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A taxonomic revision of the genus *Apostichopus* (Holothuroidea: Stichopodidae) from Japan

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Abstract

Complete redescriptions of sea cucumbers in the genus *Apostichopus* Liao, 1980 are provided using the type specimens and specimens deposited in the National Museum of Nature and Science, Tsukuba, Japan. The genus consists of *A. armatus* (Selenka, 1867) and *A. japonicus* (Selenka, 1867), which can be distinguished by some spicules in the dorsal body wall; the rim of reduced table spicules in *A. armatus* is spinous, while that in *A. japonicus* is smooth. Spicules from the tentacles, papillae, tube feet, and cloaca are similar for both species.

Key Words: Sea cucumber, Echinodermata, taxonomy, spicules, *Apostichopus*

Introduction

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, spicules, 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 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

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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 spicules (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 spicules from the dorsal body wall and from the tentacles.

In this study, a revision of the genus *Apostichopus* with full description of spicule 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.

Materials and methods

Sea cucumber specimens of the genus *Apostichopus* loaned from the Museum of Comparative Zoology, Massachusetts, USA (MCZ) and the National Museum of Nature and Science, Tsukuba, Japan (NSMT) were examined. Spicules were extracted from the tissues of the middle part of dorsal body, papilla tips, tentacles, tube feet, and cloacal wall. The tissues were dissolved using commercial bleach. The spicules were then washed several times with deionized water and 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.

Taxonomy

Order Aspidochirotida Grube, 1840

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. Spicules taking form of tables, buttons, multiperforated plates, rods, and complex plates. Dorsal body-wall spicules 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 spicules 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 East China Sea that was identified as *Apostichopus japonicus*. Judging from the drawings of the spicules, 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 spicules without pillars in the body wall (Liao 1980: fig. 1).

The absence of C-shaped spicules and the presence of reduced table spicules in the dorsal body of *Apostichopus* differentiate it from the genera *Stichopus* and *Isostichopus* Deichmann, 1958, which have abundant C-shaped spicules 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 spicules 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 spicules 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 (What constitutes published work) of the Code (International Commission on Zoological Nomenclature 1999).

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

Figs 1–5

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. 2.
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–E7045A, Tateyama Bay, Chiba, 21 April 2011; NSMT–E7047A, Aburatsubo Bay, Kanagawa, 10 July 2011, Y. Tamura; NSMT–E7047F, Aburatsubo Bay, Kanagawa, 10 July 2011, Y. Tamura; NSMT–E7047C, Aburatsubo Bay, Kanagawa, 10 July 2011, Y. Tamura; NSMT–E7047E, Aburatsubo Bay, Kanagawa, 10 July 2011.

FIGURE 1. Syntype of *Apostichopus armatus* (Selenka, 1867), MCZ HOL–741.

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. Oral opening 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.

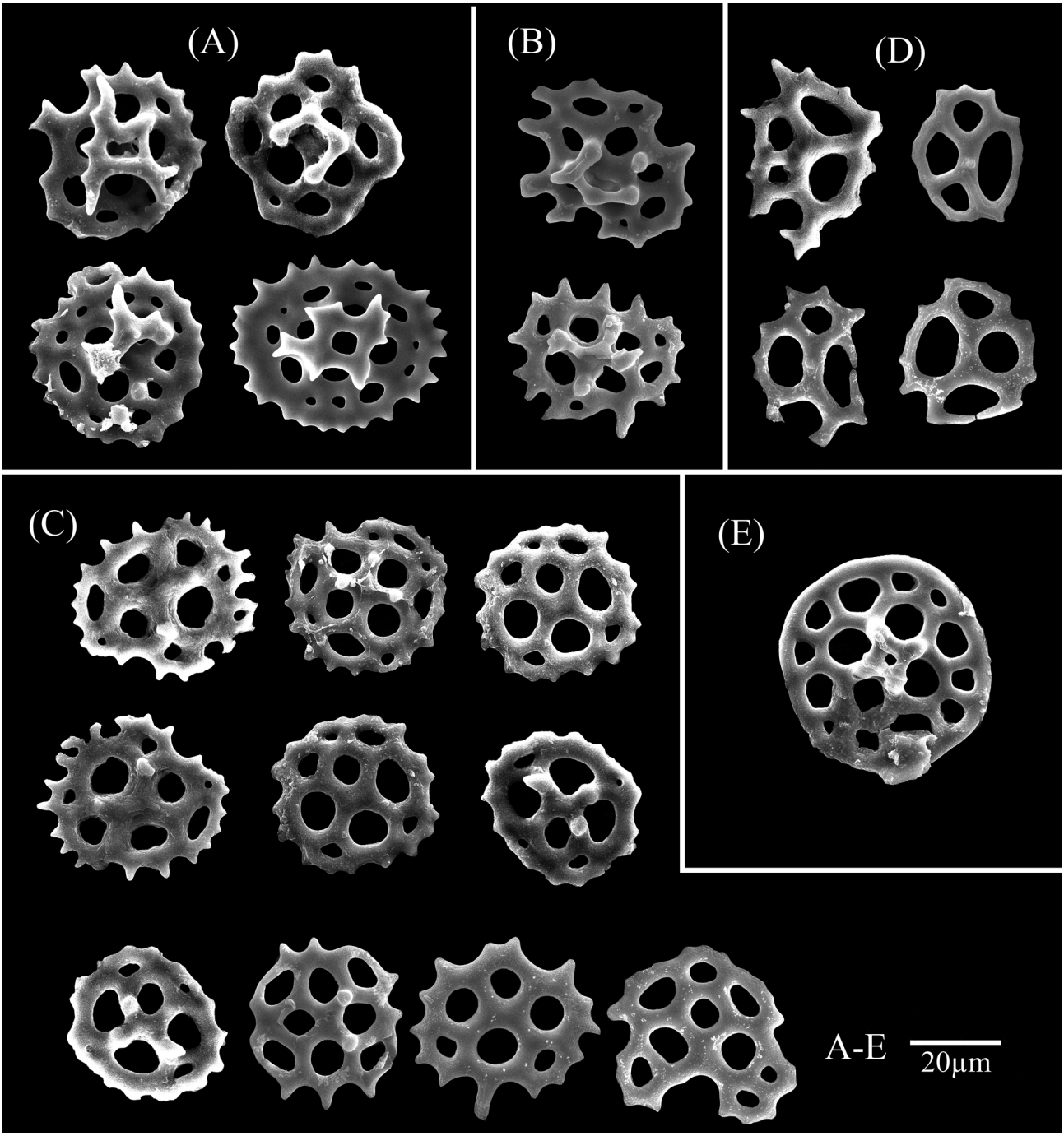

FIGURE 2. Spicules from the dorsal body wall of *Apostichopus armatus* (Selenka, 1867). A, tables with pillars and spinuous disk, NSMT–E7047E; B, tables with pillars and spinuous disk, NSMT–E7047A; C, reduced tables with spinuous disk, NSMT–E7047A; D, reduced tables with spinuous disk and 3–4 perforations, NSMT–E7047A; E, reduced tables with smooth disk, NSMT–E7047E.

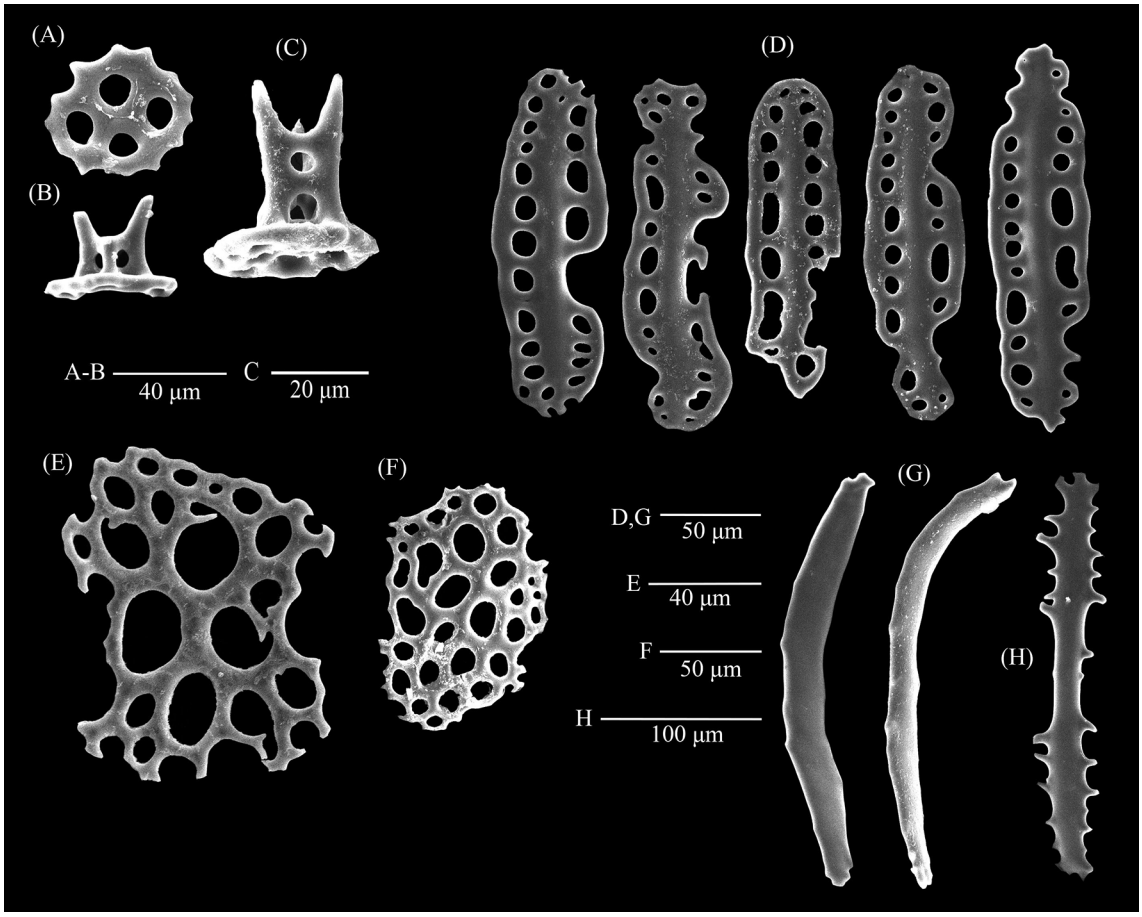

FIGURE 3. Spicules from the papillae tips of *Apostichopus armatus* (Selenka, 1867), NSMT–E7047C. A, reduced table; B–C, tables with pillars; D, elongated buttons; E–F, multiperforated plates; G, curved rods with smooth surfaces; H, straight, spiny rod.

Dorsal body wall with tables. Disk of tables rounded to oval in shape with 2–16 irregular perforations; rim of disk spinous or smooth (Fig. 2A–E). Pillar of reduced tables entirely absent or forming only a knob (Fig. 2C). Tables usually having four pillars; tip of each pillar often branched into multiple spines (Fig. 2A). Papillae containing multiperforated plates, tables, rods and elongated buttons. Multiperforated plates in papillae with numerous irregular perforations; rim uneven (Fig. 3E–F). Tables in papillae similar to those in dorsal body; pillars absent or forming knobs; rim spinous (Fig. 3A); mostly four pillars present, each having single spine on tip (Fig. 3B–C). Elongated buttons in papillae having two parallel longitudinal rows, each with 7–13 perforations (Fig. 3D). Rods in papillae variable in shape from straight to curved; surface of curved rods smooth (Fig. 3G), surface of straight rods spinous (Fig. 3H). Tentacles containing rods; curved (Fig. 4A–B); surface covered with spinelets. Tube feet containing multiperforated plates (Fig. 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 spicules in the dorsal body wall of *A. armatus.* They are similar to the spicules 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 spicules 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 spicules, and figures for the spicules from the type material. It is unfortunate that we are also unable to determine the spicules 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. 1), which now forms part of the diagnosis for *A. armatus*.

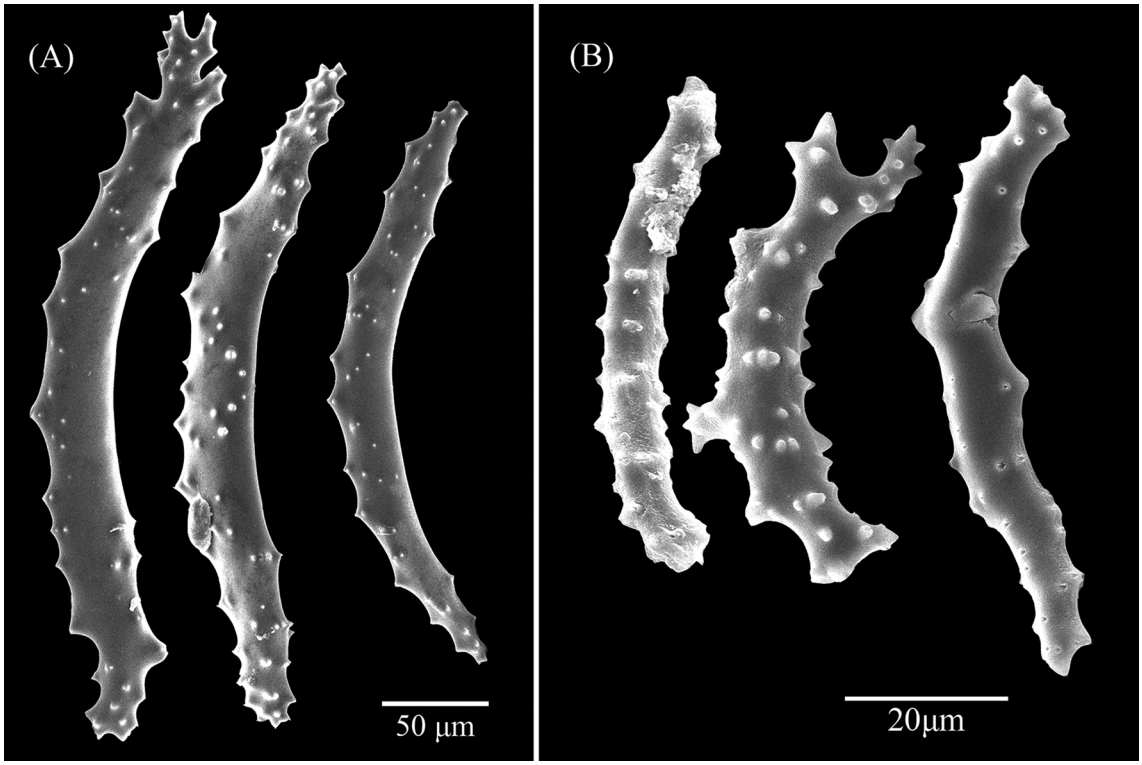

FIGURE 4. Spicules from the tentacles of *Apostichopus armatus* (Selenka, 1867), NSMT–E7047A. A, large curved rods with spinelets on surface; B, small curved rods with spinelets on surface.

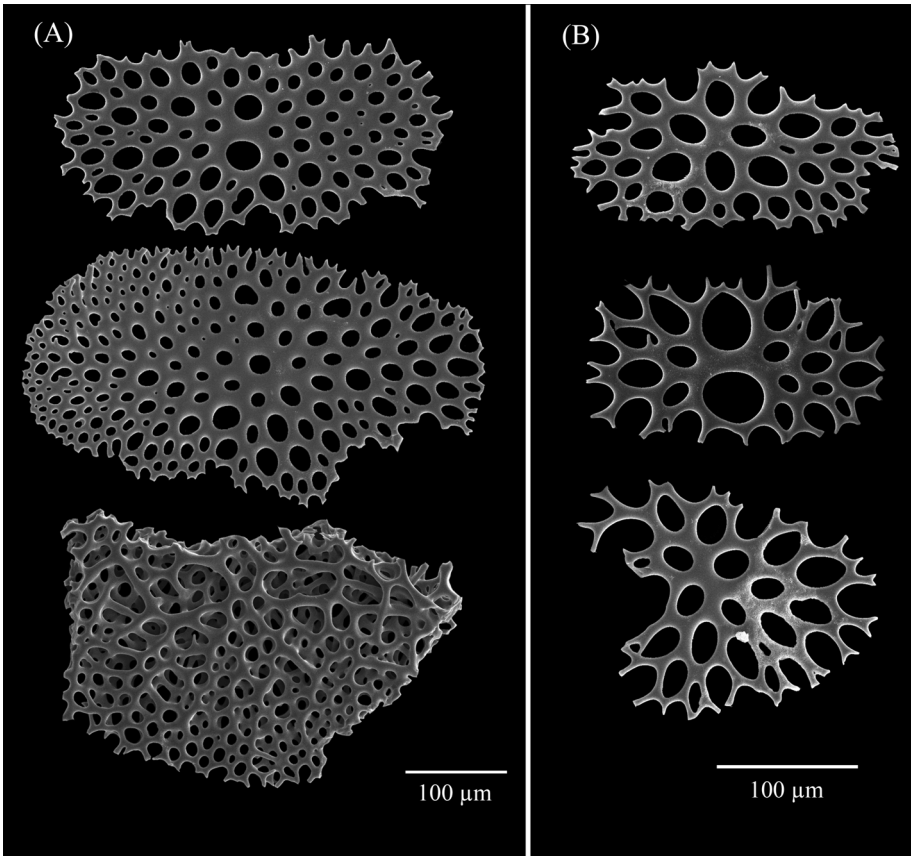

FIGURE 5. Spicules from the tube feet of *Apostichopus armatus* (Selenka, 1867), NSMT–E7047A. A–B, multiperforated plates.

Apostichopus armatus can be differentiated from *A. japonicus* by the absence of reduced table spicules with spinous rim in *A. japonicus.* Similar spinous reduced table spicules 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 and 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. Spicules from the papillae, tentacles and tube feet are similar to those of *A. japonicus.*

***Apostichopus japonicus* (Selenka, 1867)**

Figs 6–13

Stichopus japonicus Selenka, 1867: 318, pl. 18, figs 33–36; Semper, 1868: 74; von 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 typicus Théel, 1886: 196.
Stichopus (*Apostichopus*) *japonicus*–Kanno *et al*., 2006: 672–685.
Apostichopus japonicus–Kuramochi & Naganuma, 2010: 49–54, fig. 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, K. Yakiguchi, 28 September 2011; NSMT–10122A-E, 5 specimens, Takeoka, Futtsu, Chiba, K. Kawata & A. Ogawa, 24 December 2014.

Type locality. Japan.

FIGURE 6. Syntype of *Apostichopus japonicus* (Selenka, 1867), MCZ HOL–763.

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 ventor-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.

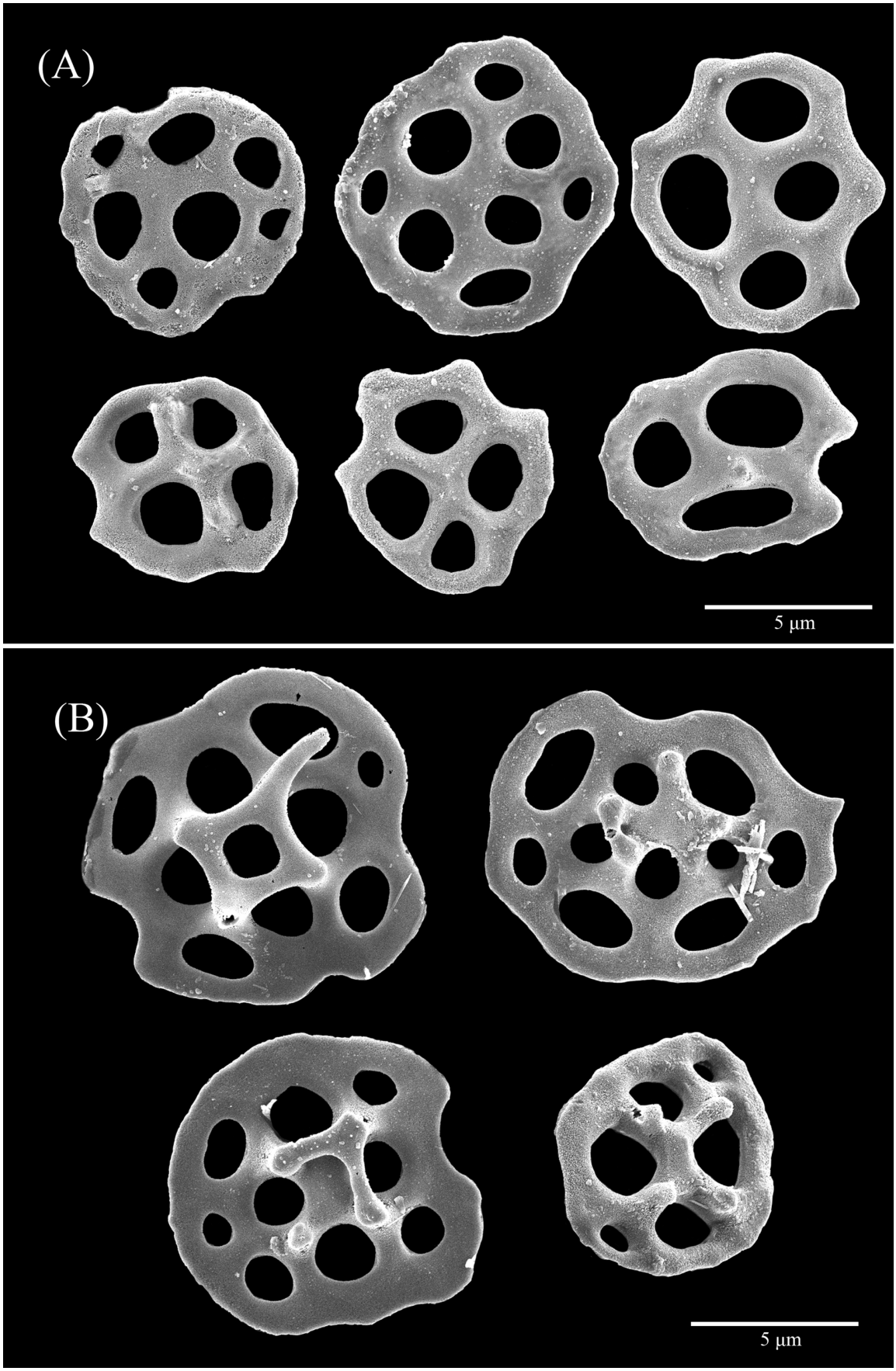

FIGURE 7. Spicules from the dorsal body wall of *Apostichopus japonicus* (Selenka, 1867), Syntype, MCZ HOL–763. A, reduced tables with smooth rims and 3–8 perforations; B, tables with pillars and smooth rims.

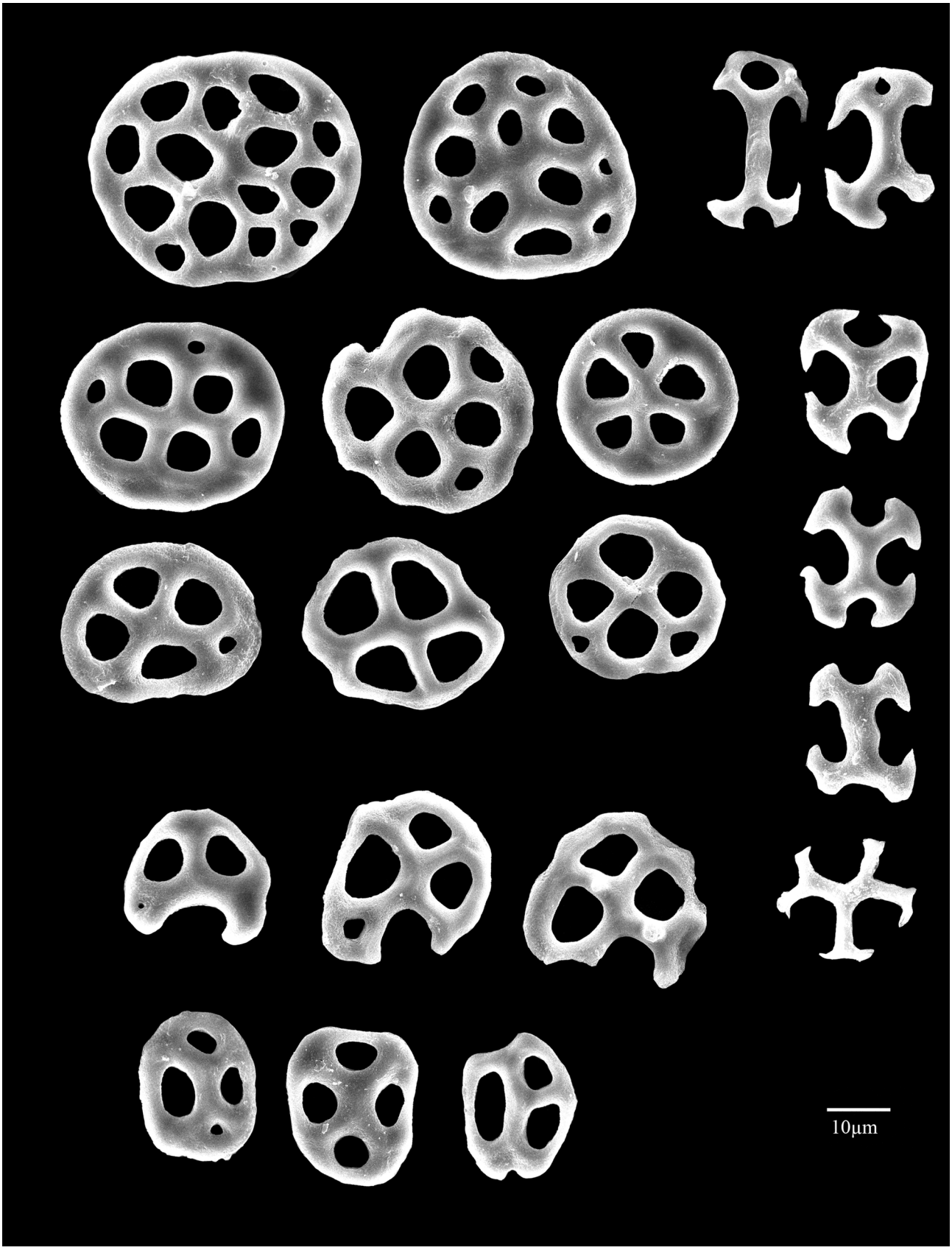

FIGURE 8. Disk of reduced table spicules with smooth rims from the dorsal body wall of *Apostichopus japonicus* (Selenka, 1867), NSMT–10122A.

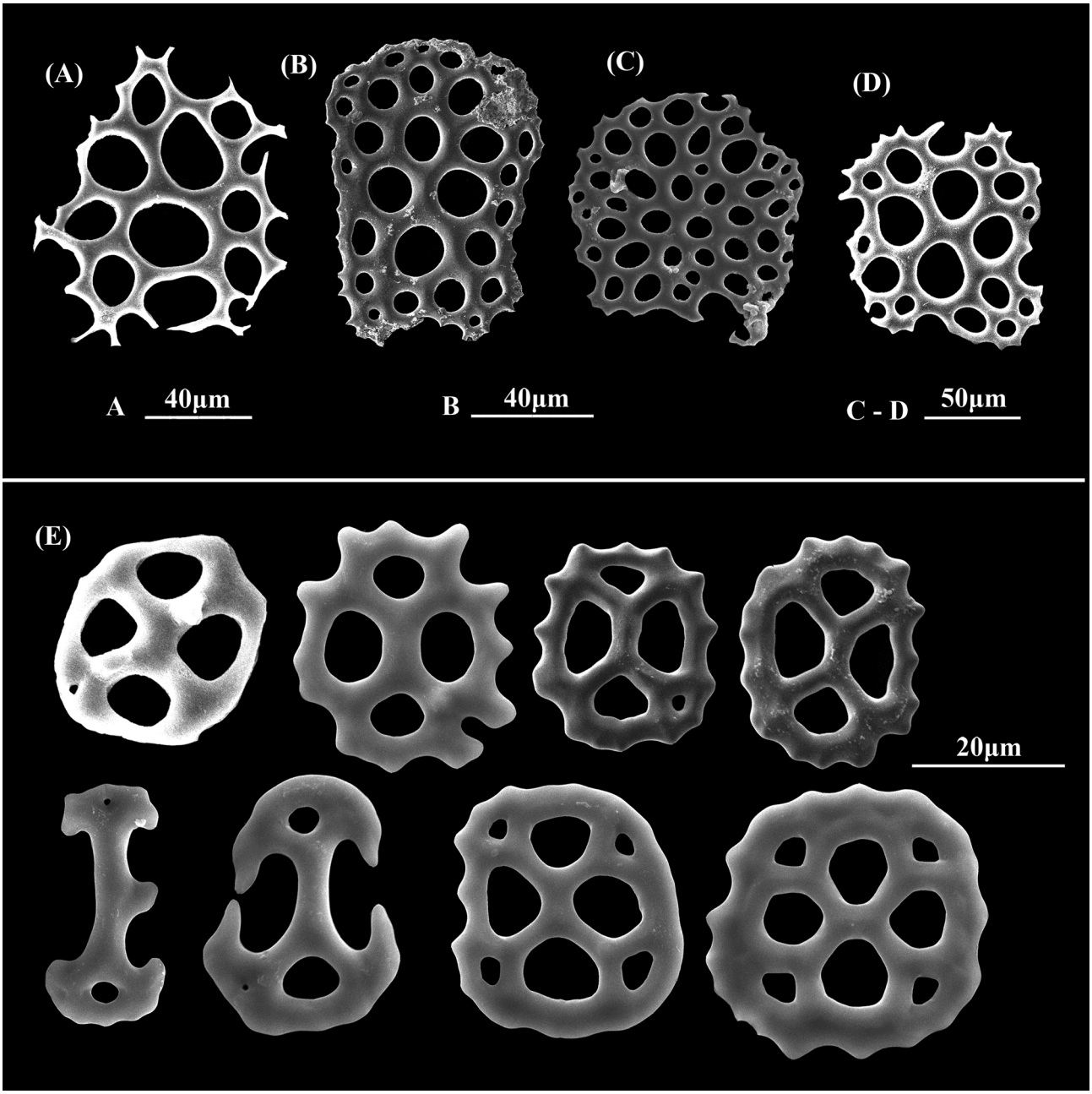

FIGURE 9. Spicules from the papillae tip of *Apostichopus japonicus* (Selenka, 1867), NSMT–10122A. A–D, multiperforated plates; E, reduced tables.

Dorsal body wall containing tables. Tables in dorsal body wall of adult reduced, pillars absent (Figs. 7, 8). 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. Multiperforated plates in papillae with four central perforation and numerous peripheral perforations; rim of uneven (Fig. 9A–D). Reduced tables in papillae similar to those in dorsal body (Fig. 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. 10A). Rods in papillae straight to curved; surface spinous (Fig. 10B). Rods in tentacles curved; surface covered with multiple spinelets (Fig. 11A–C). Tube feet with multiperforated plates (Fig. 12). Cloacal wall containing oval to rounded complex plates; surface very spinous and knobby (Fig. 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)

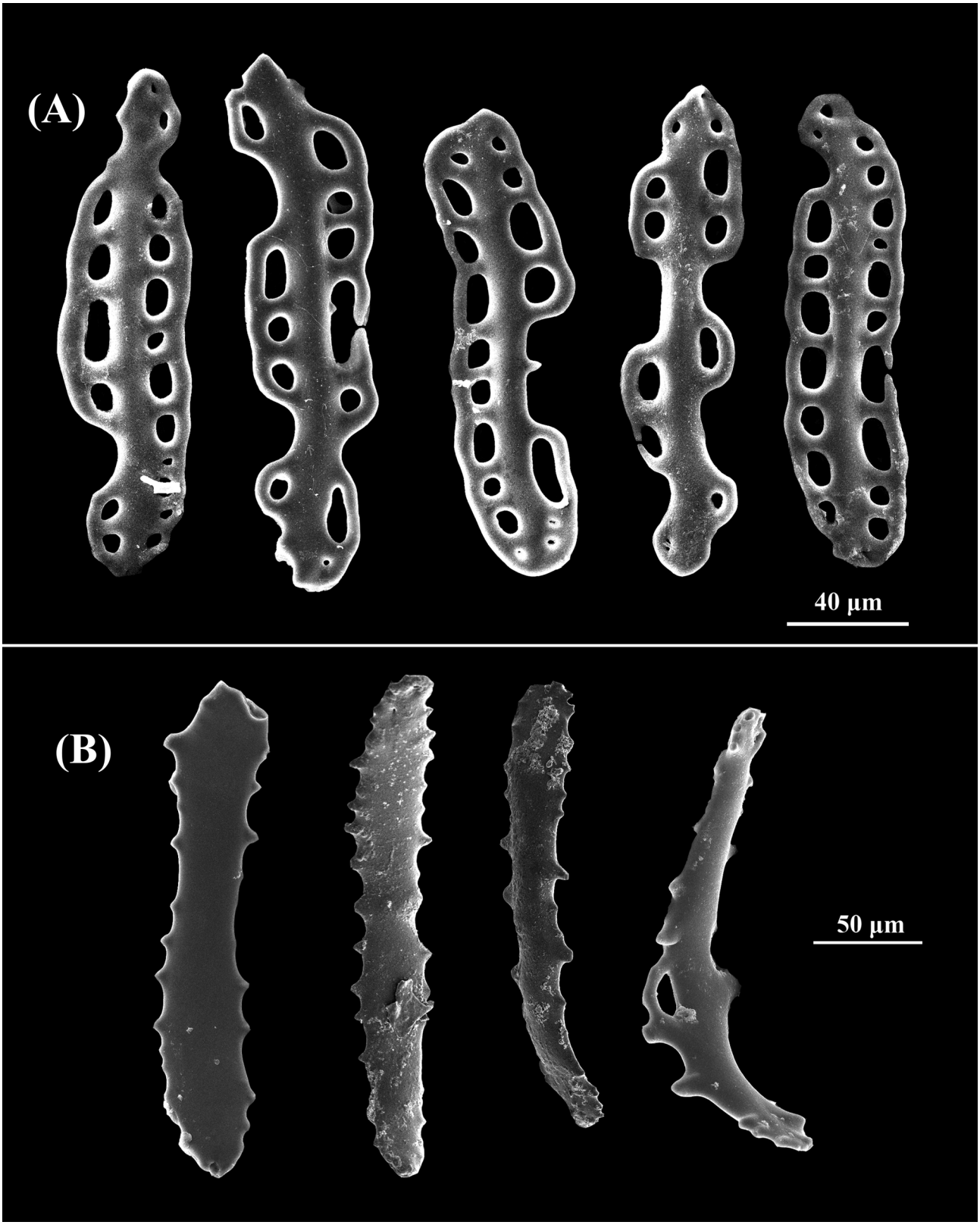

FIGURE 10. Spicules from the papillae tip of *Apostichopus japonicus* (Selenka, 1867), NSMT–10122A. A, elongated buttons; B, rods.

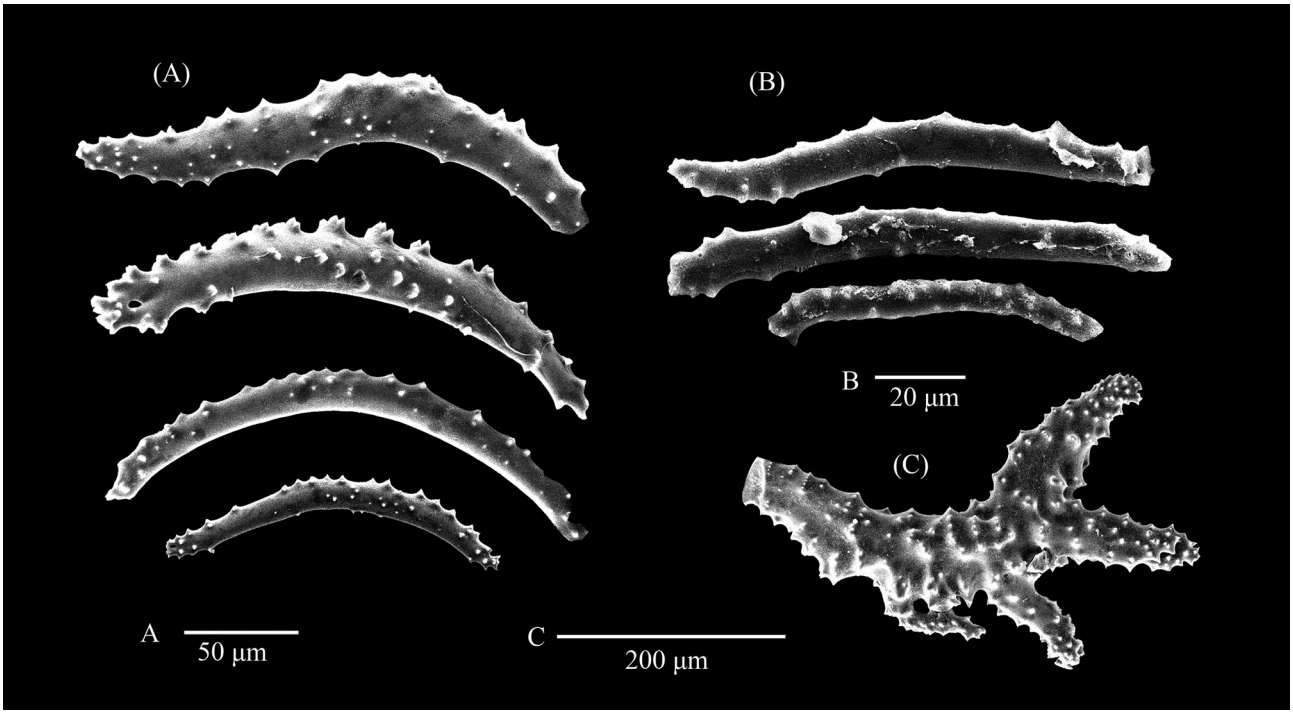

FIGURE 11. Spicules from the tentacles of *Apostichopus japonicus* (Selenka, 1867), NSMT–10122A. A–B, spiny rods. C, birfurcating rods.

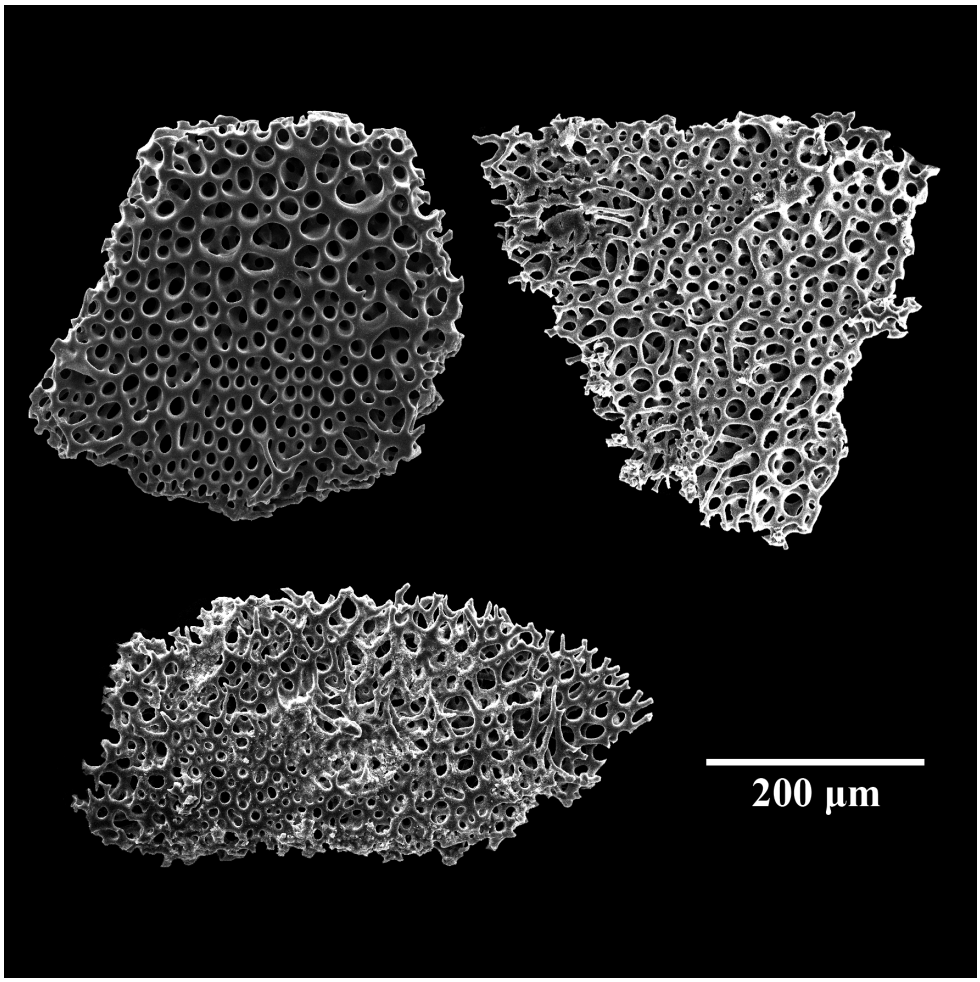

FIGURE 12. Spicules from the tube feet of *Apostichopus japonicus* (Selenka, 1867), NSMT–10122A. Large multiperforated plates.

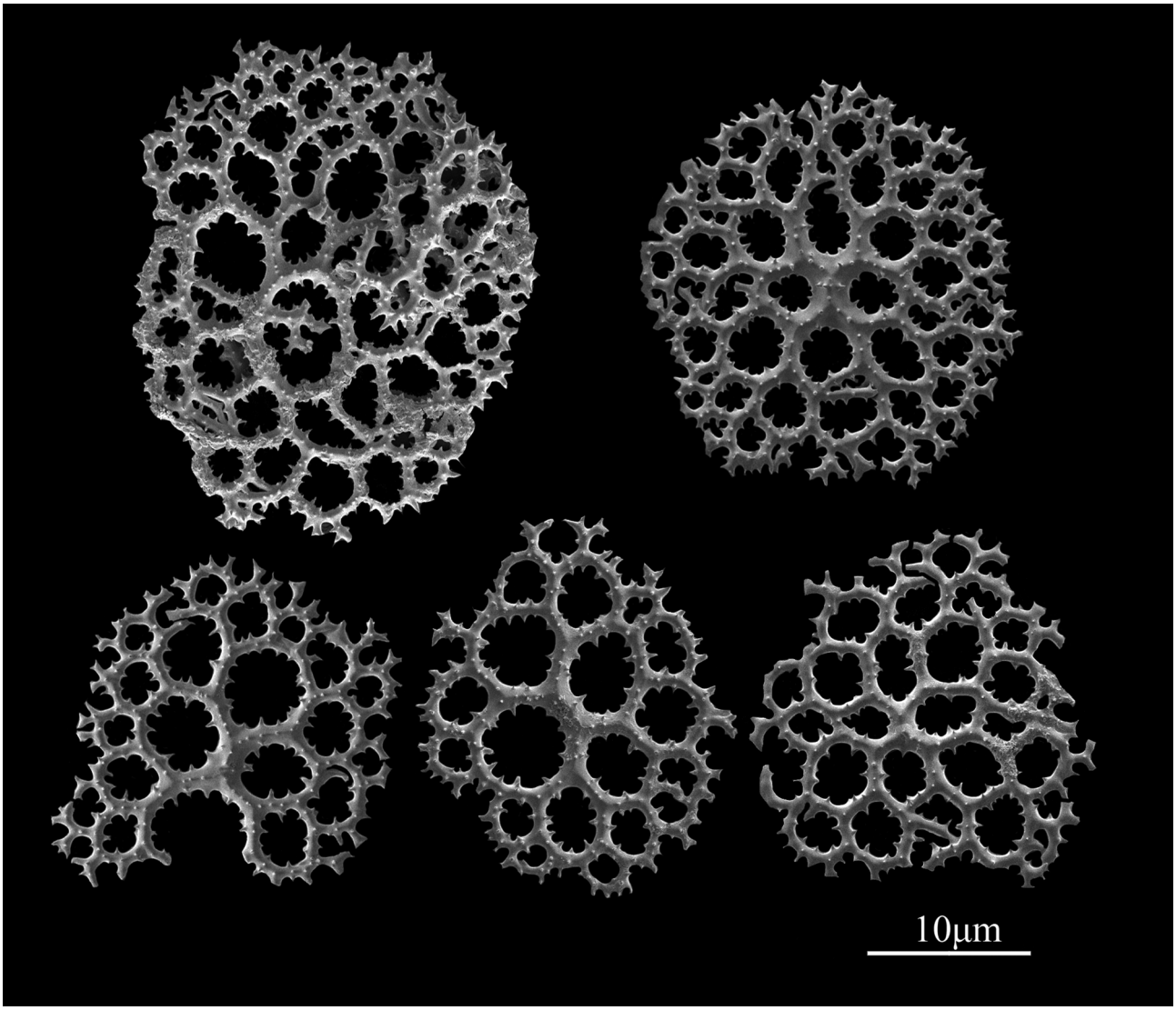

FIGURE 13. Complex plates from the cloacal wall of *Apostichopus japonicus* (Selenka, 1867), NSMT–10122A.

Remarks. The reduced table spicules of other materials examined in the dorsal body are similar to the spicules named "hemmungsbildungen" meaning table spicules 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 spicules of type material using SEM (Fig. 7) confirms that the rims of reduced table spicules 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.

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