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# Two new tropical psolid sea cucumbers from the Strait of Johor, Singapore (Echinodermata: Holothuroidea) with a key to *Psolidium* species from the tropical East Indian—West Pacific region

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Abstract. Two new psolid sea cucumber species are described from the 2010–2013 Comprehensive Marine Biodiversity Survey (CMBS) of the Johor Straits, Singapore: *Psolidium helenae* Ong & O'Loughlin, new species and *Psolidium wongae* Ong & O'Loughlin, new species. *Psolidium helenae* differs from other tropical *Psolidium* species by having: a low profile; a band of tube feet around the periphery of the sole up to six wide; an absence of smaller outer marginal tube feet on the sole; a band of mid-ventral tube feet on the sole about five wide; distally spinous cupped cross ossicles present dorsally. *Psolidium wongae* differs from other tropical *Psolidium* species by having: low profile; tube feet series around the periphery of the sole up to about 10 wide; an absence of smaller outer marginal tube feet on the sole; an absence of cupped cross ossicles dorsally and ventrally. *Psolus fimbriatus* Sluiter is referred to *Psolidium* Ludwig, as are *Psolus boholensis* Semper and *Psolus complanatus* Semper. Variety *Psolus boholensis* var. *pandanensis* Semper is raised to species status and also assigned to *Psolidium*. A key to 15 tropical East Indian–West Pacific *Psolidium* species is provided for: *Psolidium berentsae* O'Loughlin & Maric; *P. boholensis* (Semper); *P. complanatum* (Semper); *P. disjunctum* Sluiter; *P. fimbriatum* (Sluiter); *P. helenae* new species; *P. hutchingsae* O'Loughlin & Maric; *P. mccallumae* O'Loughlin & Maric; *P. minutum* (H. L. Clark); *P. pandanensis* (Semper); *P. parmatum* (Sluiter); *P. rugosum* Koehler & Vaney; *P. spinuliferum* (H. L. Clark); *P. translucidum* Koehler & Vaney; *P. spinuliferum* (H. L. Clark); *P. translucidum* Koehler & Vaney; *P. wongae* , new species.

Key words. Indo-Pacific, Psolidae, new generic referrals, new species, key

### INTRODUCTION

Ong & Wong (2015) reported upon the sea cucumbers of Singapore waters, with reference to those collected in the Johor Straits, as part of the Comprehensive Marine Biodiversity Survey (CMBS) of Singapore waters (Tan & Goh, 2015). Some ten possible new sea cucumber species were found during this survey. O'Loughlin & Ong (2015) have since described new caudinid and synaptid species, and this paper describes two new species of psolid sea cucumbers previously reported by Ong & Wong (2015) as *Psolidium* species a (ZRC.ECH 0299) and *Psolidium* species b (ZRC. ECH 0300). The new species described in this paper are the responsibility of Joo Yong Ong and P. Mark O'Loughlin. The authorship of the new species should be cited as Ong & O'Loughlin *in* Ong, Wong & O'Loughlin, 2019.

For the purpose of distinguishing our new species from other *Psolidium* species, and providing an appropriate key, we have confined our study to species reported from the Indo-Pacific biogeographical region. We define this region more precisely as the tropical East Indian—West Pacific region in which we include eastern India, Malaysia, Singapore, the South China Sea, Indonesia, the Philippines and northern Australia.

#### **MATERIAL AND METHODS**

Specimens were collected by hand along the intertidal shore during low spring tide, and by dredge and trawl offshore, in the Johor Straits between 15 October and 2 November 2012. All specimens were fixed and preserved in 75% denatured ethanol. The processing of the collections was done at the University's Tropical Marine Science Institute on St. John's Island. All specimens were deposited in the Lee Kong Chian Natural History Museum (formerly the Raffles Museum of Biodiversity Research), National University of Singapore.

Digitalised photographs of live specimens were taken using either a Nikon D90 or D800E camera equipped with a 105 mm Nikon lens. Photographs of preserved specimens were similarly taken using the same setup, or by using an Olympus DP21 digital camera mounted on an Olympus stereomicroscope SZX10. Ossicles were isolated from soft tissue with commercial bleach and washed with distilled water

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(three changes) followed by absolute ethanol (twice). The cleaned ossicles were then mounted on aluminium stubs, air dried, and sputter-coated with platinum. Scanning electron microscope (SEM) images of ossicles were taken using a JEOL JSM 6510 SEM. Measurements and photographs were made with built-in software of the JEOL JSM 6510 SEM. Ossicles were also mounted in DPX® on glass slides for observations through the light microscope. These were photographed using an Olympus DP21 digital camera mounted on an Olympus compound microscope BX43.

Tissue samples from live specimens were taken by the CMBS cryogenic collection team during the CMBS Johor Straits workshop and deposited in the cryogenic collection of LKCNHM. Tissue samples were also taken from preserved specimens and sent to Gustav Paulay at the University of Florida.

Abbrevations: CMBS, Comprehensive Marine Biodiversity Survey; LKCNHM, Lee Kong Chian Natural History Museum; ZMA, Zoological Museum Amsterdam; ZRC, Zoological Reference Collection of LKCNHM; ZRC.ECH., LKCNHM catalogue number prefix.

#### **TAXONOMY**

Order Dendrochirotida Grube, 1840 Psolidae Burmeister, 1837 Psolidium Ludwig, 1887

Diagnosis (from Davey & Whitfield, 2013, following O'Loughlin & Maric, 2008). Dendrochirotid holothuroids; small, up to 40 mm long; mid-body arched dorsally in transverse section, flat ventrally; dorsal and lateral body covered with imbricating scales, usually macroscopically conspicuous, sometimes obscured by integument, scales irregular in size and arrangement; scales decreasing in size ventro-laterally, orally and anally; lacking large oral valves; extensible oral cone with anterior, anterior—dorsal or dorsal orientation; extensible anal cone with posterior, posterior—dorsal or dorsal orientation; tube feet dorsally and laterally in mid-body, passing through scales.

Sole distinct, oval to elongate; discrete margin created by junction of small imbricating ventro-lateral scales, with thinwalled, usually calcareous sole that lacks scales; peripheral band of tube feet, may be discontinuous across the inter-radii anteriorly and posteriorly; peripheral tube feet frequently of two sizes, those of outer series smaller; mid-ventral radial series of tube feet present or absent. Calcareous ring solid, plates sub-rectangular, radial and inter-radial plates with tapered anterior projections; radial plates with deep notch posteriorly, inter-radial plates with shallow concave indentation posteriorly; 10 dendritic tentacles, ventral two smaller.

Dorsal and lateral ossicles: multilayered or single-layered perforated plates (scales), always some with tube foot canals; integument covering scales may have cupped crosses, cups,

'thorn' ossicles (irregular branched rods pointed distally), buttons, perforated plates and rosettes; tube foot small endplates, and tube foot support ossicles that are irregular rods and plates, bent and curved, variably perforated. Sole ossicles: inter-radii with small to large single-layered perforated plates (rarely with multi-layering), smooth to variably knobbed and thickened, sometimes with cupped crosses, cups, thorn ossicles and rosettes; radii with additional tube foot ossicles, large endplates and tube foot support ossicles that are irregular rods and plates, bent and curved, variably perforated.

**Remarks.** Miller et al. (2017) have provided a comprehensive molecular phylogeny of extant Holothuroidea that places Psolidium taxonomically in the Dendrochirotida. O'Loughlin & Maric (2008) have described in detail many of the species in the key below and provided illustrations for ossicles, including "thorn" ossicles. Psolus boholensis Semper, 1867 and Psolus boholensis var. pandanensis Semper, 1867 were described for specimens from different locations near Bohol in the Philippines. We judge from the descriptions by Semper (1867) that there are two discrete species involved, based on the description of tube feet on the mid-ventral ambulacrum as being "discontinuous" (P. boholensis) or "completely present" (P. boholensis var. pandanensis), and from the illustrations of ossicles with the apparent absence of cup ossicles (P. bololensis) or their presence (P. boholensis var. pandanensis, pl. 13, fig. 20b in Semper, 1867). We raise the variety to species status: Psolus pandanensis Semper, 1867. Rowe (in Rowe & Richmond, 2004) offered the opinion (page 3305) that "...almost certainly each of these species (viz. Psolus boholensis Semper and Psolus complanatus Semper, 1867) ought to be referred to the genus *Psolidium*". Based on the illustration of small scales around the mouth of Psolus boholensis (pl. 12, fig. 3 in Semper, 1867), which are not large oral valves, we agree, and confirm the referral of Psolus boholensis and the variety P. boholensis pandanensis to Psolidium. Semper (1867) examined the type and variety of P. boholensis closely and we assume he noted no difference in the form of the elongate oral cones. We also assume that if *P. complanatus* had discrete oral valves rather than small scales orally, Semper would have noted this significant diagnostic fact, and we confirm the referral of Psolus complanatus to Psolidium. From our observations of other Psolidium species we anticipate that the long anterior oral cone extension of P. boholensis is probably orientated vertically/dorsally in life.

Mark O'Loughlin has examined the Siboga expedition type specimens of *Psolus* and *Psolidium* species in the former Zoological Museum Amsterdam (ZMA) (now the Dutch Naturalis Biodiversity Centre). A dorsal body wall sample of the type of *Psolus fimbriatus* Sluiter, 1901 (ZMA specimen V.ECH.H1023) revealed the presence of small endplates and curved perforated rod-plates that could only be explained as tube foot support plates and thus indicate the dorsal presence of otherwise inconspicuous tube feet. We thus refer *Psolus fimbriatus* to *Psolidium fimbriatum* (Sluiter, 1901). An examination of the type of *Psolidium disjunctum* Sluiter, 1901 (ZMA specimen V.ECH.H1020) showed the presence of only single-layered dorsal and lateral scales.

### Key to the tropical East Indian-West Pacific species of *Psolidium* Ludwig

- Dorsal and lateral scales each covered with slightly bulbous pillars; lacking mid-ventral radial series of tube feet; branching, needle-like, "thorn" ossicles present in dorsal and ventral body wall ......Psolidium parmatum (Sluiter, 1901) (Indonesia, NW Australia, 95–487 m)

- 4. Tube feet on the mid-ventral ambulacrum discontinuous; cups absent from the body wall ..... *Psolidium boholensis* (Semper, 1867) (Philippines: Bohol; 11–31 m)
- 5. Series of inner tube feet around periphery of sole at least four wide (lacking series of smaller outer marginal tube feet).....6

- Most frequently two tube feet penetrating each scale dorsally and laterally; sole bearing irregularly oval knobbed plates only, typically 4–5 perforations; band of mid-ventral radial tube feet about five-wide.. Psolidium helenae Ong & O'Loughlin, new species (Singapore: East Johor Strait, 0.3–0.4 m)
- 9. Cups and/or cupped crosses present in the body wall......10
- Cups and/or cupped crosses not present in the body wall ..12
- Mid-ventral radial series of tube feet present; basal cross of cups and crosses not covered by fine spinelets......11
- Outer series of smaller tube feet around margin of sole; double row of inner peripheral tube feet around sole; cups and cupped

- crosses of similar size present dorsally, not in sole; rosette ossicles present dorsally and in tentacles; body rounded ventrally in transverse section.......*Psolidium berentsae* O'Loughlin & Maric, 2008 (Australia: Northeast Queensland; 6–18 m)

- Tube feet not present in mid-ventral series; ossicles in sole not thick, knobbed perforated plates......14

- 14. Dorsal and lateral scales large thick single-layered perforated plates; thin knobbed plates with large perforations in sole..... *Psolidium disjunctum* Sluiter, 1901 (Indonesia: Banda Sea; 2,798 m)
- Dorsal and lateral scales double-layered; spinous crosses in sole...... Psolidium translucidum Koehler & Vaney, 1905 (Sri Lanka; 836–1,077 m)

## Psolidium helenae Ong & O'Loughlin, new species (Figs. 1–5)

Psolidium species b.—Ong & Wong, 2015: 275, Table 1.

Material examined. Holotype: ZRC.ECH.0300, Singapore, CMBS spec. no. JS-1196, stn. SW23, East Johor Strait, Pulau Ubin, Chek Jawa, seagrass bed, collected by hand, 1°24.748′N 103°59.711′E, 0.3–0.4 m, seagrass and green algae, coll. Helen Wong et al., 17 October 2012.

Description. Preserved body 18 mm long (including posteriorly projecting anal cone 1.5 mm long), 10.5 mm wide mid-body, 8 mm high anteriorly, 6 mm high posteriorly; thick imbricating rugose scales dorsally and laterally, scales up to about 2.5 mm wide, ventral sole lacking scales; sub-rectangular form ventrally with rounded anterior and posterior ends, mouth dorso-anterior, lacking oral valves, short anal cone directed posteriorly, lacking anal valves. Cucumariid-type calcareous ring; radial plates with anterior notch for longitudinal muscle attachment, notch posteriorly, lacking posterior prolongations; inter-radial plates tapered to an anterior point, posterior indentation. Ten dendritic tentacles, eight large, two ventral smaller. Gonad tubules not branched. Up to three tube feet penetrate each scale dorsally and laterally, tube foot discs about 0.15 mm diameter; margin of the ventral sole with wide band of close-set tube feet extending to the edge of the sole, band about 6 tube feet wide, not in transverse rows, lacking discrete outer series of smaller marginal tube feet; close-set band of tube feet along mid-ventral radius, about five tube feet wide, ventral tube feet disc diameters about 0.25 mm.



Fig. 1. Dorsal view of live holotype of *Psolidium helenae* Ong & O'Loughlin, new species (ZRC.ECH.0300; 18 mm long preserved).

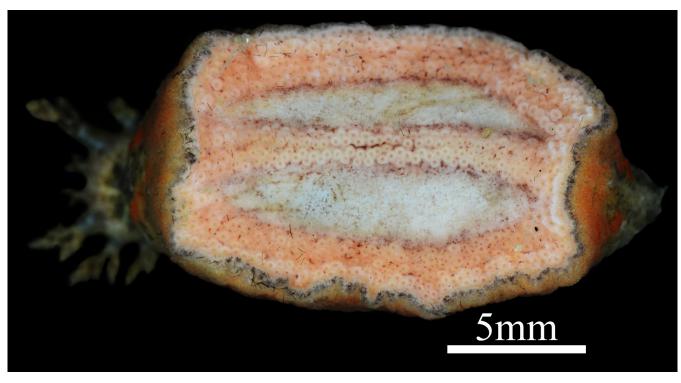


Fig. 2. Ventral view of live holotype of Psolidium helenae Ong & O'Loughlin, new species (ZRC.ECH.0300; 18 mm long preserved).

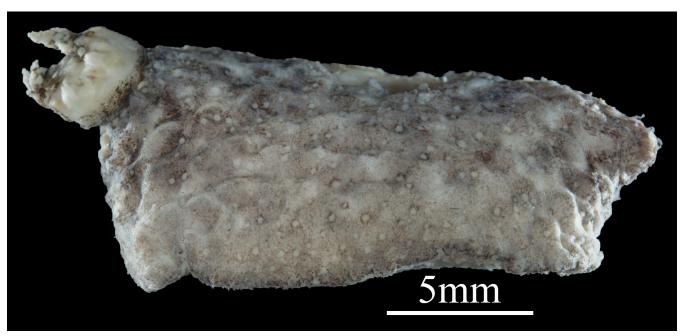


Fig. 3. Lateral view of preserved holotype of Psolidium helenae Ong & O'Loughlin, new species (ZRC.ECH.0300; 18 mm long preserved).

Dorsal and lateral multi-layered ossicles (scales), plates and distally spinous cupped crosses; small plates irregularly oval in form and size, smooth, lobed marginally, not knobbed, typically 2–6 perforations, 66–133 μm long; cupped crosses variable in form, 56-109 µm long, typically with four bifid arms that are distally finely spinous, arms joining distally on a few crosses to form cups. Dorsal tube feet with endplates and elongate perforated support plates; endplates up to 104 µm diameter; short, wide, curved, perforate, marginally spinous endplate support ossicles, up to about 114 µm long. Sole with knobbed plates only, irregularly oval, typically 4-5 perforations, large knobs around margin, some surface knobs, plates up to 157 µm long by 96 µm wide. Ventral tube feet with tube foot support plates, elongate, perforated, variably marginally knobbed, up to about 240 µm long. Tentacles with rod plates, rods and rosettes; plates usually wide mid-rod, multi-perforate over plate, variable form, up to 450 µm long, 134 µm wide mid-rod; rod-plates inter-grade with curved rods, largest perforate mid-rod and distally; rods inter-grade with fine non-perforate rods about 40 µm long; rosettes irregularly oval, about 20-45 µm long.

Live dorsal colour orange over the lateral scales becoming yellow along the upper dorsal surface; small pale yellow spots (tube feet ends) scattered over the dorsal and lateral sides; introvert and tentacles pale yellow-brown with black markings; mouth red. Preserved dorsal and lateral colour grey to pale brown with black flecking. Preserved ventral sole white, tentacles white with black markings, introvert white.

**Distribution.** Singapore: East Johor Strait, Chek Jawa, seagrass bed, 0.3–0.4 m.

**Etymology.** Named *helenae* in appreciation of the generous, gracious, considerable and much appreciated supportive contribution of Helen Pei-San Wong (of the Tropical Marine Science Institute of the National University of Singapore).

**Remarks.** Psolidium helenae Ong & O'Loughlin, new species, is distinguished from all other tropical East Indian—West Pacific region Psolidium species in the key above and by the following combination of characters: a low profile; a band of tube feet around the periphery of the sole up to six-wide; an absence of smaller outer marginal tube feet on the sole; a band of mid-ventral tube feet on the sole about five-wide; distally spinous cupped cross ossicles present dorsally.

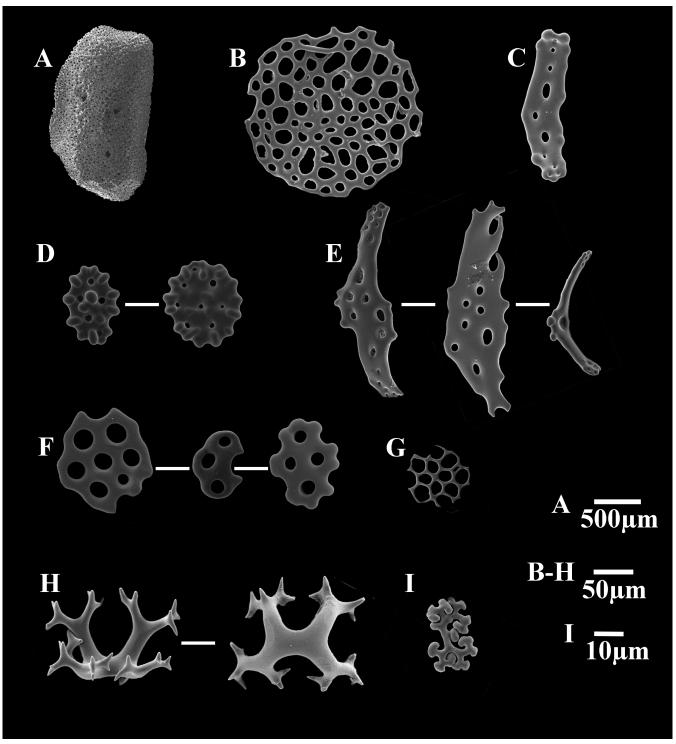


Fig. 4. SEM holotype ossicle views for *Psolidium helenae* Ong & O'Loughlin, new species (ZRC.ECH.0300). A, scale from dorsal body wall, showing canals for tube feet; B, endplate from sole tube foot; C, supporting rod from sole tube foot; D, supporting knobbed plates from sole; E, rod plates from tentacles; F, plates from dorsal body wall; G, endplate from dorsal body wall; H, cupped cross from dorsal body wall; I, rosette from tentacle.

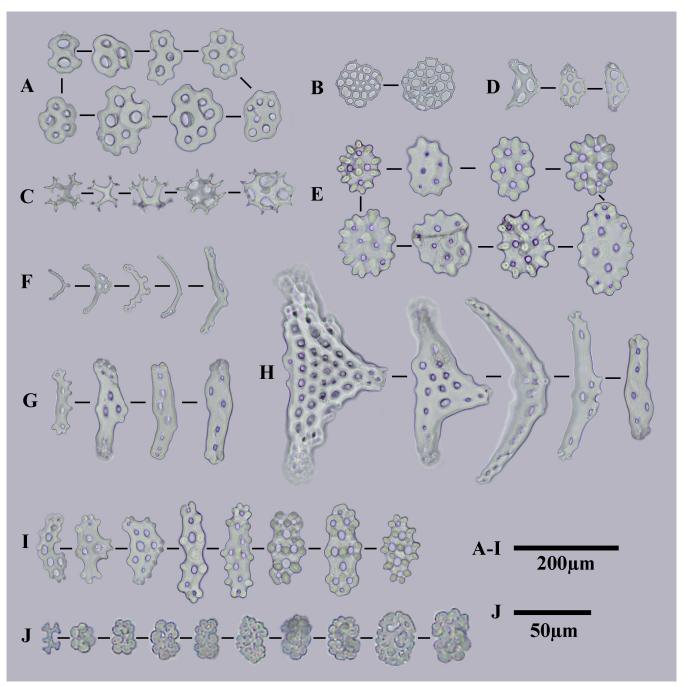


Fig. 5. Ossicles of holotype *Psolidium helenae* Ong & O'Loughlin, new species (ZRC.ECH.0300), taken by light microscope. A, plates from dorsal body wall; B, endplates from dorsal tube feet; C, cupped crosses from dorsal body wall; D, cupped plates from dorsal body wall; E, knobbed plates from sole; F, rods from tentacles; G, supporting rods from tube feet; H, rod plates from tentacles; I, supporting plates from tube feet; J, rosettes from tentacles.

### Psolidium wongae Ong & O'Loughlin, new species (Figs. 6-10)

Psolidium species a.—Ong & Wong, 2015: 275, Table 1.

**Material examined.** Holotype: ZRC.ECH.0299, Singapore, CMBS spec. no. JS-0569, stn. DW55, East Johor Strait, north east of Pulau Tekong, north of Tanjong Pasir River, collected by rectangular dredge, 1°25.003′ to 1°25.097′N, 104°05.049′ to 104°04.997′E, 11.6–13.0 m, laterite gravel and dead shells, coll. Bertrand Richer de Forges et al., 22 October 2012.

**Description.** Thick scales dorsally and laterally up to 2.0 mm wide, thin ventral sole; irregularly oval ventrally with slight taper towards anal end; body 22 mm long, 14 mm wide mid-body, 7 mm high mid-body. Slightly elevated orally, no cone, lacking oral valves, small papillae present; slightly elevated anally, no cone, lacking valves, small papillae present. Ten dendritic tentacles, eight large, two ventral small. Cucumariid-type ring; radial plates with anterior notch for longitudinal muscle attachment, notch posteriorly, lacking posterior prolongations; inter-radial plates tapered to an anterior point, posterior indentation. Up to four tube feet penetrate each scale dorsally and laterally, discs about 0.1 mm diameter. Margin of the ventral sole fluted/ribbed due to attachment to a scallop (Pectinidae) shell and with up to about 10 tube feet in a diagonal series along each fluted ridge in the mid-body, some tube feet between the ridges in the grooves; overall diagonal and not transverse fluting and tube feet series; lacking discrete outer series of smaller marginal tube feet at the edge of the sole; tube feet along mid-ventral radius in more scattered series, some on fluted ridges; ventral tube feet disc diameters about 0.2 mm.

Single polian vesicle. Gonad tubules not branched.

Multi-layered ossicles (scales) and small plates dorsally and laterally, lacking cups; multi-layered ossicles irregular in form and size, up to 1.9 mm long; plates smooth with lobed margins, not knobbed, irregularly oval in form and size, 3–11 perforations, 76–156 µm long. Sole with knobbed plates, variable in form and size, up to about 20 perforations, variably knobbed around margin and on surface, plates 91–192 µm long. Dorsal tube feet with elongate perforated support plates up to about 160 µm long. Ventral tube feet with elongate, perforated, variably knobbed, curved support plates, 129-312 µm long. Tentacles with rod plates, rods and rosettes; plates narrow, elongate, perforated, curved, irregular margins, 92-284 µm long; curved rods with short branches distally, smooth or with a few blunt knobs, up to about 170 µm long; rosettes predominantly oval, compact, up to about 60 µm long.

Live dorsal colour mottled with light and dark grey-brown predominantly on the lower sides, and white with pale and dark grey-brown over the apex; tube feet discs white. Preserved dorsal and lateral colour granular white (presence of surface plates) over grey-black. Preserved ventral sole white.



Fig. 6. Dorsal view of live holotype of *Psolidium wongae* Ong & O'Loughlin new species, (ZRC.ECH.0299; 22 mm long preserved).



Fig. 7. Lower right, ventral view of live holotype of *Psolidium wongae* Ong & O'Loughlin, new species (ZRC.ECH. 0299; 22 mm long preserved), showing artificial fluted ridges; upper left. Pectinidae shell showing hard fluted ridges

Tentacles and introvert white with close grey-black flecking.

**Distribution.** Singapore: East Johor Strait, northeast of Pulau Tekong, north of Tanjong Pasir River, 11.6–13.0 m.

**Etymology.** Named *wongae* in appreciation of the generous, gracious, considerable and much appreciated supportive contribution of Helen Pei-San Wong (of the Tropical Marine Science Institute of the National University of Singapore).

**Remarks.** Psolidium wongae Ong & O'Loughlin, new species, is distinguished from all other tropical East Indian—West Pacific Psolidium species in the key above and by the following combination of characters: a low profile; tube feet series around the periphery of the sole up to about 10 wide; an absence of smaller outer marginal tube feet on the sole; an absence of cupped cross ossicles dorsally and ventrally.

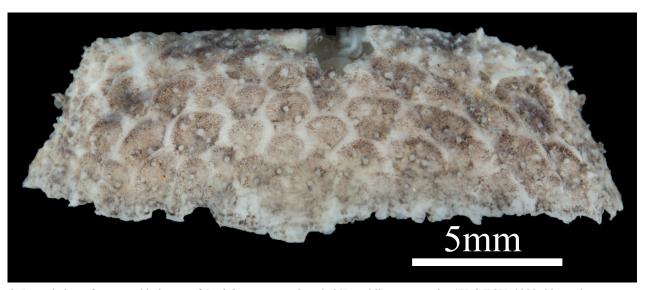


Fig. 8. Lateral view of preserved holotype of *Psolidium wongae* Ong & O'Loughlin, new species (ZRC.ECH. 0299; 22 mm long preserved).

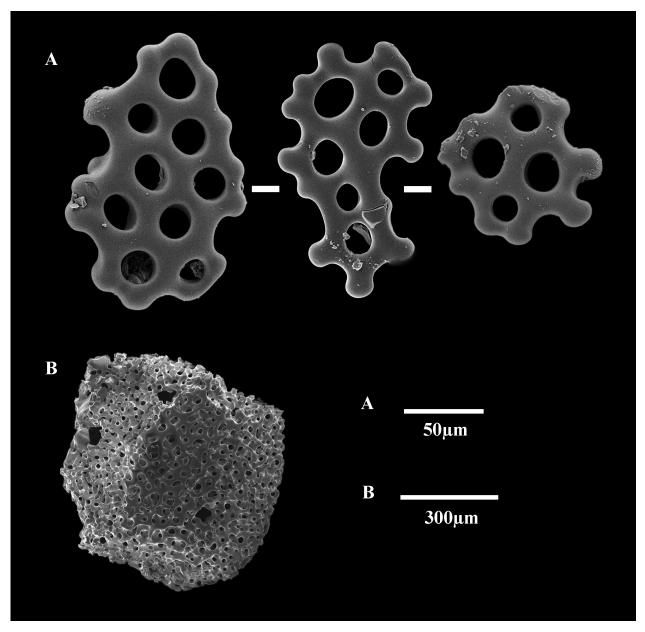


Fig. 9. SEM holotype ossicle views for *Psolidium wongae* Ong & O'Loughlin, new species (ZRC.ECH.0299). A, plates from dorsal body wall; B, scale from dorsal body wall, showing canals for tube feet.

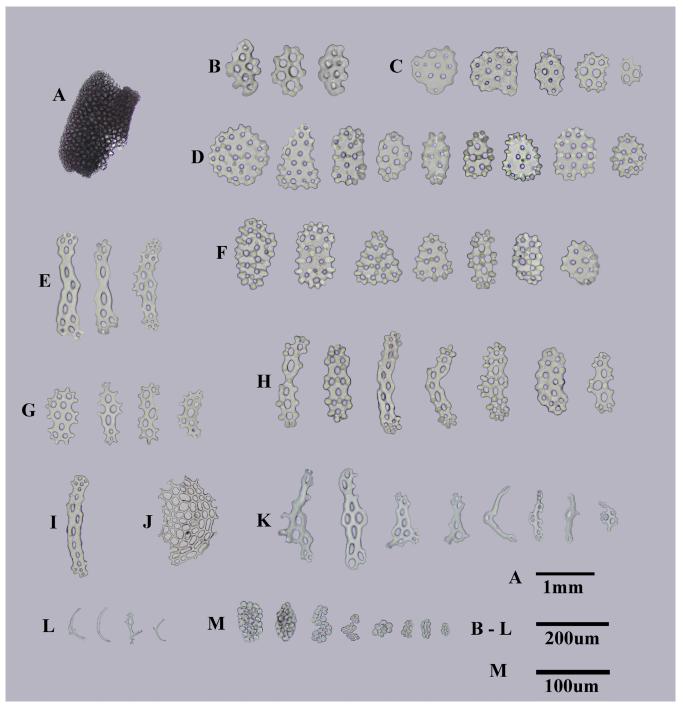


Fig. 10. Ossicles of holotype of *Psolidium wongae* Ong & O'Loughlin, new species (ZRC.ECH.0299), as viewed through the light microscope. A. Multilayered scale ossicle from dorsal wall; B, plates from dorsal body wall; C, plates from sole; D, knobbed plates from sole; E, rod plates from sole tube feet; F, knobbed plates from sole tube feet; G, plates from sole tube feet; H, supporting rod plates from sole tube feet; I, rod plate from sole tube feet; J, endplate from sole tube foot; K, supporting rod plates from tentacle; L, rod plates from tentacles; M, rosettes from tentacle.

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We are appreciative of our opportunity to work on the sea cucumbers of the Comprehensive Marine Biodiversity Survey (CMBS) organised by the National University of Singapore (NUS) and the National Parks Board (NParks). We record our recognition of the high quality of the collecting, sorting and preliminary identifying of the collections by the CMBS team, visiting scientists and NParks volunteers. The visit to TMSI by Mark O'Loughlin from Museum Victoria, Australia was supported by NUS and NParks. We also acknowledge the St. John's Island National Marine Laboratory (SJINML) for providing the facilities necessary for conducting the research. The Laboratory is a National Research Infrastructure under the National Research Foundation, Singapore. MOL acknowledges with gratitude the role of Joke Bleeker (at the then ZMA) for graciously facilitating the opportunity for him to examine the type specimens from the Siboga Expedition. We are grateful for the close and helpful reviews of our manuscript by Mariano Ignacio Martinez, Francisco A. Solis-Marin, and a third anonymous reviewer.

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