

A NEW SPECIES OF ACOTYLEAN POLYCLAD, *LEPTOSTYLOCHUS VICTORIENSIS* SP. NOV. (PLATYHELMINTHES: POLYCLADIDA) FROM VICTORIA

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ABSTRACT: A new species of *Leptostylochus* Bock, 1925 (Platyhelminthes: Polycladida), *Leptostylochus victoriensis* sp. nov., is described from an intertidal region in western Victoria, Australia. The new species is distinguished from congeners primarily by the morphology of the penis and penis pocket and by the anterior extension of the proximal vagina.

Keywords: Polycladida, morphology, Australia, *Leptostylochus*, new species

Free-living marine flatworms belonging to the order Polycladida Lang, 1884 represent a significant component of the littoral fauna of coastlines around the world. However, the polyclad fauna of south-eastern Australian coastal waters remains relatively poorly studied (Gowlett-Holmes 2008) with 18 species recorded by Prudhoe (1982). A single new species has been described since (Merory & Newman 2005) and *Idioplana australiensis* Woodworth, 1898 has been reported from Phillip Island, Victoria (Rawlinson et al. 2011). Additional records are extremely limited and Gowlett-Holmes (2008) suggested that most species in the region remain undescribed.

The stylochid genus *Leptostylochus* Bock, 1925 is currently represented in Australia only by *L. novaecambrensis* Hyman, 1959, reported from the Sydney region (Hyman 1959). The current paper reports the presence of an additional new species of this genus from the coast of western Victoria.

MATERIALS AND METHODS

Polyclads were collected from under rocks at low tide and were photographed, then fixed following the method of Newman and Cannon (1995) in which worms were coaxed onto filter paper in seawater and then placed on a block of frozen 10% formalin in seawater and left for 24 hours to harden. Specimens were then transferred to 70% ethanol for storage. For examination, specimens were dehydrated in an ethanol series, cleared in methyl salicylate and mounted on slides in Canada balsam. The genital regions of three specimens were excised using a scalpel blade, embedded in wax, and longitudinal serial sections were cut using a microtome at a thickness of 5 µm. Sections were stained

with haematoxylin and eosin. The genital region of one additional specimen was excised, and hand-cut longitudinal sections were mounted in Canada balsam. Drawings were made using a drawing tube attached to an Olympus BH2 microscope and dimensions determined using an ocular micrometer. In this paper, measurements in millimetres are presented as the range followed by the mean and the number of specimens measured in parentheses; following each parameter is the measurement of the holotype in brackets.

The holotype and the majority of the paratypes have been deposited in Museums Victoria (formerly National Museum of Victoria, NMV). Additional paratypes have been deposited in the South Australian Museum, Adelaide (SAM) and the Australian Museum, Sydney (AM).

RESULTS

Polycladida Lang, 1884

Acotylea Lang, 1884

Stylochoidea Poche, 1925

Stylochidae Stimpson, 1857

***Leptostylochus* Bock, 1925**

***Leptostylochus victoriensis* sp. nov.**

(Figures 1–8)

Material examined: Holotype, collected in intertidal zone under rocks, Separation Creek, Victoria (38.62° S, 143.90° E), 5.i.2005 (NMV 237851); 3 paratypes, same collection data (NMV 237852–4); 15 paratypes, same locality, 9.i.2002 (NMV F237855–69, SAM 36323, AM W49215); 6 paratypes, same locality, 3.i.2003 (NMV

237870–75); 3 sets of serial sections (NMV F239769–71); 2 slides of hand-cut sections (NMV F237876–7); entire specimens fixed in formalin, stored in 70% ethanol (NMV F239772–3).

Description: Small polyclads, maximum length when fixed 16–23 (19.2, n=5) [21] (all measurements in mm), maximum width 4–10 (6.8, n=5) [6]. Dorsal surface light fawn in colour, mottled with small white areas; ventral



Figure 1: *Leptostylochus victoriensis* sp. nov. Entire polyclad immediately following fixation showing coloration of dorsal surface (anterior extremity towards top of page). Scale bar = 5 mm.

surface lighter, not mottled; area around gonopores strongly suffused with pink; very slight suffusion with pink extends anteriorly along mid-region of body, slightly more pronounced in region of cerebral organ (Figure 1). Dorsal epithelium composed of columnar cells; cells of ventral epithelium cuboidal, smaller than those of dorsal epithelium; ventral musculature more strongly developed than dorsal musculature.

Cerebral organ mildly bilobed, 0.30–0.38 (0.34, n=5) [0.39] by 0.22–0.30 (0.27) [0.30], situated 2.0–2.5 (2.2, n=5) [2.4] from anterior end (Figure 2). Marginal eyes form narrow band, terminating between level of cerebral organs and pharynx (Figure 2). Cerebral eyes numerous, normally in single fan-shaped array extending halfway from cerebral organ to anterior extremity; in three specimens (Figure 3a), slight tendency to divide into two clusters both anterior and posterior to cerebral organ; in single specimen (Figure 3b), paired lateral clusters of eyes potentially definable as tentacular eyes; tentacles absent.

Pharynx elongate, 5.1–10.1 (7.2, n=5) [7.7], situated in middle of body, with c. 20 ruffles; mouth in second third of pharynx (Figure 2); intestine divaricate, non-anastomosing.

Male gonopore situated in mid-line, 0.9–2.1 (1.4, n=5) [1.6] posterior to pharynx (Figure 4), 2.3–5.8 (3.9, n=5) [7.2] from posterior extremity. Male antrum narrow, extends dorsally and enlarges; poorly developed penis sheath present; penis papilla conical, tapering, elongate; penis extends beyond penis sheath into male antrum; penis unarmed (Figure 5).

Prostatic vesicle not interpolated, pyriform, 0.32–0.92 (0.52, n=5) [0.32] long, 0.18–0.28 (0.23, n=5) [0.27] wide; prostatic vesicle with muscular wall and internal, finger-like projections; elongate, pyriform gland cells enter prostatic vesicle from external surface (Figure 6). Common vas deferens enters penis papilla distally, ventral to prostatic vesicle; runs ventral to vesicle and divides into paired vasa deferentia; spermiducal bulbs absent. Testes scattered ventrally.

Female gonopore 0.25–0.90 (0.52, n=5) [0.92] posterior to male gonopore (Figure 4). Vagina thick walled with ciliated lining; sphincter-like thickening of musculature present at distal extremity of vagina; vagina elongate, runs anteriorly, almost to level of male gonopore, then turns dorsally and runs posteriorly; proximal vagina with fewer cilia internally (Figure 7); paired oviducts enter just anterior to Lang's vesicle; uteri on either side of pharynx, apposed anterior to pharynx; short, sinuous duct leads from entry of oviducts to large, elongate Lang's vesicle, 1.2–2.1 (1.5, n=5) [2.5] long and 0.42–0.62 (0.52) [0.85] wide; Lang's vesicle lined with prominently vacuolated epithelium (Figure 8). Cement or shell glands numerous, extend laterally then both anteriorly and posteriorly from

level of female gonopore (Figure 6); glands denser on ventral surface, extending anteriorly to level of pharynx. Ovaries scattered, dorsal.

DISCUSSION

The species described above belongs to the genus *Leptostylochus* following either of the very different taxonomic systems used for the Polycladida by Faubel (1983) or Prudhoe (1985). The lack of a ventral sucker places the species in the sub-order Acotylea Lang, 1884 in both systems. In the system of Faubel (1983), the free prostatic vesicle (superfamily Craspedomatidea Bock, 1913), the ridged prostatic lining and lack of a cirrus (Stylochidae Stimpson, 1857), the unarmed penis papilla and prominent Lang's vesicle place the species in *Leptostylochus*. In the alternative system of Prudhoe (1985), the presence of marginal eyes (superfamily Stylochoidea Poche, 1926), the free prostatic vesicle lacking prostatoids, the entry of the vasa efferentia proximally, and the eyes disposed in cerebral clusters also place the species in the Stylochidae. The presence of Lang's vesicle (Idioplaninae Bresslau, 1933), the lack of a penis stylet, the shape of Lang's vesicle and the form of the vagina also lead to its placement in *Leptostylochus*. Thus while very different characters are used in the two taxonomic systems, the outcome for placing the current species is similar.

The current composition of *Leptostylochus* is unclear. Both Faubel (1983) and Prudhoe (1985) recognised *L. elongatus* Bock, 1925 (the type species), *L. capensis* Palombi, 1938, *L. gracilis* Kato, 1934, *L. novaecambrensis* Hyman, 1959 and *L. polysorus* (Schmarda 1859) as valid species. Prudhoe (1985) included *L. ovatus* Kato, 1937, which was transferred to *Plehnia* Bock, 1913 by Faubel (1983). *Leptostylochus pacificus* (Kato, 1943) (formerly *Idioplana pacifica* Kato, 1943) was included by Faubel (1983) but not by Prudhoe (1985). Differentiation of the species described herein includes all of the species considered valid by Faubel (1983).

Faubel (1983) also transferred *Idioplana australiensis* Woodworth, 1898 *sensu* Palombi (1928) to *Leptostylochus* under the new combination *L. palombii* Faubel, 1983. However, Prudhoe (1985, p. 58) considered that Palombi's specimens (from Suez) did not fit the description of any known stylochid genus. *Idioplana australiensis* has been reported from Phillip Island, Victoria (Rawlinson et al. 2011) but presumably based on the original description rather than that of Palombi (1928).

Of the above congeners, the species described above most closely resembles *L. capensis* and *L. elongatus* in possessing a sphincter-like thickening of the musculature of the distal vagina. The vaginal sphincter illustrated by Bock (1925) in this species is very small (Bock 1925, Fig.

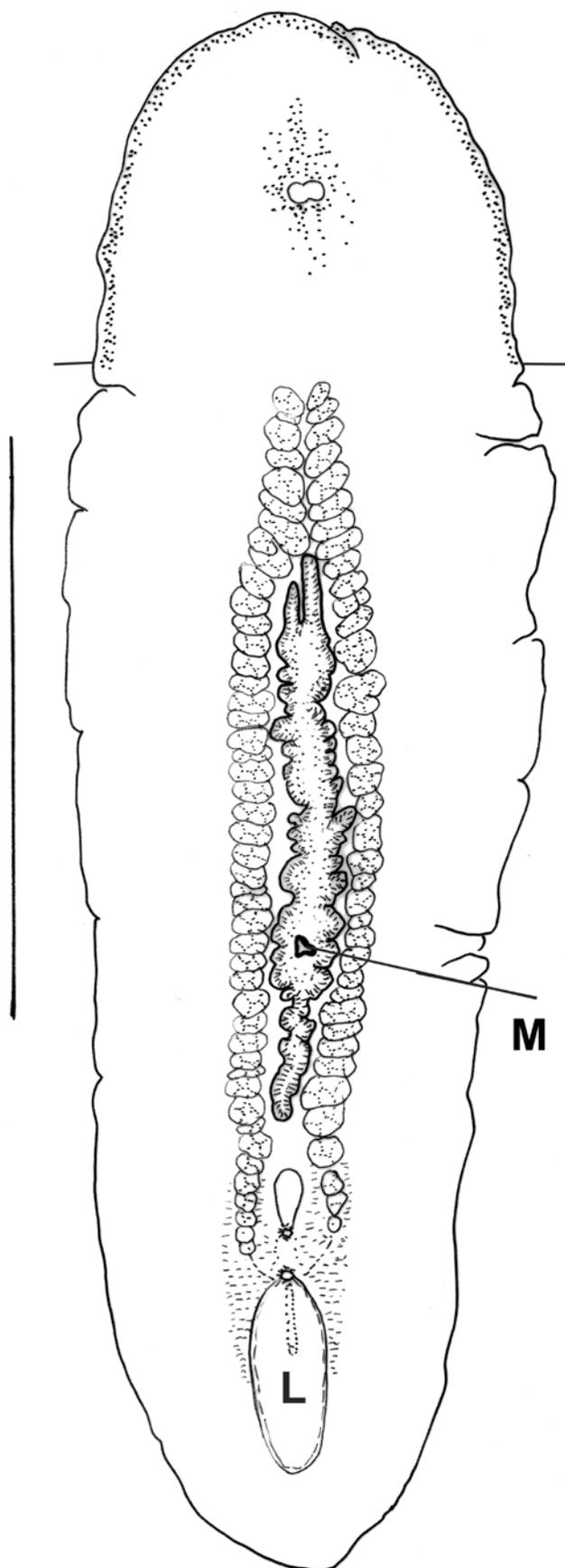


Figure 2: *Leptostylochus victoriensis* sp. nov. Entire polyclad (holotype) (horizontal lines indicate posterior extent of marginal eyes). Scale bar: 10 mm. Legend: M, mouth; L, Lang's vesicle.

13) and was not detected in a re-examination of the species by Holleman (2007). In the present species, the thickening of the musculature of the female antrum is visible in sections and resembles the 'sphincter' in *L. capensis* as illustrated by Palombi (1938, Fig. 5). It is evident as prominent oblique muscle fibres in the wall of the vagina at the female antrum, but could easily be overlooked.

In *L. elongatus*, the cerebral eyes form two more or less distinct clusters, and tentacular eyes are identifiable (Bock 1925), while in the current specimens, a slight separation into two groups was detected in only three specimens and putative clusters of tentacular eyes in only a single specimen. In *L. elongatus*, spermiducal bulbs are present (lacking in the current species) and the penis papilla is conical without an elongate projection into the male antrum (Bock 1925).

In *L. capensis*, two accessory seminal vesicles (or spermiducal bulbs) are present, and the penis is not elongate and does not project into the male antrum (Palombi 1938). In both *L. elongatus* and *L. capensis*, the vagina is relatively short, runs antero-dorsally and then recurves towards Lang's vesicle. In the species described here, the vagina is much longer and runs anteriorly and only slightly dorsally before recurving towards Lang's vesicle.

The current species differs from *L. gracilis* in lacking the slender body shape of that species, from both *L. gracilis* and *L. novaecambrensis* in lacking tentacles and in not having the marginal eyes extending as far posteriorly as the pharynx. In *L. novaecambrensis* the male and female gonopores are adjacent while they are widely separated in the species described above.

Leptostylochus polysorus has an elongate antrum masculinum, a very long penis papilla and prominent spermiducal bulbs (Stummer-Traunfels 1933), both of which the current species lacks.

Leptostylochus pacificus has a vagina which runs anteriorly, dorsal to the male genitalia, with a dorsally recurrent arm anterior to the male genitalia, so that Lang's vesicle lies dorsal to the female antrum (Kato 1943), although these differences may be due to contraction in the specimen. The species is differentiated by the presence of tentacles and a seminal vesicle.

Leptostylochus palombii differs in being pigmented, in having tentacles, a recurved Lang's vesicle and an elongate male antrum (Palombi 1928).

The species described here therefore differs from all currently described species of the genus and is considered to be new. A significant feature of the new species is the lack of a seminal vesicle or spermiducal bulbs, features found in most congeners. Although these can be subtle modifications of the vas deferens, as in *L. novaecambrensis* (see Hyman 1959, Fig. 6), in tracing the course of the vasa

efferentia in the present species, no modifications which could be designated seminal vesicles of spermiducal bulbs were observed. In addition, the new species differed from congeners in the region — *L. elongatus* from New Zealand and *L. novaecambrensis* from New South Wales — in having the cerebral eyes forming a single cluster rather than being distributed in two clusters in the congeners (Hyman 1959; Holleman 2007).

Leptostylochus victoriensis was not common and was found only occasionally, usually in pairs, under rocks at low tide (Figure 9) along a stretch of coastline examined repeatedly from about 10 km north of Separation Creek to about 5 km south of Wye River. On a single occasion, more than 20 specimens were found under a single rock. The species was not found at sites examined either in Western Port (Point Leo) or in Port Phillip Bay (Werribee South, Point Ormond, Canadian Bay). Its preferred habitat may be exposed coastlines. The full geographical distribution of this species, along with many other polyclad species described to date, remains to be determined.

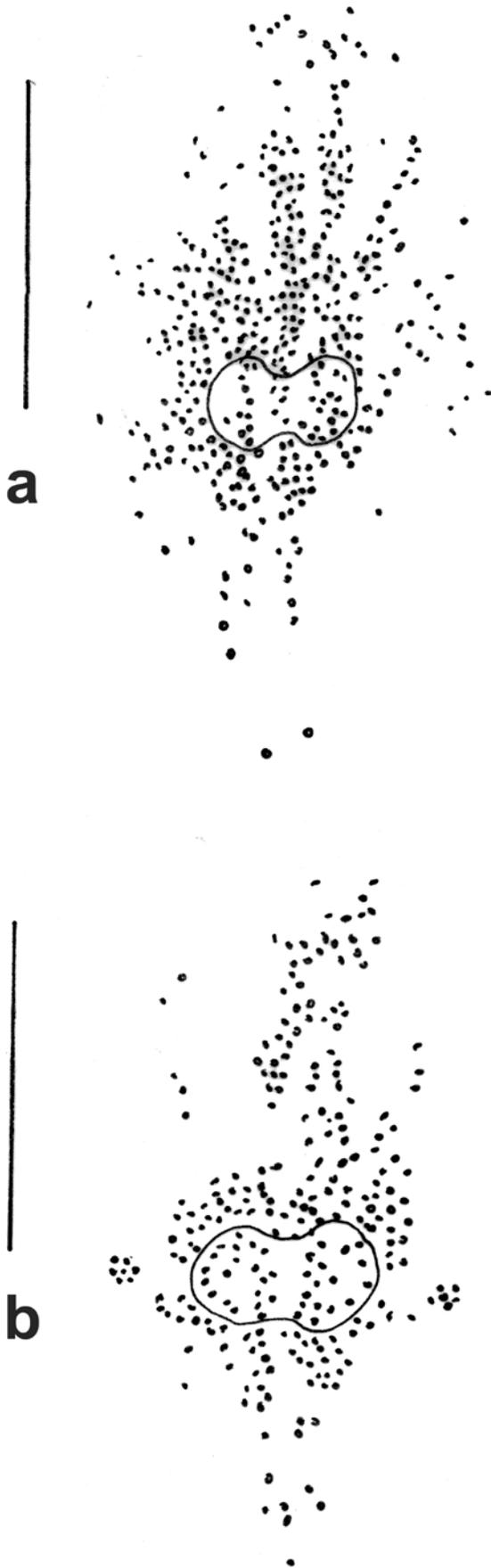


Figure 3: Cerebral organ and eyes showing lack of separation into two lateral groups (a). Cerebral organ and eyes showing possible clusters of tentacular eyes found in single specimen (b). Scale bar = 1.0 mm.

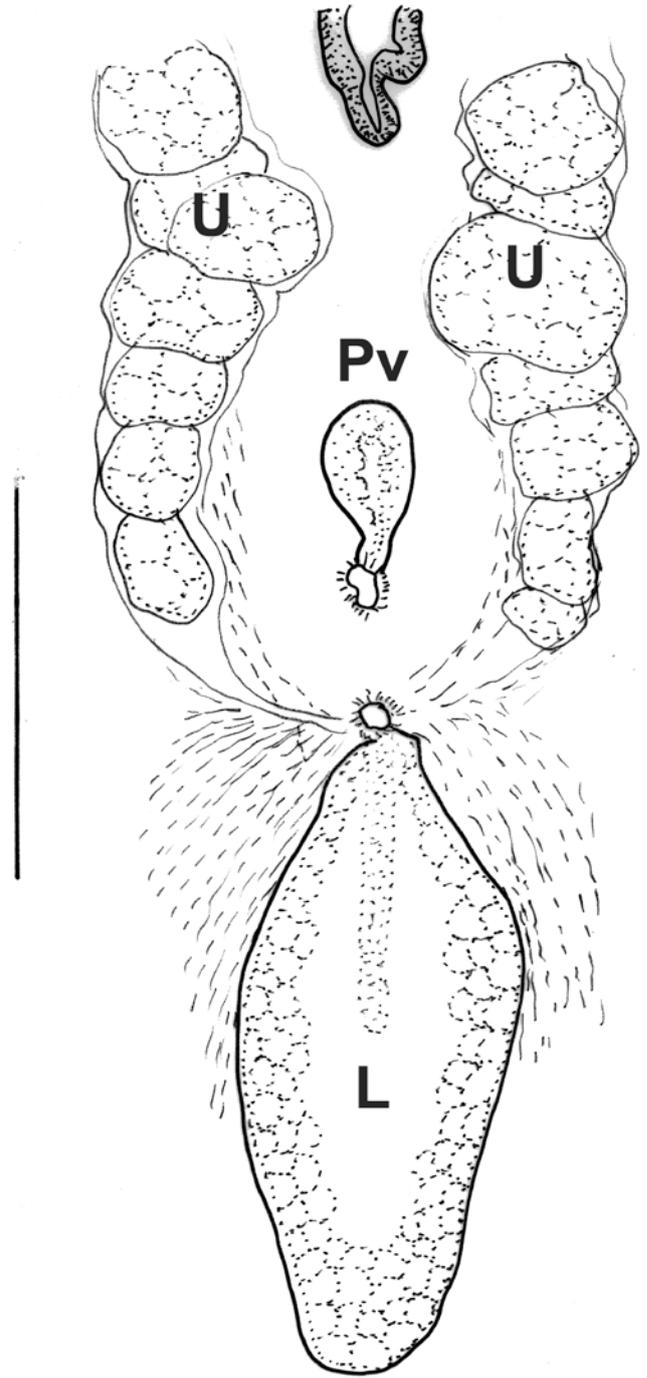


Figure 4: Ventral view of terminal genitalia. Scale bar = 0.1 mm. Legend: L, Lang's vesicle; Pv, prostatic vesicle; U, uterus.

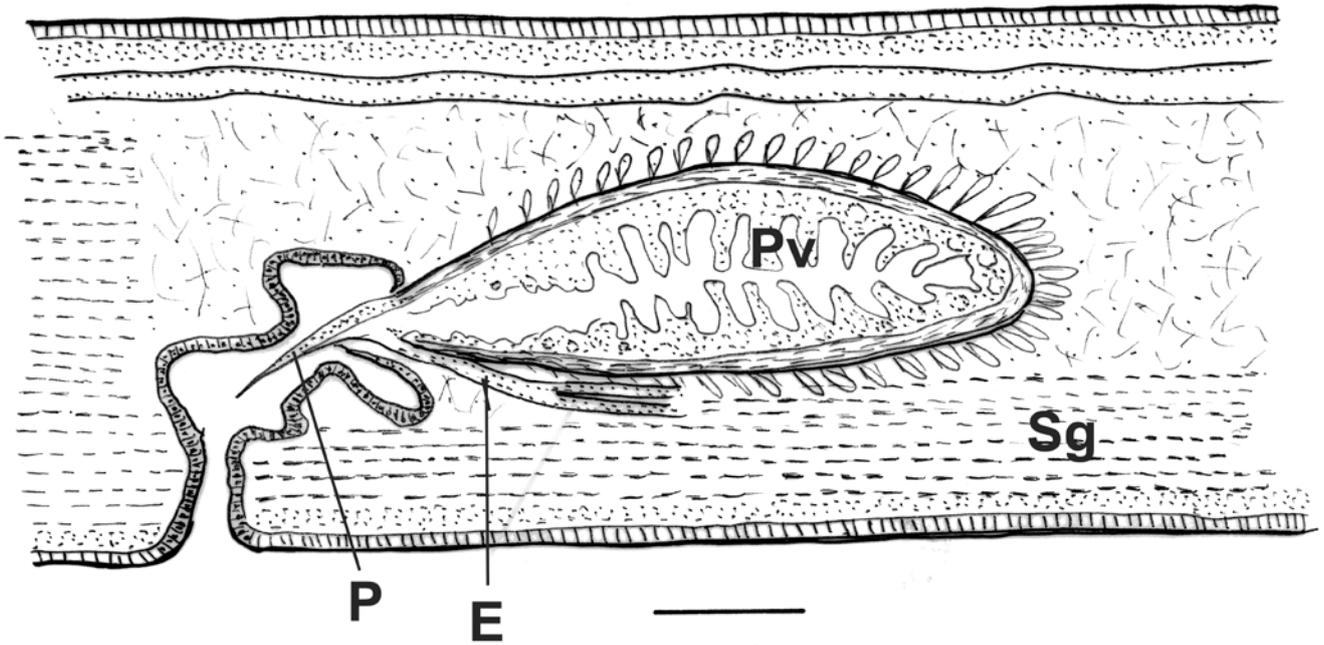


Figure 5: Longitudinal section through male terminal genitalia. Scale bar = 0.1 mm. Legend: Legend: E, ejaculatory duct; P, penis; Pv, prostatic vesicle; Sg, shell glands.

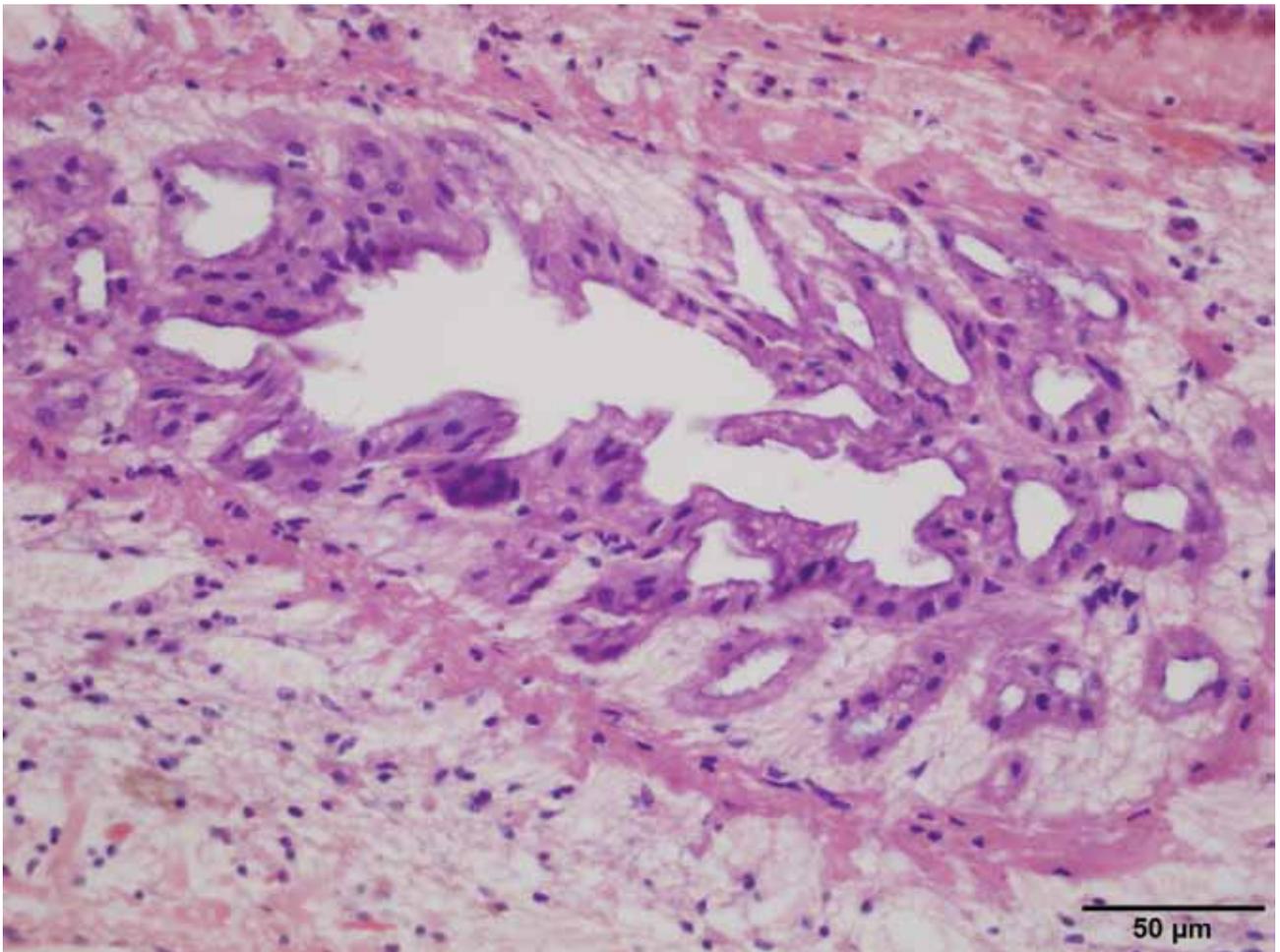


Figure 6: Histological features of the prostatic vesicle of *Leptostylochus victoriensis* sp. nov, showing tubular projections of epithelium into lumen.

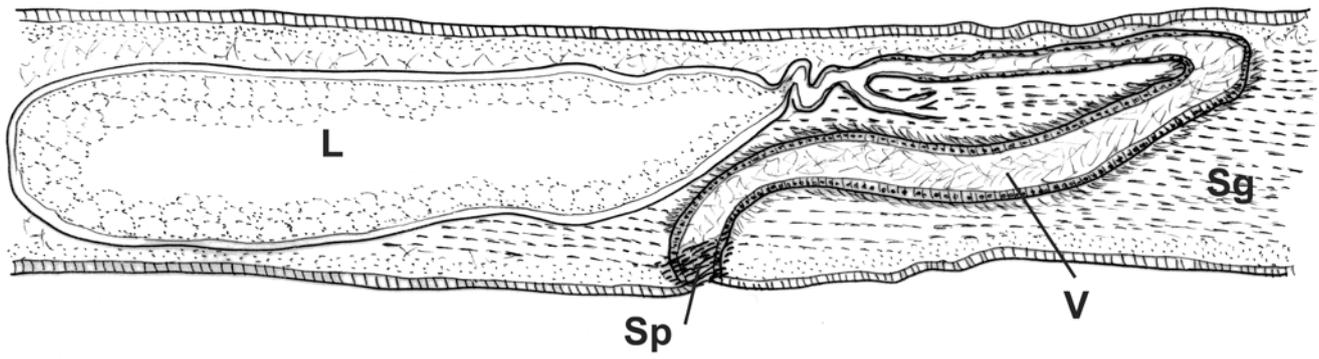


Figure 7: Longitudinal section through terminal female genitalia. Scale bar: 0.1 mm. Legend: L, Lang's vesicle; Sg, shell glands; Sp, sphincter; V, vagina.

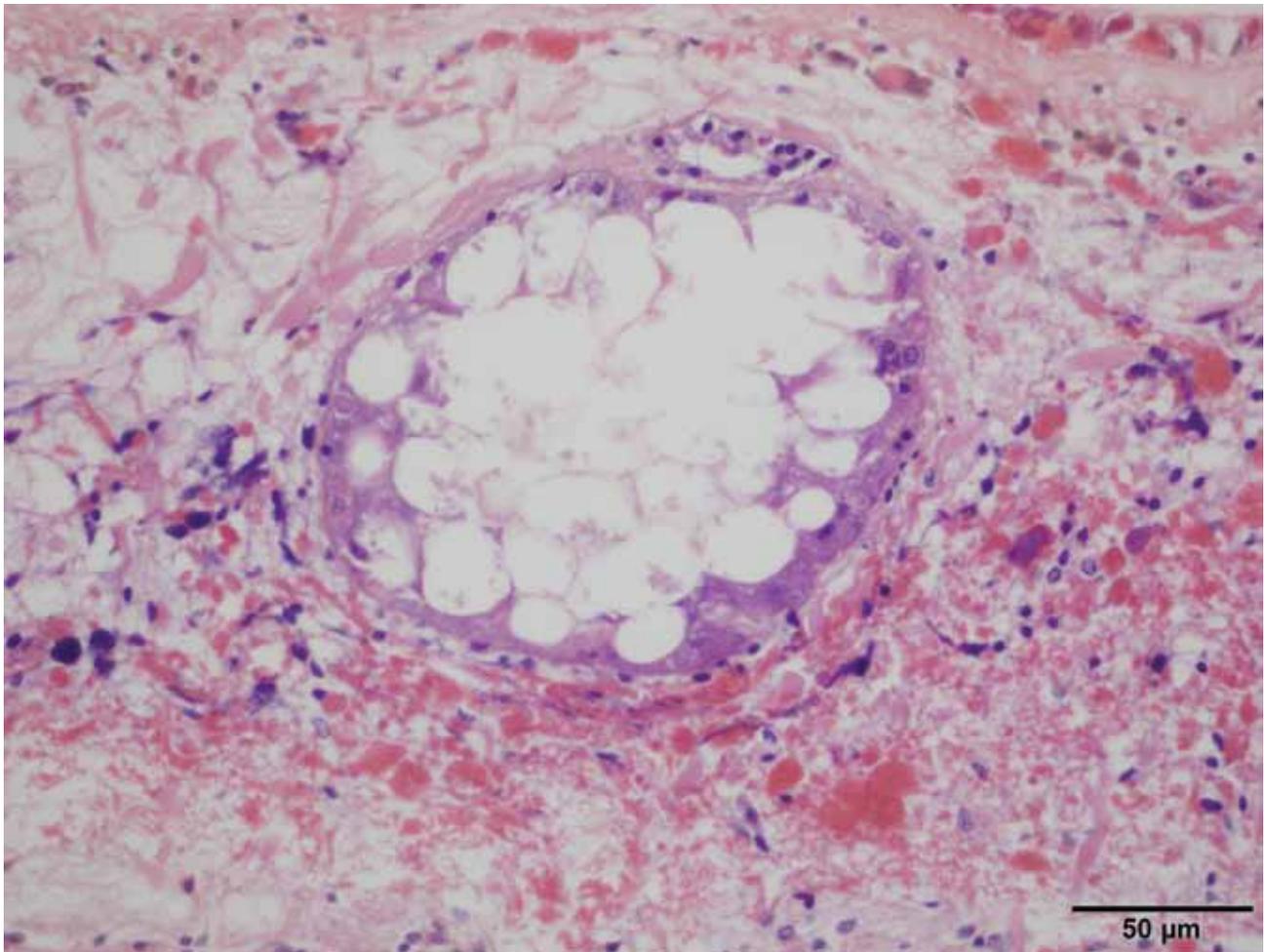


Figure 8: Histological section through Lang's vesicle of *Leptostylochus victoriensis* sp. nov, showing vacuolated epithelium characteristic of several congeners.



Figure 9: Type locality of *Leptostylochus victoriensis* sp. nov., north of Separation Creek, Victoria. Polyclads were found under rocks in pools on the rock platform.

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Conflict of interest

The author declares no conflict of interest.

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