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A new stylochid flatworm (Platyhelminthes, Polycladida) from Victoria, Australia and observations on its biology

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Abstract
There are only four polyclad flatworms currently known from temperate waters of Victoria, Australia, although these turbellarians are common inhabitants of rocky shores. A new stylochid flatworm, *Stylochus pygmaeus* sp. nov. (Platyhelminthes, Polycladida) or oyster leech is described here from Port Philip Bay, Victoria. This flatworm was observed feeding on three species of barnacles by extending its pharynx over its prey and extruding copious amounts of mucus. Worms also preferred to prey on larger-sized barnacles regardless of the species. Further observations indicated that these worms deposited eggs at night inside empty barnacle shells. Each eggmass was brooded for several days with the worms only moving off to feed. Each egg capsule contained multiple embryos and after 5 or 6 days, positively photo-tactic, four-lobed Götte's larvae emerged. Larvae metamorphosed to juvenile flatworms 1–2 weeks post-hatching but failed to settle and survive.

Keywords: *Barnacles, Götte's larvae, oyster leech, new species, Platyhelminthes, Polycladida, predators, Stylochidae,* Stylochus pygmaeus

Introduction

Little is known regarding the diversity and biology of polyclad flatworms from Australian temperate waters although these turbellarians are commonly found inshore under rocks and rubble. There are only a handful of records of polyclad flatworms from Victorian waters. Prudhoe (1982) reported three species: *Latocestus* sp., *Hoploplana rosea* Prudhoe, 1977, and *Candimboides cuneiformi*s Prudhoe, 1982 in Westernport Bay, and *Euplana gracilis* (Girard, 1850), from Port Phillip Bay. Apart from an earlier report by Laidlaw (1904) on *Notoplana australis* (Schmarda, 1859), no other flatworms from Port Phillip Bay have been described.

Much interest has been generated in acotylean polyclads of the family Stylochidae. These flatworms are commonly known as "oyster leeches" because their prey includes commercial bivalve species including rock and pearl oysters, scallops, mussels, and giant

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clams (Stead 1907; Galleni et al. 1980; Prudhoe 1985; Newman et al. 1993; O'Connor and Newman 2001, 2003). The number of stylochids identified has proliferated in the last two decades. Faubel (1983), Prudhoe (1985), and Jennings and Newman (1996a, 1996b) noted nearly 50 species of *Stylochus* Ehrenberg, 1831 and *Imogine* Giard, 1853 while Murina et al. (1995) attributed 100 species to the family Stylochidae, with predation observed in 14 species.

This account refers to the first oyster leech reported from Port Phillip Bay, Victoria. Observations on its feeding and reproductive biology are also presented here and show that it preys on three species of barnacles, and uses the empty shell of eaten barnacles to deposit its eggs. Biological observations on its feeding and reproductive behaviour are also given.

Materials and methods

Individual worms were collected in January 2002 from Breakwater Pier, Williamstown, Victoria (37°52′S, 144°55′W). All animals were found inside empty barnacle shells (*Balanus amphitrite* Darwin, 1854) (Figure 1) which were attached to perspex plates that had been submerged approximately 1–2 m below mean low water for 4 weeks. An assemblage of marine invertebrates settled on the plates during this period, including three barnacle species: *B. amphitrite*, *B. trigonus* Darwin, 1854, and *Elminius modestus* Darwin, 1854. These barnacles are typically found at this site in late spring and early summer.

Several worms were fixed and preserved by transferring them one at a time to filter paper that was then placed on the surface of a container of frozen polyclad fixative (10% formalin in non-buffered filtered seawater) (Newman and Cannon 1995). Each flatworm was gently patted flat with a soft paintbrush and the fixative was allowed to thaw. Another specimen was fixed in 95% ethanol for molecular studies. Worms were later preserved in 70% EtOH for histological preparations. Whole mounts were stained with Mayer's haemalum, dehydrated in graded alcohols, cleared in xylene, and mounted in Canada balsam. Longitudinal serial sections of the reproductive regions were prepared by embedding tissue in 56°C Paraplast, cutting at 6–8 µm, and staining with haematoxylin and eosin. Egg capsules were fixed in Bouin, and processed in the LX™ 120 Tissue Processor. They were then embedded in paraffin/polymer and 8 µm serial transverse sections were cut and then stained with Mallory's Triple Stain.

For biological observations, 36 mature worms were selected and then caged separately on perspex plates with different numbers and sizes of *B. amphitrite*, *B. trigonus*, and *E. modestus*. The number of barnacles consumed by each worm, and whether these worms laid eggs, was recorded (Figure 2).

Drawings and measurements were made by L.J.N. with the aid of a camera lucida. Measurements are expressed for the types as length (mm) × width (mm). Specimens are lodged at the Museum of Victoria (NMV) and designated as wholemounts (WM), serial sections (LS), and whole animals stored in 70% alcohol (S).

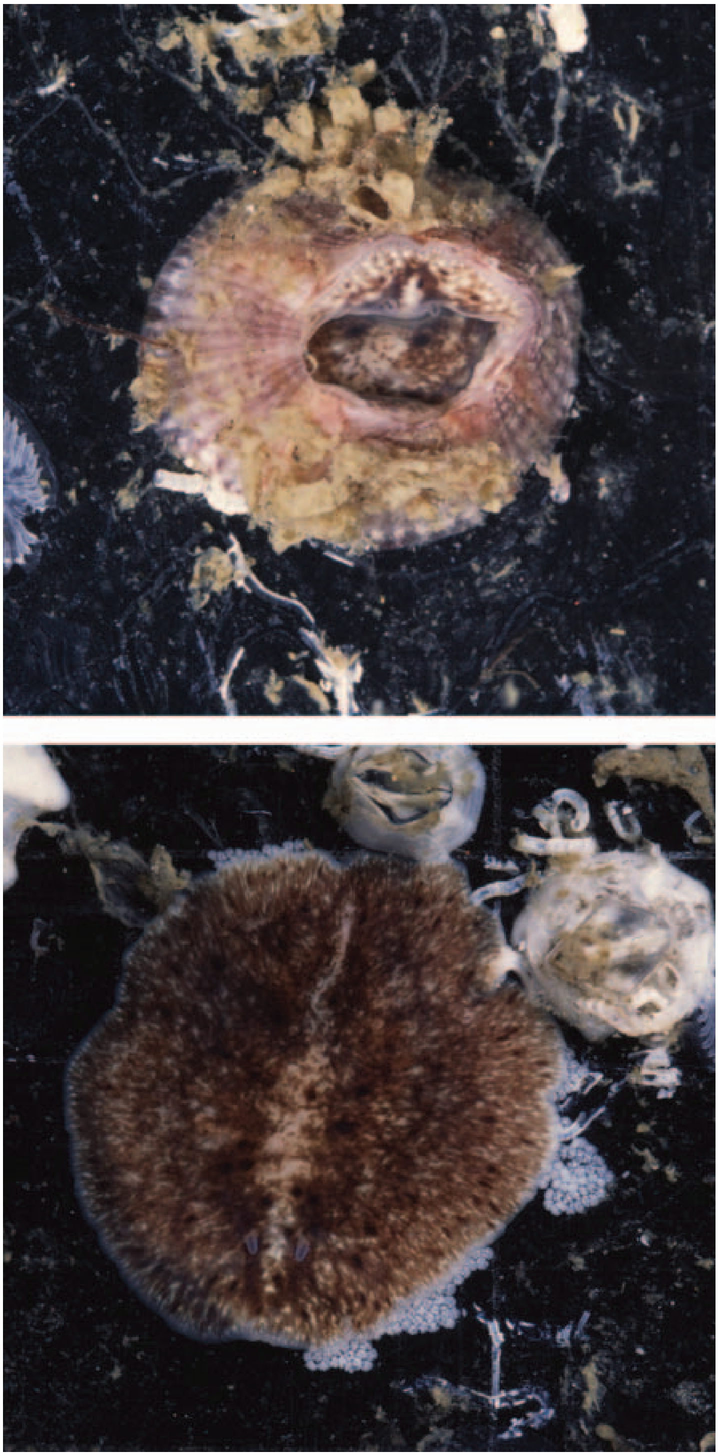
Systematics

STYLOCHIDAE Stimpson, 1857

Stylochus Ehrenberg, 1831

Stylochus pygmaeus sp. nov.

?*Stylochus zanzibaricus* Laidlaw 1903; Skerman 1960a, p 612, Figure 2.



Figures 1, 2. *Stylochus pygmaeus* sp. nov. (1) Inside the barnacle *Balanus amphitrite* Darwin, 1854. (2) With eggmass deposited on a perspex plate in the absence of barnacles of suitable size.

Material

Holotype: MV F93543, WM, 29 January 2002, Breakwater Pier, Williamstown, Victoria, Australia. Paratypes: MV F94086, WM, same data; MV F94087, LS, same data, MV F94088, same data, MV F94089, four specimens, S, same data.

Description

Animals are relatively small, body oval to elongate oval, thick and fleshy, without marginal ruffles. Smaller animals tended to be more rounded oval. Sizes ranged from 4.9×3 mm to 8.2 mm $\times 3.5$ mm.

The dorsal colour pattern is cream-beige with densely scattered dark brown and lighter brown mottling, margin without markings (Figure 2). The colour pattern is variable; larger worms are darker with denser markings. Paired, elongate retractile nuchal tentacles are located posterior to the first quarter of body length from the anterior margin (Figure 3). The ventral surface is grey-white, without markings.

There are three to four rows of scattered marginal eyes along the anterior margin, the eyes are more densely packed anteriorly, then reducing to two to three rows extending for a short distance along the margin to the level of tentacles (Figure 3). Tentacular eyes (about 10–15 each side) are present within the tentacles and there are about 20 scattered cerebral eyes between the nuchal tentacles, however, the dark markings of the worm tend to obscure the cerebral eyes.

The pharynx is central, about one-quarter the body length, highly ruffled and mid-body with complex ruffled folds (Figure 4). The mouth is in the middle of the pharynx. Gonopores are found close together but separate, posterior to the pharynx, proximal to the posterior margin. The vas deferens extend anteriorly from the male pore.

Testes are scattered throughout the body. The seminal vesicle has a single muscular lobe (0.72 mm long) and lies ventrally (Figure 5). The seminal vesicle leads into a long and narrow ejaculatory duct which joins the prostatic duct before it leads into the penis papilla. A short prostatic duct leads to a large prostate (0.80 mm long) with numerous ducts leading into the lumen which is lined with a folded epithelium. Both the seminal vesicle and prostate are of similar size. Penis papilla simple, extremely short within a shallow male antrum. Ovaries scattered dorsally throughout the body. Ova collect into the uteri on each side of the pharynx, running posteriorly to the female pore, curve dorsally and join at the distal end of the vagina. Details of the vagina were not evident from serial sections.

Diagnosis

Small, cream-beige *Stylochus* sp. with scattered brown mottling, with three to four rows of anterior marginal eyes, 10–15 tentacular eyes each side and about 20 cerebral eyes.

Etymology

Named from the Latin, *pygmaeus*=dwarf, for its relatively small size.

Remarks

Skerman (1960a) reported relatively small stylochid flatworms from the Port of Auckland, New Zealand and his specimens were identified by L. Hyman as *Stylochus zanzibaricus*

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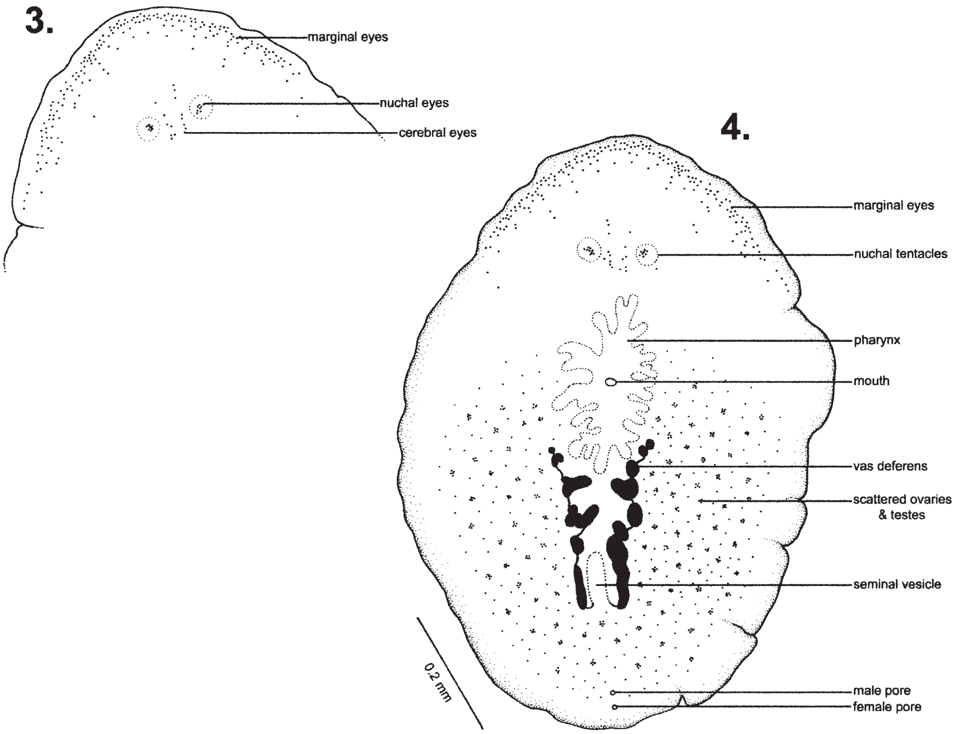

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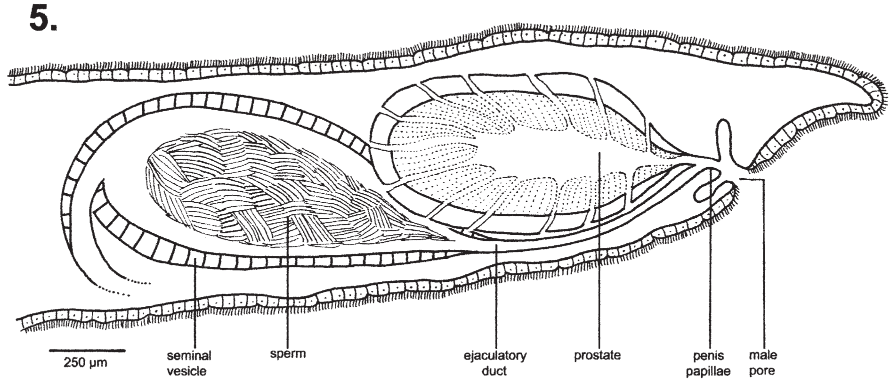

Figures 3–5. *Stylochus pygmaeus* sp. nov. (3) Details of the eyes arrangement. (4) Morphology of the holotype from ventral view. (5) Reconstruction of the reproductive anatomy of a paratype.

Laidlaw, 1903. A black and white photograph (Skerman 1960a, Figure 2) indicates that these stylochids are similar to *Stylochus pygmaeus* sp. nov. They were also thought to prey on the same barnacle species.

Although the present species bears some resemblance to the worms described briefly by Laidlaw (1903) as *Stylochus zanzibaricus* from Zanzibar (Tanzania), his original description, from a single fixed specimen, is without colour notes or any details of the reproductive anatomy. Both Laidlaw (1903) and Meixner (1907) believed that the copulatory structures of *S. zanzibaricus* were identical to *S. neapolitanus* Lang, 1884 from the Mediterranean and that these species are "probably identical". Furthermore, the colour figure (Lang 1884, Plate 1, Figure 7) of *S. neapolitanus* Lang, 1884 is clearly different to our specimens since this stylochid is large and elongate (not small and rounded oval), with long striped nuchal tentacles and distinct transverse marginal banding. These features are clearly not found in *S. pygmaeus* sp. nov.

Moreover, there are distinct morphological differences between Skerman's (1960a) specimens and *S. pygmaeus* sp. nov. Laidlaw (1903) reported that the marginal eyes are in a band of equal width that does not continue around the anterior margin as far as the tentacles, whereas in the present specimens the band of eyes continues posterior to the tentacles. In *S. pygmaeus* sp. nov. there are three to four rows of eyes at the anterior margin and two to three rows laterally. Nor does Skerman document the presence of cerebral eyes that are embedded in the epidermis between the tentacles. Furthermore, the tentacular eyes are clearly clustered within the tentacles, not on them, as stated by Laidlaw (1903). In our specimens, the nuchal tentacles are more elongate that those shown by Laidlaw, although this may be due to the fact that he examined a fixed specimen and we are describing live animals. The diminutive size at sexual maturity of this new species sets this species aside from any congenerics.

Distribution

Common during summer months within barnacles attached to pier pilings in the greater Melbourne ports area, Victoria, Australia.

Biology

Feeding

Flatworms were observed to prey on three species of barnacles (*B. amphitrite*, *B. trigonus*, and *E. modestus*) but appeared to select prey according to size, rather than species, with larger barnacles preferentially consumed (Merory, unpublished data). Worms did not feed every day. During feeding the pharynx was extended between the wall of the barnacle and the opercular valve in the area of the opercular muscle in a similar manner to that described by Hurley (1976) for *Imogine triparitus* (Hyman, 1953), when flatworms are feeding on either *B. amphitrite* or *E. modestus*. However, to enter larger *B. trigonus*, the pharynx was inserted directly between the opercular plates and the worm remained in place until the operculum was forced apart, a far more lengthy process than that observed by Hurley (1976). One worm was observed with its pharynx extended between the opercular plates of a large *B. trigonus* for almost 48 h before entering and consuming the barnacle. During this time, copious amounts of clear mucus were extruded on the worm's dorsal surface and passed anteriorly towards the extended pharynx and then along the pharynx on to the closed opercular plates. Also, contrary to Hurley's assertion that flatworms only consume barnacles once inside them, one flatworm was observed consuming a large *E. modestus* by extending only its pharynx and anterior end inside the opercular plates of the barnacle for the 2 h taken to consume the barnacle.

Larval development

Most worms deposited eggs within 15 days from onset of the experiment. Oviposition occurred at night, usually over two successive nights. Egg capsules were deposited in a single layer on to the calcareous base of dead barnacles and on the insides of the shell plates. Occasionally they were deposited on the upper margin of the external side of the shell plates or in an adjoining empty barnacle shell as well. Once the initial egg laying was completed, no subsequent eggs were deposited during 21 days of observations. Eggs were only deposited in *B. amphitrit*e or *B. trigonus* shells although *E. modestus* barnacles of the same size were consumed. This is possibly because *E. modestus* is not cemented to the substratum by a thick calcareous base as found in both *Balanus* species, and so after death, *E. modestus* may be easily dislodged.

All worms brooded their egg masses by covering them until the eggs had hatched. However, most worms left their eggs in order to feed and subsequently returned to brood them. Hatching success was almost 100%, regardless of whether eggs were brooded, or whether they were transferred to a petri dish the morning after they were deposited, and regularly flushed with fresh seawater.

The egg mass was white and opaque when first deposited, but became translucent after 3–4 days. Each egg capsule contained eight embryos, which were seen rotating by their cilia, and which usually hatched 5 days after oviposition, but could take as long as 14 days. Towards hatching, the speed of rotation of embryos within the capsule increased, until one embryo broke through the capsule. Within the next few minutes, the remaining embryos escaped through the same exit point. The capsule collapsed inwards, occasionally trapping the last embryo, which was sometimes observed still rotating within the capsule days later.

Ciliated, four-lobed Götte's larvae emerged and swam towards the light, indicating they are positively phototatic. Despite the lack of algal food (they were kept in 23 µm filtered seawater and no food was offered), they metamorphosed directly into juvenile flatworms 7–12 days post-hatching. Larvae became less active and more likely to be found on the bottom of the petri dish in the 48 h prior to metamorphosis. Juvenile flatworms failed to survive more than 7 days in the laboratory although they were offered small live barnacles and the seawater was changed every second day.

Discussion

Surprisingly few polyclad flatworms have been formally recorded from temperate Australian waters although they are frequently sighted and are known predators of a variety of inshore invertebrates including bivalves and barnacles. Identification of polyclad flatworms remains problematic and there is still much confusion in the literature over validity of many polyclad names, especially since few specimens have been examined alive or their colour patterns accurately recorded. Furthermore, few specimens have been fixed flat for histological preparations such as serial sectioning of the reproductive structures.

Attempts to locate the original specimens reported by Skerman (1960a) have been unsuccessful and we remain uncertain as to whether *S. pygmaeus* sp. nov. is the same as the species recorded by Skerman in New Zealand, especially since they seemingly prey on the same barnacle species.

It is apparent from anecdotal evidence that *S. pygmaeus* sp. nov. has been commonly associated with these three species of barnacles at Williamstown, Victoria for at least 3 years (E. Johnston, personal communication). As these flatworms infest samples of fouling barnacles found on ships (Skerman 1960b), it is possible that they have been transported around the world together with their hosts. This would explain their occurrence at Williamstown, which is in close proximity to the main shipping channel and the busy docklands area to the port of Melbourne.

It has been assumed that stylochids enter bivalves and barnacles by digesting the muscles effecting closure of the shell (Hurley 1976; Galleni et al. 1980). This is not unreasonable given that the pharyngeal glands that open on to the tip of the pharynx produce proteolytic enzymes (Barnes 1987) and the pharynx is inserted in the region of the adductor muscles of the prey species (Hurley 1976). However, this was not the strategy that *S. pygmaeus* sp. nov. used to enter large *B. trigonus*. Instead, the operculum was covered with copious amounts of mucus, presumably to kill or stun the barnacle.

Hyman (1951) suggested the mucus may be toxic, but it is also possible that it paralyses the barnacle, a possibility suggested by Galleni et al. (1980) who noted an earlier observation by Koopowitz (1970) of the reduction in heart rate of a snail preyed on by another flatworm species. This is consistent with our observations of several large *B. trigonus* individuals that had their cirri covered with mucus. The beating of the cirri slowed markedly, and barnacles failed to respond in the normal manner (closure) when we tapped the operculum with a probe. They were unable to fully retract their cirri and could only half close the operculum. However, the possibility that barnacles were smothered cannot be ruled out, as the mantle and cirri are the principal sites of gas exchange (Anderson 1994), and possible suffocation of prey by another flatworm species was raised by Newman et al. (2000).

We had no difficulty in maintaining larvae in the laboratory prior to their metamorphosis into juvenile flatworms. Their subsequent mortality, despite the provision of live young barnacles as a possible food source, raises the question of whether they may have another food source when they are young. It is also unknown if they can prey switch from barnacles to bivalve species like some other stylochids (Pearse and Wharton 1938; Galleni et al. 1980; Murina et al. 1995). Because of their high reproductive success, and their probable transport to new locations via shipping or ballast waters, it is likely that they could pose a threat to commercial species such as oysters if either juvenile or adult worms of this species are able to attack bivalves.

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