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Doctoral Dissertation

Systematic Studies on Sea Cucumbers of the Family Stichopodidae (Echinodermata: Holothuroidea)

（シカクナマコ科（棘皮動物：海鼠類）の体系学的研究）

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March 2018

Systematic Studies on Sea Cucumbers of the Family Stichopodidae (Echinodermata: Holothuroidea)
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A PhD dissertation

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DISCLAIMER

This dissertation should not be deemed to be ‘published’ within the meaning of the International Code of Zoological Nomenclature (Article 8.2). New taxon names and nomenclatural acts referred to in this dissertation are disclaimed and ‘unavailable’ (Article 8.3).

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ABSTRACT

Sea cucumbers in the family Stichopodidae are predominantly distributed in a wide range of depths, both in the tropics as well as the temperate waters. Despite their ecological and economic importance, the systematic and phylogenetic position of this family remains problematic and unresolved. Therefore, this dissertation presents a collection of some studies done on the systematics of the family Stichopodidae with the aim to further understand the relationships and systematics of the family. Resulting from these studies, one new genus, one new species, two new species combinations, and one resurrected species name are proposed in this dissertation.

A taxonomic study of species in the genus *Stichopus* was done using specimens collected from the Straits of Malacca. Five species, including one new species, were reported. The new species, *Stichopus fusiformiossa*, has unusual fusiform ossicles in the tentacles, which are not found in the other species of the genus. Pseudo-tables and large perforated plates are newly recorded for *Stichopus herrmanni* and *Stichopus vastus*, respectively.

The taxonomic status of the genus *Apostichopus* has been in a constant change due to the confusions arising from the existence of four nominal taxa, three colour morphs, and two valid species. A revision of this genus was done using the type specimens, as well as additional specimens from Japan, to clarify the taxonomic status by detailed ossicle analysis. Scanning electron micrographs of complete ossicle assemblages from dorsal body, papillae, tentacles, tube feet and cloaca are reported for the first time. This resulted in redescription of this genus with two member species including one resurrected species name (*A. armatus*). Both *A. japonicus* and *A. armatus* can be differentiated by the character of either smooth or spinous rim of the reduced table ossicle in dorsal body.

Lastly, a new genus is proposed in this dissertation to accommodate two species already described from Australia that were previously placed in the genus *Stichopus*. The erection of the new genus, *Notostichopus*, is based on morphology, phylogenetic, genetic distance, and geographical distribution. *Notostichopus* is characterized by the absence of rosette ossicles, and table ossicles only have a single or no crossbeam in dorsal body. This genus is represented by two species revised

here (*Notostichopus ludwigi* and *N. ellipes*), distributed in the shallow, subtropical and temperate waters of Australia.

Following the outcome of this dissertation, the family Stichopodidae at present consists of a total of 10 genera and 34 described species after incorporating changes proposed by these studies.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction to the Family Stichopodidae

Sea cucumbers are one of the members of the phylum Echinodermata with cylindrical and elongated body form. Unlike the other echinoderm classes having the pentamerous radial symmetry in vertical axis, sea cucumbers (Holothuroidea) have their pentamerous radial symmetry axis horizontally (Smirnov 2014). The five ambulacral areas which run longitudinally across the oral to aboral area are in 2+3 arrangement (two in the dorsal and three in the ventral) in most of the groups but less apparent in the orders Dendrochirotida and Apodida. Ambulacral appendages generally are divided into two types, namely papillae and tube feet (Clark 1922). Papillae occur primarily along the dorsal ambulacra as well as the lateroventral margin of the body (Clark 1922). Tube feet are ambulacral appendages at the ventral area that are arranged in three rows, used mainly for locomotion. Oral opening is typically surrounded by 20 tentacles, but the number of tentacles may vary.

Sea cucumbers exhibit global distribution in the marine environment. They are found ranging from the shallow intertidal area to the depths of about 10,000 m (Bruun 1951). Apart from wide variation of depths, sea cucumbers also occur in all seas and latitudes, including the Arctic and Antarctic (Clark & Rowe 1971; Smirnov 1994; O'Loughlin et al. 2011). In addition to a few species that exhibit pelagic and benthopelagic lifestyles (Rogacheva et al. 2012), almost all sea cucumbers are categorized as benthos that spends most of their entire adult life utilizing a wide range of substrates on the sea bottom (Brusca & Brusca 2003).

The family Stichopodidae was first proposed by Haeckel (1896) with the name "Stichopodida" to accommodate the genera *Stichopus* and *Thelenota*. The defining character that separates this family from the other families is the unique two tuft gonads (Haeckel 1896: 443). Following is the current taxonomic classification of the family Stichopodidae:

Class Holothuroidea
  Subclass Holothuriacea
    Order Synallactida
      Family Stichopodidae
        Genus *Apostichopus*
        Genus *Astichopus*
        Genus *Australostichopus*
        Genus *Eostichopus*
        Genus *Isostichopus*
        Genus *Neostichopus*
        Genus *Parastichopus*
        Genus *Stichopus*
        Genus *Thelenota*

The taxonomic position of the family Stichopodidae within the order Aspidochirotida (now *nomen dubium*) was first proposed by Grube (1840) based on the peltate-shaped tentacles, along with another family Holothuriidae. Later, Perrier (1902) included five families (Deimatidae, Théel 1882; Elpidiidae, Théel 1882; Psychropotidae, Théel 1882; Pelagothuriidae, Ludwig 1894; Synallactidae, Ludwig 1894) in the order Aspidochirotida but failed to mention the presence of Stichopodidae in the order. Then, Smirnov (2012) added one new family (Mesothuriidae, Smirnov 2012) into the order and removed three families making it a total of five families within the order which includes Stichopodidae. The placement of Stichopodidae within the order Aspirochirotida based on morphology (see Grube 1840; Smirnov 2012) remains unchanged before a comprehensive phylogenetic study. The molecular phylogenetic study of extant sea cucumbers done by Miller et al. (2017) revealed that the order Aspidochirotida is polyphyletic, while families Stichopodidae, Deimatidae and Synallactidae form a monophyletic clade. Therefore, Miller et al. (2017) erected a new order named Synallactida based on their phylogenetic analysis, to accommodate the three families although it is still unclear of which morphological synapomorphies supports this order.

Currently, the family Stichopodidae consists of nine genera and 35 described species distributed globally (Paulay & Hansson 2013a). The genera *Stichopus* Brandt, 1835 and *Thelenota* Brandt, 1835 were the first two genera included in the family. Currently, *Thelenota* consists of three valid species. *Stichopus* Brandt, 1835 is the most speciose genus in the family, containing 14 described species (Paulay & Hansson 2013b). A major revision of *Stichopus* was done by Clark (1922) resulting in erection of another two genera named *Parastichopus* and *Astichopus*. Although Clark (1922) merely fixed the type species for the proposed genus *Parastichopus* without giving a description, Deichmann (1937) later consolidated this genus by defining the genus, and transferred some species of *Stichopus* from the coast of California into it. She would later continue to describe three more genera namely: 1) the monotypic genus of *Neostichopus* Deichmann, 1948 from South Africa; 2) *Isostichopus* Deichmann, 1958 from the coast of America; 3) *Eostichopus* Deichmann, 1958, but designation of type species was not given rendering this genus *nomen nudum* (Clark & Rowe 1967). However, Cutress & Miller (1982) validated the genus by designating a new species, *E. arnesoni* Cutress & Miller, 1982 as type species for the genus. In the course of studying holothurians in China, Liao (1980) transferred the species *Stichopus japonicus* Selenka, 1867 to a new genus *Apostichopus* based on the morphological features, distribution, and temperature tolerance differences. The genus *Australostichopus* Levin in Moraes, Norhcote, Kalinin, Avilov, Silchenko, Dmitrenok, Stonik & Levin, 2004 is the latest addition to the family, differentiated primarily by the structures of glycoside. As for phylogenetic studies that involves the family Stichopodidae, apart from the phylogenetic study done by Miller et al. (2017) that deals with the higher taxon level of sea cucumber, another phylogenetic study of Stichopodidae was done by Byrne et al. (2010) focusing on the genus *Stichopus*.

As the phylogenetic and taxonomic status of the family Stichopodidae are yet to be fully understood, a comprehensive revision of the family is intended in this research. In order to achieve this goal, this dissertation addresses some systematic aspects of sea cucumber in the family Stichopodidae. In Chapter 2, a study of diversity and taxonomy of stichopodids based on materials collected from the Straits of Malacca is done. This study yielded description of a new species. Chapter 3 deals with the taxonomic revision of commercially important sea cucumbers of the genus *Apostichopus* from Japan. The existence of different colour morphs with several conferred names existing in the genus has caused taxonomic complications. Examination of type specimens and additional specimens of *Apostichopus* enabled me to conclude the taxonomic status of species in the genus. Chapter 4 of this dissertation describes a new genus and two new combinations of stichopodids from Australia based on morphology and molecular phylogeny.

1.2 Materials and Methods

Specimens used in this study

Fresh specimens and preserved museum specimens were used in this study. Details of specimens used are outlined in “Material and Method” of each chapter.

Dissection and morphological examination

Ossicle extraction, preparation, and observation

Ossicles are extracted from tissues of the mid part of dorsal body, papilla tips, tentacles, tube feet, and cloacal wall. A small piece (about 2 × 2 mm) of tissue is cut and placed on a clean glass slide. A few drops of weak commercial bleach were added using a pipette to dissolve the tissue. Tissues were left to dissolve for about 5–15 minutes (or more depending on size of tissue and strength of bleach). The ossicles were then washed several times with deionized water and finally with absolute alcohol. Extracted spicules were air dried and mounted on SEM stubs. The spicules were coated with gold–palladium alloy and observed under a JEOL JSM-6380LV SEM with the acceleration voltage of 20 kV.

Definition of terms used for ossicle descriptions

The terms used to describe different types of ossicles (Fig. 1.1) are outlined here:

a) Buttons — oblong shaped ossicle with variable number of holes arranged in pairs. Size and shape of holes varies. Surface of buttons smooth.

b) Fusiform — spindle-liked ossicle, tapering at both ends; with dense spines interconnected to each other forming some hollow spaces.

c) Multiperforated plates — fully formed plates are circular in outline, rim can be smooth or jagged. Number, size, and arrangement of perforation on plates varies.

d) Rods — elongated bar-like ossicle which can be thickened centrally and/or distally. Some rods are perforated on the main stalk; branching or not. Rods can form curves to C-shaped or S-shaped.

e) Rosettes — ossicles formed from dichotomously branched rods (terminal and lateral). Branching may variously anastomose forming perforated deposits

f) Tables — ossicle that is formed from multi-armed (usually four) calcareous deposits which branched distally. These branches anastomosing to from one perforated disk of different sizes; usually four central perforations are larger, surrounded by smaller peripheral perforations. Variable number of vertical pillars/spires arise from the central of the plate. Pillars connected to each other by crossbeam in various numbers. Tip of the pillar ends either with single spine or numerous spines forming a crown.

DNA extraction, PCR, and sequencing

Total genomic DNA were extracted from approximately 10–20 mg of tissue from the tube feet using DNeasy Blood & Tissue Kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's specification. Sections of the cytochrome *c* oxidase subunit I (COI) and the small subunit 16S ribosomal DNA (16S rDNA) genes were amplified using the primers COIe-F and COIe-R (Arndt et al. 1996) and 16Ss-F (5′-GTAGCATAATCACTTGTCTCTTAAA-3′) and 16Ss-R (5′-TTATTTAGAAGATAGAAGCTGACCT-3′), respectively. PCR amplification was conducted following Uthicke & Benzie (2003), Uthicke et al. (2010), and Byrne et al. (2010). PCR reaction with TaKaRa Ex Taq DNA polymerase (TaKaRa Bio, Shiga, Japan) consisted of initial denaturation at 95 °C for 60 s followed by 40 cycles of denaturation at 95 °C for 30s, annealing at 50 °C for 30 s, extension at 72 °C for 80 s, and final extension at 72 °C for 10 min. All nucleotide sequences were determined using BigDye Terminator Kit ver. 3.1 and ABI 3730 Genetic Analyzer (Life Technologies, California, USA).

Phylogenetic analyses

The 16S and COI sequences were aligned using the MAFFT ver. 7 (Katoh & Standley 2013) and the alignment reliability was tested using GUIDANCE2 (Sela et al. 2015). Unreliable and ambiguous sites (GUIDANCE Score <0.92) were then removed and the aligned sequences were trimmed in MEGA ver. 5.2 (Tamura et al. 2011). Concatenated 16S and COI dataset (881bp) was obtained by joining the aligned sequences using SequenceMatrix (Vaidya et al. 2010). The optimal nucleotide substitution model was determined by PartitionFinder ver. 2 (Lanfear et al. 2016) under the Akaike information criterion (Akaike 1974) and the model GTR+I+G was chosen for both 16S and COI.

Phylogenetic analyses were performed with maximum-likelihood (ML) and Bayesian inference (BI) methods using the concatenated dataset that were partitioned into 16S and COI regions. Codon position was also partitioned in the COI region. The ML analysis was performed with the program RAxML ver. 8.2.3 (Stamakis 2014) on the Cipres Portal (www.phylo.org). Nodal support estimation within ML tree was performed by 1,000 bootstrap replications (Felsenstein 1985). Bayesian inference was performed with MrBayes ver. 3.2.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Four independent Markov chain Monte Carlo (MCMC) runs were sampled every 500 generations for 1,000,000 generations. The first 10,000 trees were discarded as burn-ins. Genetic distances were calculated based on uncorrected *p*-distance using MEGA ver. 5.2 (Tamura et al. 2011).

CHAPTER 2

STICHOPODIDS IN THE STRAITS OF MALACCA WITH DESCRIPTION OF A NEW SPECIES[1]

2.1 Introduction

One of the earliest comprehensive records on the diversity and distribution of holothurians in Malaysian waters was done by Ridzwan & Che Bashah (1985). Then, Zulfigar et al. (2008) produced a field guide to sea cucumbers in shallow water and coral reefs in Malaysia. However, taxonomic studies of the genus *Stichopus* in Malaysia were scarce and done only at the southern part of the South China Sea (Siti et al. 1999; Massin et al. 2002). Massin et al. (2002) described two new species, *Stichopus rubermaculosus* and *Stichopus ocellatus* there. The genus *Stichopus* is one of the dominant genera in tropical shallow waters which is an important fishery commodity. Although Straits of Malacca is a major area for stichopodid fisheries, there has not been a comprehensive taxonomic study done on stichopodids in that area. Furthermore, the genus *Stichopus* is taxonomically very confusing due to their similarity between each species in outer appearance and the presence of variations (Massin et al. 2002). In this study, detailed morphological descriptions were done on the species of the genus *Stichopus* including a new species found from the shallow reefs of the Straits of Malacca.

2.2 Materials and Methods

Sea cucumbers were collected from the shallow coral reef areas of Pulau Payar (6°26′2.7″N, 99°40′54.8″E), Pulau Songsong (5°48′31.2″N, 100°17′38.0″E) and Pulau Sembilan (4°1′46.8″N, 100°32′39.7″E) in the Straits of Malacca as shown in Figure 2.1. The sampling areas were situated in highly sedimented waters of the Straits of Malacca (Chua et al. 2000) with poor reef framework formation (Pillai & Scheer 1974). All sampling areas exhibited similar shallow reef flat at depths about 10–15m with gradual slope of sandy substrate extending to 30m depth. SCUBA

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[1] The contents of this chapter have been published in:

Woo SP, Zulfigar Y, Tan SH, Kajihara H, Fujita T (2015) Sea cucumbers of the genus *Stichopus* Brandt, 1835 (Holothuroidea, Stichopodidae) in Straits of Malacca with description of a new species. ZooKeys 545: 1–26.

diving was employed in collecting specimens using wandering transect covering an area of about 150–200 m^2, up to 30 m water depth during day and night. The sea cucumber specimens were fixed in absolute ethanol for two weeks and stored in 70% ethanol. Ossicles were extracted from the tissues of the dorsal body, tip of the papillae, tentacles, and tube feet. The tissue was dissolved using commercial bleach and ossicles were then washed several times with distilled water before transferring them to a glass slide to be observed under microscope. Pencil drawing of the ossicles were done using a drawing tube attached to the microscope. The pencil drawings were then traced on a tracing paper using fine technical pens and digitized by scanning.

2.3 Taxonomy

Genus *Stichopus* Brandt, 1835

Type species. *Stichopus chloronotus* Brandt, 1835

Diagnosis. Peltate-shaped tentacles at ventral mouth with quadrangular shaped body. Flattened ventral sole with tube feet in ambulacra area. Papillae conspicuous. No cuvierien tubules and no anal teeth or traceable papillae around the cloacal opening. Gonads in two tufts, one at each sides of the dorsal mesentery. Ossicles primarily tables, branched rods and C-shaped rods (Clark & Rowe 1971).

Species composition. This genus consists of 14 species. *Stichopus chloronotus* Brandt, 1835; *Stichous ellipes* Clark, 1938; *Stichopus fusiformiossa* Woo in Woo et al., 2015; *Stichopus herrmanni* Semper, 1868; *Stichopus horrens* Selenka, 1867; *Stichopus ludwigi* Erwe, 1913; *Stichopus monotuberculatus* (Quoy & Qaimard, 1833); *Stichopus naso* Semper, 1868; *Stichopus noctivagus* Cherbonnier, 1980; *Stichopus ocellatus* Massin, Zulfigar, Hwai & Boss, 2002; *Stichopus pseudohorrens* Cherbonnier, 1967; *Stichopus quadrifasciatus* Massin, 1999; *Stichopus rubermaculosus* Massin, Zulfigar, Hwai & Boss, 2002; *Stichopus vastus* Sluiter, 1887

Remarks. The common characteristics of this genus include gonads with two branching tufts (which is a taxonomic character for the family Stichopodidae) and the presence of tables, C-shaped, and S-shaped rod ossicles in the tissue (Clark &

Rowe 1971). The distribution of the genus *Stichopus* was throughout the tropical and subtropical waters of the Indo-West Pacific region (Clark & Rowe 1971).

Stichopus chloronotus **Brandt, 1835**

Figs 2.2–2.3

Holothuria (*Holothuria*) *quadrangularis* Lesson, 1830: 90, pl. 31, fig. 1.

Stichopus (*Perideris*) *chloronotus* Brandt, 1835: 250.

Stichopus chloronotus; Selenka 1867: 315, pl. 17, figs 20–24; 18, fig. 25; Pearson 1903: 204; Panning 1944: 30, fig. 3a–e; Loi & Sach 1963: 238, pl. 1, fig. A, pl. VI, fig. 1; Clark & Rowe 1971: 178, pl. 27, fig. 18; Mary Bai 1980: 16, fig. 101; Tan Tiu 1981: 65, pl. 7, figs 1–3; Clark 1984: 99; Féral & Cherbonnier 1986: 94; Cannon & Silver 1986: 27, fig. 4h; Cherbonnier 1988: 146, fig. 60A–O; James & James 1994: 12, pl. VI; Kerr 1994: 163; Rowe & Gates 1995: 323; Massin et al. 2002: 74, figs 1–2, pl. 1A.

Stichopus cylindricus Haacke, 1880: 47.

Stichopus chloronotus var. *fuscus* Pearson, 1903: 204.

Stichopus hirotai Mitsukuri, 1912: 161.

Holothuria viridis Cherbonnier, 1952: 19–21, fig. 7.

Material examined. Five specimens: USM/MSL/PB004, USM/MSL/PB005, USM/MSL/PB006, USM/MSL/PB007, USM/MSL/PP005.

Type locality. Lugunor Islands, Guam.

Description. Body quadrangular in cross-section with four distinctive sides; smooth, firm, and hard, indicating thick integument; dark blue in colour underwater and almost black out of water (Fig. 2.2). Large and long papillae at dorso-lateral edge running from collar of tentacles toward anus in two rows; similar papillae at ventro-lateral edge but in one single row; tip to base of papillae yellow to ochre in colour. Ambulacral areas with tube feet and narrow interambulacra; central ambulacrum wider compared to other two ambulacra. Oral opening with 20 peltate tentacles on ventral side; anus at terminal.

Dorsal body wall with tables, C-shaped rods, and S-shaped rods (Fig. 2.3A–C). Tables abundant in dorsal body wall; base smooth with four large central perforations and 4–10 smaller peripheral holes; four pillars forming spires, joined with one crossbeam; tip of each pillar spiny. C-shaped rods smooth with sharp endings; some being irregular in form. S-shaped rods derived from C-shaped rods present. Dorsal papillae bearing tables, C-shaped rods, S-shaped rods, and rosettes, (Fig. 2.3D–G). Tables in dorsal papillae with large disc, four central perforations, and multiple peripheral holes; pillars joined by one, sometimes incomplete, crossbeam; tip of pillars with multiple large spines. Ventral tube feet bearing tables, rods, C-shaped rods, and multiperforated plates, (Fig. 2.3H–K). Large plates in ventral tube feet having distinctive larger central perforations; numerous smaller peripheral holes distributed over plates. Other smaller plates in ventral tube feet having perforations similar in size to each other. Edges of plates in ventral tube feet jagged but less pointy and sharp. Tables in ventral tube feet having reduced pillars or no pillar; one crossbeam incompletely joining all pillars; four central perforations with less peripheral holes compared to tables from dorsal body. Rods in ventral tube feet with 0–3 (mostly one) perforation(s) at central extension (Fig. 2.3I). Tentacles with tables and rods (Fig. 2.M, N). Tables in tentacles reduced to only disk without pillars, with four central perforations surrounded by smaller peripheral holes; similar to tables in dorsal body wall. Rods in tentacles in various sizes, with spiny and rough surface; smaller rods straight while larger rods slightly curved (Fig. 2.3M).

Remarks. *Stichopus chloronotus* is fairly easy to be identified in situ due to the bright, distinctive green-blue colouration on the body with yellowish coloured papillae (Fig. 2.2A), although there are some colour variations in the papillae across different localities (Massin et al. 2002). This species is distinguished from the other congeneric species also by its smooth body.

Large ossicles with elaborated sculpted surface in the tentacles reported from Madagascar by Cherbonnier (1988) were not found in specimens of this study. This type of ossicle is also missing from other descriptions by Théel (1886) using specimens from Fiji and Tonga, Massin (1996) using specimens from Ambon, Indonesia, and Massin et al. (2002) using specimens from Johor , Malaysia.

Distribution. This is a common species found throughout the Indo-West Pacific area (Clark & Rowe 1971).

Stichopus herrmanni Semper, 1868

Figs 2.4–2.5

Stichopus variegatus Herrmanni Semper, 1868: 73.

Stichopus variegatus; Cherbonnier 1947: 187–189, fig. a–c; Féral & Cherbonnier 1986: 98.

Stichopus herrmanni; Massin 1999: 63, fig. 52.

Material examined. Four specimens: USM/MSL/PSEM 001, USM/MSL/PSEM002, USM/MSL/PSEM003, USM/MSL/PP004.

Type locality. Philippines.

Description. Large body with quadrangular cross-section with four distinctive sides; firm, rugose, and having thick folding surfaces. Uniformly greyish brown on dorsal side; light brown to yellowish on ventral body (Fig. 2.4) with an orange patch spreading from mid ventral body to anterior ventral body. Two rows of small and short papillae on dorso-lateral edges; papillae absent on ventro-lateral edges; tip of papillae brown; base grey-coloured. Numerous, smaller, brown-tipped papillae spreading across dorsal body. Laterally, papillae being lesser in number and density. Ring of minute papillae surrounding oral opening. Tube feet numerous in ambulacra areas. Central ambulacrum occupying more rows of tube feet compared to other two ambulacra areas. Narrow interambulacra areas without tube feet. Twenty peltate-shaped tentacles. Anus terminal.

Dorsal body mainly tables, pseudo tables, rosettes, and C-shaped rods (Fig. 2.5A–D). Table ossicles in dorsal body have rounded to quadrangular bases, with four central perforations and numerous peripheral holes; three or four short pillars forming spires connected by a cross beam; tip of pillars with thorny crown endings (Fig. 2.5A). Rosettes in dorsal body abundant with various shapes and sizes; simple to complex bifurcation on both ends (Fig. 2.5C). Pseudo tables in dorsal body have four pillars extending from reduced base; no disk formed at base (Fig. 2.5B). Papillae consist of tables, C-shaped rods, and rosettes (Fig. 2.5E–G). Base of tables in papillae with rough rims; four pillars with multiple spines on the tip erected from disc; four central holes on the disc with 2–3 peripheral holes. C-shaped rods and rosettes in papillae similar to those in dorsal body. Tube feet have large perforated plates, reduced tables, and rods (Fig. 2.5H–J). Perforated plates in tube feet in rectangular and square shapes, jagged and pointy rims (Fig. 2.5H). Rods in tube feet with central extended perforations (Fig. 2.5J); surface covered with spinelets. Reduced tables of tube feet have base with four central perforations and 5–8 peripheral holes; reduced pillars liken knobs formed at central of base; rim of base being smooth (Fig. 2.5I). Tentacles containing rods of different sizes with rough surfaces covered with spinelets; slightly bended (Fig. 2.5K).

Remarks. *Stichopus herrmanni* is originally described as a subspecies of *Stichopus variegatus* before Rowe & Gates (1995) reclassify and accorded *S. herrmanni* a species status. *Stichopus herrmanni* is closely related to *Stichopus monotuberculatus* (Quoy & Gaimard, 1833) but the papillae of *S. herrmanni* are clearly smaller and less conspicuous than those of *S. monotuberculatus*. Massin et al. (2002) noted that rosette ossicles in *S. herrmanni* were highly variable in size and abundance. The presence of pseudo-tables found in the dorsal body of *S. herrmanni* specimens in this study was the first to be reported for the genus *Stichopus*. Pseudo-tables have only been found in *Thelenota* within the family Stichopodidae (Cherbonnier & Féral 1984; Cherbonnier 1988; Massin & Lane 1991; Massin 1999). It is not certain whether this ossicle is commonly occurring in this species of different localities. Pseudo-tables can be used to distinguish *S. herrmanni* from other congeners if they are consistently present in specimens from other localities.

Distribution. Throughout the Indo-West Pacific (Clark & Rowe 1971).

***Stichopus horrens* Selenka, 1867**

Figs 2.6–2.7

Stichopus horrens Selenka, 1867: 316; Panning 1944: 35; Loi & Sach 1963: 238, pl. 1, figs B, C, pl. VI, fig. 2; Cannon & Silver 1986: 27, figs 2d, 7g; Féral & Cherbonnier 1986: 96; Cherbonnier 1988: 147, fig. 61A–P; Rowe & Gates 1995: 324; Gosliner et al. 1996: 281, fig. 1033; Byrne et al. 2010: 1077, figs 2A–D, 3B–D.

Stichopus godeffroyi Semper, 1868: 75, pl. 30, fig. 4; Sluiter 1901: 31.

Stichopus godeffroyi var. *pygmaeus* Semper, 1868: 75; Lampert 1885: 105; Ludwig 1888: 812.

Stichopus tropicalis Fisher, 1907: 676, pl. 70, fig. 1a–i.

Material examined. Four specimens: USM/MSL/PP001, USM/MSL/PP002, USM/MSL/PP003, USM/MSL/PP006.

Type locality. Society Islands, French Polynesia

Description. Body slightly sub-quadrangular in cross-section, with firm and rough surface; wrinkles on mid-dorsal area. Dominantly dark brown and yellowish in colour, with sporadic whitish-brown patches (Fig. 2.6). Papillae scattered across dorsal surface with larger papillae along dorso- and ventro-lateral areas; base of papillae dark grey, apex white. Ring of small papillae surrounding oral opening. Tube feet restricted in three ambulacra areas, with mid ambulacrum being wider with more rows of tube feet but a gap of lesser tube feet density in middle area of mid ambulacrum. Cream-white background colour with patches of brown dots across ventral body. Twenty peltate tentacles. Anus terminal.

In dorsal body, numerous tables and C-shaped rods (Fig. 2.7A, B). Tables in dorsal body with four central perforations, 9–21 peripheral holes around smooth-surfaced base; four pillars with moderate heights forming spires joined with one crossbeam, tip of spires with large spines. Ossicles in dorsal papillae comprised of tack-liked tables, perforated plates, rods, and C-shaped rods (Fig 2.7C–F). Four pillars of table erected and fused at tips forming tall spire from middle of base (Fig. 2.7C). Rods have rough surface and tiny spines on surface, especially at both ends; central perforations can be elaborate or simple (Fig. 2.7E). Perforated plates in papillae have jagged rims and 6–12 irregular holes; smaller in size than those of tube feet. Tube feet with large rods, multiperforated plates, and tables (Fig. 2.7G–I). Large rods have central plate with perforations; rod surfaces rough and covered with spinelets (Fig. 2.7G). In tentacles, ossicles consist of rods in different size and thickness (Fig. 2.7J). All rods have rough surface and spinelets; slight curves in larger rods while smaller appeared straight.

Remarks. This species exhibits diverse body colours in specimens from different localities. Clark (1922) noted specimens from the Hawaiian Islands were dark olive-green, mottled with deep brownish-green in colour, which were not seen in specimens from the Torres Strait. Domantay (1953) remarked that the colour of this species changes with age. The species *S. horrens* look very similar to *S. fusiformiossa* but are readily distinguishable by the presence of tack-like table ossicles on the papillae of *S. horrens* and the absence of fusiform ossicles on the tentacles in *S. horrens.* Detailed characters distinguishing these species are provided in the section describing *S. fusiformiossa*.

Distribution. Society Islands, Galapagos, Indian Ocean, Bay of Bengal, South China Sea, Southern Japan, Papua New Guinea, Philippines, Australia, Hawaii to New Caledonia (Clark & Rowe, 1971).

***Stichopus vastus* Sluiter, 1887**

Figs 2.8–2.9

Stichopus vastus Sluiter, 1887: 198, pl. 2, figs 46–48; Rowe & Gates 1995: 326; Massin 1999: 71, figs 57a–l, 58a–m, 29a–g, 60a–d, 61, 112d,e; James 1998: 13, fig. 1; Massin et al. 2002: 92, pl. 2E, F, figs 12, 13.

Stichopus spec.; Colin & Arneson 1995: 262, fig. 1242; Gosliner et al. 1996: 282, fig. 1039.

Stichopus "variegatus"; Colin & Arneson 1995: 262, fig. 1240.

Material examined. One specimen, USM/MSL/PLAN001.

Type locality. Java, Indonesia.

Description. Body slightly quadrangular in cross-section, without distinct edges. Body surface smooth and tough. Black deep depression lines on dorsal body; dorsal background brown colour with numerous darker brown strips transverse and encircles the base of papillae (Fig. 2.8). Two rows of large papillae with very low protrusion on dorsal body. Tube feet only in ambulacral areas; median tube feet twice wider than those in other two ambulacral areas; narrow interambulacral areas clearly separates each ambulacrum area. Reddish brown background colour on ventral side with lighter yellowish-brown on interambulacral areas. Ventral mouth with 18–20 peltate tentacles surrounded by ring of minute papillae at oral opening (Fig. 2.8).

Tables, rosette, C-shaped rods, and reduced tables present in dorsal body (Fig. 2.9A– D). Tables in dorsal body with large quadrangular base, four central perforations, and 15–27 smaller peripheral holes; four pillars forming spire from the central of the base with a cross beam connecting them; tip of pillars with multiple thorny spines (Fig. 2.9A). Reduced tables in dorsal body having similar structure to the base of tables but lack pillars; slightly raised surface on the central (Fig. 2.9D). Ossicle of papillae consists of tables, reduced tables, C-shaped rods, S-shaped rods, rosettes, and perforated plates (Fig. 2.9I–M). Tables in papillae similar to those in dorsal body; some with larger thorns on pillar apexes (Fig. 2.9I). In ventral tube feet, rods, multiperforated plates, tables, and C-shaped rods (Fig. 2.9E–H) present. Rods in tube feet have conspicuous large central plate and multiple perforations of different sizes; surface rough and covered with spinelets (Fig. 2.9E). Oval- to square-shaped perforated plates; rims were not smooth and jagged (Fig. 2.9F). Tables in tube feet have small base disc with four main central holes and 5–8 peripheral holes; four pillars erected from central of base with thorny crowns on the tip, but do not extend out of base (Fig. 2.9G). Small C-shaped rods in tube feet (Fig. 2.9H). Ossicles of tentacles consists of rods, C-shaped rod, multiperforated plates, and tables (Fig. 2.9N–O, Q). Curved rods in tentacles in multiple different sizes; rough surface covered with spinelets especially dense at both ends. Perforated plates in tentacles spiny, rough surface covered with spinelets; some likens dichotomous rods (Fig. 2.9M) found in dorsal papillae. Tables and C-shaped rods in tentacles similar to ones found in the papillae (Fig. 2.9O, Q).

Remarks. *Stichopus vastus* is the only species in *Stichopus* that have elaborate reticulated stripes covering the dorsal body with different density and intensity (Fig. 2.8). Massin (1999) and Massin et al. (2002) demonstrated this coloration varies for specimens from across different localities. The black transverse line in the area between bulges is also unique to this species. Massin et al. (2002) mentioned S-shaped rods were found in the dorsal body in small specimens, and the absent in larger specimens (>160 mm in body length). C-shaped rods in the tentacles of the present specimens were also mentioned by Cherbonnier & Féral (1984) but not by Massin (1999) and Massin et al. (2002). Prominent large perforated plates found in the dorsal papillae have not been reported from this species and they look like dichotomous branching rods found in the tentacles.

Distribution. Indian Ocean, Andaman Islands, South China Sea, Indonesia, Thailand, Flores Sea, Great Barrier Reef Australia, Micronesia, Palau, Papua New Guinea (Clark & Rowe 1971; Massin et al. 2002).

Stichopus fusiformiossa Woo in Woo et al., 2015

Figs 2.10–2.14

Material examined. Three specimens: Holotype, USM/MSL/PSS001, collected from Pulau Songsong (5°48′31.2″N, 100°17′38.0″E), Kedah, Malaysia, 6m depth, on sand, fixed in 99% ethanol. Paratype 1, USM/MSL/PSS002, collected from Pulau Songsong (5°48′31.2″N, 100°17′38.0″E), Kedah, Malaysia, 8m depth, on sand, fixed in 99% ethanol. Paratype 2, USM/MSL/PP003, collected from Pulau Songsong (5°48′31.2″N, 100°17′38.0″E), Kedah, Malaysia, 7m depth, on sand, fixed in 99% ethanol.

Type locality. Straits of Malacca, Malaysia: Pulau Songsong, Kedah, 05°48′31.2″N, 100°17′38.0″E, on sandy substrate adjacent to a reef area, at a depth of 6–8 m, collected by Woo SP and Zulfigar Y.

Description. External morphology: Body quadrangular in cross-section with slight rounded four sides. Body wall firm, rugose, and wrinkled surface; variously-sized tiny warts regularly-arranged on dorsal body (Fig. 2.10). Dorsal body brown in background colour, with patchy beige areas and black patches; latter usually running from anterior to posterior ends and concentrated in middle part of body, and absent in some specimens. Ventral body wall light beige background with two orange, narrow lines spreading from oral to aboral. Large dorsal papillae 0.5–1.0 cm in width at base in the holotype, but highly variable between specimens, more or less arranged in two continuous rows; smaller dorsal papillae scattered on dorsal body; colour of dorsal papillae black, with apex always white. Two rows of large (about 2 cm), white-coloured papillae aligned and arranged in straight line along ventro-lateral edges. Minute papillae forming ring surrounding oral opening. Tube feet in ambulacral areas, more numerous in central ambulacral areas than in lateral ones. Two interambulacral areas very narrow, with lesser number of tube feet. Tube feet fairly long, 3–5 mm in length. All specimens examined having 20 peltate-shaped tentacles. Anus terminal.

Ossicles in dorsal body consisting tables, rosettes, and reduced tables (Fig. 2.11A–D). Many tables in dorsal body having large base with four central perforations and multiple peripheral perforations; four pillars erected from base forming a spire connected by a cross beam, with spines at the tip (Fig. 2.11A). Some tables in dorsal body having smaller base with less peripheral perforations; pillars connected incompletely with a crossbeam, tip of spire without crown of spines (Fig. 2.11D). Reduced tables in dorsal body sometimes with pillars reduced to knobs and disc with four central perforations and limited peripheral perforations (Fig. 2.11B). Papillae consisting tables, reduced tables, C-shape rods, X-shaped rods, rosettes, and rods (Fig. 2.13A–I). Large tables in papillae with multiperforated base disc; four pillars forming spire connected with a crossbeam, tip of pillar very spiny (Fig.

2.13A). Reduced tables in papillae larger compared to extensive bifurcations at both ends (Fig. 2.13D); smaller rosettes more complex in bifurcations (Fig. 2.13G). C-shaped rods in papillae simple, some modified to S-shaped (Fig. 2.13E). X-shaped rods in papillae have bifurcate endings in each arm; some with five arms (Fig. 2.13H). Rods in papillae with rough surface (Fig. 2.13I). Ossicles in tube feet mainly rods with perforated central plate, multiperforated plates, and tables (Fig. 2.12E–G). Surface of rods in tube feet rough, covered with spinelets; numbers and sizes of perforations on central plates of tube feet vary between rods. Tables in tube feet simpler compared to tables in dorsal body and papillae; tip of pillars less spinous and forming small crowns. Ossicles in the tentacles consisting of rods and fusiform ossicles (Fig. 2.14A, B). Rods in tentacles rough, slightly curved, and densely covered with spinelets at both ends. Fusiform ossicles spindle-like in shape with dense spines interconnected to each other forming some hollow spaces in between (Fig. 2.14B).

Remarks. This new species looks very much like *S. horrens* in its body colouration. They both have similar, grey-brown background with irregular grey and black spots in colour. The live specimens of this species do not have very long papillae as observed in *S. horrens* reported by Rowe & Gates (1995) and Massin et al. (2002). *Stichopus fusiformiossa* also lacks tack-like table ossicles in the papillae which are the definitive taxonomic feature for *S. horrens*. The prominent white and black colour of the papillae is distinctive to separate *S. fusiformiossa* from *S. rubermaculosus* with the red-coloured papillae, *S. quadrifasciatus* that has brown to red papillae tip, and from *S. chloronotus* with yellow-coloured papillae. Furthermore, *S. quadrifasciatus* has four transverse black-grey bands on the dorsal body wall, which are not seen in *S. fusiformiossa*. The sporadic arrangement of papillae on the dorsal body of *S. fusiformiossa* do not have consistent nor specific patterns as similarly observed in *S. horrens* in this study. However, the two rows of large, black papillae with white tip, arranged in a straight line along ventro-lateral edges are not seen in any other species.

Stichopus fusiformiossa lacks C-shaped rods in the dorsal body, which is commonly seen in the other *Stichopus* species. Reduced tables are found in the dorsal body in *S. fusiformiossa* and *S. herrmanni*, but the former lacks rosettes and C-shaped ossicles that are present in the dorsal body of *S. herrmanni*. The C-shaped rods in the papillae of *S. fusiformiossa* are strongly modified and do not resemble to any ossicles observed in the other *Stichopus* species except the C-shaped rods of *S. chloronotus* (Théel 1886; Sluiter 1887; Ludwig 1888; Mitsukuri 1912). But the colouration and arrangement of papillae easily separate *S. fusiformiossa* from *S. chloronotus*.

The X-shaped rods in the papillae of *S. fusiformiossa* have been only found in *S. variegatus* (now *nomen dubium*) by Cherbonnier (1988). The X-shaped rods of *S. variegatus* are derived from rosettes (Cherbonnier 1988). Careful examination of his drawings (Cherbonnier 1988: fig. 62H) suggested that X-shaped rods of *S. fusiformiossa* were more rigid and angled compared to the curvy and slender ones of *S. variegatus*.

The rods with rough surfaces seen in *S. fusiformiossa* are commonly found in any other species of *Stichopus*. Fusiform and spindle-liked ossicles instead are rare and only shared with *S. variegatus* (Cherbonnier 1947, fig. C). Since *S. variegatus* had been separated to either *S. herrmanni* and *S. monotuberculatus* in the nomenclatural act by Rowe & Gates (1995), the presence of fusiform and spindle-like ossicles can be accorded to *S. fusiformiossa* as a character differentiating it from both *S. herrmanni* and *S. monotuberculatus* because both *S. herrmanni* and *S. monotuberculatus* lack the presence of this ossicle. A reexamination of specimens of Cherbonnier (1947) collected from the Gulf of Oman, Madagascar, and the Red Sea are necessary to establish the correct species name of those specimens.

Etymology. The new specific name is a compound descriptive name from the combination of adjective-noun derived from the Latin words of fusiform (*fusiformis*) and bone (*ossa*). The name is nominative, neuter, and plural; referring to the distinctive fusiform and spindle-liked ossicles found in the tentacles.

2.4 Key to Species of *Stichopus* in the Straits of Malacca

1 Tack-liked tables present in dorsal papillae.................................. ***Stichopus horrens***
Tack-liked tables absent in dorsal papillae...**2**
2 Fusiform ossicles present in tentacles................................. ***Stichopus fusiformiossa***
Fusiform ossicles absent in tentacles..**3**
3 Uniform blue green colouration on dorsal body wall.............. ***Stichopus chloronotus***
Beige, brownish to yellowish colouration on dorsal body. **4**
4 Elaborate reticulated stripes on the dorsal body wall........................ ***Stichopus vastus***
No stripy body wall... ***Stichopus herrmanni***

CHAPTER 3

REVISION OF THE GENUS *APOSTICHOPUS* FROM JAPAN[2]

3.1 Introduction

Sea cucumber is one of the most important fisheries product in Japan. Commonly known as ‘manamako’, sea cucumbers from the genus *Apostichopus* are the most commonly harvested species which consists of in three different colour variants (Zhao 2015).

The taxonomy of the sea cucumber genus *Apostichopus* Liao, 1980 has been in flux, and involves four nominal taxa, three colour morphs, and two valid species. The four nominal taxa are: *Stichopus japonicus* Selenka, 1867, *Holothuria armata* Selenka, 1867, *Stichopus japonicus* var. *typicus* Théel, 1886, and *Stichopus roseus* Augustin, 1908; all have been established based on specimens from Japanese waters. The three colour morphs (red, green, and black) were first recognized by Mitsukuri (1912) by examining specimens from Japan, Far East Russia, and Korea. Mitsukuri (1912) regarded these colour morphs as representing a single species, to which he applied the name *Stichopus japonicus*, synonymizing *Holothuria armata* and *S. j.* var. *typicus*. *Stichopus roseus*, described by Augustin (1908), was not included in Mitsukuri (1912), which was published posthumously by H. Ohshima (Mitsukuri 1912: 2, footnote) after Mitsukuri’s death in 1907. Choe & Ohshima (1961) showed that the green and red morphs might represent different species due to the differences in morphology (Polian vesicles, ossicles, and gelatinous layer in mature eggs), spawning and aestivation periods, and preferences for sediment type and salinity.

The idea of the presence of the two different biological entities, each represented by the red morph and the green/black morphs, has been supported by allozyme (Kanno & Kijima 2003) and microsatellite (Kanno et al. 2006) data. However, Sun et al. (2010) concluded that all the colour morphs belonged to a single

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[2] The contents of this chapter have been published in:

Woo SP, Ogawa A, Tan SH, Yasin Z, Kajihara H, Fujita T (2017) A taxonomic revision of the genus *Apostichopus* (Holothuroidea: Stichopodidae) from Japan. Zootaxa 4350: 121–135.

species by showing uncorrected *p*-distances of CO1 gene sequences between different colour morphs; the maximum value reached 1.48%. On the other hand, Ward et al. (2008) and Uthicke et al. (2009) found that intraspecific variation in CO1 was 0.6–0.72% for holothuroids including *Stichopus*. Therefore, the genetic distances of CO1 shown by Sun et al. (2010) is not in contradiction to Kanno & Kijima (2003) and Kanno et al. (2006) who argued the existence of two valid species in *Apostichopus*.

Kuramochi & Naganuma (2010) put an end to this taxonomic suspension by applying the names *Apostichopus japonicus* (Selenka, 1867) to the red morph and *A. armatus* (Selenka, 1867) to the green/black forms. However, their figures illustrating the ossicles (Kuramochi & Naganuma 2010: figs 2, 3) were of low resolution, without scanning electron microscope (SEM) images showing detailed features that are essential in differentiating the two species. Furthermore, the description provided by Kuramochi & Naganuma (2010) only included ossicles from the dorsal body wall and from the tentacles.

In this study, a revision of the genus *Apostichopus* with full description of ossicle assemblages from dorsal body, papillae, tentacles, tube feet, and cloacal wall is done by examination of type specimens and additional specimens of *Apostichopus* using SEM.

3.2 Materials and Methods

A total of 19 specimens (including two syntype) of sea cucumber specimens from the genus *Apostichopus* used in this study, were loaned from the Museum of Comparative Zoology, Massachusetts, USA (MCZ) and the National Museum of Nature and Science, Tsukuba, Japan (NSMT).

The methods for extraction, preparation, and observation of ossicles were described in Chapter 1.

3.3 Taxonomy

Order Synallactida

Family Stichopodidae Haeckel, 1896

Genus *Apostichopus* Liao, 1980

Type species. *Stichopus japonicus* Selenka, 1867 (but see Remarks below).

Type locality. Japan

Diagnosis. Mouth ventral, surrounded by twenty peltate-shaped tentacles. Body stout and cylindrical. Conical papillae conspicuous or less conspicuous, protruding at dorsal and lateral sides of body; similar conical papillae arranged in one line at ventro-lateral sides; smaller papillae scattered on dorsal and lateral surfaces. Background body colour highly variable from red to brown and green to black. Gonads in two tufts. Ossicles taking form of tables, buttons, multiperforated plates, rods, and complex plates. Dorsal body-wall ossicles composed of tables; often reduced with pillars absent, but simple and not spinous if present; rims smooth or spinous. Dorsal body wall of juvenile contains tables with four spires, each having multiple spines on its end, with two to three crossbeams. Rosette and C-shaped ossicles absent in dorsal body wall of both adult and juveniles. Papillae containing elongated buttons, rods, tables, and multiperforated plates. Rods in tentacles. Ventral tube feet containing multiperforated plates and tables, the latter without pillars. Spinous complex plates present in cloacal walls both in adult and juvenile.

Species composition. *Apostichopus armatus* (Selenka, 1867); *Apostichopus japonicus* (Selenka, 1867) (see Remarks).

Remarks. Liao (1980) erected this genus based on material from the northern China Sea that was identified as *Apostichopus japonicus*. Judging from the drawings of the ossicles, Liao's (1980) material was most likely to represent *Apostichopus armatus* (Liao 1980: fig. 1), to which Article 70.3 (Misidentified type species) of the Code (International Commission on Zoological Nomenclature 1999) applies. In order to serve stability and universality of the name, we take the option stipulated under Article 70.3.1, in which the nominal species previously cited as the type species is to be retained, because alteration of the type species will not affect the genus name usage. The diagnosis for *Apostichopus* given by Liao (1980) sufficiently illustrated clear separation of *Apostichopus* from other genera based on reduced table ossicles without pillars in the body wall (Liao 1980: fig. 1).

The absence of C-shaped ossicles and the presence of reduced table ossicles in the dorsal body of *Apostichopus* differentiate it from the genera *Stichopus* and *Isostichopus* Deichmann, 1958, which have abundant C-shaped ossicles and no reduced table in the dorsal body (Clark 1922; Deichmann 1958). The genus *Apostichopus* also can be easily distinguished from the genera *Thelenota* Brandt, 1835 and *Astichopus* Clark, 1922 by the presence of table ossicles that are absent in the dorsal body of the two genera (Clark 1922). Complex plates in the cloacal walls and elongated buttons in the papillae are observed in *Apostichopus* (Théel 1886; Liao 1980) and *Parastichopus* Clark, 1922 (Clark 1922; Lambert 1986). The well-developed table ossicles with complete spires in the dorsal body of *Parastichopus* are absent in adult *Apostichopus* (body length greater than 70 mm) (Mitsukuri 1897). Furthermore, *Apostichopus* can be found from the intertidal zone to the depth of about 20–40 m (Zhao 2015), while *Parastichopus* is distributed only in deeper waters from 30 m down to the depth of 600 m (Deichmann 1937; Lambert 1986; Imaoka et al. 1990).

We only included two species in the species list of *Apostichopus*. This is contrary to the inclusion of *Parastichopus californicus* (Stimpson, 1857), *Stichopus johnsoni* Théel 1886, *Parastichopus leukhothele* Lambert, 1986, *Parastichopus multidentis* Imaoka, 1991, *Stichopus nigripunctatus* Augustin, 1908, *Parastichopus nipponensis* Imaoka, 1990 (synonym of *P. nigripunctatus*), and *Stichopus parvimensis* Clark, 1913 by Paulay (2013) as part of the species list in *Apostichopus*. The inclusion is deemed invalid because the nomenclatural act of transferring these species to the genus *Apostichopus* did not fulfil the requirements of Article 8 of the Code (International Commission on Zoological Nomenclature 1999).

***Apostichopus armatus* (Selenka, 1867)**

Figs 3.1–3.5

Holothuria armata Selenka, 1867: 330, pl. XVIII, fig. 66; Lampert 1885: 91.
Stichopus armatus (*Holothuria*); Théel 1886: 196
Stichopus (Holothuria) armatus; Mitsukuri 1896: 408; Mitsukuri 1912: 164
Stichopus japonicus var. *typicus* Théel, 1886: 161, pl. VIII, fig. 3.
Apostichopus japonicus; Liao 1980: 116, fig. 1; Sun et al. 2010: 280–285.
Apostichopus armata [sic]; Kuramochi & Naganuma 2010: 50, figs 1[2a, 2b]–3 [the ossicles in figs 2 and 3 were erroneously given under the name ‘*A. japonicus*’ on the figures].

Type material examined. Syntype MCZ HOL–741, Hakodate, Japan.

Additional materials examined. NSMT–E7045abe1, Tateyama Bay, Chiba, 21 April 2011; NSMT–E7047abe1, Aburatsubo Bay, Kanagawa, 10 July 2011, Y. Tamura; NSMT–E7047abe1.1, Aburatsubo Bay, Kanagawa, 10 July 2011, Y.

Tamura; NSMT–E7047abe3, Aburatsubo Bay, Kanagawa, 10 July 2011, Y. Tamura; NSMT–E7047abe5, Aburatsubo Bay, Kanagawa, 10 July 2011, Y. Tamura.

Type locality. Hakodate [misspelled as Hakodadi by Selenka (1867)], Japan.

Description. Twenty peltate tentacles. Two rows of dorsal papillae, each arranged alternately in zig-zag pattern along dorso-lateral radius. Single row of conical papillae along each ventro-lateral radius. Numerous, loosely arranged, small papillae scattered on dorsal and lateral body. Ventral side flattened. Tube feet arranged in 5–6 rows in central ambulacral area, 4–5 rows in both lateral ambulacral areas. Interambulacra narrow. Mouth subventral, surrounded by ring of small papillae. Dorsal and ventral body surfaces uniformly light green to yellowish in alcohol. Gonads in two tufts; one on each side of dorsal mesentery. Free-hanging tentacular ampullae 20 in number. Polian vesicle single, in various shapes and forms. Intestine connected to dorsal mesentery with rete mirabile.

Dorsal body wall with tables and reduced tables (Fig. 3.2). Disk of tables rounded to oval in shape with 2–16 irregular perforations; rim of disk spinous or smooth (Fig. 3.2A–E). Pillar of reduced tables entirely absent or forming only a knob (Fig. 3.2C). Tables usually having four pillars; tip of each pillar often branched into multiple spines (Fig. 3.2A). Papillae containing multiperforated plates, tables, reduced table, rods and elongated buttons (Fig. 3.3). Multiperforated plates in papillae with numerous irregular perforations; rim uneven (Fig. 3.3E–F). Tables in papillae similar to those in dorsal body; pillars absent or forming knobs; rim spinous (Fig. 3.3A); mostly four pillars present, each having single spine on tip (Fig. 3.3B–C). Elongated buttons in papillae having two parallel longitudinal rows, each with 7–13 perforations (Fig. 3.3D). Rods in papillae variable in shape from straight to curved; surface of curved rods smooth (Fig. 3.3G), surface of straight rods spinous (Fig. 3.3H) Tentacles containing rods; curved (Fig. 3.4A–B); surface covered with spinelets. Tube feet containing multiperforated plates (Fig. 3.5A–B). Cloacal wall containing oval to rounded complex plates; surface very spinous surface.

Distribution. Shallow temperate waters in the coasts of China, Japan, Korea and Russia. Northern distribution limit at Sakhalin Island, Russia and Southern limit in the Kagoshima prefecture in Japan (see Zhao 2015).

Remarks. Reduced tables are the dominant ossicles in the dorsal body wall of *A. armatus.* They are similar to the ossicles found in the type material of *H. armata* in which Selenka (1867) described as “Spärlich durchlöcherte plättchen”

[sic] or sparsely perforated plates which he compared to the ossicles found in *Holothuria (Halodeima) floridana* (Pourtalès, 1851) (Selenka 1867: fig. 49). However, Selenka (1867) did not include the details of both the rims in the platelet ossicles, and figures for the ossicles from the type material. It is unfortunate that we are also unable to determine the ossicles from the type material in this study because they appeared to be absent or completely dissolved. Incidentally, Selenka's (1867) *H. armata* is "schwarz" (black) coloured, which is clearly still visible upon examining the specimen (Fig. 3.1), which now forms part of the diagnosis for *A. armatus*.

Apostichopus armatus can be differentiated from *A. japonicus* by the absence of reduced table ossicles with spinous rim in *A. japonicus.* Similar spinous reduced table ossicles are also clearly illustrated in *S. j.* var. *typicus* by Théel (1886: pl. 8, fig. 2), *S. japonicus* in Liao (1980: fig. 1), and *A. armata* in Kuramochi & Naganuma (2010: fig.2), which all represents *A. armatus*. Although some reduced tables with smooth rim (similar to those found in the dorsal body of *A. japonicus*) are also present in *A. armatus,* they are only present in small amount compared to the dominant number of spinous ones. Ossicles from the papillae, tentacles and tube feet are similar to those of *A. japonicus.*

Apostichopus japonicus **(Selenka, 1867)**

Figs 3.6–3.13

Stichopus japonicus Selenka, 1867: 318, pl. 18, figs 33–36; Semper 1868: 74; Marenzeller 1882: 136–137, taf V, fig 11; Théel 1886: 160, pl. VII, fig 3; Mitsukuri 1896: 408; Augustin 1908: 6–7, fig 4; Mitsukuri 1912: 163, pl. 4, figs 32–44; Ohshima 1915: 247–248; Clark 1922: 61; Choe & Ohshima 1961: 97–105; Levin 1983:1–45, figs 1–12.

Stichopus japonicus var. *typicus* Théel, 1886: 196.

Stichopus (Apostichopus) japonicus; Kanno et al. 2006: 672–685.

Apostichopus japonicus; Kuramochi & Naganuma 2010: 49–54, figs 1a, 1b, 2.

Stichopus roseus Augustin, 1908: 13–14, fig. 10.

Type material examined. Syntype MCZ HOL–763, Northwest Pacific Ocean, Japan.

Additional materials examined. NSMT–E3673, Fukue Island, Nagasaki, 14 March 1998, T. Fujita; NSMT–7045A, Tateyama Bay, Chiba, 21 April 2011, Y. Tamura; NSMT–E7046A, Futtsu, Chiba,4 29 June 2011, Y. Tamura; NSMT–E7046C, Futtsu, Chiba, 29 June 2011, Y. Tamura; NSMT–E7047B, Aburatsubo Bay, Kanagawa, 10 July 2011, Y. Tamura; NSMT–E7047D, Aburatsubo Bay, Kanagawa, 10 July 2011, Y. Tamura; NSMT–E7048, Uchiura Bay, Kanagawa, 28 September 2011, K. Yakiguchi; NSMT–10122A-E, 5 specimens, Takeoka, Futtsu, Chiba, 24 December 2014, K. Kawata & A. Ogawa.

Type locality. Japan.

Descriptions. Twenty peltate tentacles. Two rows of dorsal papillae, each arranged alternately in zig-zag pattern along the dorso-lateral radii. Single row of conical papillae along each ventro-lateral radius. Similar conical papillae, but larger, arranged single row at both ventro-lateral radii. Numerous, loosely arranged, small papillae scattered on dorsal and lateral body. Ventral side flattened. Tube feet arranged in 5–6 rows in central ambulacral area; 4–5 rows in both lateral ambulacra areas. Interambulcra narrow. Mouth subventral, surrounded by a ring of small papillae. Background body colour in alcohol dark reddish brown on dorsal, lighter brown on both lateral and ventral body. Gonads in two tufts; one each side of dorsal mesentery. Twenty free-hanging tentacular ampullae. Polian vesicle single, in various shapes and forms. Intestine connected to dorsal mesentery with rete mirabile.

Dorsal body wall containing tables and reduced tables (Figs 3.7, 3.8). Tables in dorsal body wall of adult reduced, pillars absent. Disk of table oval to round in shape with smooth rims; 2–14 irregular perforations on disk. Tables in dorsal body of juvenile with four pillars; single spine at tip of each pillar. Papillae containing multiperforated plates, reduced tables, rods, and elongated buttons (Figs 3.9, 3.10). Multiperforated plates in papillae with four central perforation and numerous peripheral perforations; rim of uneven (Fig. 3.9A–D). Reduced tables in papillae similar to those in dorsal body (Fig. 3.9E), size larger and more perforations (2–14 perforations); rims smooth to uneven, but not spinous. Elongated buttons in papillae having two longitudinal rows of 5–11 perforations (Fig. 3.10A). Rods in papillae straight to curved; surface spinous (Fig. 3.10B). Rods in tentacles curved; surface covered with multiple spinelets (Fig. 3.11A–C). Tube feet with multiperforated plates (Fig. 3.12). Cloacal wall containing oval to rounded complex plates; surface very spinous and knobby (Fig. 3.13).

Distribution. Shallow temperate waters in the coasts of China, Japan, Korea and Russia. Northern distribution limit at Sakhalin Island, Russia and Southern limit in the Kagoshima prefecture in Japan (see Zhao 2015)

Remarks. The reduced table ossicles of other materials examined in the dorsal body are similar to the ossicles named “hemmungsbildungen” meaning table ossicles reduced to the ring in Selenka (1876: pl 18, fig. 36). The most contrasting difference between *A. japonicus* and *A. armatus* can be seen in the rims of reduced tables found on the dorsal body. The reduced tables have only smooth and non-spinous rims in *A. japonicus* compared to spinous disk rim tables in *A. armatus*. Observations on the reduced table ossicles of type material using SEM (Fig. 3.7) confirms that the rims of reduced table ossicles in the dorsal body are smooth and non-spinous. Kuramochi & Naganuma (2010) also observed similar characters at rim of tables in materials they examined. Although the figures of reduced tables of *A. japonicus* observed under compound microscope in Kuramochi & Naganuma (2010: fig. 2) showed that the rim of disks in tables appear to be spinous, greater resolution observation using scanning electron microscope in this study revealed that the rim on the disks of the reduced tables are merely undulating on the rims but do not form spines.

CHAPTER 4

A NEW GENUS AND TWO NEW COMBINATIONS OF STICHOPODIDS FROM AUSTRALIA[3]

4.1 Introduction

The genus *Stichopus* Brandt, 1835 is the most speciose in the family stichopodidae, containing 14 described species (Paulay & Hansson 2013b). *Stichopus* species are predominantly distributed in the tropical waters of Indo-Western Pacific (Clark & Rowe 1971) with the exception of two species, *Stichopus ellipes* Clark, 1938 and *Stichopus ludwigi* Erwe, 1913, which are found in the shallow waters of south and southwestern Australia, and in south and southwestern Australia, respectively (locality record in Australian Museum's collection).

The taxonomic and phylogenetic status of both *S. ellipes* and *S. ludwigi* has not been revised after they were first described. In the study done by Byrne et al. (2010), they found that the genus *Stichopus* is monophyletic with the exception of *S. ellipes*, which is clustered outside of the *Stichopus* clade in their analysis using a single gene marker (16S rDNA). The phylogenetic position of the closely related species, *S. ludwigi*, was unfortunately not able to be ascertained as it is not included in the analysis. Therefore, a revision of the taxonomy and phylogenetic status of both species is timely.

In this study, specimens of *S. ellipes* and *S. ludwigi* loaned from the Australian Museum are used for the revision. Full description of ossicle assemblages from dorsal body, papillae, tentacles, and tube feet are done using scanning electron microscope (SEM), and phylogenetic analysis is done using two mitochondrial gene markers (16S and COI).

4.2 Materials and Methods

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[3] The contents of this chapter are based on the following manuscript submitted to the journal *Marine Biodiversity* on 5 October 2017:

Woo SP, Kajihara H, Byrne M, Fujita T. Description and phylogenetic relationships of a new genus, with two new combinations of sea cucumbers (Holothuroidea, Stichopodidae) from Australia.

A total of seven specimens of sea cucumber (*S. ludwigi* and *S. ellipes*) used in this study, were loaned from the Australian Museum (AM). The methods for extraction, preparation, and observation of ossicles were described in Chapter 1. Molecular analyses were carried out as described in Chapter 1 as well. Total DNA of the five specimens of *S. ludwigi* and two specimen of *S. ellipes* were extracted and sequenced. Sections of the cyctochrome oxidase subunit I (COI) and the small subunit 16S ribosomal (16S rDNA) genes for two specimens of *S. ellipes*, and one specimen of *S. ludwigi* were succcesfully sequenced.

4.3 Taxonomy

Order Synallactida

Family Stichopodidae Haeckel, 1896

Genus *Notostichopus* gen. nov.

Type species *Stichopus ludwigi* Erwe, 1913

Etymology The new generic name is derived from the combination of adjective-noun derived from the Greek word of south (νότος, *notos*) and the sister genus name *Stichopus*. The name is nominative, singular and masculine.

Distribution Temperate and subtropical, shallow coastal waters of Australia.

Diagnosis Body cylindrical. Mouth ventral, surrounded by twenty tentacles. Dorsal papillae at dorsolateral radii, or distributed across dorsal side. Tube feet crowded on ventral side. Dorsal body ossicles consisting of tables and C-shaped rods; rosettes and buttons absent. Tables with single crossbeam. Rod ossicles in tube feet with peripheral extension; with or without perforations.

Remarks This new genus is established based on the isolated phylogenetic position (see Molecular Phylogeny of *Notostichopus* sp. nov.) and ossicles composition. The presence of C-shaped ossicles and well-developed tables in dorsal body of *Notostichopus* differentiates this genus from the genera *Apostichopus* Liao, 1980, *Astichopus* Clark, 1922, *Australostichopus* Levin in Moraes, Norhcote, Kalinin, Avilov, Silchenko, Dmitrenok, Stonik & Levin, 2004, *Neostichopus* Deichmann, 1948, *Parastichopus* Clark, 1922, and *Thelenota* Brandt, 1835, where both of these ossicles are not found.

The genus *Notostichopus* can be differentiated from *Isostichopus* Deichmann, 1958 and *Eostichopus* Cutress & Miller, 1982 by having only a single or lacking crossbeam in the table ossicles in the *Notostichopus*. In contrast, *Isostichopus* and *Eostichopus* have at least two, or up to 10 crossbeams in the table ossicles. Rosette ossicles are absent in *Notostichopus* and exclusively found in *Stichopus* Selenka, 1867.

Notostichopus ludwigi **(Erwe, 1913) comb. nov.**

Figs 4.1–4.3

Stichopus ludwigi Erwe 1913: 388–389, fig. 1, pl. 8, fig. 24a–f; Clark 1922: 52, 63.

Type material. MNB 6170 (not examined), 1–6 m depth, collected by Michelsen and Hartmeyer, Hamburger SW-Australien [sic] Expedition 1905, in ethanol, deposited in Museum für Naturkunde, Berlin, Germany.

Type locality. Southwest Australia.

New materials examined. Two specimens. AM J24986, Billy Lights Point, Port Lincoln, South Australia, Australia, 34°45′06″S, 135°53′34″E, 6 m depth, on sand and seagrass substrate, 9 February 2012, collected by B. Stobart and M. Byrne; AM J8422, Ludlow, Geographe Bay, Western Australia, Australia, 33°37′S, 115°18′E, 9 m depth, in sand and reef substrate, 23 November 1971, collected by N. Coleman.

Distribution. Southwest Australia and south Australia.

Diagnosis Dorsal body containing tables; disc of tables having four central perforations and 3–4 peripheral perforations; rims of disc spinous, especially at four corners. Presence of modified tables in dorsal body. Absence of peripheral perforations in rods from tube feet.

Description. Morphology: Mouth ventral, surrounded by twenty tentacles. Body cylindrical, strongly contracted and folded. Papillae at dorsal body contracted, arranged in 2–3 lines at dorsolateral radii. Dorsal and ventral body uniformly light brown, with patches of darker brown on dorsal side. Black retracted papillae forming conspicuous, 2–3 mm diameter spots on dorsal body. Tube feet retracted; arrangements undetermined. Anus terminal. Gonad in two tufts.

Ossicles: Dorsal body wall containing tables, modified tables, reduced tables, and C-shaped rods (Fig. 4.1). Disc of tables in dorsal body square with spinous rim at four corners (Fig. 4.1B). Four large central perforations and 3–4 smaller peripheral perforations on disc of tables. Four spires, with 1–2 spines ending erected at central part of table disc; single crossbeam connecting three or four spires. Some tables with reduced spires, forming knobs; or spires absent (Fig. 4.1C). Modified tables in dorsal body with multiple spines on rims of disc, interconnected to spire (Fig. 4.1A). Tube feet containing multiperforated plates, rods, and tables (Fig. 4.2). Multiperforated plates measuring 200–300 µm (Fig. 4.2A). Rods spinous, with peripheral extensions along stalk (Fig. 4.2B). Disc of tables in tube feet having four central perforations and 1–4 peripheral perforations; rim spinous (Fig. 4.2C). Four short spires shorter than those found in tables of dorsal body, ending with 1–2 small spines, erected at central part of table disc; crossbeam wanting. Tentacles containing rods; slightly curved (Fig. 4.3); surface covered with spinelets.

Remarks. Tables and C-shaped rods in the dorsal body are consistent with the original description and figures by Erwe (1913: pl. 8, fig. 24a–b). Greater resolution observation of the tables in dorsal body using SEM in this study revealed that the rim on the disks of the tables are much more spinous compared to the drawings done by Erwe (1913: pl. 8, fig. 24a). In addition to tables in dorsal body, modified tables with spines on rims of disc interconnected to spire are first recorded here. Although Erwe (1913) remarked that C-shaped rods differs in *N. ludwigi* from those of *Stichopus variegatus*, Semper 1868 [now either *Stichopus herrmanni* Semper, 1868 or *Stichopus monotuberculatus* (Quoy & Gaimard, 1886)] in terms of size, it is known that the length of the rods varies interspecifically and intraspecifically (Cherbonnier 1988; Massin 1996; Massin 1999; Massin et al. 2002; Woo et al. 2015). Therefore, the size of C-shaped rods are less useful as taxonomic character for separating different species in the family.

The presence of modified tables in the dorsal body differentiates *N. ludwigi* from *N. ellipes*, where the latter possesses tables with large disc and very spinous spire endings. Furthermore, extension in rods in the tube feet of *N. ludwigi* do not form perforations compared to rods in the tube feet of *N. ellipes* where large perforations and multiple peripheral perforations form in the extension.

Notostichopus ellipes (Clark, 1938) comb. nov.

Figs 4.4–4.8

Stichopus ellipes; Clark 1938: 514–515, fig. 54; Clark & Rowe 1971: 178; Byrne et al. 2010: 1068–1081.

Type material. MCZ HOL-1553 (not examined), 10–12 m depth, wild caught, collected by Carnegie Australian Harvard Expedition, in ethanol, deposited in Museum of Comparative Zoology, Cambridge, USA.

New materials examined. Five specimens. AM J12946, Jervis Bay, New South Wales, Australia, 35°03′S, 150°44′E, 28 October 1979, collected by J. Marshal; AM J13118, Long Reef, New South Wales, Australia, 33°44′S, 151°19′E, 10 January 1963, collected by J.W. Evans; AM J13119, Glaisher Point, Cornulla, New South Wales Australia, 34°04′24″S, 151°09′24″E, 10 August 1965, collected by C. Lawler; AM J14808, Batemans Bay, New South Wales, Australia, 35°43′S, 150°15′E, 19.8 m depth, 10 March 1981 collected by J. Marshal; AM J20266, Castle Hill Bay, New Castle, New South Wales, Australia, 32°55′S, 151°46′E, 30 April 1987, collected by T. Cowling.

Type locality. Eighty Mile Beach, Broome, Western Australia

Distribution. Western Australia southeastern Australia.

Diagnosis. Dorsal body containing table ossicles; disc of tables having four central perforations and multiple peripheral perforations; large disc and rims of disc smooth. Four spire connected by single crossbeam; spire endings spinous. Large and peripheral perforations in the extension of rod ossicles in tube feet.

Description. Morphology: Mouth ventral, surrounded by twenty tentacles. Body cylindrical, compressed and contracted. Papillae at dorsal body retracted. Background of dorsal and ventral body uniformly light brown, with patches of darker brown on dorsal and ventral sides; minute black dots scattered across dorsal body. Tube feet crowded on ventral side; distributed in radii and interradii. Anus terminal.

Ossicles: Dorsal body consisting tables, C-shaped rods and S-shaped rods (Fig. 4.4). Disc of tables in dorsal body large (55–70 μm), with four central perforations and 9–25 peripheral perforations; rim of disc smooth (Fig. 4.4A). Four spires, with multiple spines endings, erected at central part of table disc; single crossbeam connecting adjacent spires. Some spines on spire tip fused with spines of adjacent spires. Some tables with reduced spires, forming knobs. Some C-shaped rods modified forming S-shaped rods (Fig. 4.4B). Papillae containing rods and table ossicles (Fig. 4.5). Two types of rods: 1) rods with central perforations, number of perforation varies; some without perforations (Fig. 4.5A). Surface smooth with occasional spinelets. 2) rods curved with some spinelets on surface (Fig. 4.5B). Tables in papillae with large disc; rim of disc smooth; four central perforations with multiple peripheral perforations on disc (Fig 4.5C). Four spires with spinous endings erected from the central part of disc; single cross beam connecting adjacent spires. Tube feet containing multiperforated plates, tables, and rods (Fig 4.6, 4.7). Tables in tube feet similar to those in papillae; spires more spinous; single spinous crossbeam (Fig 4.6C). Rods in tube feet with large, peripheral extension along main stalk with multiple perforation; perforations larger adjacent main stalk, smaller perforations in peripheral (Fig. 4.7) Tentacle containing curved rods; surface covered with spinelets (Fig. 4.8).

Remarks. The table ossicles in the dorsal body and papillae of *N. ellipes* observed in this study were very similar to those observed by Clark (1938: fig. 54). Although Clark (1938: 515) recorded the diameter of disc of tables (length of specimen 163 mm) as 80–85 µm, the diameter may vary from each individual as the diameter of disc of tables in this study (length of preserved specimens 150–180 mm) ranges from 55–70 µm. In addition to tables, C-shaped rods, commonly found this genus and other confamilial genera (*Stichopus* and *Isostichopus*), were observed to be present in the dorsal body wall of *N. ellipes*. This is in contrast to the observation of Clark (1938) which may have overlooked on the presence of C-shaped rods in *N. ellipes*.

Notostichopus ellipes can be differentiated from *N. ludwigi* in the difference of table ossicles in the dorsal body. The diameter of the disc of tables in *N. ellipes* are much larger compared to those found in *N. ludwigi* which can accommodate more numbers of peripheral perforations. The spire endings of tables in *N. ellipes* are also more spinous compared to the spire endings of tables in *N. ludwigi*. Furthermore, all tables in *N. ellipes* are not modified as seen in *N. ludwigi*. Rod ossicles found in the tube feet also showed differences between these two species.

The extension along the stalk rods in the tube feet in *N. ellipes* forms perforations and extends to the whole of the stalk.

4.4 Molecular Phylogeny of *Notostichopus* gen. nov.

The resulting ML and BI trees produced identical topology (Fig. 4.9). All the genera included in the analysis formed distinct clades supported by moderate to high values for both bootstrap (70–100) and posterior probability (0.9–1.00). The species *N. ellipes* and *N. ludwigi* were clustered together forming a clade sister to the one consisting of *Stichopus* and *Isostichopus* clades. This corroborates the suggestions of Byrne et al. (2010) where both these “warm-temperate water” [subtropical] species from Australia belong to a separate genus from *Stichopus* although Byrne et al. (2010) likened these species morphologically to the genus *Stichopus* by the presence of tables and C-shaped ossicles. Moreover, removing *N. ellipes* and *N. ludwigi* from the genus *Stichopus* would seem justifiable, where it will restrict the distribution of *Stichopus* only to warm tropical waters of Indo-West Pacific region (Deichmann 1958; Byrne et al. 2010). Our phylogeny analysis agrees with Byrne et al. (2010), suggesting each genus of the family Stichopodidae are more or less separated and restricted geographically: three tropical genera, *Stichopus* in the Indo-West Pacific, *Isostichopus* in the eastern Pacific/Caribbean,and *Eostichopus* in the Caribbean, a subtropical/temperate genus *Notostichopus* in central to southern Australia, and two temperate genera of *Apostichopus* in North Pacific and *Australostichopus* in Southwestern Pacific. The phylogenetic position and geographic extension of the remaining two genera *Parastichopus* (Atlantic) and *Neostichopus* (South Africa/Western Indian Ocean) remains undetermined. Our phylogenetic analysis also agrees with the inclusion of *Parastichopus californicus* Stimpson, 1857 within the genus *Apostichopus* by Paulay (2017), although this transfer is deemed invalid in nomenclature because it did not fulfil the publication requirements of the Code (International Commission on Zoological Nomenclature 1999: Article 8). Since specimens of *P. californicus* were not obtained in this study, there is insufficient morphological information to support the combination of this species to the genus *Apostichopus*. A revision to the species in genus *Parastichopus* is required to determine their status.

The genetic distances within and between genera (uncorrected *p*-distance values) for COI and 16S sequences are shown in Table 2. The average intergeneric genetic distances for COI and 16S in the family are 0.187 and 0.120 respectively. The genetic distances of 16S and COI gene fragments between *Notostichopus* and other genera are always larger than the average between genera except for the *Isostichopus* (0.144) and *Stichopus* (0.162) in COI, and *Isostichopus* (0.090) in 16S. Although *Notostichopus* forms a sister clade to the one comprised of *Stichopus* and *Isostichopus*, the genetic distances (in terms of both COI and 16S) between *Notostichopus* and *Isostichopus* (0.144, 0.090) are smaller than that between *Notostichopus* and *Stichopus* (0.162, 0.121).

CHAPTER 5

SUMMARY

Since the erection of the family Stichopodidae, there were only two major revisions that consolidated this family done by Clark (1922) based on morphology, and by Byrne et al. (2010) based on phylogenetics. As both revisions are centred on the type genus *Stichopus*, which incidentally is the most speciose genus in the family, both revisions have yet to be able to completely resolve the many systematic complications of this family. Therefore, this dissertation aims to study the systematics of the sea cucumber from the family Stichopodidae.

Chapter 2 reports a study that was done on the diversity and taxonomy of stichopodids found in the Straits of Malacca. In this study, one new species is described based on specimens found in Pulau Songsong, Malaysia, which possess unique ossicle that is found in tentacles of this species. The fusiform and spindle-liked ossicles found in the tentacles are defining characters accorded to the new species. Same ossicles were found previously in *Stichopus variegatus* Semper, 1868, which, however, is no longer a valid species name. Besides description of a new species, this chapter also presented two newly recorded ossicles for two species of the genus *Stichopus* that is useful as diagnostic characters.

In the third chapter, a revision of the genus *Apostichopus* was done to resolve the taxonomic complications that involve four nominal taxa, three colour morphs (red, green and black), and two valid species. The four nominal taxa involved are: *Stichopus japonicus* Selenka, 1867, *Holothuria armata* Selenka, 1867, *Stichopus japonicus* var. *typicus* Théel, 1886, and *Stichopus roseus* Augustin, 1908; all have been established based on specimens from Japanese waters. Examination of the type specimens and additional specimens from Japan done in this study concluded that there are two valid species with the name *Apostichopus japonicus* and *Apostichopus armata* based on evidences of ossicles and genetic data done by previous studies. Furthermore, full description of ossicle assemblages of both species from dorsal body, papillae, tentacles, tube feet, and cloacal wall are given for the first time.

Chapter 4 in this dissertation proposed a new genus to accommodate two species (*Notostichopus ellipes* and *Notostichopus ludwigi*) from Australia. The erection of this genus is based on description of ossicles, phylogenetic relationships, and also genetic distances between the genera in the family. Full description of ossicle assemblages from dorsal body, papillae, tentacles, and tube feet are also presented. The transfer of *N. ellipes* and *N. ludiwigi* from the genus *Stichopus* to this new genus resulted in the restriction of geographical distribution of the genus *Stichopus* to tropical waters of the Indo-west Pacific. The phylogenetic analysis in this study agrees with Byrne et al. (2010), suggesting that each genus of the family Stichopodidae are more or less separated and restricted geographically.

Recent phylogenetic study on extant sea cucumbers has significant impacts and changes to the higher taxon of the family Stichopodidae. Moreover, some members of the family Synallactidae (*nomen dubium*) are found to form a monophyletic clade within the family Stichopodidae. Therefore, the taxonomic and phylogenetic relationship status of the family Stichopodidae remains unclear and a comprehensive revision is warranted. Future phylogenetic studies in this family may include gene markers of 18S, 16S, COI, H3, 12S and 28S from all representative species and genera to effectively resolve the phylogenetic relationship of the family both at higher and lower taxon level. Along with phylogenetic studies, morphological studies on the complete ossicle assemblages should be accompanied to determine morphological synapomorphies that support each level of taxa in Stichopodidae.

As a conclusion, a total of one new genus, one new species, two new combinations, and one resurrected species name is proposed through this dissertation. Therefore, this family at present consists of 10 genera and 34 described species after incorporating changes proposed in this dissertation.

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FIGURES AND TABLES

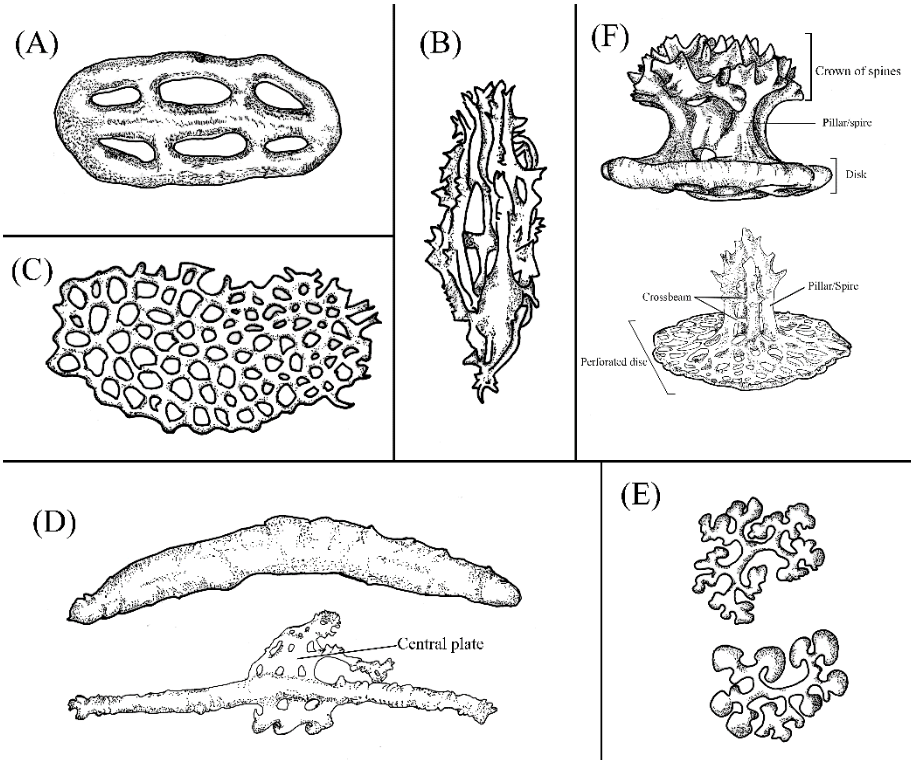

FIGURE 1.1 Different types of ossicles found in sea cucumbers of the family Stichopodidae. A, buttons; B, fusiform; C, multiperforated plate; D, rods; E, rosettes; F, tables.

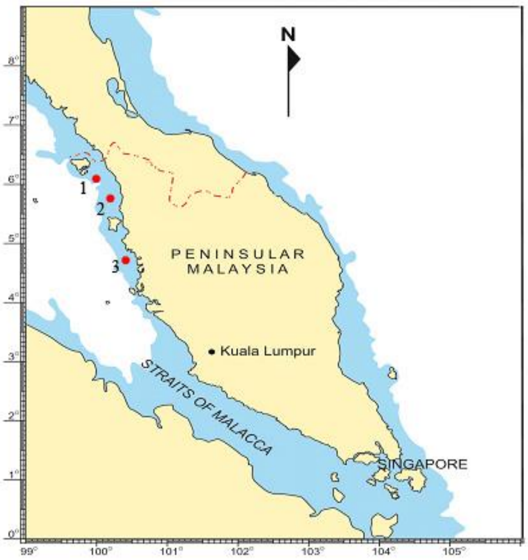

Figure 2.1 Map of study area in the Straits of Malacca. 1, Pulau Payar; 2, Pulau Songsong; 3, Pulau Sembilan.

Figure 2.2 Dorsal and ventral view of *Stichopus chloronotus* Brandt, 1835.

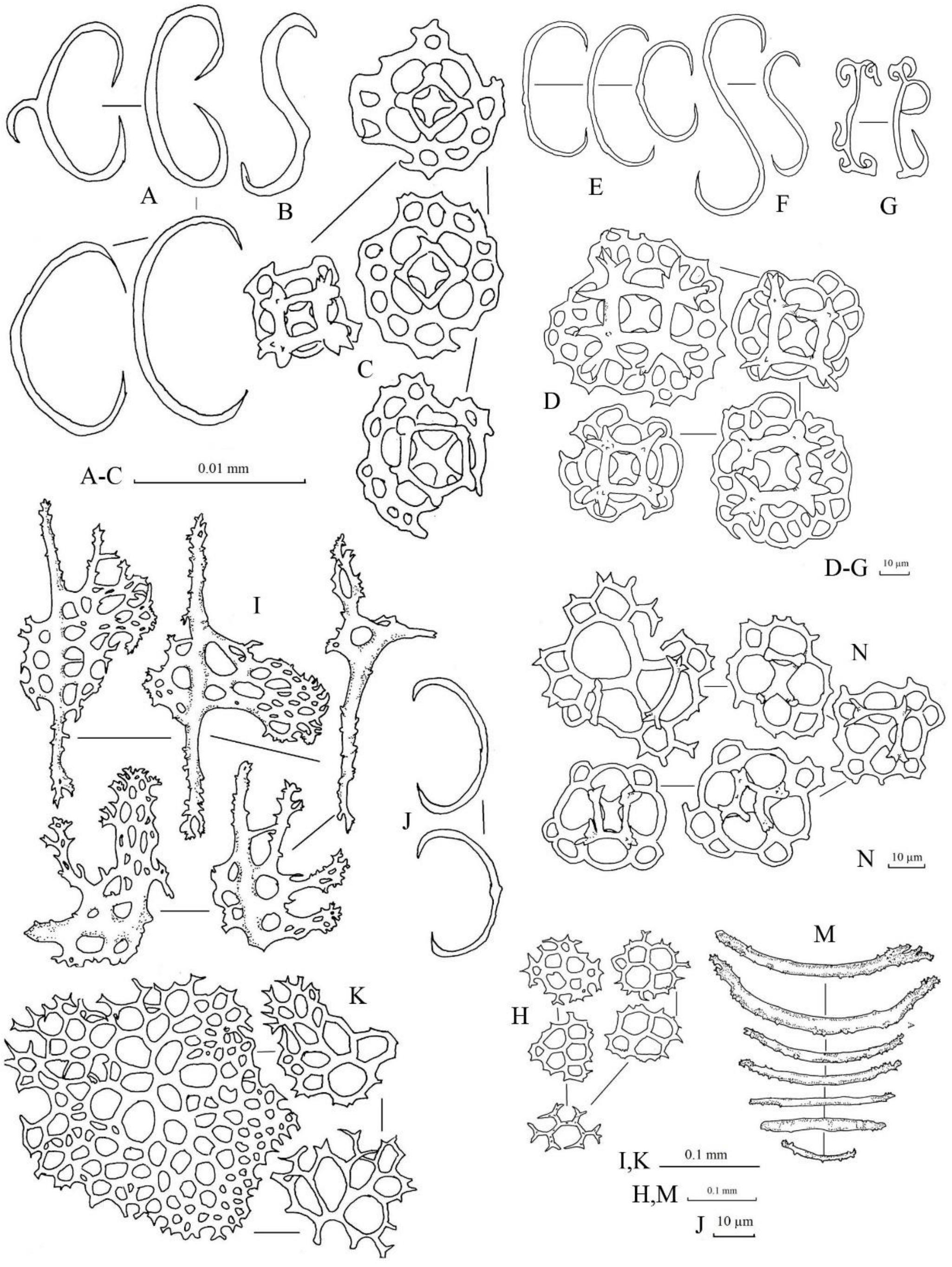

Figure 2.3 Spicules of *Stichopus chloronotus* Brandt, 1835. A, C-shaped rods from the dorsal body; B, S-shaped rods from the dorsal body wall; C, tables from the dorsal body wall; D, table with large base from the dorsal papillae; E, C-shaped rods form the dorsal papillae; F, S-shaped rods from the dorsal papillae; G rosettes from the dorsal papillae; H, tables from the tube feet; I, large rods with central perforations from the tube feet; J: C-shaped rods from the tube feet; K, perforated plates from the tube feet; M, rods from the tentacles; N, tables from the tentacles

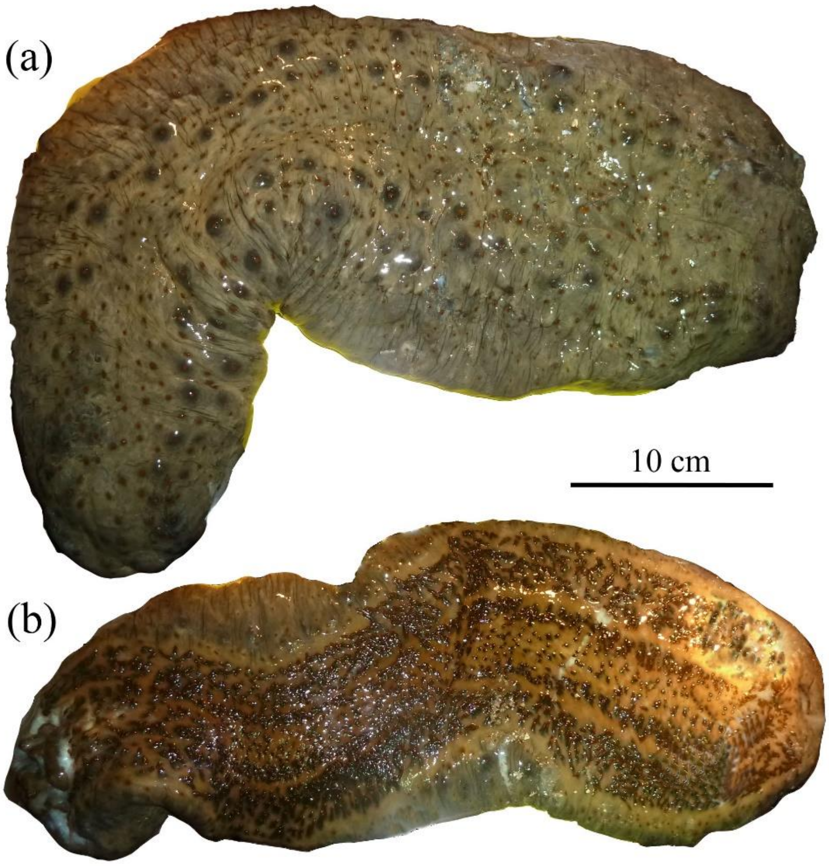

Figure 2.4 Dorsal and ventral view of *Stichopus herrmanni* Semper, 1868.

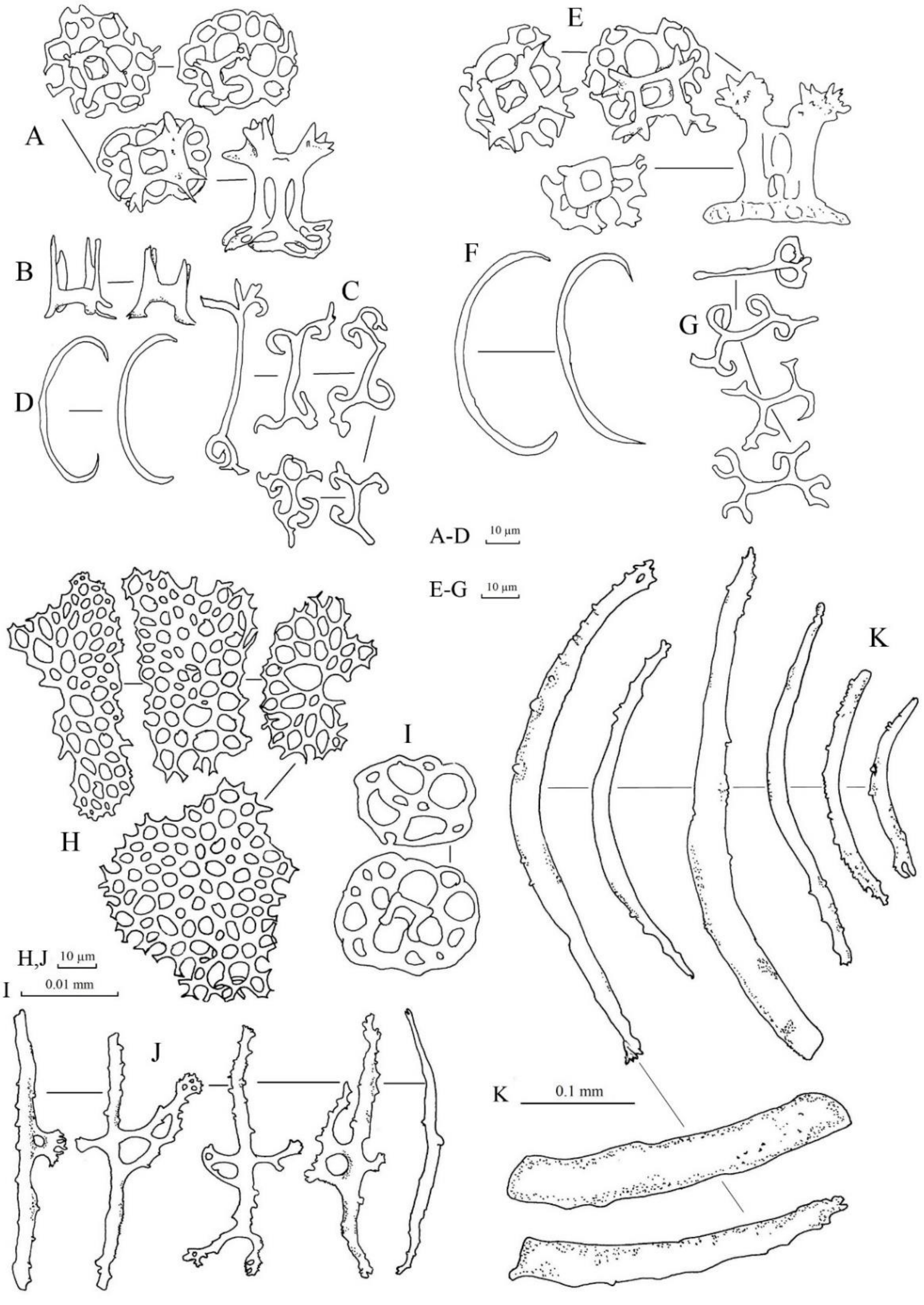

Figure 2.5 Spicules of *Stichopus herrmanni* Semper, 1868. A, tables from the dorsal body; B, pseudo tables from the dorsal body wall; C, rosettes from the dorsal body wall; D, C-shaped rods from the dorsal body wall; E, tables from the dorsal papillae; F, C-shaped rods from the dorsal papillae; G, rosettes from the dorsal papillae; H, large multiperforated plates from the tube feet; I, reduced tables from the tube feet; J, rods with central perforations from the tube feet; K, rods of different sizes from the tentacles.

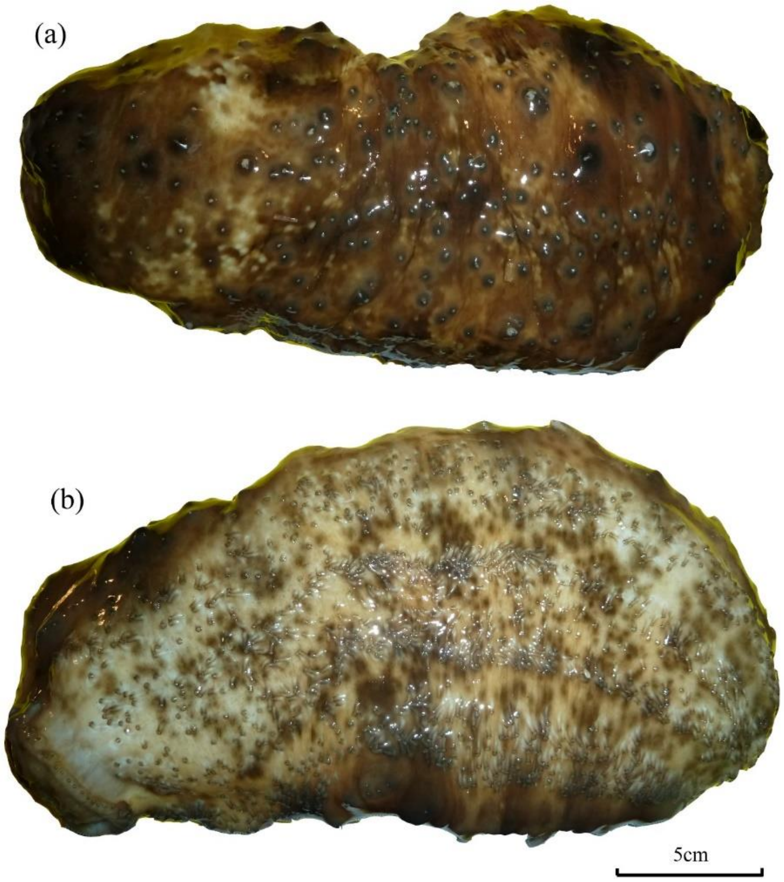

Figure 2.6 Dorsal and ventral view of *Stichopus horrens* Selenka, 1867.

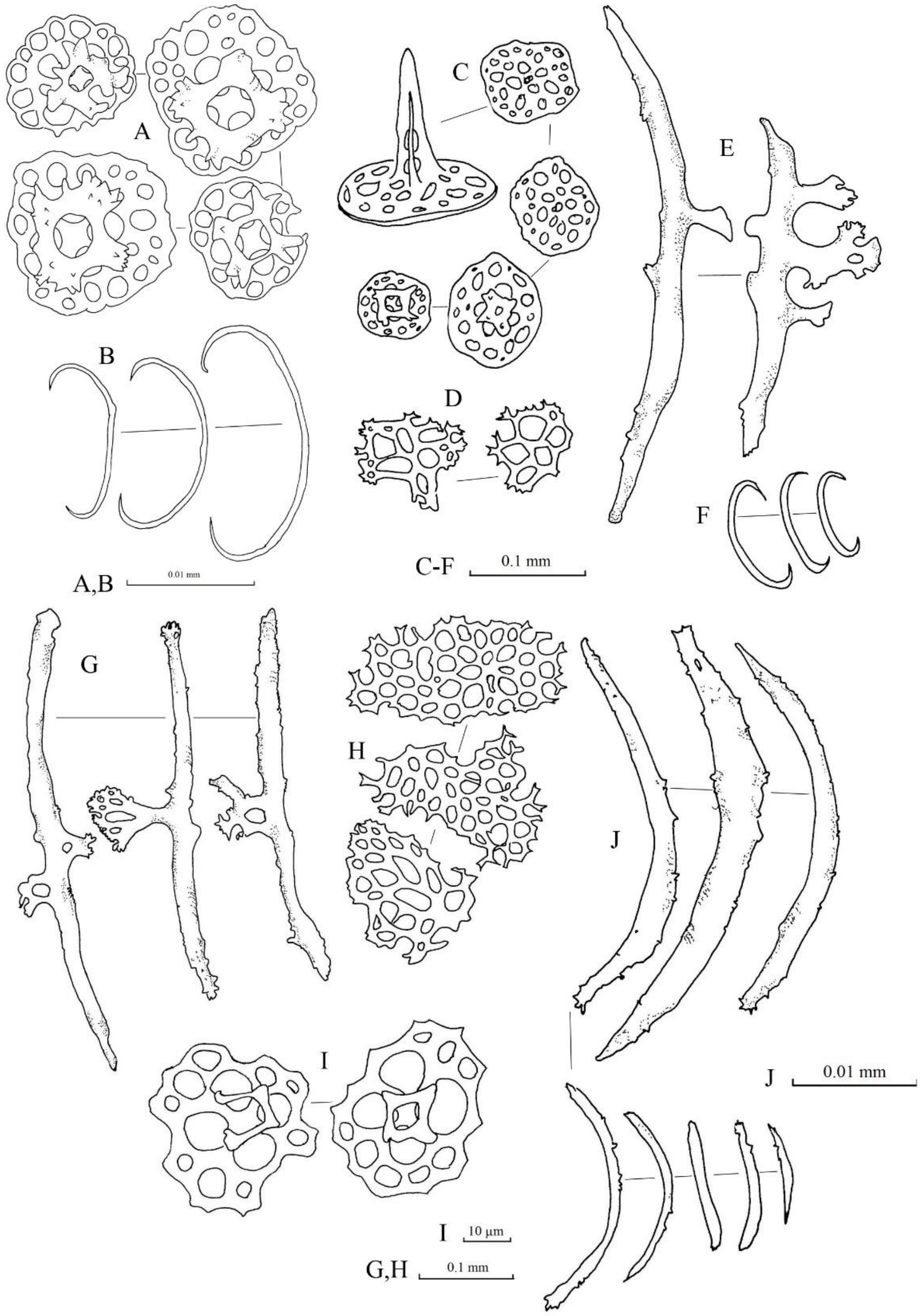

Figure 2.7 Spicules of *Stichopus horrens* Selenka, 1867. A, tables from the dorsal body wall; B, C-shaped rods from the dorsal body wall; C, tack-liked tables from the dorsal papillae; D, perforated plates from the dorsal papillae; E, rods from the dorsal papillae; F, C-shaped rods from the dorsal papillae; G, large rods from the tube feet; H, multiperforated plates from the tube feet; I, tables from the tube feet; J, Rods of different sizes from the tentacles.

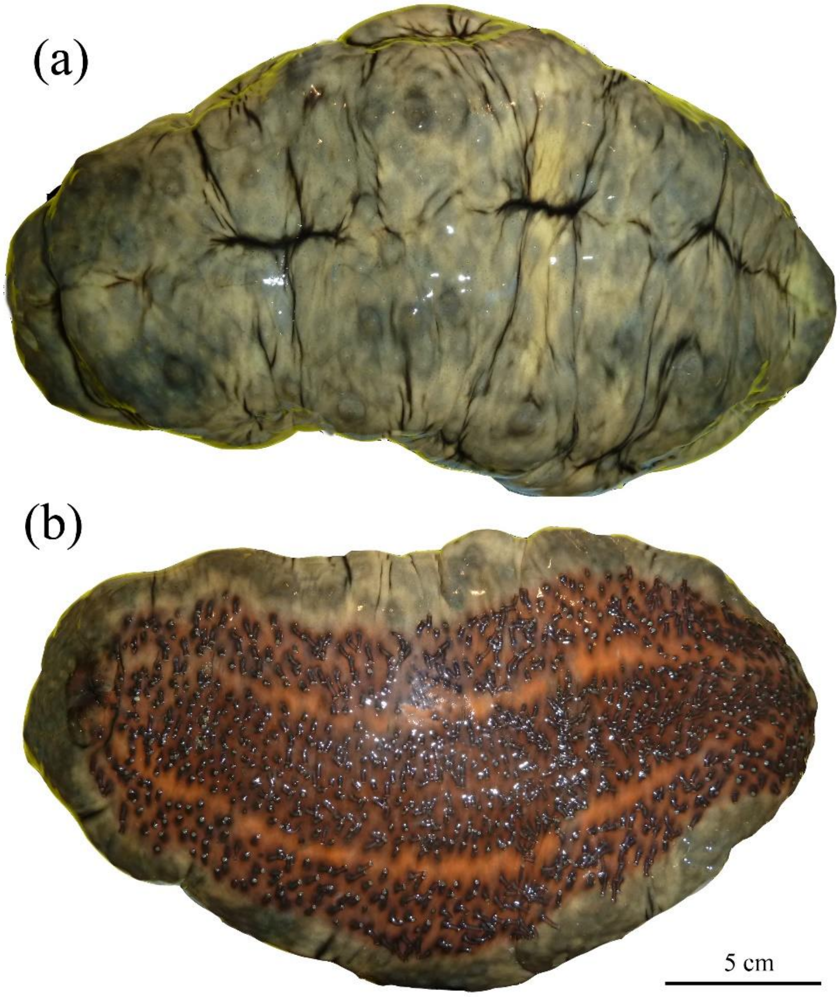

Figure 2.8 Dorsal and ventral view of *Stichopus vastus* Sluiter, 1887.

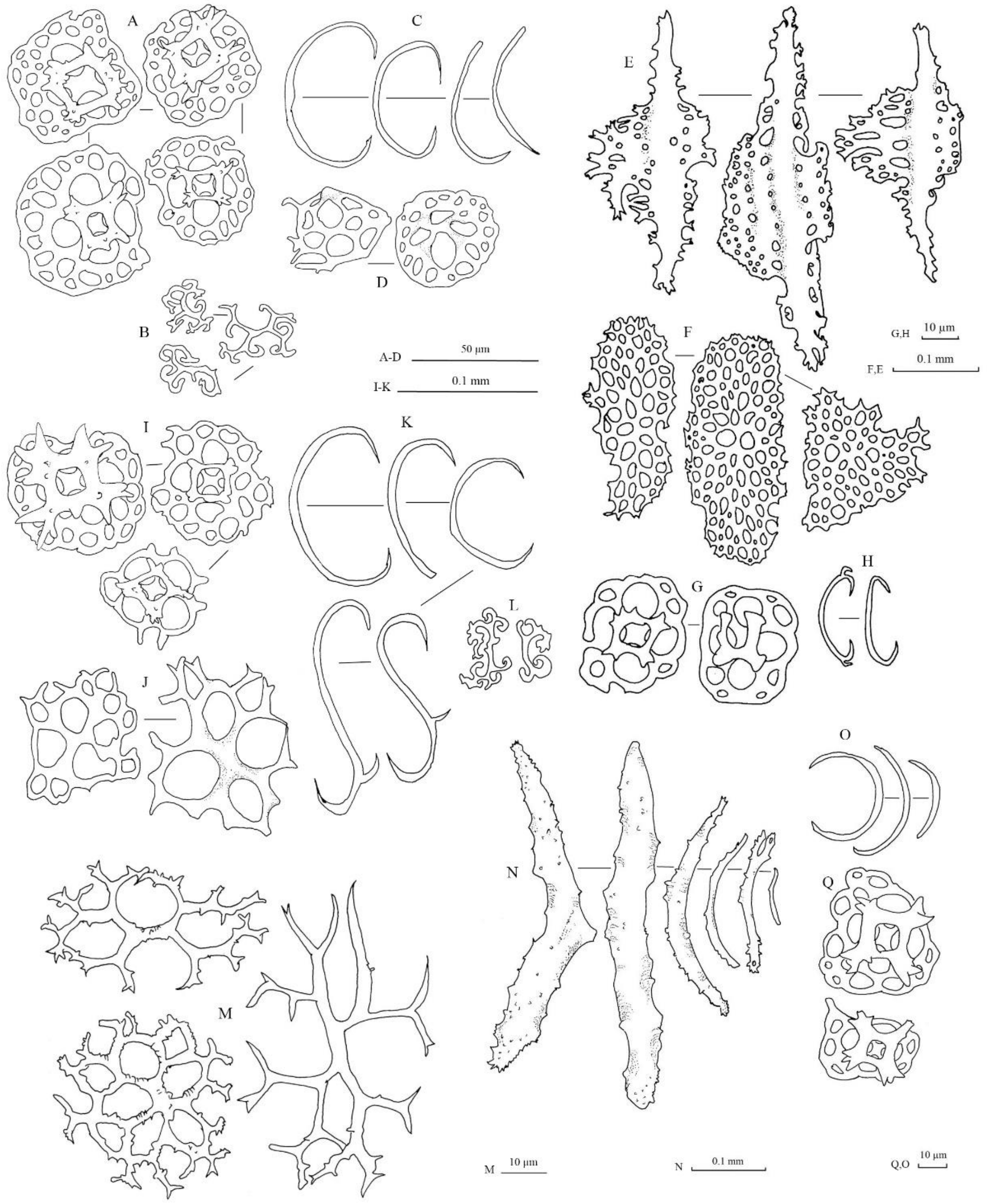

Figure 2.9 Spicules of *Stichopus vastus* Sluiter, 1887. A, Large tables from the dorsal body wall; B, rosettes from the dorsal body wall; C, C-shaped rods from the dorsal body wall; D, reduced tables from the dorsal body wall; E, rods with large perforated central plate from the tube feet; F, multiperforated plates from the tube feet; G, tables from the tube feet; H, C-shaped rods from the tube feet; I, tables from the dorsal papillae; J, large reduced tables from the dorsal papillae; K, C-shaped and S-shaped rods from the dorsal papillae; L, rosettes found from the dorsal papillae; M, large perforated plates from the dorsal papillae; N, rods from the tentacles; O, C-shaped rods from the tentacles; Q, tables from the tentacles.

Figure 2.10 Dorsal and ventral view of *Stichopus fusiformiossa* Woo in Woo et al., 2015.

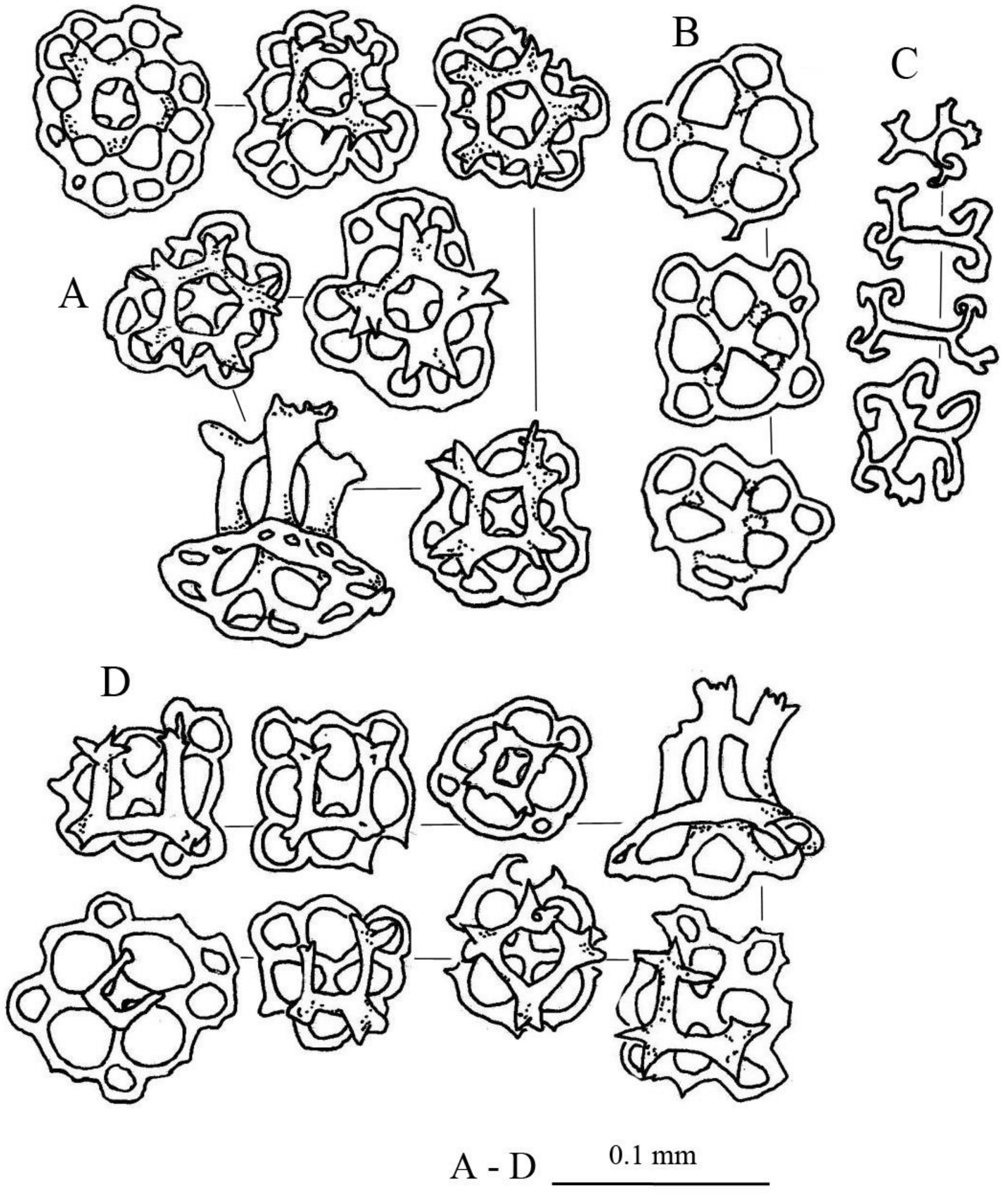

Figure 2.11 Spicules from the dorsal body wall of *Stichopus fusiformiossa* Woo in Woo et al., 2015. A, tables with multiple perforations from the base of the dorsal body wall; B, tables with limited number of perforations from the base of the dorsal body wall; C, rosettes; D, reduced tables.

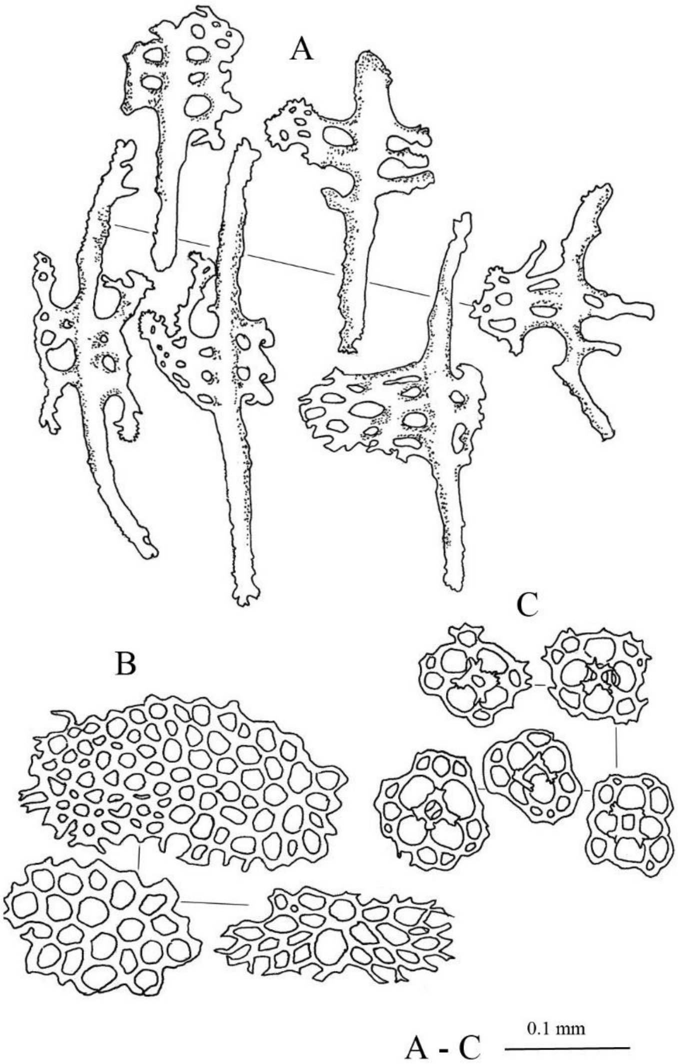

Figure 2.12 Spicules from the tube feet of *Stichopus fusiformiossa* Woo in Woo et al., 2015. A, rods with large central perforated plates; B, large perforated plates; C, tables.

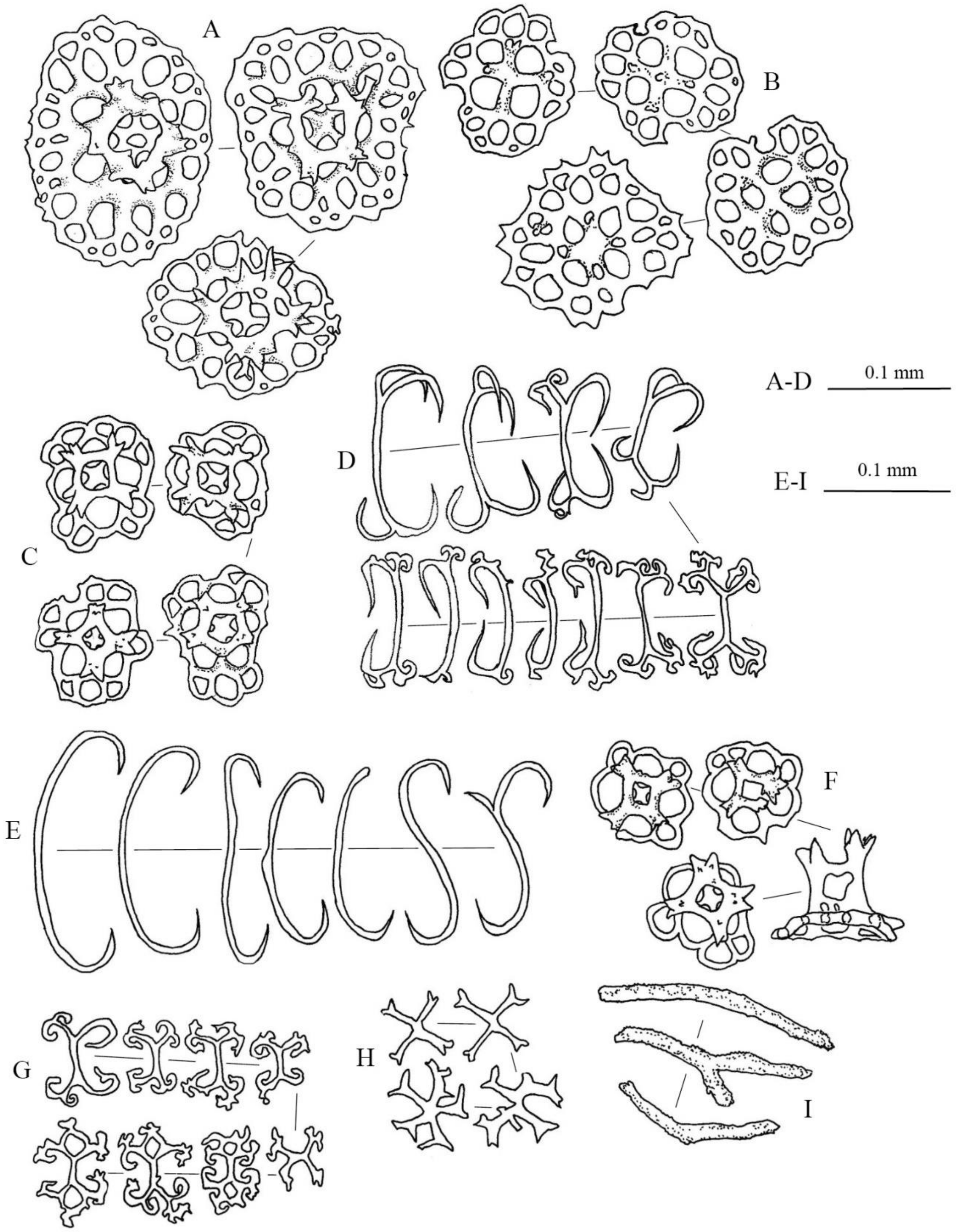

Figure 2.13 Spicules from papillae of *Stichopus fusiformiossa* Woo in Woo et al., 2015. A, table with large base plates; B, reduced tables; C, tables; D, modified C-shaped rods; E, C-shaped rods; F, tables with smaller base plates and number of perforations; G, rosettes; H, X-shaped rods; I, rods.

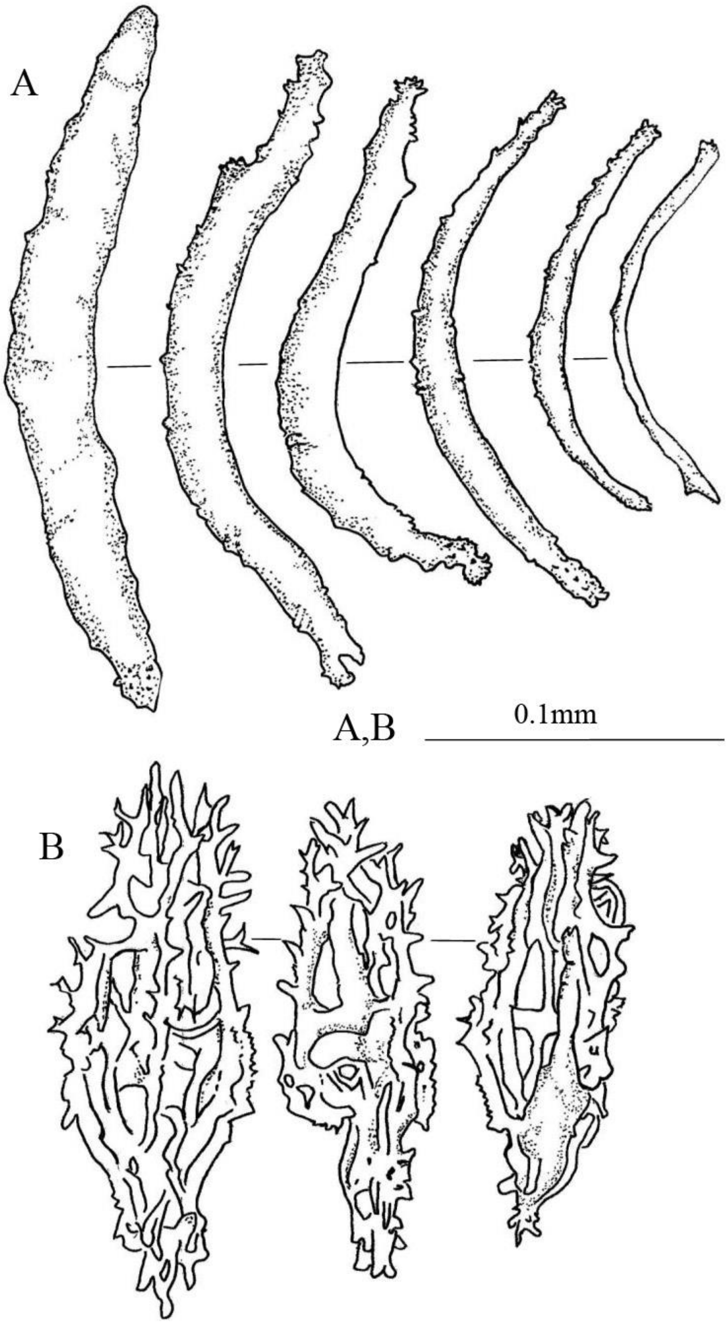

Figure 2.14 Spicules from the tentacles of *Stichopus fusiformiossa* Woo in Woo et al., 2015. A, curved rods; B, fusiform spicules.

FIGURE 3.1 Syntype of *Apostichopus armatus* (Selenka, 1867), MCZ HOL–741.

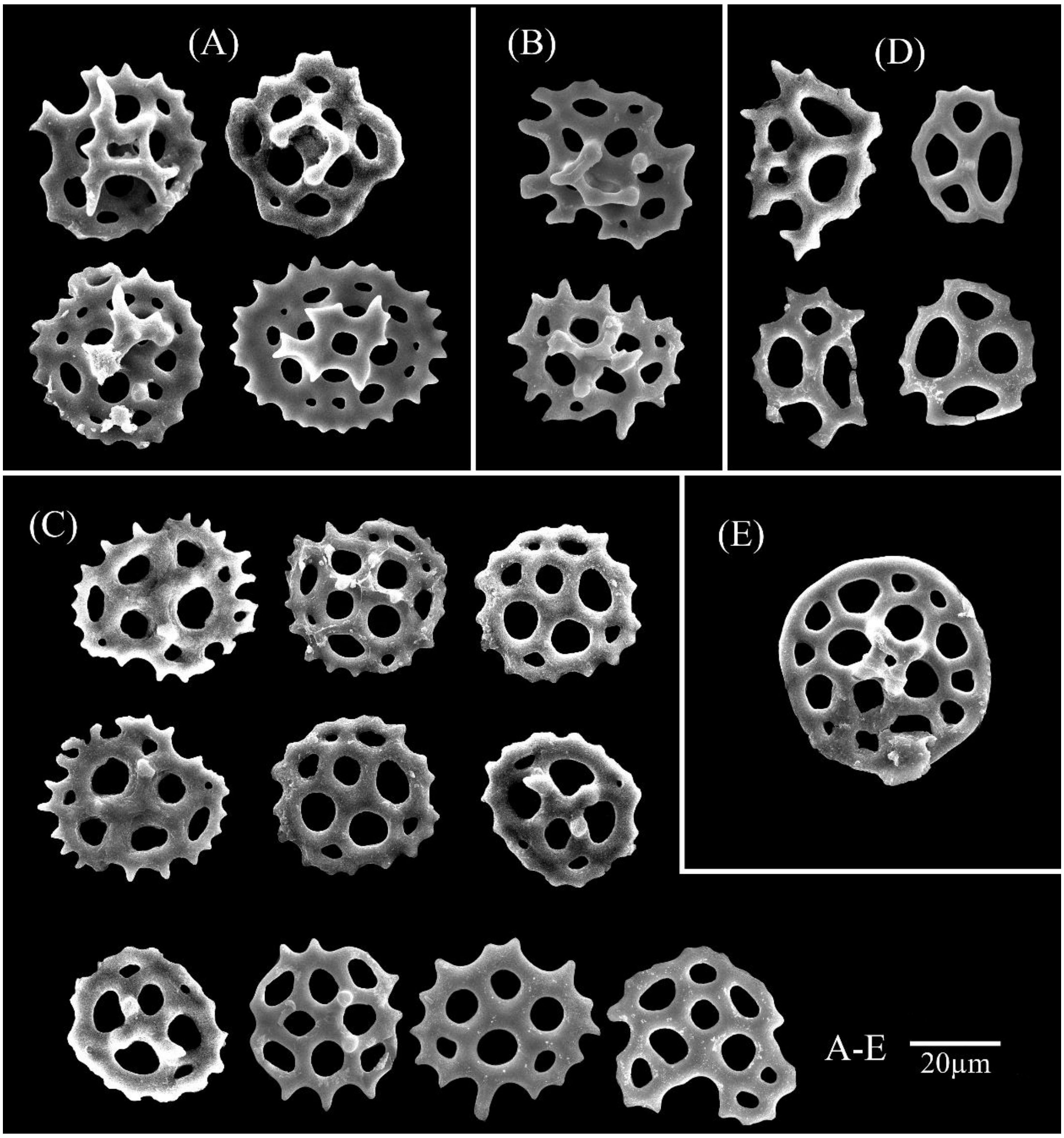

FIGURE 3.2 Spicules from the dorsal body wall of *Apostichopus armatus* (Selenka, 1867). A, tables with pillars and spinuous disk, NSMT–E7047E; B, tables with pillars and spinuous disk, NSMT–E7047A; C, reduced tables with spinuous disk, NSMT–E7047A; D, reduced tables with spinuous disk and 3–4 perforations, NSMT–E7047A; E, reduced tables with smooth disk, NSMT–E7047E.

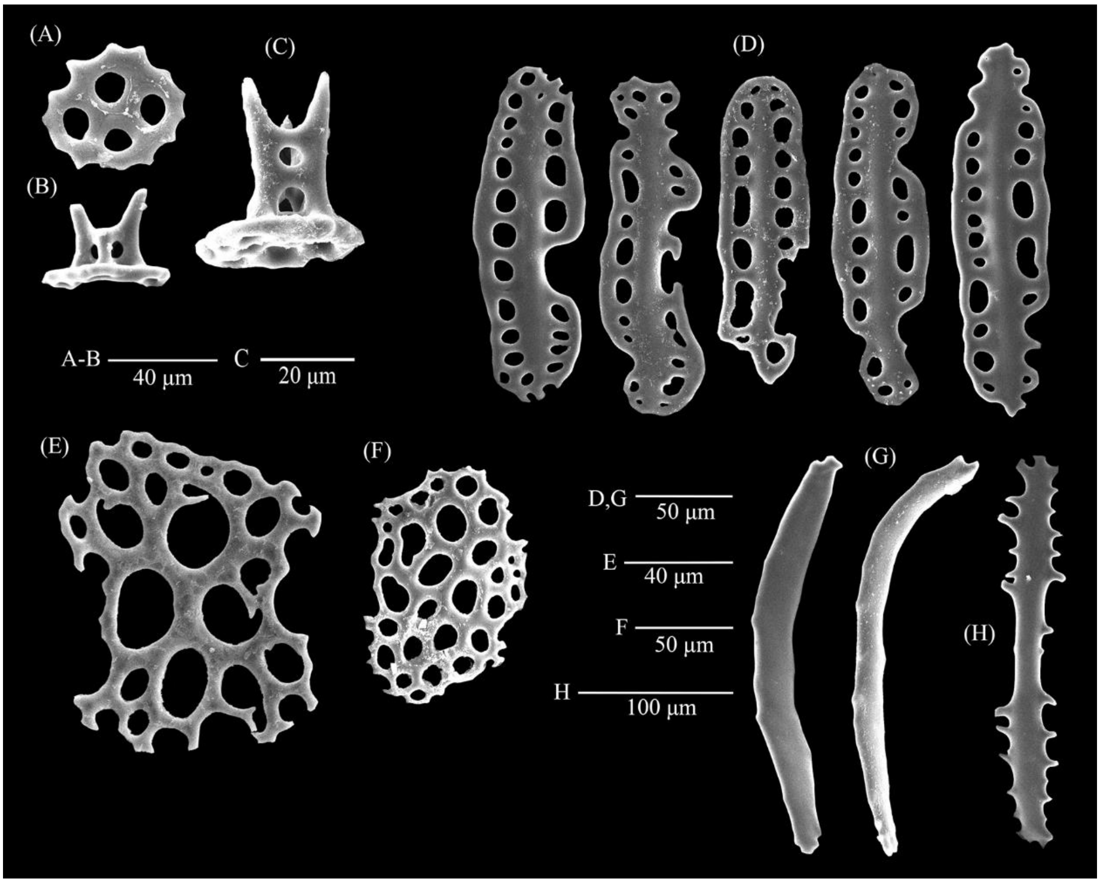

FIGURE 3.3 Spicules from the papillae tips of *Apostichopus armatus* (Selenka, 1867), NSMT–E7047C. A, reduced table; B–C, Tables with pillars; D, elongated buttons; E–F, multiperforated plates; G, curved rods with smooth surfaces; H, straight, spiny rod.

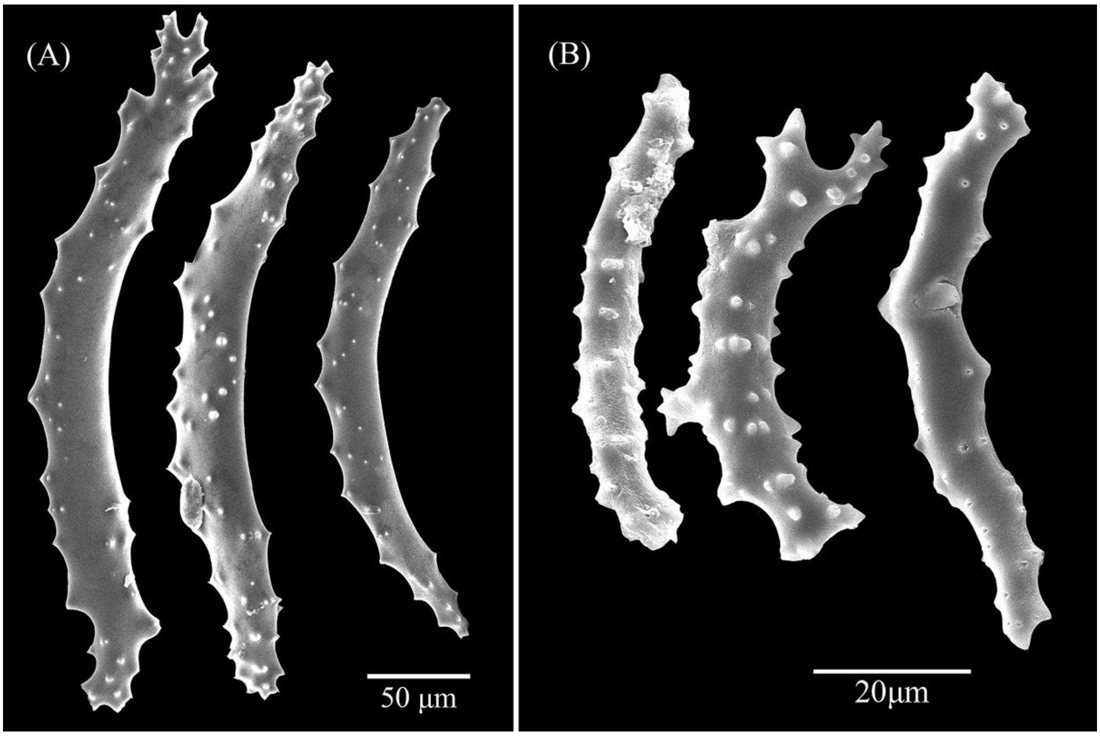

FIGURE 3.4 Spicules from the tentacles of *Apostichopus armatus* (Selenka, 1867), NSMT–E7047A. A, Large curved rods with spinelets on surface; B, small curved rods with spinelets on surface.

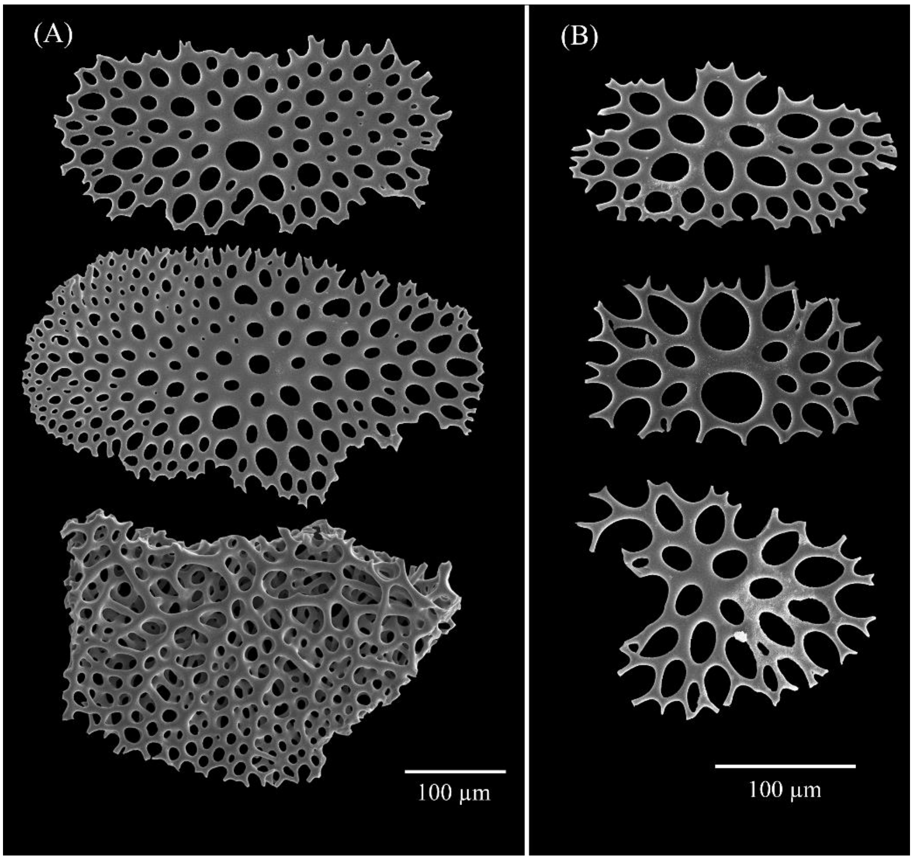

FIGURE 3.5 Spicules from the tube feet of *Apostichopus armatus* (Selenka, 1867), NSMT–E7047A. A–B multiperforated plates.

FIGURE 3.6 Syntype of *Apostichopus japonicus* (Selenka, 1867), MCZ HOL–763.

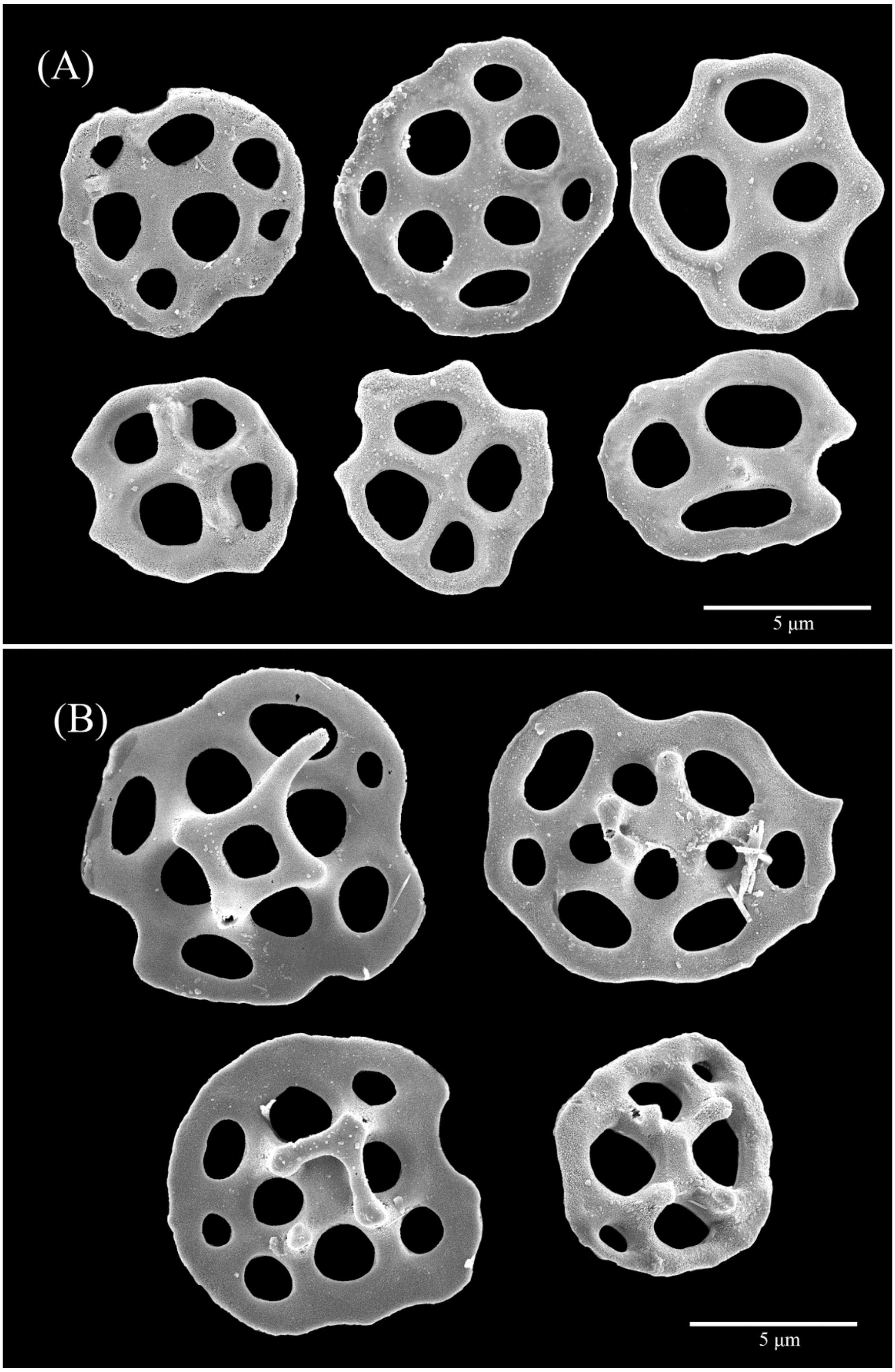

FIGURE 3.7 Spicules from the dorsal body wall of *Apostichopus japonicus* (Selenka, 1867), syntype, MCZ HOL–763. A, reduced tables with smooth rims and 3–8 perforations; B, tables with pillars and smooth rims.

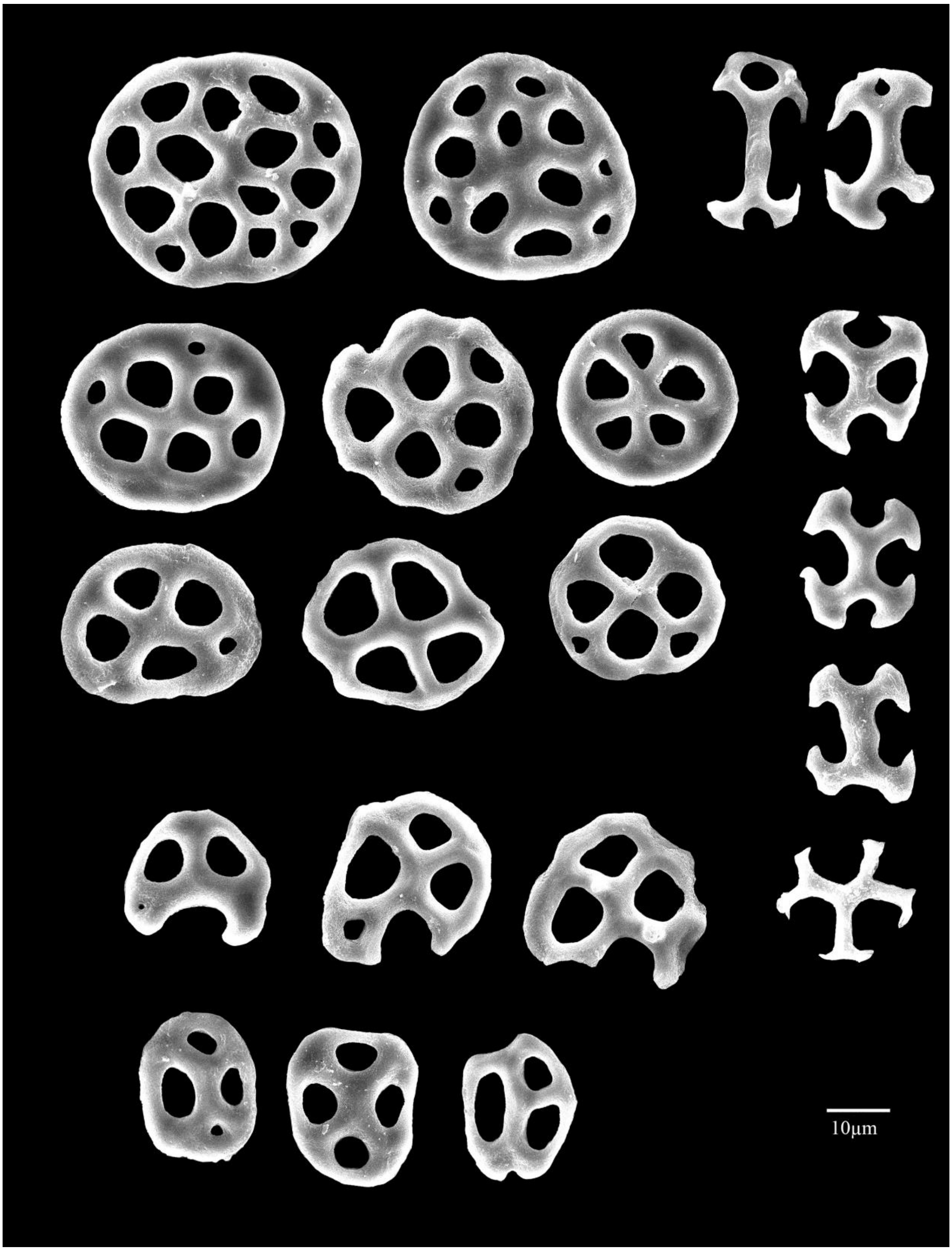

FIGURE 3.8 Disk of reduced table spicules with smooth rims from the dorsal body wall of *Apostichopus japonicus* (Selenka, 1867), NSMT–10122A.

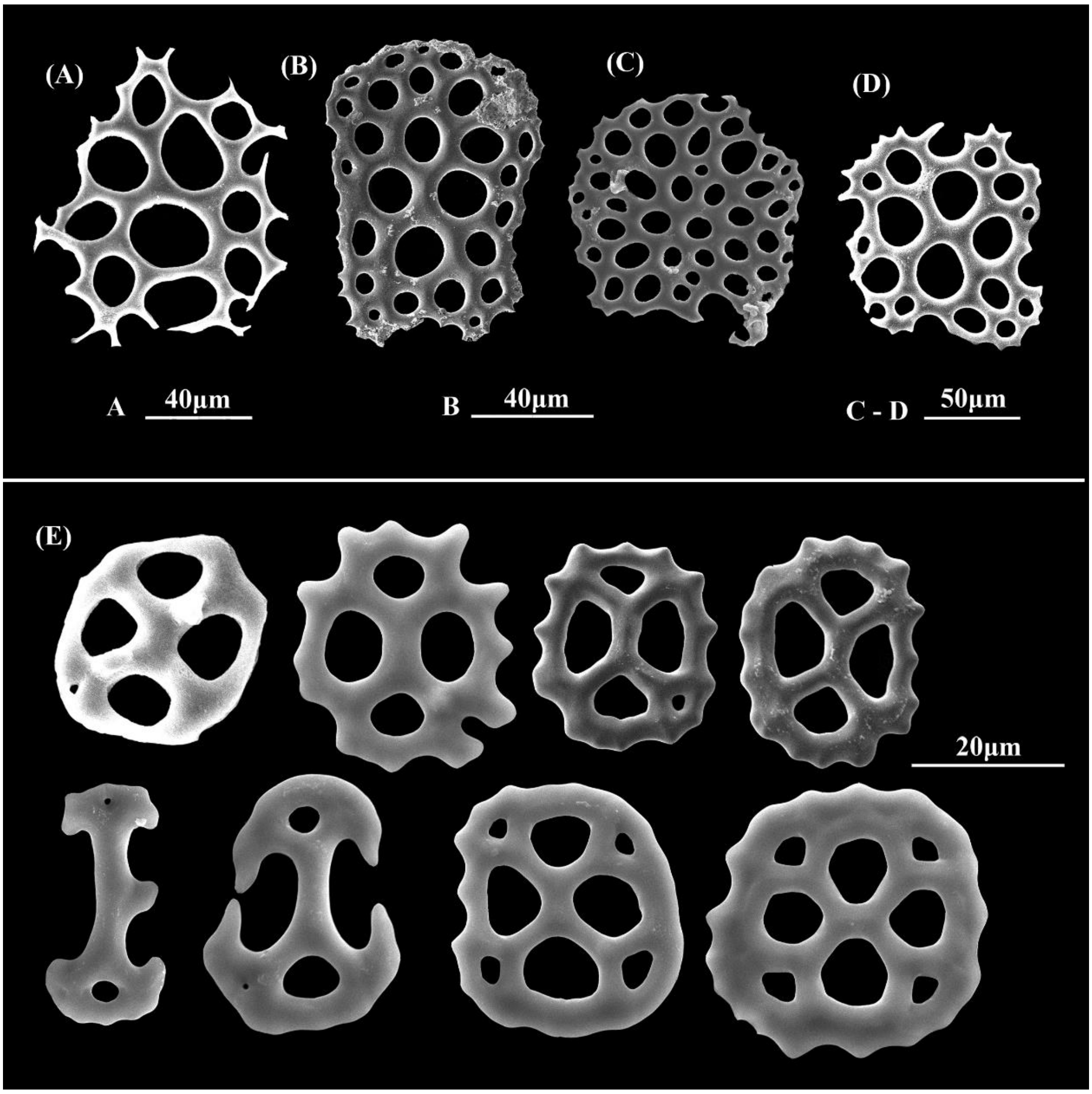

FIGURE 3.9 Spicules from the papillae tip of *Apostichopus japonicus* (Selenka, 1867), NSMT–10122A. A–D, multiperforated plates; E, reduced tables.

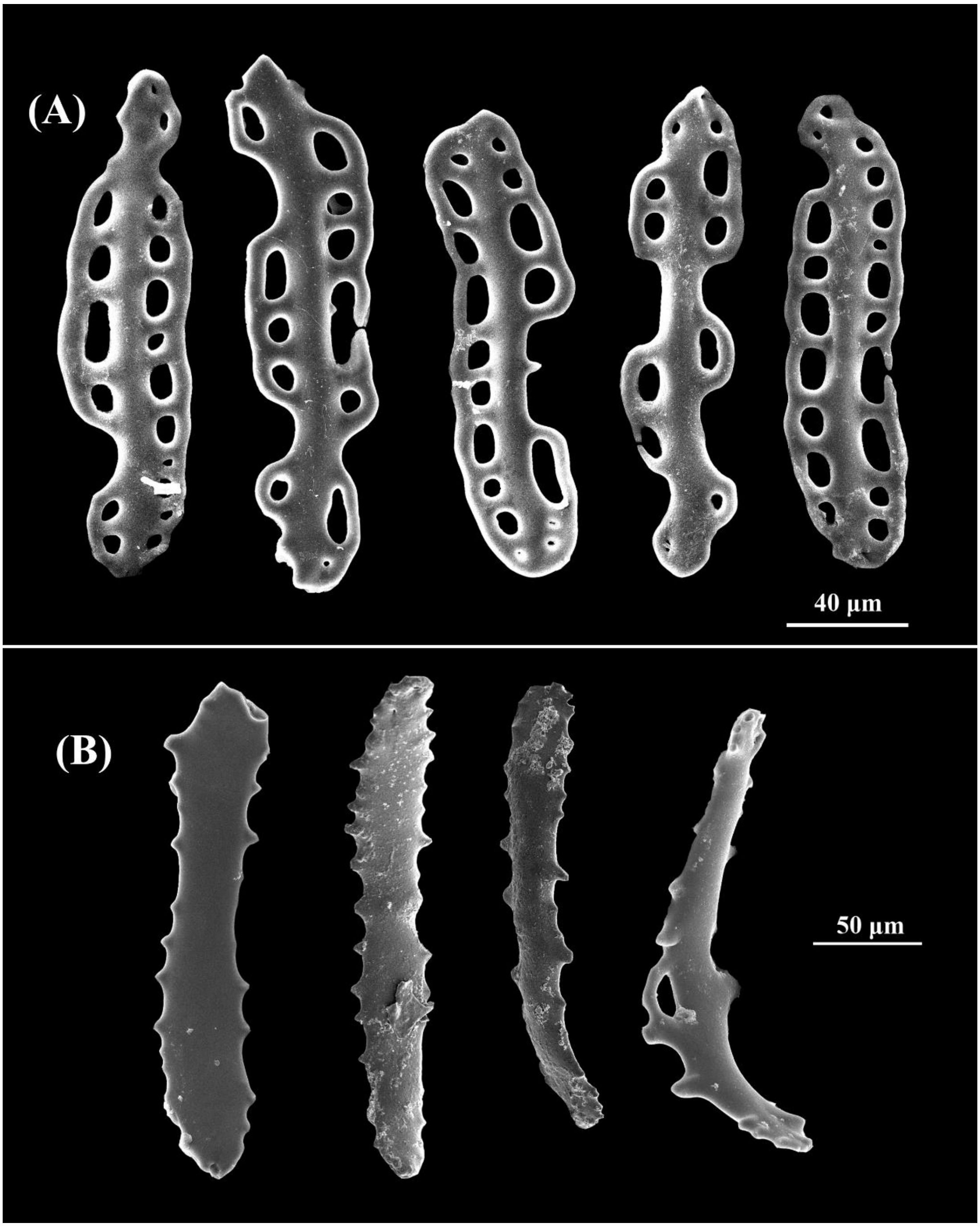

FIGURE 3.10 Spicules from the papillae tip of *Apostichopus japonicus* (Selenka, 1867), NSMT–10122A. A, elongated buttons; B, rods.

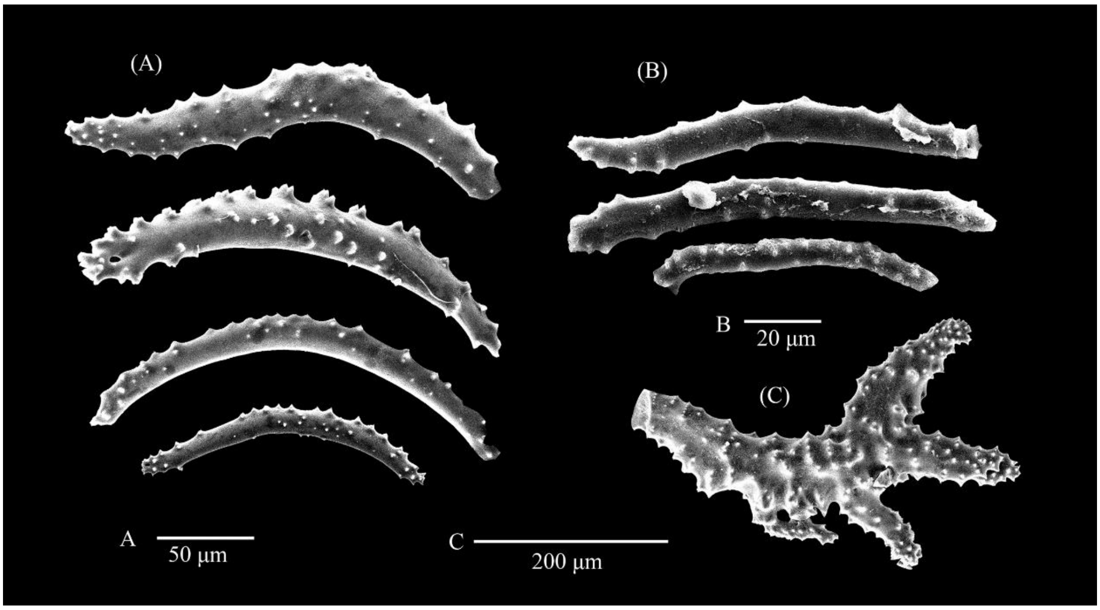

FIGURE 3.11. Spicules from the tentacles of *Apostichopus japonicus* (Selenka, 1867), NSMT–10122A. A–B, spiny rods; C, bifurcating rods.

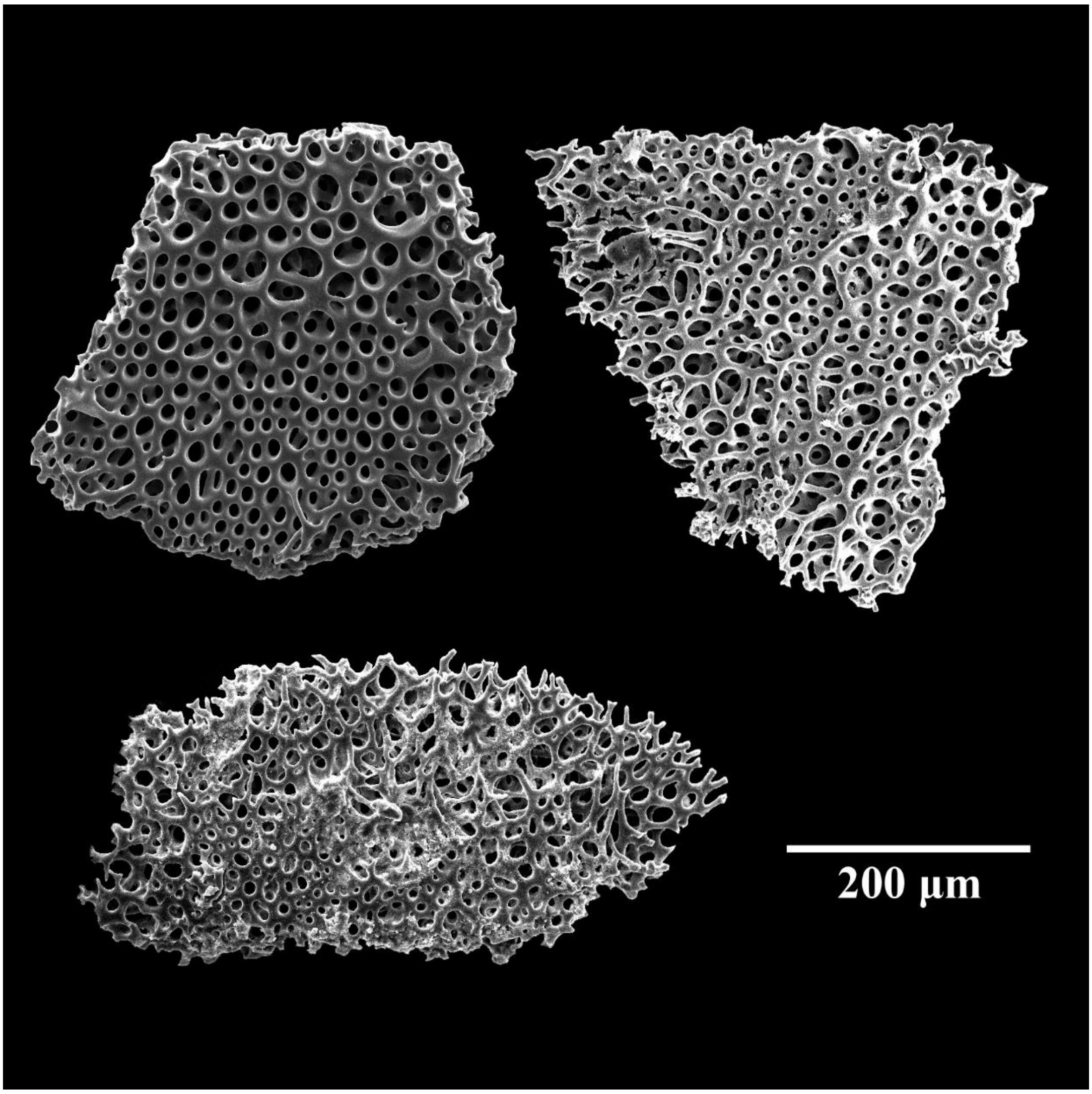

FIGURE 3.12 Spicules from the tube feet of *Apostichopus japonicus* (Selenka, 1867), NSMT–10122A. Large multiperforated plates.

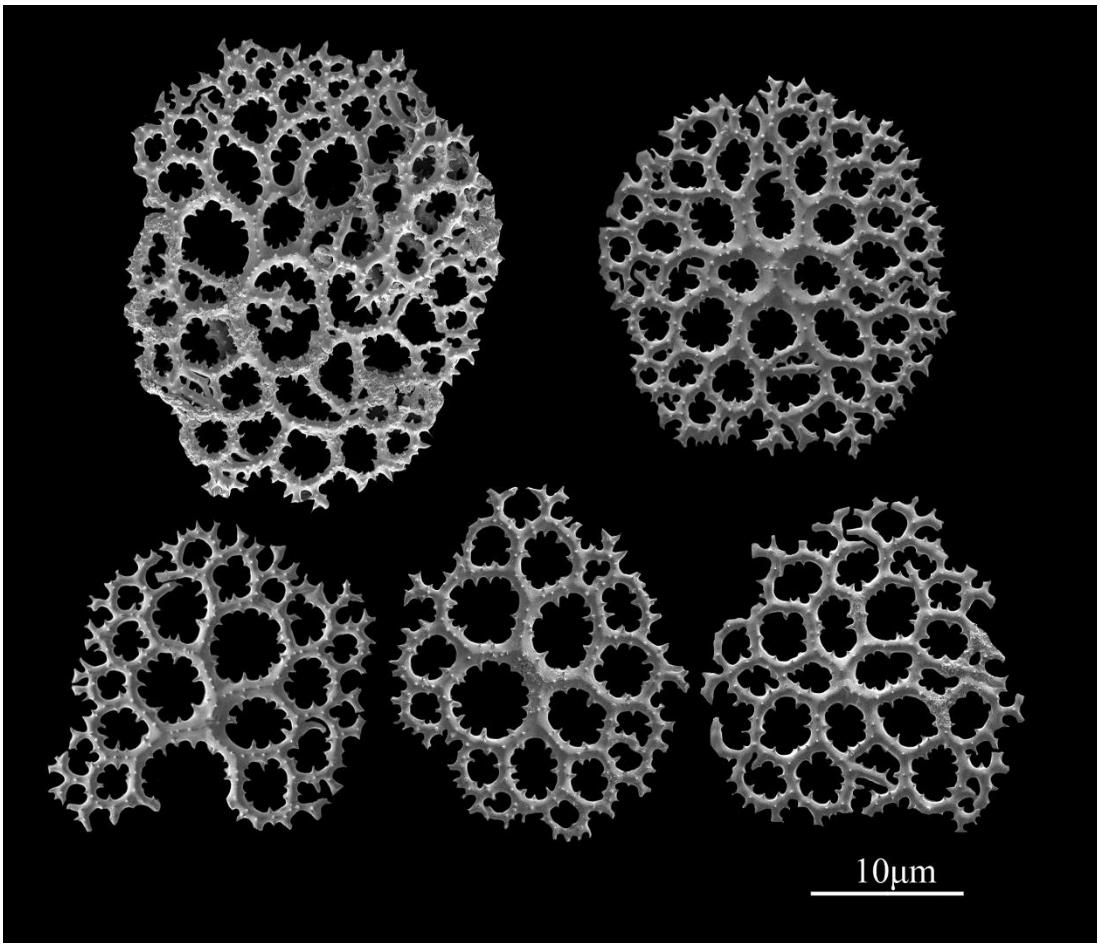

FIGURE 3.13 Complex plates from the cloacal wall of *Apostichopus japonicus* (Selenka, 1867), NSMT–10122A.

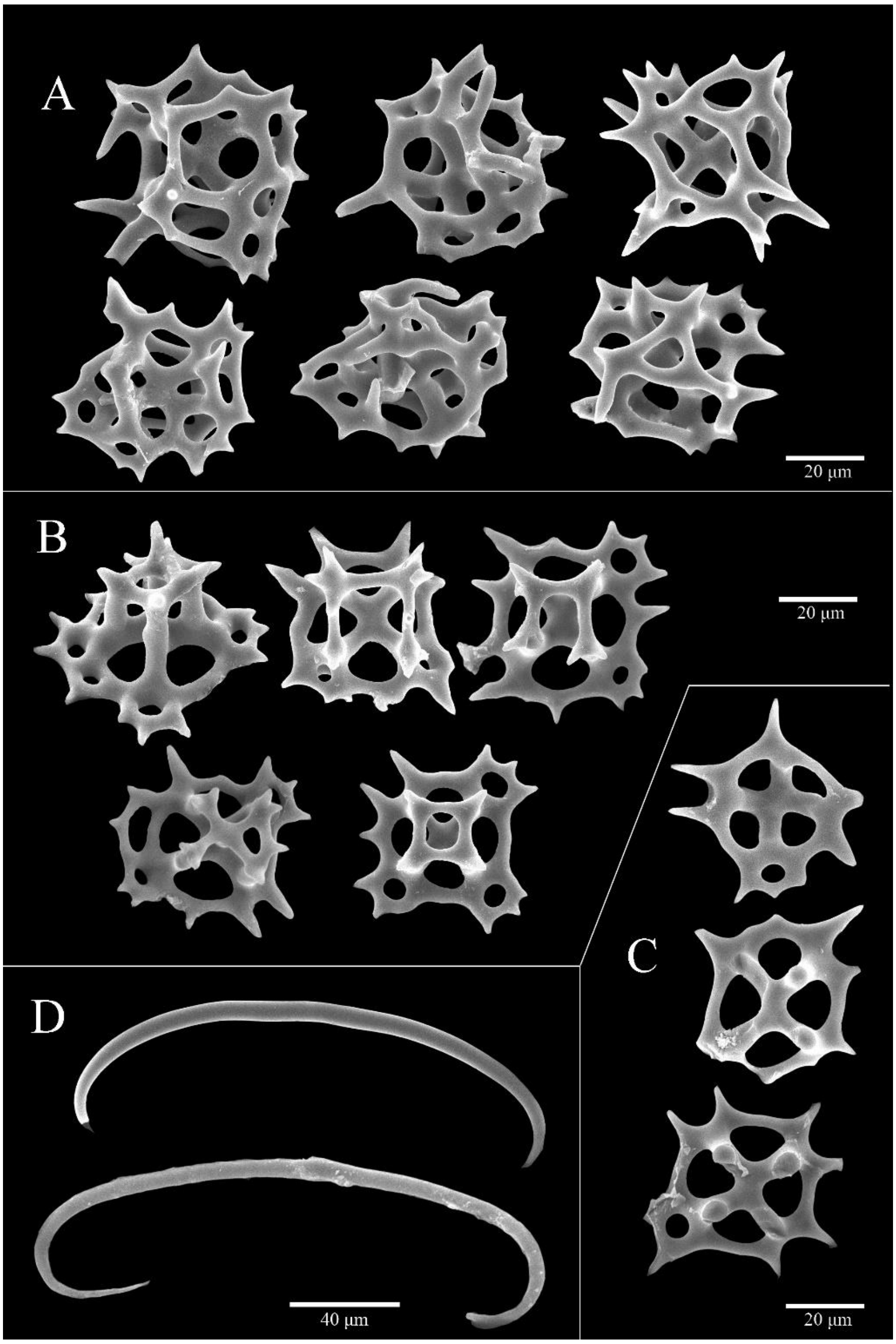

Figure 4.1 Ossicles from the dorsal body wall of *Notostichopus ludwigi* (Erwe, 1913), AM J24986. A, Modified tables; B, tables with pillars and spinous disk; C, reduced tables with spinous disk; D, C-shaped rods.

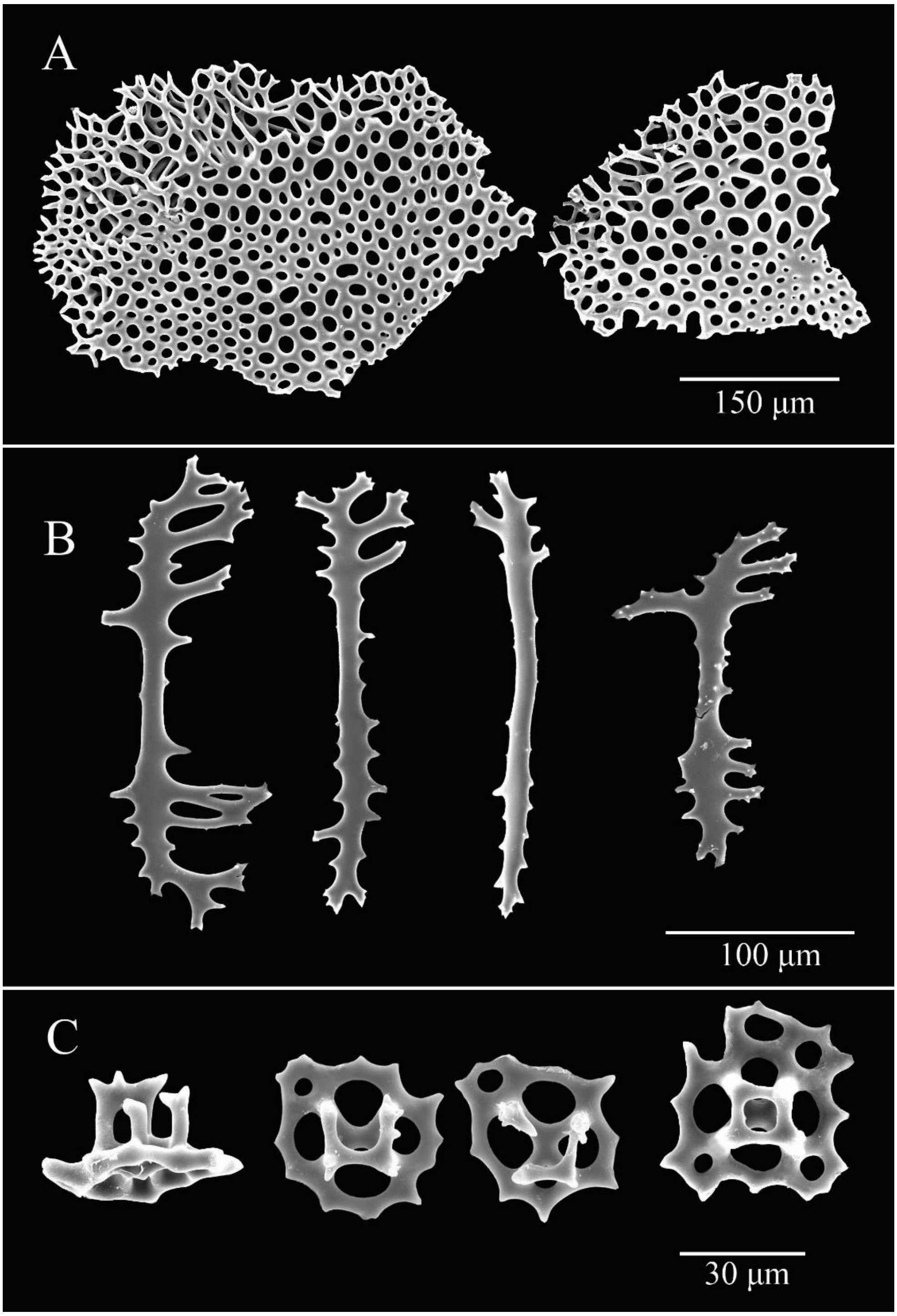

Figure 4.2 Ossicles from the tube feet of *Notostichopus ludwigi* (Erwe, 1913), AM J24986. A, Multiperforated plates; B, Spinous rods with peripheral extensions; C, tables with four short spires.

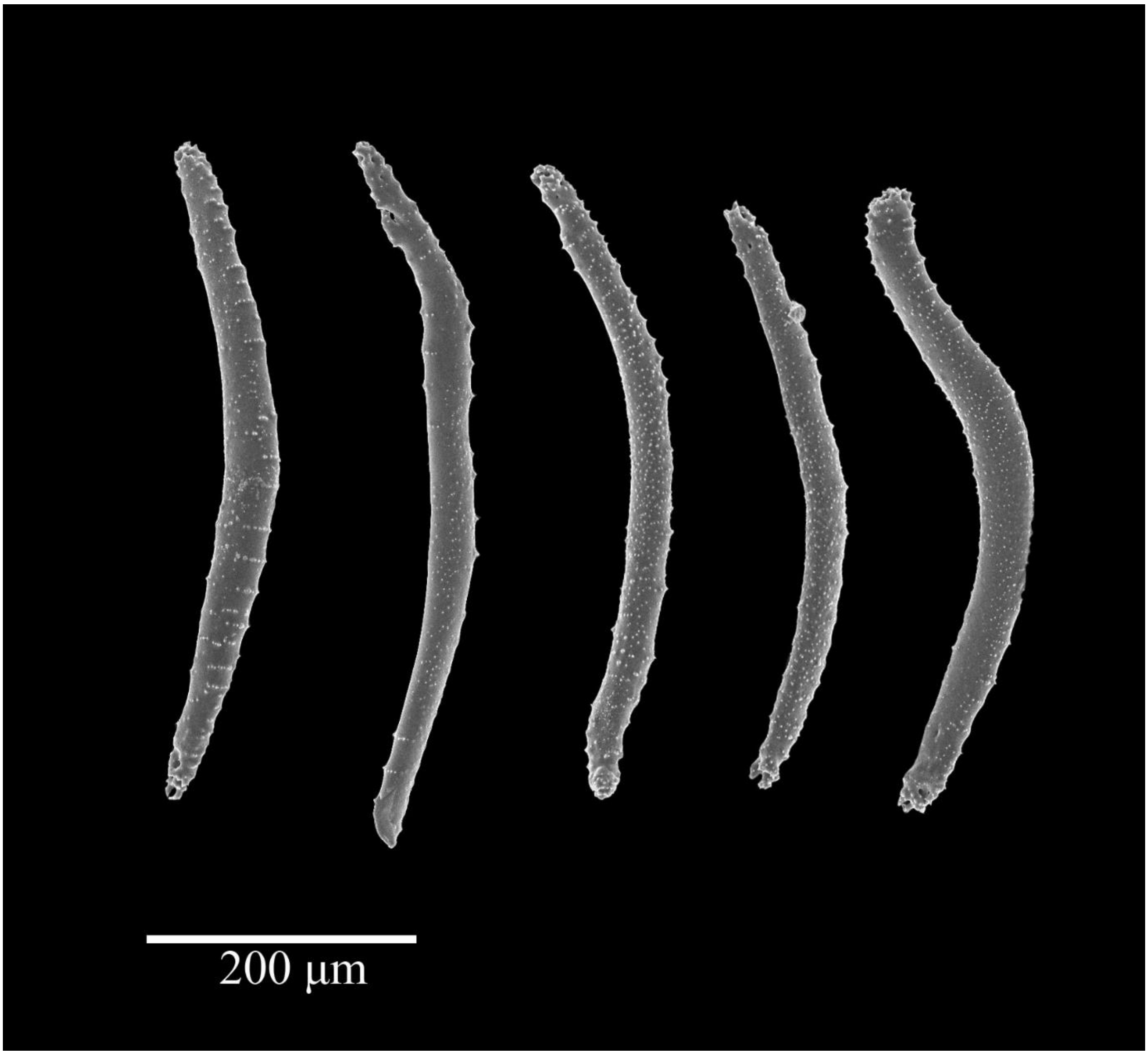

Figure 4.3 Rod from the tentacles of *Notostichopus ludwigi* (Erwe, 1913), AM J24986.

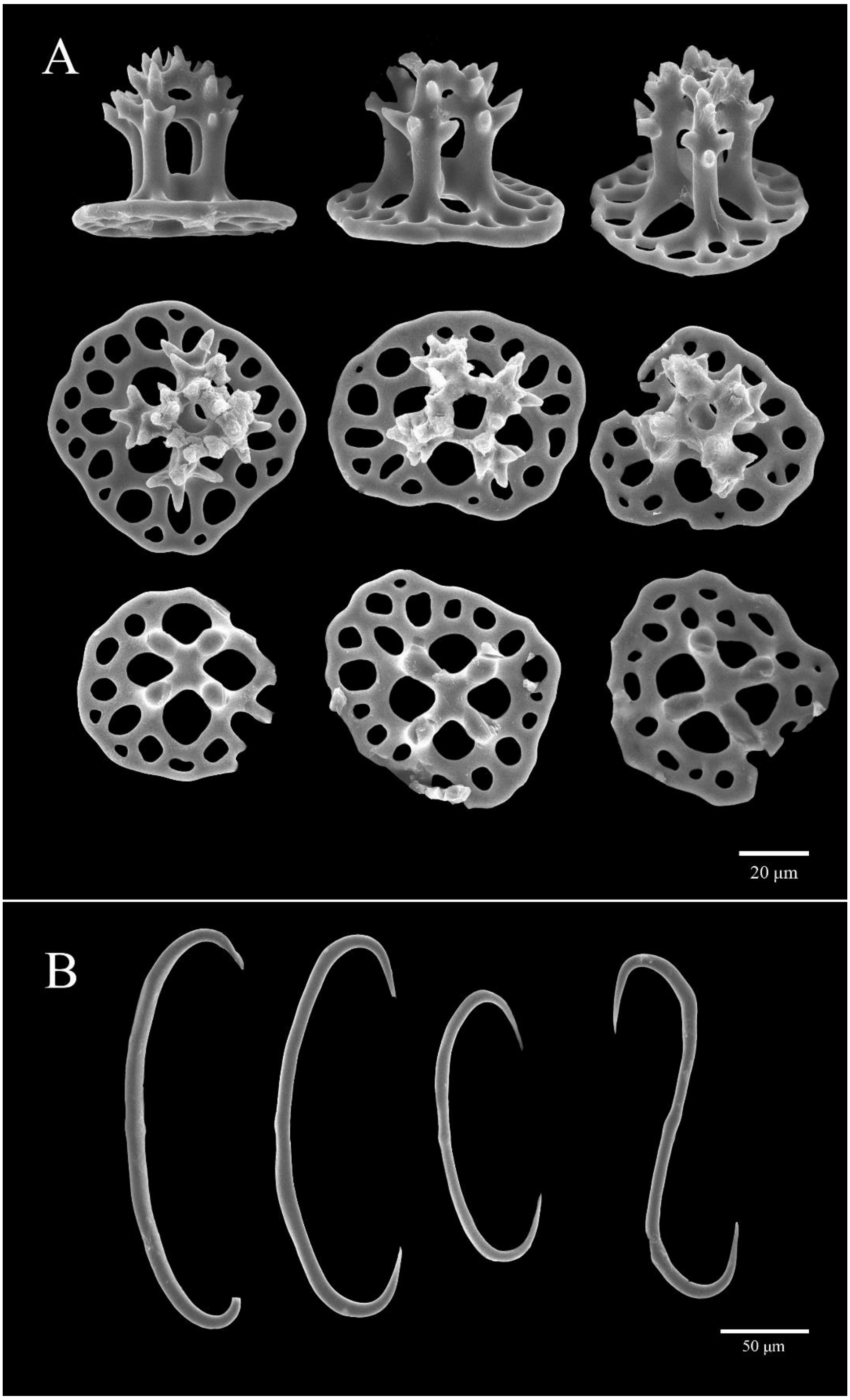

Figure 4.4 Ossicles from the dorsal body of *Notostichopus ellipes* (Clark, 1938), AM J13119. A, tables with four spires ending with multiple spines; B, C-shaped and S-shaped rods.

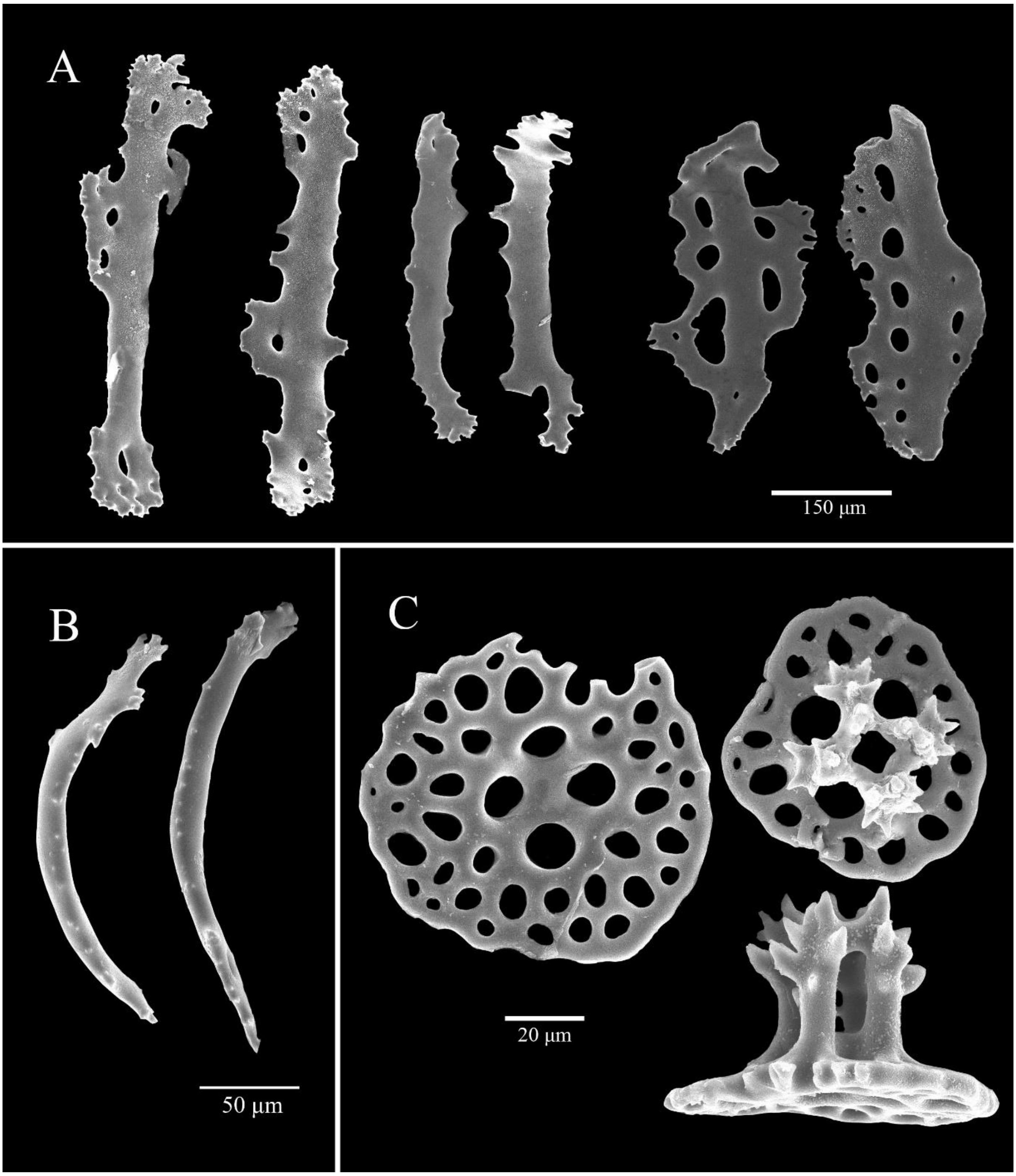

Figure 4.5 Ossicles from the papillae tip of *Notostichopus ellipes* (Clark, 1938), AM J13119. A, Rods with/without perforations; B, curved rods with spinelets; C, tables with large disc and four spires.

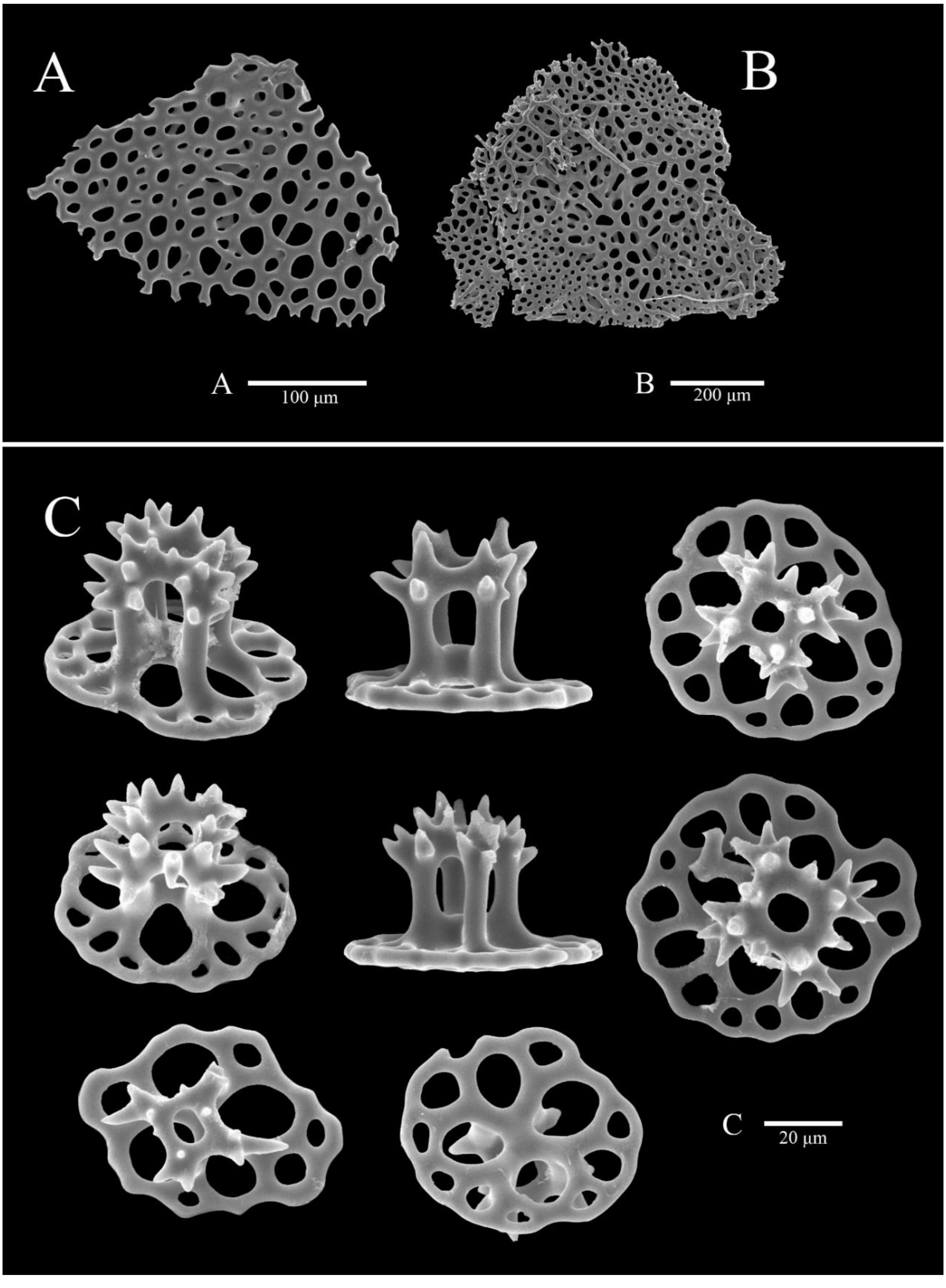

Figure 4.6 Ossicles from the tube feet of *Notostichopus ellipes* (Clark, 1938), AM J13119. A–B, multiperforated plates; C, tables.

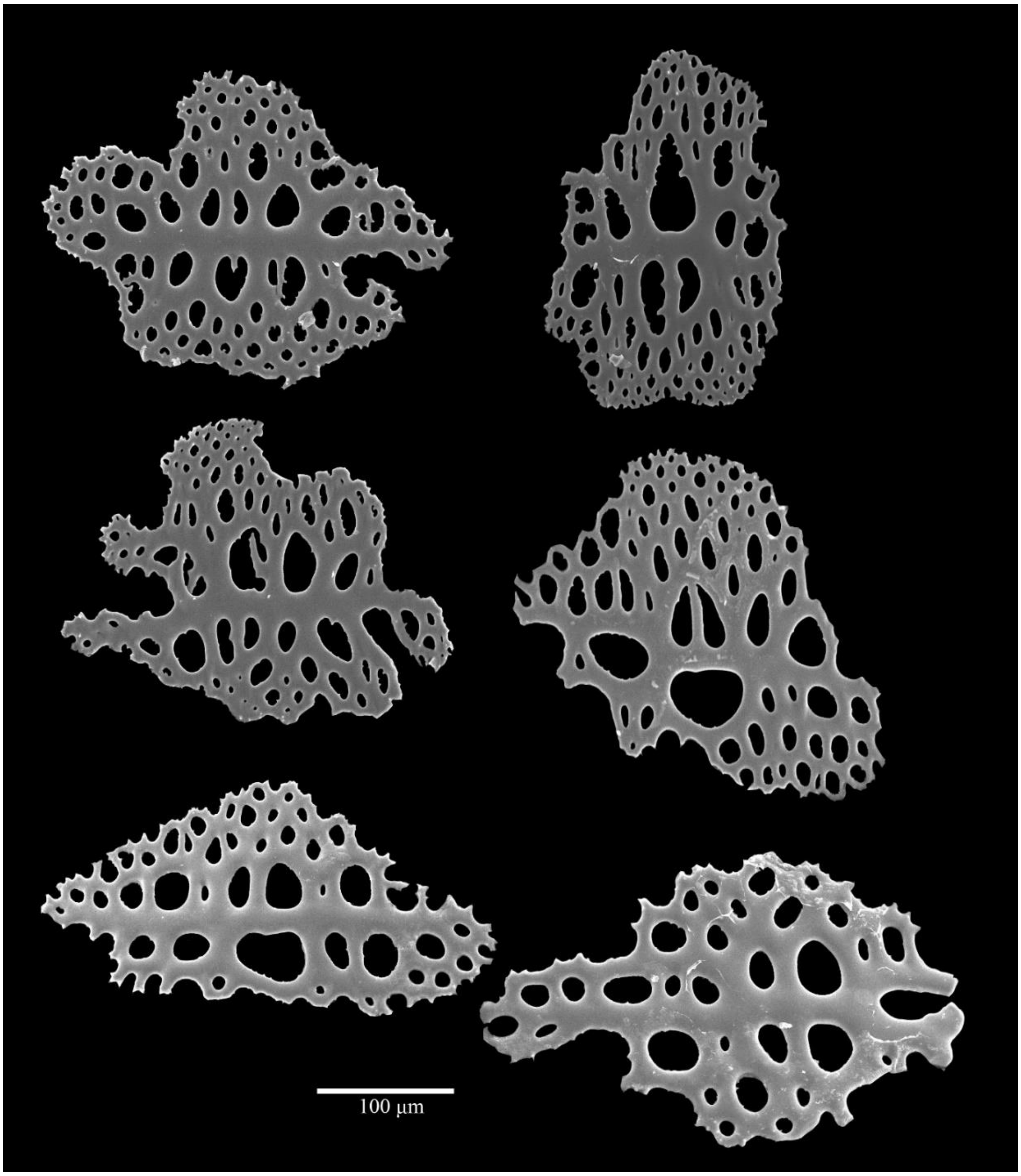

Figure 4.7 Rod with peripheral extension and perforations from the tentacles of *Notostichopus ellipes* (Clark, 1938), AM J13119.

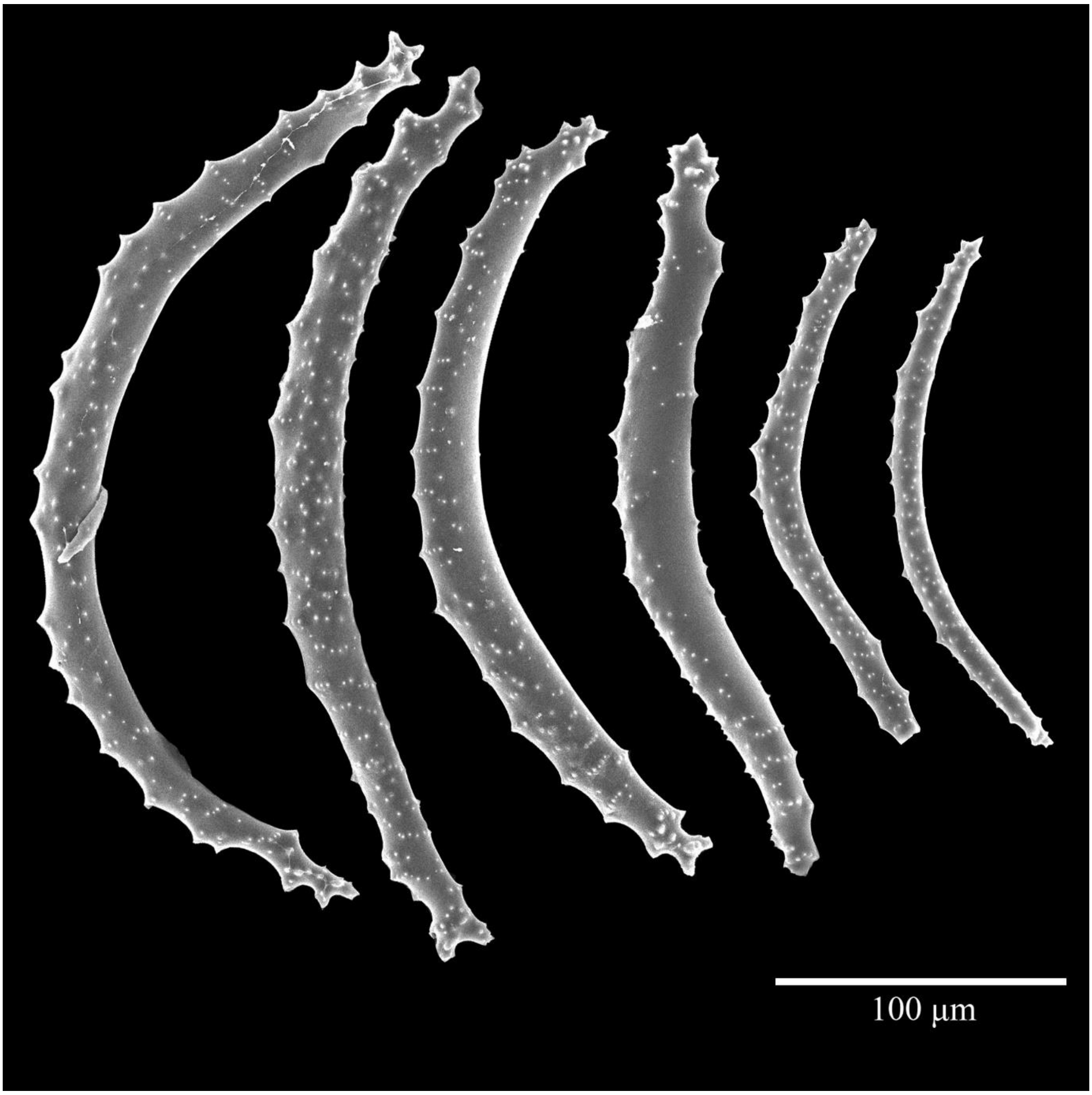

Figure 4.8 Rod from the tentacles of *Notostichopus ellipes* (Clark, 1938), AM J13119.

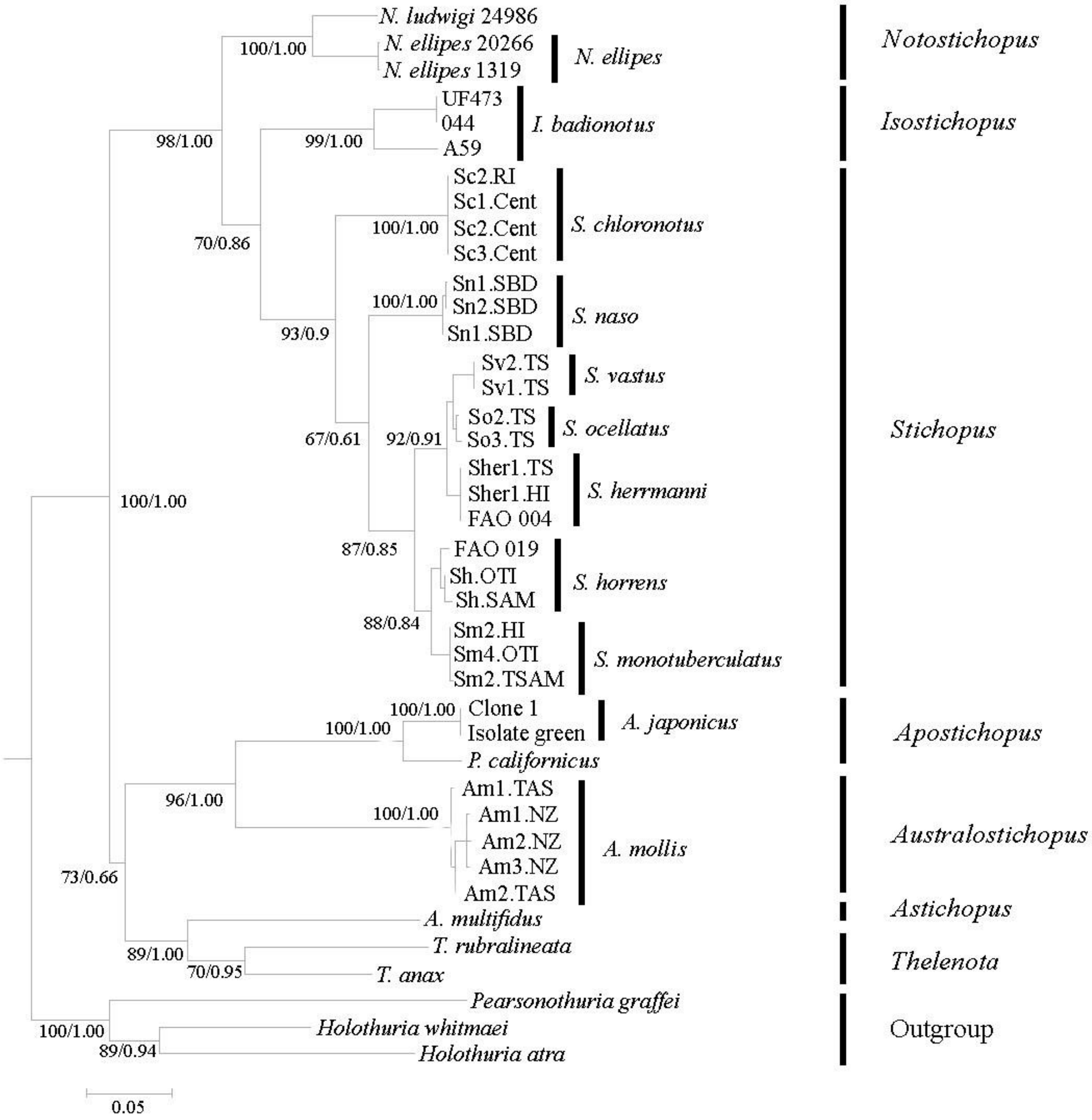

Figure 4.9 Bayesian consensus tree based on concatenated 16S and CO1 sequences. The numbers near nodes are bootstrap values for maximum likelihood analysis and Bayesian posterior probability values

Table 4.1 List of 16S and COI sequences used in the phylogenetic analyses with their corresponding species name and GenBank/DDBJ accession numbers. Sequences generated from this study are denoted (*).

Species	Tree ID	16S	COI
Stichopus chloronotus	Sc2.RI	EU856691	EU855619
	Sc1.Cent	EU856682	EU856610
	Sc2.Cent	EU856683	EU856611
	Sc3.Cent	EU856684	EU856612
Notostichopus ellipes	*N. ellipes* 20266	LC333903*	LC334345*
	N. ellipes 13119	LC333902*	LC334344*
Stichopus herrmanni	Sher1.TS	EU856629	EU856545
	Sher1.HI	EU856628	EU856544
	FAO 004	EU822451	EU848281
Stichopus horrens	FAO 019	EU822434	EU848282
	Sh.OTI	EU856638	EU856554
	Sh.SAM	EU856637	EU856555
Notostichopus ludwigi	*N. ludwigi* 24986	LC24986*	LC334346*
Stichopus naso	Sn1.SBD	EU856661	EU856587
	Sn2.SBD	EU856662	EU856588
	Sn1.MOR	EU856666	EU856586
Stichopus monotuberculatus	Sm2.HI	EU856695	EU856577
	Sm4.OTI	EU856654	EU856571
	Sm2.TSAM	EU856650	EU856567
Stichopus ocellatus	So2.TS	EU856680	EU856608
	So3.TS	EU856681	EU856609
Stichopus vastus	Sv1.TS	EU856707	EU856622
	Sv2.TS	EU856708	EU856623
Apostichopus japonicus	Clone1	AY852282	AY852280
	Isolate green	KT724357	KT625444
Astichopus multifidus	*A. multifidus*	EU822453	EU848293
Australostichopus mollis	Am1.TAS	EU856670	EU856599
	Am2.TAS	EU856672	EU856601
	Am1.NZ	EU856669	EU856598
	Am2.NZ	EU856671	EU856600
	Am3.NZ	EU856673	EU856602
Isostichopus badionotus	UF473	KX856756	EU848276
	FAO 044	EU822435	EU848276
	A59	JN207495	JN207564
Parastichopus californicus	*P. californicus*	DQ777096.	HM542319
Thelenota rubralineata	*T. rubralineata*	EU822452	EU848260
Thelenota anax	*T. anax*	KX640821	KX640822
Holothuria whitmaei	*H. whitmaei*	AY509147	AY177134
Holothuria atra	*H. atra*	JN120767	GQ920737
Pearsonothuria graeffei	*P. graeffei*	EU822440	EU848285

Table 4.2 Genetic distances (uncorrected *p*-distances) for COI (above diagonal) and 16S (below diagonal) gene fragments within and between genera of Stichopodidae. Numbers in bold along the diagonal represent intraspecific distances (16S/CO1).

	Apostichopus	*Australostichopus*	*Isostichopus*	*Stichopus*	*Notostichopus*	*Thelenota*	*Astichopus*
Apostichopus	**0.006/0.068**	0.200	0.214	0.207	0.214	0.209	0.209
Australostichopus	0.088	**0.001/0.017**	0.231	0.214	0.216	0.211	0.227
Isostichopus	0.129	0.122	**0.028/0.060**	0.117	0.144	0.199	0.199
Stichopus	0.120	0.119	0.078	**0.036/0.085**	0.162	0.191	0.191
Notostichopus	0.124	0.126	0.090	0.121	**0.011/0.074**	0.192	0.199
Thelenota	0.120	0.128	0.140	0.125	0.126	**0.061/0.172**	0.190
Astichopus	0.129	0.113	0.134	0.120	0.133	0.097	**N/A**
COI	Range	0.117–0.231					
	Average	0.197					
16S	Range	0.078–0.140					
	Average	0.118					