg	Lead mg/Kg	Phosphorus mg/Kg	Magnesium mg/Kg	Calcium mg/Kg	Sodium mg/Kg	Potassium mg/Kg
Keith, SA	27/10/1998	*R. beveridgei*	6.7	0.25	2.17	4.1	1.31	0.14	0.56	9570	2300	5110	2540	8300
		R. beveridgei	3.9	0.16	1.31	3.1	1.58	0.41	0.68	9430	1940	5720	2370	9660
		R. beveridgei	8.5	0.29	4.01	6.7	1.87	0.68	1.04	14500	5840	12500	2100	9340
		R. beveridgei	7.1	0.22	2.31	6.0	1.37	0.34	0.73	14000	5380	10100	1840	9280
	9/12/1998	*R. beveridgei*	6.5	0.11	0.75	3.6	0.73	0.23	0.33	*	*	*	*	*
		R. beveridgei	7.5	0.10	0.72	3.5	0.71	0.22	0.33	*	*	*	*	*
	29/07/1999	*R. dromaius*	6.1	0.14	2.44	5.4	1.00	0.14	0.56	15900	3780	14000	1200	6590
		R. beveridgei	2.8	0.14	0.63	2.2	1.10	0.41	0.68	7020	1708	2020	720	2560
		R. beveridgei	5.3	0.10	2.08	6.1	1.04	0.68	1.04	12700	1450	7580	1070	3270
		Both	3.9	0.22	1.08	3.6	1.01	0.34	0.73	7940	1110	2760	848	1790
		Both	6.8	0.24	3.12	6.4	0.90	0.34	0.73	13200	2890	9690	977	3240
		R. beveridgei	3.5	0.43	0.78	4.0	1.75	0.34	0.13	8770	1170	3970	850	3590
Glossop, SA	20/10/2000	*R. beveridgei*	10.5	0.15	*	3.7	*	0.25	*	6653	618	1237	*	*
Wild #1	29/06/1999	*R. beveridgei*	2.5	0.14	0.75	2.9	0.99	0.14	0.56	8200	1890	3370	1160	4950
Mean			5.8	0.19	1.70	4.4	1.18	0.33	0.62	10657	2506	6505	1425	5688
Range			2.5-10.5	0.10-0.43	0.63-4.01	2.2-6.7	0.71-1.87	0.14-0.68	0.13-1.04	6653-15900	618-5840	1237-14000	720-2370	1790-9660
no. samples			n=14	n=14	n=13	n=14	n=13	n=14	n=13	n=12	n=12	n=12	n=11	n=11

* No result

If a reduction in intensity of an individual parasite species occurs in the presence of another parasite species, then competition may be taking place. Such a reduction may be a result of the parasite species changing its resource use in the presence of the competitive species (Poulin, 1998). This phenomenon can be measured by a shift in the site of infection - a competitive interaction (Thompson, 1980).

Poulin (1998) emphasised that parasite competition may be influenced by the order in which parasites become established in the host. The species that suffers most (from competition) will perform better if given a head start. When moderate numbers of parasites of different species occur in the same host, they can alter their resource use and avoid the numerical effect of competition (Poulin, 1998). Because of these interactions, parasites become restricted to a particular site or niche which is defined as the potential distribution in the host (Poulin, 1998).

It is not accepted here that the low intensities of *R. australis* or *R. chiltoni* are related to the presence of a primary infection of the species. Luttermoser (1938) was able to superimpose *Skrjabinia cesticillus* infections in chickens. Chandler (1939), however, showed some evidence of a primary infection of *Hymenolepis diminuta* in rats precluding the establishment of a secondary infection. Additional studies (see Roberts and Mong, 1968) failed to support Chandler's findings in relation to establishment of cestode species and showed that secondary infections can establish and attain normal size, albeit, at a slower rate. Furthermore, there is no evidence here that *Raillietina* infection in emus is subject to the protective immunity known to occur for some cestode species (Weinmann, 1966).

The potential intestinal distribution of a cestode species or the range of intestine in which a species can develop can be defined as the "fundamental niche" (Poulin, 1998), that is, the intestinal distribution of the species summed across all birds in which it has occurred

(Bush and Holmes, 1986). For four species of *Raillietina, R. australis, R. chiltoni, R. dromaius* and *R. mitchellii,* the anterior intestine is the fundamental niche. However, the posterior SI is the fundamental niche for *R. beveridgei.*

Realised niches are subsets of the fundamental niche and represent the optimal part available to species subjected to interactions with other helminths (Pianka, 1975; Stock and Holmes, 1988). In the intestinal tracts examined in this study, when cestode species occurred in moderate to high intensities in the anterior portion of the S.I., each species occupied a predictable, preferred site. Pojmanska (1982) also found a tendency to specific site segregation when European coots (*Fulica atra*) harboured heavy infections of three species of *Diorchis,* which was not present when small numbers of cestodes occurred. She concluded that some regulatory mechanism determined the distribution of cestodes in the host gut.

Morphological, physical and/or biochemical mechanisms may be involved in excystment, evagination and establishment and individual parasites may have very specific nutritional requirements even though they utilise the same site in the same host (Smyth and McManus, 1989). Physical factors include the intestinal sites most favourable to absorption (Smyth and McManus, 1989) and intestinal surface topography. Other factors may be the host's physiology or antibody response (Crompton, 1973). Crompton (1973) points out that although a helminth is usually found in a particular zone in the alimentary tract, it does not necessarily mean that it is dependent upon the conditions there.

Raillietina beveridgei did not share its preferred site of attachment with other species. Although the influence of the species occupying the anterior portion of SI on *R. beveridgei* cannot be measured here, those taxa may preclude its establishment in that region of the SI. Increased burdens of *R. beveridgei* increased posteriorly in the SI rather than anteriorly, i.e. away from the competitor's niche. This extension in site is an example

of the "crowding effect" (Crompton, 1973), that is, the response to competition for a limiting environmental resource resulting from an increase in population density (Crompton and Nesheim, 1976). A similar posterior expansion was observed for higher intensities of *R. chiltoni* in wild birds. Conversely, the distribution of *R. dromaius* expanded anteriorly in the same birds. Additionally, the distribution of *R. mitchelli,* appears to move distally when in high intensity and occurring with other species (see data from wild bird #3, Table 18). This would suggest that the number of cestodes competing for available attachment sites influences the distribution of these cestode species, or their site of attachment. Thus the density at which individuals interfere with each other may have been reached (Poulin, 1998).

Cestodes absorb nutrients through their body surfaces. "Small absorbers" are primarily associated with the mucosal surface and "large absorbers" are mid-luminal and attach their scoleces to the mucosal surface but retain the majority of their biomass in the lumen (Bush and Holmes, 1986; Stock and Holmes, 1988). Large lumen-dwelling absorbers were lacking in the anterior intestine and small mucosal-dwelling absorbers were lacking in the posterior intestine of grebes examined by Stock and Holmes (1988). Macroscopically, there appeared to be mucosal-dwelling species inhabiting anterior SI portions that were yellow-cream, consistent with the colour of intestinal mucus, whilst *R. beveridgei* and *R. chiltoni* were whiter. These observations indicate that *R. mitchelli, R. australis* and *R. dromaius* appear to have a close association with the mucosal surface whilst *R. beveridgei* and *R. chiltoni* appear to be more lumen dwelling.

Herd and Dawson (1984) reported a reduction in wall thickness from the jejunum to the distal intestine (ileum) in emus because of a reduction in muscle layers of the muscularis externa. This enabled the macroscopic visualisation of large numbers of *R. beveridgei* in distal sections of unopened intestines at collection. Herd and Dawson (1984)

also reported that the ileum contained a large volume of fluid. This may be favourable to *R. beveridgei* and *R. chiltoni* as lumen-dwelling absorbers and consequently influence the site of scolex attachment.

In addition, niche restriction may serve to maintain or reinforce reproductive barriers between similar parasite species (Poulin, 1998). Many types of parasites achieve greater reproductive success in some portions of their fundamental niche than in others (Sukhdeo, 1990a; Chilton *et al.,* 1992). This may be difficult to accept in cestodes because they are hermaphrodite, however both self and cross insemination is known to occur (Smyth and McManus, 1989). In digenean trematodes, it has been demonstrated that even in self-fertilisers, cross fertilisation is necessary (Nollen, 1993; Rohde, 1994). Reproductive barriers are known to occur between similar congeneric parasite species, especially where they have similar copulatory organs (Rohde, 1991, 1994; Poulin, 1998) as do the species of *Raillietina* described in this study. *Raillietina dromaius* has a copulatory organ that differ most from congeners in the Dromaiidae, and this species appears to share portions of intestine with *R. australis* and *R. mitchelli* whereas the species with morphologically similar copulatory organs to *R. mitchelli* have less overlap in distribution, particularly when cestodes are present in high intensities.

Cestode species occurred in different proportions in emus from different farms indicating that the proportion of species harboured may be related to locality. This observation is supported by differing proportions of species in the wild birds, even though two of these birds were from relatively close localities and emus are known to walk up to 12 km per day in winter and 25 km per day in summer (Dawson *et al.* 1984). With an increased range, definitive hosts come into contact with potentially more infected intermediate hosts and with other definitive hosts from which they may capture species through intermediate host transfer (Edwards and Bush, 1989; Bush *et al.*, 1990). Some

species, however, never reach isolated sites (Simberloff and Moore, 1997), such as, in this case, a farm. This may explain the absence of *R. mitchelli* at Glossop given that the cestode occurs at low prevalence and may not have been introduced onto the property with infected hosts. The absence may alternatively be a consequence of some interaction with the intermediate host.

Rohde (1991) suggested that interactions between commonly occurring congeneric parasite species were not strong enough to restrict the number of morphologically and ecologically similar species using the same host. Exposure to cestode species is not even and in equally susceptible hosts, the proportion of *R. australis* and *R. dromaius* may simply reflect the distribution of parasites in space and time (Poulin, 1998). Individual hosts differ in behaviour which may account for variation in exposure and consequently, prevalence of infection (Simberloff and Moore, 1997).

In parasite transmission patterns where passive entry occurs from eating an infected intermediate host, the occurrence of species may be influenced by foraging patterns of the host or the activity of the intermediate host (Simberloff and Moore, 1997). The proportions of all of the cestode species in the definitive host could merely reflect associations existing in the intermediate host. Parasite communities in the definitive host can only be understood by examining the way in which new parasites are recruited, i.e. the way in which parasites are acquired by their hosts (Poulin, 1998).

Evidence from several studies of chickens infected with *Skrjabinia cesticillus* (Ackert and Reid, 1937; Sinha and Srivastava, 1958) suggested that ageing of the avian host results in increased resistance to infection. Gray (1972a) questioned whether destrobilisation was a manifestation of host ageing or a result of a physiological interaction. Experiments later showed that increased resistance to infection with *S. cesticillus* occurred in older chickens, manifested by increased destrobilisation and loss of

scoleces (Gray, 1972b). The destrobilisation had greater longevity in male birds. In addition, cestodes were distributed over a greater length of intestine in older (more resistant) birds (Gray, 1972a). Based on these studies, the data collected here suggest the greater number of destrobilised worms and the more posterior distribution of *R. beveridgei* observed in one bird, (Keith #1), could be related to the bird's age and/or sex; however this cannot be confirmed. The more posterior distribution of cestodes may also be an artefact associated with this bird's greater intestinal length (364 cm). Parasite burdens in other emus indicate that destrobilisation is not related to parasite intensity or the subsequent unavailability of attachment sites.

Scoleces of destrobilised *R. beveridgei* were smaller than established worms indicating the possible link between reductions in size and ageing as suggested by Wardle (1932a). The reduction in size may be part of the process in which these scoleces become detached from the intestinal mucosa.

The possibility that the destrobilised cestodes could be new infections beginning development prior to a migration forward to attachment sites cannot be excluded. Scoleces grow rapidly and reach maximum size before strobilisation begins (Smyth, 1969) . Where high intensities of *R. beveridgei* occurred in posterior segments, migration forward is considered unlikely because of competition for attachment sites. Studies investigating the initial site of establishment of each species would require worm free, juvenile birds which was considered outside of the scope of this study.

There was a marked reduction in wet and dry weight with increased population density but because considerable variation occurred in the weight of *Hymenolepis diminuta* in 40-200 worm infections in rats, Hesselberg and Andreassen (1975) only provided data for low intensity (1-20 worms). Ghazal and Avery (1974) also reported a considerable variation in the size of *H. nana* in infections in rats and suggested for a complete analysis,

it would be necessary to measure all worms. Clearly this was impractical here and the data collected on weight of worms and intensity of infection are similarly variable. A trend towards a decrease in weight with higher intensity of infection is not evident. It has been suggested that substantial variation occurs in the size of cestodes in an infection and size can also be influenced by the age of the worm (Ghazal and Avery, 1974).

There is, however, a reduction in the diameter of the scolex of *R. australis* and *R. beveridgei* with increases in intensity of infection. The mean diameter of scoleces in an infection of 19 *R. australis* with 448 other cestodes is significantly different from scoleces of that species measured in other infections. One explanation might be that the scoleces failed to reach normal size because of competition with large numbers of *R. dromaius* in that infection. Although an interaction between *R. australis* and *R. dromaius* has been inferred in other aspects of this study, to suggest that an interaction is also responsible for a reduction in scolex diameter cannot be confirmed here. The difference between the mean scolex diameter in low and high infections of *R. beveridgei* is also significant. The significant difference between the mean scolex diameter of *R. dromaius* in a high intensity is not consistent with a reduction but rather an increase in size. In addition, there was no verification of a reduction in the size of the scoleces of *R. dromaius* when it occurred with large numbers of other cestode species (Table 25). Consequently, the measure of scolex diameter shows some evidence of a relationship between reduced size and intensity of infection but it is not consistent for all species. Furthermore, there are certain to be distortions of scoleces, as a result of fixation and mounting, which have not been measured here.

The reduction in scolex diameter is consistent with other measures of growth affected by crowding such as length, bulk and proglottis production (Jones and Tan, 1971).

If an aspect of the size of *R. dromaius* is affected by crowding, further studies are required to determine if other parts of the tapeworm are affected independently.

Egg production was drastically reduced with increasing burdens of *H. microstoma* (see Jones and Tan, 1971), *H. diminuta* (see Hesselberg and Andreassen, 1975) and *H. nana* (see Ghazal and Avery, 1974). In the Hymenolepididae, eggs leave the strobila singly or occasionally connected in chains or packets (Czaplinski and Vaucher, 1994). The data collected here show that in some *Raillietina* species, the number of egg capsules per gravid proglottis decreases with intensity of infection, but the number of eggs per capsule remains constant. Although there are differences in the number of capsules in low and high intensities, further data are required to determine if they are real, given that equal variation occurred in cestodes from infections of low intensities. The reduction in egg capsules may be a consequence of the reduction in worm size (Ghazal and Avery, 1974) or a result of nutritional competition (Read and Simmons, 1963; Jones and Tan, 1971).

Plasma analyses are unremarkable and are in agreement with assays performed on ostrich (Brown and Jones, 1996; Mushi *et al.*, 1998) and emus (Levy *et al.,* 1989; Okotie-Eboh *et al.*, 1992; Costa *et al.*, 1993). Liver analyses also reflect normal values although the cadmium levels of emus at Keith are generally high, which may be a result of contamination and warrant further investigation (Puls, 1994a). Lead levels are lower than previously recorded in emus (Puls, 1994a). Other variation appears to be related only to on-farm dietary supplementation.

This study indicates that zinc, manganese, lead, magnesium, calcium, and phosphorous may be accumulated by *Raillietina* species, however, the role of calcareous corpuscles in the accumulation of these, and other, cestode metabolites awaits further study. There was no apparent association between accumulations of calcium, magnesium

and phosphorous in cestodes with any deficiencies in emus which could lead to leg weakness.

4.5 Summary.

The cestodes infecting emus are an interactive community with interspecific associations. Niche separation was dependent on population density and may facilitate reproduction. In heavy infestations, cestode species occupied a predictable portion of intestine and there was a tendency to specific site segregation, not observed when small numbers of worms were present. Intestinal distribution appeared to be related to an association between cestode species as a mucosal-dwelling parasite or as a lumen-dwelling parasite. Different proportions of species occurred in hosts from different localities and the highest burden occurred in a wild bird. *Raillietina* species appear to have little detrimental effect on hosts, particularly farmed birds with good nutritional status.

Chapter 5.

THE LIFE CYCLE OF *RAILLIETINA* SPECIES INFECTING THE EMU

The solving of a helminth life-cycle is a daunting task that requires a rather unscientific mix of insight and good luck, in addition to rigorous and systematic exploration of the possible succession of hosts, based on habitat and feeding biology. (Janovy, 1997).

5.1 Introduction

Previously described intermediate stages of *Raillietina* species are cysticercoids (Chervy, 2002), which are small, encysted forms with a well-formed, inverted scolex and no bladder. In cysticercoids, the well-formed scolex does not achieve adult size, however, rostellar hooks are usually fully formed (Wardle and Mcleod, 1952; Freeman, 1973) although this is not the case in cestodes belonging to some families (Jarecka, 1958, 1970; Hobbs *et al.*, 1990). In the Davaineidae, the cysticercoid hook is not known to differ from the hook of the adult worm which makes it possible to identify the cysticercoid occurring in the intermediate host to species by means of morphological comparison (Jarecka, 1958).

Artyukh (1966) surveyed the available information on life cycles of davaineid cestodes and reported 13 species with known intermediate hosts. The range of intermediate hosts included insects, particularly beetles and ants, molluscs and polychaetes. According to Schmidt (1986), Davaineidae usually have insects and gastropods as intermediate hosts.

The abundance of parasite species varies in space and time in response to variations in the suitability of habitat and parasites are not uniformly distributed. Parasites become aggregated amongst the available hosts in that most hosts harbour few parasites and few hosts harbour many parasites (Poulin, 1998). Keymer and Anderson (1979) showed that

aggregated distributions of the infective stages of *Hymenolepis diminuta* in beetles accentuated the aggregation of parasites in the definitive host population. To measure aggregation is to quantify the variability in intensity of infection amongst hosts (Poulin, 1998).

5.2 Materials and Methods

5.2.1 Collection of organisms

Soft-hair paintbrushes, approximately 1 cm in breadth, were used to brush ants into small plastic containers. Additional insects were collected fortuitously or in "pit-traps", a plastic container 26 mm in diameter and 80 mm deep. Pit traps containing 70% ethanol were dug into and set at ground level and left for periods of eight to nine days on three separate occasions at an emu farm at Keith, SA. Insects and other organisms were fixed and stored in 70% ethanol and later separated from debris using a stereomicroscope.

The temperature at the time of collection was recorded on a "Maxima/Minima" thermometer (DGBM 6915023 West Germany) placed at ground level in full sun.

5.2.2 Organisms examined

The total number of organisms examined appears in Table 29. Ants and beetles were the most common insects gathered. Most of the remainder were collected in pit traps.

5.2.3 Identification of ants and beetles

Ants were identified to genus using the keys provided by Shattuck (1999) and A. McArthur and R. Simms (SAM), confirmed the generic identities from representative specimens. If distinct (dimorphic) size ranges occurred in worker ants they were divided

into small or minor and large or major workers (Shattuck1999). The number and identity of ants and ant-mimicking wasps collected at each sampling site appears in Table 30.

Table 29. Number and identity of all organisms collected and dissected for cysticercoids.

Organism		Number
Hymenoptera	Ants, Wasp	7432
Coleoptera	Beetles	464
Collembola	Springtails	189
Diptera	Flies, Mosquitoes	188
Acarina	Mites	22
Nematoda		12
Araneid	Spiders	10
Embioptera	Embiids	7
Crustacea	Slaters	6
Psocoptera	Book lice	5
Diplopoda	Millipedes	3
Dermaptera	Earwigs	2
Hemiptera	Aphids	1

Table 30. Number and identity of ants and ant-mimicking wasps collected at emu farms and dissected for cysticercoids.

Identification	Keith	Glossop	Avenue
Pheidole spp.	2474	1666	64
Adlerzia spp.	1852	442	209
Iridomyrex spp.	625	88	38
Rhytidoponera spp.	54	16	-
Notonchus spp.	5	21	-
Melophorus spp.	25	33	-
Camponotus spp.	-	33	-
Anonychomyrma spp.	-	35	-
Wingless wasp (unidentified)	2	-	-

K. Henry, SARDI Entomology, identified the beetles from representative specimens. The number and identification of the beetles collected at Keith and Glossop appears in Table 31.

Table 31. Number and identity of beetles collected at emu farms and dissected for cysticercoids.

Identification	Keith	Glossop
Cryptophagidae *Atomaria* sp.	310	1
Carabidae *Mecylothrax* sp.	126	-
Anthicidae *Anthicus* sp.	7	1
Tenebrionidae *Adelium brevicorne*	20	10
Tenebrionidae *Chalcopteroides* sp.	-	18
Scarabaeidae *Aphodius* sp.	1	-

5.2.4 Collection of cysticercoids

Individual insects were identified and dissected with fine forceps under low magnification using a stereomicroscope. Cysticercoids were removed using a fine glass pipette, mounted and cleared in De Fauré's medium together with the remainder of the host. On occasion, where multiple numbers of cyticercoids were present, single specimens were mounted separately and crushed with gentle pressure to describe and measure rostellar and sucker hooks. Voucher specimens of cysticercoids mounted on slides have been deposited in the Australian Helminth Collection (AHC) at the South Australian Museum, Adelaide (SAMA)

5.2.5 Statistical analysis

Statistical analysis was performed with Statistix 7 for Windows. Aggregation was measured by $SD^2/\bar{x}$. The index of discrepancy was calculated as:

$$1\text{-}(2\sum_{i=1}^{N}\left(\sum_{j=1}^{i} xj\right) / xN(N=1))$$

where N = number of hosts and x = the number of parasites in hosts j (Poulin, 1998).

5.3 Results

5.3.1 Intermediate host

Cysticercoids were recovered from ants collected at two emu farms. Only ants of the genus *Pheidole* Westwood were found to harbour cestode cysticercoids, which were recovered from both minor and major workers. The number of *Pheidole* sp. positive for cysticercoids at Keith and Glossop appears in Tables 32 and 33. A total of 67 (1.6%) of 4204 *Pheidole* species were infected, 2.2% at Keith and 0.7% at Glossop. No ants collected on one occasion at Avenue, SA were found to contain cysticercoids.

Table 32. The proportion of *Pheidole* species positive for cysticercoids collected on an emu farm at Keith. *Denotes ants collected in pit traps.

Date	*Pheidole* (minor worker)	*Pheidole* (major worker)	Temperature °C
16.iii.1999	0/1	0/0	22
1.iv.1999	3/89	2/17	16
6.v.1999	0/113	0/3	25
25.v.1999	14/412	0/33	16
29.vi.1999	5/107	0/6	21
8.vii.1999	12/72	0/2	17
8.vii.1999*	2/57	0/2	17
29.vii.1999	1/34	0/0	16
8.ix.1999	8/161	1/10	17
16.ix.1999	0/15	0/0	15
16.ix.1999*	2/154	0/2	15
27.x.1999	0/52	0/2	22
2.xii.1999	0/1	0/0	46
9.xii.1999	0/2	0/0	25
29.xii.1999	0/102	0/2	23
6.i.2000*	0/87	0/1	30
10.ii.2000	0/1	0/0	40
31.v.2000	4/82	0/15	14
7.vi.2000	0/313	0/4	15
6.vii.2000	2/201	0/9	17
9.viii.2000	1/292	0/18	13

Table 33. The proportion of *Pheidole* species positive for cysticercoids collected on an emu farm at Glossop.

Date	*Pheidole* (minor worker)	*Pheidole* (major worker)	Temperature °C
20.x.2000	0/33	0/2	35
1.iii.2001	0/200	0/5	37
30.iv.2001	6/845	0/14	21
1.v.2001	5/321	1/13	17

5.3.2 Description of cysticercoids

5.3.2.1 ***Raillietina australis*** (Krabbe, 1869)

FIGS 111-113,129

Material examined: Glossop, SA (34° 16' S, 140° 32' E), 30.iv.2001, SAMA AHC S28417.

Description

Mature cysticercoid ovoid. Cysticercoids from light infection (n= 3) 0.365 long x 0.288 wide. Size range - mean length 0.352-0.400 (0.372), mean width 0.248-0.296 (0.268, n=12). Cysticercoid contains outer and inner pouch. Outer pouch with notch at anterior end, inner pouch ovoid, 0.321-0.344 (0.323) long x 0.192-0.232 (0.209, n=10) wide. Larval scolex 0.176-0.212 (0.190, n=10) in diameter. Rostellum 0.108-0.132 (0.122, n=5) with circlet of hooks 0.120-0.180 (0.150, n=10) in diameter. Hooks number 290-370 (328, n=5) arranged in two rows, larger hooks 0.024-0.026 (0.025, n=10) long, smaller hooks 0.019-0.021 (0.020, n=10) long. Suckers spherical 0.080-0.084 (0.081, n=10) in diameter armed with 13-17 rows of hooks 0.006-0.014 long.

Host: Pheidole species.

Location in host: Haemocoele of gaster.

5.3.2.2 ***Raillietina beveridgei*** O'Callaghan, Davies and Andrews, 2000

FIGS 114-116, 127

Material examined: Keith, SA (36° 06'S, 140° 19'E), 25.v.1999, SAMA AHC S28418; Glossop SA, 30.iv.2001, 1.v.2001.

Mature cysticercoid ovoid containing calcareous corpuscles 0.006- 0.010 in diameter. Cysticercoids from single infections 0.376-0.544 (0.451) long x 0.256-0.400 (0.320, n=3) wide. Size range - mean length 0.232-0.544 (0.352), mean width 0.176-0.400 (0.254, n=70). Cysticercoid contains outer and inner pouch. Outer pouch with notch at anterior end, inner pouch ovoid, 0.216-0.300 (0.251) long x 0.128-0.172 (0.143, n=10) wide. Larval scolex 0.116-0.156 (0.139, n=10) in diameter. Rostellum 0.068-0.101 (0.082, n=10) with circlet of hooks 0.080-0.116 (0.092, n=10) in diameter, compressed in appearance. Hooks number 332-424 (368, n=10) arranged in two rows, larger hooks 0.017-0.019 (0.018 n=20) long, smaller hooks 0.015-0.016 (0.016, n=20) long. Suckers spherical, 0.068-0.100 (0.084) x 0.060-0.068 (0.064, n=10), armed with 15-18 rows of hooks 0.006-0.010 long.

Host: Pheidole species.

Location in host: Haemocoele of gaster.

5.3.2.3 ***Raillietina chiltoni*** O'Callaghan, Davies and Andrews, 2000

FIGS 117-119, 130

Material examined: Keith, SA, 8.vii.1999 SAMA AHC S28419; Keith, SA, 1.iv.1999; 25.v.1999; 29.vi.1999; 8.ix.1999; 31.v.2000.

Mature cysticercoid ovoid occasionally containing calcareous corpuscles 0.003-0.014 in diameter. Cysticercoid from single infection 0.616 long x 0.464 wide. Size range - mean length 0.200-0.616 (0.294), mean width 0.152-0.464 (0.223, n=121). Cysticercoid contains outer and inner pouch. Outer pouch with notch at anterior end, inner pouch ovoid 0.156-0.256 (0.208) long x 0.128- 0.228 (0.169, n=20) wide. Larval scolex 0.126-0.224 (0.162, n=30) in diameter. Rostellum 0.088-0.112 (0.105, n=5) frequently obscured by circlet of hooks 0.112-0.172 (0.136, n=20) in diameter, compressed in appearance. Hooks number 280-374 (320, n=25) arranged in two rows, larger hooks 0.029-0.033 (0.031, n=10) long, smaller hooks 0.023-0.026 (0.025, n=10) long. Suckers 0.064-0.080 (0.072) x 0.048-0.064 (0.056, n=10) with circlet of hooklets 0.040-0.057 (0.053) x 0.034-0.048 (0.037, n=10). Hooks 0.004-0.012 long arranged in 12-18 rows.

Host: Pheidole species.

Location in host: Haemocoele of gaster.

5.3.2.4 ***Raillietina dromaius*** O'Callaghan, Davies and Andrews, 2000

FIGS 120-123, 128

Material examined: Keith, SA, 1.iv.1999 SAMA AHC S28420; Glossop, SA, 1.iv.2001.

Mature cysticercoid ovoid containing calcareous corpuscles 0.006-0.015 in diameter. Cysticercoids from single infections 0.0.584-0.800 (0.692) long x 0.432-0.600

(0.516, n=2) wide. Size range - mean length 0.520-0.800 (0.592), mean width 0.304-0.600 (0.366, n=10). Cysticercoid contains outer and inner pouch. Outer pouch with notch at anterior end, inner pouch ovoid, 0.296-0.432 (0.397) long x 0.216-0.296 (0.261, n=10) wide. Larval scolex 0.248-0.392 (0.298, n=10) in diameter. Rostellum 0.160-0.180 (0.170, n=2) usually obscured by circlet of hooks 0.172-0.220 (0.203, n=10) in diameter. Hooks number 124-150 (135, n=6) arranged in two rows, larger hooks 0.057-0.063 (0.060, n=10) long, smaller hooks 0.046-0.051 (0.049, n=10) long. Accessory rostellar spines present, 0.006-0.010 long. Suckers evident only by circlet of hooklets 0.089-0.119 (0.099, n=10) in diameter. Hooks 0.006-0.018 long arranged in 8-12 rows.

Host: Pheidole species.

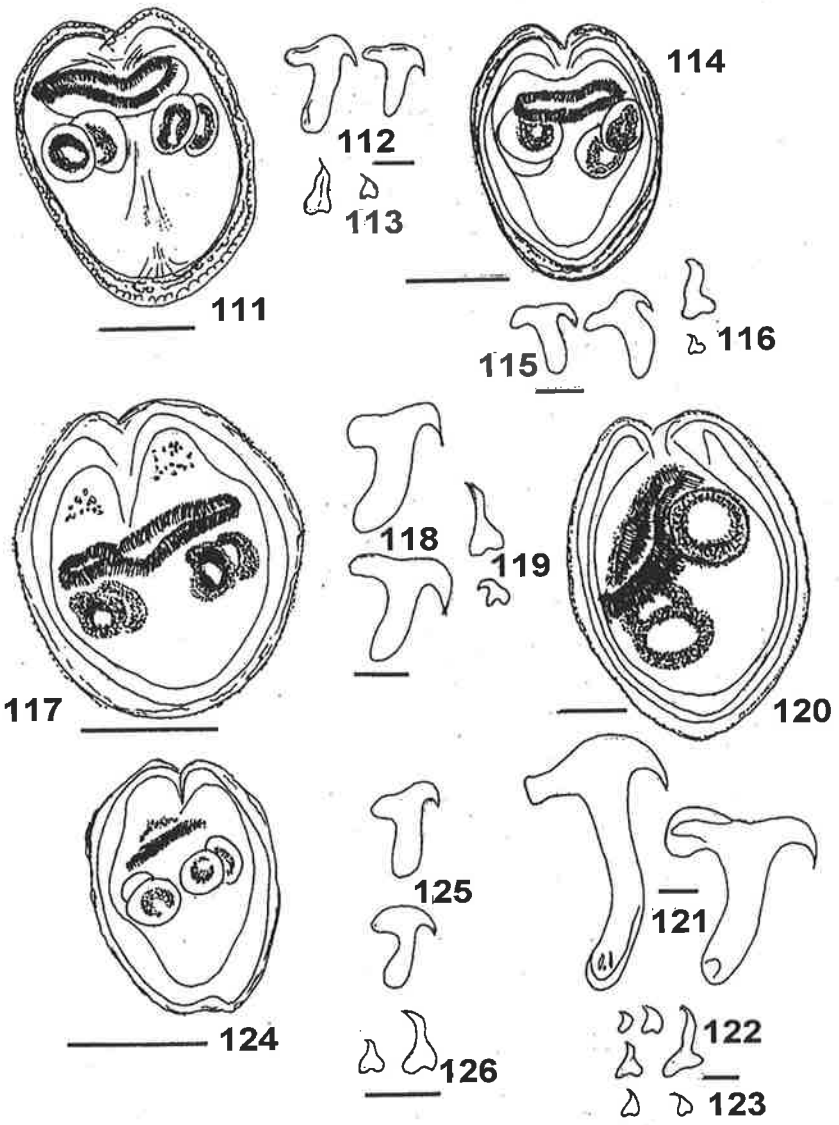
Location in host: Haemocoele of gaster.

5.3.2.5 ***Raillietina mitchelli*** O'Callaghan, Davies and Andrews, 2000

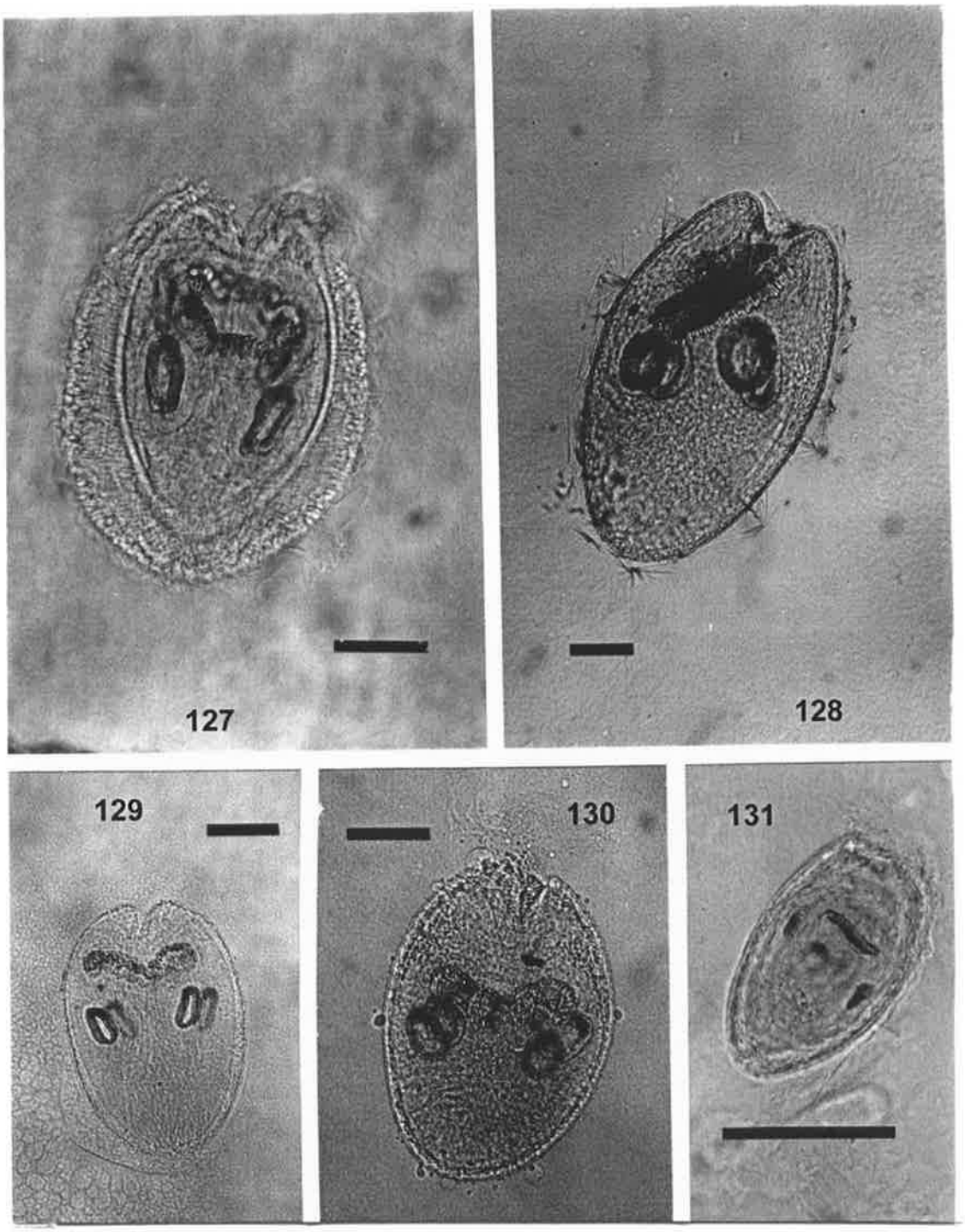
FIGS 124-126, 131

Material examined: Keith, SA, 1.iv.1999 SAMA AHC S28421; Keith SA, 25.v.1999; 8.ix.1999; 16.ix.1999.

Mature cysticercoid ovoid containing calcareous corpuscles 0.003-0.005 in diameter. Cysticercoids from light infections (n=2) 0.168-0.176 (0.172) long x 0.128-0.132 (0.130) wide. Size range - mean length 0.160-0.232 (0.190), mean width 0.104-0.160 (0.122, n=44). Cysticercoid contains outer and inner pouch. Outer pouch with notch at anterior end, inner pouch ovoid, 0.120-0.140 (0.129) long x 0.080-0.100 (0.088, n=10) wide also with anterior notch. Larval scolex 0.080-0.106 (0.088, n=10) in diameter.



Figs 111-126. Cysticercoid of *Raillietina australis.* 111. Cysticercoid. 112. Rostellar hooks. 113. Sucker hooks. 114-116. Cysticercoid of *R. beveridgei.* 114. Cysticercoid. 115. Rostellar hooks. 116. Sucker hooks. 117-119. Cysticercoid of *R. chiltoni.* 117. Cysticercoid. 118. Rostellar hooks. 119. Sucker hooks. 120-123. Cysticercoid of *R. dromaius.* 120. Cysticercoid. 121. Rostellar hooks. 122. Sucker hooks. 123. Accessory spines. 124-126. Cysticercoid of *R. mitchelli.* 124. Cysticercoid. 125. Rostellar hooks. 126. Sucker hooks. Scale bars = 0.1 mm, 111,114,117,120,124; 0.01 mm, 112,113,115,116,118,119,121-123,125,126.



Figs 127-131. Cysticercoids from *Pheidole* species. 127. *Raillietina beveridgei.*128. *R. dromaius.*129. *R. australis.*130. *R. chiltoni.* 131. *R. mitchelli.* Scale bars = 100 μm.

Table 34. Cysticercoids of *Raillietina* species recovered from *Pheidole* species at Keith, SA. Number of positive ants followed, in parentheses, by number of cysticercoids in each ant. Number of positive ants in total number of *Pheidole* species examined
* = cysticercoid of two species from single ant.

Date		*Raillietina australis*	*R. beveridgei*	*R. chiltoni*	*R. dromaius*	*R. mitchelli*
1.iv.1999	Minor			2 (5,12)		1 (34)
5+/106	Major				2 (5,4)	
25.v.1999	Minor		6 (5,16,1,6,17,30)	7 (17,6,2,4,26*,5,6)		2 (5*,5)
14+/445						
29.vi.1999	Minor		2 (6,9)	3 (9,32,1)		
5+/113						
8.vii.199	Minor		1 (6)	13 (11,15,2,9,9,8,18,4,6,6,34,23,3)		
14+/133						
29.vii.1999	Minor			1 (4)		
1+/34						
8.ix.1999	Minor		1 (2)	5 (19,20,8*,3,11)		1 (5*)
7+/171	Major			1 (9)		
16.ix.1999	Minor					2 (4,2)
2+/171						
31.v.2000	Minor		1 (2)	3 (1,5,20)		
4+97						
6.vii.2000	Minor		2 (5,7)			
2+210						
9.viii.2000	Minor				1 (2)	
1+/310						

Table 35. Cysticercoids of *Raillietina* species recovered from *Pheidole* species at Glossop, SA. Number of positive ants followed, in parentheses, by number of cysticercoids in each ant. Number of positive ants in total number of *Pheidole* species examined
* = cysticercoid of two species from single ant.

Date		*Raillietina australis*	*R. beveridgei*	*R. chiltoni*	*R. dromaius*	*R. mitchelli*
30.iv.2001	Minor	2 (12,5)	4 (2,1,10,11*)	1 (6*)		
6+/859						
1.v.2001	Minor		4 (9,2,1,13)		1 (1)	
6+/314	Major		1 (20)			

Rostellum detected only by circlet of hooks 0.046-0.058 (0.052, n=10) in diameter. Hooks number 284-342 (336, n=10) arranged in two rows, larger hooks 0.010-0.011 (0.011, n=10) long, smaller hooks 0.008-0.010 (0.009, n=10) long. Suckers 0.027-0.037 (0.033, n=10) in diameter armed with hooks 0.003-0.011 long arranged in 6-7 rows.

Host: Pheidole species.

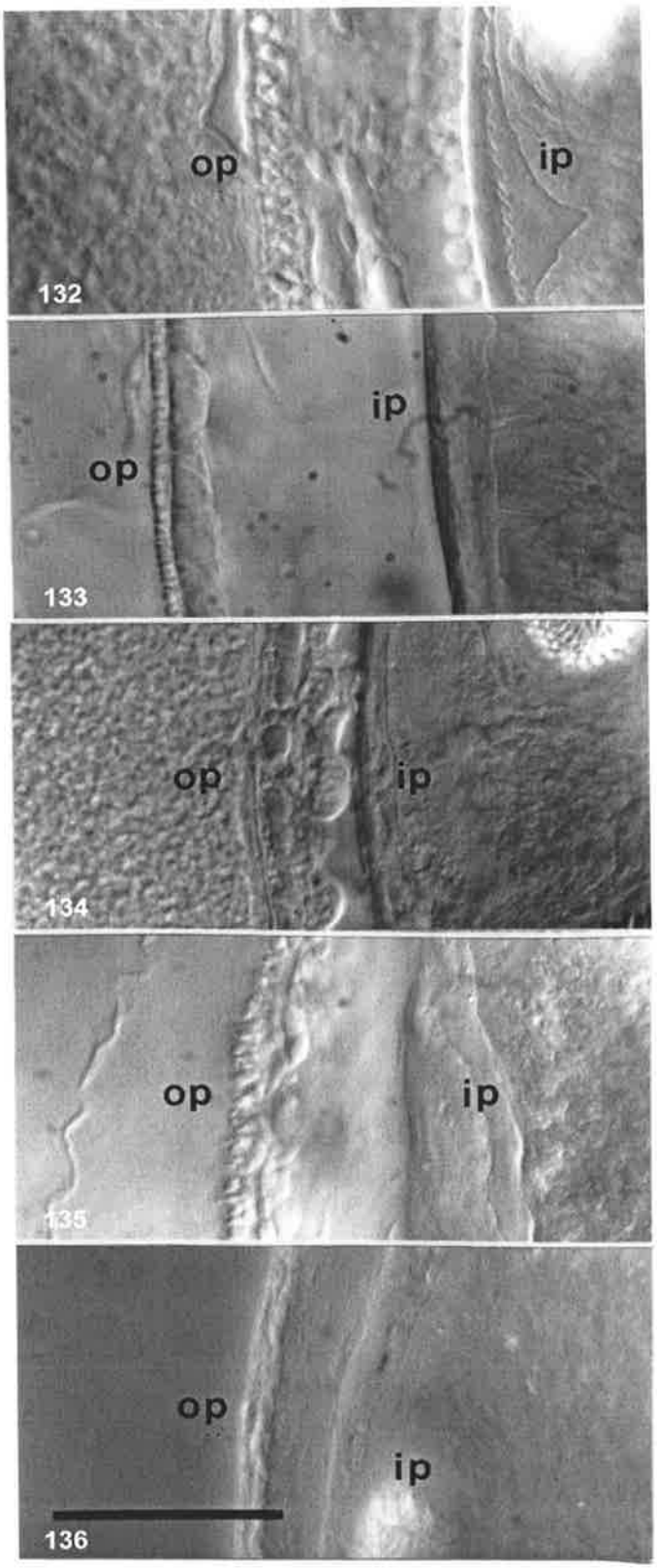
Location in host: Haemocoele of gaster.

5.3.3. Number of cysticercoids recovered

The number of infected ants and the number of cysticercoids recovered from each ant are shown in Tables 34 and 35. Six hundred and forty-four cysticercoids were recovered from 67 (1.6%) ants of the total number of *Pheidole* species examined. The maximum number of cysticercoids recovered from an ant was 34 and the mean number was 9.6. High numbers of cysticercoids were found in both minor and major workers. In the majority of cases (95.5%), ants harboured single species infections. Mixed infections comprising *R. chiltoni* and *R. mitchelli* occurred in two ants at Keith whilst one ant was infected with cysticercoids of both *R. chiltoni* and *R. beveridgei* at Glossop.

5.3.4 Appearance of the cysticercoid wall

In addition to the characteristic morphological features of the cysticercoids of each *Raillietina* species already noted, differences were also observed in the appearance of the wall of each cysticercoid (Figs 132-136). The appearance of the cysticercoid surface under high magnification using the light microscope varied for each cestode species and there were morphological differences in the cross-sectional image of the inner



Figs 132-136. Cysticercoid wall of *Raillietina* species.132. *Raillietina beveridgei.* 133. *R. dromaius.* 134. *R. australis.* 135. *R. chiltoni.* 136. *R. mitchelli* op = outer pouch; ip = inner pouch. Scale bar = 25 μm. Nomarski differential interference contrast microscopy.

and outer pouch. The cysticercoid tegument of *R. australis, R. beveridgei* and *R. chiltoni* was nodular in appearance, but appeared to have circular striations in *R. dromaius* (Fig 133) and *R. mitchelli* (Fig 136). Consequently, a detailed examination and comparison of the pattern formed by the thin outer membrane and the complex structural composition of the inner and outer pouch wall enabled further distinction of the cysticercoids of each cestode species.

5.3.5 Size of cysticercoids

The size of cysticercoids differed at the two localities. For example, cysticercoids of *R. beveridgei* were consistently larger at Glossop than Keith (Table 36).

Table 36. Mean length and width of cysticercoids of *Raillietina beveridgei* at Keith and Glossop, SA.

Size of Infection	Keith	Glossop
<5	368 µm x 278 µm n=4	444* m x 332 µm n=2
<10	364 µm x 252 µm n=14	389 m x 273 µm n=6
<20	298 µm x 203 µm n=8	311 m x 262* µm n=8

*significantly different $p < .001$

Conversely, *R. dromaius* was larger at Keith than at Glossop (800 x 600 µm vs 584 x 432 µm), however, only a single parasite could be measured from each locality. There was no detectable difference in the size, particularly the size of the gaster, of the infected ants at the two localities.

There was a trend towards an inverse relationship between the size of cysticercoids and the parasite burden in *Pheidole* species. This decrease in size is depicted for *R. beveridgei* in Fig. 137. At Glossop, an increase in length of cysticercoids was recorded for an infection of 20 parasites, which occurred in a major worker.

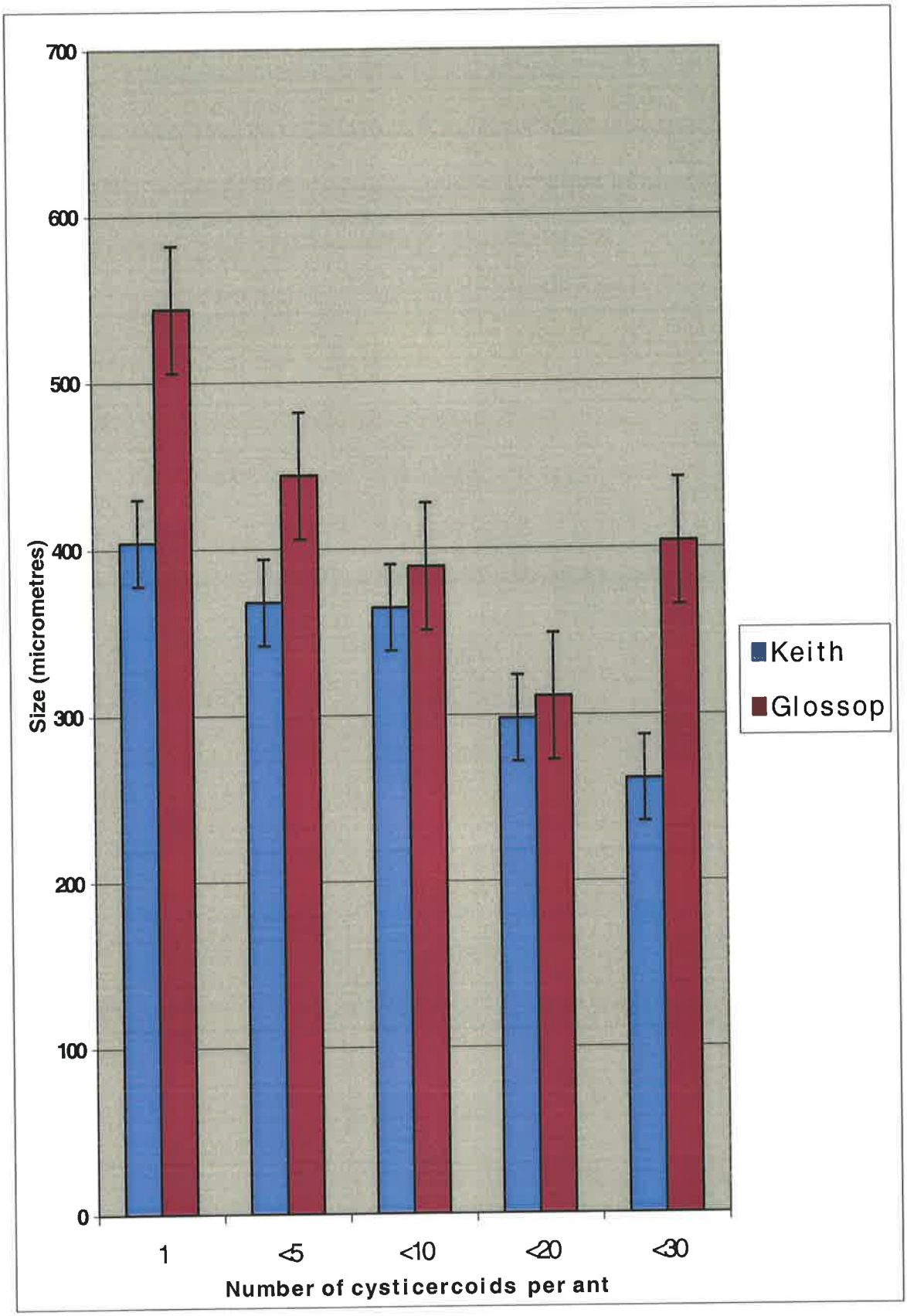


Fig. 137. Mean length (± SE) of *Raillietina beveridgei* cysticercoids from Keith and Glossop, (<5, number measured n=4,2; <10, n=14,6; <20, n=8,8; <30, n=10,[14 major worker]).

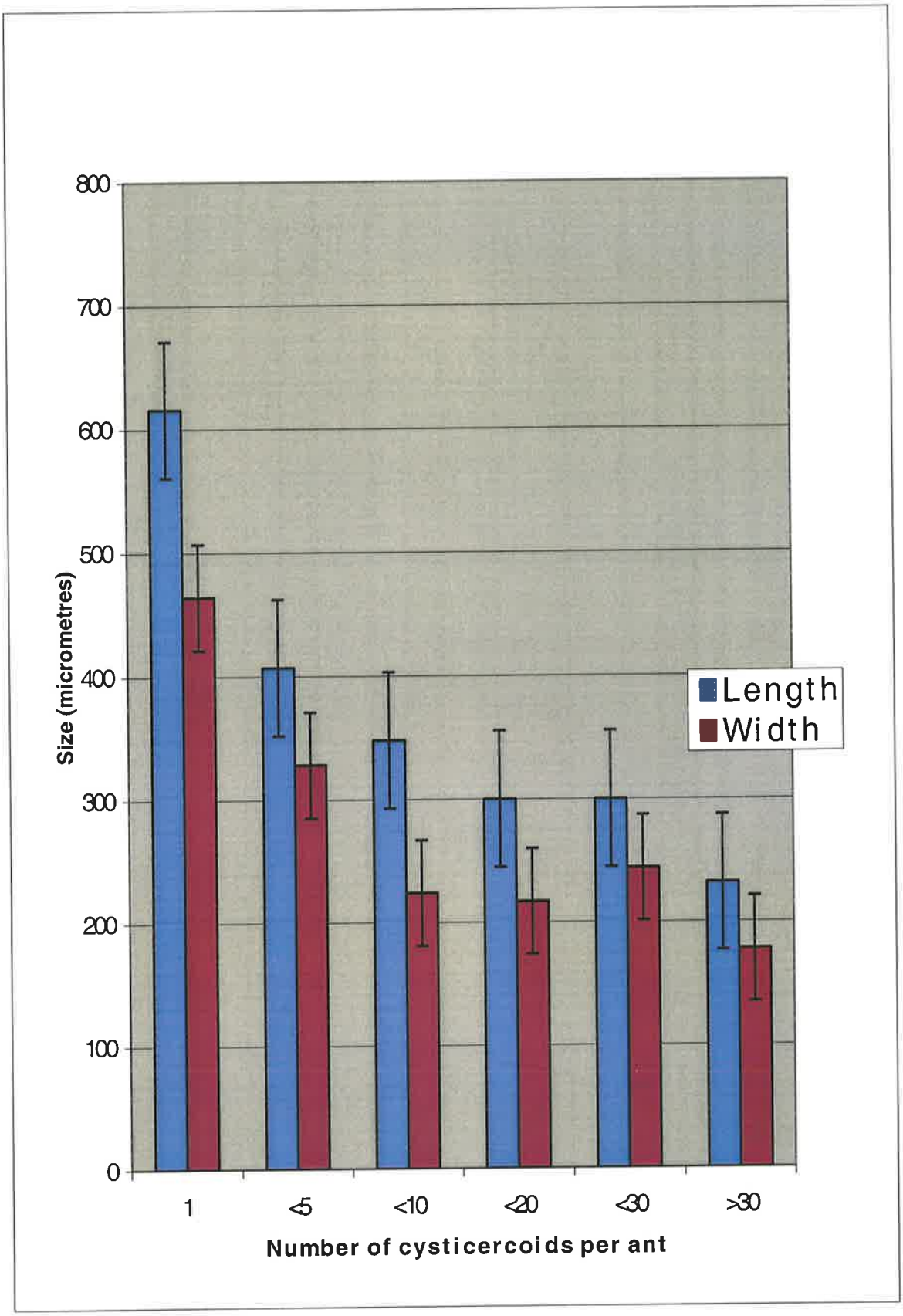


Fig. 138. Mean length and width (± SE) of cysticercoids of *Raillietina chiltoni* from ants at Keith. (<5 number measured n=9, <10 n=15, <20 n=19, <30 n= 30, >30 n=41)

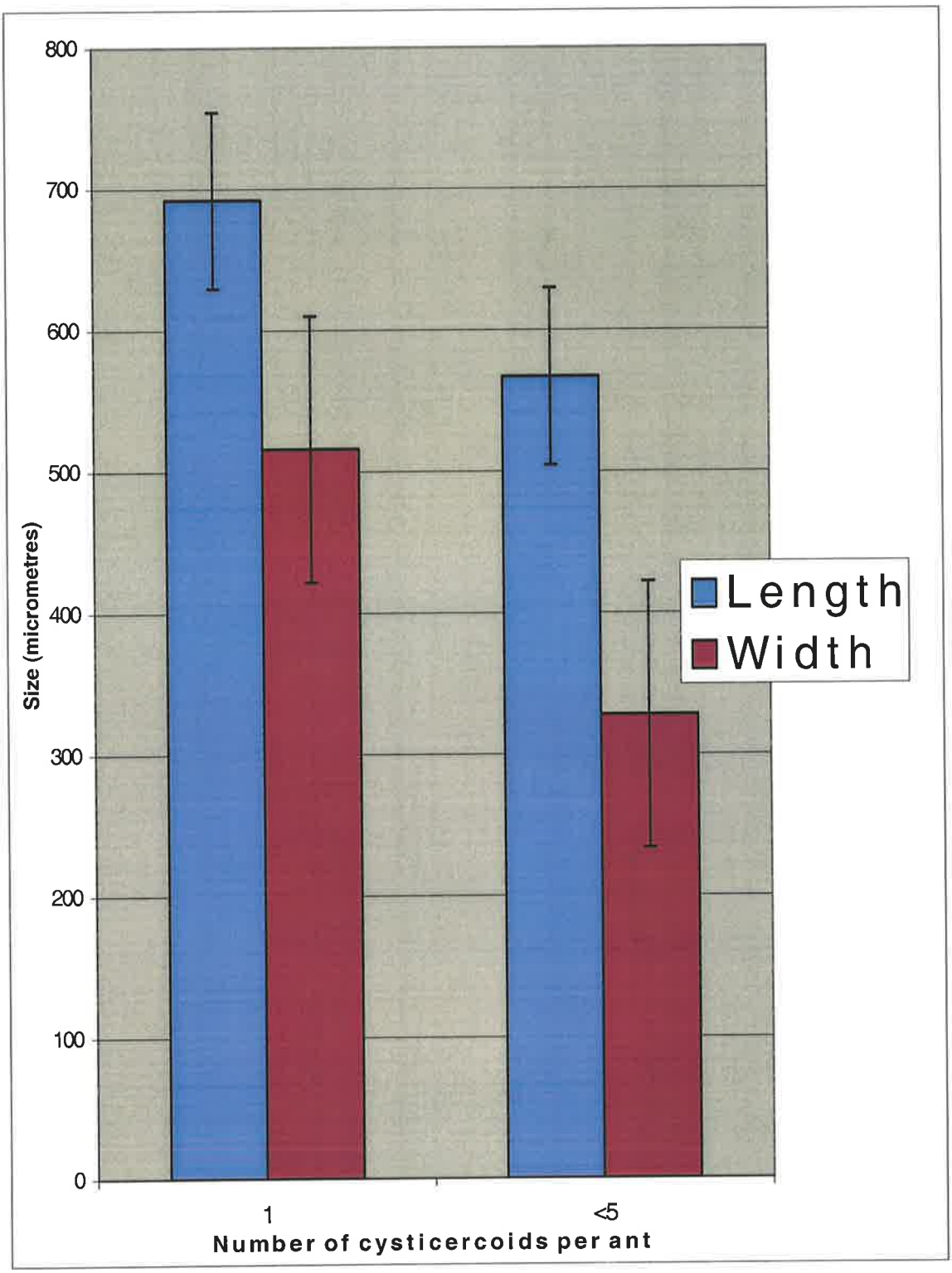


Fig. 139. Size of *Raillietina dromaius* cysticercoids in ants from Keith. Mean length and width (± SE) of single cysticercoid infection (number of ants n=2) and <5 cysticercoids (n=8).

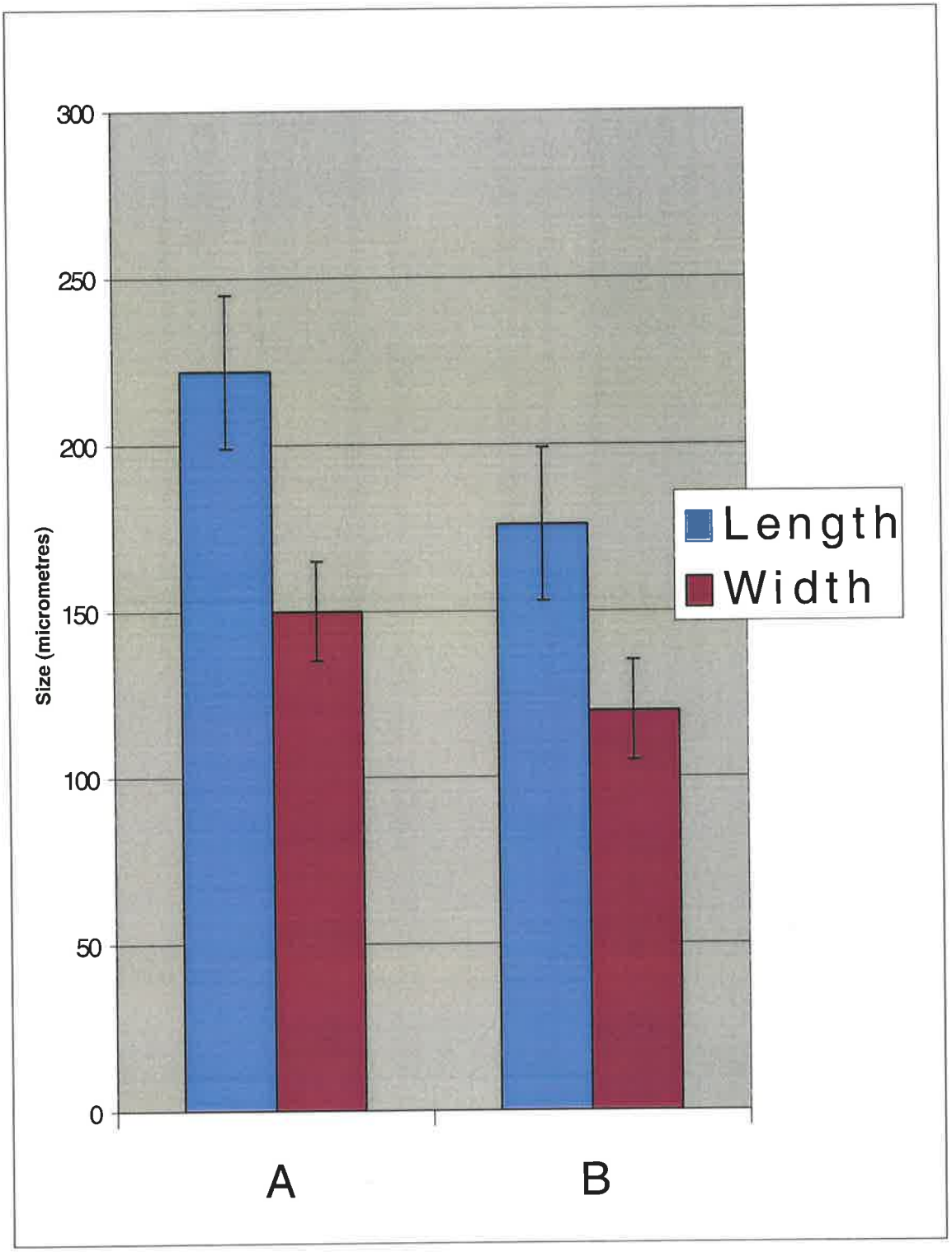


Fig. 140. Mean length and width (± SE) of five cysticercoids of *Raillietina mitchelli* recovered from 1 ant. "A" = single species infection, "B" = *R. mitchelli* with 26 *R. chiltoni*.

Similar patterns were observed for *R. chiltoni* (Fig. 138), *R. dromaius* (Fig. 139) and *R. mitchelli* (Fig. 140). The data for *R. mitchelli* indicate that the decrease in size occurs in both single species and mixed species infections. Where data could be compared between similar-sized infections of the same cestode species in major and minor workers, it was evident that cysticercoids were larger in major workers (Table 37).

Table 37. Mean length and width of cysticercoids of *Raillietina chiltoni* and *R. beveridgei* in major and minor workers of *Pheidole* species.

Raillietina species	Major	Minor
R. chiltoni (Keith)	444.0* x 333.3	298.5* x 234.7
R. beveridgei (Glossop)	403.0* x 275.0	309.0* x 246.0

Significant * $p < .001$

5.4 Discussion

The cysticercoids described here possess a scolex, an inner and an outer pouch, a rostellum with two circular rows of hooks and suckers armed with hooks. These are characters consistent with those described for other species of *Raillietina* (see Meggitt, 1924; Moghe, 1925; Malviya and Dutt, 1971a, b). The diagnostic characters in cysticercoids are the size and number of rostellar hooks consistent with the five species of adult worms found in emus.

The length of sucker hooks and accessory spines (microtriches) (particularly evident in the cysticercoid of *R. dromaius*) is also consistent with adult forms indicating that they attain full size in larval forms. The scolex and rostellum do not reach adult size and continue to grow after reaching the site of attachment in the final host as suggested by Fourie *et al.* (1997) for *Houttuynia struthionis*. Maximum dimensions of larval scoleces varied from 31-82% of adult forms and similarly the maximum dimensions of the rostellum varied from 33-66% of adult forms.

No scoleces of larval dimensions were recovered in emu intestines indicating that scoleces, in conjunction with rostellum and suckers, grow rapidly and reach maximum size even before strobilisation begins (Smyth, 1969). Reid *et al.* (1938) reported rapid development of adult worms and found gravid proglottides 11 days after experimental infection of chickens with *Skrjabinia cesticillus* (Molin, 1858). Data here indicate that suckers have developed approximately 60-79% of adult size in cysticercoids and therefore grow and/or develop less markedly as the scolex grows in the final host.

Data collected on the infections in ants confirmed the absence of *R. mitchelli* in birds at Glossop. In addition, cysticercoids of *R. australis* were not found in ants at Keith where the adult cestode was uncommon (Table 38).

Cysticercoids were not uniformly distributed in the population of ants examined. Parasites showed an aggregated distribution (Shaw and Dobson, 1995; Goater and Holmes, 1997) that was measured by the variance to mean ratio (7.76) (Poulin, 1998). A variance to mean ratio greater than unity indicates a departure from randomness and a tendency to aggregation. The aggregated distribution was exhibited by some ants containing more parasites than the average (9.6) and others fewer. As expected in this aggregated distribution, most ants harboured no parasites and few (1.6%) harboured one to many cysticercoids.

Exposure would not be constant throughout the ant population since the spatial distribution of transmission stages would not be random relative to the ants, particularly as these stages (i.e. eggs in capsules in proglottides) leave the emus in faeces as pulses and the intermediate host are themselves aggregated within the bird's habitat (Hudson and Dobson, 1997).

Table 38. Percentage of *Raillietina* species found in five emus at Keith and Glossop together with the percentage of ants infected with a species and the relationship of the cysticercoid species to the total number of cysticercoids recovered.

Keith	*R. australis*	*R. beveridgei*	*R. chiltoni*	*R. dromaius*	*R. mitchelli*
Adults	2.1%	67.1%	10.5%	18.3%	2.0%
Ants	0	24.0%	64.8%	5.6%	11.0%
Cysticercoids	0	20.3%	67.7%	2.0%	10.0%
Glossop					
Adults	45.3%	49.2%	1.2%	4.4%	0
Ants	16.7%	75.0%	8.3%	8.3%	0
Cysticercoids	18.3%	74.2%	6.5%	1.1%	0

The processes acting to facilitate aggregation here are difficult to determine. In calculating the variance to mean ratio, negative hosts were not included and technically should be (Poulin, 1998), although this would only give evidence of greater aggregation (variance: mean = 493.0 if negative hosts included). Hosts that are equally susceptible to infection may acquire varying numbers of parasites simply because of an uneven distribution of parasites in the sampling environment (Poulin, 1998). Ants were not collected in a systematic fashion but in varying numbers at entrances of nests or in randomly dispersed pit traps.

The specific identity of *Pheidole* was not attempted so which of the approximately 53 species known to occur in Australia (Shattuck, 1999) can act as an intermediate host remains to be determined. Consequently, any variation of susceptibility within *Pheidole* species cannot be measured.

Ants are the intermediate host of seven of the 13 species of davaineid cestodes surveyed by Artyukh (1966). *Pheidole* species were reported as hosts of *Raillietina* (*R.*) *tetragona* (Molin, 1858), *R.* (*R.*) *echinobothrida* (Megnin, 1880), *R.* (*Paroniella*) *georgiensis* Reid & Nagara, 1961 and *R.* (*Skrjabinia*) *circumvallata* var. *sibirica* Fedyuschin, 1953. Furthermore, *R.* (*R.*) *singhi* Malviya & Dutt, 1971 and *R.* (*R.*) *mehrai*

Malviya & Dutt, 1971 have *Pheidole* sp. as their intermediate host (Malviya and Dutt, 1971a, 1971b).

Pheidole species are general predators and scavengers and feed on a variety of food and seeds (Greenslade, 1979; Shattuck, 1999; Johnson, 2001). The collection of seeds and proglottides excreted in emu dung fortuitously coincide, providing efficient transmission to the intermediate host as *Pheidole* spp. are known to remove seeds from vertebrate faeces (Pizo and Oliveira, 1999). The gravid proglottides are collected and are a likely source of food for ant larvae (Naumann, 1991; Shattuck, 1999). Infection of the intermediate host is likely to occur when ant larvae feed on capsules and eggs. Individual ants gaining access to proglottides also access large numbers of cestode eggs at that time. Cysticercoids then develop as the ant matures and only mature, fully-formed cysticercoids are found in adult ants.

To acquire the minimum infection of a farmed bird, 89 ants harbouring the mean infection of cysticercoids would need to be ingested. For a wild emu to acquire an infection of 3367 worms, 350 ants harbouring the mean number of cysticercoids would need to be ingested. This indicates that the food chain may provide the means to increase the frequency of ingestion of infected intermediate hosts by emus.

Pheidole spp. were more commonly found during the cooler months when temperatures were lower. This ant genus is known to prefer cooler temperatures when foraging (Shattuck, 1999; McArthur pers. com.). Emus spend the day in active food collection except in extreme temperatures and settle for rest about 20-40 min after sunset (Dawson *et al.*, 1984). Thus seasonality in infection might occur with higher ingestion rates occurring in the cooler months, i.e. April to October in southern Australia. Future areas of study would be to investigate the association between *Pheidole* and the food of emus, particularly the berries and seeds of native bushes and also grasses. *Pheidole* species

are known to prey on eggs of insects in the canopy of various crops (Mansfield *et al.*, 2003). In addition, there may be a limited number of species of *Pheidole* that act as intermediate hosts and the behaviour of infected ants may be altered to favour ingestion by emus.

In describing cysticercoids of two species of *Raillietina*, Malviya and Dutt (1971a) reported the wall of the cysticercoid of *R. mehrai* as ruffled in appearance. *Raillietina singhi* (Malviya and Dutt, 1971b) was recovered from the same ants but the appearance of the cysticercoid wall was not recorded. These two species of cysticercoids were differentiated from congeners by size and shape of the cysticercoid, scolex and rostellum and the number and length of rostellar hooks. In this study, cysticercoids of the species of *Raillietina* infecting emus could also be differentiated by the appearance of the cysticercoid wall and by the wall of the outer and the inner pouch. These may represent additional characters for identification of cysticercoids of *Raillietina* species, particularly when the adult worms occur in the same or closely related hosts.

The trend towards an inverse relationship between the size of cysticercoids and the parasite burden indicates that competition for resource use; space and nutrients may limit the size of cysticercoids in the intermediate host. The difference in size was most evident when comparing cysticercoids from large, major workers and small, minor workers and may also be influenced by a variation in size of the species of *Pheidole* infected. This phenomenon has been described for *Hymenolepis diminuta* (see Keymer, 1981) and *S. cesticillus* (see Reid *et al.*, 1938), but has not been recorded in *Raillietina*. It seems relevant, therefore, that if size of cysticercoids is used diagnostically, cysts from single and multiple infections must be recorded separately in species descriptions.

5.5 Summary

Cysticercoids of the five species of *Raillietina* known to infect emus were recovered from the intermediate host, viz. ants belonging to *Pheidole*, and were described. Key features separating the cysticercoids appear in Table 39. In addition to these characters, morphological differences were also detected in the structure of the cysticercoid wall. The growth of the cysticercoids of *Raillietina* species in the intermediate host may be influenced by competition for space and nutrients.

Addendum

In describing cysticercoids, the terminology of Malviya and Dutt (1971a, b) is used and is comparable to Voge (1960). Where applicable, the outermost layer or external membrane = the cysticercoid wall or capsule (Ubelaker *et al.*, 1970); the outer pouch = the peripheral layer and the inner pouch = the intermediate layer.

Table 39. Key features of the cysticercoids of *Raillietina* species in *Pheidole* species. *Cysticercoids from single infection. †Cysticercoids from light infections. Measuremnets are in micrometres.

		Raillietina australis	*Raillietina beveridgei*	*Raillietina chiltoni*	*Raillietina dromaius*	*Raillietina mitchelli*
Size	Length	365†	451*	616*	692*	172†
	Width	288†	320*	464*	516*	130†
Size Range						
	Length	352-400 (372)	232-544 (352)	200-616 (294)	520-800 (592)	160-232 (190)
	Width	248-296 (268)	176-400 (254)	156-464 (223)	304-600 (366)	104-160 (122)
Inner Pouch						
	Length	321-344 (323)	216-300 (251)	156-256 (208)	296-432 (397)	120-140 (129)
	Width	192-232 (209)	128-172 (143)	128-228 (169)	216-296 (261)	80-100 (88)
Scolex Diameter		176-212 (190)	116-156 (139)	126-224 (162)	248-392 (298)	80-106 (88)
Rostellum		108-132 (122)	68-101 (82)	88-112 (105)	172-220 (203)	46-58 (52)
Rostellar hooks number		290-370 (328)	332-424 (368)	280-374 (320)	124-150 (135)	284-342 (336)
Rostellar hooks						
	Large	24-26 (25)	17-19 (18)	29-33 (31)	57-63 (60)	10-11 (11)
	Small	19-21 (20)	15-16 (16)	23-26 (25)	46-51 (49)	8-10 (9)
Suckers		80 x 84	84 x 64	72 x 56	119 x 89	37 x 27

Chapter 6.

SOME ASPECTS OF THE FINE STRUCTURE OF *RAILLIETINA* SPECIES

In worms of this family the head is usually armed with large numbers (several hundreds) of minute hammer-shaped hooks, so small that they can only be seen with difficulty under the oil immersion lens. (Southwell and Kirshner, 1938).

6.1 Introduction

The rostellum is the most readily recognised feature of most davaineids (Schmidt, 1986) and the family characteristically has numerous, small, hammer-shaped rostellar hooks (Yamaguti, 1959; Jones and Bray, 1994). The rostellum and associated features of the scolex often have a more complicated structure incorporating "accessory rostellar spines" which have been reported from light microscopy studies of several davaineid genera (see Schmidt, 1986; Jones and Bray, 1994). Scanning electron microscopy studies (Gijon-Botella *et al.*, 1989; Bâ *et al.*, 1995) described scale-like spines in davaineids that, from a transmission electron microscopy study, are now considered to be tegumental microtriches with complicated morphology (Stoitsova *et al.*, 2001). I consider, as has been emphasised by de Chambrier and Vaucher (1997) and Scholz *et al.* (1999), that microtriches of different types have been referred to erroneously as spines. Tegumental projections or microtriches are characteristic features of the cestode surface (Rothman, 1959) and have been described on the scoleces of cestodes for which ultrastructural studies have been conducted (e.g. Lee, 1966; Slais, 1973; Lumsden, 1975; Jones, 1998).

MacKinnon and Burt (1983) described the microtriches in the cysticercoid of the davaineid cestode *Ophryocotyle insignis* Lonnberg, 1890 and suggested that microtriches may be used as a taxonomic (micro)character for distinguishing species. More recently, the fine structure of the rostellar apparatus of a davaineid cestode, *Fernandezia spinosissima*

von Linstow, 1894, was described (Stoitsova *et al.*, 2001) and it was suggested that the microtriches were much more widespread among davaineids than had been observed on whole mounts and that they are an obligatory element of the davaineid rostellar apparatus. The microtriches have a structural variation (Holy and Oaks, 1986; Palm *et al.*, 1998; Faliex *et al.*, 2000) and numerous functions, such as host attachment, agitation of the environment, locomotion, uptake of nutrients and protection (Thompson *et al.*, 1980; Jones, 1998).

In this study, microtriches have been identified anterior to the rostellum (Fig. 141) and also posterior to rostellar hooks in the *Raillietina* species infecting emus. At high magnification using light microscopy, small (1-4 μm long) thorn-shaped, microtriches have been observed on the scolex of four species, *R. australis, R. beveridgei, R. chiltoni* and *R. mitchelli.* However, a characteristic feature of the scolex of *R. dromaius* is the presence of large (9 μm long) rose-thorn-shaped microtriches posterior to the rostellar hooks (Fig. 142), also observed in the infective stage (see Chapter 5, Fig. 123). The exact location of these microtriches on the scolex of species, other than *R. dromaius,* was difficult to determine with light microscopy and there was considerable variation due to distortion as a result of fixation, dissection and mounting. The region of the scolex surrounding or underlying the everted rostellum has been termed the 'rostellar cavity' (Siddiqi, 1961), the 'rostellar sheath' (Movsesyan, 1977), the 'rostellar base' (Beveridge, 1981; Bâ *et al.,* 1995), the 'rostellar pouch' (Mariaux and Vaucher, 1989), the'rostellar collar' (Bâ *et al.,* 1995), the 'rostellar lip' (Fourie *et al.,* 1997) and the 'pseudoproboscis' (Stoitsova *et al.,* 2001). One aim of my ultrastructural study was to examine the features of the scolex and confirm the presence of and identify microtriches on the scolex of the *Raillietina* species described in this study.

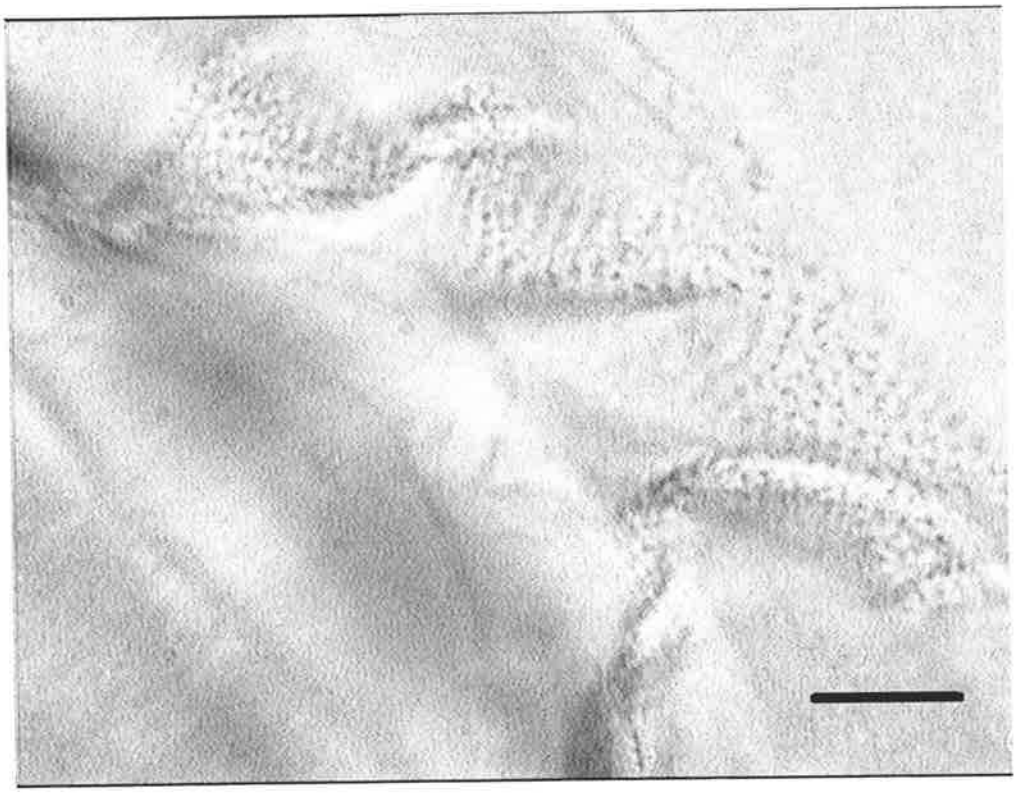


Fig. 141. Light photomicrograph of "scale-like spines" anterior to rostellum of *Raillietina mitchelli.* Scale bar = 10 μm. Bright-field microscopy.

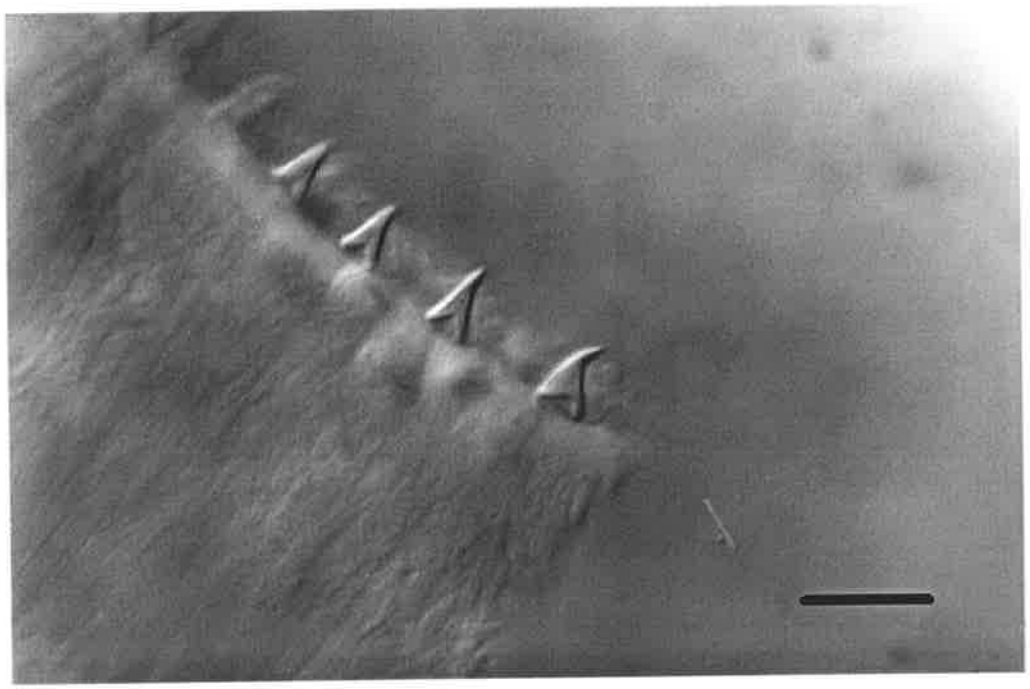


Fig. 142. Light photomicrograph of *Raillietina dromaius* showing rose-thorn-shaped accessory spines/microtriches. Scale bar = 10 μm. Nomarski differential interference contrast microscopy.

The nature and origin of the egg capsule is not clear in davaineids and no transmission electron microscopy observations on the embryonic membranes have been conducted (Jones and Bray, 1994). Membranes around the oncosphere appeared to be connected by 'tube-like' or 'funnel-like' filaments in light microscopy studies of *Skrjabinia cesticillus* (see Reid *et al.*, 1938; Reid, 1962). The outer membranes observed were thought to be remains of the uterine wall. Singh and Baugh (1984) described concentric layers of membrane and envelope and suggested their origin was derived from vitelline cells because no trace of uterus could be seen in stained sections. Consequently, these light microscope studies conflict and are limited in value (Jones and Bray, 1994). An additional aim of my study was to conduct a transmission electron microscopy study of the egg of a *Raillietina* species.

6.2 Material and methods

6.2.1 Histological examination

The scoleces of several different *Raillietina* species for comparison were obtained from formalin-fixed material collected from the intestine of emus at Keith, SA. Scoleces were processed and sectioned as previously described (4.3.3). Scoleces were sectioned longitudinally and are described in sagittal section.

6.2.2 Scanning Electron Microscopy (SEM)

Cestodes were collected from fresh intestines and relaxed in tap water. Scoleces were removed and fixed in 2.5% glutaraldehyde in 0.05M phosphate buffer for 48 h and then washed in buffer overnight. Specimens were post-fixed in 2% osmium tetroxide in buffer for 2 h, washed twice in buffer for 2 h and placed into tannic acid in buffer for 2 h.

After further washes in buffer, specimens were further fixed in 2% aqueous uranyl acetate for 2 h, twice rinsed in water for 15 min and dehydrated through graded ethanol, infiltrated with a transition fluid (dry acetone, dried over a molecular sieve) twice for 30 min. Cestode scoleces were placed into a Critical Point Dryer (Balzers) in acetone. Acetone was replaced with liquid CO_2 with 8 flushes over 1 h. Specimens were mounted on a metal stub with adhesive tape, sputter coated with 0.02 μm of gold and desiccated. SEM was performed on a Phillips XL20 and a XL30 scanning electron microscope at an accelerating voltage of 2 kV and digitally photographed at the Centre for Electron Microscopy and Microstructure Analysis (Adelaide Microscopy) at Adelaide University.

6.2.3 Transmission Electron Microscopy (TEM)

Cestode scoleces were dissected from strobila at low magnification using a stereomicroscope (Wild™ type M8), fixed in 4% paraformaldehyde, 1.25% glutaraldehyde in phosphate buffered saline (PBS), 4% sucrose at pH 7.2 overnight; washed twice in buffer (PBS and 4% sucrose) for 10 min and post fixed in 1% osmium tetroxide in PBS for 2 h on a rotator (TAAB™ type N, 10 RPM). Scoleces were then dehydrated in a graded ethanol series, (75%, 90%, 95%, 100%, 3 times for 20 min each) and an additional 1 h at 100%. Specimens were infiltrated with resin (Epoxy Procure Araldite ProSCiTech) in a 1/1 mixture of 100% ethanol and resin overnight followed by 3 changes of 100% resin for 8 h and finally embedded in fresh resin.

Thick and thin sections were cut with glass knives and a Dupont diamond knife using an LKB ultramicrotome. Thin sections were collected on slotted grids coated with a film of collodion and carbon and stained with 4% aqueous uranyl acetate for 20 min and Reynolds lead acetate stain for 20 min. Sections were examined on a Phillips CM 100

transmission electron microscope operated at an accelerating voltage of 80 kV and images were recorded digitally at Adelaide Microscopy.

6.2.4 Measurements

Measurements of specific features were made by direct comparison to the scale bar on electron micrographs. The most suitable electron micrograph, representing each feature, was selected for measurements and may not be reproduced here.

6.2.5 Specimens examined

Cestodes examined by SEM originated from Keith, SA. Cestodes examined using TEM originated from Glossop, SA except for *R. mitchelli* that originated from Keith. A minimum of ten scoleces of each species were processed and viewed for photography.

6.2.6 Release of eggs from capsules

A fresh cestode was removed from an intestine collected at Keith and washed in tapwater. Using a stereomicroscope, whole gravid proglottides were removed and further washed in tap water. The scolex was removed, fixed, mounted and cleared in De Faurés medium for identification. Egg capsules were released mechanically from the proglottides and placed onto a glass slide in half-strength phosphate buffered saline (PBS) or water. Eggs were released from the capsule by applying gentle pressure to a glass coverslip.

6.3 Results

6.3.1 Histological features of the scolex

In general, two longitudinal muscle layers (LM) are uniformly distributed in a ring around the proglottides and are continuous throughout the strobila (e.g. Fig. 143).

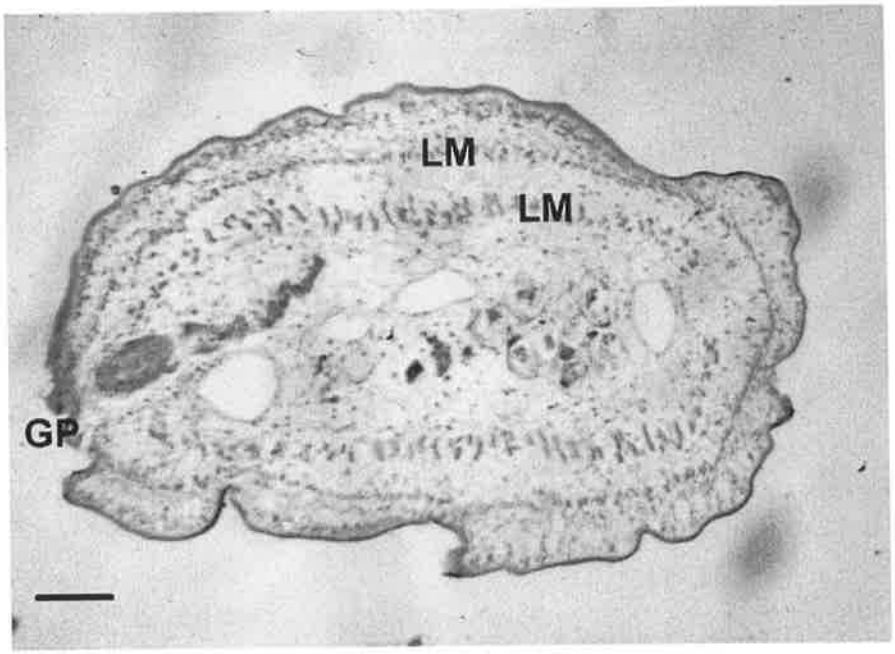


Fig. 143. Light micrograph of a histological cross-section of a proglottis of *Raillietina beveridgei* showing the longitudinal muscle fibres. Longitudinal muscle (LM), genital pore (GP). Scale bar = 100 μm.

6.3.1.1 *Raillietina australis*

The larger LM layer extends to the base of the scolex where it interlaces with five to six oblique muscle bands giving the base of the scolex a muscular appearance (Fig. 144). The LM extends further from the base of the suckers to the rostellar hooks and anterior to the rostellum. The muscular suckers and retracted rostellum contain longitudinally-arranged fibres and an accumulation of nuclei in the central field (Fig.144, arrows). In section, there is some thinning of the anterior region of the sucker consistent with sucker shape. Posterior to the sucker is an accumulation of nuclei and some transverse muscle (TM) fibres. Similarly, another accumulation of nuclei and TM fibres underlies the rostellum, anterior to parenchymatous proglottis tissue (Fig. 144, arrow).

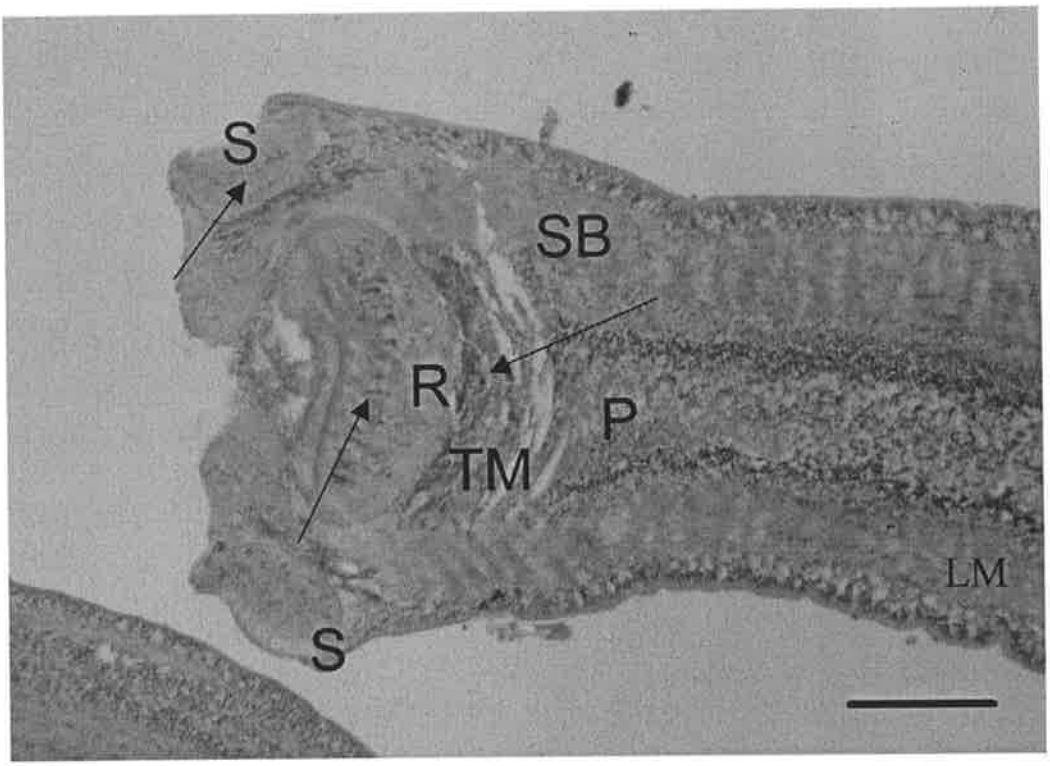


Fig. 144. Light micrograph of a sagittal histological section through the scolex of *Raillietina australis*. Sucker (S), scolex base (SB), retracted rostellum (R), transverse muscle (TM), parenchymatous tissue (P), accumulations of nuclei (arrows), longitudinal muscle (LM). Scale bar = 100μm.

6.3.1.2 *Raillietina beveridgei*

A thin band of LM composed of two to three muscle fibres extends to the base of the scolex. At the base of the rostellum, the band consists of five to six separated muscle fibres that extend further around the rostellum to the rostellar hooks and also around the suckers (Fig. 145). The tegument thins slightly at the base of the scolex. Poorly-defined TM fibres are present in the central region of the posterior scolex. TM fibres also form a strong band posterior to the rostellum. The rostellum and suckers are muscular with longitudinally-arranged fibres and an accumulation of nuclei and vacuolation in the central field. LM fibres of the rostellum are most prominent between rostellar hooks (Fig. 146). Anterior to the rostellum is a complex array of transverse and radial muscle (RM) fibres (Fig. 145, arrow) extending beneath the tegument of the anterior scolex to the suckers and

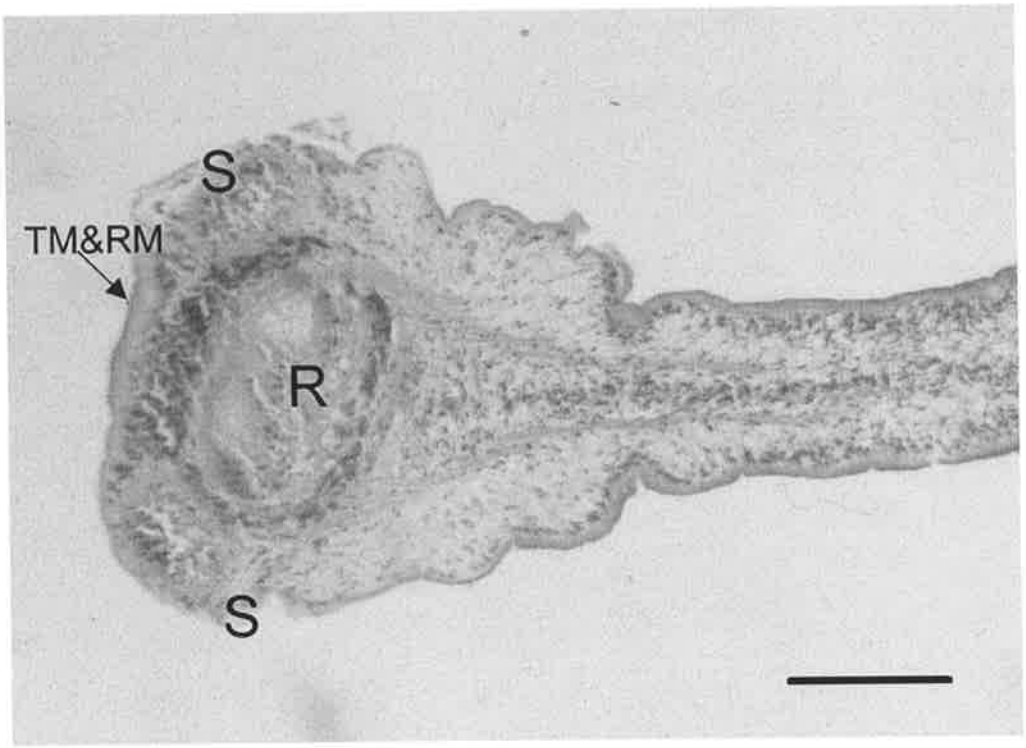


Fig. 145. Light micrograph of a sagittal histological section through the scolex of *Raillietina beveridgei*. Sucker (S), rostellum (R), transverse and radial muscle (TM&RM) beneath tegument. Note the thinner tegument of scolex. Scale bar = 100 μm.

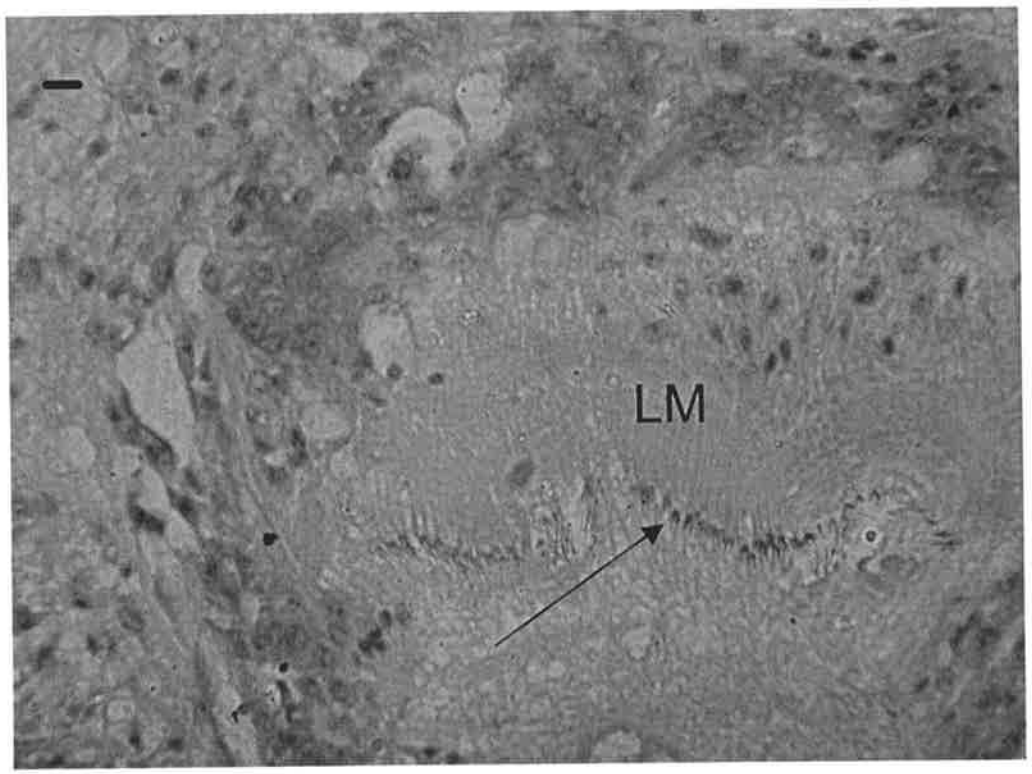


Fig. 146. Light micrograph of a sagittal histological section through the rostellum of *Raillietina beveridgei*. Longitudinal muscle (LM) between rostellar hooks (arrow). Scale bar = 10 μm.

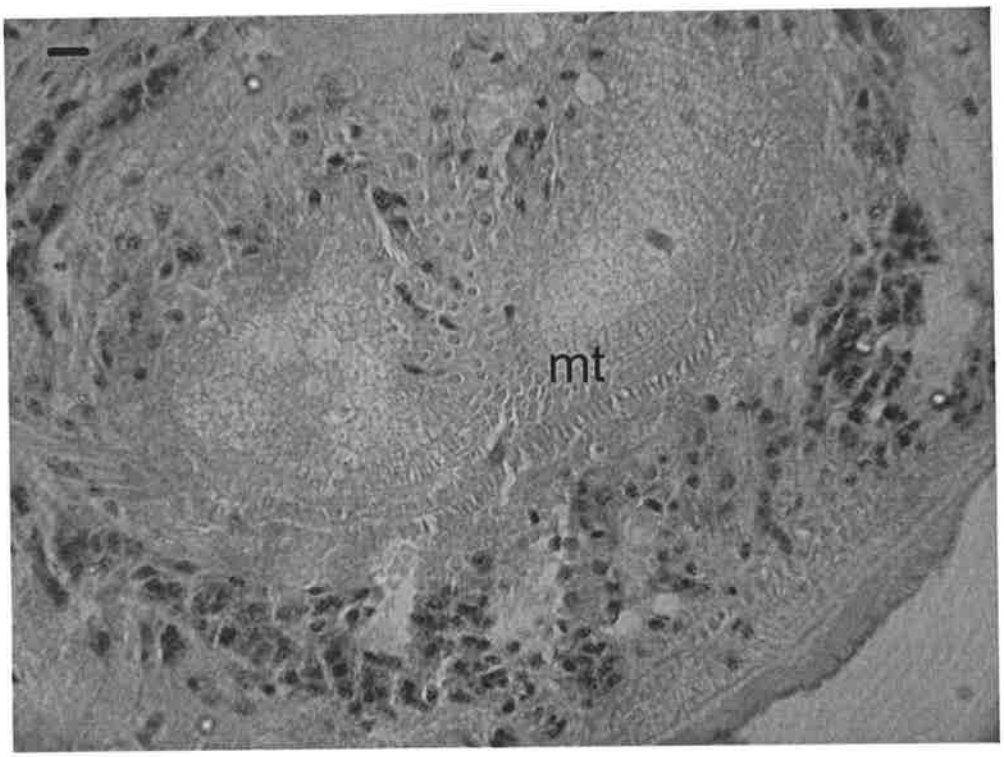


Fig. 147 Light micrograph of a sagittal histological section through the rostellum of *Raillietina beveridgei.* Microtriches (mt) anterior to rostellum. Scale bar = 10μm.

rostellum. There is an accumulation of nuclei both posterior and anterior to the rostellum. Microtriches are evident anterior to the rostellum (Fig. 147).

6.3.1.3 *Raillietina chiltoni*

The LM layer is composed of 10-15 muscle fibres and extends from the proglottides into the scolex forming a ring around the base of the everted rostellum (Fig. 148). Some fibres separate and extend laterally and underlie the tegument of the scolex between the suckers but also reach the base of the rostellum. TM fibres extend across the scolex underlying the rostellum to the suckers. The rostellum contains longitudinally-arranged fibres that extend to layers of RM fibres and TM fibres at its base. The longitudinally-arranged fibres are most prominent at the rostellar hooks where fibres separate each hook and extend posteriorly. Suckers and the rostellum contain nuclei and vacuolation in the central field. Accumulations of nuclei also occur at the base of the

rostellum and below the suckers. Microtriches are evident on the scolex tegument posterior to the everted rostellum (Fig. 149).

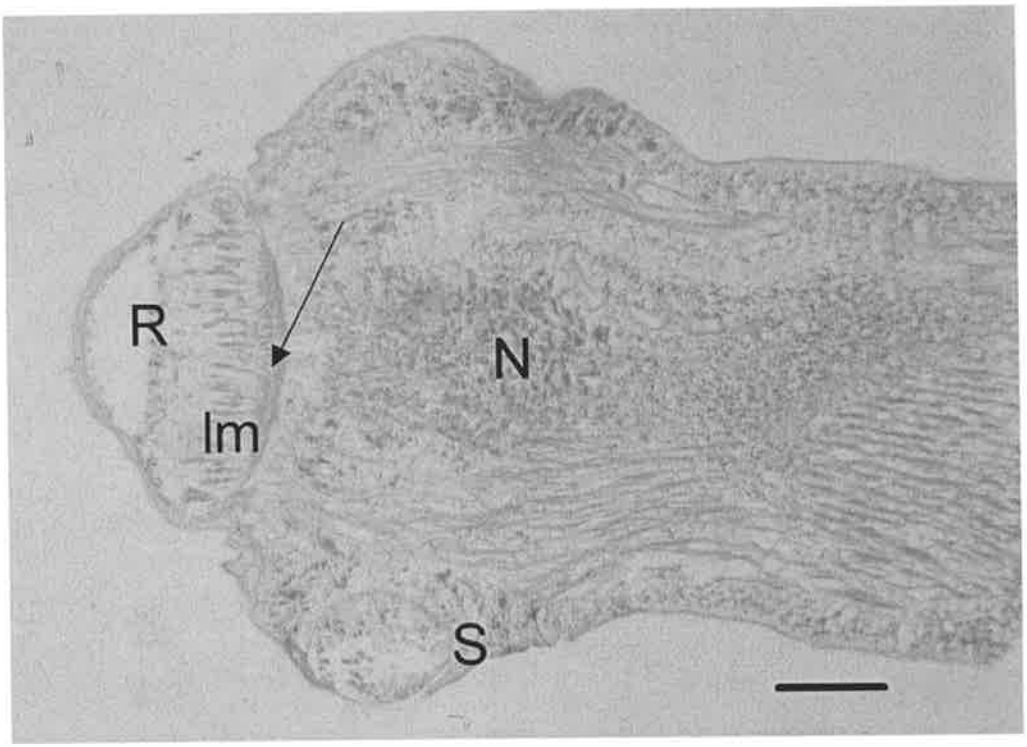


Fig. 148. Light micrograph of a sagittal histological section through the scolex of *Raillietina chiltoni* with everted rostellum. Sucker (S), rostellum (R), accumulation of nuclei (N), longitudinal muscle fibres (lm) separating rostellar hooks, radial and transverse fibres at base of rostellum (arrow). Scale bar = 100 μm.

6.3.1.4 *Raillietina dromaius*

A band of LM, consisting of three to ten fibres, extends from the proglottides into the neck and then the scolex where it expands into a thicker band of 12 or more fibres (Fig. 150). Fibres of this muscle band interlace with RM fibres at the base of the scolex. The thicker band of LM extends to the base of the rostellum and rostellar hook guards. RM fibres are interlaced with LM fibres and nuclei throughout the central region of the scolex and give the scolex a muscular appearance. A small region of TM fibres and nuclei extend across the base of the scolex inside the LM layer. Another LM layer of up to four fibres

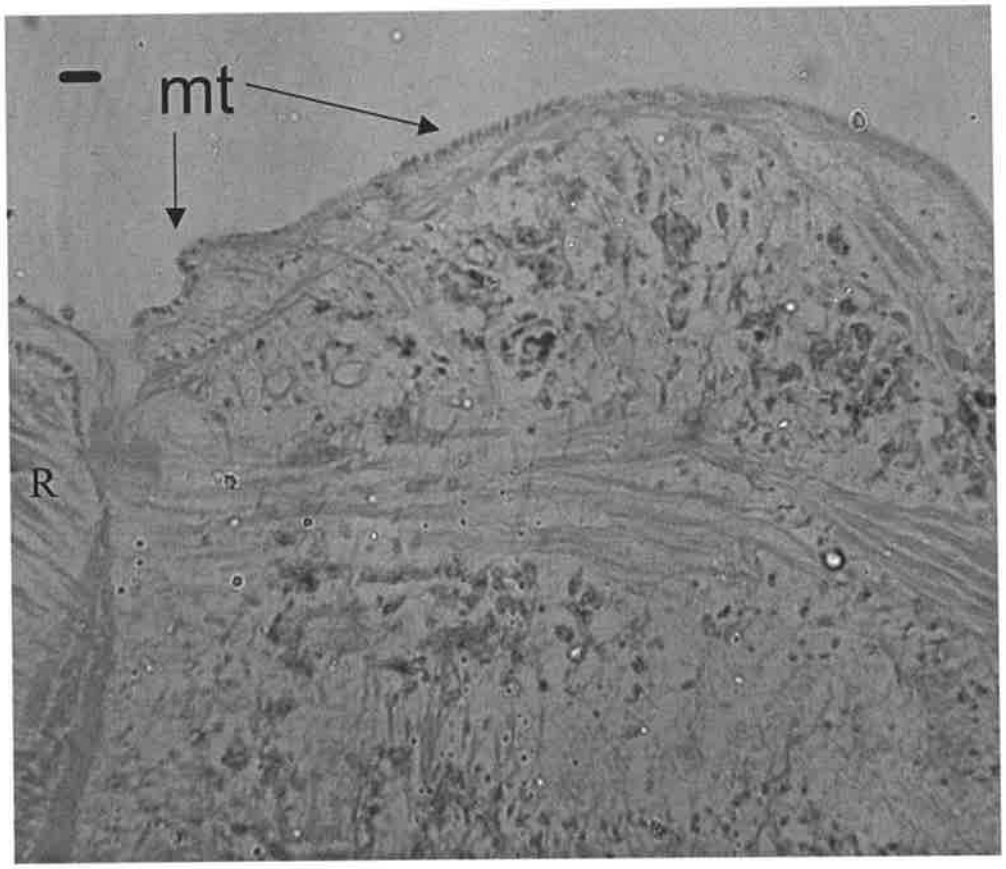


Fig. 149. Light micrograph of a sagittal histological section of microtriches (mt) on the scolex tegument of *Raillietina chiltoni* posterior to the everted rostellum (R). Scale bar = 10μm.

extends anteriorly from beneath the tegument at the middle of the scolex to the rostellar hooks. A TM layer runs from the rostellum to each rostellar hook connecting with the hook guard. The rostellum has longitudinally arranged fibres that meet a layer of muscle fibres consisting of both RM and TM fibres at the base of the rostellum giving it a muscular appearance. Microtriches are visible on the tegument anterior to the rostellum and posterior to the suckers in addition to the larger rose-thorn-shaped accessory spines/microtriches at the base of the rostellum (Fig. 151). A few small RM fibres are associated with the accessory spines.

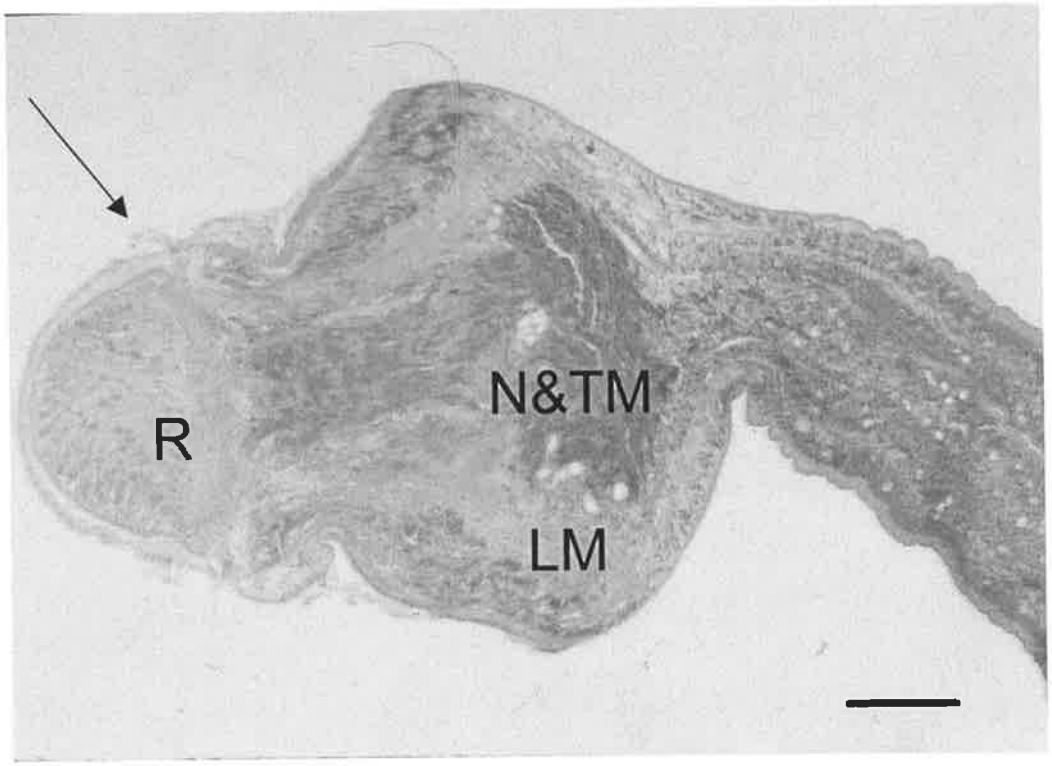


Fig. 150. Light micrograph of a sagittal histological section through the scolex of *Raillietina dromaius* with everted rostellum. Rostellum (R), longitudinal muscle (LM), nuclei and transverse muscle (N&TM). Arrow indicates region enlarged in Fig. 151. Scale bar = 100 μm.

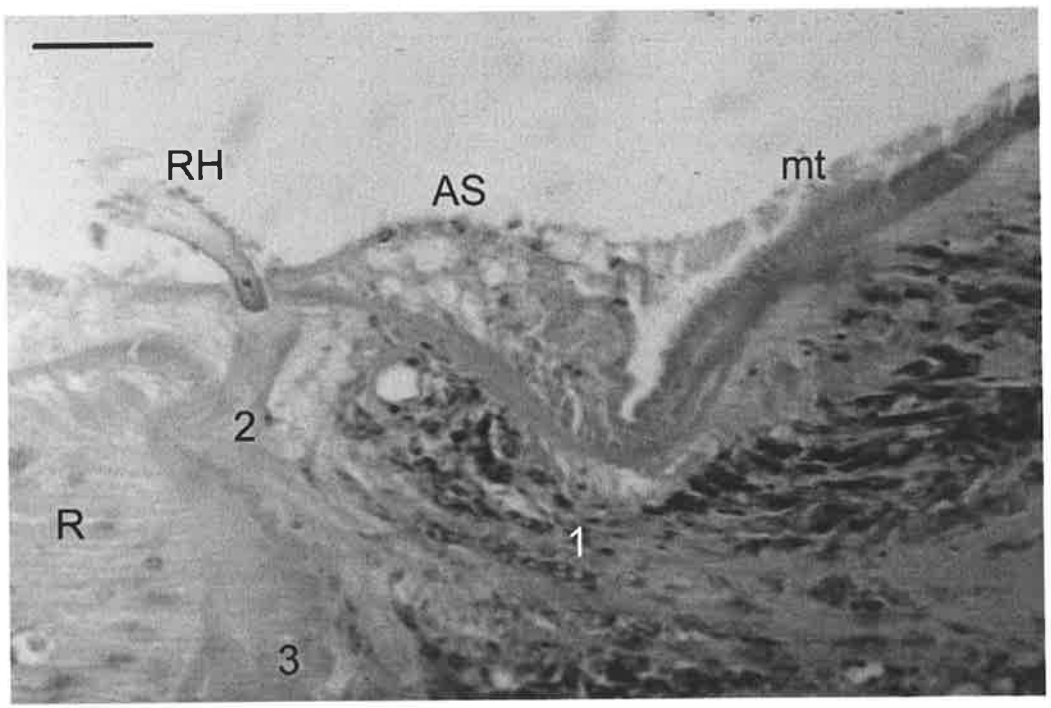


Fig. 151. Light micrograph of a sagittal histological section of the scolex of *Raillietina dromaius*. Rostellum (R), rostellar hook (RH), microtriches (mt), accessory spines (AS), longitudinal muscle fibres (1), transverse muscle layer (2), muscle fibres at base of rostellum (3). Scale bar = 10 μm.

6.3.1.5 *Raillietina mitchelli*

The musculature of the scolex is feeble (Fig. 152) in comparison to that of the other species described here. The LM layer consists of one to four short irregular fibres, discontinuously extending to the base of the rostellum. An occasional LM fibre is present lateral to this LM layer in the neck. Poorly-developed longitudinally-arranged fibres are present in the rostellum, most prominent between the rostellar hooks. There are a few nuclei surrounding the rostellum but not evident in it. The suckers contain a few LM fibres and nuclei.

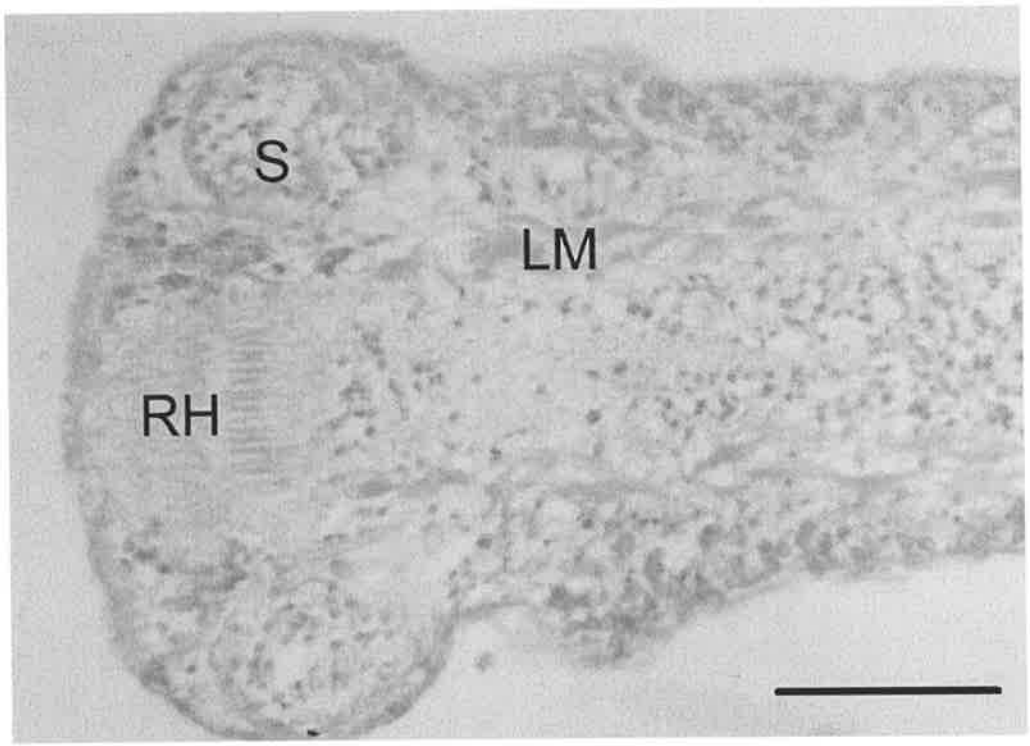


Fig. 152. Light micrograph of a sagittal histological section through the scolex of *Raillietina mitchelli* with retracted rostellum. Sucker (S), rostellar hooks (RH), longitudinal muscle (LM). Scale bar = 50 μm.

6.3.2 SEM of the scoleces of *Raillietina dromaius* and *Raillietina beveridgei*

6.3.2.1 *Raillietina dromaius*

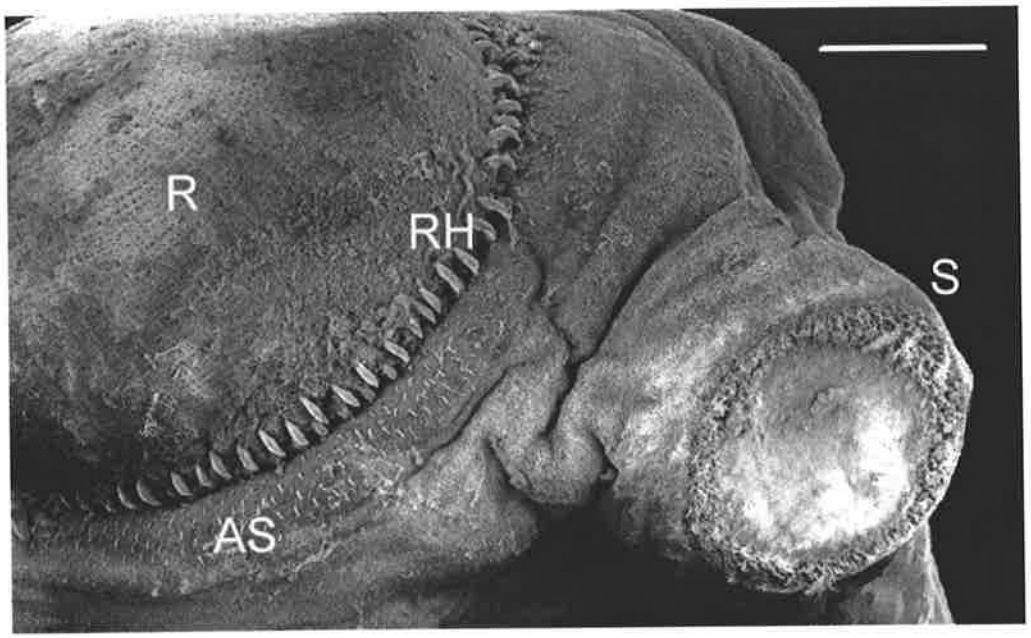


Fig. 153. Scanning electron micrograph of the scolex of *Raillietina dromaius.* Sucker (S), rostellum (R), rostellar hooks (RH), 'accessory spines'/microtriches (AS). Scale bar = 100 μm.

The significant characters of the scolex for the genus *Rallietina* are evident - a circular row of rostellar hooks and armed suckers (Fig. 153). The blade of each rostellar hook is exposed. 'Scale-like rostellar spines' (Gijon-Botella *et al.*, 1989; Bâ *et al.*, 1995), microtriches, are present in rows posterior to the rostellar hooks with maximum dimensions of 10.4 μm long x 7.2 μm wide (Fig. 154). These microtriches were easily dislodged during relaxation and fixation. The suckers are circular, 205 μm in diameter armed with hooklets in a complete circle around the circumference of the sucker (Fig. 153).

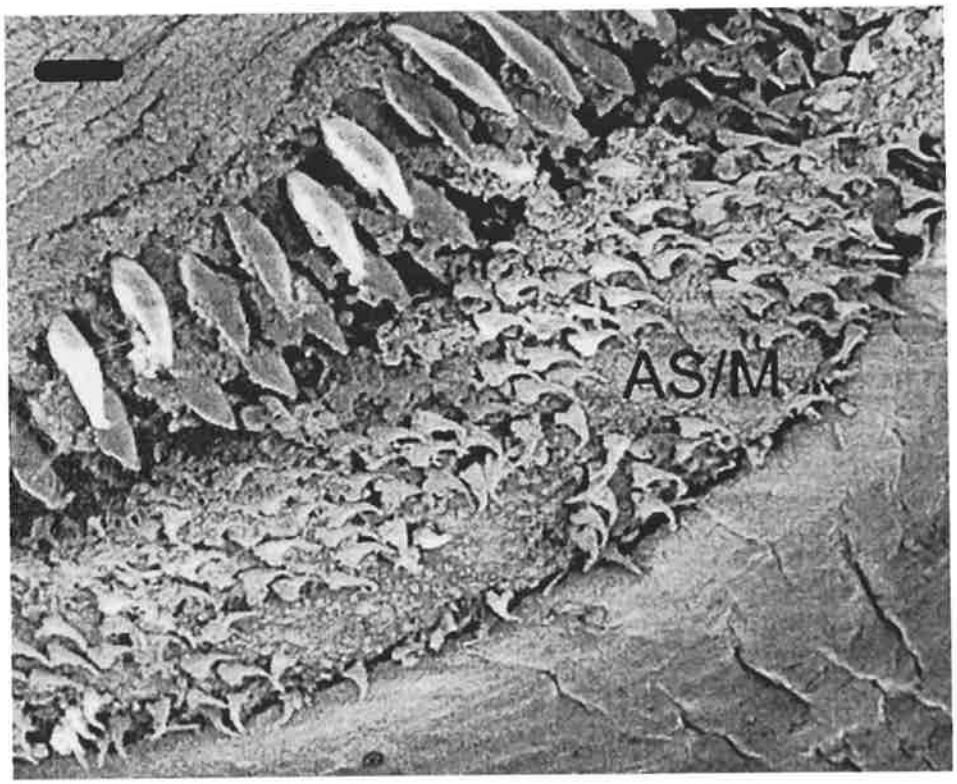


Fig. 154. Scanning electron micrograph of *Raillietina dromaius* showing rose-thorn shaped spines/microtriches (AS/M) posterior to the rostellar hooks. Scale bar = 8 μm.

6.3.2.2 *Raillietina beveridgei*

The scolex exhibits similar characters to other *Raillietina* species. It appears rectangular with equally-spaced and exposed suckers. The maximum dimension of the scolex from the outer edge of the suckers is 527 μm. Suckers are 133 μm in diameter with rows of hooklets on the anterior edge. The everted rostellum is 285 μm in diameter with a circular row of rostellar hooks at the base (Fig. 155). Tegumental microtriches are not evident using SEM.

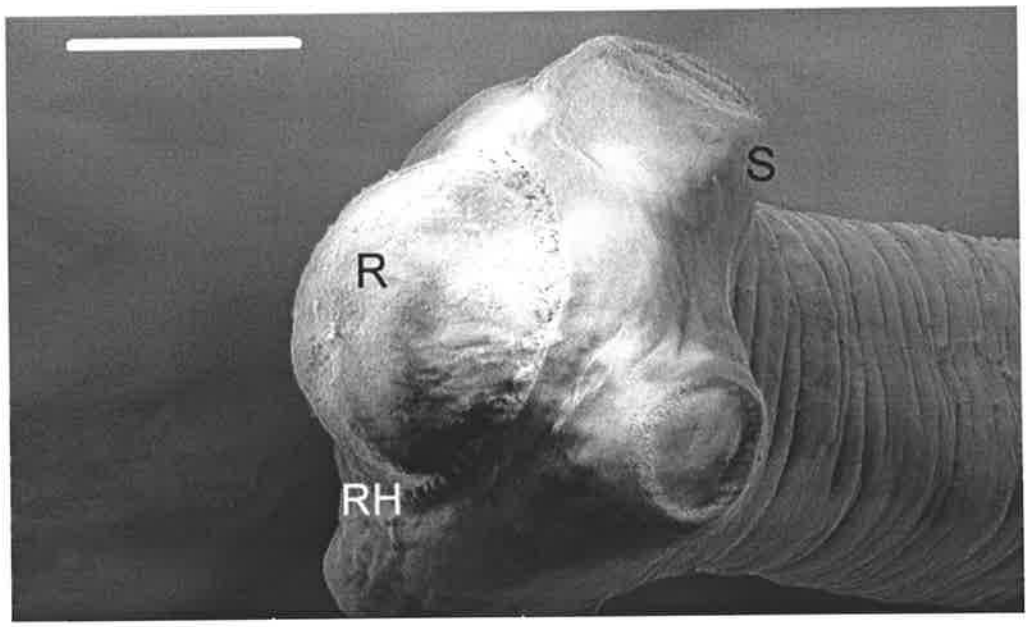


Fig. 155. Scanning electron micrograph of the scolex of *Raillietina beveridgei* with everted rostellum. The majority of rostellar hooks and sucker hooklets have been dislodged. Sucker (S), rostellum (R), rostellar hooks (RH). Scale bar = 200 μm.

6.3.3 Transmission Electron Microscopy (TEM

6.3.3.1 The scolex of *Raillietina dromaius.*

Three forms of microtriches were found on the surface of the scolex. Descriptive terminology of microtriches follows that of Thompson *et al.* (1980). 'Peg-like' microtriches are present posterior to the rostellar hooks. From light microscopy observations, these microtriches (see Fig. 156) have a strongly-pointed and posteriorly-angled shaft. In transverse section the base is ellipsoidal to cylindrical in shape, slightly acute at one end, with an electron-lucid matrix and four strongly electron-dense areas, two apical and two on each side (Fig. 159) (Jha and Smyth, 1969). Rostellar hooks are circular in transverse section and electron dense (Fig. 159). LM and TM fibres are present in the rostellum. The rostellum is surrounded by vacuolated parenchymatous tissue that increases the dimensions of the anterior scolex and everted rostellum.

'Spine-like' microtriches are up to 1.8 μm long (Fig. 156), angled posteriorly with a strongly-pointed, electron dense, elongated shaft mounted on a base inserted into the distal cytoplasm of the tegument (see Fig. 151) are present caudal to the 'peg-like' microtriches 'Blade-like' microtriches up to 600 nm long were present on the tegument of the scolex (Fig. 157). Towards the base of the scolex and on the neck, these microtriches are 0.450-0.950 μm in length (Fig. 158).

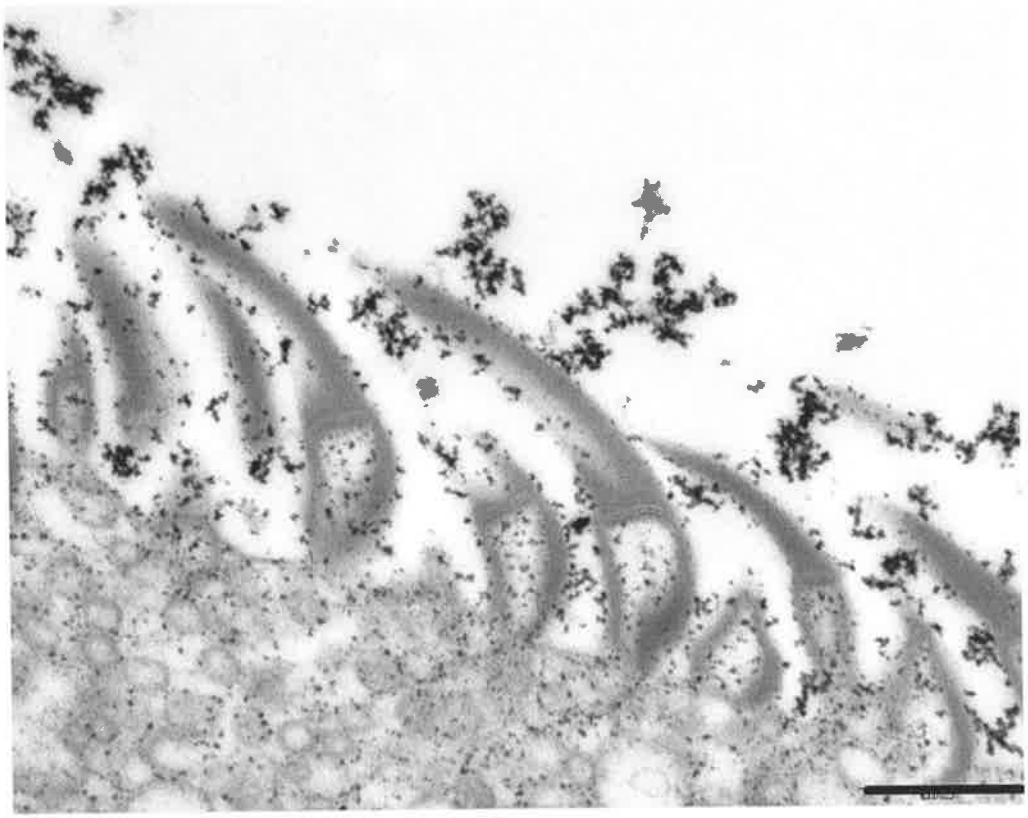


Fig. 156. Transmission electron micrograph of *Raillietina dromaius*. 'Spine-like' microtriches posterior to rostellar hooks and 'peg-like' microtriches. Scale bar = 500 nm.

Fig. 157. Transmission electron micrograph of *Raillietina dromaius*. 'Blade-like' microtriches on rostellar tegument. Scale bar = 1μm.

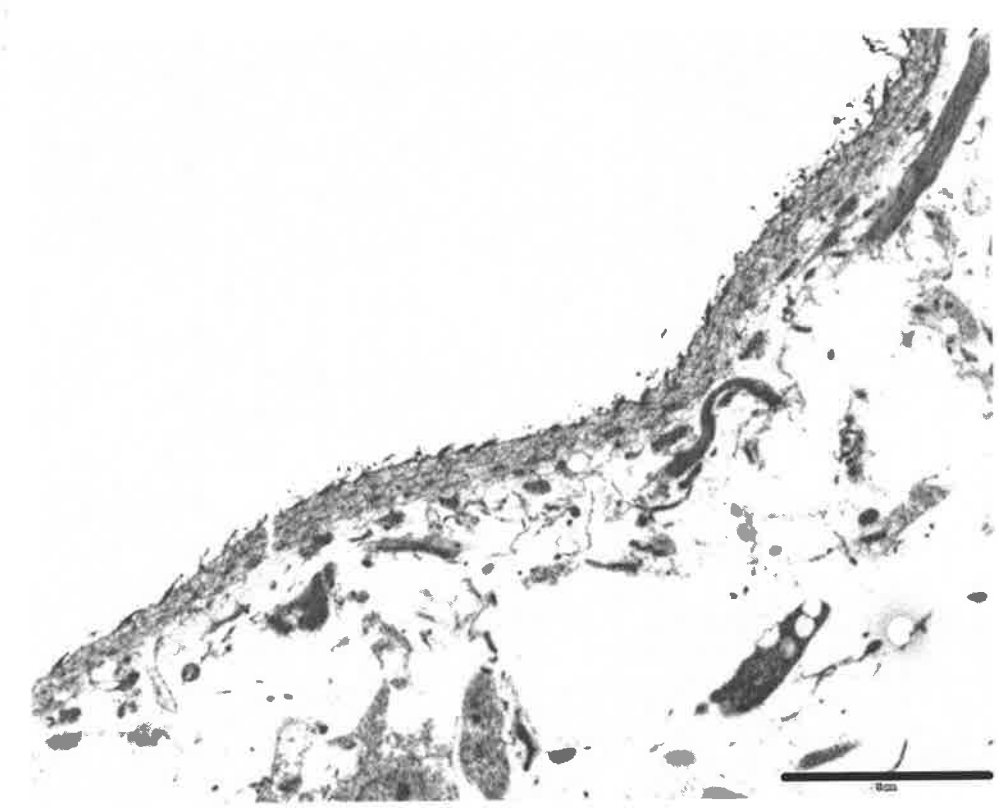


Fig. 158. Transmission electron micrograph of *Raillietina dromaius*. 'Blade-like' microtriches on the tegument of the posterior scolex and neck. Scale bar = 5 μm.

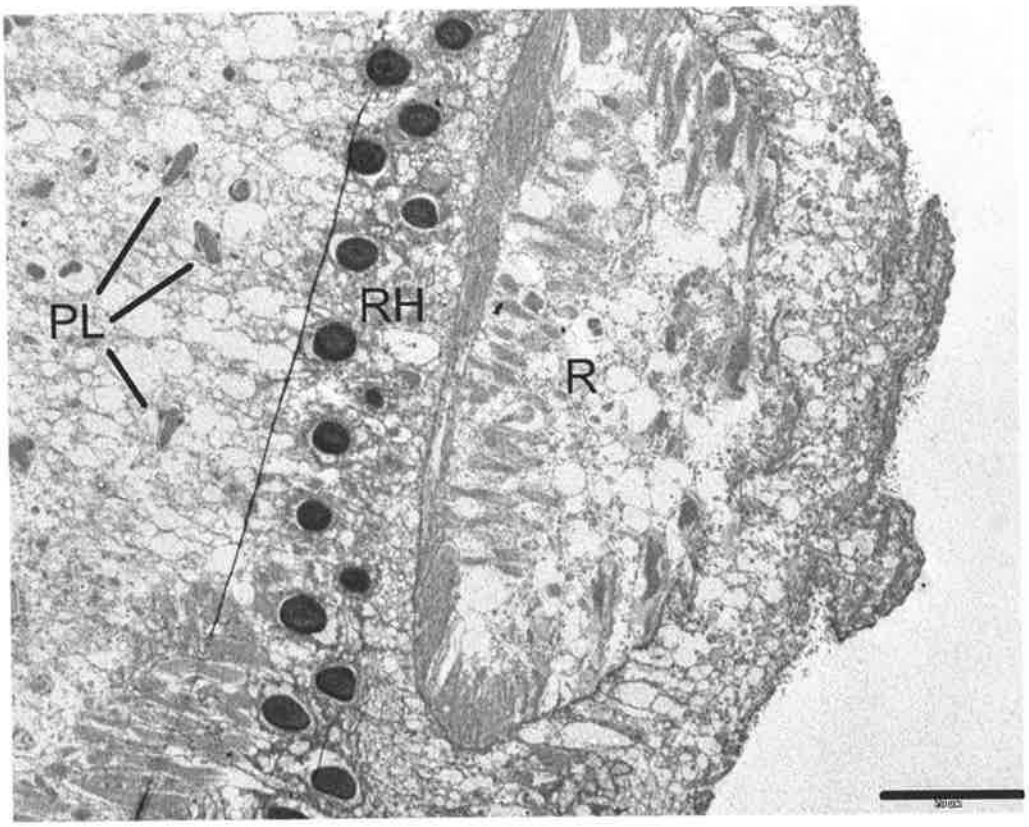


Fig. 159. Transmission electron micrograph of *Raillietina dromaius*. Glancing section through apical cytoplasm of the tegument. 'Peg-like' microtriches (PL) in transverse section, rostellar hooks (RH) and rostellum (R). Scale bar = 20 μm.

6.3.3.2 The sucker of *Raillietina australis*

Filamentous microtriches of varying size are present on the muscular sucker of *R. australis* and the tegument posterior to the sucker (Fig. 160). The tegumental microtriches are densely packed, between 0.8 and 1.4 μm in length and embedded in a granular cytoplasm (Fig. 161). The shaft is electron dense and curved posteriorly. RM and LM fibres are present in the sub-tegumental layer. The tegument and muscle fibres extend around and partly cover the retracted sucker.

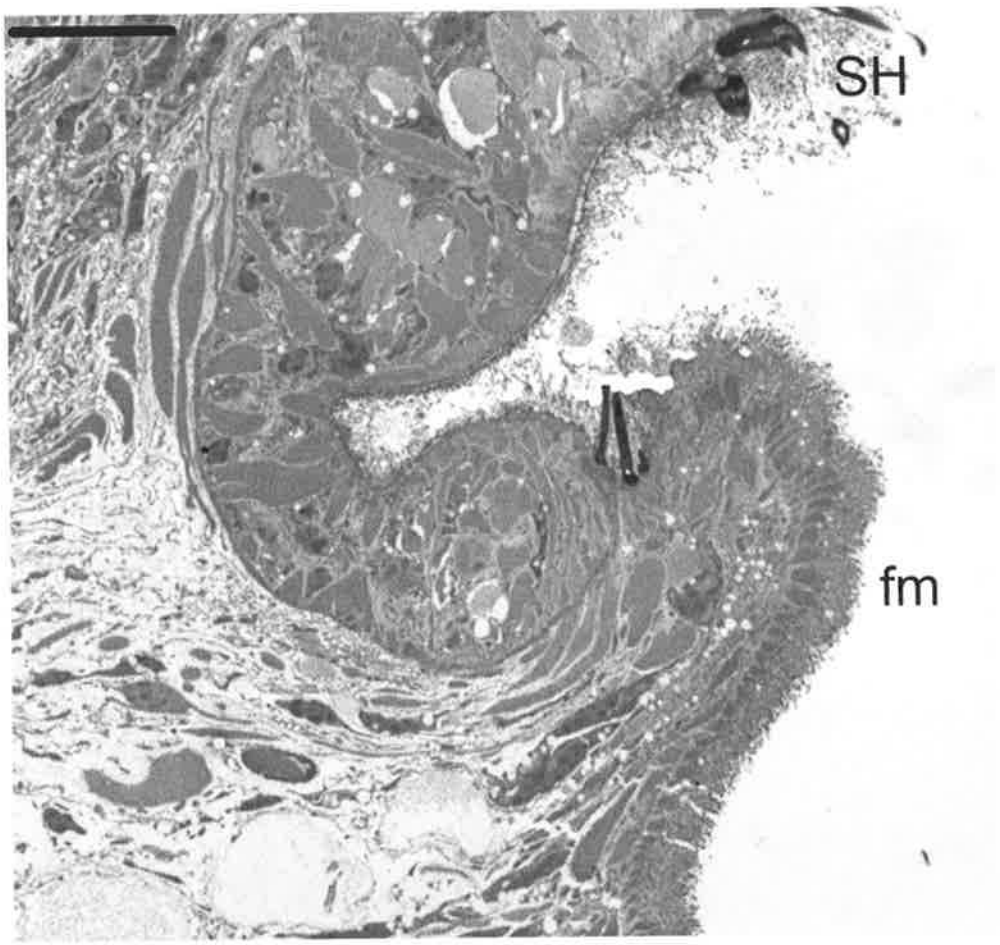


Fig. 160. Transmission electron micrograph of the sucker of *Raillietina australis.* Sucker hooklet (SH), filamentous microtriches (fm). Scale bar = 10 μm.

6.3.3.3 The scolex of *Raillietina mitchelli*

Rostellar hooks are 8.2-10.1 μm long *en face* and have an electron-dense hook guard (Fig. 162). Longitudinal muscle fibres run parallel to each rostellar hook and extend to the muscle fibres at the base of the rostellum. The specimen of *R. mitchelli*, represented here, has a retracted rostellum. The outer layer of the scolex completely encloses the rostellum. Peg-like microtriches, 1.0-2.0 μm in length, extend between the rostellar hooks but are anterior to the retracted rostellum (Figs 163, 164). These microtriches form a band posterior to the everted rostellum. A complex group of muscle fibres, comprising inner longitudinal muscle and oblique muscle fibres, is present in the scolex anterior to the

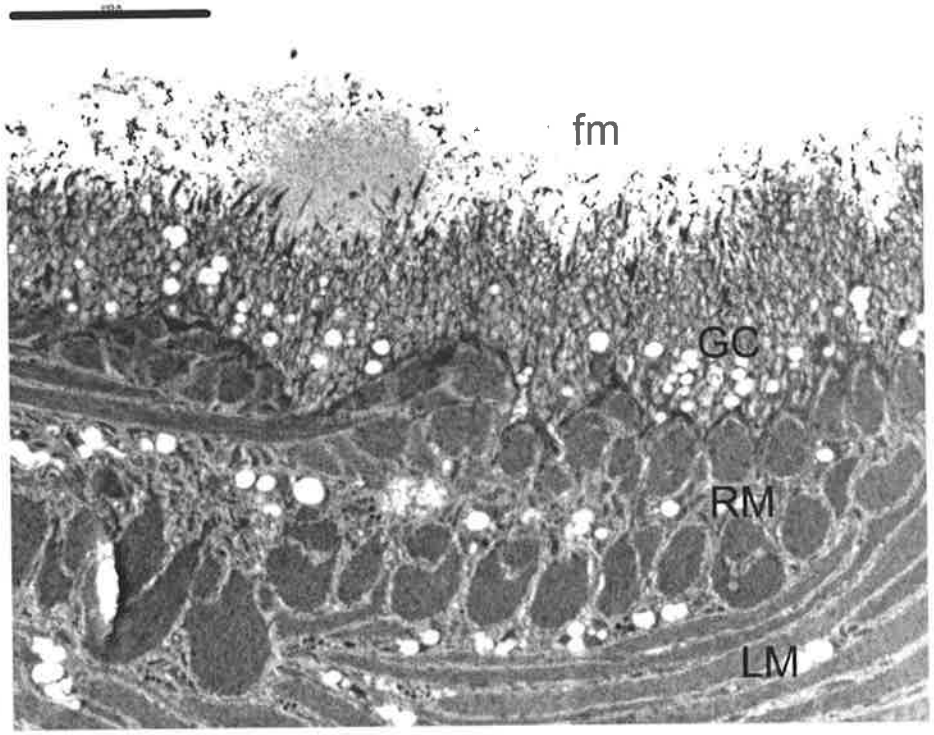


Fig. 161. Transmission electron micrograph of posteriorly-angled tegumental microtriches and muscle layers adjacent to the sucker of *Raillietina australis*. Filamentous microtriches (fm), granular cytoplasm (GC), radial muscle fibres (RM), longitudinal muscle fibres (LM). Scale bar = 5 μm.

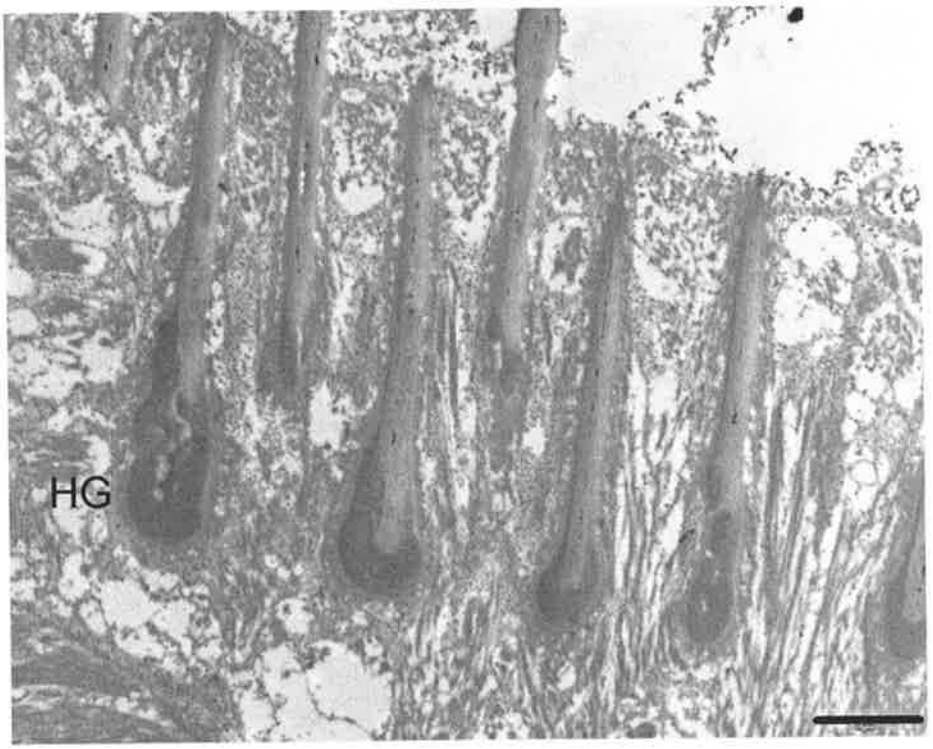


Fig. 162. Transmission electron micrograph of the rostellar hooks of *Raillietina mitchelli*. Rostellar hook guard (HG). Scale bar = 2 μm.

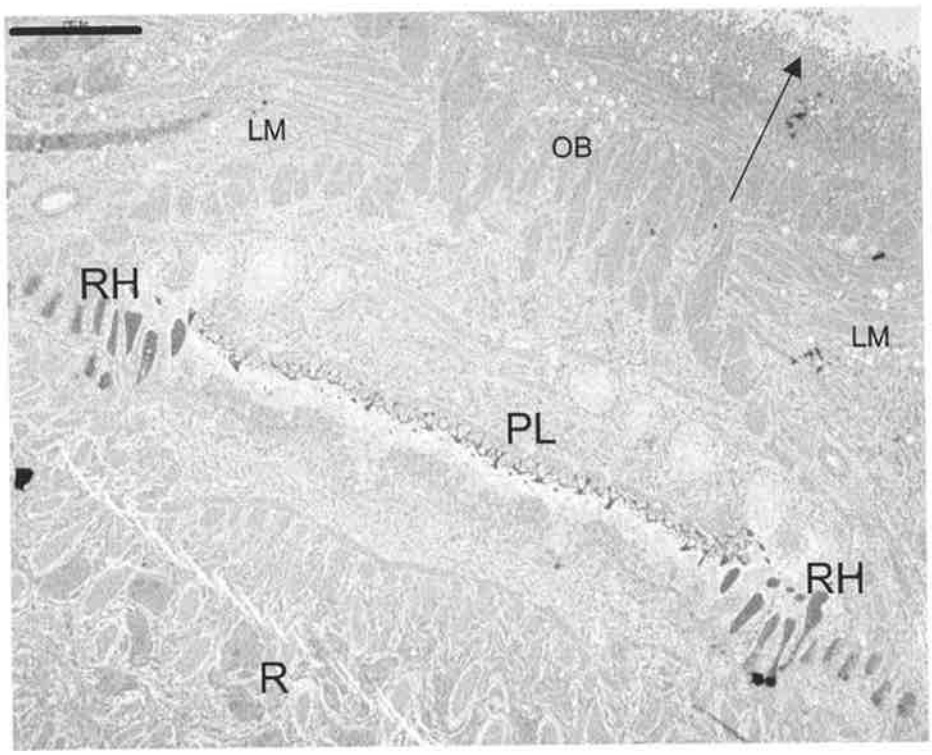


Fig. 163. Transmission electron micrograph of the scolex of *Raillietina mitchelli* with retracted rostellum. Rostellum (R), rostellar hooks (RH), 'peg-like" microtriches (PL), filamentous microtriches (arrow), oblique muscle fibres (OB) and longitudinal muscle fibres (LM). Scale bar = 10 μm.

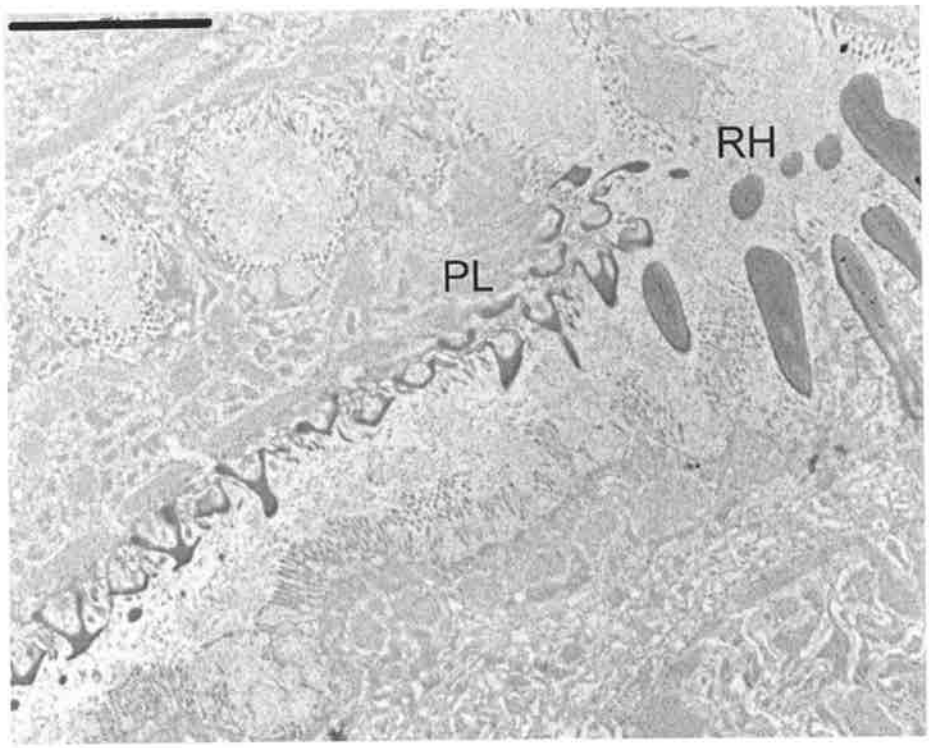


Fig. 164. Transmission electron micrograph of the scolex of *Raillietina mitchelli* with retracted rostellum. Rostellar hooks (RH) and peg-like microtriches (PL). Scale bar = 5 μm.

retracted rostellum. Small filamentous microtriches are present on the tegument of the anterior scolex (Fig. 163, arrow).

6.3.4 Fine structure of the egg-capsule of *Raillietina beveridgei*

Gravid proglottides contain capsules embedded in parenchymatous tissue (Figs 165, 166). Fully-formed eggs are contained in capsules with a thickened wall composed of compact capsular material (Fig. 167). A thin, membranous layer separates each capsule (Fig. 167). Eggs are dispersed in parenchymatous tissue and glandular-like cells of unidentified composition and of unknown origin. Membranes connected to those forming the outer membrane of each egg radiate and penetrate the parenchyma and are connected to those that enclose the glandular-like cells (Fig. 168). The branching membranes permeate the parenchymatous tissue of the capsule forming a close association between the cytoplasm of the egg, the parenchymatous tissue of the capsule and the glandular-like cells.

A single, continuous and flexible membrane (embryophore) surrounds the oncosphere and oncospheral membrane, which at low magnification, appears to be multiple membranes. The oncospheral membrane extends to, but remains distinctly separate from, the outer membrane (Fig. 169) giving the appearance of filaments (Ransom, 1905) or tube-like structures (Reid *et al.*, 1938) and is separated from the oncospheral membrane by the inner envelope. The embryophore is composed of electron-dense material. The internal surface appears to be composed of an accumulation of microvilli or microtubules (Figs 171, 172). The oncospheral membrane appears as a thin membrane surrounding the oncosphere (Figs 169, 170, 171). Oncospheral hooks in cross-section are electron dense (Figs 169, 170).

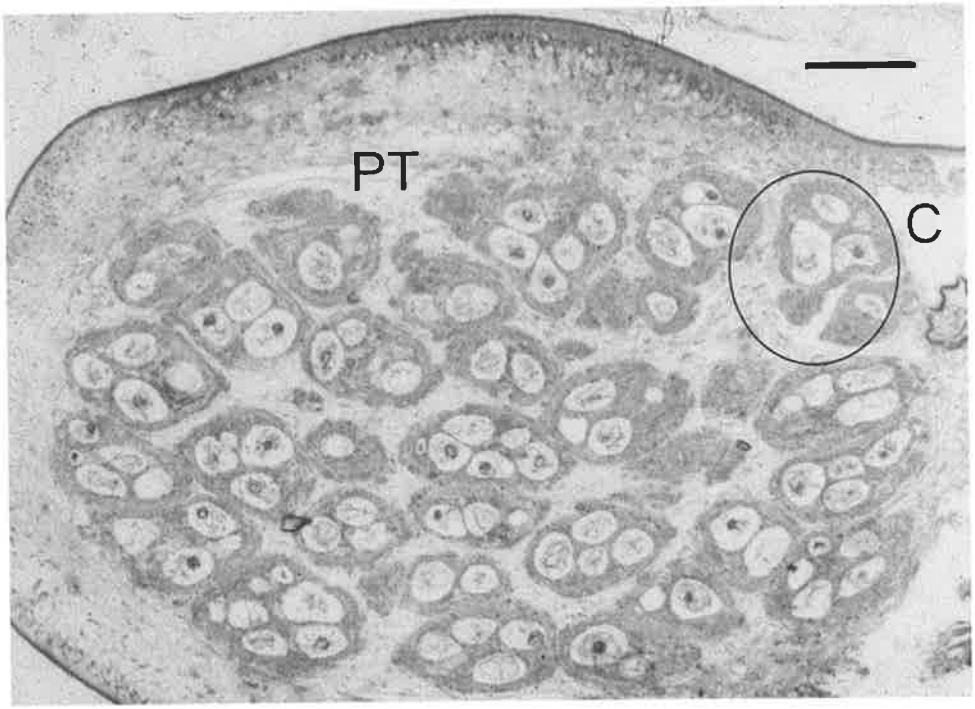


Fig. 165. Light micrograph of a histological section of gravid proglottis of *Raillietina beveridgei* showing capsules (C) and parenchymatous tissue (PT). Scale bar = 100 μm.

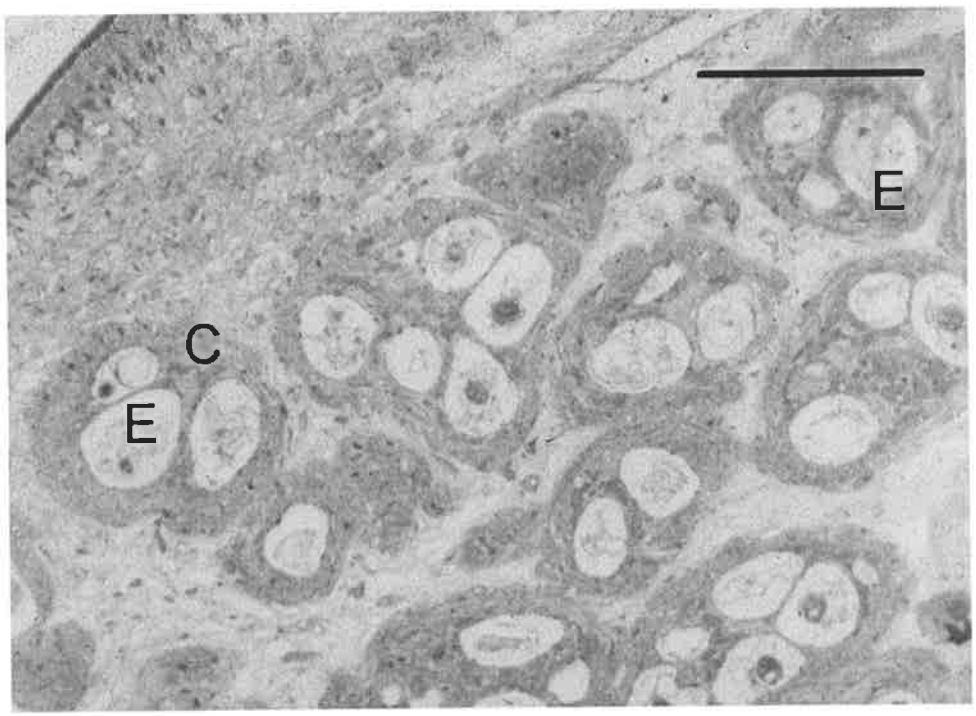


Fig. 166. Light micrograph of a histological section of gravid proglottis of *Raillietina beveridgei* showing capsules (C) and eggs (E). Scale bar = 100 μm.

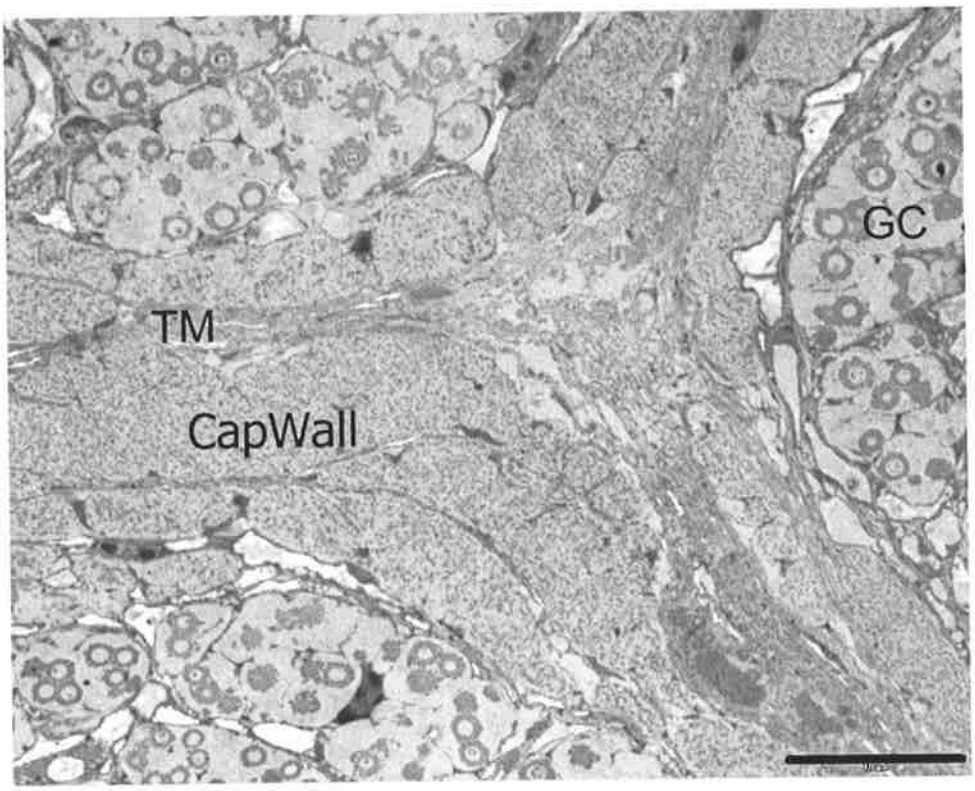


Fig. 167. Transmission electron micrograph of *Raillietina beveridgei* showing capsule wall (CapWall), thin membraneous layer (TM) separating capsules and glandular cells (GC). Scale bar = 10 μm

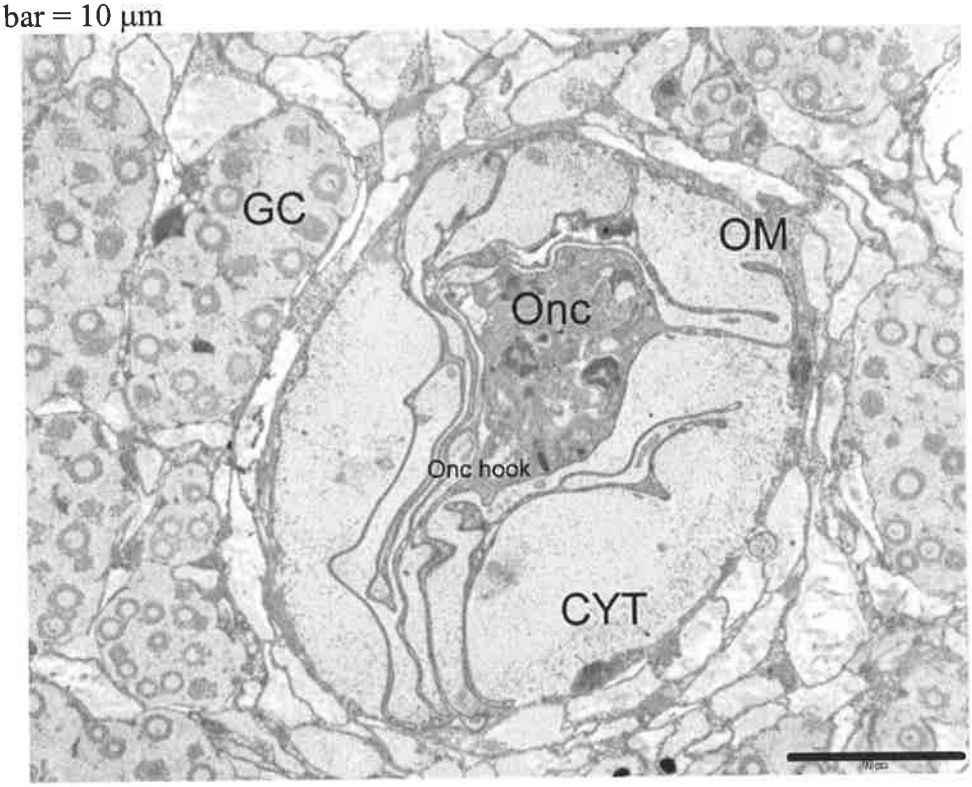


Fig. 168. Transmission electron micrograph of *Raillietina beveridgei.* Egg embedded in glandular-like cells, parenchyma and cellular membranes. Oncosphere (Onc), outer membrane (OM), envelope cytoplasm (CYT), glandular cells (GC) and section of oncospheral hook (Onc hook). Scale bar = 10 μm.

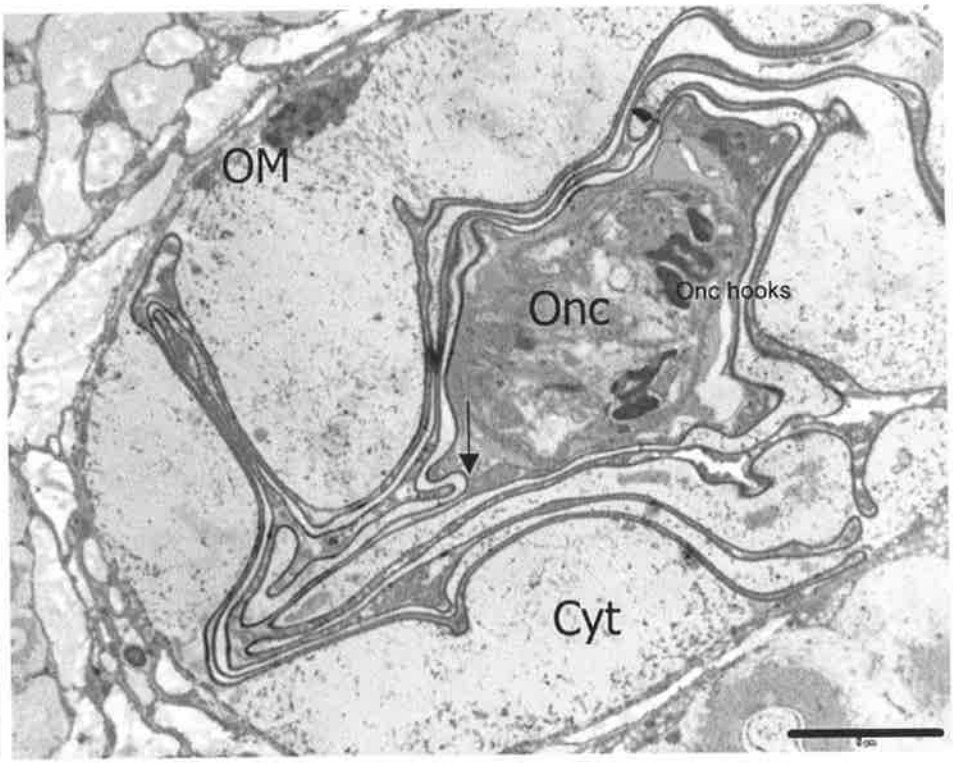


Fig. 169. Transmission electron micrograph of an egg of *Raillietina beveridgei* showing oncosphere (Onc), outer membrane (OM), cytoplasm (Cyt) and oncospheral membrane (arrow). Scale bar = 5 μm.

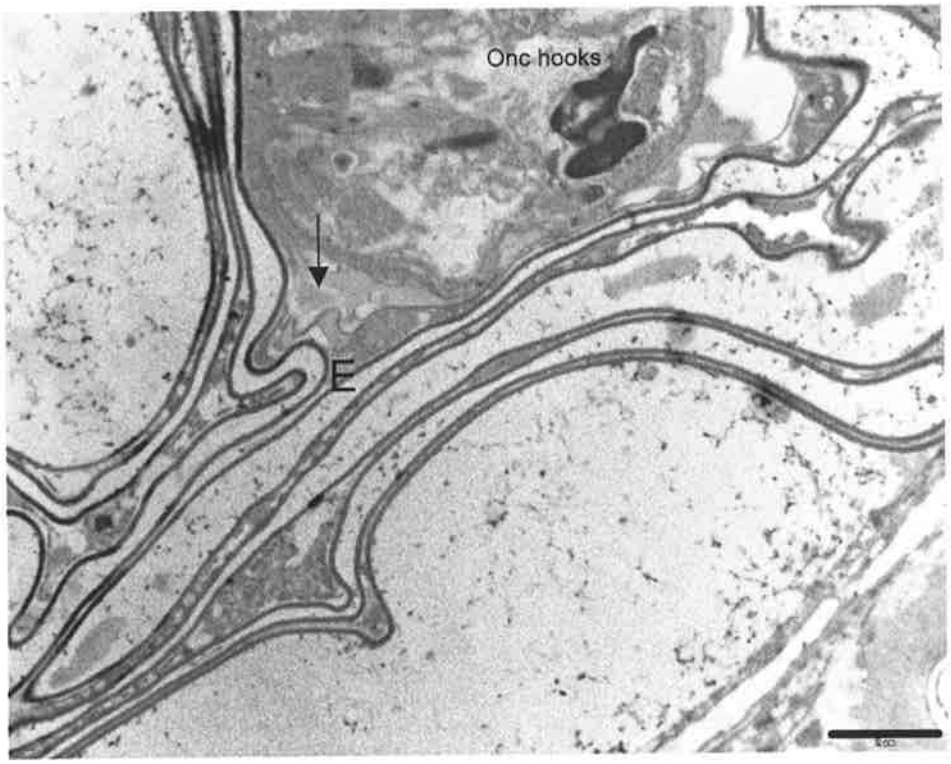


Fig. 170. Transmission electron micrograph of the oncosphere and embryophore of *Raillietina beveridgei* at higher magnification. Oncospheral hook (Onc hooks), oncospheral membrane (arrow) and embryophore (E). Scale bar = 2μm.

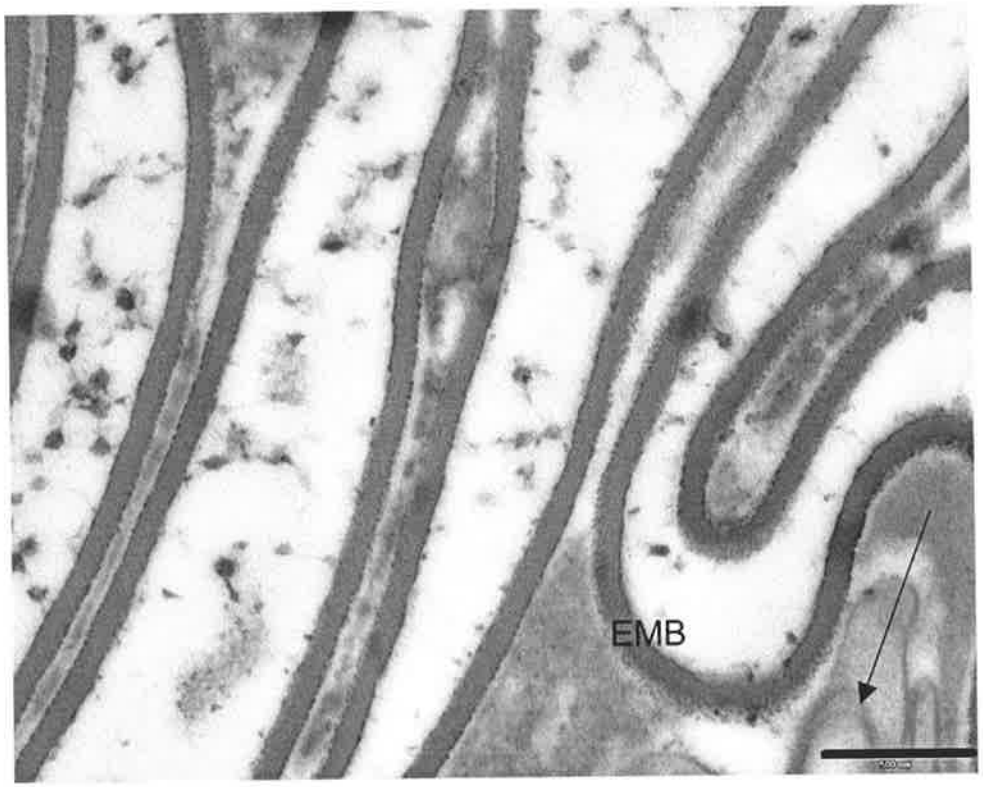


Fig. 171. Transmission electron micrograph of the flexible embryophore (EMB) and oncospheral membrane (arrow) of *Raillietina beveridgei*. Scale bar = 500 nm.

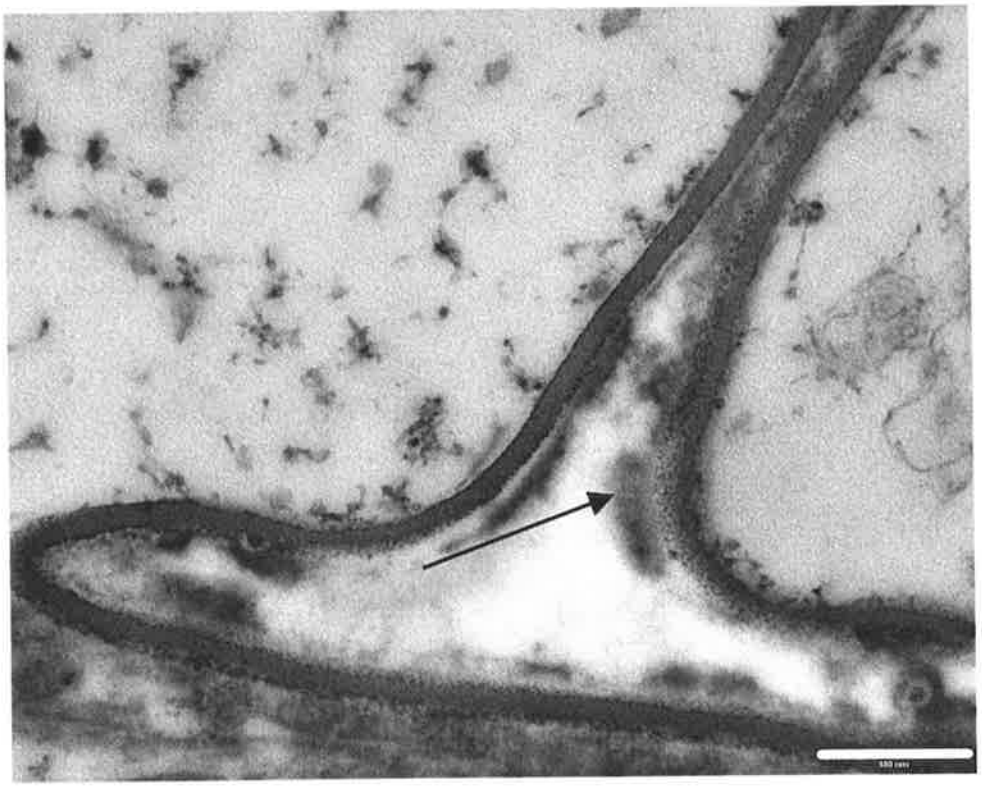


Fig. 172. Transmission electron micrograph of the embryophore of *Raillietina beveridgei* showing the microvilli or microtubules (arrow). Scale bar = 500 nm.

6.3.5 Observations of the embryophore in released eggs of *Raillietina beveridgei*

When eggs were mechanically released from egg capsules, the folded embryophore expanded to encircle the activated oncosphere that was in turn, encircled by the oncospheral membrane (Fig. 173). Remnants of the thin outer membrane were evident and appear to remain as part of the capsule parenchyma (see page 158). The process occurred in approximately 30 min in both tap water and PBS. Hook movement in the activated oncosphere was infrequently observed and apparently generated by contractile movement or pumping action of oncospheral muscles from the base of the oncosphere upward to the hooks. This caused the oncospheral hooks to exhibit an upward and outward motion.

6.4 Discussion

Differences are observed in the morphology of the scoleces when examined histologically, particularly differences in the scolex musculature. The shape of the scolex appears to be dependent on the complexity and extent of the musculature. The scoleces of *R. australis* and *R. dromaius* contain strong LM layers with muscular suckers and rostellum. The scolex musculature of other species is less developed (Figs 145, 148, 152). The musculature of the scolex of *R. dromaius* appears to be associated with maintaining the everted rostellum and protrusion of the rostellar hooks.

Strong musculature in the posterior scolex appears to support the integrity of the scolex and the suckers. The suckers of *R. australis* are also well supported by musculature and their anterior positioning suggests that they are strong contributors to attachment (Figs 144, 160). The less-developed musculature of the remaining species, viz. *R. beveridgei, R. chiltoni* and *R. mitchelli*, suggests that they may rely principally on the rostellar hooks and sucker hooklets for attachment.

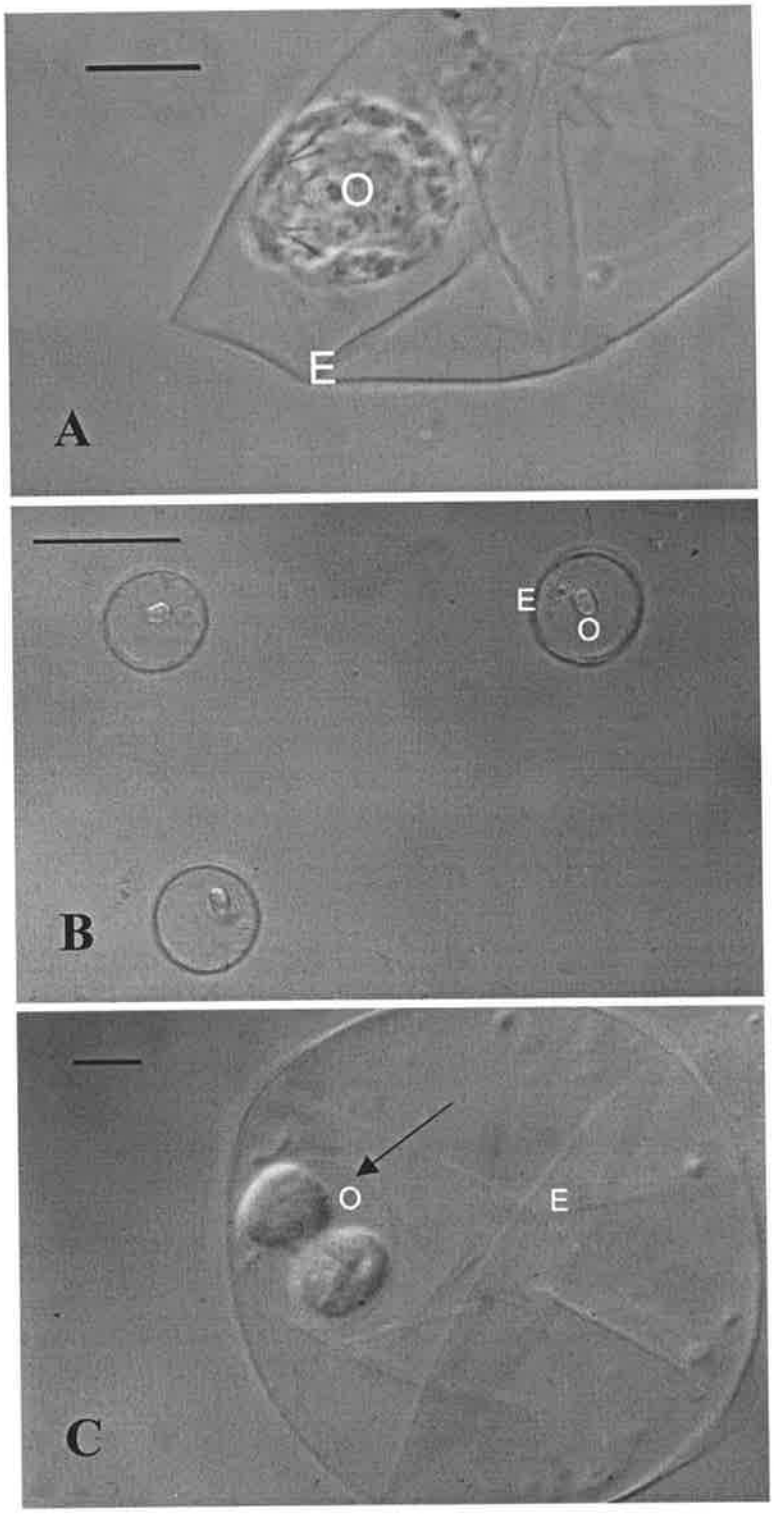


Fig. 173. Light micrographs (Nomarski differential interference contrast) of the eggs released from egg capsules of *Raillietina beveridgei*. A: unexpanded embryophore (E) with oncosphere (O); B: expanded embryophores; C: activated oncosphere with expanding embryophore (E) and oncospheral membrane (arrow). Scale bars = A, 10 μm; B, 100 μm; C 10 μm.

The muscle layers and fibres of the scolex of *Raillietina* species, particularly the LM layer and those posterior to the rostellum, are believed to be responsible for retraction of the rostellum (Blitz and Smyth, 1973). However, *in vivo*, it seems certain that the scolex musculature supports the everted rostellum and orientation of rostellar hooks. The latter feature did not change in the retracted rostellum of the *Raillietina* species examined here. The hook blade always extends horizontally outwards. LM fibres running parallel to hooks appear to be responsible for maintaining the vertical orientation of the hook guard. The strength and detail of the musculature seen in *R. dromaius* is consistent with this suggestion because the rostellum of that species appears not to retract and the rostellar hooks are firmly supported in protrusion. Nevertheless, the ability to retract a rostellum suggests that it is a vulnerable and sensitive region of the parasite (Blitz and Smyth, 1973) that is also dependent on the musculature of the scolex. In this study, rostellar hooks were lost more frequently from specimens with everted rostella. It seems plausible, therefore, that retraction of the rostellum in the unattached cestode aids in the protection of the rostellar hooks.

Blitz and Smyth (1973) found thinning of the tegument on the scolex of *S. cesticillus* in comparison to proglottides and suggested that the unique property of rostellar retraction would be difficult if the tegument was thick as in the proglottides. Histologically, the distal cytoplasm appears to be thicker in the neck and anterior proglottides than in the scolex of some species of *Raillietina* (Fig. 145) but these observations are have not been confirmed with TEM.

The microtriches observed in this study do not differ from those reported on the scolex of other davaineids (Blitz and Smyth, 1973; Bâ *et al.* 1995; Fourie *et al.*, 1997; Stoitsova *et al.*, 2001). An absorptive role has been attributed to the rostellar tegument and

microtriches (Smyth *et al.*, 1967; Smyth, 1969). Microtriches that lack an electron-dense cap (Blitz and Smyth, 1973) are considered to favour an absorptive function but microtriches with this morphology were not detected here. 'Spine-like' and 'blade-like' microtriches with an electron-dense spine and angled posteriorly, as seen in this limited study (e.g. Figs 156, 157), are considered to be involved principally in attachment (Rothman, 1963; Blitz and Smyth, 1973; Thompson *et al.,* 1980). In addition, the orientation of the rostellar hook handle to the apical centre of the protruding rostellum, the parallel guard and the exposed blade, support this role (Stoitsova *et al.*, 2001). The orientation and size of the 'peg-like' microtriches on the base of the rostellum of *R. dromaius*, posterior to the rostellar hooks, also suggest a role in attachment, however, Thompson *et al.*, (1980) considered that they may also be involved in abrading the mucosal surface of the host.

Histological observations indicate that the orientation of the rostellar hooks and peg-like microtriches is dependent on the position of the rostellum and the consequent retraction of the rostellar pouch. The detection of minute spines anterior to the rostellum by light microscopy in the taxonomic study of *R. mitchelli* (see p. 38 and Fig. 141) is a result of an apical examination of the scolex with a retracted rostellum in a cleared specimen. In normal circumstances, the everted rostellum would protrude the rostellar hooks, retract the rostellar pouch and some of the tegumental layer and expose the peg-like microtriches at the base of the rostellum. This is consistent with the location of these spines on the scoleces of the other cestode species examined here.

Thompson *et al.* (1980), together with other authors (Lee, 1966; Lumsden, 1975), stated that microtriches appear to be an integral component of the cestode tegument. Although rostellar spines had been reported in several species of the Davaineinae, Bâ *et al.* (1995) believed that the scale-like spines they examined represented a character common

to all of the davaineids. Later, Fourie *et al.* (1997) reported accessory spines on a 'rostellar collar' of *Houttuynia struthionis* and considered their shape to be of specific significance to the cestode. More recently, Stoitsova *et al.* (2001) confirmed that the scolex of *Fernandezia spinosissima* possessed modified microtriches with complicated morphology that appeared to be 'scale-like' and corresponded to accessory spines previously mentioned in taxonomic descriptions of davaineids. In this study, peg-like and spine-like microtriches on the scolex of *Raillietina* species have been described (Figs. 141, 142, 154, 157, 159). It is concluded that the peg-like microtriches reported here correspond to accessory spines on the base on the rostellum (Scholz *et al.*, 1998) and represent one of the different types of the microtriches described by Thompson *et al.* (1980) and it is agreed that structural and dimensional variations of microtriches occur not only in different regions of the scolex of individual cestode species, but also within the same region in different cestode species. Furthermore, future evaluation of tegumental microtriches of the scolex may be useful in taxonomic and phylogenetic analysis of the genus *Raillietina* (de Chambrier and Vaucher, 1997; Rego *et al.*, 1998; Scholz *et al.*, 1999).

An outer membrane, an outer envelope, an embryophore, an inner envelope and an oncospheral membrane surround the oncosphere. Cyclophyllidean cestodes have these three membrane-bound, primary layers (Lethbridge, 1980; Fairweather and Threadgold, 1981) separated by outer and inner envelopes. All of the membranes here appear to be flexible and thin and comparable to cyclophyllideans such as *Nematotaenia dispar* (see Tkach and Swiderski, 1997). Although there are many variations in embryonic ultrastructure in the cyclophyllideans (Coil, 1975; Lethbridge, 1980), the ultrastructural features of the embryonic membranes of *R. beveridgei*, particularly the folded embrophore, are to the best of my knowledge, unlike any of the limited number of cestode species previously studied.

Reid *et al.* (1938) described the basic structure of the embryo of *S. cesticillus* using light microscopy. In that study, several membranes were identified including small tube-like membranes or filaments connecting the oncospheral membrane and outer membranes with other smaller membranes crossing and re-crossing the apparently thicker outermost membrane. The filaments formed a funnel shape that was considered to be a potentially diagnostic character for the species.

In another light microscopy study, Singh and Baugh (1984) described the embryonic envelopes of *Cotugnia digonopora.* A thin, delicate, usually undetected membrane surrounds the oncosphere with a heavy protective covering or shell outside it. An additional, thin, irregular membrane separated the inner and outer envelopes and the outer capsule was also a thin membrane. The membranes of the embryo of *R. echinobothrida* were also described in the same study. A spherical and rigid membrane surrounded the oncosphere that was described as the inner capsule. No true oncospheral membrane could be detected. A thin, flexible outer capsule enclosed the cytoplasmic layer.

The function of the outer capsule is to protect the egg (e.g. from desiccation and bacterial attack) and aid survival outside the host (Conn, 1985). The outer capsule and embryonic membranes of *R. beveridgei* are delicate and would not allow for extensive survival periods in the environment. Survival would be extended in the higher humidity of ant nests. It is feasible that adult ants detect and carry whole gravid proglottides to nests where the intermediate hosts then become infected after ingesting eggs.

Solid prey, mostly carried by worker ants, is intended as food for larvae that feed avidly upon it although they have reduced mouthparts (Naumann, 1991; Shattuck, 1999). Consequently, the ant larva may mechanically break or digest the embryonic membranes to assist with the release of the oncosphere. The structure of the embryonic membranes thus may be an adaptation well suited to the behaviour of the intermediate host. It seems likely

that the occurrence of fully-formed cysticercoids in the haemocoele of the gaster of adult ants is a result of the earlier infection of larval ant stages (Case and Ackert, 1940). In this study (Chapter 5), the examination of ants was restricted to adult forms and consequently, the inability to find a hexacanth embryo in infected intermediate hosts can be explained.

As shown in my study, mechanical removal of the egg from the capsule appears to be the first step to facilitate activation of the remaining embryonic envelopes and expose them to the host digestive system (Smyth, 1969). Oncospheral motion described here is typical of the clawing movement previously described (Ogren, 1961; Ogren and Magill, 1962; Rybicka, 1966; Collin, 1968). Sawada (1967) applied a variety of methods to artificially hatch the oncosphere of *R. echinobothrida*, describing the motion as 'amoeboid movement'. In that study, oncospheres emerged from what is described here as the oncospheral membrane to remain enclosed by the embryophore.

Adjustment of the salt/water balance may be an important factor in hatching (Sawada, 1967; Lethbridge, 1980). Limited and sporadic movement of *H. nana* oncospheres, whilst retained in their embryonic envelopes, was observed by Schiller (1959) and in intact eggs of *S. cesticillus* (see Reid *et al.*, 1949) and *H. microstoma* (see Collings and Hutchins, 1965). This may have been a result of agitation or temperature change (Lethbridge, 1980). Activated oncospheres are known to emerge from intact eggs (Reid *et al.*, 1949; Sawada, 1960a, 1967; Holmes and Fairweather, 1982; Smyth and McManus, 1989). Lethbridge (1980) considered it is impossible to draw general conclusions from these studies and the role of enzymatic alteration of embryonic membranes has not been determined *in vivo*. The stimulation of this activity has not been fully determined but may involve various ions (Sawada, 1960b, 1967).

Enzymatic alteration of the embryonic envelopes by the host's digestive system is regarded as essential to *in vivo* hatching systems (Lethbridge, 1980). It appears from this

and previous studies (Sawada, 1960b, 1967) that the embrophore is the only membrane that remains to be affected by this enzymatic alteration in *Raillietina* species and closely related taxa.

6.5 Summary

Histologically, there are discrete differences in the musculature of the scolex of *Raillietina* species that appear to be associated with scolex integrity, projection of the rostellum and the suckers as well as orientation of rostellar hooks. SEM and TEM studies have established the position and structure of some of the microtriches observed in the light microscopy studies and have identified others not resolved by light microscopy. The location on the scolex of the 'peg-like' microtriches that were observed and described in the taxonomic study (Chapter 3) has been clarified. The embryophore and oncospheral membrane of a davaineid cestode, *R. beveridgei*, has been examined and further studies will determine if there is morphological relatedness in other species of *Raillietina.*

Chapter 7.

GENETIC ANALYSIS OF *RAILLIETINA* SPECIES FROM EMUS

Regardless of his specialisation, every biologist working with some specific zoological aim must first know which species of animal he is dealing with and must present an exact zoological species diagnosis. (Skrjabin, 1949).

7.1 Introduction

Where difficulties associated with the identification of cestodes occur because of a lack of morphologically informative characters, other sources of evidence may be considered. Combining as many sources of information as possible maximises information, explanation and stability (Hillis, 1987; Spakulova, 2002). In the case of an absence of morphological characters, the use of molecularly-defined units has become applicable (Mariaux, 1996) and may be revealing where morphological variation is limited or the homology of morphological features is unclear (Moritz and Hillis, 1996). The majority of cestode taxa are only marginally defined and are difficult to compare because of a lack of workable characters and a history of poor descriptions (Mariaux, 1996). Molecular methods have been applied to more well-known or medically important groups of cestodes (Bowles *et al.*, 1994; Kokaze *et al.*, 1997; Okamoto *et al.*, 1997; Hancock *et al.*, 2001). These methodologies can also be applied to study particular problems or specific taxa (Mariaux, 1996). Recently, *Raillietina* has been identified as an exceedingly complex genus with approximately 200 species differentiated only by a scant number of definitive characters and containing certain synonomies (Mariaux, 1996; Janovy, 1997).

7.1.1 Molecular analysis using nucleotide sequence data

The advent of molecular techniques has opened new avenues for parasite identification and the study of their systematics and epidemiology (Gasser, 1999). Nucleotide sequence analyses provide large numbers of characters for analysis although only a small fraction of the maximum number of characters has been examined for any organism (Hillis, 1987). The data are absolute and sequences obtained previously or subsequently in different laboratories, using different methods, are directly comparable (McManus and Bowles, 1996). Information from molecular techniques has, more often than not, substantiated earlier work by morphologists (Hillis, 1987).

The 18S nuclear ribosomal (rDNA) gene was chosen on the basis that the gene region has wide phylogenetic utility at many levels including taxonomic studies; regions of relatively high sequence variability are framed by regions of high sequence conservation, allowing easy alignment and the establishment of base position homology between taxa (Hillis *et al.*, 1996; Littlewood *et al.*, 1998). Sequence similarity (i.e. homogeneity) is greater within a species than between species and consequently rDNA can provide useful genetic markers (Gasser, 1999). Molecular analysis of 18S rDNA has proven useful for phylogenetic analyses of the Cestoda (Okamoto *et al.*, 1997; Mariaux, 1998; Zender and Mariaux, 1999; Olson and Caira, 1999; Olson *et al.*, 1999; Kodedova *et al.*, 2000; Skeríková *et al.*, 2001).

Internal transcribed spacers (ITS) rDNA also provide accurate species markers in helminths (Gasser, 1999) and are useful for examining relationships between closely-related species (Hillis *et al.*, 1996). Although the number of studies is limited, ITS2 rDNA has been used successfully to establish PCR-based diagnostic systems in the Cestoda (Bowles and McManus, 1993; Bowles *et al.*, 1995; Gasser and Chilton, 1995; Okamoto *et al.*, 1997; van Herwerden *et al.*, 2000; Král'ová *et al.*, 2001). Intraspecific variation,

comprising either significant sequence and/or length heterogeneity, occurred in the ITS region in these studies, reflecting population variation. In spite of an apparent variation, Gasser and Chilton (1995) suggested that the degree of sequence differences within a species of taeniid tapeworm appeared to be similar or the same and concluded that a PCR-restriction fragment length polymorphism (RFLP) technique of ITS2 rDNA would be useful to differentiate a range of taeniid cestode species.

The mitochondrial DNA Cytochrome Oxidase (CO1) gene is considered to be a neutral marker for revealing relationships because it evolves rapidly and most of the substitutions occur at neutral sites and examination of partial sequences of this gene has been performed successfully on cestodes (Okamoto *et al.,* 1997; Hancock *et al.*, 2001).

The aim of this study is to determine the level of interspecific differences in the 18S, ITS2 and CO1 sequences of morphologically distinct but closely related species of *Raillietina*. Successful use of these techniques will corroborate the morphological and biological characters of *Raillietina* species (Chapter 3) and identify species-specific molecular features (Spakulova, 2002). While it is appreciated that some of these molecular data could be expanded and used, in conjunction with sequence data already accumulated and available on GenBank, to infer a phylogeny, such an undertaking was considered outside the scope of my study.

7.2 Materials and Methods

7.2.1 DNA based characterisation

Five species of *Raillietina* infecting emus were collected as previously described. Collection details appear in Table 40. DNA was extracted from cestode samples using the QIAamp DNA Mini Kit according to the manufacturer's instructions and following the

Table 40. Identity of cestodes used for 18, ITS2 and CO1 molecular analysis. Single specimens unless stated otherwise.

Parasite	Locality	Host	Collection date
Raillietina beveridgei	Keith , SA	Emu	25/v/1998
R. australis	Glossop, SA	Emu	20/x/2000
R. dromaius	Glossop, SA	Emu	20/x/2000
R. mitchelli	Keith, SA	Emu	25/v/1998
R. mitchelli	Keith, SA	Emu	30/vii/1998
R. chiltoni (2 specimens)	Keith, SA	Emu	25/v/1998
Skrjabinia cesticillus	Angle Vale, SA	Fowl	29/xi/1999

tissue protocol and eluted in 150 µl QIAGEN AE buffer of which 1 µl was used in a PCR reaction.

For PCR, primers SSUA: 5'-AMCTGGTTGATCCTGCCAG-3' and SSUBR: 5-TGATCCATCTGCAGGTTCACCT-3' (Littlewood *et al.*, 1998) were used to amplify the 18S rDNA of each cestode in a 50 µl reaction containing 50mM KCl, 10 MM tris-HCl pH 8.3, 2 mM $MgCl_2$, 200µM each dNTP, 100 pmol each primer and 1.5U Amplitaq Gold (Roche Molecular systems). Amplification was performed in a GeneAmp PCR System 2400 (Perkin-Elmer) in 0.2 ml thin-walled tubes containing 50 µl reaction volumes. Cycling conditions were 95°C for 9 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min. PCR products were run on a 1.2% agarose gel, stained in 4 µg/ml ethidium bromide and visualised under UV light. *Zeuxapta seriolae*, a monogenean parasite (Axinidae) of marine kingfish, *Seriola lalandi*, was used as a DNA control in accordance with the origin of the primers (Littlewood *et al.*, 1998).

Following agarose gel analysis, bands were excised from the gel and the PCR product purified using the UltraClean™ 15 DNA Purification Kit (MoBio Laboratories Inc.) and reconstituted in 50 µl of nuclease-free water. The SSU primers above were used

along with primers ZF: 5'-AATTGGAGGGCAAGTCTGGTG-3' and ZR: -AACTAAGAACGGCCATGCACC-3' (based on a ClustalX alignment of a selection of helminth species 18S sequences in GenBank (Littlewood *et al.*, 1998) for sequencing the 18S region in both directions.

DNA sequencing was performed using the ABI Prism® Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). The sequencing reaction was then isopropanol precipitated and analysed on an ABI Prism® 3700 DNA Analyser.

In addition, a short region (151-291 bp, position 685-902; Fig. 170) of DNA sequence data was obtained from PCR products sequenced using fmol DNA cycle sequencing kit (Promega, USA) according to the manufacturers instructions. The 18SrDNA forward primer (10μM) was used to sequence clones of three isolates and the 18SrDNA forward primer 5' –AAGCTCGTAGTTGGATCT-3' was used to sequence the six PCR products. The 18SrDNA forward and 18SrDNA forward2 cycle sequencing reactions were labelled with α-^{33}P-dATP on a Robocycler PCR machine (Stratagene) using the following cycling conditions, 94°C for 30 s, followed by 40 cycles of 50°C for 60 s and 70°C for 30 s. The samples were denatured at 94°C for 5 min, snap chilled on ice and fractionated on a 6% polyacrylamide gel at 60W for 2 h. The sequencing gel was dried on a gel dryer (Model 583, BioRad) and the DNA sequence visualised by autoradiography.

ITS2 PCR primers are as follows: 3S 5' – CGGTGGATCACTCGGCTCGTG and A28 5' - CCTGGTTAGTTTCTTTTCCTCCGC (Okamoto *et al.*, 1997) and R12F 5' – CGGCTTCTTCCTAATATGTGG and R12F 5' – ACCACAGCATCCACAGTTCAC and ITS2Rseq 5' – GACTGATCCGAGGTCAG. The internal sequencing primers were designed from sequences obtained from alignments of 3S/A28 PCR products. DNA was extracted from excised bands of buffered agarose gels using Bresaclean™ (*Raillietina.*

chiltoni) and from PCR reaction (*R. beveridgei, R. australis, R. dromaius, R. mitchelli* and *S. cesticillus*). *Raillietina mitchelli* was processed separately.

CO1 primer design was based on those used by Okamoto *et al.* (1997) and Hancock *et al.* (2001), CO1F 5'- TTTTTTGTGCATCCTGAGGTTTAT AND CO1r 5'- TAAAGAAAGAACGTAATGAAAATG. PCR reactions were performed with the same amplification program altering only the annealing temperature.

7.3 Results

7.3.1 18S rDNA

PCR products on agarose gel are shown in Fig. 174. The amplified 18S rDNA fragments were directly sequenced for each cestode and aligned using ClustalX version 1.64b. Final alignment is shown in Fig. 175 and includes sequence data for *Skrjabinia cesticillus*. A published 18S rDNA sequence of *Raillietina australis* (see Olsen *et al.*, 2001) was obtained from GenBank database and is also included. Nucleotide sequence data were analysed by the neighbour-joining method to obtain a phylogenetic tree shown in Fig. 176. The 18S sequences of the five species of *Raillietina* were 2221 to 2242 nucleotides in length (Table 41) and their GC content was approximately 52%.

The five species of *Raillietina* shared 2091 (92.7%) of the nucleotides over the 2256 alignment positions. Most differences were observed in two regions (alignment positions 709-945 and 1704-1834). Multiple changes, comprising an indel or a substitutional change, occurred at 123 positions in these regions. The length of alignment gaps ranged from 1 to 8 nucleotides. In a total of 193 variable positions between all *Raillietina* species, single base substitutions occurred at 118 alignment positions for which there were no alignment gaps. Most (80%) of the point mutations were transitions involving substitutions between purines (A with G; n= 42) or between pyrimidines (C with

T; n= 52) rather than transversions, i.e. substitutions between a purine and a pyrimidine (n= 24).

Table 41. Length of nucleotide sequences for five species of *Raillietina* and *Skjabinia cesticillus* obtained in this study.

Cestode species	Sequence length
Raillietina beveridgei	2221
R. mitchelli	2221
R. australis	2235
R. chiltoni	2242
R. dromaius	2220
S. cesticillus	2042

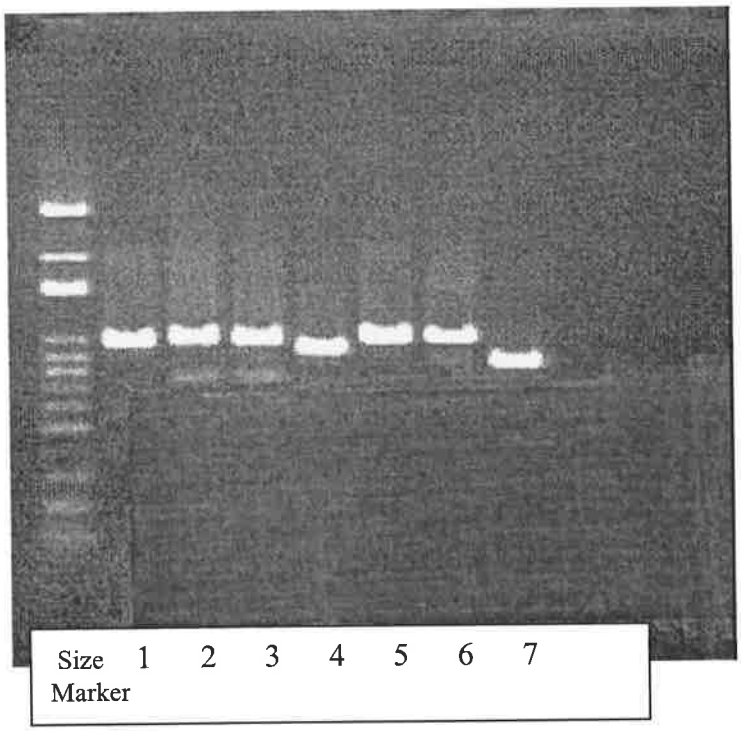


Fig. 174. 18S rDNA amplification, agarose gel. Lane 1, *Raillietina beveridgei*; lane 2 , *R. australis*; lane 3, *R dromaius*; lane 4, *Skrjabinia cesticillus*; lane 5, *R. chiltoni*; lane 6, *R. chiltoni*; lane 7, Control, *Zeuxapta* species (positive control).

Differences between the five *Raillietina* species ranged from 31 to 88 bases (Table 42) where nucleotide positions with gaps were excluded. The 18S sequences of the two specimens of *R. chiltoni* were identical. Short regions of sequence data read from an

autoradiograph were identical to those produced by the ABI Prism® analyser. The GenBank sequence for *R. australis* was most similar to the 18S sequence of *R. beveridgei* obtained here.

Table 42. Pairwise comparisons of the number of nucleotide differences in the 18S sequence among the five *Raillietina* species.

	R. beveridgei	*R. mitchellii*	*R. australis*	*R. chiltoni*
Raillietina. beveridgei	-			
R. mitchelli	65 (2.9%)	-		
R. australis	81 (3.6%)	63 (2.8%)	-	
R. chiltoni	88 (3.9%)	56 (2.5%)	31 (1.3%)	-
R. dromaius	87 (3.9%)	62 (2.8%)	42 (2.8%)	33 (1.5%)

```
#R.beveridgei   TTAAGCCATG CATGTCTCAG TACAGGCCTT CATACGGTGA AACCGCGAAT GGCTCATTAA ATCAGCTATG GTTTATTGGA
#AF286980       .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli    .......... .......... .......... .......... .......... .......... .......... ..........
#R.australis    .......... .......... .......... .......... .......... .......... .......... ..........
#R.chiltoni     .......... .......... .......... .......... .......... .......... .......... ..........
#R.dromaius     .......... .......... .......... .......... .......... .......... .......... ..........
#S.cesticillus  .......... .......... .......... A......... .......... .......... .......... ..........

#R.beveridgei   TCGTACCCGT TAAATGGATA ACTGTAATAA CTCTAGAGCT AATACATGCC TCGATGCCCT GACCCTGTCC TCTTGCGGCT
#AF286980       .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli    ..A....... .......... .......... .......... .......... .......... .......... ..C..T....
#R.australis    ..A....... .......... .......... .......... .......... .......... .......... .....T....
#R.chiltoni     ..A....... .......... .......... .......... .......... .......... .......... .....T....
#R.dromaius     ..A....... .......... .......... .......... .......... .......... ....T..... .....T....
#S.cesticillus  ..A....... .......... .......... .......... .......... ....A..... ..-------.. ----------

#R.beveridgei   CCTTGTAGTT GCAGTTGGGG GCAGGGGAGG GGTGCACTTA TTAGATCAGA AGCCAACCGG CGTTGCGTGT GTAAACACGT
#AF286980       .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli    .G......C. .......T.. .......... .......... .......... .......... .......... ...TT.....
#R.australis    .T......C. .......T.. ...A...... .......... .......... .......... .......... ...TT.....
#R.chiltoni     .T......C. .......T.. .......... .......... .......... .......... .......... ...TT.....
#R.dromaius     ........C. .......T.. .......... .......... .......... .......... .......... ...TT.....
#S.cesticillus  ---------- -......-TC. ..T...A.T. .......... .......... .......TA. T----..G.. TC.CG.T..-

#R.beveridgei   GCGTTGAAGC ACTTCTGGTG ACTCTGGATA ATTGTTACAG ATCGCAGTCG GCCTT-GAGT CGGCGACGGG TCCTTCAAAT
#AF286980       .......... .......... .......... .......... .......... .....-.... .......... ..........
#R.mitchelli    ...C...... .......... .......... .......... .......... .....-.... .......... ..........
#R.australis    ...C...... .......... .......... .......... .......... .....-.... .......... ..........
#R.chiltoni     ...C...... .......... .......... .......... .......... .....-.... .......... ..........
#R.dromaius     ...C...... .......... .......... .......... .......... .....-.... .......... ..........
#S.cesticillus  --.C.A.... .......... .......... .......... .......... .....T.... .......... ..........
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#R.beveridgei  GTCTGCCCTA TCAACTTTCG ATGGTAGGTG ATCTGCCTAC CATGGTGATA ACGGGTAACG GGGAATCAGG GTTCGATTCC
#AF286980      .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli   .......... .......... .......... .C........ .......... .......... .......... ..........
#R.australis   .......... .......... .......... .C........ .......... .......... .......... ..........
#R.chiltoni    .......... .......... .......... .C........ .......... .......... .......... ..........
#R.dromaius    .......... .......... .......... .C........ .......... .......... .......... ..........
#S.cesticillus .......... .......... .......... .C........ .......... .......... .......... ..........

#R.beveridgei  GGAGAGGGAG CCTGAGAAAC GGCTACCACT TCCAAGGGAG GCAGCAGGCG CGTAAATTAC CCACTCCCAG TACGGGGAGG
#AF286980      .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli   .......... .......... .......... .......... .....C.... ..C....... .......... C.........
#R.australis   .......... .......... .......... .......... .......... ..C....... .......... C.........
#R.chiltoni    .......... .......... .......... .......... .......... ..C....... .......... C.........
#R.dromaius    .......... .......... .......... .......... .......... ..C....... .......... C.........
#S.cesticillus .......... .......... .......... .......... .......... ..C....... .......... C.........

#R.beveridgei  TGGTGACGAA AAATACCGAT GCGGGACTCC TTCAAGAGG- CTCCGTAATC GGAATGAGTG GACTCTAAAT CCTTTCACGA
#AF286980      .......... .......... .......... .........- .......... .......... .......... ..........
#R.mitchelli   .......... .......... .......... .........T .......... .......... .......... ..........
#R.australis   .......... .......... .......... .........- .......... .......... .......... ..........
#R.chiltoni    .......... .......... .......... .........- .......... .......... .......... ..........
#R.dromaius    .......... .......... .......... .........- .......... .......... .......... ..........
#S.cesticillus .......... .......... .......... .--.T....- .......... .......... .......... ..........

#R.beveridgei  GGATCAATTG GAGGGCAAGT CTGGTGCCAG CAGCCGCGGT AACTCCAGCT CCAATAGCGT ATATTAAAGT TGCTGCAGTT
#AF286980      .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli   .......... .......... .......... .......... .......... .......... .......... ..........
#R.australis   .......... .......... .......... .......... .......... .......... .......... ..........
#R.chiltoni    .......... .......... .......... .......... .......... .......... .......... ..........
#R.dromaius    .......... .......... .......... .......... .......... .......... .......... ..........
#S.cesticillus .......... .......... .......... .......... .......... .......... .......... ..........
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#R.beveridgei   AAAAAGCTCG TAGTTGGATC TCGGTGGCGT TGTTGCCTGC CGGTATT--T GAGCGGCTGG TGTGTGGTTA GCGGCGCATA
#AF286980       .......... .......... .......... .......... .......-- .......... .......... ..........
#R.mitchelli    .......... .......... .......... .....T.... .......-- .......... .........G .....A....
#R.australis    .......... .......... .......... .......... .......-- .......... .........G .....T....
#R.chiltoni     .......... .......... .......... .......... .......-- .......... .........G ..........
#R.dromaius     .......... .......... .......... .......... .......-- .......... .........G ..........
#S.cesticillus  .......... .......... .....C..A. .T.......T T......AT. .G.....-.. ..CTA..C.G C.....TG.C

#R.beveridgei   GTCGTTGTGC CAGTCTACTA CCTGT----C GGTGTGCCTC ATCC--TC-G CC---CTGTG GCAAA----- ---TCA----
#AF286980       .......... .......... .....----. .......... ....--..-. ..---..... .....----- ---.......
#R.mitchelli    ..T.C..... .TA..... .....GCCT. A......... GG.T--..-. ..---..... A.....---- ---.......
#R.australis    ..TCC..... .T.C..G..G T....----. A..T..... GGTTAG..T. ..---..... ATTG----CT CTG...GTTG
#R.chiltoni     ..T.C....T .T.C..G..G T....----. A..T..... GGTTAG..C. ..AGT..... ..TG.CTACT CTG...GTCG
#R.dromaius     ..T.C....T .T.C.....G T....----. A..T..... GGT.AG..T. .AG--.C... A---.----- ---A------
#S.cesticillus  ..GC.....- --------.. ..G..----- ---------- .--------- ..------C. .----A---- ----------

#R.beveridgei   CGGCGGTGG- -GCTGGGTAC CTGGCATCCT GGTGGTGGGT GGAGCAGTGG CTGTGTTGTC GTTGCCATTG AAAAGCACTG
#AF286980       .........- -......... .......... .......... .......... .......... .......... ..........
#R.mitchelli    .........- -.T......G ........T. .......... .AGC.G.... .......... .......C.. ..........
#R.australis    ...T.....C TA.C.....G .....G.AT. ..C......C ..T..G.... .......... .......... ..........
#R.chiltoni     ..AT.....C GATC.....G .....G.GT. ..C......C ..T..G.... .......... .......... ..........
#R.dromaius     ...TA....C T.TC.....G .....G.GT. ..C......C ..T..G.... .......... .........A ..........
#S.cesticillus  ---------- ----....-. .GC....G-- ---....C.. .---.GTG.. .A..C.---- ....------ -..T..G.A.

#R.beveridgei   TCGTATCGAG CTGGCAAGGT GGTGGCGTCA CCTTTAAGCC ATGTCTGTGG TCTGGCAACA GCCACAGGTG TAGGCGGGTG
#AF286980       .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli    .......A.. .......... .A........ .......... .......... .......... ...G...... ..........
#R.australis    .......A.. .......... .A...T.... .......... .......... .......... ...G...... ..........
#R.chiltoni     .......A.. .......... .A...T.... .......... .......... .......... ...G...... ..........
#R.dromaius     .......A.. .......... .A...T.... .......... .......... .......... ...G...... ..........
#S.cesticillus  ....CC.A.. ......T..C .AG.AT..A. ...G...... .......... A---.T.... T......... ..T.......
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#R.beveridgei   TTGGACAGTG CTCTACACAC GCTGTGGGGT CTGTCAGCTC GTCTGCATGC CTT-TAGATG CCCTTCGAAA GGTGTCTGTG
#AF286980       .......... .......... .......... .......... .......... ...-...... .......... ..........
#R.mitchelli    .......... .C........ .......... .....G.... .......... ...-.G.... .......... ..........
#R.australis    .......... .C........ .......... .....G.... .......... ...-.G.... .......... ..........
#R.chiltoni     .C........ .C........ .......... .....G.... ..T....... ...-.G.... .......... ..........
#R.dromaius     .......... .C........ .......... .....G.... ..T....... ...-.G.... .......... ..........
#S.cesticillus  .C..G..... .A..G...TT .T.....A.C .....G..C. ..G....... .C.C.T.G.. .....AA... ......G.G.

#R.beveridgei   GGCGGATGGC ACGTTTACTT TGAACAAATT TGAGTGCTCA AATCAGGCCG ACGTTGCCTG AAAAGTTTTG CATGGAATAA
#AF286980       .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli    .......... .......... .......... .......... .......... .T........ .......... ..........
#R.australis    .......... .......... .......... .......... .......... .......... .......... ..........
#R.chiltoni     .......... .......... .......... .......... .......... .......... .......... ..........
#R.dromaius     .......... .......... .......... .......... .......... .......... .......... ..........
#S.cesticillus  .......... .......... .......... .......... .......... .T........ ..T.C..... ..........

#R.beveridgei   TGGAATAGGA CTTCGGTTCT ATTTCGTTGG TTTTTGGATC CGAAGTAATG ATCAAAAGGG ACAGGCGGGG ACGTTTGTAT
#AF286980       .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli    .......... .......... .......... .......... .......... .......... .......... ..........
#R.australis    .......... .......... .......... .......... .......... .......... .......... ..........
#R.chiltoni     .......... .......... .......... .......... .......... .......... .......... ..........
#R.dromaius     .......... ....C..... .......... .......... .......... .......... .......... ..........
#S.cesticillus  .......... .......... .......... ....C..... .......... ........A. .......... ..........

#R.beveridgei   GGCTGCGCTA GAGGTGAAAT TCGTGGACCG TAGCCAGACA AACTAAAGCG AAAGCATTCG TCAAGCATGT TTTCATTGAC
#AF286980       .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli    .......... .......... .......... .......... .......... .......... .......... ..........
#R.australis    .......... .......... .......... .......... .......... .......... .......... ..........
#R.chiltoni     .......... .......... .......... .......... .......... .......... .......... ..........
#R.dromaius     .......... .......... .......... .......... .......... .......... .......... ..........
#S.cesticillus  .......... .......... .......... .......... .......... .......... .......... ..........
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#R.beveridgei  CATGAGCGAA AGTCAGAGGC TCGAAGACGA TCAGATACCG TCCTAGTTCT GACCATAAAC GATGCCAACT GACGATCCGT
#AF286980      .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli   .......... .......... .......... .......... .......... .......... .......... ..........
#R.australis   .......... .......... .......... .......... .......... .......... .......... ..........
#R.chiltoni    .......... .......... .......... .......... .......... .......... .......... ..........
#R.dromaius    .......... .......... .......... .......... .......... .......... .......... ..........
#S.cesticillus .......... .......... .......... .......... .......... .......... .......... ..........

#R.beveridgei  GGTGGTAGTT ATAAAACCTT CCCCACGGGC AGTCCCCGGG AAACCCTTAA GTCTTTGGGT TCCGGGGGAA GTATGGTTGC
#AF286980      .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli   .......... .......... .......... .......... .....T.... .......... .......... ..........
#R.australis   .......... .......... .......... .......... .....T.... .......... .......... ..........
#R.chiltoni    .......... .......... .......... .......... .....T.... .......... .......... ..........
#R.dromaius    .......... .......... .......... .......... .....T.... .......... .......... ..........
#S.cesticillus .......... ...--..... .......... .......... .....T.... .......... .......... ..........

#R.beveridgei  AAAGCTGAAA CTTAAAGGAA TTGACGGAAG GGCACCACCA GGAGTGGAGC CTGCGGCTTA ATTTGACTCA ACACGGGAAA
#AF286980      .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli   .......... .......... .......... .......... .......... .......... .......... ..........
#R.australis   .......... .......... .......... .......... .......... .......... .......... ..........
#R.chiltoni    .......... .......... .......... .......... .......... .......... .......... ..........
#R.dromaius    .......... .......... .......... .......... .......... .......... .......... ..........
#S.cesticillus .......... .......... .......... .......... .......... .......... .......... ..........

#R.beveridgei  ACTCACCCGG GCCGGACACT ATGAGGATTG ACAGATTGAT AGCTCTTTCT TGATTTGGTG GTTGGTGGTG CATGCCC-GT
#AF286980      .......... .......... .......... .......... .......... .......... .......... ....G..-..
#R.mitchelli   .......... .......... ........G. .......... .......... .......... .......... .......C..
#R.australis   .......... .......... .......... .........C .......... .......... .......... .......-..
#R.chiltoni    .......... .......... .......... .......... .......... .A........ .......... ....G..-..
#R.dromaius    .......... .......... .......... .......... .......... .......... .......... ....G..-..
#S.cesticillus .......... .......... .......... .......... .......... .......... .......... .......-..
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#R.beveridgei   TCTTAGTTGG TGGAGCGATT TGTCTGGTTA ATTCCGATAA CGAACGAGAC TCCGGCCTGC TAATTAGTGC ATTTGTCCAC
#AF286980       .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli    .......... .......... .......... .......... .......... .......... .......... ..........
#R.australis    .......... .......... .......... .......... .......... .......... .......... ..........
#R.chiltoni     .......... .......... .......... .......... .......... .......... .......... ..........
#R.dromaius     .......... .......... .......... .......... .......... ...A...... .......... G.........
#S.cesticillus  .......... .......... .......... .......... .......... ...A...... .......... G.........

#R.beveridgei   TGCATCTGTG TAGGCGGCGT TGGACGAGGC TGCTGTTACT GGGTTTTGTG GGTGTC--TA GTGGTGTTGC CGCTTTCGGT
#AF286980       .......... .......... .......... .......... .......... ......--.. .......... ..........
#R.mitchelli    .......... .......... .......... .........C .C.A.....A ......--.T ..A---.... TA.C..T...
#R.australis    .......... .......... ......G... .....C.... .C.G.A.... .ACTA.AG.C ...-.A.... TA.CC.T...
#R.chiltoni     .......... .......... ...G...... .......... .CAG.A..C. ..CTA.AG.C ...-.A.... .ATCC.AC..
#R.dromaius     .......... .......... .......... ......CG.. ACAGC..... ..CTA.AG.C ...-.A.... T..CC.TA..
#S.cesticillus  ...CC...C- .G.A...... ..A.T.C... ....A----- -------... ...T..---- ---------- ----------

#R.beveridgei   TGAACTC--- AGCTGAGATG TGGCGGCGCA TTGGATGCCT GCATGCTCGT GTGTACGGGG TTGTCAGTCC GATGTTAGCC
#AF286980       .......--- .......... .......... .......... .......... ........T- .......... ..........
#R.mitchelli    ..........- .......G.. ....A.T... ...A...... .......... ........C- .....G..T. .G........
#R.australis    ....T..TTC .AA....GC. ...T..T... ...AC..T.. ......CTC. T.......T- .......... .G........
#R.chiltoni     ...GT..TTC .AT....GC. ...T..T... ...AC...T. ......CT.. ........T- .....G.... .G........
#R.dromaius     ..-GT..--C .AT....GC. ...T..T... ...AC..... .......T.. ........T- .....G.... .........T
#S.cesticillus  ---------- ---------- -------.-- ------.... ..-------- -.C.G...C- .A.....A-. .GC.....T.

#R.beveridgei   TCCGGGTGTG GCGCGAATGC CTACTTCTTA GAGGGACAAG CGGGAGAAGC CGCACGAAAT AGAGCAATAA CAGGTCTGTG
#AF286980       .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli    .......... .......... T......... .......... .......... .......... .......... ..........
#R.australis    .......... .......... .......... .......... .......... .......... .......... ..........
#R.chiltoni     .......... .......... .......... .......... .......... .......... .......... ..........
#R.dromaius     .......... .......... T......... .......... .......... .......... .......... ..........
#S.cesticillus  CT....G.C. .......... TC........ .......... .......... .......... .......... ..........
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#R.beveridgei  ATGCCCTTAG ATGTCCGGGG CCGCACGCGC GCTACAATGA CGGTGTCAAC GAGTCAGACC TTCTGGCCCG AAAGGGTTGG
#AF286980      .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli   .......... .......... .......... .......... ........G. .......... .......... ..........
#R.australis   .......... .......... .......... .......... .......... .......... .......... ..........
#R.chiltoni    .......... .......... .......... .......... .......... .......... .......... ..........
#R.dromaius    .......... .......... .......... .........G .......... ....A..... .......... ..........
#S.cesticillus .......... .......... .......... .......... .......... .......... .......... ..........

#R.beveridgei  GCAAACTGGT CAATCACCGT CATGACAGGG ATCGGGGCTT GGAATTGTTC CCCGTGAACG AGGAATTCCT AGTAAGTGCA
#AF286980      .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli   .......... .......... .......... .......... .C........ .......... .......... ..........
#R.australis   .......... .......... .......... .......... .......... .......... .......... ..........
#R.chiltoni    .......... .......... .......... .......... .C........ .......... .......... ..........
#R.dromaius    .......... .......... .......... .......... .......... .......... .......... ..........
#S.cesticillus AT........ .......... .......... .......... .......... .......... .......... ..........

#R.beveridgei  AGTCATAAGC TTGCGCTGAT TACGTCCCTG CCCTTTGTAC ACACCGCCCG TCGCTACTAC CGATTGAATG GTTTAGCAAG
#AF286980      .......... .......... .......... .......... .......... .......... .......... ..........
#R.mitchelli   .......... .......... .......... .......... .......... .......... .......... ..........
#R.australis   .......... .......... .......... .......... .......... .......... .......... ..........
#R.chiltoni    .......... .......... .......... .......... .......... .......... .......... ..........
#R.dromaius    .......... .......... .......... .......... .......... .......... .......... ..........
#S.cesticillus .......... .......... .......... .......... .......... .......... .......... ......T...

#R.beveridgei  GTCCTTGGAT TGGTGCCATA ATGGTGGTAT CCGTGAGGT- TACTGTCAAA CTGGT-ACTG AGAAGAAGAC CAAACTTGAT
#AF286980      .......... .......... .......... .........- ...W...... .....C...C .......A.. S...-...--
#R.mitchelli   .......... .......... ........G. .........- .....C.... .....-.... .......... ..........
#R.australis   .......... .......... ........G. .........- .....C.... .....-.... .......... ..........
#R.chiltoni    .......... .......... ........G. .........- .....C.... .....-.... .......... ..........
#R.dromaius    .......... .......G.. ........G. .........- .....C.... .....-.... .......... ..........
#S.cesticillus .......... C.AC...T.T G.A.CT.CCA ...AA....G .GTA..TCGT ....C-GT.. ......C... ..........
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```
#R.beveridgei   CATTTAGAGG AAGTAA
#AF286980       ---------- ------
#R.mitchelli    .......... ......
#R.australis    .......... ......
#R.chiltoni     .......... ......
#R.dromaius     .......... ......
#S.cesticillus  .......... ......
```

Fig. 175. Nucleotide sequences of 18S rDNA. Dots denote homology with the *Railietina beveridgei* sequence.

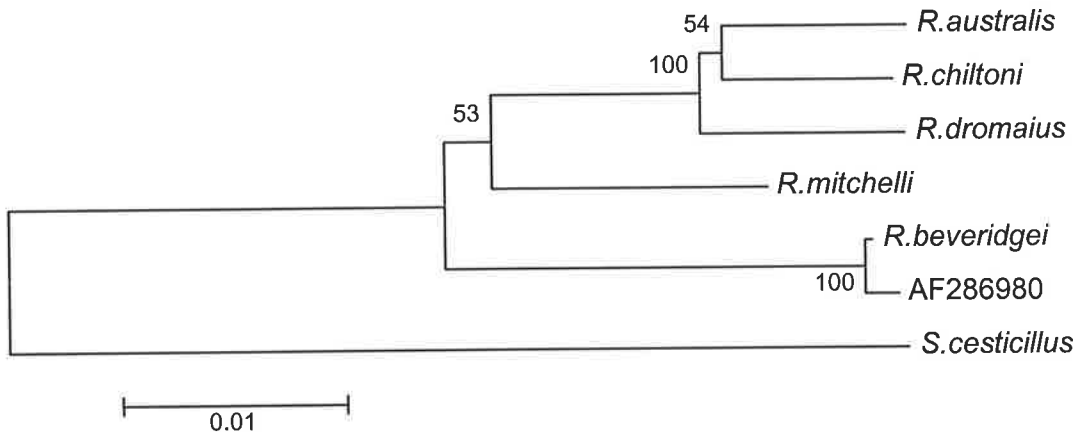

Fig. 176. Neighbour-joining analysis of partial 18S rRNA sequences of *Railletina* species. AF286980 = GenBank accession number for '*Raillietina australis*' (see Olson *et al.*, 2001). Bootstrap support figures are shown at branches.

7.3.2 ITS2 rDNA

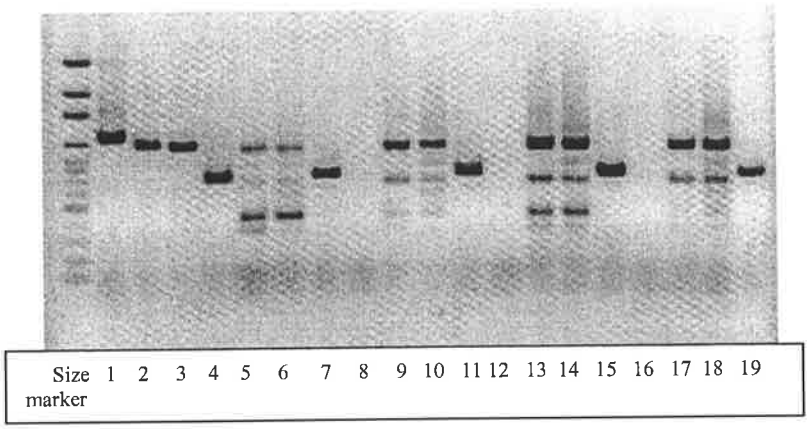

Fig. 177. ITS2 amplification, Agarose gel. Lane 1, *Raillietina beveridgei*, lane 2, *R. australis*, lane 3, *R. dromaius*, lane 4, *Skrjabinia cesticillus*, lanes 5&6, *R. chiltoni,* lane 7, *Zeuxapta* sp., lane 8, negative control, lanes 9,10,13,14,17,18, *R. chiltoni* at annealing temperatures 57°C, 58°and 59°C, lanes 11,15,19, *Zeuxapta* sp., lanes 12,16, negative control.

PCR products on agarose gel appear in Fig. 177. ITS2 sequences were produced for five species of *Raillietina,* however, it was not possible to align the sequence data for *R. mitchelli* with confidence and consequently these data have been excluded from the analysis. The remaining four species could be distinguished and their ITS2 sequences were aligned using ClustalX version 1.64b and appear in Fig. 178. The sequences were 923 to 1081 nucleotides in length (Table 43) and their GC content varied from 49% to 63%.

Table 43. Length of nucleotide sequences for four species of *Raillietina*

Cestode species	Sequence length
Raillietina australis	987
R. chiltoni	923
R. dromaius	974
R. beveridgei	1081

The four cestode species shared 564 (48.9%) of the nucleotides over the 1154 alignment positions. Length of alignment gaps ranged from one to 19 nucleotides. In a total of 288 variable positions between the *Raillietina* species, single base substitutions occurred at 244 alignment positions for which there were no alignment gaps. Point mutations involving substitutions between purines totalled 155 (A with G, n= 83) or between pyrimidines (C with T, n = 72) and those between a purine and a pyrimidine totalled 89. Differences between the *Raillietina* species ranged from 83 to 211 bases where nucleotide positions with gaps were excluded (Table 44). Nucleotide sequence data were analysed by the neighbour-joining method (Fig. 179).

Table 42. Pairwise comparisons of the number of nucleotide differences in the ITS2 sequence among the five *Raillietina* species.

	R. australis	*R. chiltoni*	*R. dromaius*
Raillietina australis	-		
R. chiltoni	83 (8.4%)	-	
R. dromaius	138 (14.0%)	143 (14.5%)	-
R. beveridgei	211 (21.4%)	204 (20.7%)	210 (21.3%)

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#R. australis  TGAATTAGTG TGAATCGCAG ACTGCTTTGA ACATCGACAT CTTGAACGCA TATTGCGGCC ATAGGCTTGC CTGTGGCCAC
#R. chiltoni   .......T.. .......... .......... .......... .......... .......... .......... ..........
#R. dromauis   .......... .......... .......... .......... .......... .......... .......... ..........
#R. beveridgei .......... .......... .......... .......... .......... .......... .......... ..........

#R. australis  GTCTGTCCGA GCGTCGGCTT ATAAACTATC ACTGCGCGTA ATAAGCAGTG GCTTGGGAGA ATGTCGGGTA ATGTGAA---
#R. chiltoni   .......... .......... G......... .......... .......... .......... ...C..A..T ...C...---
#R. dromauis   .......... .......... G......... .........T .......... .......... T..C..CAC. .....G.---
#R. beveridgei .......... .......... G......... .........T .......... .......... T..C..T.C. G.A..GCGGC

#R. australis  -TAGT----C CGCTCTCCCT AGGCTATCGC AGCTGCTG-- ----TCCTCA TGTGCTGTAG T--------- TCCATGTGGG
#R. chiltoni   -....TATG. ..G..... C......... .........-- ----.....- .........-- ---------- -----.....
#R. dromauis   -...C----. T.GCT..T.. C......T.. .A......CC C---.....- ........GC .GTGCTATAG .GTT......
#R. beveridgei C.G..TGTCT .CGAT.AT.A CA.GCG...T C...TTA.AC CATG.....T CCACGCAATC .TTAAAGGTG .A.G......

#R. australis  TGTGCG---- ---------- -----TATTC T------CCT TATTCGC-TC CGC-----CT CTCCTCT-TA TGC-------
#R. chiltoni   .....AATT- ---------- -----.G... A------T.. .G.....-.T ........-. ...T...-.. .C.-------
#R. dromaius   .....ATAT- ---------- -----.CG.A .G------... .G..T..-.. ...GCCGC.. .....T.-.G .C.-------
#R. beveridgei ....T.TATA TTGGCTTTGT TAGCC.C..A .CCGTAT..A .GCGT..G.G ..TGCGTG.A TG.G.G.G.G ...GTGCGTG

#R. australis  C-TATGCGCA TACCTATGTC CCT------G TGTCCCTGTG GTCATGTGTG ---TTTGTG- --TGTGGGCG TCCACG----
#R. chiltoni   .-...TT--. ....CG.... T..------. ..GA.A.T.. ---------- ---.G..C.- --..C.TC.. .....A----
#R. dromauis   .C...T.TGT G...C-...G A..------C C.C.TA.... TGTG..G..T ---.A.....- --.AC.T... .T...A----
#R. beveridgei .G.GC.T..G .G..CGCC.. ...ACCGAT. ...G.A.A.T .CTT...... CGA......G GA.T..C.T. .A...ATAGC

#R. australis  -----GGGTG ACCAGTGT-G AAT------- ---------G GG---GGCTA GGGCGGTTCA CGGATAAGCA AGTGAATGC-
#R. chiltoni   -----..... ----.....- ...------- ---------A .A---..... A..T...... ...G.TGA.. .......A.-
#R. dromauis   ------..A.. .AT..A.GA. G..------- ---------. A.ATG..G.. ..AT..A... G.AG.GG... ....T..A.-
#R. beveridgei GAGTA..A.. .GAT.G.GC. C..TGCAGTG CGGCGCAACA ..ATA..A.. ATTTT...T. T..C.G.... ...CGG...C
```

```
#R. australis  -----GA-TG A----CGAGG ATGGCGTGAG GTGCGGAT-- ---------A GTCGGGATGG AGAGGCATTT GCTGCTGCGG
#R. chiltoni   -------..C. .----..... ......... .........-- ---------. ....A.C... ......G... .........
#R. dromauis   -----.GG.. .GGTG..... G..AGA.... .........-- ---------. .C..A..... GT....G... A..AT..T..
#R. beveridgei TGCGT.GA.. .TGGG..GT. GAT.G....C .GAT.A..GT GAGTGACTG. .A..TAT... T.....G... A.........

#R. australis  CTTCTTCCTA ATATGTGGTC ACTCGTGGTG GCGTAGAGCT GGTGGTTGTG CCATGACTAC AGCATTATGC ATGTGTATAT
#R. chiltoni   .......... .......... ..C....... .......... .......... ....T..... ........A. .........
#R. dromauis   .......... ..G....... ..CT.C.... .......... .......... .......... ..T.....A. ........T.
#R. beveridgei .......... ..G....... G.CTC..... A...C..... .......... .......... ..T..G-.A. ....A.....

#R. australis  ATGTGCATAT GCGTATGCGT GTATGTGTGT ATGTGTGTGT GTGTGGGTAT TATACATGCA TACGTATGTG TGTTTGTGTG
#R. chiltoni   ...C....-- ---------- A......... GCA...A... ---------- -----..A.. .......... ..C......A
#R. dromauis   G.A.A...-- --...C.T.. ..GC...... T......C.. ..A.------ -----..... ....C.CA.. .A..AA...A
#R. beveridgei T.......-- ------AT.. A....C.... G......G.. .C.A------ -----..AT. ..T.C..A.. CAC--..A.A

#R. australis  CGTATGCATA TGACTGCAGT TCACAAACCG TGGCCTAGTG TTCATTTATA TTGA-AGGTG TATGA---GA GTGAGAGTGA
#R. chiltoni   .A....T... ..G....... ......G... .......... .GT..A.... .AT.T.TA.A ...ATATT.. AG.T.T....
#R. dromauis   .A....T... ..G....... .......... .......... .AT.-A.... ....------ ------AG.T ...T......
#R. beveridgei ...G...GCG .AGT...... .......... ....T..A.. .GT.------ ....GG.... CGC..----.T .G.T.TTATC

#R. australis  GAGTGCGAGG GTGTCCATCA CTCCTTCAAT GT--TGTGCT ATACACGTTG CGTGAACTGT GGATGCTGTG GTCAGTGAGT
#R. chiltoni   .G...T.... ...CG..--- .......... ..--....T. ..G....... .......... .......... .......G..
#R. dromauis   ....---.TT ....G..-.. ........G. ..AT.T..TG .AG.....G. .......... .......... .......G..
#R. beveridgei TT...T.T.T .G..G....C ..TGA.GT.. -.AT.A..TA G.......A. A......... ..C....... .......T..

#R. australis  GTGGTGG-TG TGTGTTTA-- ----TGGTAC TCCATGAAGC CACTT---CA TGAAGTAAGT GCAAATGCCT TGATGTGTGT
#R. chiltoni   ...A..---.. ...------- -------... A....A.... ..-------- .......... ..CG...... CA.....--.
#R. dromauis   ...C..CG.. .....G..CG TGTA...C.. AA...T.CA. ..TGAAGC.T ...G.C.... .......TA. CA.....--
#R. beveridgei .CC....-.A CA..------ ----CC..G- -T..ACC... ..G.----.G ...G...G.G C.......GC ----.....--
```

```
#R. australis  GTGTGCGTGT GTGTGGCATT TGTGCTTGCT TCTTTTTTTA A-----TAGT CGGGCTCAGC AAAATCTGGC TTATATTTGT
#R. chiltoni   ---------- --........ ...A...... .........T .ATTGA.... .......... .......... .....G....
#R. dromauis   ---------- --........ .........C ....C...C. TT----.... ......T..T G......... .....A....
#R. beveridgei ---------- --........ ...A...... ....C.AA.C ------..A. G...TCG... .........T .C..G..G..

#R. australis  G----GGTGG GTGGGTTGGT GTGTGTT--- ---------- ------GAAG CGAGAGAGTA GCTGCCCTGA CCTCGGATCA
#R. chiltoni   .-------.T .------C.. ..A.....-- ---------- ------..GC .A........ .......... ..........
#R. dromauis   .-------.T .---------- ---.....-- ---------- ------..GC .A........ ...A...... ..........
#R. beveridgei .TATT.A..T .CT.C..T.. .G..A..TAT TGAATTCAAG AGCTAA..GC .A........ ...A...... ..........

#R. australis  GTCGTGATTA CCCGCTGAAC TTAAGCATAT CAAT
#R. chiltoni   ....C...-. .......... .-........ ....
#R. dromauis   .......... ...A...... .......... ....
#R. beveridgei .......... .......... .......... ....
```

Fig. 178. Nucleotide sequences of Second Internal Transcribed Spacer (ITS2) of Ribosomal DNA. Dots denote homology with the *Raillietina australis* sequence.

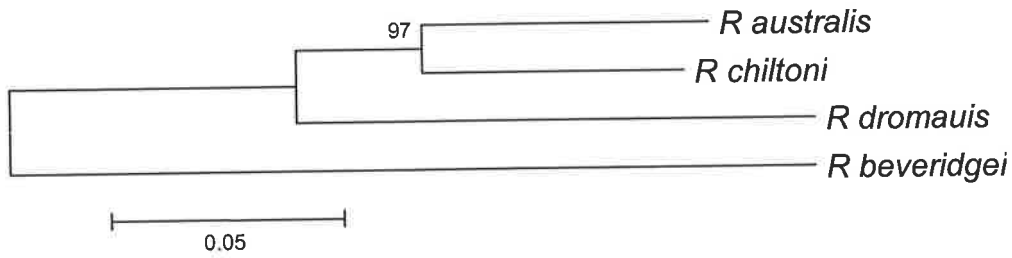

Fig. 179. Neighbour-joining analysis of partial ITS2 sequences of four *Railletina* species.

7.3.3 CO1 mtDNA

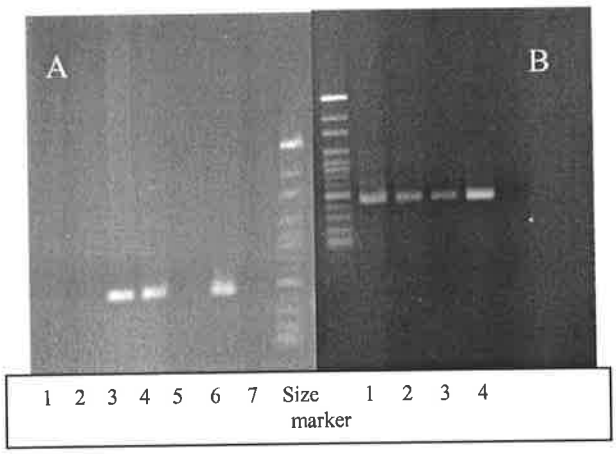

Fig. 180 A. COI amplification, Agarose gel. Annealing temperature 55°C, lane 1, *Raillietina beveridgei*; lane 2, *R. australis*; lane 3, *R. dromaius*; lane 4, *Skrjabinia cesticillus*; lane 5, *R. mitchelli*; lane 6, *R. chiltoni*; lane 7, negative contol; lane 8, size marker. **B.** Annealing temperature 45°C. Lane 1, size marker; lane 2, *R. beveridgei*; lane 3, *R. australis*; lane 4, *R. mitchelli*; lane 5, *S. cesticillus.*

PCR products on agarose gel appear in Fig. 180. The CO1 sequences were aligned using ClustalX version 6.4b (Fig. 181) and were 409 to 424 nucleotides in length (Table 45). Their GC content ranged from 36% to 50%. The *Raillietina* species shared 249 nucleotides over the 424 alignment positions. Alignment gaps ranged from two to eight nucleotides in length.

Table 45. Length of CO1 nucleotide sequences for five species of *Raillietina* and *Skrjabinia cesticillus*.

Cestode species	Sequence length
Raillietina beveridgei	409
R. mitchelli	420
R. australis	424
R. chiltoni	414
R. dromaius	414
S. cesticillus	415

Differences between the five species of *Raillietina* ranged from 53 to 100 nucleotide positions where gaps were excluded (Table 46). Nucleotide sequences were analysed by the neighbour-joining method (Fig.182).

Table 46. Pairwise comparisons of the number of nucleotide differences in the CO1 sequence among the five species of *Raillietina*.

	R. chiltoni	*R. australis*	*R. dromaius*	*R. mitchelli*
Raillietina chiltoni	-			
R. australis	54 (12.7%)	-		
R. dromaius	57 (13.8%)	56 (13.2%)	-	
R. mtichelli	61 (14.7%)	63 (14.9%)	53 (12.8%)	-
R. beveridgei	98 (23.7%)	100 (23.6%)	93 (22.5%)	75 (17.9%)

The nucleotide sequence data reported here are available in GenBank with the following accession numbers: *Raillietina australis* CO1, AY377442; 18S, AY382311; ITS2, AY382317; *R. beveridgei* CO1, AY379526; 18S, AY382312; ITS2, AY382318; *R. chiltoni* CO1, AY379527; 18S, AY382313; ITS2, AY382319; *R. dromaius* CO!, AY379528; 18S, AY382314; ITS2, AY382320; *R. mitchelli* CO1, AY379529; 18S, AY382315; *Skrjabinia cesticillus* CO1, AY379530; 18S, AY382316; ITS2, AY382321.

```
#R. chiltoni       CTGAG--TTA TGTATTGATT TTGCCAGGGT TTGGTATAGT TAGTCATGTG TGTTTAAGGA TAAGGATGAC AGATGATGCT
#R. australis      .....GT... ...G...... ..A..GAAA. .......T.. .......... .......AA. .........T G........G
#R. dromaius       .....--... ...G..A... ..A..T.... .......T.. .G.......A ...A.G..T. ....A...TT G..A......
#S. cesticillus    .....--... ...T.....A ..A..T..A. ....A..TA. .G.....A.T .....G..T. .G..TT.AT. C..G......
#R. mitchelli      .....GT... ...G...... ..A..T.... .......... .G.A..CA.A .....G.AT. .T..T...TT ...G.....G
#R. beveridgei     .....GT... ...T.A.... ..A..T.... ...A...GA. CG.GT.C... .......ATG .T..A.A.TT .A.A......

#R. chiltoni       TTTGGTTTTT ATGGTTTATT GTTTGCTATG TTTTCTATAG TTTGTTTAGG TTCTAGTGTG TGAGGGCATC ATATGTTTAC
#R. australis      .....A.... ..TT.C.G.. A......... .......... .......... ......C..A ..G..T.... ..........
#R. dromaius       .....G.... .......G.. A......... .......... .......... .........A ..G..A.... ..........
#S. cesticilus     .....G.... ........A. T......... ...G...... .....C.T.. AAGA..G..T .....T.... ..........
#R. mitchelli      .....A.... .......... A......... .......... .C........ G.G......T ..G..A.... ..........
#R. beveridgei     .....A..C. .......... AA......CT ...A....TA ...A...... G.G.G....T C.C..TT.C. ..........

#R. chiltoni       TGTCGGATTG GATGTGAAGA CTGCAGTTTT TTTTAGTTCT GTTACTATGA TTATTGGTGT CCCAACTGGT ATAAAGGTTT
#R. australis      A..T..T..A .....T.... ....G..... .......... .......... .A.....G.. A..C...... .......A..
#R. dromaius       A..T.....A .....A.... .......... .......... ..A....... .A..A..G.. T..T..A... ........A.
#S. cesticillus    A..T...... .....T.... ....T..A.. ......A... ..A..A.... .......... T..T..A... ........A.
#R. mitchelli      ...T.....A .....T.... .G.....A.. .......... .....G.... .A........ T.....A... ..........
#R. beveridgei     GC.T..G..A ..A....... .A.....A.. ........A. .C.....A.. .A........ A...CTA..C ........GC

#R. chiltoni       TTACTTGGTT ATATATGTTG ATGAAATCTG GTGTTGAGAA GGGGGAGCCA ATAGTGTGAT GAGTAATTTC TTTTATAGTG
#R. australis      .......A.. .......... .........A A.A..A.A.. .A.A....TT G.T....... .......... .........A
#R. dromaius       .......... G......... .........A .....A.T.. .A.T.....T ..TT.A..G. .GA.T..... ....G....A
#S. cestillus      ....A..A.. .........A T....TAG.C AGA..A.T.. .TCT..T... ..TA.T.... .G...G.A.. ......T..T
#R. mitchelli      .......A.. .......... .........A A....A.T.. .A.T..A... G..A.A.... ..A.TG.... A......A.A
#R. beveridgei     .......A.. .........A .C.......A A....A.T.. .A.T..A... G.T..A.... ..A.TGC... A......A.T

#R. chiltoni       TTATTTACTT TTGGGGGTGT CACCGGTATT GTATTATCGG CTTGTGTGCT TGATAAAGTT TTACATGATA CTTGATT---
#R. australis      .......... .......G.. T..A...... ..GC....T. .......A.. .........C ..G....... .......TGT
#R. dromaius       .......... ....T..G.. A..T...... .....G..T. .......A.. .........G .......... .A.....---
#S. cesticillus    C.T....... ....T..... T..T..A... ........T. .A.....TT. A........G .......... .......T--
#R. mitchelli      ..G....... .C..A..... G..T...... ..T.....T. .A.....A.. .......... .......... .......T--
#R. beveridgei     ......G.A. C.A.T...A. GG.A..C..A .....G.TT. .C..------ --------.. ..G...T... T...G..T--
```

```
#R. chiltoni       --GTGGTTGC ACATT--CAT -ACG
#R. australis      TT..A..... .....TT... T...
#R. dromaius       --..T..... .....--... -...
#S. cesticillus    --..T..A.. .....--... -...
#R. mitchelli      --..A..... T....TT... T...
#R. beveridgei     --........ .....TT... -...
```

Fig. 181. Nucleotide sequences for CO1. Dots denote homology with *Raillietina chiltoni* sequence.

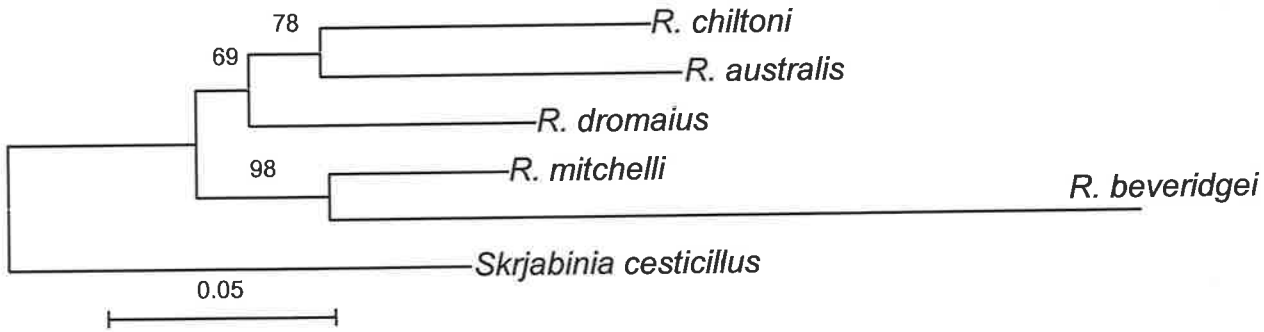

Fig. 182. Neighbour-joining partial sequence analysis of the COI gene.

7.4 Discussion

Molecular techniques have been applied to distinguish the species of *Raillietina* identified *a priori* using more traditional techniques. This study has confirmed the efficacy of the morphological characters used for species diagnosis and has provided an additional aid to overcome problems in morphological variation of life cycle stages, environmental and host-induced modifications (McManus and Bowles, 1996) and morphological variation, such as the increase in size as a result of fixation and the influence of artificial media, known to occur when employing traditional methods for cestode identification (Wardle, 1932a, b).

The inter-specific differences of the 18S gene (Table 42) were smaller than those of the ITS2 and CO1 gene. The 18S gene sequence contained two regions of high sequence variability (approximately 16% of the sequence) and large regions of sequence homology similar to that recorded in previous studies on cestodes (Hillis *et al.*, 1996; Littlewood *et al.*, 1998). The 18S sequences were of similar length (2220-2242) as were the CO1 sequences (414-424), however, the length of the ITS2 sequences showed greater variation. Okamoto *et al.* (1997) found similar results, in an analysis of *Hymenolepis* species and it was suggested that the alignment of the ITS2 by ClustralX might not always be reliable. In this study, ITS2 sequence data for *R. mitchelli* could not be aligned. Chilton (pers. com.) suggested that these difficulties occur because there are multiple variants of ITS in some cestode species and that the sequence data can vary. Different sequences can be obtained from an individual cestode (Bowles and McManus, 1993; Bowles *et al.*, 1994; Bowles *et al.*, 1995; Gasser and Chilton, 1995). For many flatworms, ITS sequences are poorly conserved and difficult to align (Morgan and Blair, 1998a;b; van Herwerden *et al.*, 1998). There are multiple repeats that vary in number among individual species (van Herwerden *et al.*, 1999; van Herwerden *et al.*, 2003) and there are size and sequence variations within

individuals (van Herwerden *et al.*, 1998: Macnish *et al.*, 2002; van Herwerden *et al.*, 2003). In some instances, intra-individual sequence variation is greater than between individuals (van Herwerden *et al.*, 1999). However, ITS1 and ITS2 provide reliable genetic markers for a range of nematode species. The level of intraspecific variation in sequences is low and the differences between morphologically-defined species are consistent (van Herwerden *et al.*, 2000). ITS is therefore regarded as an excellent tool for identifying nematode species that are already known to be different (Blouin, 2002). Because of the difficulties encountered here, it appears that analysis of mitochondrial DNA may be more useful in distinguishing closely related cestode species.

The level of intraspecific variation is not known apart from the examination of the 18S gene of two specimens of *R. chiltoni* and awaits further study. This level of inter-individual variation should be assessed before taxonomic study or characterisation of a population can be undertaken (McManus and Bowles, 1996). The 18S sequence obtained from GenBank was 98.8% similar to the 18S sequence of *R. beveridgei* obtained in this study and confirms that *Raillietina* species can be accurately identified with this methodology but questions its value without accurate identification of the test specimen. It should be remembered that in this case, *Raillietina beveridgei* was only recently recognised and cestodes collected from emus earlier were considered to be *R. australis.*

The results of my study provide data for the selection of target sequences to investigate taxonomic relationships in the genus and closely related taxa. Furthermore, these preliminary studies provide data for direct comparison in larger phylogenetic studies of the family (McManus and Bowles, 1996). There are also practical implications in resolving taxonomic questions such as the validity of closely-related species identified elsewhere in this study, for example *R. geraldschmidti* and *R. infrequens* in cassowaries and the undescribed *Raillietina* species reported from closely related hosts such as the

Kiwi, *Apteryx* sp. (see Clark, 1981). Future studies of this nature are clearly dependent on the availability of suitable material.

7.5 Summary

Differences were detected in the 18S, ITS2 and CO1 sequences of five species of *Raillietina* infecting the emu, *Dromaius novaehollandiae.* The level of interspecific variation ranged from 1.3% to 3.9% for 18S, 8.4% to 21.4% for ITS and 12.7% to 23.7% for CO1. Comparison of limited sequence data indicated that intra-individual variation was minimal for 18S but considerable for ITS2, which complicated analysis. The level of inter-specific differences were consistent for each species and are greater than those reported in other studies of cestodes (Bowles and McManus, 1994; Okamoto *et al.*, 1995; van Herwerden *et al.*, 2000). Intraspecific differences reported in cestodes elsewhere (Hancock *et al.*, 2001) are low and it would be expected that the interspecific differences seen here are significant enough to be useful in the differentiation of *Raillietina* species.

Chapter 8

GENERAL DISCUSSION.

It may not be out of place to mention that this paper is part of a scheme to more fully investigate our bird life (Johnston, 1910).

It has taken a considerable period of time to confirm the presence in Australia of a cestode species described more than 130 years ago collected from an Australian bird in a foreign zoo. This probably reflects the lack of study of the cestode fauna of endemic birds (Beveridge and Jones, 2002). It is not surprising then, that in my study, four new species have been described in the emu and it is conceivable that a considerable number of cestode species in other Australian birds still remain to be documented. Beveridge and Jones (2002) noted, however, that there are relatively few endemic cestode genera, an observation supported by the presence in this study of the cosmopolitan genus, *Raillietina.*

Having established that there are five morphologically distinct species of *Raillietina* in emus in Australia, it became possible to gather additional evidence to support the taxonomic conclusions. In determining the key diagnostic features, the length of the rostellar hooks became the primary feature in diagnosis, enabling accurate identification of species that was supported by statistical analysis. Using this method for species separation, the distribution of each cestode species in the intestine was mapped and patterns became evident. Cestode species occupied a favoured and predictable portion of intestine and examination of the whole gastro-intestinal tract, or at least the entire anterior intestine and a portion of distal intestine, was essential to determine the number of cestode species present in any one bird. The interaction between cestode species and intestinal location was

also confirmed statistically and the number of cestodes present in any intestinal region differed and was dependent on the species inhabiting that region. This observation explained the absence of some combination of *Raillietina* species in archived material.

One of the interesting aspects of the taxonomic study is the similarity of species in closely-related Australian fauna. The cestodes described from the Casuariidae are morphologically similar to those found in the Dromaiidae which supports the suggestion of Beveridge and Jones (2002) that the cestodes of Australian birds are candidates for the study of parasite evolution. When reviewing the cestode fauna of the Dromaiidae and Casuariidae, it was evident that there is considerable similarity in the characters of the strobila but not in the size and number of less variable characters, such as rostellar hooks (Beveridge, 1974) and other characters of the scolex. The morphological differences, for example, between *R. geraldschmidti* and congeners in the Dromaiidae are considered to reflect the difficulties, expressed by others (Mariaux, 1996; Janovy, 1997), in the assessment of the subtle morpho-anatomical differences between closely-related taxa. Further, for *Raillietina*, the validity of the morphological identifications may be disputed because a small number of workable characters and assumptions of host specificity (Wardle, 1932; Mariaux, 1996)

The separation of *Raillietina* species in the Struthioniformes came further into question with the recovery of *R. australis* in an ostrich. This cestode could not be separated on appearance and differed primarily in size from *R. australis* in emus. There are many examples of a cestode species capable of infecting different hosts, developing at different rates or failing to remain established (Bona, 1983; Smyth and McManus, 1989). Furthermore, differences in the size of cestodes are related to the definitive host and size of infection (Stunkard, 1948; Beveridge, 1974). Consequently, the relationship of the species

within closely-related Australian ratites requires further investigation in order to evaluate phenotypic variability.

The spatial boundaries of the *Raillietina* species in the emu indicate that interactions occur between cestodes of different species in this host. Congeneric helminth species typically have niches with minimal overlap (Stock and Holmes, 1988) that can be explained as evolutionary niche divergence (Poulin, 1998), however, reproduction may also have a role in restricted niches (Rohde, 1991; 1994; Poulin, 1998). The spatial segregation identified here may be a result of competition for attachment sites but is considered to favour reproduction (Poulin, 1998) and may be precipitated by size and shape of copulatory organs as previously observed (Rohde, 1991; 1994).

Although the intensities of cestodes in some cases appeared staggering, there is little evidence that there was any loss of condition or depletion of nutrients in the birds examined. One can extrapolate from the biochemical analysis of the liver of wild birds harbouring high intensities that some vitamin and nutrient levels are depleted, but no conclusions can be drawn from the limited data especially given that farmed birds are likely to have received dietary supplementation.

The reduction in size of scoleces and a reduction in the number of capsules per proglottis in high intensities are suggestive of a crowding effect. The investigation set out to determine if growth and fecundity were dependent on cestode intensity. Accepting that there are gross inequalities in size and fecundity in individual cestodes (Poulin, 1998), the analysis suggests an interaction that may only be demonstrated by artificial infection.

The identification of *Pheidole* species complements previous studies in which this ant genus has been identified as the intermediate host for other species of *Raillietina* and closely-related taxa (Artyukh, 1966; Malviya and Dutt, 1971a, b). This ant genus is the second largest, is found worldwide, is easily accessible to birds and probably has had a

long and close association with the cestode genus. As more life cycles are elucidated, the extent of this association will become evident. There appears to be scope for further study relating to the species of *Pheidole* involved, their distribution and also the mechanism of infection of the emu, which at this time is believed to be coincidental rather than an association with seeds.

The cysticercoids described in this study could be distinguished by the length and number of rostellar hooks that correspond to those of the adult tapeworm and further justified the validity of species. It was also possible to discriminate between species by closely examining the structure of the cysticercoid wall which may be of taxonomic value in interpreting specific differences in closely-related taxa. The data collected in this study show that the size of cysticercoids of *Rallietina* species is affected by competition for space and food and complements earlier studies (Reid *et al.*, 1938; Keymer, 1981). The presence of full-size microtriches and rostellar hooks in cysticercoids suggests that they form very early in scolex differentiation or that rostellar hooks are not formed from microtriches, unlike the rostellar hooks of *Taenia* (see Mount, 1970). It appears that examining the very early stages of cysticercoid development in *Raillietina* will provide further information on the development and structural relationships of cestodes.

Microtriches are ubiquitous amongst the Eucestoda and those encountered in this study do not differ from those reported on scoleces of other taxa. The demonstration that the orientation of microtriches is dependent on the eversion or retraction of the rostellum confirms that the 'peg-like' mictrotriches observed on the scolex of *Raillietina* species in light microscopy studies are present on the region known as the rostellar pouch (Mariaux and Vaucher, 1989) or rostellar sheath (Movsesyan, 1977). Aberrations are observed on specimens when the rostellum is retracted.

The results of the TEM study of the egg and capsule of *R. beveridgei* show that the embryonic membranes are delicate and appear to offer little protection to the oncosphere. Proglottides are likely to be gathered relatively quickly by adult ants and returned to a humid underground chamber. Ant larvae are probably infected by eating eggs from whole gravid proglottides. It has been suggested that dispersion of the whole gravid proglottides in this manner may facilitate group infection of intermediate hosts and enable completion of the life cycle in arid environments (Tkach and Swiderski, 1997). In these circumstances, the egg requires little protection. These results expand the information available for the comparison of cestode egg structure, however, because of its remarkable variability and the implication that virtually no two species are exactly alike, further work is needed (Conn, 1999; Swiderski *et al.*, 2001). The mechanism of egg hatching appears, not unsurprisingly, to be identical to that of *R. echinobothrida* (see Sawada, 1967) and suggests that it is consistant for the genus.

The sequence data provide strong evidence in support of the morphological and biological data in distinguishing the *Raillietina* species described in the study. Comparative morphology is of fundamental importance in taxonomy but the study of helminths presents difficulties because of a lack of variation in workable characters (Wardle, 1932a; Mariaux, 1996; Spakulova, 2002). The combination of applications has not previously been employed (although recommended) in *Raillietina* which is recognised as taxonomically complex (Mariaux, 1996; Janovy, 1997). There is intra-individual variation in ITS sequences that has been detected in a variety of flatworms (see van Herwerden *et al.*, 2000). Nevertheless, DNA sequence data has proved useful in further differentiating the cestode species revealed in this study.

The sequence data provide little support for any phylogenetic relationship that might be proposed on morphological or biological grounds. Molecular data indicate that

Raillietina australis and *R. chiltoni* are closely related but distantly related to *R. beveridgei*. *Raillietina beveridgei* is morphologically similar to *R. chiltoni* when comparison of characters (Wardle, 1932a) such as reproductive organs is considered. No associations can be made from comparison of the size and shape of rostellar hooks where *R. beveridgei* is more morphologically similar to *R. australis* than *R. australis* is to *R. chiltoni.* In order to consider phylogenetic relationships further, it would be advisable to analyse a number of genes as well as include morphological data (McManus and Bowles, 1996).

In conclusion, my study has applied a comprehensive range of analyses by combining different methodologies to collect a variety of corroborating data so that taxonomic judgements could be justified. There is confidence that this work has enhanced the historically poor state of knowledge of the cestode fauna of Australian birds (Beveridge and Jones, 2002) and provides a foundation for the investigations to follow.

Appendix A.
Table 47. An updated list of the species of *Fuhrmannetta, Raillietina, Paroniella* and *Skrjabinia* not recorded by or reported since Schmidt (1986).

The species of *Fuhrmannetta*

Species	Host	Locality
R. (F). lophoceri	*Lophocerus erythrorhynchus*	Sth. Africa
R. (F). talourensis	*Gallus gallus*	India

The Species of *Raillietina*

Species	Host	Locality
R. (R). angusta	*Numida meleagridis*	Sth. Africa
R. (R). mathevossiane	*Ammoperdix griseogularis*	Uzbek S.S.R.
R. (R). namaquensis	*Rattus (Aethomys) namaquensis*	Rhodesia
R. (R). thapari	*Picus squamatus*	India
R. (R). bembezi	*Bubo africanus*	Rhodesia
R. (R). bumi	*Bubo africanus*	Rhodesia
R. (R). ortleppi	*Vinago waahli*	Africa
R. (R). douceti	*Turacus* sp.	Ivory Coast
R. (R). loeweni	*Lepus californicus melanotis*	USA
R. (R). apivori	*Pernis aviporus*	USSR
R. (R). coturnix	*Coturnix* sp.	USSR
R. (R). erschovi	*Columba livia*	USSR
R. (R). kirghizica	*Columba livia*	USSR
R. (R). buckleyi	*Streptopelia senegalensis*	India
R. (R). streptopeliae	*Streptopelia t. tranquebarica*	?
R. (R). carneostrobilata	Turkey & Pheasant	Bulgaria
R. (R). gvosdevi	*Streptopelia turtur*	Kazakh SSR
R. (R). macracanthos	*Picus viridus*	Bulgaria
R. (R). somaliensis	*Acryllium ualturinum*	Somalia
R. (R). garciai	*Quiscalus niger brachypterus*	Puerto Rico
R. (R). inda	*Streptopelia chinensis suratensis*	India
R. (R). mehrai	*Columba livia intermedia*	India
R. (R). singhi	*C. l. intermedia*	India
R. (R). toyohashiensis	*Numida galeata*	Japan
R. (R). kaimonjiensis	*Columba livia domestica*	Japan
R. (R). gauricanae	*Oryzomys*	Brazil
R. (R). kyushuensis	*Columba livia domestica*	Japan
R. (R). japonensis	*C. l. domestica*	Japan
R. (R). beppuensis	*C. l. domestica*	Japan
R. (R). oligocapsulata	*Sylvilagus brasiliensis*	Venezuela
R. (R). alectoris	*Alectoris graeca*	Israel
R. (R). bungoensis	*C. l. domestica*	Japan
R. (R). kirghizica	*C. l. domestica*	Japan
R. (R). waltairensis	*Streptopelia chinensis suratensis*	India
R. (R). canabia	*C. l. domestica*	Saudi Arabia
R. (R). zahratis	*C. l. domestica*	Saudi Arabia
R. (R). petronica	*Gallus gallus*	Vietnam

R. (R). teetari	*Francolinus pondicerianus*	India
R. (R). gevreyi	*Tyto alba affinis*	Congo
R. (R). hardyali	*Gallus gallus*	India
R. (R). garmi	*Columba livia*	Tadzhikistan
R. (R). rybickae	*Gallus gallus*	India
R. (R). sonini	*Dendrocopos major*	USSR
R. (R). raillietina	*Gallus gallus*	India
R. (R). oitensis	*Accipiter g. gularis*	Japan
R. (R). kunisakiensis	*Sphenurus s. sieboldii*	Japan
R. (R). selfi	*Sylvilagus auduboni*	USA
R. (R). caballeroi	*Zenaida aurita*	Cuba
R. (R). afghana	*Blanfordimus afghanus*	Afghanistan
R. (R). palawanensis	*Chacophaps indica*	Philipines
R. (R). moldavica	*Picus viridis, P. canus, Junx torquilla*	USSR
R. (R). turnixae	*Turnix tanki*	Vietnam
R.(R?). melomyos	*Melomys rufescens*	Papua NG

The Species of *Paroniella*

Species	Host	Locality
R. (P). tenuiformis	*Gallus gallus domesticus*	Sudan
R. (P). singapurensis	*Oriolus chinensis maculatus*	Malaya
R. (P). barmeriensis	*Corvus splendens*	India
R. (P). japonica	*Corvus levaillanti*	Japan
R. (P). orientalis	*C. corone & C. coronoides*	Japan
R. (P). oitaensis	*Corvus levaillantii*	Japan
R. (P). yapoensis	*Campethera n. nivosa*	Africa
R. (P). assamensis	*Gallus gallus*	India
R. (P). delhiensis	*Numida* sp.	India
R. (P). nedumangadensis	*C. l. domestica*	India
R. (P). capoori	*Francolinus pondicerianus*	India
R. (P). kratochvili	*Pica pica*	Afghanistan
R. (P). beppuensis	*Corvus levaillantii*	Japan

The species of *Skrjabinia*

Species	Host	Locality
R. (S). sudanica	*Gallus gallus domesticus*	Sudan
R. (S). caucasica	Turkey	U.S.S.R.
R. (S). hiyodori	*Hypsipetes a. amaurotis*	Japan
R. (S). doggaddaensis	*Gallus gallus*	India
R. (S). polyhamata	*Numenius phaeophus*	Japan
R. (S). maplestone	*Coturnix coturnix*	Japan

Schmidt (1986) listed 14 species of unknown (sub)generic status, 11 of these were not recorded by Sawada (1964). Schmidt (1986) also listed 23 species of (*R*) *Raillietina* not listed by Sawada (1964); 21 of these were reported prior to 1964 and two before 1986.

Three species of (*R*) *Raillietina* recorded by Sawada (1964) were transferred to other genera prior to 1986.

Sources: Helminthological abstracts, CAB International, Wallingford Oxon, UK
Current contents, OVID Technologies, Inc. ISI® Pyrmont, NSW. Australia
PC-SPIRS™, SilverPlatter International, Norwood, MA USA.

Appendix B.

Table 48. Mean length of the large rostellar hooks from *Raillietina beveridgei* measured in Lactophenol and De Fauré's medium both *en face* and on side.

	En face		On side	
	De Fauré's	Lactophenol	De Fauré's	Lactophenol
Number	20	20	20	20
Mean	18.7	18.9	18.9	19.3
S.D.	0.61	0.60	0.54	0.73
Range	17.6-20	18.4-20	18.4-20	18.4-20

t-test p = 0.489, 0.095 Not significant.

Appendix C. Size of large and small rostellar hooks

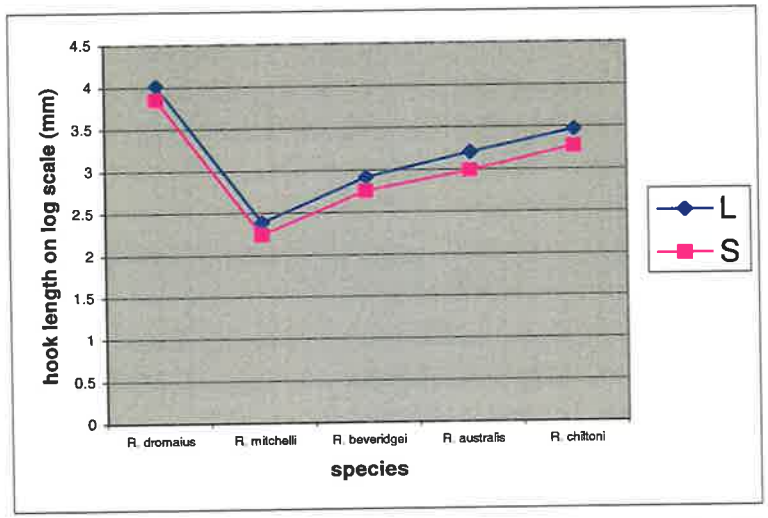

Fig. 183. The means of large (L) and small (S) rostellar hooks are significantly different from one another across 5 *raillietina* species using Tukey and least significant difference (LSD) comparison of means.

Table 49. Size of large and small rostellar hooks (µm)

		Length	Range	S.D.	Confidence Intervals	Number measured
Raillietina australis	Large hook	24.8	20.8-29.6	1.50	24.6-25.0	250
	Small hook	20.1	16.0-23.2	1.24	19.9-20.2	250
Raillietina beveridgei	Large hook	18.6	16.0-20.8	0.98	18.5-18.8	250
	Small hook	15.8	13.6-19.2	1.02	15.7-16.0	250
Raillietina chiltoni	Large Hook	32.3	25.6-39.2	2.48	32.0-32.6	250
	Small hook	26.6	22.4-33.6	2.20	26.4-27.0	250
Raillietina dromaius	Large hook	55.9	50.2-63.2	2.95	55.3-56.5	110
	Small hook	47.8	43.2-53.6	2.39	47.3-48.2	110
Raillietina mitchelli	Large hook	11.0	8.8-12.0	0.76	10.8-11.2	70
	Small hook	9.5	8.8-10.4	0.52	9.4-9.6	70

Appendix D. Statistical representation of rostellar hook length and the distribution of cestode species.

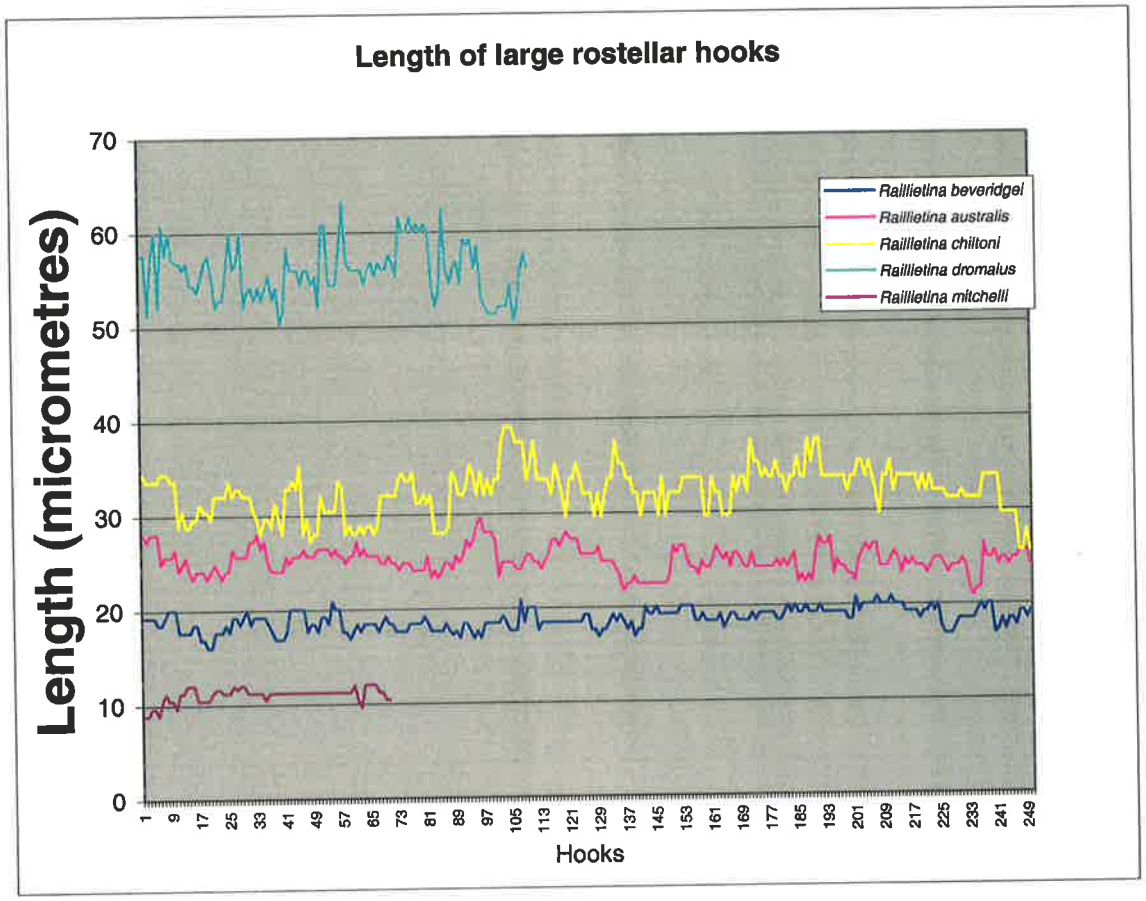

Fig. 184. Length of large rostellar hooks of 5 species of *Raillietina*

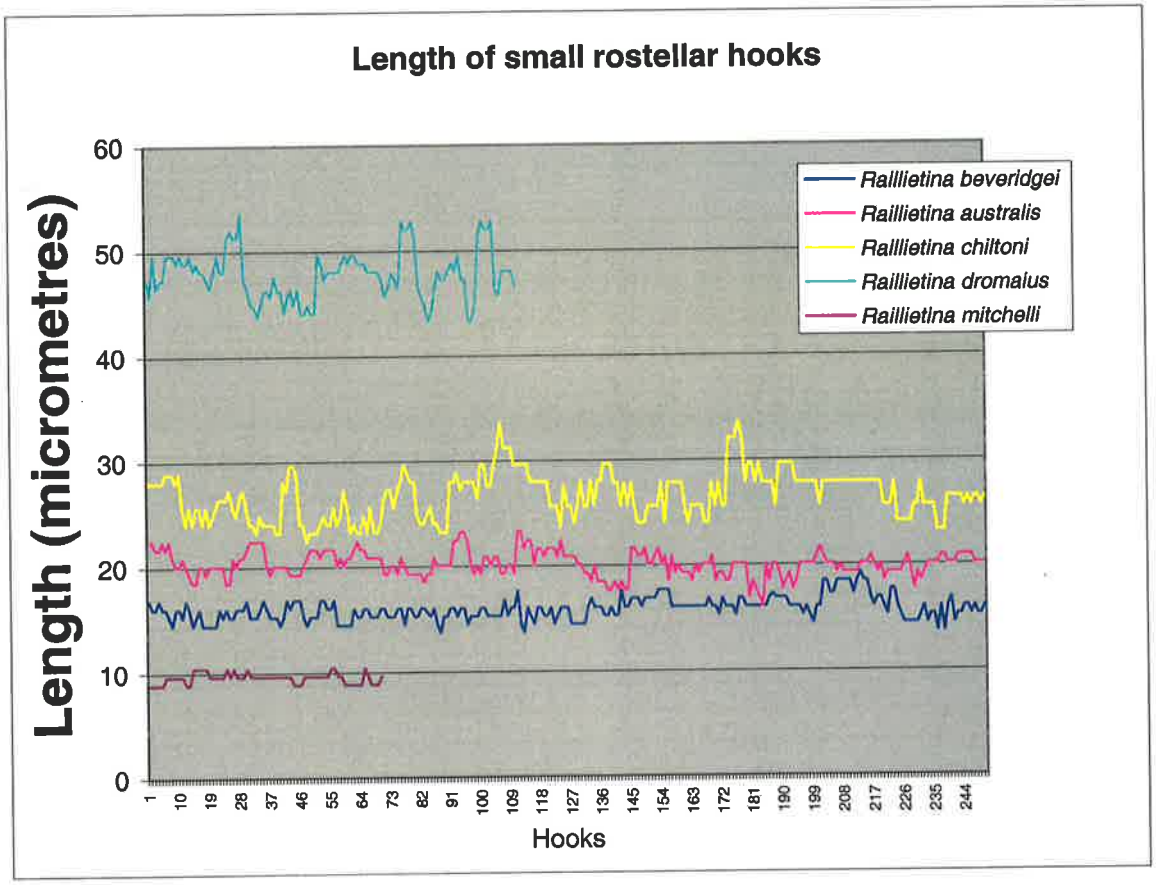

Fig. 185. Length of small rostellar hooks of 5 species of *Raillietina*

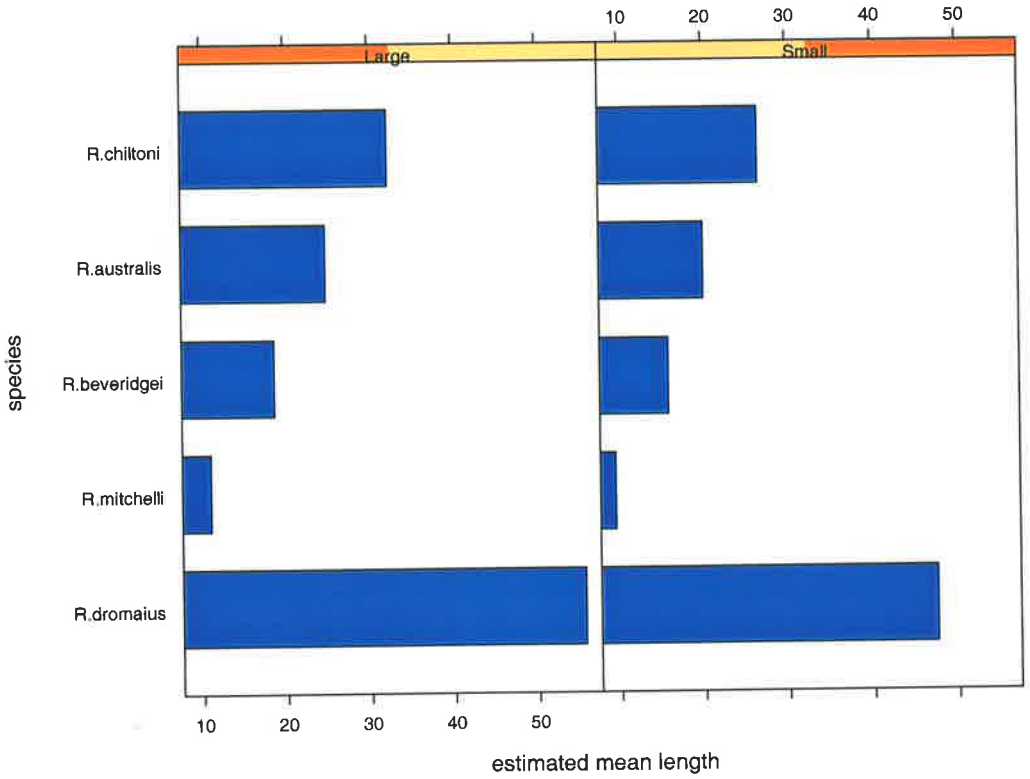

Fig. 186. Back-transformed estimated means for species of *Raillietina* by size interaction effect on hook length ($p<0.001$).

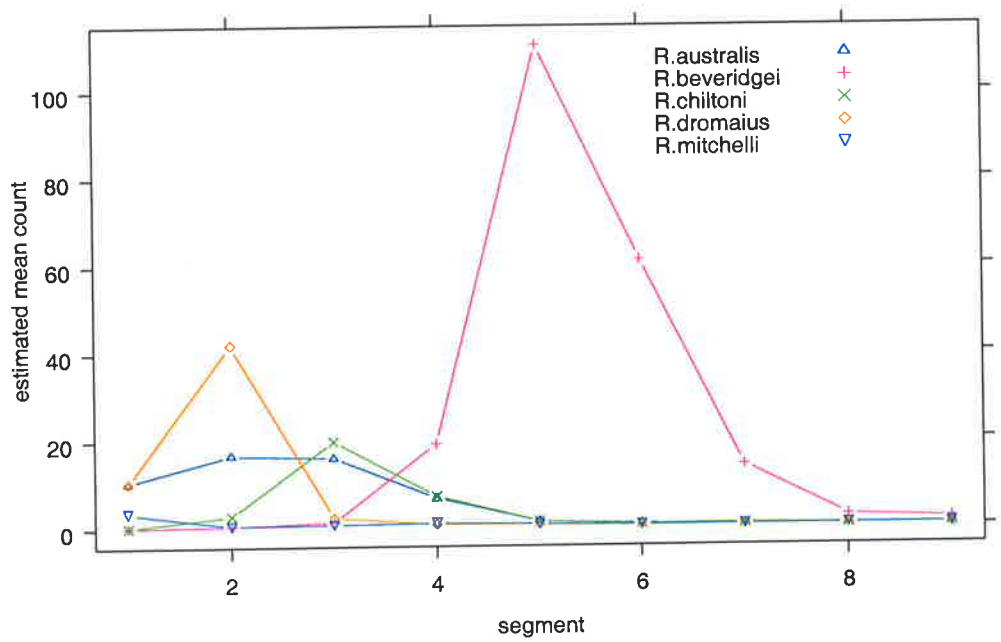

Fig 187. Back-transformed estimated means for segment by species interaction effect of tapeworms of the genus *Raillietina* ($p<0.001$). Segment 1 immediately behind stomach.

Appendix E.

Table 50. Intensity of helminths, other than cestodes, recovered from intestinal tracts of wild birds.

	Wild bird #1	Wild bird #2	Wild bird #3
Dromaeostrongylus bicuspis	370	1960†	5385‡
Trichostrongylus tenuis	60	200	75
Brachylaima cribbi	-	12	-

† Includes 490 immature nematodes. ‡ Includes 4,100 immature nematodes.

Appendix F. Table 51. Biochemical analyses of emu liver. *no result

Locality	Liver	Selenium	Copper	Iron	Zinc	Manganese	Cadmium	Lead	Phosphorus	Magnesium	Calcium	Sodium	Potassium	Vitamin B12
	Date Collected	umol/Kg	mmol/Kg	mmol/Kg	mmol/Kg	mmol/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	nmol/Kg Wet Wt.
Keith, SA	27/10/1998	47.5	0.28	15.0	4.0	0.09	30	0.11	6300	480	168	3740	8130	*
		44.8	0.42	12.0	2.1	0.08	25	0.06	4955	421	118	3140	7680	*
		43.4	0.41	8.0	1.9	0.05	26	0.08	9170	343	80	2300	6630	*
		46.2	0.64	25.0	*	0.11	45	0.06	9570	588	144	3750	10900	*
	9/12/1998	45.8	0.15	10.0	1.7	0.14	1.9	0.13	10000	756	195	3140	11000	*
		46.5	0.22	11.0	2.0	0.11	1.6	0.11	9720	684	169	3030	10200	*
	29/07/1999	27.2	0.13	21.0	1.7	0.09	30	0.11	9610	686	316	4540	9500	*
		30.6	0.12	10.0	1.3	0.09	25	0.06	10400	615	138	3210	9940	348
		47.0	0.19	13.0	2.1	0.14	26	0.08	10000	708	243	5060	11200	962
		26.6	0.13	16.0	1.5	0.04	45	0.06	10100	599	204	3980	10800	985
		37.7	0.19	29.0	2.0	0.11	45	0.06	10300	672	156	3970	10100	882
		26.1	0.14	10.0	1.6	0.09	45	0.06	10600	698	192	3920	10700	1172
	29/06/1999	19.3	0.19	20.0	1.4	0.12	30	0.11	9520	548	76	3360	10500	1538
Kingston, SA	11/08/1998	33.7	0.13	40.0	2.5	0.13	4.7	0.25	*	*	*	*	*	*
Glossop, SA	20/10/2000	31.0	0.06	*	2.1	*	1.6	*	9087	662	197	*	*	876
		25.0	0.18	*	2.3	*	2.2	*	11170	651	211	*	*	851
		27.0	0.24	*	2.9	*	4.8	*	*	*	*	*	*	810
		20.0	0.10	*	2.9	*	1.9	*	12013	692	245	*	*	934
		27.0	0.08	*	2.7	*	4	*	9700	709	203	*	*	900
		24.0	0.20	*	2.6	*	1.7	*	7043	687	192	*	*	580
		28.0	0.15	*	3.1	*	2.2	*	9677	674	189	*	*	909
Wild #1	29/06/1999	32.3	0.20	32.0	3.1	0.15	25	0.06	10400	491	194	6710	9140	280
Wild #2	17/11/1999	13.9	0.14	10.0	1.2	0.09	1.9	0.13	8520	551	165	4370	9680	153
Mean from Keith		37.6	0.22	15.3	2.2	0.09	28.9	0.16	9250	600	169	3626	9791	981
		n=13	n=13	n=13	n=13	n=13	n=13	n=13	n=13	n=13	n=13	n=13	n=13	n=6
Mean from Glossop		26.0	0.14	*	2.7	*	2.6	*	9782	679	206			837
		n=7	n=7		n=7		n=7		n=6	n=6	n=6			n=7
Mean Total		32.6	0.2	17.6	2.2	0.09	18.5	0.16	9422	615	181	3881	9040	805
		n=23	n=23	n=16	n=22	n=16	n=23	n=16	n=21	n=21	n=21	n=15	n=15	n=15
Range	13.9-47.5	0.12-0.64	8.0-40.0	1.2-4.0	0.04-0.15	1.6-45.0	0.06-0.25		4955-11170	343-756	76-316	2300-6710	6630-11000	153-1538

Appendix G. Table 52. Biochemical analyses of emu plasma.

Locality	Plasma Date Collected	Selenium µmol/L	Copper µmol/L	Phosphorus mmol/L	Magnesium mmol/L	Calcium mmol/L	Vitamin B12 pmol/L
Keith, SA	25/05/1999	1.11	7	3.9	3.0	2.8	1490
		*	10	2.0	2.0	2.9	1885
		1.10	9	3.4	2.5	5.1	1724
		1.35	10	3.3	2.3	6.2	2104
		1.44	7	2.5	2.2	2.9	2903
		0.97	6	3.3	2.3	2.7	1681
		1.20	6	2.2	1.6	2.7	2021
		1.31	9	3.3	2.1	3.8	3252
	29/07/1999	*	*	2.2	1.4	2.1	660
		*	*	1.5	1.2	3.8	1425
		*	*	2.9	1.9	3.6	2190
		*	*	1.3	1.1	2.5	1376
		*	*	1.5	1.0	2.5	1707
		*	*	2.4	1.6	3.1	2357
		*	*	1.4	1.0	2.7	1418
		*	*	2.0	1.5	3.3	1321
Glossop, SA	20/10/2000	*	8	1.5	1.3	2.5	3485
		*	9	1.7	1.5	2.8	1074
		*	10	1.6	1.6	3.1	1612
		1.23	8	1.4	1.2	2.6	3499
		*	*	1.7	1.3	2.8	3704
		1.19	7	1.4	1.1	2.6	1148
		1.23	12	2.3	1.5	2.9	1785
		1.30	8	1.6	1.4	2.9	2813
		1.19	7	1.3	1.6	2.7	2889
		1.23	8	1.6	1.3	2.9	2217
Mean		1.22	8.3	2.1	1.6	3.0	2067
Range		0.97-1.44	6-12	1.3-3.9	1.0-3.0	2.1-6.2	660-3799
no. samples		n=14	n=17	n=26	n=26	n=26	n=26

Apendices H.

O'Callaghan, M.G., Davies, M. and Andrews, R.H. (2000). Species of *Raillietina* Fuhrmann, 1920 (Cestoda: Davaineidae) from the emu, *Dromaius novaehollandiae*. *Transactions of the Royal Society of South Australia*, **124**, 105-116.

O'Callaghan, M., Davies, M., and Andrews, R.H., (2000) Species of raillietina fuhrmann, 1920 (Cestoda: Davaineidae) from the emu, dromaius novaehollandiae. *Transactions of the Royal Society of South Australia, v. 124, pp. 105-116.*

Appendix I.

O'Callaghan, M.G., Andrews, R.H., Davies, M. and Spratt, D.M. (2001). Species of *Raillietina* Fuhrmann, 1920 (Cestoda: Davaineidae) from the southern cassowary (*Casuarius casuarius*). *Transactions of the Royal Society of South Australia,* **125**, 133-139.

O'Callaghan, M. G., Andrews, R.H., Davies, M., and Spratt, D.M., (2001) Species of raillietina fuhrmann, 1920 (Cestoda : Davaineidae) from the southern cassowary (casuarius casuarius).
Transactions of the Royal Society of South Australia, v. 125, pp. 133-139.

Appendix J.

O'Callaghan, M.G., Davies, M. and Andrews, R.H. (2003). Cysticercoids from five species of *Raillietina* Fuhrmann, 1920 (Cestoda: Davaineidae) in ants, *Pheidole* sp., from emu farms in Australia. *Systematic Parasitology,* **55**, 19-24.

O'Callaghan, M.G., Davies, M., and Andrews, R.H., (2003) Cysticercoids of five species of raillietina fuhrmann, 1920 (Cestoda: Davaineidae) in ants, pheidole sp., from emu farms in Australia.
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