

A New Species of *Thyone* (Echinodermata: Holothuroidea: Dendrochirotida: Phyllophoridae) from Wakayama, Japan

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A new phylloporid sea cucumber, *Thyone susamiensis* sp. nov., is described from the intertidal zone of the south coast of Wakayama Prefecture, western Japan. It is distinguished from the most of its congeners by the absence of ossicles in the body wall. *Thyone susamiensis* has only rosette-like ossicles in the introvert, where table-like ossicles are usually observed in other *Thyone* species. The usual table-like ossicles were observed only in the peri-oral skin in *T. susamiensis*. Among congeners, this condition has been reported only in *Thyone flindersi* O'Loughlin, Barmos, and VandenSpiegel, 2012. However, *T. flindersi* occasionally has table-like ossicles in the anal appendages, where *T. susamiensis* usually only has rod-like ossicles. The general body colouration and the shape of the calcareous ring are also distinctive, separating species *T. susamiensis* from *T. discolor* Sluiter, 1901, *T. roscovita* Hérouard, 1889, and *T. venusta* Selenka, 1867, species for which detailed description of ossicles are lacking.

Key Words: Holothuroidea, Dendrochirotida, Phyllophoridae, *Thyone susamiensis* sp. nov., new species, Japan.

Introduction

The dendrochirotid sea-cucumber genus *Thyone* Oken, 1815 (type species: *Holothuria fusus* Müller, 1776) is one of the oldest in the class Holothuroidea and has a complicated nomenclatural history. Names established in Oken (1815–1816), including *Thyone*, were once ruled to be unavailable since the works did not strictly follow the Principle of Binominal Nomenclature (International Commission on Zoological Nomenclature [ICZN] 1956). While Jaeger (1833) subsequently made the dendrochirotid *Thyone* available, Lesson (1830) had earlier erected an aspidochirotid holothurian genus with the same name: *i.e.*, the aspidochirotid *Thyone* Lesson, 1830 had precedence over the dendrochirotid *Thyone* Jaeger, 1833. Despite the above ICZN ruling, however, *Thyone* has been in prevailing usage in the sense of Dendrochirotidae, rather than Aspidochirotidae. This prompted Paulay and O'Loughlin (2012) to ask the ICZN to make *Thyone* Oken, 1815 available under its plenary power. Recently, a ruling on Paulay and O'Loughlin's (2012) Case 3598 was published as OPINION 2367 (ICZN 2015) to make *Thyone* Oken, 1815 an available and valid name within the Dendrochirotidae, thereby maintaining current usage in accordance with Article 82.1 of the International Code of Zoological Nomenclature (ICZN 1999).

Being the most speciose genus in the class Holothuroidea, *Thyone* is problematic not only nomenclaturally, but also taxonomically. Oken's (1815) original diagnosis was quite simple, based on the number of expansible tentacles (10) and the shape of the composite calcareous ring (thin and tubular). Because this combination of features applies to many

other holothurians even in different families (*e.g.*, species in the sclerodactyliid genus *Havelockia* Pearson, 1903), a number of species once placed in *Thyone* have been transferred to other taxa, such as *Stolus* Selenka, 1867. Still, 61 species and two subspecies are currently contained in *Thyone* (Paulay 2014), many of which require scrutiny to ascertain their precise taxonomic placement.

Although no full redescription of the type species, *T. fusus* (Müller, 1776), has been published, detailed information concerning some of its features was provided by Madsen (1941). According to this and other publications, many of the currently recognized species of *Thyone* share certain common features, which are presently understood as defining features of the genus: 10 tentacles comprising eight large and two small (ventral) ones; a long, tubular composite calcareous ring, in which the anterior first piece of each radial and inter-radial element is broad with anterior projections, more posterior to which a mosaic pattern of 1–3 rows of pieces is usually formed; a bifid posterior part of the radial elements, usually with the beginning of the fork situated posterior (sometimes barely anterior) to the rear end of the adjacent inter-radial element; one Polian vesicle and one (sometimes two) stone canals; pedicels covering the entire body; no pedicels on the introvert; the body elongate and cylindrical or tapered tapered both ends; multi-perforate plate-like ossicles or rosette-like ossicles in the tentacles; and usually two-pillared table-like ossicles in the body wall (these sometimes lacking).

The following six nominal species of *Thyone sensu lato* have been reported from Japanese waters: *Thyone benti* Deichmann, 1937 from Sagami Bay (Kuramochi 2006); *T. bicornis* Ohshima, 1915 from Suruga Bay (Ohshima 1915);

T. multipes Augustin, 1908 from Uraga Strait and Sagami Bay (Augustin 1908) and Suruga Bay (Ohshima 1915); *T. parva* Ohshima, 1915 from off the Ojika Peninsula (Ohshima 1915); *T. punctata* Ohshima, 1915 from off Cape Sata (Ohshima 1915); and *T. sacellus* (Selenka, 1867) from off Kyushu (Mitsukuri 1912). Today, *T. multipes*, *T. parva*, and *T. punctata* are accepted as *Allothyone multipes*, *Paracucumaria parva*, and *Stolus punctatus*, respectively (Panning 1949), and *T. sacellus* is considered a synonym of *Stolus buccalis* (Stimpson, 1855) (Thandar 1990). However, according to Augustin's (1908) description, *Allothyone multipes* actually does not display the diagnostic features of *Allothyone* Panning, 1949 (*q.v.*), so it may really belong to *Thyone*. Furthermore, *Thyone benti* actually should be included in *Eupentacta* Deichmann, 1938 or *Havelockia* Pearson, 1903 (see Discussion). Therefore, currently, only *Thyone bicornis* and *Allothyone multipes* are the only confirmed or suspected representatives of *Thyone* in Japanese waters. *Thyone bicornis* was found at a depth of 86 m in Suruga Bay, Shizuoka, during the North-western Pacific survey by the U.S. Fish Commission Steamer *Albatross* (Ohshima 1915). *Allothyone multipes* was found at a depth of 350 m in the Uraga Strait between Chiba and Kanagawa, and at a depth of 150 m in Sagami Bay, Kanagawa (Augustin 1908). Recently, we found a species of *Thyone* that is distinct from both *T. bicornis* and *A. multipes* on an intertidal shore of the Pacific Ocean farther southwest in Japan. We compared this species to all its congeners through a careful survey of the literature, which resulted in the conclusion that this shore-dwelling species is new to science.

Materials and Methods

Ten specimens of the new species were collected by the authors between December, 2013, and January, 2014, from the type locality, *viz.*, the intertidal zone of a boulder shore (33°30'21"N, 135°35'24"E) in the town of Susami on the south coast of Wakayama Prefecture, Japan.

Five specimens were fixed in 80% ethanol after anaesthesia; the remaining five were fixed without anaesthesia. Anaesthesia was performed in a menthol solution: 0.37 g of menthol was dissolved into 100 mL of 99% ethanol, which was diluted to 3.0% with seawater. The specimens were injected with 0.5–2.5 mL of this diluted solution and immersed in the diluted solution for 2.0–6.0 h; when the anaesthesia had taken effect, the tentacles emerged in response to a gentle squeeze of the body. Before fixation, hydrated magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) was added to the solution at a concentration of 3.0 g per 100 mL for further anaesthesia.

All specimens were dissected; the tentacles, anal appendages, Polian vesicles, stone canals, and calcareous rings were examined under a stereoscopic dissecting microscope (Nikon SMZ). In order to observe the ossicles' morphology, small pieces of tissue were removed from the following parts of the body (Figs 1D, E, 2A, B): 1) the mid-parts of the inter-radial tentacle on the abdominal-lateral side; 2) the peri-

oral skin on the abdominal-lateral side; 3) the skin of the oesophagus on the abdominal-lateral side; 4) the anterior, 5) middle, and 6) posterior parts of the skin of the introvert in the inter-radius, on the abdominal-lateral side; 7) the anterior, 8) middle, and 9) posterior parts of the body-wall integument in the inter-radius, on the abdominal side; 10–12) the integument of the same regions of the body on the dorsal side; 13) pedicels of the middle parts of the abdominal side; and 14) the body-wall integument and 15) appendages around the anus, both on the abdominal-lateral side. Examination of ossicles from parts 2) and 3) was done only for three specimens because the necessary dissection resulted in major damage to the specimens.

The tissue samples were dissolved in sodium hypochlorite (NaClO , 5%) under a compound microscope (Nikon Optiphot). Because no obvious differences were found among the ossicles of the 10 specimens examined, those of three specimens were counted and measured on the basis of photographs. We classified the ossicles into five types: two types of 'rosette' (*i.e.*, rosette-like ossicle, extremely branched and perforated), one type of 'table' (ossicles consisting of a multi-perforate 'disc' and a skeleton-constructing 'spire' that grows perpendicularly from the disc), one type of 'rod' (rod-like ossicles armed distally with lumps or branches or few perforations), and one type of 'plate' (multi-perforate flat ossicles). For every tissue sample, we measured a total of 10–100 ossicles to the nearest micrometer. The type specimens of the new species and glass slides of their ossicles have been deposited in the Invertebrate Collection (INV) of the Wakayama Prefectural Museum of Natural History (WMNH), in Kainan, Wakayama, Japan.

Thyone susamiensis sp. nov.

(Figs 1–7)

Material examined. Holotype, WMNH-2014-INV-2 (anaesthetized; length 127 mm, width 20 mm). Paratypes: WMNH-2014-INV-1 (anaesthetized; length 111 mm, width 17 mm); WMNH-2014-INV-3 (anaesthetized; length 121 mm, width 19 mm); WMNH-2014-INV-4 (anaesthetized; length 94 mm, width 18 mm); WMNH-2014-INV-5 (anaesthetized; length 74 mm, width 8 mm); WMNH-2014-INV-6 (unanaesthetized; length 54 mm, width 22 mm); WMNH-2014-INV-7 (unanaesthetized; length 51 mm, width 9 mm); WMNH-2014-INV-8 (unanaesthetized; length 71 mm, width 23 mm); WMNH-2014-INV-9 (unanaesthetized; length 58 mm, width 25 mm); WMNH-2014-INV-10 (unanaesthetized; length 65 mm, width 22 mm). All specimens from type locality; collection details given in Materials and Methods section above.

Description. Appearance of anaesthetized specimens not drastically altered by fixation. General colour of specimens also not seriously affected by fixation/preservation (Fig. 1A–C). Body slightly curved or U-shaped, with both ends tapered and turned upwards; body wall soft and thin. Body whitish-yellow or cream, with blackish-purple marbling. Anterior and posterior ends with blackish-purple pigmentation in most specimens.

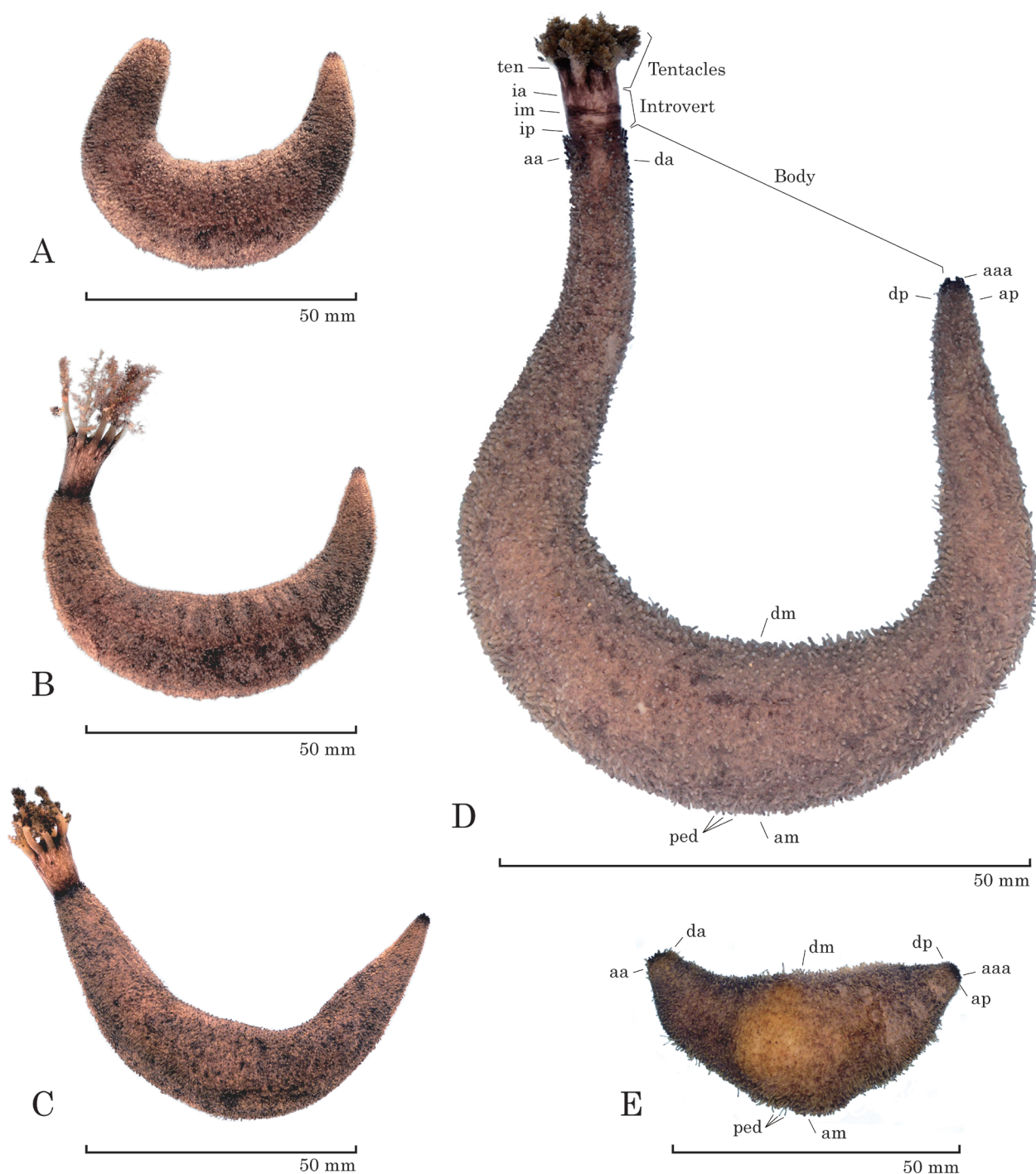


Fig. 1. *Thyone susamiensis* sp. nov., lateral views, A–C, WMNH-2014-INV-4 (paratype). A, B, Live specimen, before (A) and after (B) anaesthesia; C, fixed with anaesthesia; D, WMNH-2014-INV-2 (holotype), fixed with anaesthesia; E, WMNH-2014-INV-9 (paratype), fixed without anaesthesia, tentacles and introvert entirely retracted. Abbreviations indicate sites of tissue sampling for ossicle examination: aaa, anal appendage on abdominal-lateral side; aa, anterior part of body on abdominal side; am, middle part of body on abdominal side; ap, posterior part of body on abdominal side; da, anterior part of body on dorsal side; dm, middle part of body on dorsal side; dp, posterior part of body on dorsal side; ia, anterior part of introvert; im, middle part of introvert; ip, posterior part of introvert; ped, pedicel; ten, tentacle.

Tentacles arranged in single circle; two tentacles on ventral side smaller (Fig. 2A). Colour of tentacles and introvert whitish-yellow or cream, and generally pigmented with brown or blackish-purple markings, occasionally variegated. Tentacle tips (nodules) tinged with brown or blackish-purple. Short processes present, surrounding entrance of oesophagus.

Non-retractile pedicels covering entire body at constant density. On each side of narrow longitudinal muscles, pedicels forming ambiguous, contiguous row. Pedicels generally paler than adjacent body. Pedicels becoming gradually smaller anterior and posterior to those in middle portion of body, absent in introvert region (Fig. 1B–D, Table 1). Pedicels blackish-purple on both ends of body in most speci-

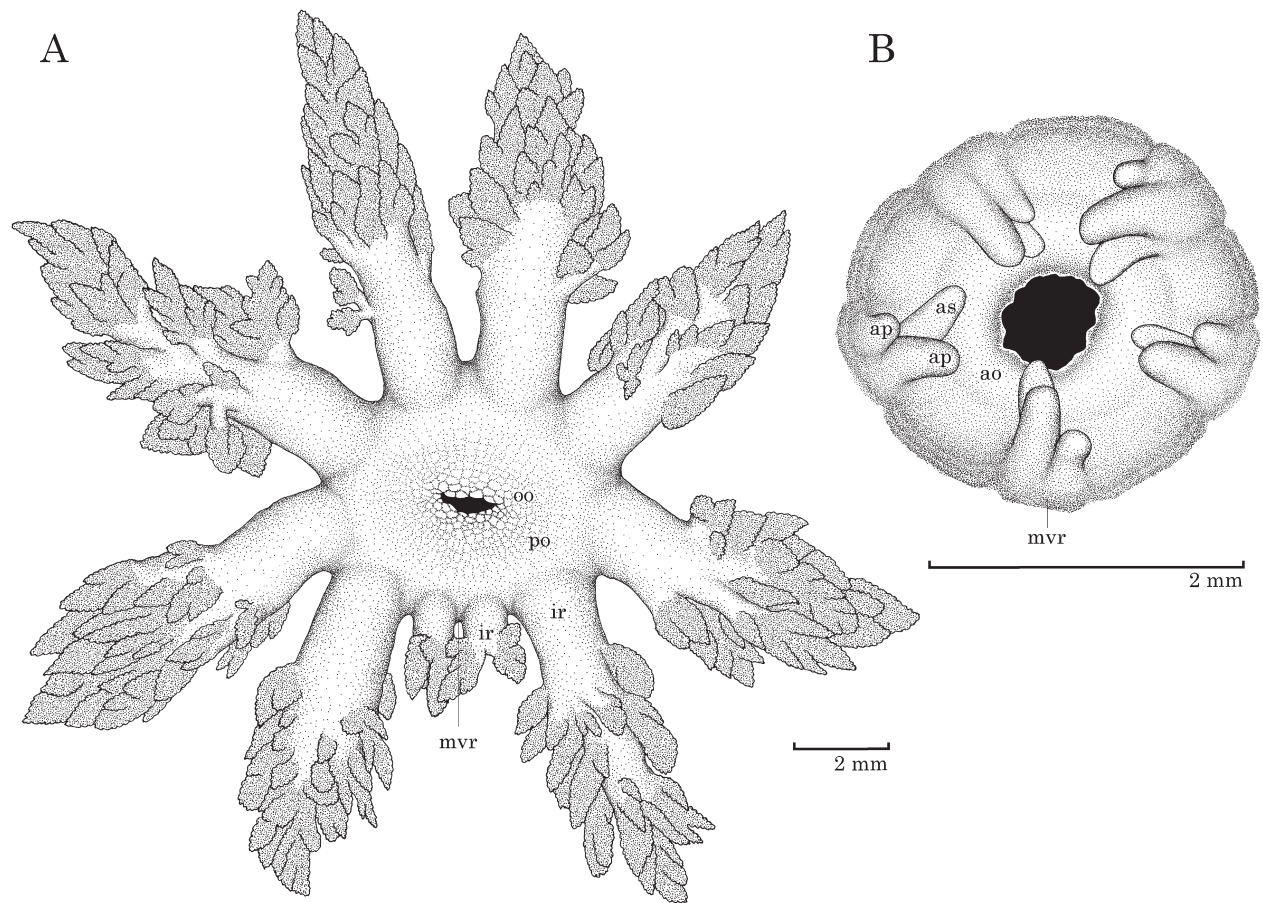


Fig. 2. *Thyone susamiensis* sp. nov., A and B, WMNH-2014-INV-3 (paratype), front view of oral opening and circumoral tentacles (A), front view of anal opening and anal appendages (B). Abbreviations: ao, anal opening; ap, anal papilla; as, anal scale; ir, inter-radial tentacle; mvr, mid-ventral radial part; oo, oral opening; po, peri-oral part.

Table 1. Summary of measurements and counts for 10 specimens of *Thyone susamiensis* sp. nov.

| No. ^a | Type status ^b | Body state ^c | Gonad state ^d | Body size (mm) | | Size of calcareous ring element in abdominal-lateral side (mm) | | | | Counts | | | | | | |
|------------------|--------------------------|-------------------------|--------------------------|----------------|-------|--|-------|--------------|-------|--------------------------------|-----------|-----------|---------------|------------|--------------|-----------------|
| | | | | | | Radial | | Inter-radial | | Pedicels in a row ^e | | Tentacles | Anal papillae | Anal scale | Stone canals | Polian vesicles |
| | | | | Length | Width | Length | Width | Length | Width | Body | Introvert | | | | | |
| 1 | p | a | t | 111 | 17 | 15.4 | 1.4 | 9.5 | 1.4 | >150 | 0 | 10 | 10 | 7 | 1 | 1 |
| 2 | h | a | t | 127 | 20 | 21.3 | 1.4 | 13.7 | 1.4 | >180 | 0 | 10 | 10 | 5 | 1 | 1 |
| 3 | p | a | o | 121 | 19 | 17.4 | 1.3 | 11.5 | 1.3 | >190 | 0 | 10 | 10 | 5 | 1 | 1 |
| 4 | p | a | o | 94 | 18 | 16.5 | 1.6 | 8.5 | 1.5 | >170 | 0 | 10 | 10 | 5 | 1 | 1 |
| 5 | p | a | i | 74 | 8 | 13.8 | 1.4 | 6.1 | 1.1 | >120 | 0 | 12 | 7 | 3 | 1 | 1 |
| 6 | p | u | o | 54 | 22 | 18.2 | 1.6 | 10.2 | 1.0 | ? | 0 | 10 | 10 | 5 | 1 | 1 |
| 7 | p | u | i | 51 | 9 | 13.4 | 1.0 | 6.0 | 1.0 | ? | 0 | 10 | 10 | 5 | 1 | 1 |
| 8 | p | u | o | 71 | 23 | 16.7 | 1.4 | 9.7 | 1.9 | ? | 0 | 10 | 10 | 5 | 2 | 1 |
| 9 | p | u | o | 58 | 25 | 18.5 | 1.5 | 9.8 | 1.2 | ? | 0 | 10 | 10 | 5 | 1 | 1 |
| 10 | p | u | t | 65 | 22 | 21.2 | 1.7 | 9.4 | 1.5 | ? | 0 | 10 | 10 | 5 | 2 | 1 |

^aRegistration No. Wakayama Prefectural Museum of Natural History, Invertebrate Collection of 2014 (WMNH-2014-INV-). ^bp: paratype, h: holotype. ^ca: anaesthetized, u: unanaesthetized. ^di: immature, o: ovarium, t: testis. ^eNumber of pedicels in a row along to longitudinal muscle in abdominal side.

mens. Ten anal papillae and five anal scales in most radii (five pairs of anal papillae in outer circle and five anal scales in inner circle; Fig. 2B, Table 1), generally pigmented brown or blackish-purple. Each anal scale containing a long calcare-

ous structure. Base of anal scale connected to thin calcareous plate forming delicate calcareous ring surrounding anal region. Tubular composite calcareous ring present, encircling oesophagus. Pieces of radial and inter-radial elements forming

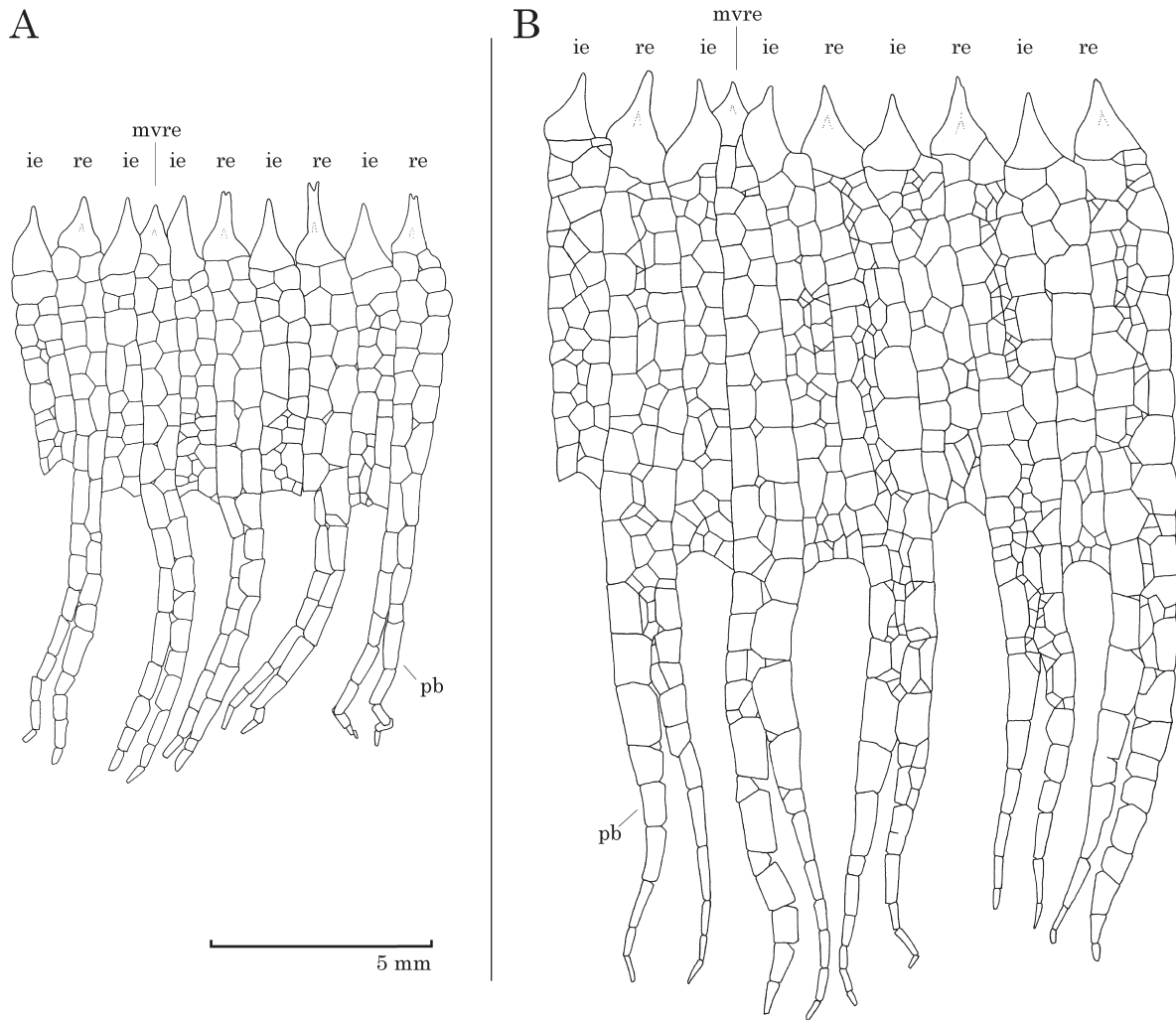


Fig. 3. *Thyone susamiensis* sp. nov., elements of calcareous rings, viewed from body cavity. A, WMNH-2014-INV-7 (paratype); B, WMNH-2014-INV-10 (paratype). Abbreviations: ie, inter-radial element; mvre, mid-ventral radial element; pb, posterior bifurcation; re, radial element.

mosaic pattern (*i.e.*, not lined up). Radial elements bifurcate posteriorly, approximately twice as long as inter-radials, posterior forks of radial elements starting posterior to ends of adjacent inter-radial elements (Fig. 3A, B, Table 1). Radials and inter-radials with sharp anterior projections; some projections on radials bifid at tip; anterior first piece of each radial and inter-radial element broad, pieces after that narrow, and usually aligned roughly in one to three (mostly two) longitudinal row. Most specimens ($n=8$, including holotype) with one Polian vesicle and one stone canal (Table 1). Tubules of gonads unbranched.

Two types of ossicles found in tentacles, *viz.*, ‘rosettes’ and ‘rods’, as well as full series of intermediate forms; these ossicles, irrespective of form, with mean length and mean central width $61\text{--}78\text{ }\mu\text{m}$ and $17\text{--}20\text{ }\mu\text{m}$ (depending on specimen), respectively (Fig. 4A, B, Table 2). Ossicle size not significantly different among three specimens examined (Kruskal-Wallis test, $P_s>0.05$).

Two types of ossicles found in peri-oral skin: 1) ‘rosettes’, with mean length and mean central width $32\text{--}35\text{ }\mu\text{m}$ and $19\text{--}21\text{ }\mu\text{m}$ (depending on specimen), respectively; size not significantly different among three specimens examined

Table 2. Abundance (%) and size (μm) of ossicles from inter-radial tentacle of *Thyone susamiensis* sp. nov. (Fig. 4A, B).

| | % | Length | Width ^a |
|--------------------------------|--------------|--------------------------|--------------------------|
| | | Mean \pm sd (Range) | Mean \pm sd (Range) |
| WMNH-2014-INV-2 | | | |
| Rosettes and rods ^b | 100 (100) | 62 ± 25 (24–177) | 20 ± 11 (2–56) |
| WMNH-2014-INV-7 | | | |
| Rosettes and rods ^b | 100 (100) | 61 ± 19 (26–114) | 18 ± 8 (2–43) |
| WMNH-2014-INV-8 | | | |
| Rosettes and rods ^b | 100 (100) | 78 ± 36 (31–195) | 17 ± 10 (2–37) |

^aWidth in the central part, not the maximum value. ^bBecause it is difficult to draw a definite line of demarcation between rosettes and rods, both types were measured without distinguishing them.

(Kruskal-Wallis test, $P_s>0.05$); and 2) ‘tables’ with short spires and large discs, spires with two pillars and zero to two cross-beam connections, discs chiefly oval. Mean length

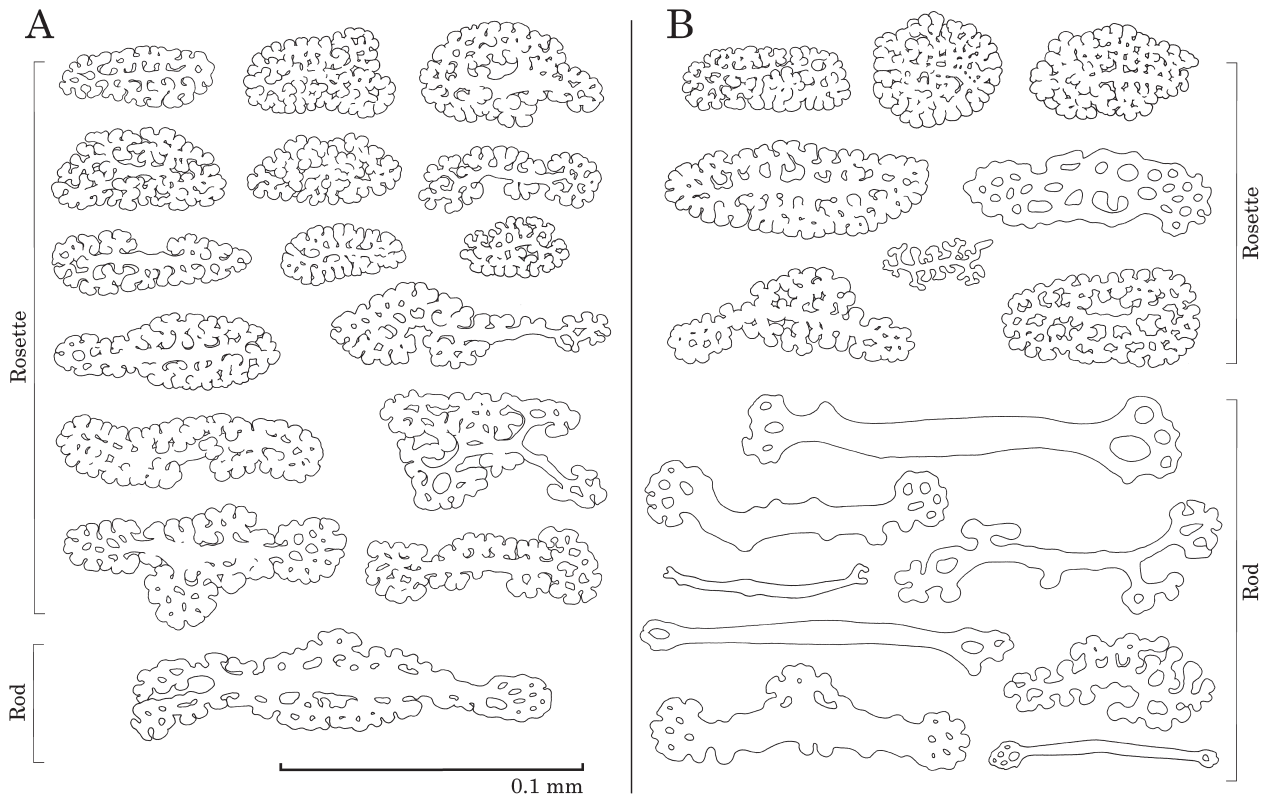


Fig. 4. *Thyone susamiensis* sp. nov., ossicles of inter-radial tentacle on abdominal-lateral side. A, WMNH-2014-INV-7 (paratype); B, WMNH-2014-INV-8 (paratype).

and mean central width of discs 64–70 μm and 39–57 μm (depending on specimen), respectively (Fig. 5A, B, Table 3); number of perforations in each disc 8–40, with mean of 18–27 holes (depending on specimen). Statistical analysis not applied to this type of ossicle, due to their very low frequency of occurrence.

Single type of ossicle (*viz.*, ‘small rosettes’) found in processes of oesophagus, mean length and mean central width 22–25 μm and 9–12 μm (depending on specimen), respectively (Fig. 5A, B, Table 3). Measurements not different significantly among three examined specimens (Kruskal-Wallis test, $P_s > 0.05$).

Single type of ossicle (*viz.*, ‘rosettes’) found in introvert throughout its anterior, middle, and posterior parts; mean length and mean central width 31–39 μm and 21–27 μm (depending on specimen), respectively (Fig. 5A, B, Table 3). These sizes not differing significantly among the three parts and three examined specimens (Kruskal-Wallis test, $P_s > 0.05$). Ossicles in posterior part not dense.

Integument of body wall lacking ossicles throughout anterior, middle, and posterior parts, on both abdominal and dorsal sides.

Pedicels displaying only one type of ossicle (*viz.*, ‘endplates’). In middle part of abdominal side, diameter of endplates 117–195 μm (mean 149–180 μm , depending on specimen) and approximately 47–144 holes (mean 81–123, depending on specimen) (Fig. 6A, B, Table 4). Diameter of endplates significantly different among three specimens (Kruskal-Wallis test, $P < 0.01$), smaller in smaller specimens.

Approximately three different forms of hole arranged in three concentric areas on each endplate, *viz.*, latitudinally elongated holes near rim, longitudinally elongated holes in mid region, and almost circular holes in central area.

Body-wall around anus lacking ossicles, except for fragments of calcareous plates surrounding anal region. Usually, anal appendages with one type of ossicle (*viz.*, ‘rods’) in anal papillae (Fig. 7A, B, Table 5), mean length and mean central width 91–96 μm and 4.4–6.3 μm (depending on specimen), respectively, not significantly different in size among three examined specimens (Kruskal-Wallis test, $P_s > 0.05$). Only one of these three specimens displaying, in low frequency, another type of ossicle (*viz.*, ‘rosettes’) in anal appendages, with mean length of 39 μm and mean central width of 23 μm (Fig. 7B, Table 5).

Distribution. So far known only from type locality.

Ecology. At the type locality, individuals of *Thyone susamiensis* were found burrowing in sand and pebbles that filled up spaces between boulders, often spreading their tentacles into the water (at least at night). We collected the following other species of sea cucumber nearby: *Holothuria* (*Lessonothuria*) *pardalis* Selenka, 1867; *H. (Mertensiothuria)* *leucospilota* Brandt, 1835; *H. (Platyperona)* sp.; *Afroccumis africana* (Semper, 1867); *Lipotrachea* sp.; *Polycheira* sp.; and *Chiridota* sp.

Etymology. The specific name *susamiensis* is an adjective, derived from the type locality, Susami town.

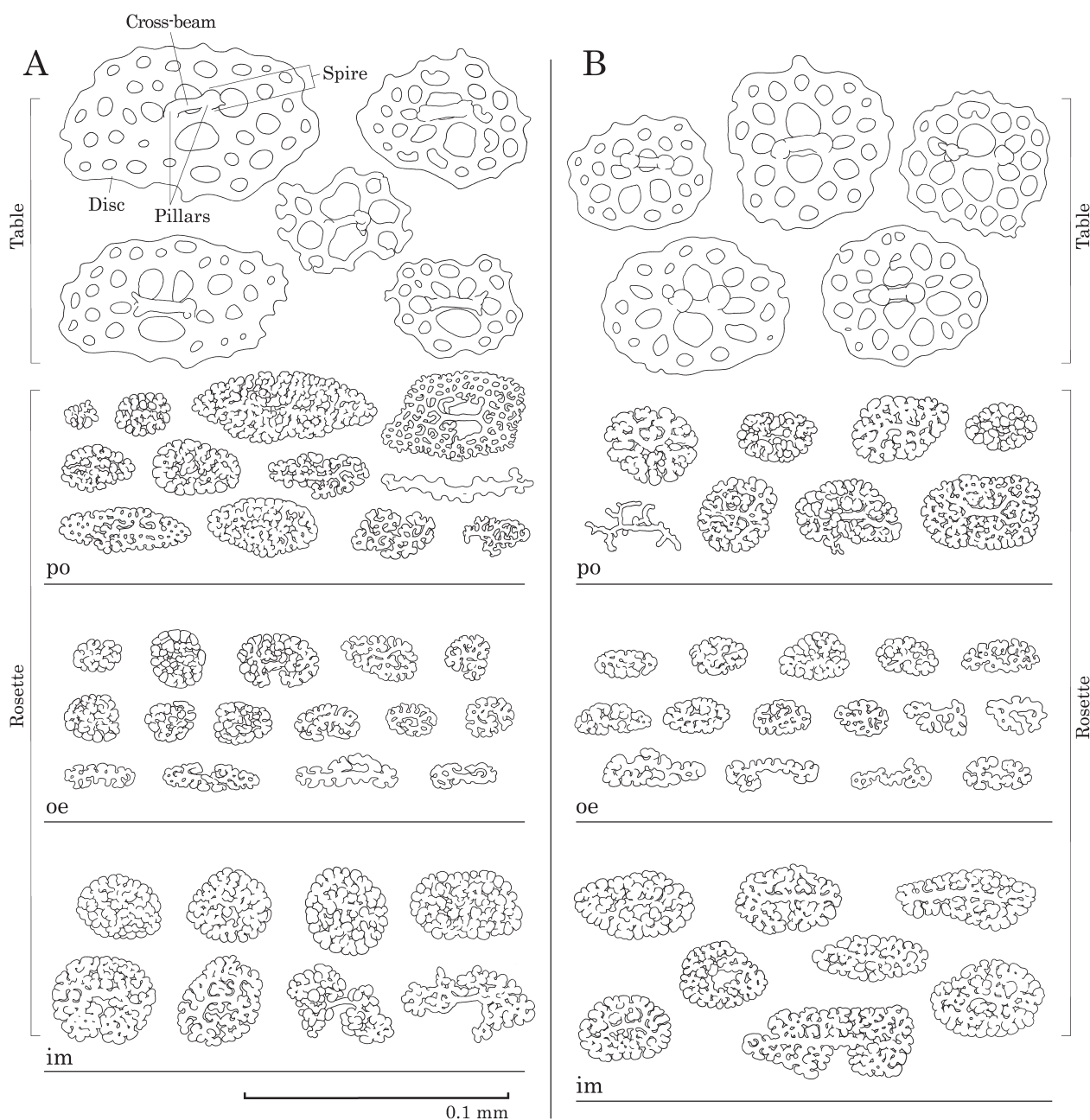


Fig. 5. *Thyone susamiensis* sp. nov., ossicles of peri-oral skin (po), oesophagus skin (oe), and middle part of introvert skin (im) on abdominal-lateral side. A, WMNH-2014-INV-7 (paratype); B, WMNH-2014-INV-8 (paratype).

Discussion

The present new species must be included in *Thyone*, as it agrees with the diagnosis of *Thyone* by Oken (1815) for the most part, and also displays the common characteristic features of congeners that are currently understood as defining features of this genus, except for having no ossicles in the integument of the body wall. Most species now included in *Thyone* have table-like ossicle in the body wall, but it has been recognized that some species do not (e.g., *Thyone flindersi* O'Loughlin, Barmos, and VandenSpiegel, 2012 (q.v.)).

In some species of holothurians, table-like ossicles in the

body-wall integument degenerate after growth, and even disappear completely, as in *Apostichopus japonicus* (Selenka, 1867) (Mitsukuri 1897). Changes in ossicles in many littoral sea cucumbers occur in juvenile stages of under approximately 30 mm in length (e.g., Cutress 1996). In *T. susamiensis*, no ossicles were found in either large (e.g., WMNH-2014-INV-2, length 127 mm) or small (e.g., WMNH-2014-INV-7, length 51 mm) specimens, although all were over this threshold size. Because the body size of congeners in which such ossicles are found is almost the same as in our specimens, a lack of ossicles in the body-wall integument appears to be a consistent feature of *T. susamiensis*, at least in specimens that are longer than approximately 50 mm, and can serve as one of its discriminating features.

Table 3. Abundance (%) and size (μm) of ossicles from peri-oral skin, oesophagus skin, and introvert skin of *Thyone susamiensis* sp. nov. (Fig. 5A, B).

| | Peri-oral | | | Oesophagus | | | Introvert (anterior) | | | Introvert (middle) | | | Introvert (posterior) | | |
|-----------------|------------------------|--------------------|--------------------|--------------|--------------------|--------------------|----------------------|--------------------|--------------------|--------------------|--------------------|--------------------|-----------------------|--------------------|--------------------|
| | Length | | Width ^a | Length | | Width ^a | Length | | Width ^a | Length | | Width ^a | Length | | Width ^a |
| | % (n) | Mean±sd (Range) | Mean±sd (Range) | % (n) | Mean±sd (Range) | Mean±sd (Range) | % (n) | Mean±sd (Range) | Mean±sd (Range) | % (n) | Mean±sd (Range) | Mean±sd (Range) | % (n) | Mean±sd (Range) | Mean±sd (Range) |
| WMNH-2014-INV-2 | | | | | | | | | | | | | | | |
| Tables | 3 (4) ^b | 70±8 (60–79) | 57±11 (47–70) | 0 (0) | | | 0 (0) | | | 0 (0) | | | 0 (0) | | |
| Rosettes | 97 (100) | 32±7 (11–50) | 20±4 (6–32) | 100 (100) | 22±6 (9–41) | 12±3 (4–21) | 100 (100) | 39±7 (20–58) | 27±5 (15–38) | 100 (100) | 39±8 (21–71) | 26±4 (16–38) | 100 (100) | 34±6 (20–49) | 25±4 (15–40) |
| WMNH-2014-INV-7 | | | | | | | | | | | | | | | |
| Tables | 3 (16) ^b | 64±14 (47–97) | 39±9 (23–55) | 0 (0) | | | 0 (0) | | | 0 (0) | | | 0 (0) | | |
| Rosettes | 97 (100) | 32±11 (15–69) | 19±6 (8–39) | 100 (100) | 25±7 (13–48) | 12±4 (3–24) | 100 (100) | 33±9 (12–58) | 22±6 (8–34) | 100 (100) | 31±8 (7–53) | 21±6 (5–36) | 100 (76) | 32±6 (15–44) | 22±5 (9–33) |
| WMNH-2014-INV-8 | | | | | | | | | | | | | | | |
| Tables | 4 (10) ^b | 64±11 (52–89) | 51±9 (36–68) | 0 (0) | | | 0 (0) | | | 0 (0) | | | 0 (0) | | |
| Rosettes | 96 (100) | 35±10 (16–73) | 21±6 (3–43) | 100 (100) | 25±6 (8–38) | 9±4 (2–21) | 100 (100) | 37±8 (21–64) | 22±5 (10–34) | 100 (100) | 37±11 (18–90) | 22±6 (5–41) | 100 (10) | 33±5 (24–42) | 23±4 (15–28) |

^aWidth in the central part, not the maximum value. ^bBecause the abundance of tables was very low, all the tables were measured, while 100 rosettes were randomly measured.

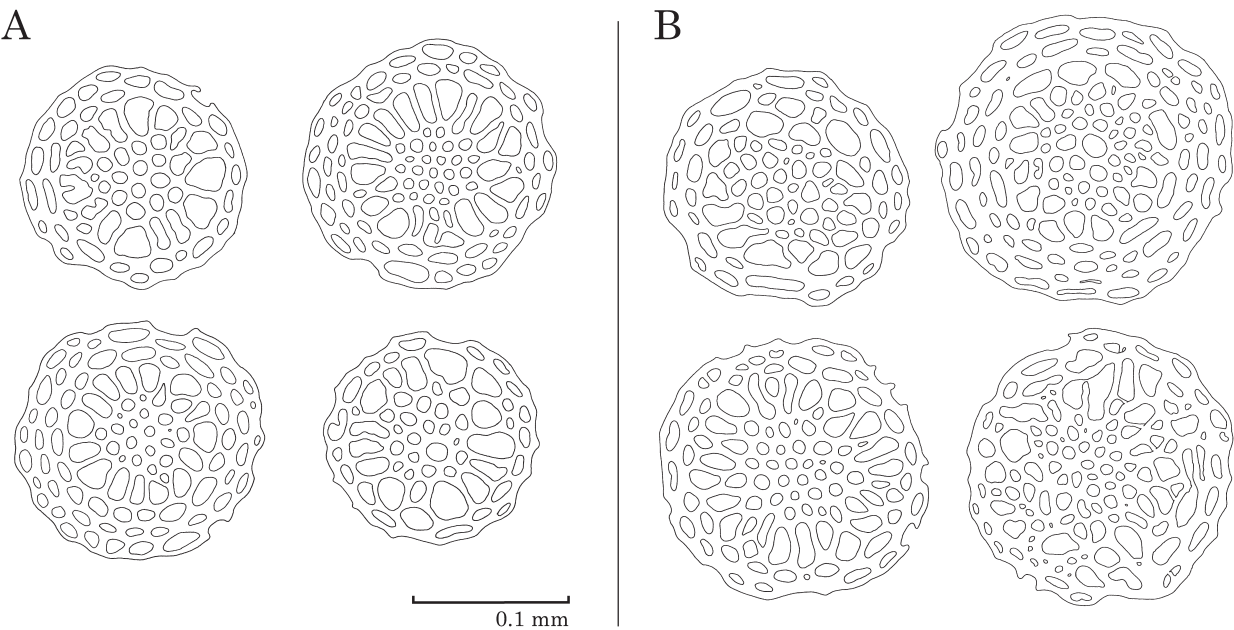


Fig. 6. *Thyone susamiensis* sp. nov., ossicles in pedicels from middle part of body on abdominal side. A, WMNH-2014-INV-7 (paratype); B, WMNH-2014-INV-8 (paratype).

Possession of 10 dendritic tentacles and a long composite calcareous ring is currently known only among phylloporids and is limited to species in the following six genera: *Allothyone*; *Ekmanothyone* Massin, 1993; *Pentamera*; *Stolus*; *Thorsonia* Heding, 1940; and *Thyone* Oken, 1815. In *Allothyone* and *Thorsonia*, table-like ossicles (with genus-specific structure) are present in the body wall (Heding 1940; Panning 1949). In *Ekmanothyone* and *Stolus*, ‘buttons’ (mostly four-perforated ossicles with many knobs) are present in the body wall (Selenka 1867; Massin 1993). Thus, species

in these four genera are clearly different from the present species. In *Pentamera*, calcareous deposits are also abundant in the integument of the body wall (Ayres 1852), where they consist of numerous inseparable layered plates. Ayres (1852) remarked that the calcareous ring is shaped like that of sclerodactylid species, which is different from that of *Thyone*.

Furthermore, we think that the species currently assigned to *Thyone* should be split into at least four groups: 1) the species that display all the common characteristics

of congeners such as the type species *T. fusus*; 2) the species with four or more Polian vesicles and stone canals, such as *T. montoucheti* Tommasi, 1971; 3) the species that lack ossicles or have just a few ossicles in the body wall and have

Table 4. Abundance (%) and size (μm) of ossicles from mid-body pedicels of *Thyone susamiensis* sp. nov. (Fig. 6A, B) and number of holes in plates of ossicles.

| | % | Diameter ^a | Number of holes |
|-----------------|-------------|---------------------------|--------------------------|
| | | Mean \pm sd (Range) | Mean \pm sd (Range) |
| WMNH-2014-INV-2 | | | |
| Endplates | 100 (10) | 180 \pm 8 (165–195) | 123 \pm 17 (90–140) |
| WMNH-2014-INV-7 | | | |
| Endplates | 100 (13) | 149 \pm 13 (117–165) | 81 \pm 18 (47–101) |
| WMNH-2014-INV-8 | | | |
| Endplates | 100 (10) | 175 \pm 12 (153–188) | 106 \pm 20 (81–144) |

^aDiameter along the major axis.

multi-perforated plate-like ossicles in the tentacles, such as *Allothyone multipes*; and 4) the species lacking ossicles in the body wall but having rosette-like ossicles in the tentacles, a group including *T. susamiensis*.

From a literature survey on *Thyone sensu lato*, it seems that six species lack body-wall ossicles: *Thyone bacescoi* Cherbonnier, 1972 (*q.v.*); *T. carens* Cherbonnier, 1988 (*q.v.*); *T. flindersi*; *T. okenii* Bell, 1884 (*q.v.*); *T. vadosa* Cherbonnier, 1988 (*q.v.*); and *Allothyone multipes* (see Augustin 1908). Furthermore, the existence of ossicles in the body-wall integument has not been reported in the following three species: *T. discolor* Sluiter, 1901 (*q.v.*); *T. roscovita* Hérourard, 1889 (*q.v.*); and *T. venusta* Selenka, 1867 (*q.v.*). Among these, however, the four species *T. bacescoi*, *T. carens*, *T. okenii*, and *T. vadosa* have table-like ossicles at least in the introvert (and possibly in other parts of the body), and *A. multipes* has multi-perforate plate-like ossicles (not rosette-like ones) in the tentacles, these features not being observed in *T. susamiensis*.

Thyone flindersi has table-like ossicles in the peri-oral skin and sometimes in the anal appendage, but not in the

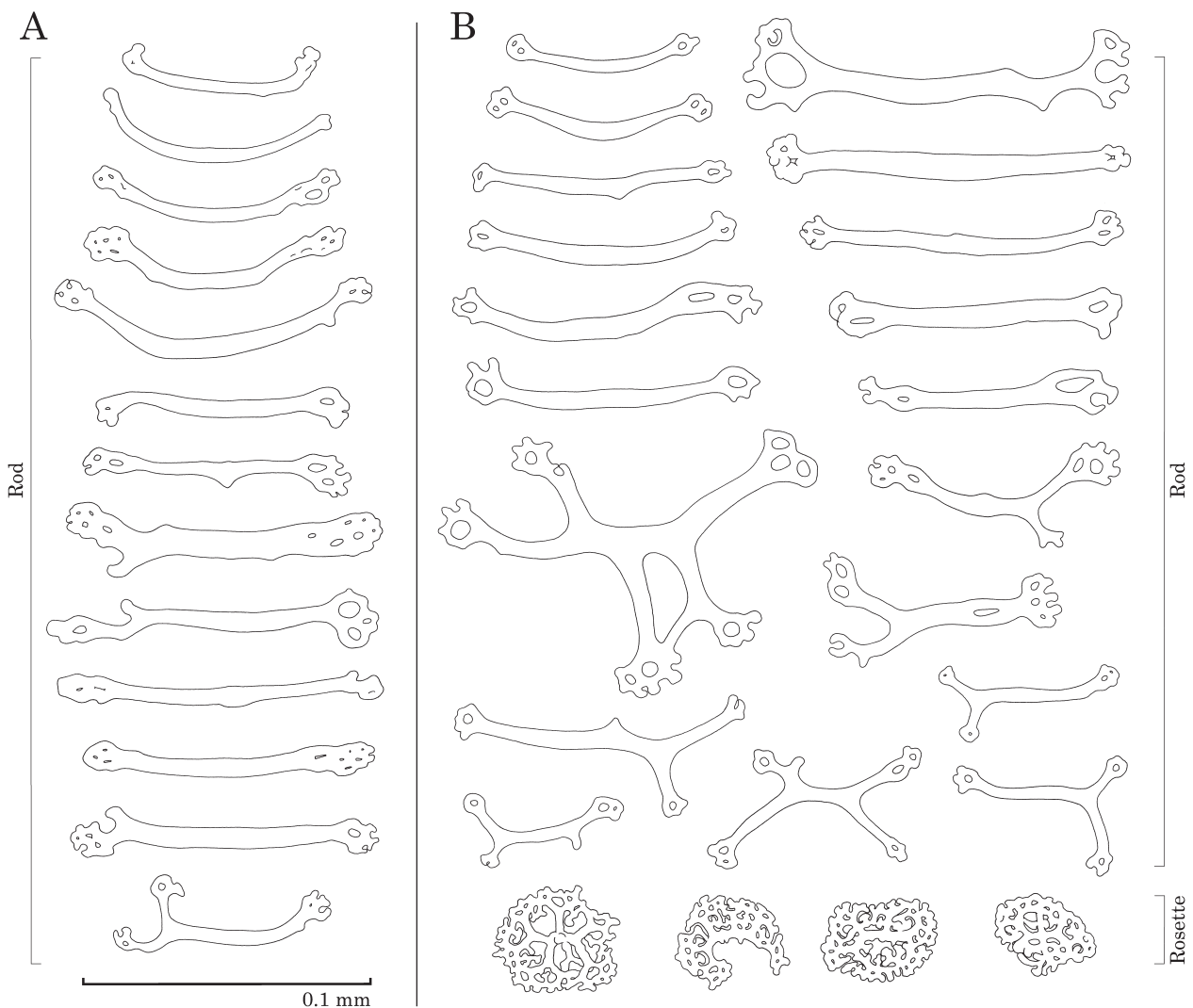


Fig. 7. *Thyone susamiensis* sp. nov., ossicles in anal appendages on abdominal side. A, WMNH-2014-INV-7 (paratype); B, WMNH-2014-INV-8 (paratype).

Table 5. Abundance (%) and size (μm) of ossicles from anal papillae of *Thyone susamiensis* sp. nov. (Fig. 7A, B).

| | % | Length | Width ^a |
|-----------------|-------------|--------------------------|--------------------------|
| | | Mean \pm sd (Range) | Mean \pm sd (Range) |
| WMNH-2014-INV-2 | | | |
| Rods | 100 (19) | 91 \pm 16 (53–114) | 5.4 \pm 1.8 (2–9) |
| Rosettes | 0 (0) | | |
| WMNH-2014-INV-7 | | | |
| Rods | 100 (16) | 96 \pm 13 (8–113) | 4.4 \pm 1.4 (2–7) |
| Rosettes | 0 (0) | | |
| WMNH-2014-INV-8 | | | |
| Rods | 88 (88) | 91 \pm 20 (35–137) | 6.3 \pm 2.1 (3–11) |
| Rosettes | 12 (12) | 39 \pm 8 (28–55) | 23 \pm 8 (8–35) |

^a Width in the central part, not the maximum value.

introvert. In having table-like ossicles in the peri-oral skin, *T. susamiensis* resembles *T. flindersi*, but, *T. susamiensis* is whitish-yellow or cream, and generally marbled with blackish-purple (Fig. 1), while *T. flindersi* ranges from brown to dark reddish brown (O'Loughlin *et al.* 2012). The two species also differ in the colour of the tentacles, introvert, and pedicels. The posterior ends of the radial elements of the calcareous ring always bifurcate only once in *T. susamiensis* (Fig. 3), but in *T. flindersi*, they are doubly bifurcate, with four posterior points (O'Loughlin *et al.* 2012). Furthermore, *T. flindersi* sometimes has table-like ossicles in the anal appendages, a feature not observed in *T. susamiensis*.

Thyone susamiensis can also be distinguished from *T. discolor*, *T. roscovita*, and *T. venusta*, although the latter three lack detailed descriptions. *Thyone discolor* was established based on specimens from the Molo Strait and Aru Islands. While the species was poorly illustrated, it has long, thin pedicels and incomplete calcification of the calcareous ring (Sluiter 1901), thus being different from *T. susamiensis*, in which the pedicels are relatively thin compared to those of the other holothurians, but not very long. In addition, the calcareous ring in *T. susamiensis* is well calcified even in the smallest specimen (Fig. 3). In the European form *T. roscovita*, the posterior part of the radial elements of the calcareous ring is not very long (Hérourard 1889), while in *T. susamiensis*, the radial elements are approximately twice as long as the inter-radial elements. The body of *T. roscovita* was not illustrated by Hérourard (1889), but based on the description provided, it is cylindrical (and not curved) and pinkish with brown spots. In contrast, the body of *T. susamiensis* is U-shaped with tapered ends, having a whitish-yellow or cream background colour marbled with dark purple. In *Thyone venusta* (type locality: Red Sea), the body is not pigmented (Selenka, 1867) and the endplates are shorter than those of *T. susamiensis*.

Several species currently assigned to *Thyone* should be transferred to other genera. *Thyone benti* Deichmann, 1937, in which each radial and inter-radial element of the calcareous ring consists of one large piece and two posterior prolongations of composed of a few small, narrow pieces, not forming a mosaic pattern, and in which the pedicels are restricted to the radials, should be transferred to *Eupentacta* Deichmann, 1938 or *Havelockia* Pearson, 1903. *Thyone propinqua* Cherbonnier, 1970, in which the elements of the calcareous ring are lined up, not forming a mosaic pattern, and the pedicels are arrayed in five series and not scattered, should be transferred to *Pentamera* Ayres, 1852. *Thyone anomala* Östergren, 1898, in which the calcareous ring was described as being similar to that of *Stolus sacellus* Selenka, 1867, presently accepted as *Stolus buccalis* (Simpson, 1855)—*i.e.*, tubular, formed by numerous elements as a mosaic with one narrow anterior piece followed by usually 4–8 rows of pieces—and in which there is no distinction between the radial and inter-radial elements, and knobbed button-like ossicles are present in the body wall, should be transferred to *Stolus* Selenka, 1867. The proper generic assignment of many species for which detailed information is lacking is left to be resolved in future studies.

Ossicle size did not significantly vary among individuals of *T. susamiensis*, except that the size of the endplates that usually depended on the size of the pedicels (presumably depend on body size). From this, it is assumed that mean ossicle size of *T. susamiensis* does not vary widely intraspecifically. Ossicle size is usually not considered as a characteristic in *Thyone*. However, since there are only few ossicle types to observe in ossicle-poor species such as *T. flindersi* and *T. susamiensis*, ossicle size might come to be useful in distinguishing *T. susamiensis* from other ossicle-poor congeners discovered in future studies.

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