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Ka-Man Lee ^a; Michel A. Beal ^a; Emma L. Johnston ^a

^a School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, Australia

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A new predatory flatworm (Platyhelminthes, Polycladida) from Botany Bay, New South Wales, Australia

KA-MAN LEE, A. MICHEL BEAL, & EMMA L. JOHNSTON

School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, Australia

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Abstract

A new species of Stylochidae flatworm *Imogine lateotentare* is described from Botany Bay, eastern New South Wales, Australia. This flatworm is distinguished from other species in the same genus mainly by having small, transparent and inconspicuous tentacles, densely packed purplish pink flecks at the posterior of the dorsal surface, distinctive purplish red colour gonopores and continuous bands of numerous frontal and cerebral eyes. Feeding and reproductive behaviour in the laboratory are described. This flatworm was found closely associated with the barnacle *Balanus variegatus* (Darwin, 1854) on which it fed by extending its pharynx over the barnacle opercular and sucking out the flesh but ejecting the cirri. It consumed one *Balanus variegatus* in a 14-day observation period and it was observed feeding exclusively at night.

Keywords: Barnacles, *Imogine*, *Platyhelminthes*, *Polycladida*, predators, *Stylochidae*

Introduction

Free-living polyclad flatworms of the family Stylochidae, commonly referred to as “oyster leeches”, are typically carnivores and are well-known predators of bivalves, molluscs and barnacles all over the world (Pearse and Wharton 1938; Landers and Rhodes 1970; Littlewood and Marsbe 1990; Jennings and Newman 1996b; O’Connor and Newman 2001). Interest in the diversity, ecology, and especially the feeding behaviour of the family Stylochidae has been spurred by the recognition of stylochids as the cause of great economic loss of commercial bivalves (Pearse and Wharton 1938; Skerman 1960; Chen et al. 1990). Despite this, little is known regarding the biology and ecology of Stylochidae in Australian waters as only five *Imogine* spp.: *Imogine lesteri* (Jennings and Newman 1996a), *Imogine kimaie*, *Imogine mcgrathi*, *Imogine meganae*, and *Imogine pardalotus* (Jennings and Newman 1996b) have been formally reported from the entire east coast of Australia.

Identification of marine flatworms remains difficult because of a lack of literature and reports containing detailed descriptions, as well as the rarity of well-preserved specimens

Correspondence: Ka-Man Lee, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052, Australia. Email: kal@student.unsw.edu.au

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for histological preparation (Newman and Cannon 2003). A new fixation method, developed in the last decade, preserves colour patterns of the flatworms with improved authenticity (Newman and Cannon 1995) and the widespread use of this technique will no doubt lead to a rise in the number of polyclads described. The family Stylochidae has been revised and is subdivided into two major genera, *Stylochus*, which have a single-lobed seminal vesicle, and *Imogine*, with a tripartite seminal vesicle (Newman et al. 1993; Newman and Cannon 2003).

Materials and methods

Specimen collection

The study site was located at Kurnell Pier, Botany Bay, New South Wales (33°59.92'S, 151°12.62'E), 15 km south of Sydney. The pier extends 1.3 km from the southern shore of the bay and has restricted public access. Naturally occurring sessile assemblages on the pier include anthozoans, ascidians, bryozoans, hydrozoans, macroalgae, polychaetes and sponges (Clark and Johnston 2005). To assist in specimen collection, assemblages of sessile marine invertebrates were first allowed to develop on artificial substrata. Settlement plates consisting of 6 × 6 cm black Perspex tiles attached underneath two 60 × 60 cm PVC backing plates were deployed at Kurnell Pier on 4 May 2004. Backing plates were suspended horizontally at a depth of 3 m below the low water mark. After 12 weeks settlement plates were retrieved, and any flatworms found between settlement and backing plate were collected immediately using a small paintbrush and spatula. Settlement plates were retained and brought back to the laboratory in aerated water and placed in a dark constant-temperature room (23.5°C), in a well-aerated transparent plastic container (15 × 8 × 8 cm) with 1 litre of field seawater. After 24 h, any flatworms emerging from the assemblages were collected using a paintbrush. Collection of flatworms for taxonomic studies continued for a year.

Specimen processing

Collection and preservation of flatworms was made difficult by the mobility and extreme delicacy of the worms. The fixative solution used was 10% formalin in seawater. The fixative solution was frozen and the polyclads were fixed by coaxing on to a filter paper and placed on to the frozen fixative. Cold fixative was added to just cover the flatworm in order to prevent the specimen being dried out. A soft brush was used to ensure the flatworm remained flat under the fixative and then the flatworm was left for 24 h without disturbance. The fixative was replaced by 70% ethanol for long-term preservation. This method ensures that the flatworms remained flat with the colour pattern preserved for histological preparation and microscopical examination (Newman and Cannon 1995, 2003).

Whole mounts of two of the *Imogine lateotentare* were prepared by staining flatworms in Mayer's haematoxylin for 5 min in order to obtain the best staining results. Specimens were then dehydrated in graded alcohols, cleared in xylene and mounted in Canada balsam. Longitudinal serial sections of the reproductive regions were prepared by embedding excised tissue in 56°C Paraplast, cutting at 6 µm, and staining with haematoxylin and eosin as described by Newman and Cannon (1995, 2003).

Drawings and measurements were made with the aid of a micro-projector (Ken-A-Vision, MFG Inc., USA). Measurements of the body were taken from live animals in a

quiescent state and expressed as length (mm) \times width (mm) for the type material only. These measurements can only be used as a guide because of the plasticity of the polyclads. Diagrammatic reconstructions of the reproductive systems are given. Descriptions of colours are based on the living animals and the colour descriptions were written in numbers referring to the Pantone® Colour chart. All material is lodged at the Australian Museum: whole mounts are designated as WM, longitudinal serial sections as LS and whole animals stored in 70% alcohol as S.

Predatory behaviour

Size of barnacle opercular openings and length of flatworms were measured prior to the experiment. Barnacle size was tested using a one-factor analysis of variance (one-way ANOVA) with the presence of flatworms as a fixed factor to ensure that the size of barnacle prey tested in the treatments and controls were consistent. Transparent plastic containers (15 \times 8 \times 8 cm) were used as experimental containers. Each container was filled with 1.5 litres of field seawater, continuously aerated, and changed once every 24 h. Of the 15 *I. lateotentare* collected during the study, six were used for a predatory behavioural study. One settlement plate with five *Balanus variegatus* recruits was placed in each predation treatment container. One flatworm was placed in each of the six predation treatment containers and a further six containers holding barnacles only were used as controls. All other organisms were scraped from the settlement plate. Observations of flatworm predation on barnacles were taken once every 4–6 h during both day and night for 2 weeks. A record was made of the number of barnacles consumed, and the activity and conditions of flatworms and barnacles.

Systematics

STYLOCHIDAE Stimpson, 1857

Imogine Girard, 1853

Imogine lateotentare sp. nov.

(Figures 1, 2)

Material examined

Holotype: W 29330, WM, 4 May 2004, Kurnell Pier, Botany Bay, New South Wales, Australia. Paratypes: W 29331, WM, 16 July 2004, Kurnell Pier, Botany Bay, New South Wales, Australia; W 29332, LS, same data; W 29333, S, same data.

Description

The size of flatworms measured live ranged from 9.5 \times 4.7 to 19.2 \times 8.0 (SE \pm 0.7 \times 0.2) mm ($n=15$). Body rounded oval, thick and fleshy, blunt posterior, without marginal ruffles. Background of dorsal surface cream-beige (721) with scattered brown (731) mottling and light brown (722) flecking towards the margin (Figure 1A). Purplish pink (507) flecks densely packed at the posterior of dorsal surface (Figure 2A). Gonopores purplish red (216) at posterior end on ventral surface (Figure 2B). Nuchal tentacles small and transparent, about 0.21 mm wide and 0.88 mm apart with 30–40 eyes aggregated at the tip of each nuchal tentacle. Four to five rows of scattered marginal eyes along the anterior

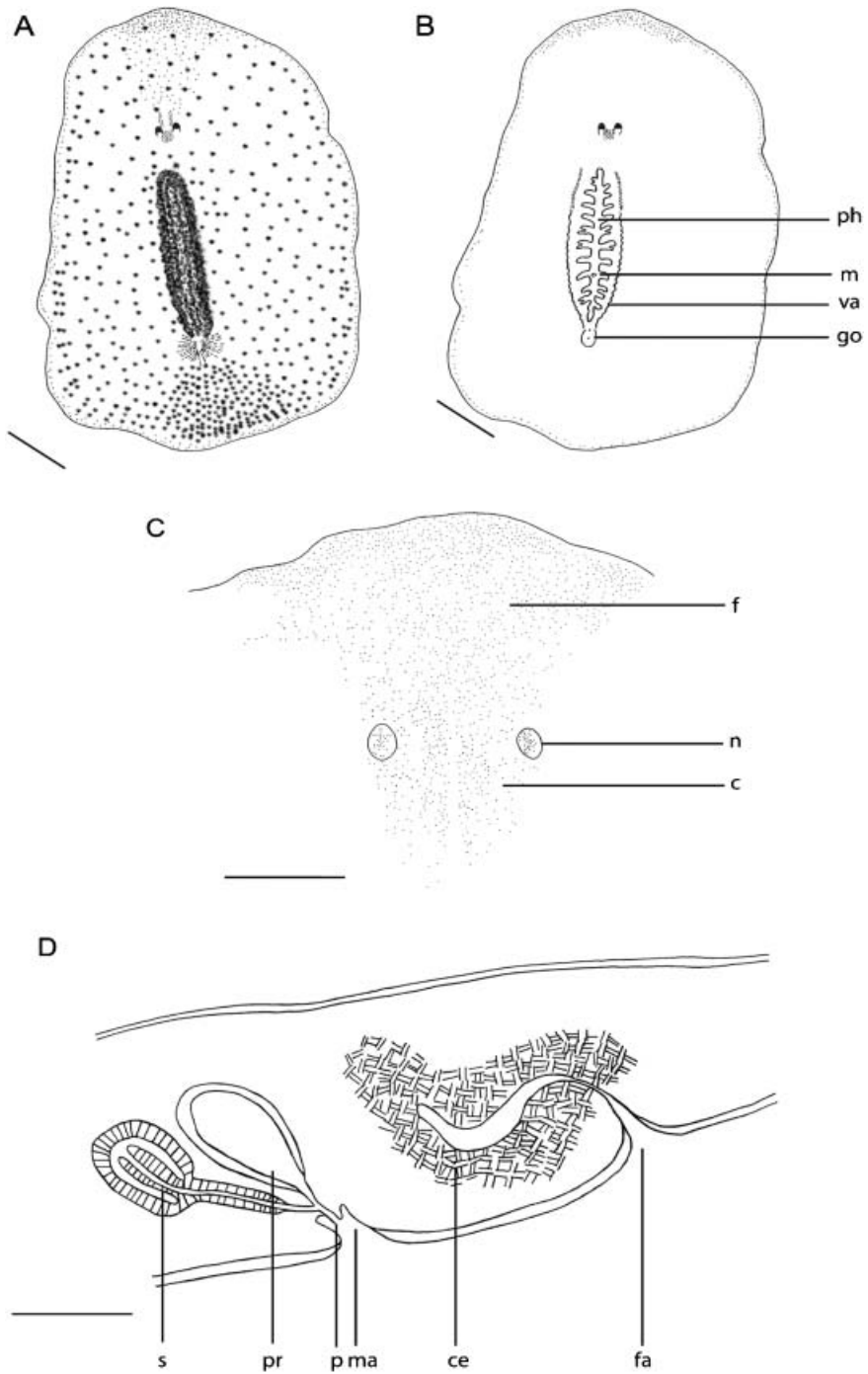


Figure 1. *Imogine lateotentare* sp. nov., preserved. (A) Diagram of the dorsal surface; (B) morphology of the ventral surface; (C) arrangement of the dorsal eyes; (D) diagrammatic reconstruction of the reproductive system. c, cerebral eyes; ce, cement glands; f, frontal eyes; fa, female antrum; go, gonopores; m, mouth; ma, male antrum; n, nuchal tentacle; p, penis papillae; ph, pharynx; pr, prostatic vesicle; s, seminal vesicle; va, vasa deferentia. Scale bars: 1.5 mm (A, B); 0.9 mm (C); 0.6 mm (D).

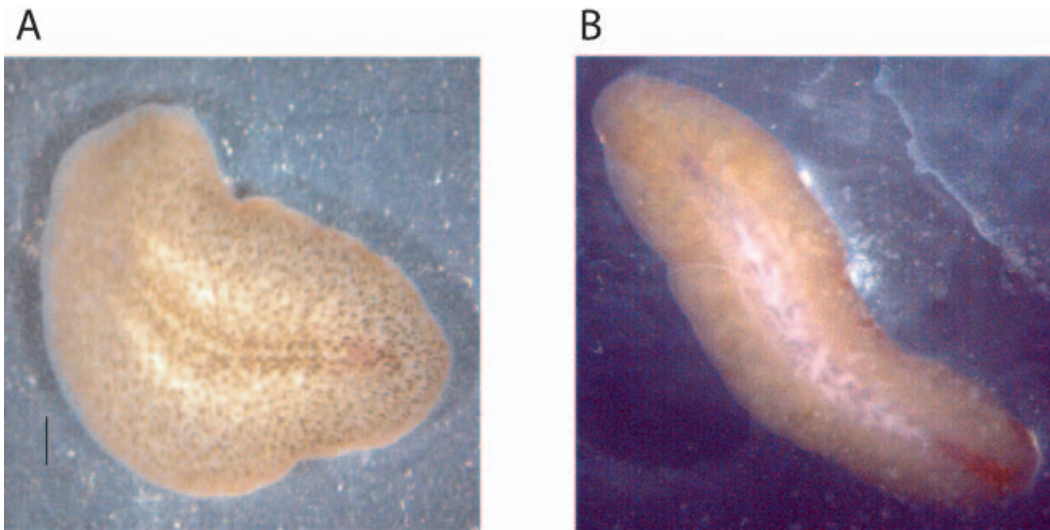


Figure 2. Living *Imogine lateotentare* sp. nov. from Kurnell Pier, Botany Bay, New South Wales, Australia. (A) Colour pattern on dorsal surface; (B) ventral view showing pharynx, gonopores and vas deferens. Scale bar: 1.4 mm (A).

margin, more densely packed anteriorly, reducing to two to three rows on both sides. Cerebral eyes numerous, embedded in the epidermis, aggregated in two bands lying between the tentacles, scattered to some distance to the back end of the tentacles, extending anteriorly into frontal eyes which are extremely numerous and scattered. Frontal eyes merge into anterior marginal eyes (Figure 1C). Pharynx long, narrow and ruffled, in middle of body, three-quarters of body length, with about 22 complex pharyngeal folds. Mouth at two-thirds of pharynx (Figure 1B). Intestinal branches are non-anastomosing. Gonopores close but well-separated posterior to pharynx. Female gonopore close and posterior to male pore. Vasa deferentia extend anteriorly from pores, originate laterally to pharynx, lying along the entire length of pharynx.

Testes scattered throughout body with tripartite globular seminal vesicle. Ventral, lateral and central lobes of seminal vesicle equally sized, about 0.5 mm long \times 0.43 mm wide. Central lobe of seminal vesicle passes posteriorly, joins the prostatic duct at the proximal end of penis. Prostatic duct short joins dorsally to the mid-penis from prostatic organ. Prostatic organ large, muscular, about 0.78 mm long \times 0.43 mm wide, horizontal to ejaculatory duct. Penis is simple, papilla small, within deep male antrum.

Ovaries scattered throughout the body. Vagina is long, muscular and narrow, with a shallow female antrum, accompanied by numerous cement glands (Figure 1D). Life history is indirect through Gotte's larva.

Diagnosis

Relatively small size compared to other *Imogine* spp. Cream-beige and light brown with irregular pattern of dark brown flecks over the dorsal surface and densely packed medially. Purplish pink flecks densely packed at the posterior of the dorsal surface and purplish red gonopores at the posterior of the ventral surface. Nuchal tentacles are not obvious.

Numerous and scattered cerebral and frontal eyes. Prostatic vesicle is relatively larger than seminal vesicle. Numerous cement glands.

Etymology

Named from the Latin, *lateo*=hidden, *tentare*=tentacles, for its inconspicuous and transparent nuchal tentacles.

Distribution

Imogine lateotentare was more common in spring and summer within emptied barnacle shells attached on the settlement plates deployed at Kurnell Pier. It also has been found associated with barnacles attached on the settlement plates at Port Kembla, New South Wales, Australia.

Biology

Egg deposition of *I. lateotentare* was observed in spring. Flatworms were observed to lay eggs within 5 days of solitary confinement in the laboratory (K.-M. Lee and E. L. Johnston, unpublished data). Thousands of eggs were deposited, mainly within empty barnacle shells and also in the corners of the container. The egg mass was white and opaque when first deposited, laid in zig-zag chains, covered with sticky gelatinous substance fastening the eggs firmly on the substrata. The egg mass became yellowish brown after 3–4 days. Hatching began 5–7 days after the eggs were laid. Gotte's larva emerged, black in colour, with four ciliated lobes, anterior and posterior cilia tufts, and were positively phototactic. Emptied egg capsules remained on the substrata after hatching.

Ecology

Predatory behaviour and feeding rate. Size of opercula of *B. variegatus* recruits ranged between 4.5 and 7.6 mm, with a mean size of 5.5 mm (SE ± 0.07). Length of flatworms ranged between 9.5 and 19.2 mm, with a mean length of 12.8 mm (SE ± 1.4). Flatworms were observed to prey on *B. variegatus* exclusively at night. One *B. variegatus* was eaten in each of the six treatment replicates during the 2-week observation period. The flatworm was observed to glide across the barnacle to the opercular valves and to insert its pharynx between the tergum and scutum when the barnacle started to feed. The flatworm then stayed in that position for 15–30 min. The barnacle was observed trying to avoid the flatworm by closing or scraping its opercular valve around the edge of the opercular opening but that was not successful. The flatworm sucked out all the barnacle flesh using its pharynx, leaving the cirri untouched outside the shell. The flatworm then remained inside the dead barnacle shell for approximately 2–3 min. Dead barnacles eaten by *Imogine* were recognized by the presence of open unmoving opercular plates. White food particles showed up clearly in the gut of the worm. After feeding, the flatworm emerged from the empty barnacle shell and moved to a position beneath the settlement plate in the container. It remained there stationary for about 3 h, presumably in the process of digesting the barnacle. Thereafter, the flatworm recommenced activities, spending short periods of time moving around the container interspersed with longer periods of resting under the settlement plate.

The size of barnacle opercular openings did not differ between control and predation treatment containers ($F_{1,58}=2.8$, $P=0.10$) and no barnacle died in the control containers. It is apparent that the replicate flatworms show similar behaviour. Flatworms spent approximately 80% of their time hiding underneath the settlement plate with occasional exploration of the container. Feeding was observed at night between days 6 and 8, with the flatworms staying underneath the plate with flesh seen in the gut for a few hours immediately after consumption. Further exploratory behaviour was observed for intermittent periods (2–4 days) after consumption of the barnacle.

Discussion

Stylochids are common voracious mobile predators on natural and artificial substrata, however, only a few of them have been formally recorded from temperate Australian waters. Identification of acotylea species based on their colour pattern only can be especially difficult as they may vary their colour pattern according to the colour of their prey items. *Imogine* spp. are usually confused with *Stylochus* spp., necessitating the use of sectioning of the reproductive structures to distinguish between them. *Imogine* spp. differ from *Stylochus* spp. in having tripartite seminal vesicles (Jennings and Newman 1996b; Newman and Cannon 2003). *Imogine lateotentare* is distinguished superficially from other Australian and worldwide *Imogine* sp. by having small and inconspicuous nuchal tentacles, reddish pink flecks densely packed at the posterior on its dorsal surface, distinctive purplish red gonopores clearly seen on its ventral surface, extremely numerous frontal and cerebral eyes, relatively larger prostatic vesicle compared to the seminal vesicle, and numerous cement glands.

In recent decades particular attention has been paid to the feeding behaviour of stylochids since they are common predators of barnacles and mussels (e.g. Ferrero et al. 1980; Chintala and Kennedy 1993), and are a well-known pest of commercial bivalve production throughout the world (Pearse and Wharton 1938; Landers and Rhodes 1970; Galleni et al. 1980; Newman et al. 1993; Jennings and Newman 1996b). Our observations indicate that *I. lateotentare* only feeds at night, and this has not been reported in other studies of feeding behaviour of stylochids (e.g. Lytwyn and McDermott 1976; Murina et al. 1995). Such feeding behaviour may allow the flatworm to avoid potential visual predators, e.g. fish. *I. lateotentare* caused no physical damage to the shell of its prey, *B. variegates*. However, the barnacle was unable to close its valves properly once the flatworm had inserted its pharynx. It has been suggested that the polyclads may paralyse the barnacles through the toxins in their tissues (Newman and Cannon 2003) or in the mucus (Hyman 1951).

Predation is an important factor influencing the characteristics of species, populations and communities, and is one of the most important factors to determine the distribution and abundance of organisms (Connell 1970; Rand 1985; Sih et al. 1985; Menge 1995). Polyclads are one of the most common mobile predators found on artificial and natural hard substrata and are closely associated with sessile assemblages (Butler and Connolly 1996; Brown and Swaeringen 1998; Newman 2001). Flatworms occurring at high densities, such as the typhloplanid flatworms, are able to reduce their prey populations and hence alter community structure (Blaustein and Dumont 1990). The predation rate of *I. lateotentare* in this study was relatively low when compared to other members of the family Stylochidae preying on other barnacle species in the field (e.g. 5–10 *B. improvisus* barnacles were consumed by one *Stylochus tauricus* in a month (Murina et al. 1995). Possible

explanations are the small size of *I. lateotentare* and the reduced energy expenditure of flatworms in well-controlled laboratory conditions that are free of predators and other environmental stresses. *Imagine lateotentare* is a potential agent shaping assemblage structure if it occurs at high densities in the field. Considerably more ecological studies of flatworms, such as *I. lateotentare*, are required before we understand the dynamics of sessile assemblages and their mobile predators.

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