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A Study of the Freshwater Sponges, *Eunapius carteri* (Bowerbank, 1863), *Eunapius crassissimus* (Annandale, 1907), and *Trochospongilla latouchiana* Annandale, 1907, Spongillidae (Demospongiae) from Lake Tonle Sap in Cambodia

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Abstract

Some sponges were found in Lake Tonle Sap in 2003 and 2004 under an International Scientific Field Research Program between Cambodia and Japan. They were identified as belonging to three species; *Eunapius carteri*, *E. crassissimus*, and *Trochospongilla latouchiana*. These records of occurrences in Cambodia should contribute to resolving questions regarding biogeographical sponge conditions in Southeast Asia. Key words: freshwater sponge, Lake Tonle Sap, Cambodia

Introduction

To date, there has been little study of the freshwater sponges of the Indochinese Peninsula, even though Evans [20], Annandale [4, 5, 8, 9], and Gee [35] described some species of freshwater sponges from several stations. With little being known of freshwater sponges in Cambodia, this area can be considered a biogeographical blank region.

Between 2002 and 2004, an International Scientific Field Research Program between Cambodia and Japan entitled "Evaluation of Mechanisms Sustaining the Biodiversity of Lake Tonle Sap, Cambodia" was carried out. A. Otaka collected some sponges from Lake Tonle Sap which were used in this study.

Material and Method

The sponges collected from Tonle Sap were identified as being of three species; *Eunapius carteri*, *E. crassissimus*, *Trochospongilla latouchiana*. The dates and sites of collection are shown in Table 1 and Fig. 1 and 2.

The sponges were fixed with 10% formalin for preservation after collecting. The three species were identified by light microscopy and an attempt was made to examine

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them by scanning electron microscopy (SEM). The sample of one of the three species, *Trochospongilla latouchiana*, however, was too small, had a very small number of gemmules, and could not be observed by SEM. Specimens of the other species were made for SEM by the following method.

Spicules: Part of the sponge was rinsed with distilled water in a test tube and then concentrated nitric acid was added. The spicules were freed from the specimen by boiling, rinsed with distilled water, and then rinsed with 95% ethanol. One drop of the specimen solution was pipetted from the test tube onto a cover glass mounted on an aluminum stub, after which the stub was placed in a decicater and allowed to dry.

Gemmule coats and micropyles: The gemmules were fixed in 1% osmium tetroxide in a 0.1M phosphate buffer. After fixation, the specimen was dehydrated within an ethanol series, replaced with isoamyl acetate, and dried by the critical point drying method with a Hitachi HCP-1. For observation of the gemmule sections, some of the treated gemmules were cut in two through the micropyles with a double- edged razor blade.

All the specimens were coated with gold-palladium alloy and observed with a

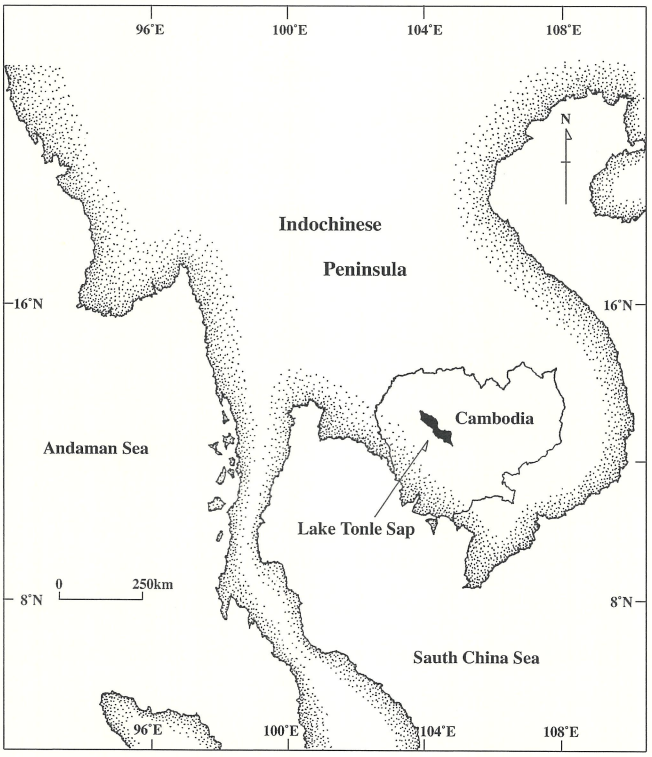

Fig. 1 A map showing the locality of Lake Tonle Sap.

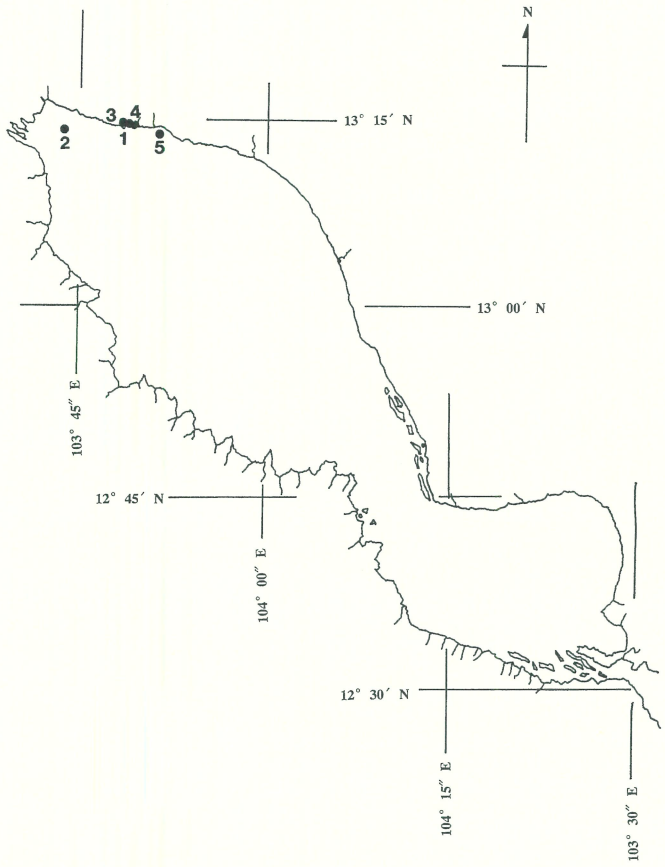

Fig. 2 Site of sampling stations of freshwater sponges in Lake Tonle Sap.

Table. 1 List of sampling station and dates of collection of freshwater sponges in Lake Tonle Sap

	Station 1	Station 2	Station 3	Station 4	Station 5
E. carteri			8, Vov., 2003		
E. crassisisma	11, Nov., 2003	5, Nov., 2003			18, May., 2004
T. latouchiana	11, Nov., 2003		8, Nov., 2003	5, Nov., 2003	

Hitachi S-570 scanning electron microscope.

Measurement for spicules and gemmules: To determine the dimensions of the spicules, the IBAS analysis system (Zeiss) was used morphometrically.

Results

***Eunapius carteri* (Bowerbank, 1863)**

Spongilla carteri Bowerbank, 1863, p.469. -Carter, 1881a, p.86. -Weltner, 1895, p.117; 1913, p.475. -Annandale, 1906b, p.187; 1907c, p.24; 1908b, p.157; 1911c, p.87; 1912c, p.137;

1919c, p.87. -Kirkpatrick, 1906, p.218. -Rezvoj, 1926a, p.108; 1928, p.219. -Vorstman, 1927, p.184. -Gee, 1929d, p.297; 1930a, p.70; 1931e, p.43; 1932g, p.302; 1932h, p.185; 1932f, p.507; 1932d, p.53; 1932c; p.36. -Burton, 1929, p.157. -Arndt, 1932c, p.552; 1936, p.14. -Topsent, 1932a, p.568. -De Laubenfels, 1936, p.36. -Schröder, 1942, p.247. -Jewell, 1952, p.448. -Penny, 1960, p.14. -Sasaki, 1967, p.31.

Eunapius carteri Gray, 1867, p.552.

Spongilla carteri var. *cava* Annandale, 1911c, p.88. -Gee, 1931e, p.35; 1932h, p.188.

Spongilla carteri var. *lobosa* Annandale, 1911c, p.89; 1918a, p.211. -Gee, 1931e, p.41; 1932h, p.188.

Spongilla carteri var. *mollis* Annandale, 1911c, p.88; 1918a, p.211. -Gee, 1931e, p.43; 1932h, p.187.

Spongilla carteri var. *balatonensis* Arndt, 1923, p.79; 1926, p.342. -Gee, 1931e, p.33; 1932h, p.189.

Spongilla carteri var. *melli* Arndt, 1923, p.80. -Gee, 1926c, p.110; 1927c, p.184; 1928, p.221; 1930e, p.29; 1931e, p.42; 1932h, p.189.

?*Spongilla aetheriae* Annandale, 1913b, p.237.

?*Spongilla rotundacula* Rezvoj, 1925, p.567. -Arndt, 1926, p.342.

?*Spongilla carteri* var. *rotundacuta* Gee, 1931e, p.48; 1932h, p.193.

Spongilla friabilitis Carteri, 1849, p.83.

Eunapius carteri Penney and Racek, 1968, p.23.

Sponges: The sponges were attached to the surface of submerged wood, twigs, and the stems of water plants and formed a flattish thin crust. The surface of the sponges was almost smooth and tips of the vertical skeleton protruded slightly from the epidermis as short bristles. As for consistency, the sponges were not so hard, but not so fragile either.

Megascleres: The megascleres were entirely smooth, rather stout, fusiform, gradually sharply pointed and slightly curved amphioxea (Fig. 5a). They ranged from 244 to 340 μm in length (mean value 290 μm, SD = 20.0) and from 13.4 to 21.7 μm in width(mean value 17.8 μm, SD = 1.7).

Microscleres: Microscleres were absent.

Gemmoscleres: In shape and structure, the gemmoscleres were similar to the megascleres (Fig. 5a, b). They were entirely smooth amphioxea. But they were shorter, thinner, more curved, and sharply pointed. They ranged from 170 to 270 μm in length (mean value 224 μm, SD = 19.8) and from 6.3 to 12.1 μm in width (mean value 9.3 μm, SD = 1.0).

Gemmules: The gemmules were moderately abundant, scattered freely and singly, not in groups, throughout the skeletal network. They had a round conical outline and an almost flattened base (Fig. 3a, b). The greatest diameter ranged from 341 to 499 μm (mean value 435 μm). The gemmule coat consisted of a pneumatic layer and outer and inner gemmular membranes. The pneumatic layer consisted of stacks of low polygonal prismatic alveoli. Several pores interconnecting alveoli were present on the upper and lower walls of individual alveoli (Fig. 3e). On the surface of the gemmules, there were many small uniform depressions, each of which was situated at the top of an alveoli stack (Fig. 3a, c). Most of the gemmmoscleres were attached tangentially to the whole surface of the gemmule. A few gemmoscleres were embedded in the pneumatic layer (Fig. 3e).

Micropyles: The micropyles were situated singly at the top of the gemmule (Fig. 3b). They were rather long and tubular, and traversed the thick pneumatic layer and distally protruded slightly from a funnel-shaped depression of the gemmule coat (Fig. 3d).

***Eunapius crasissimus* (Annandale, 1907)**

Spongilla crassissima Annandale, 1907c, p.17; 1911c, p.98; 1918a, p.212. -Gee, 1931e, p.36; 1932c, p.37. -Penney, 1960, p.16. -Sasaki, 1967,p.31. -Racek,1968, p.62. -Rützler, 1968, p.62.

Spongilla crassissima var. *bigemmulata* Annandale, 1907c, p.18.

Spongilla crassior Annandale, 1907b, p.389.

Spongilla crassissima var. *crassior* Annandale, 1911c, p.98; 1918a, p.212.-Stephens, 1919, p.97. -Gee, 1931e, p.36; 1932c, p.37. -Penney, 1960, p.16. -Sasaki, 1967, p.33.

Eunapius crasissimus Penney and Racek, 1968, p.30. -Racek, 1969, p.276.

Sponges: The sponges were attached to the surface of submerged wood, twigs, and the stems of water plants and formed a flattish crust. The surface of the sponges was smooth. The sponges were hard in consistency.

Megascleres: The megascleres were entirely smooth, invariably stout, cylindrical, and slightly curved amphistrongyla (Fig. 6a). Some megascleres were thinner, shorter, and were amphioxea. The amphistrongyla megascleres ranged from 186 to 256 μm in length (mean value 220 μm, SD = 16.2) and from 21.1 to 38.8 μm in width (mean value 28.8 μm, SD = 2.7).

Microscleres: Microscleres were absent.

Gemmoscleres: The gemmoscleres were cylindrical amphistrongyla, or abruptly pointed amphioxea (Fig. 6b, c). They were slightly bent and entirely covered with small and

irregular spines. Most of the spines curved inward from their tips to approximately the middle one-third of the spicules. Most of the spines on the middle third of the surface of the spicules were directly conical spines. The spicules often bore a large number of minute spines at both ends, and ranged from 83 to 144 μm in length (mean value 109 μm, SD = 19.8).

Gemmules: The gemmules were more abundant at the base of the sponges, but did not form a distinct basement layer. They were occasionally observed in free groups of two to four (Fig. 4a). Each gemmule had a distorted spherical body (Fig. 4b). Their greatest diameter ranged from 302 to 506 μm (mean value 390 μm). The gemmule coat consisted of a pneumatic layer and outer and inner gemmular membranes. The pneumatic layer was sometimes irregular in thickness (Fig. 4d), but at the base of the gemmules, it was usually thin. The pneumatic layer consisted of stacks of low polygonal prismatic alveoli. Several pores interconnecting alveoli were present on the upper and lower walls of individual alveoli (Fig. 4g). On the surface of the gemmules, there were many shallow depressions, each of which was situated at the top of an alveoli stack (Fig. 4b, g). The gemmoscleres were arranged in two layers (Fig. 4g). One layer, which had more gemmoscleres, was attached tangentially to the whole surface of the gemmule. The second layer was embedded in the pneumatic layer rather near the inner gemmular membrane.

Micropyles: The micropyles were often invisible under a dissecting microscope. Even under the scanning electron microscope, it was not very easy to find the micropyles, because the gemmule coat often almost covered them. The micropyles were long bent tubes and distally protruded slightly from the gemmule coat (Fig. 4e, f).

***Trochospongilla latouchiana* (Annandale, 1907)**

Trochospongilla latouchiana Annandale, 1907c, p.21; 1908b, p.157; 1911c, p.115; 1918a, p.201. -Gee, 1926c, p.110; 1926a, p.181; 1927b, p.60; 1928, p.225; 1929d, p.297; 1930a, p.98; 1930e, p.27; 1931e, p.41; 1932b, p.10; 1932f, p.507; 1932c, p.42. -Vorstman, 1927, p.184; 1928, p.116. -Rao, 1929, p.269. -Arndt, 1932c, p.566; 1936, p.10. -Schröder, 1935, p.104. -Penny, 1960, p.56. -Sasaki, 1967, p.41. -Penney and Racek, 1968, p.23. -Masuda and Satoh, 1989, p.81.

Trochospongilla latouchiana subsp. *sinnensis* Annandale, 1919b, p.457. -Gee and Wu, 1925a, p.226. -Gee, 1926a, p.181; 1931e, p.49; 1932b, p.13.

Trochospongilla latouchiana var. *pasigensis* Gee, 1932b, p.14; 1932f, p.534; 1932c, p.42. -Penney, 1960, p.56.

Sponges: The sponges were attached to the surface of submerged wood, twigs, and the stems of water plants and formed a flattish thin crust. The surface of the sponges was almost smooth and tips of the vertical skeleton protruded slightly from the epithelium as short bristles. The sponges were soft and fragile in consistency.

Megascleres: The megascleres were entirely smooth, distinctly fusiform, and slightly curved amphioxea (Fig. 7a). A small number of megascleres had some microspines (Fig. 7b). They ranged from 286 to 446 μm in length(mean value 394 μm, SD = 26.0) and from 10.5 to 20.7 μm in width (mean value 14.9 μm, SD = 1.7).

Microscleres: Microscleres were absent.

Gemmoscleres: The gemmoscleres were minute biotulates with a smooth shaft (Fig. 7c, d). They had terminally circular rotules of unequal diameter. The upper rotules were smaller than the lower ones. Both the upper and lower rotules slightly curved at the marginal portions to form a shallow bowllike structure. The gemmoscleres ranged from 13.4 to 18.4 μm in length (mean value 16.3 μm, SD = 1.0). The diameter of the upper rotules ranged from 14.5 to 20.0 μm (mean value 17.2 μm, SD = 1.1), and that of the ones ranged from 17.9 to 24.0 μm (mean value 20.8 μm, SD = 1.2). The diameter of the shafts at the middle portion ranged from 2.4 to 5.2 μm (mean value 3.6 μm).

Gemmules: The diameter of gemmules ranged from 205 to 240 μm.

DISCUSSION

1) Distribution

***Eunapius carteri* (Bowerbank, 1863)**

India: Carter, 1849; 1881-Bowerbank, 1863-Gray, 1867-Weltner, 1895-Kirkpatrik, 1906-Annandale, 1906; 1907, 1912c-Gee, 1929d; 1930a; 1932c; 1932h. **Sri Lanka**: Annandale, 1911c-Gee, 1932c. **Mauritius**: Weltner, 1895-Kirkpatrick, 1906; Annandale, 1911c; 1918a-Gee, 1932c; 1932h. **Indoneia**: Weltner, 1895-Kirkpatrik, 1906, Annandale, 1911c; 1918a-Arndt, 1923-Vorstman, 1927-Gee, 1930a; 1932h. **Burma**: Annandale, 1908b; 1911c. **Thailand**: Gee, 1930a; **Philippines**: Gee, 1932c; 1932f. **China**: Gee, 1926c; 1927; 1929d; 1930e; 1932c. **Taiwan (Formosa)**: Sasaki, 1967. **Hungary**: Weltner, 1895-Annandale, 1911c, Gee, 1932c. **Iran-Afghan Frontier**: Annandale, 1919c-Arndt, 1926-Rezvoj, 1925; 1928-Gee, 1932c; 1932h. **Egypt**: Annandale, 1913b. **Uganda**?: Kirkpatrick, 1906-Weltner, 1913-Annandale, 1914-Arndt, 1931, Gee, 1932c. **-Cameroon**?: Arndt, 1931.

***Eunapius crasissimus* (Annandale, 1907)**

India: Annanadale, 1907c, 1911c, 1918a, Gee, 1931e, 1932c. **China**: Gee, 1932c.

Taiwan (Formosa): Sasaki, 1967.

***Trochospongilla latouchiana* Annandale, 1907**

India: Annandale, 1907; 1911; 1918-Gee and Wu, 1925-Gee, 1926a; 1930a; 1930e; 1931e; 1932b; 1932c; 1932d; 1932f-Schröder, 1935-Arndt, 1936-Penney, 1960-Sasaki, 1967-Penney and Racek, 1968- Masuda and Satoh, 1989. **Burma**: Annandale, 1908; 1911; 1918-Rao, 1929-Gee, 1926a; 1930a; 1930e; 1932c; 1932b; 1932f-Schröder, 1935-Arndt, 1936-Penney, 1960-Sasaki, 1967-Masuda and Satoh, 1989. **China**: Annandale, 1918-Gee and Wu, 1925; 1927-Gee, 1926a; 1926c; 1927; 1928; 1930a; 1930e; 1932b; 1932c; 1932f-Schröder, 1935-Arndt, 1936-Penney, 1960-Sasaki, 1967-Penney and Racek, 1968-Masuda and Satoh, 1989. **Indonesia**: Gee, 1929; 1930a; 1930e; 1932b; 1932c; 1932f-Arndt, 1932; 1936-Schröder, 1935-Penney, 1960-Sasaki, 1967-Masuda and Satoh, 1989. **Philippines**: Gee, 1932b; 1932c; 1932f-Arndt, 1936-Penney, 1960-Masuda and Satoh. **Taiwan (Formosa)**: Sasaki, 1967-Masuda and Satoh. **Japan**: Masuda and Satoh, 1989. **Australia**: Penney and Racek, 1968-Racek, 1969. **Angola**: Arndt, 1936-Penney, 1960-Masuda and Satoh, 1989.

Our collection of *Eunapius carteri*, *E. crassissmus* and *Trochospongilla latouchiana* from Lake Tonle Sap is the first record of occurrence of these species in Cambodia. However, the finding of *E. carteri* was an expected result to some degree as occurrence of this species has successively been reported in Japan, China, South Asia, India, Iran-Afghan Frontier to North Africa.

E. crassissimus has been reported in Taiwan, China, and India. Our record will contribute to the drawing of a map of distribution when more samples of this species are found in the Indochinese Peninsula.

The finding of *T. latouchiana* was also expected to some degree as occurrence of this has successively been reported in Japan, China, South Asia, Australia and India.

In the Indochinese Peninsula, the following 11 species of freshwater sponges are known:

1) *Spongilla alba* Carter, 1849: Thailand (Annandale, 1918a)
2) *Eunapius carteri* (Bowerbank, 1843): Burma (Annandale, 1911c), Thailand (Gee, 1930f)
3) *Eunapius potamolepis* (Annandale, 1918): Thailand (Annandale, 1918a)
4) *Radioispongilla crateriformis* (Potts, 1882): Burma (Annandale, 1911c)
5) *Radiospongilla cerebellata* (Bowerbank, 1863): Burma (Annandale, 1911c)
6) *Ephydatia fluviatilis* (Linnaeus, 1758): Thailand (Evans, 1901), Burma (Annandale, 1918a)

7) *Trochospongilla latouchiana* Annandale, 1907: Burma (Annandale, 1911c)
8) *Trochospongilla phillottiana* Annandale, 1907: Burma (Annandale, 1911c)
9) *Corvospongilla burmanica* (Kirkpatrik, 1908): Burma (Annandale, 1911c)
10) *Unborotula bogorensis* (Weber, 1890): Thailand (Annandale, 1918a, Gee, 1931e)
11) *Metania vesparioides* (Annandale, 1908): Burma (Annandale, 1918a)

From Lake Tole Sap, only three species were collected. On the other hand, the occurrence of seven species has been reported in Burma. More investigations in this lake and other areas in Cambodia should reveal additional members of other species and contribute to resolving questions regarding biogeographical sponge conditions in Southeast Asia.

2) Morphology

Most of the *Eunapius* species exhibit a large range of morphological variations in their spicular components as well as in their gemmules. All the megascleres of *E. carter* from Lake Cambodia were amphioxea. In the case of *E. crassisssmus*, on the other hand, most of them were amphistrongyla and a small number of megascleres were amphioxea. The amphioxea ones were young megascleres. We must pay close attention to the degree of maturity of spicules when we observe them. The representative species, *E. fragilis*, is a cosmopolitan species and also shows a large range of morphological variations in its megascleres. Some subspecies have amphioxea and others have both amphistrongyla and amphioxea. The ratio of the two types and the degree of sharpness of their tips in the *Eunapius* species may be taxonomical characteristics and deserve detailed ecological and zoogeographical studies.

The gemmules of *E. carteri* were scattered freely and singly, not in groups, throughout the skeletal network. The gemmules of *E. crassissimus* were more abundant at the base of the sponges, but did not form a distinct basement layer. They were occasionally observed in free groups of two to four. The situation of gemmules in the sponges and the degree of coupling of the gemmules may be also taxonomical characteristics and deserve detailed ecological and zoogeographical studies.

The further collection of as much material as possible in Cambodia should contribute to resolving questions regarding biogeographical sponge conditions in Southeast Asia.

Acknowledgements

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Explanation of Figure 3-7

Fig. 3 Gemmules of *Eunapius carteri* (Bowerbank)

a. Apical view of a gemmule. Many gemmoscleres are attached to the surface of the gemmule tangentially. It has a round conical outline and an almost flattened base. Many small uniform depressions are seen on the surface of a gemmule. ×110

b. A cross-section of a gemmule cut through a micropyle. The gemmule coat is evenly developed. ×110

c. An enlarged view of Fig. 3a showing a micropyle protruding slightly from a funnel-shaped depression of the gemmule coat. ×240

d. An enlarged view of Fig 3b. The tubular micropyle protrudes slightly from a funnel-shaped depression of the gemmule coat. ×240

e. A part of a cross-section of a gemmule coat consisting of outer and inner gemmular membranes and a pneumatic layer. The pneumatic layer consists of alveloli arranged in many stacks. Several pores which interconnect the alveoli are seen on the upper and lower walls of individual alveoli. ×380

Fig. 4 Gemmules of *Eunapius crassisimus* (Annandale)

a. Three gemmules are combined and covered with a continuous gemmule coat. Many gemmoscleres are attached to the surface of the gemmule tangentially. Many small uniform depressions are seen on the surface of a gemmule. ×94

b. A single gemmule. A micropyle is seen at the center of a gemmule. ×110

c. An enlarged view of Fig. 4b showing a micropyle protruding slightly from the gemmule coat. ×300

d. A cross-section of a gemmule cut through a micropyle. The gemmule coat is evenly developed. ×110

e. An enlarged view of Fig. 4d. The tubular micropyle slightly bends and protrudes slightly from the gemmule coat. ×280

f. A long micropyle bends significantly. ×280

g. A part of a cross-section of a gemmule coat consisting of outer and inner gemmular membranes and a pneumatic layer. The pneumatic layer consists of alveloli arranged in many stacks. Several pores which interconnect the alveoli

are seen on the upper and lower walls of individual alveoli. Some gemmoscleres are seen on the surface and others are near the inner gemmular membrane. ×510

Fig. 5 Spicules of *Eunapius carteri* (Bowerbank)

a. A megasclere. This spicule is entirely smooth, slightly curved, fusiform and sharp at both ends (Amphioxea type). ×210

b. A gemmosclere. This spicule is entirely smooth, curves, and has sharper ends than those of the megascleres. ×280

c. A gemmmosclere. This spicule bends slightly near both ends. ×280

Fig. 6 Spicules of *Eunapius crassisimus* (Annandale)

a. A megasclere. This spicule is entirely smooth, thick, only slightly curved, fusiform and has slightly swollen blunt ends (Amphistrongyla type). ×320

b. A gemmosclere. This spicule is cylindrical (Amphistrongyla type), slightly bends and is covered with small irregular spines. Most of the spines curve inwards from their tips to approximately the middle one third of the spicules. Most of the spines on the middle third of the surface of the spicules are directly conical spines. This spicule bears more spines at both ends. ×580

c. This spicule resembles the spicule of Fig. 6b except for the ends, which come to abrupt points (Amphistrongyla type). ×580

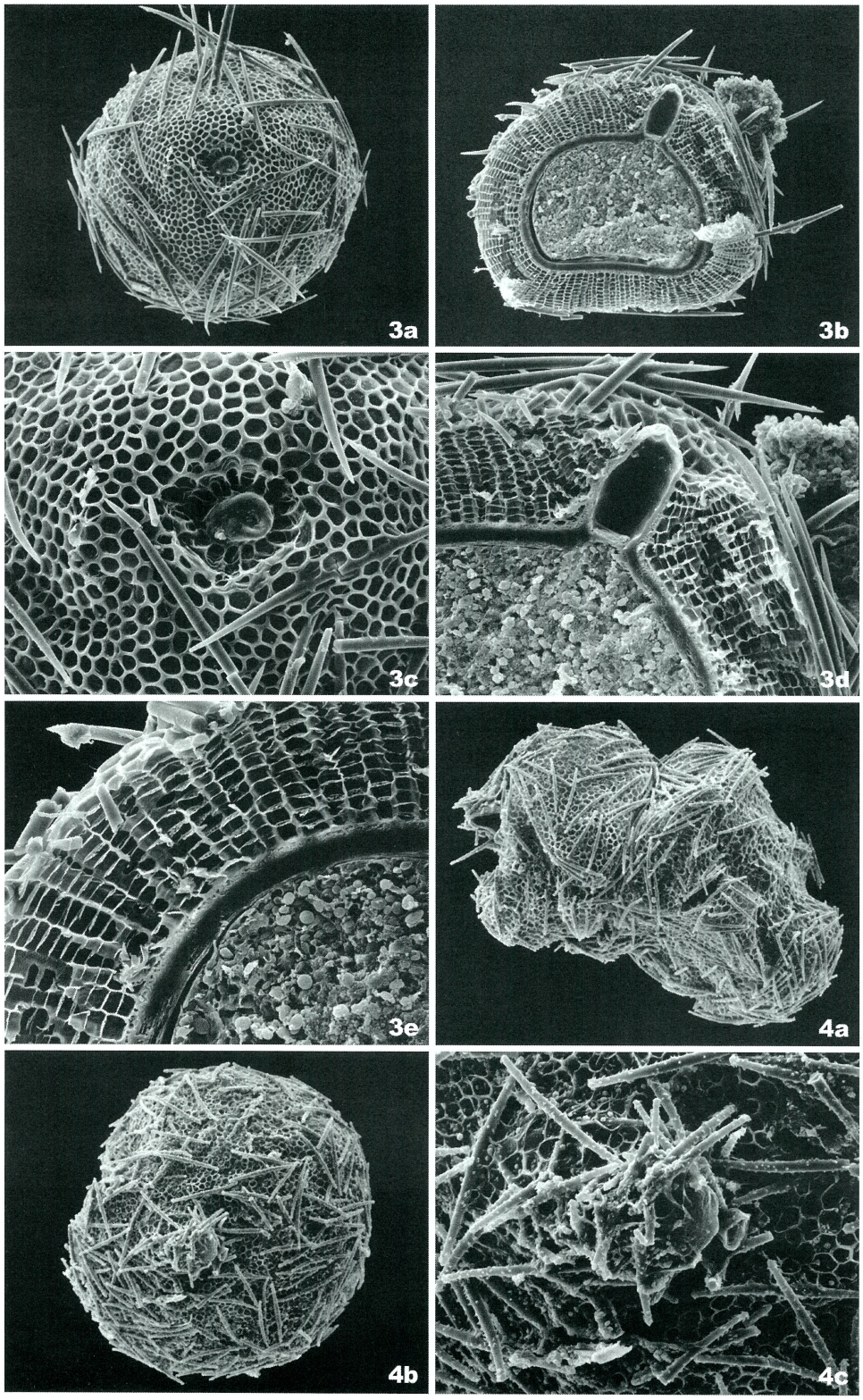
Fig. 7 Spicules of *Trochospongilla latouchiana* Annandale

a. Megascleres. These spicules are entirely smooth and slightly curved amphioxea. ×110

b. Megascleres. Some of these spicules have a small number of microspines (arrows). ×110

c. Gemmoscleres. These spicules are minute biotules with a smooth shaft and have terminal rotules of unequal diameter. ×110

d. Many gemmoscleres and some megascleres. The rotules of the gemmoscleres are circular but of unequal diameter. ×110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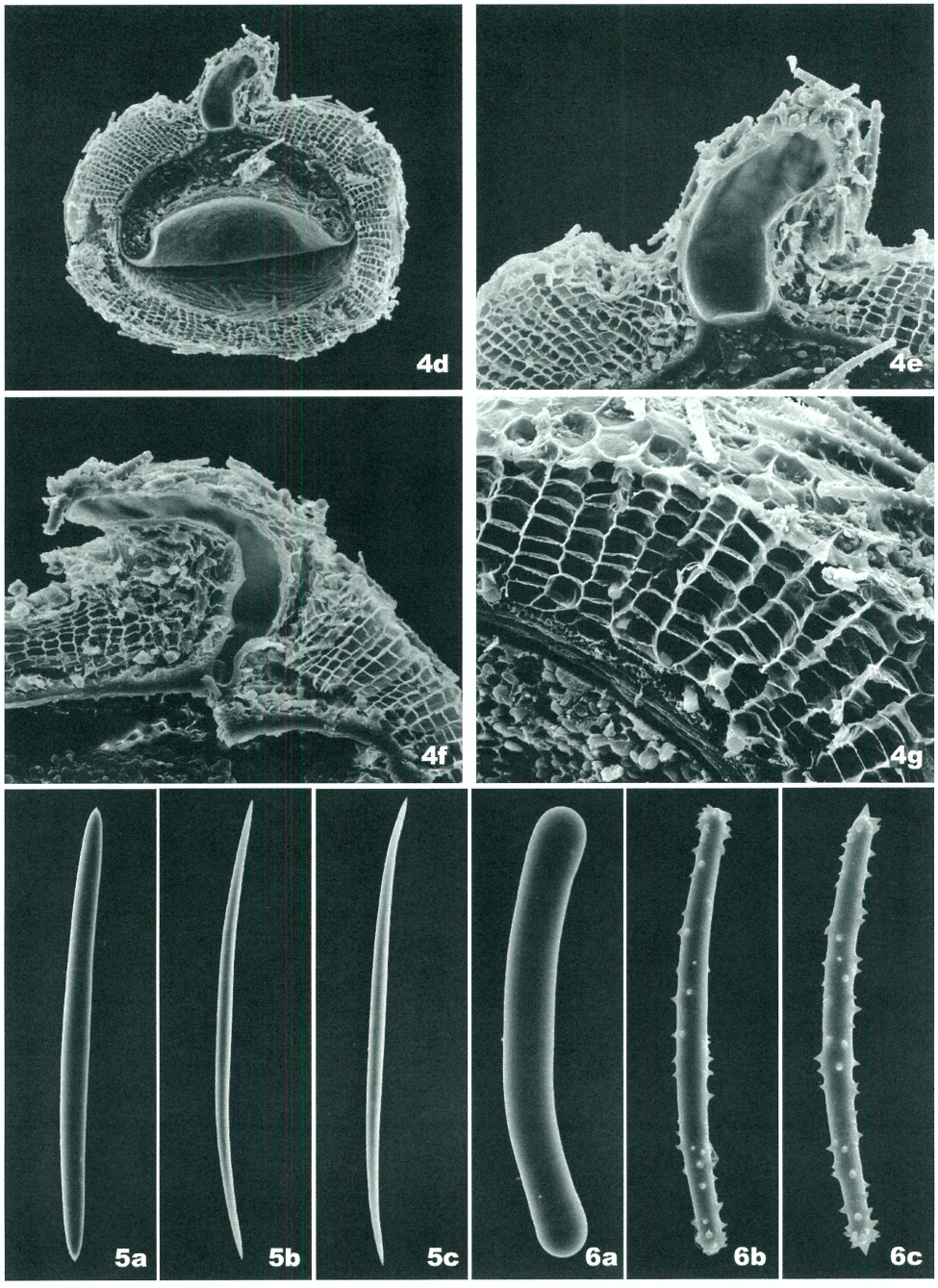
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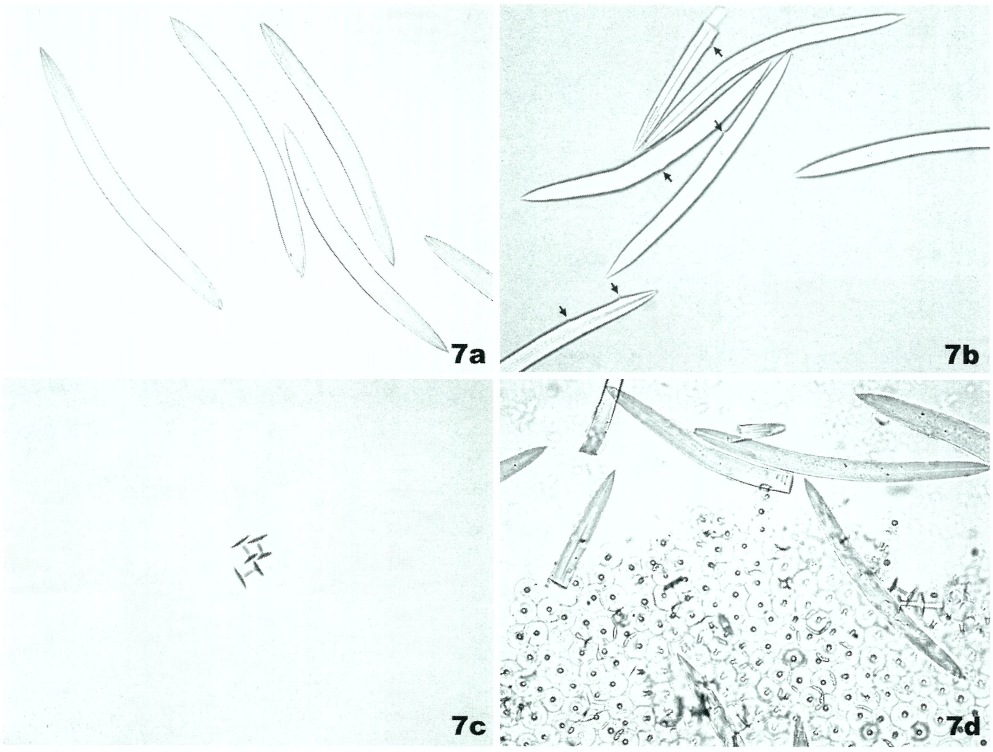

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