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VERSITA

Pseudoleptobothrium aptychotremae Young, 1967 (Monogenea, Microbothriidae) redescribed from a new host, *Trygonorrhina fasciata* (Rhinobatidae) in South Australia with a description of the larva and post-larval development

Vanessa Glennon[1]*, Leslie A. Chisholm[1] and Ian D. Whittington[1,2]

[1]Marine Parasitology Laboratory, School of Earth and Environmental Sciences, DP418, University of Adelaide, Adelaide, South Australia 5005; [2]Monogenean Research Laboratory, Parasitology Section, South Australian Museum, North Terrace, Adelaide, South Australia 5000; Australia

Abstract

The adult of *Pseudoleptobothrium aptychotremae* Young, 1967 (Monogenea, Microbothriidae) is redescribed from the dermal denticles of the southern fiddler ray, *Trygonorrhina fasciata* (Rhinobatidae) collected off Adelaide, South Australia. This is a new host and locality record. The anatomy of the larva is described from observations of live larvae and the presence of six needle-like spicules in the larval haptor is confirmed. The development of *P. aptychotremae* is also described.

Key words

Pseudoleptobothrium aptychotremae, Microbothriidae, Monogenea, *Trygonorrhina fasciata*, Rhinobatidae, Australia

Introduction

The southern fiddler ray, *Trygonorrhina fasciata* Müller et Henle, 1841 (Rhinobatidae), is common in coastal waters off Adelaide. Recent detailed examination of *T. fasciata* specimens revealed monogeneans on the skin which matched the description of the microbothriid *Pseudoleptobothrium aptychotremae* Young, 1967 which was originally described from the skin of a different rhinobatid host, *Aptychotrema rostrata* (Shaw et Nodder, 1794) [= *A. banksii* (Müller et Henle, 1841)] in Moreton Bay, Queensland (see Young 1967). *P. aptychotremae* was the first, and until now the only, record of a microbothriid on a member of the Rhinobatidae. No details of the egg or larva were provided by Young (1967). In fact, the larva of just one microbothriid species, *Leptocotyle minor* from the dogfish *Scyliorhinus canicula*, has been described (see Kearn 1965). It was hypothesised that microbothriids might not belong in the Monogenea due to the absence of hooks in the adult haptor (see Bychowsky 1957). However, Kearn (1965) and Kearn and Gowing (1990) confirmed the presence of six curved spicules in the larval haptor of *L. minor* and suggested that these might be vestigial hooklets. Kearn and Gowing (1990) also reported the existence of at least six similar spicules in the larva of *P. aptychotremae*. However, the latter observations were of unhatched larvae and required further verification.

The present study redescribes *P. aptychotremae* from a new host species in a new locality. The anatomy of the larva is described and the development of *P. aptychotremae* is also documented.

Materials and methods

Seven southern fiddler rays (*T. fasciata*) were caught by hand in shallow water at Kingston Point, Seacliff (35°1′59″S, 138°31′29″E), near Adelaide, South Australia between April and May, 2003. Rays were transported alive to the University of Adelaide (UA) and transferred to a 1000 L aquarium containing recirculating, aerated seawater. One piece of plastic fly wire (60 × 50 cm) was secured in the tank to promote a continuous and heavy parasite infection by trapping monogenean eggs (see Ernst and Whittington 1996). Rays were fed daily on chopped pilchard.

Adult and post-larval *P. aptychotremae* were obtained by scraping the dorsal and ventral surfaces of two freshly pithed

*Corresponding author: vanessa.glennon@adelaide.edu.au

rays with a scalpel. Scrapings were brushed promptly, using a soft paintbrush, into glass Petri dishes containing fresh filtered seawater (FSW) filtered through Whatman qualitative paper. The contents of the dishes were then examined for parasites using a dissecting microscope with incident light. Several adult specimens were lightly compressed in a drop of FSW on a slide beneath a coverslip and studied alive using phase contrast and differential interference (Nomarski) microscopes. The presence and position of ducts and glands, which are often seen more easily in live material, were determined. Parasites were then flattened and fixed in 10% formalin under a coverslip. Specimens were either left unstained or were stained in acetocarmine, dehydrated in a graded ethanol series, cleared in cedarwood oil and mounted on microscope slides in Canada balsam beneath a coverslip. Mounted specimens were examined using a compound microscope with phase contrast or Nomarski optics and drawings made with the aid of a drawing tube. Measurements were taken using a computerised digitising system, similar to that described by Roff and Hopcroft (1986). Measurements of the male copulatory organ follow the curve. All measurements are given in micrometres as the mean, with the range and sample size in parentheses.

Larval anatomy was described by studying live larvae. *P. aptychotremae* eggs were collected by isolating individual infected rays kept in the UA aquarium for periods of 2 to 5 h in a 60 L bin containing ~ 40 L of FSW aerated by an air stone. Following isolation, rays were returned to the aquarium. The water from each 60 L bin was filtered through a 63 µm Nitex mesh sieve and the residue examined in a Petri dish containing FSW for eggs laid by parasites *in vivo*. The distinctive tetrahedral eggs of *P. aptychotremae* were easy to distinguish from the eggs of two other monogenean species that also parasitise *T. fasciata*: *Calicotyle australis* (Monocotylidae) and *Branchotenthes octohamatus* (Hexabothriidae).

Pseudoleptobothrium aptychotremae eggs were transferred to glass crystallising dishes (volume approximately 30 ml) and incubated under a LD 12:12 light regime (light on, 07.00 h; light off, 19.00 h) in a controlled light cabinet at ambient room temperature (average daily minima and maxima: 17 to 21°C). Illumination was provided by a 7 W bulb covered with a blue Cinemoid filter inside the cabinet. The desired light regime was achieved by a programmed timer. The FSW for each egg batch was replaced daily. After hatching, individual larvae were transferred to a glass slide in a small drop of seawater then lightly compressed beneath a coverslip. Each larva was viewed using phase contrast optics under an oil immersion lens. Measurements of total larval length and width and pharynx length and width were made when larvae were compressed sufficiently to prevent swimming.

Institutions from which specimens were borrowed or in which they were deposited include: The Natural History Museum (BMNH) Cromwell Road, London SW7 5BD, U.K. (contact: Eileen Harris); South Australian Museum (SAMA), Australian Helminthological Collection (AHC), North Terrace, Adelaide, South Australia 5000, Australia (contact: David Stemmer); United States National Parasite Collection (USNPC), Beltsville, Maryland 20705, U.S.A. (contact: Eric Hoberg).

The following specimens were obtained for comparative purposes: *P. aptychotremae* from *Aptychotrema rostrata* (= *A. banksii*) from Moreton Bay, Queensland, USNPC 61768 (paratypes, 2 slides). An additional microbothriid specimen from the skin of *A. rostrata* collected in 2003 by Dr Bronwen Cribb (University of Queensland) from Moreton Bay was prepared and mounted as previously described and deposited at SAMA (AHC 28917). Eleven microbothriid voucher specimens (AHC 2517), collected from the dorsal surface of *T. fasciata* from Gulf St. Vincent, South Australia by T. Harvey Johnston in 1939 and stored in ethanol, were borrowed from SAMA. Specimens were dehydrated and mounted (six stained, five unstained) in Canada balsam and deposited in SAMA as mounted vouchers (AHC 28918-28).

Results

Microbothriidae Price, 1936
Pseudoleptobothrium Young, 1967

***Pseudoleptobothrium aptychotremae* Young, 1967** (Figs 1–3)

Type host and locality: *Aptychotrema rostrata* (Shaw et Nodder, 1794) (= *A. banksii*) (Rhinobatidae); Moreton Bay, Queensland, Australia (see Young 1967).

Additional host and localities: *Trygonorrhina fasciata* Müller et Henle, 1841 (Rhinobatidae), Kingston Point, Seacliff (35°1′59″S, 138°31′29″E), near Adelaide, South Australia, Australia (present study); Gulf St. Vincent, South Australia, Australia (material collected by T.H. Johnston 1939 but record previously unpublished).

Site on host: Attached to dermal denticles, primarily on anterior dorsal surface.

Prevalence: 14% (1 of 7 rays from Kingston Point, Seacliff infected at time of capture).

Material deposited: 2 vouchers BMNH 2006.1.19.1-2; 4 vouchers SAMA AHC 28929-32; 2 vouchers USNPC 97436.

Redescription based on two live and eight whole mounted adult specimens from *Trygonorrhina fasciata* (Rhinobatidae): Body elliptical, total length 2,019 (1,520–2,899, n = 7). Maximum width 1,044 (888–1,266, n = 8) near middle of body at level of ovary (Fig. 1A). Haptor continuous with body; appears as small posterior depression with thick muscular walls; hamuli and hooklets absent (Fig. 1A). Longitudinal muscle bands fan out anteriorly from haptor into body. Glandular tissue between haptor and posteriormost region of intestine (Fig. 1A).

Mouth terminal, V-shaped. Pigmented eyespots absent. Pharynx cup-like, layered internally 261 (201–305, n = 8) long, 234 (172–288, n = 8) wide. Oesophagus short. Intestinal caeca with 9–12 non-dendritic lateral diverticula (Fig. 1A).

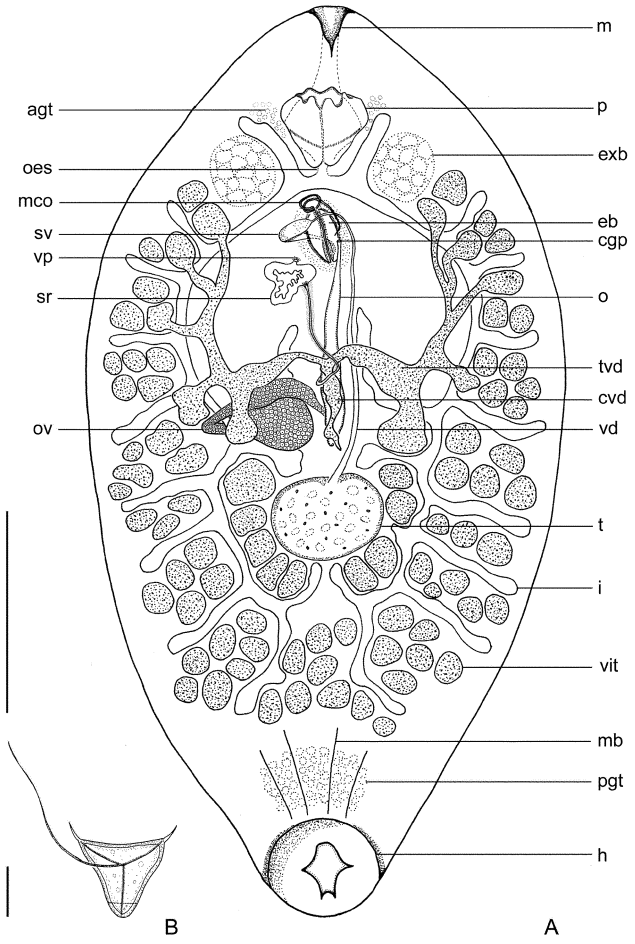

Fig. 1. *Pseudoleptobothrium aptychotremae*: **A** – whole body composite drawing of adult, ventral view; **B** – egg; agt – anterior glandular tissue, cgp – common genital pore, cvd – common vitelline duct, eb – ejaculatory bulb, exb – excretory bladder, h – haptor, i – intestine, m – mouth, mb – muscle band, mco – male copulatory organ, o – ootype, oes – oesophagus, ov – ovary, p – pharynx, pgt – posterior glandular tissue, sr – seminal receptacle, sv – seminal vesicle, t – testis, tvd – transverse vitelline duct, vd – vas deferens, vit – vitellarium, vp – vaginal pore. Scale bars = 500 μm (A), 100 μm (B)

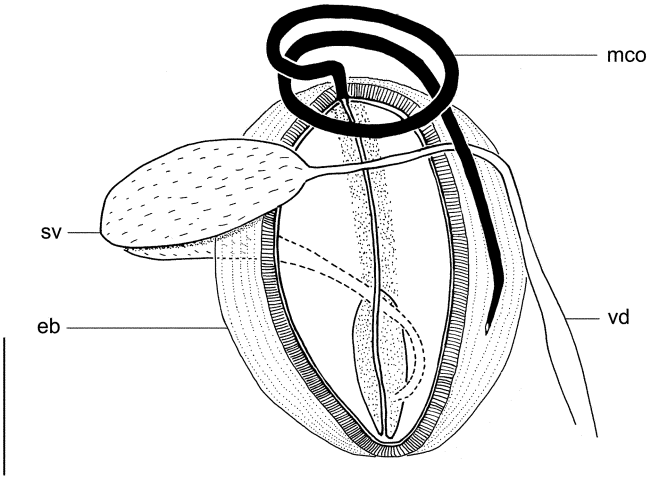

Fig. 2. Male copulatory apparatus of *Pseudoleptobothrium aptychotremae*. Abbreviations as in Figure 1. Scale bar = 50 μm

Excretory bladder present on either side of anteriormost gut branches at level of pharynx (Fig. 1A).

Testis 240 (179–350, n = 8) long, 394 (319–467, n = 8) wide; dorsoventral muscle bundles present (Fig. 1A); occupies intercaecal space posterior to ovary, encircled by vitellarium. Vas deferens runs anteriorly, dorsal to transverse vitelline duct, just left of midline, then narrows, runs medially and expands to form seminal vesicle. Seminal vesicle elongate; loops dorsal to ejaculatory bulb, entering bulb near base (Fig. 2). Ejaculatory bulb 99 (95–104, n = 8) long, 82 (78–85, n = 8) wide; longitudinal muscle fibres visible in muscular wall; conspicuous lumen runs length of bulb surrounded by glandular tissue in basal region where seminal vesicle joins (Fig. 2). Male copulatory organ sclerotised, hollow, 386 (353–417, n = 8) long (Figs 2 and 3A) arises at anterior of ejaculatory bulb, turns sharply, loops dorsoventrally, terminating near common genital pore ventrally at midline (Fig. 1A); distal region narrows and bends slightly (Figs 2 and 3A).

Extensive follicular vitellarium occupies area between lateral body margins and intestinal caeca from level of gut bifurcation, confluent posteriorly; also present within intercaecal space, particularly apparent in posterior half of body, surrounding testis (Fig. 1A). Vitelline ducts from anterior and posterior body regions merge just anterior and ventral to ovary, forming transverse vitelline ducts which meet medially; common vitelline duct posteriorly directed (Fig. 1A). Vaginal pore located just right of midline, approximately level with base of ejaculatory bulb; joins convoluted seminal receptacle (Fig. 1A); long, sinuous duct runs posteriorly from seminal receptacle (Fig. 1A), crosses common vitelline duct obliquely, ventrally then runs dorsal to ootype; path taken dorsal to ootype obscured. Ovary on right side of body (Fig. 1A); proximal region spherical, 211 (153–232, n = 8) in diameter; distal region loops right intestinal caecum dorsoventrally then travels medially. Ootype elongate, thick-walled (Fig. 1A). Eggs tetrahedral, side length 77 (75–80, n = 10); filamentous appendage at abopercular pole (maximum length observed 381); two additional horn-shaped appendages 37 (32–40, n = 10) long (Fig. 1B); measurements based on eggs laid *in vivo*.

Remarks: Specimens from *T. fasciata* (present study) were compared with type material from *A. rostrata* and with unidentified microbothriids from *T. fasciata* collected by T.H. Johnston in 1939 (Table I, Fig. 3). All specimens from *T. fasciata* were generally larger than those from *A. rostrata*, although the range of measurements for most structures overlapped between specimens from both host species (Table I). However, the specimens collected by T.H. Johnston from *T. fasciata* had a male copulatory organ which was much shorter and lacked the dorsoventral loop (Table I, Fig. 3B).

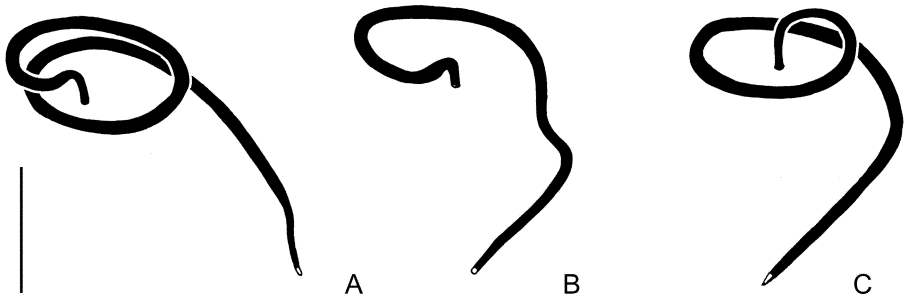

Fig. 3. Morphological variation in the male copulatory organ of adult specimens of *Pseudoleptobothrium aptychotremae*: **A** – from *Trygonorrhina fasciata* (present study). **B** – from *T. fasciata* (specimens collected by T.H. Johnston from Gulf St. Vincent, South Australia, 1939). **C** – from *Aptychotrema rostrata* (Young's 1967 specimens from Moreton Bay, Queensland). Scale bar = 50 µm

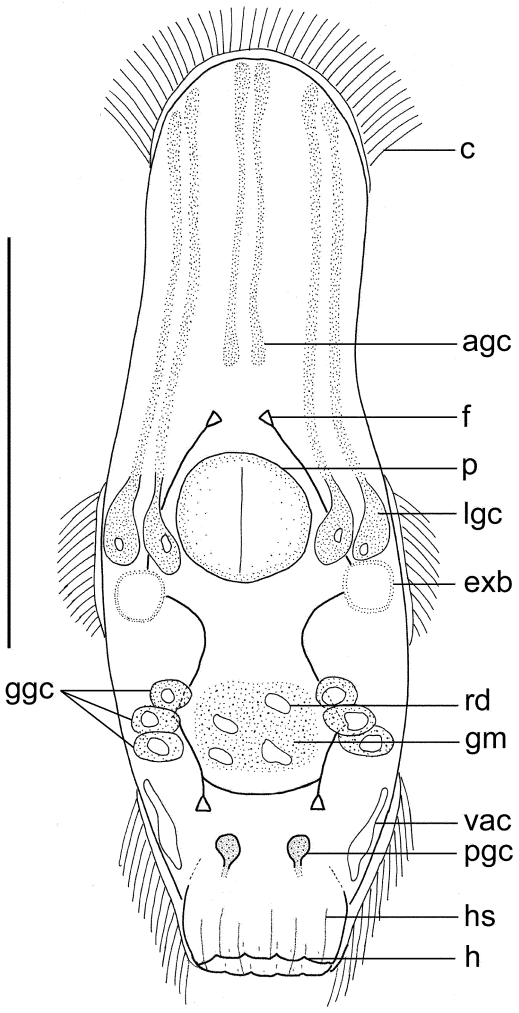

Fig. 4. Larva of *Pseudoleptobothrium aptychotremae*. Whole body, ventral view: agc – anteromedian gland cell containing granular secretion, c – locomotory cilium, exb – excretory bladder, f – flame bulb, ggc – group of three gland cells containing granular secretion, gm – granular mass, h – haptor, hs – haptoral spicule, lgc – lateral gland cell containing granular secretion, p – pharynx, pgc – posterior gland cell containing granular secretion (haptoral cement gland?), rd – refringent droplet, vac – vacuole. Scale bar = 50 µm

The duct leading from the seminal receptacle was illustrated by Young (1967) as crossing the left transverse vitelline duct dorsally, however, examination of type material and the specimens from *T. fasciata* in this study indicates the path to be ventral (see Fig. 1A). Excretory bladders were not described by Young (1967).

Description of larva (Fig. 4)

Observations based on 10 live larvae. Larva 112 (110–115, n = 10) long, 88 (45–60, n = 10) wide. Three distinct ciliated zones: One anterior zone, two lateral, median patches and one posterior zone surrounding haptor. Refringent droplets not observed in ciliated epidermal cells. Pigmented eyespots absent. Pharynx 19 (15–20, n = 10) long, 18 (15–20, n = 10) wide. Mouth not visible. Large darkened mass containing granular secretion and refringent droplets posteromedian to pharynx.

At least two median gland cells containing granular secretion anterior to pharynx; single duct runs anteriorly from each gland cell, opening at anterior end with slightly swollen terminus. Two gland cells containing granular secretion present on either side of body approximately level with pharynx; single duct travels anterolaterally from each gland cell, opening at lateral margins of head with slightly swollen terminus. Group of three gland cells containing dark, granular secretion located on either side of large granular mass; ducts associated with these gland cells not seen. Two gland cells containing granular secretion observed anterior to haptor (possibly haptoral cement glands?). Single elongated vacuole with prominent outline present on either side of body just anterior to haptor.

Two pairs of flame bulbs visible: One pair just anterior to pharynx; one pair in posterior region of body between haptor and darkened, granular mass; ducts connecting flame bulbs (where observed) follow path as shown on the figure. Conspicuous excretory bladders on either side of body level with posterior region of pharynx.

Haptor terminal, cup-like; six needle-like spicules deeply embedded in haptor margin.

Table I. Comparative measurements of *Pseudoleptobothrium aptychotremae* (Microbothriidae) from the skin of two rhinobatid species in Australian waters

	Trygonorrhina fasciata		*Aptychotrema rostrata**
	Kingston Point, Seacliff, South Australia	Gulf St. Vincent, South Australia	Moreton Bay, Queensland
Source of specimens	present study	SAMA specimens (AHC 28918-28)	2 paratypes USNPC 61768 + 1 voucher SAMA (AHC 28917)
Number of specimens	8	11	3
Total length	2,019** (1,520–2,899)	2,214 (1,933–2,346)	1,715 (1,582–1,833)
Body width	1,044 (888–1,266)	1,059 (966–1,123)	758 (626–866)
Pharynx length	261 (201–305)	176 (157–194)	168 (146–181)
Pharynx width	234 (172–288)	179 (163–191)	193 (192–195)
Male copulatory organ length	386 (353–417)	235 (203–249)	326 (313–335)
Ejaculatory bulb length	99 (95–104)	117 (112–128)	55 (49–63)
Ejaculatory bulb width	82 (78–85)	69 (62–82)	26 (22–31)
Testis length	240 (179–350)	170 (147–201)	126 (114–140)
Testis width	394 (319–467)	263 (216–303)	230 (195–263)
Egg side length	77*** (75–80)	–	–

*Young (1967) reported *Aptychotrema rostrata* as *A. banksii*; **only 7 specimens measured for total length; ***10 eggs measured; all values in micrometres, mean followed by range in parentheses.

Post-larval development (Fig. 5)

Eight juvenile *P. aptychotremae* specimens (vouchers SAMA AHC 28933-40) recovered from the skin of *T. fasciata* from Kingston Point, Seacliff during dissections were studied to determine their developmental sequence. The smallest post-larva found was 321 µm long, three times the length of the larva (Fig. 5A). By this size, the intestinal caeca had started to form and early differentiation of tissue into reproductive structures was evident within the intercaecal space (Fig. 5A). Four bands of longitudinal muscle were visible in the haptoral region and gland cells with ducts leading to the haptor margin were also present (Fig. 5A). The lateral gland cells observed in the larva (see Fig. 4) were still clearly visible (Fig. 5A). At 360 µm long (Fig. 5B), the anteriormost intestinal diverticula had formed. The testis, ejaculatory bulb and ovary were more clearly defined and the male copulatory organ and ootype were also discernible by this stage (Fig. 5B). Development of the vitellarium had commenced along the lateral anterior margin (Fig. 5B). At 410 µm long (Fig. 5C), all reproductive structures were well formed including the ovary. Further proliferation of the vitellarium and of the lateral diverticula of the intestine was apparent, as well as elongation of the male copulatory organ (Fig. 5C).

Discussion

Young (1967) proposed *Pseudoleptobothrium* to accommodate the microbothriid species *P. aptychotremae* attached to the dermal denticles of the shovelnose ray *A. rostrata* (= *A. banksii*) from Moreton Bay, Queensland, Australia. Members of *Pseudoleptobothrium* are distinguished from all other microbothriids by the ovary looping the right intestinal caecum. Comparison of the microbothriids collected from *T. fasciata* at Kingston Point, Seacliff with type specimens of *P. aptychotremae* from *A. rostrata* from Moreton Bay, Queensland, revealed only slight differences in body size and male copulatory organ morphology (Table I, cf. Fig. 3A, C). Some variation was also observed in the size and morphology of the male copulatory organ between specimens from *T. fasciata* (present study) and the previously unidentified microbothriids from *T. fasciata* collected by T.H. Johnston (Table I, cf. Fig. 3A, B). However, these morphometric differences may not be definitive. Factors including parasite age (e.g. Kearn 1987), geographical location (e.g. Whittington 1990, Rohde *et al*. 1992), sample size and method of preparation, can all influence morphology. The method of preparation may be particularly relevant in explaining the shorter length of the male

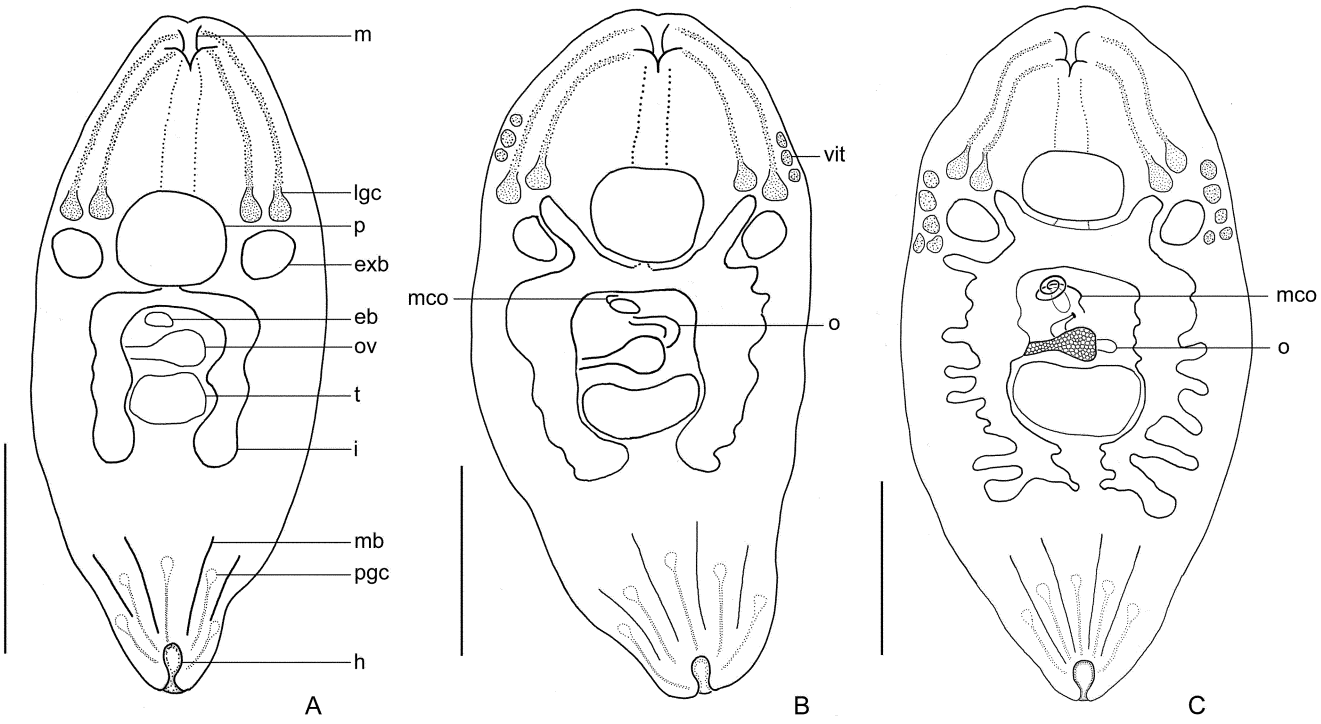

Fig. 5. Post-larval development of *Pseudoleptobothrium aptychotremae*: **A** – early formation of intestinal caeca and differentiation of tissue into reproductive structures (post-larva 320 μm). **B** – anteriormost intestinal diverticula visible; greater definition of testis, ejaculatory bulb and ovary; early stages of male copulatory organ and ootype present; vitellarium visible along lateral anterior margin (juvenile 360 μm). **C** – all reproductive structures well formed; further development of the vitellarium and lateral diverticula of the intestine (juvenile 410 μm). Abbreviations as in Figures 1 and 4. Scale bars = 100 μm

copulatory organ in the microbothriids collected by T.H. Johnston. While these parasite specimens were straight, they were not flattened, so the organ could not be measured accurately and this is likely to have resulted in an underestimate of total length. Overall, the differences we observed between specimens from the two host species are too indistinct to warrant their separation. *T. fasciata* is therefore considered a new host record and South Australia, a new locality record for *P. aptychotremae*.

Examination of the larva of *P. aptychotremae* has confirmed the presence of six spicules in the haptor which is consistent with that observed by Kearn (1965) in the larva of *L. minor*. Kearn (1965) explained the reduction in number and structure of haptoral sclerites in *L. minor* compared to other monogeneans, as a likely consequence of the mode and place of attachment on the host. Post-larval and adult *L. minor* attach directly to the denticle with a cement-like substance (Kearn 1965) and we have observed the same mechanism of attachment for adult *P. aptychotremae* on *T. fasciata*. Post-larval *P. aptychotremae* may similarly attach to the denticle promptly after locating a host. The gland cells we identified near the base of the haptor in the larva (see 'pgc' in Fig. 4) persist throughout parasite development (see Fig. 5) and are likely to be cement glands. All parasite stages were firmly attached to the host and could only be removed by scraping. Therefore hooklets that are incapable of piercing the hard surface of a denticle would be redundant. This supports the interpretation by Kearn (1965) that the six haptoral spicules are not functional larval organs but ancestral vestiges.

The morphology of the eggs of *P. aptychotremae* (tetrahedral) and *L. minor* (ovoid) are different but we could find no obvious morphological features to distinguish between the hatched larvae of these two microbothriid species. Kearn (1965) reported three pairs of flame bulbs in *L. minor*, whereas only two pairs were visible in *P. aptychotremae*. However, the paths of the ducts connecting the anterior and posteromedian pairs of flame bulbs in *P. aptychotremae* were obscured by gland cells (see 'ggc' in Fig. 4). It is therefore possible that a third pair may be present in this region which also corresponds to the position of a pair of flame bulbs in the larva of *L. minor*.

Kearn (1965) described a sac-shaped gut communicating with the pharynx in the larva of *L. minor*. Within the same region in the larva of *P. aptychotremae*, we observed a large, granular mass (see 'gm' in Fig. 4), but we did not see any connection between the mass and the pharynx. However, during early post-larval development in *P. aptychotremae*, the area posterior to the pharynx containing the granular mass differ-

entiates to form the reproductive structures, flanked on either side by intestinal caeca (Fig. 5). The granular mass in the larva of *P. aptychotremae* may therefore be involved in the generation of germ tissue.

On hatching, the pharynx and excretory bladders occupy a median position in the body of *P. aptychotremae* larva (see Fig. 4). As the larva grows, the region posterior to the pharynx elongates to accommodate the developing reproductive organs and intestine (Fig. 5). Sexual development in most monogeneans is widely acknowledged as protandrous (e.g. Whittington 2005) and *P. aptychotremae* conforms to this general rule. The male reproductive organs appear to be fully formed well before the vitellarium completes development (see Fig. 5C). However, despite this apparent maturity, it is unknown whether the male reproductive system is capable of producing sperm at this stage.

Further studies of microbothriid larvae are needed to facilitate a greater understanding of the biology of these monogeneans and to allow more robust comparisons between species. The number and distribution of ciliated cells and sensilla, revealed by staining larvae with silver nitrate, have been of taxonomic value among other monogenean larvae (e.g. Lambert 1978, 1980; Chisholm 1998) and may also prove useful in future for the Microbothriidae.

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