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A Study of the Freshwater Sponge, *Ephydatia fluviatilis* (L.), Spongillidae (Demospongiae) from Lake Sampaloc, the Philippines

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Abstract

A detailed study of a sponge which certainly belongs to the genus *Ephydatia*, which was collected from Lake Sampaloc in the Philippines in 1991, was performed. Regarding the genus *Ephydatia* in the Philippines, de Drago found that only the existence of *Ephydatia fluviatilis ramsay* had been recorded there (de Drago, 1975). Our sample, the taxonomical characteristics of which were measured, was observed under a scanning electron microscope, and its specific status was discussed in this study. Until additional data on water quality and the detailed results of observations of the *Ephydatia* group worldwide can be obtained, it seems advisable to treat our sponge from Lake Sampaloc as *Ephydatia fluviatilis* (L.), and we suggest that freshwater sponges of the Philippines now be classified into 7 genera and 15 species.

Introduction

The freshwater sponges of the Philippines have been studied by Annandale N. (1909, 1909),[1-2] Gee N. G. (1931, 1932, 1932, 1933),[3-6] Penney J. T. and Racek A. A. (1968),[7] Racek A. A. (1969),[8] and de Drago I. (1975).[10] Based on their results, these sponges have been classified into 7 genera, 14 species. Since Annandale and Gee's study, however, no additional samples from the Philippines have been collected and recorded. We collected one sample, which certainly belongs to the genus *Ephydatia*, from Lake Sampaloc in 1991. de Drago determined that *Ephydatia fortis* is a synonym for *Ephydatia fluviatilis ramsay*, and that the latter was the only member of the genus *Ephydatia* to have been recorded in the Philippines. In this study, our sample, the taxonomical characteristics of which were measured and which was observed under a scanning electron microscope, is discussed with regard to its specific status.

Materials and Methods

The sponge used in this study was obtained from Lake Sampaloc in the Philippines

(14°15′N, 121°64′E) on March 27, 1991 (Fig. 1), and was fixed with 10% formalin for preservation.

Water parameters: Transparency, air and water temperatute, salinity, electric conductivity and pH were measured in the field, and other parameters were measured in the laboratory using surface water samples. Transparency was measured with a Secchi disk (diameter: 30cm), pH with a pH meter (Yokogawa YEW-PH51), air temperature with a digi-thermo (Sansho CT-2000), and water temperature, salinity and conductivity with the YSI model 33 conductivity meter. The sample water was filtered through Whatman GF/F and then brought to the laboratory in Japan with no more treatment. Measurements of cation concentrations (Na^+ and K^+) were determined by emission spectrophotometry, and Si^{4+}, Ca^{2+} and Mg^{2+} were determined by ICP emission spectrophotometry. Nutrients (NO_3-N and PO_4-P) and F^-, Cl^- and SO_4^{2-} were analyzed by ion chromatography (Dionex QIC).

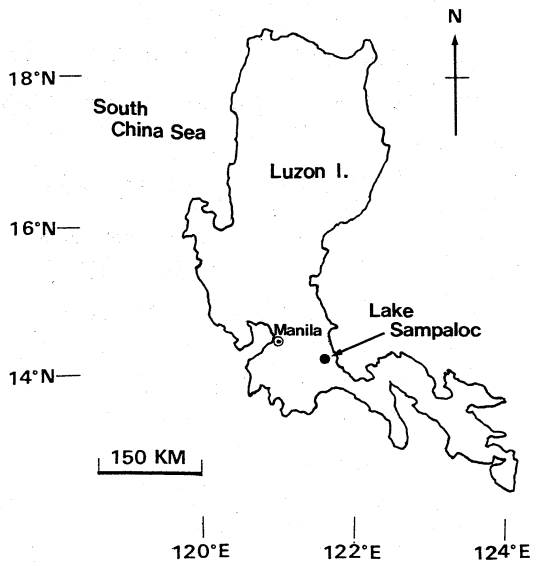


Fig. 1 A map showing the locality of Lake Sampaloc.

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

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

All specimens were coated with gold-palladium alloy and observed with a Hitachi S-570 scanning electron microscope.

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

Results

***Ephydatia fluviatilis* (Linnaeus, 1758)**

Megascleres: Most megascleres were entirely smooth, but a few were covered with small spines except at their tips (Fig. 3, 4, 5). Spines were imperceptible under the low magnification of a light microscope. All the megascleres were slightly curved amphioxea, and ranged from 270 to 365μm in length (mean value 306μm, SD=16.7) and from 12.6 to 19.3μm in width (mean value 16.1μm, SD=1.3).

Microscleres : Microscleres were absent.

Gemmoscleres : Gemmoscleres were biotulates of one class, with rotules of equal diameter (Fig. 7, 8). They ranged from 26.2 to 39.1μm in length (mean value 32.5μm, SD=2.7), as shown in Fig. 2. Their rotules were incised irregularly but not too deeply

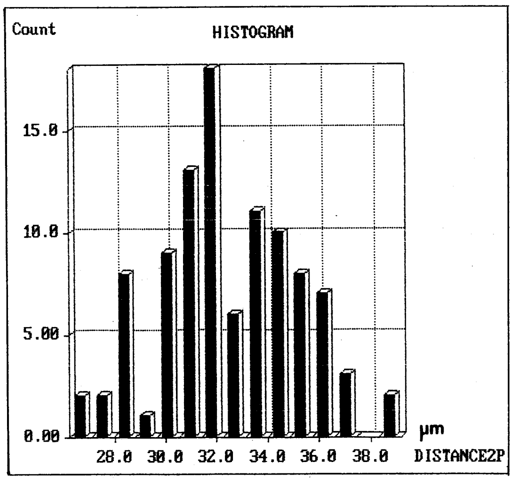


Fig. 2a Length of gemmoscleres.

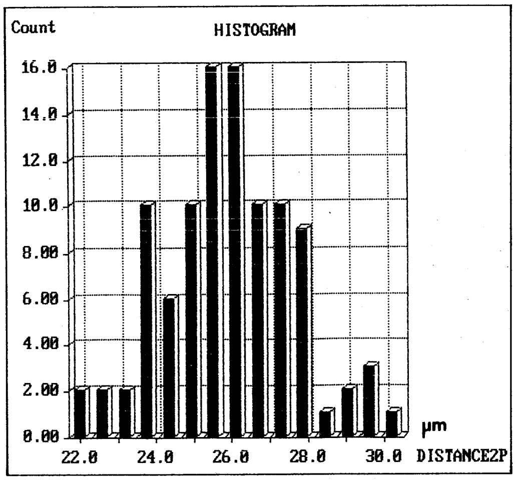


Fig. 2b Diameter of rotules.

(Fig. 6). The outer surfaces of the rotules were flattened and sometimes had microspines on them (Fig. 8). The rotules ranged from 21.7 to 30.4μm (mean value 25.9μm, SD=1.7) in diameter. Most of the shafts had some large spines with some microspines at the middle portion (Fig. 7, 8). A few shafts had no large spines. The diameter of the shaft at the middle portion of those having no spines was a little smaller than near the rotules. The diameter of shafts at the middle portion ranged from 3.9 to 7.5μm (mean value 6.0μm, SD=0.66).

Gemmules : Gemmules were rather abundant and occurred singly and scattered throughout the skeletal meshwork. They were a light tan tinged light yellowish, being only a little yellowish, and were only a little darker than the sponge itself. The gemmules were subspherical and a little depressed from the side of the micropyles (Fig. 9). The greatest diameter range from 397 to 597μm (mean value 513μm, SD=46.8). The thickness of the gemmule coat was regular except for a part of the micropyle. The gemmule coat consisted of a pneumatic layer and outer and inner gemmular layers (Fig. 12). The pneumatic layer was well developed and consisted of subspherical alveoli and a small amount of reticulated thin fibers which existed near the inner gemmular layer and in the interstices surrounded by alveoli (Fig. 13, 14). Individual alveoli were interconnected by some pores (Fig. 14).

Gemmoscleres were embedded in the gemmule coat in one layer and were strictly radial, resting with one rotule on the inner gemmular layer, with the other reaching the outer gemmular layer (Fig. 12).

Table 1 Some water parameters of Lake Sampaloc (16 : 20, 27March, 1991)

	Transparency m	Air temp. °C	Water temp. °C	Salinity ‰	Electric Conduc. μS/cm at 25°C	pH	F^- mg/l	Cl^- mg/l
0m water depth	1.15	29.7	30.0	0	288	8.32	0.277	5.6
10m water depth		—	26.5	0	295	7.6	—	—

	NO_3-N mg/l	PO_4-P mg/l	SO_4^{2-} mg/l	Na^+ mg/l	K^+ mg/l	Ca^{2+} mg/l	Mg^{2+} mg/l	Si^{4+} mg/l
0m water depth	0.015	0.201	2	128	16.2	15.2	18.3	14.9
10m water depth	—	—	—	—	—	—	—	—

Micropyles : Each micropyle was singly situated, slightly elevated, and surrounded by a very narrow collar (Fig. 10, 11). The micropyles were not tubular.

Water parameters

Some of the water parameters of Lake Sampaloc are shown in Table 1. The water was alkaline freshwater, not brackish water. Some species of water plants also inhabited this lake ; that is, *Eichhornia crassipes* (Mart.) Solm-Laub., *Pistia stratiotes* L., *Lemna* sp. and *Nymphaea* sp..

Discussion

There are still some taxonomical problems with the *Ephydatia* group, especially *E. fluviatilis*. In 1968, Penney and Racek comprehensively revised worldwide collections of freshwater sponges of Spongillidae.[7] According to their monograph, the *Ehydatia* group consists of the following nine species; *E. fluviatilis, E. meyeni, E. muelleri, E. japonica, E. ramsay, E. robusta, E. fucunda, E. fortis, and E. millsii*. Based on his experiment in which the morph of the gemmosclere of *E. robusta* was changed to those of *E. robusta* and *E. fluviatilis* under different chemical regimes[9], Poirrier (1974) suggested that *E. robusta* should be considered as a synonym of *E. fluviatilis*. He determined that the 'robusta' morph was the result of a morphological response to the presence of chloride salts and high alkalinity.[9] In 1975, de Drago advocated a new scheme in which the genus *Ephydatia* consisted of the following species : *E. fluviatilis fluviatilis, E. fluviatilis ramsayi, E. muelleri, E. meyeni and E. millsii*.[10] According to his new scheme, *E. ramsay, E. robusta, E. fucunda*, and *E. fortis* should be considered as a synonym of *E. fluviatilis ramsayi* and the dimension and form of gemmosclere of *E. fluviatilis ramsay* should be considered to have great variety. Furthermore, de Drago proposed the recognition of two subspecies, *E. f. fluviatilis* with gemmoscleres$<30\mu$m in length, generally restricted to the northern hemisphere, and *E. f. ramsayi* with gemmoscleres$>$ 30μm in length, restricted to tropical regions including the Philippine Islands and the Southern hemisphere. In reporting on a freshwater sponge from the West Indies, Smith (1994) indicated that his sponge best fit Penney and Racek's description (1968) of *Ephydatia robusta*. But, considering the opinions of Poirrier (1974)[9] and de Drago, he considered that it would be better to treat his sponge as *E. fluviatilis* until more information becomes available.

The *Ephydatia* spp. in the Philippine Islands has been studied by some sponge

Table 2 Historical list of Philippines freshwater sponges

This study(1999)	Annandale(1909)	Gee(1931～33)	Penney and Racek(1968)	Racek(1969)	Drago(1975)
1. *Spongilla alba*	*S. microsclerifera*	*S. microsclerifera*	*S. alba*		
2. *Stratospongilla clementis*	*Spongilla clementis*	*Spongilla clementis*	*Stratospongilla clementis*		
3. *Eunapius carteri*		*Spongilla carteri*	*E. carteri*		
4. *Eunapius fragilis*		*Spongilla fragilis* var. *decipiens*	*E. fragilis*		
5. *Eunapius tinei*		*Spongilla tinei*	*E. tinei*		
6. *Radiospongilla cerebellata*		*Spongilla poliference*	*R. cerebellata*		
7. *Radiospongilla philippinensis*	*Spongilla philippinensis*	*S. philippinensis*	*R. sceptroides ?*	*R. philippinensis*	
8. *Radiospongilla crateriformis*		*Ephydatia crateriformis*	*R. crateriformis*		
9. *Radiospongilla luzonensis*		*Spongilla luzonensis*	*Radiospongilla* sp.		
10. *Ephydatia fluviatilis*					
11. *Ephydatia fortis*		*E. fortis*	*E. fortis*		*E. fluviatilis ramsayi*
Ephydatia fortis		*E. fortis* var. *vorstmani*	*E. fortis*		*E. fluviatilis ramsayi*
12. *Ephydatia meyeni*		*E. fluviatilis* var. *meyeni*	*E. meyeni*		
13. *Dosilia plumosa*		*D. plumosa*	*D. plumosa*		
14. *Trochospongilla latouchiana*		*T. latouchiana* var. *pasignensis*	*T. latouchiana*		
Trochospongilla latouchiana		*T. latouchiana*	*T. latouchiana*		
15. *Trochospongilla phillottiana*		*T. phillottiana* var. *tunguensis*	*T. philottiana*		

scientists (Table 2). Gee first recorded *E. fortis, E. fortis* var. *vorstmani* and *E. fluviatilis* var. *meyeni*.[3-6] Penney and Racek elevated Gee's *E. fluviatilis* var. *meyeni* to *E. meyeni*.[7] According to de Drago's new scheme, the genus *Ephydatia* in the Philippines consists of *E. fluviatilis ramsayi* and *E. meyeni*.

Our sponge can be considered to be closely allied to *E. fortis* of Penney and Racek's description (1968), if the diameters of rotules and gemmules are taken into consideration. However, our sponge differs from *E. fortis* and *E. fortis* var. *vorstmani* with regard to the extent of the length of gemmoscleres. If only the length of the gemmoscleres is taken into consideration, our gemmoscleres could be considered to be closely allied to *E. ramsay* of Penney and Racek's description, but the gemmoscleres have not as many spines on their shaft and the diameters of the our rotules are larger than those of their description. The gemmoscleres of our sponge are closely allied to the typical *Ephydatia* of Penney and Racek's description (1968) in their shape, but the genlmules are a little bigger than those of *Ephydatia*.

According to de Drago's scheme for length of gemmoscleres and geographical distribution, our sponge may fit the classification *E. fluviatilis ramsay*. de Drago mentioned that *E. fluviatilis ramsay*, containing *E. fortis* of Penney and Racek, is restricted to tropical regions and the Southern hemisphere. But we (1993) collected *E. fortis* from the Ryukyu Islands[12] and Sasaki (1941) collected *E. fluviatilis etorohuensis* from the Kuril Islands. Penney and Racek treated the latter as *E. fotis* in their monograph.[13] Typical *E. fluviatilis* was also presented in both island groups.[13]

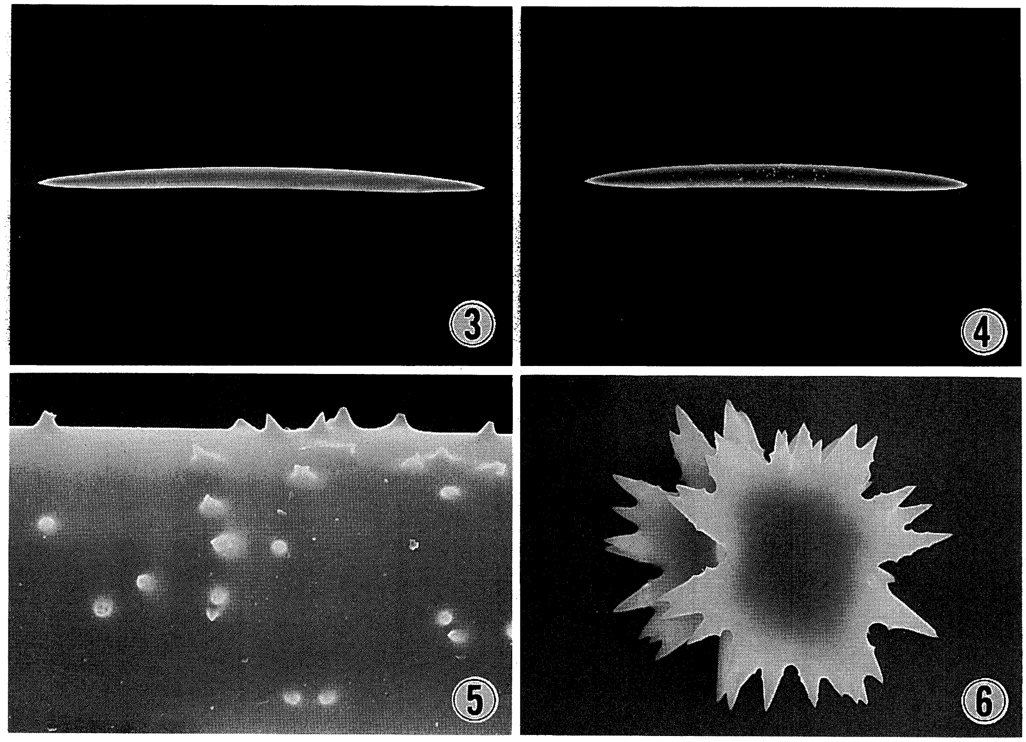
Typical *E. fluviatilis* in Japan has reticulated fibers near the inner gemmular layer. On the other hand, *E. fortis* of Japan has none. Our sponge resembled *E. f fluviatilis* closely in this characteristic.[14]

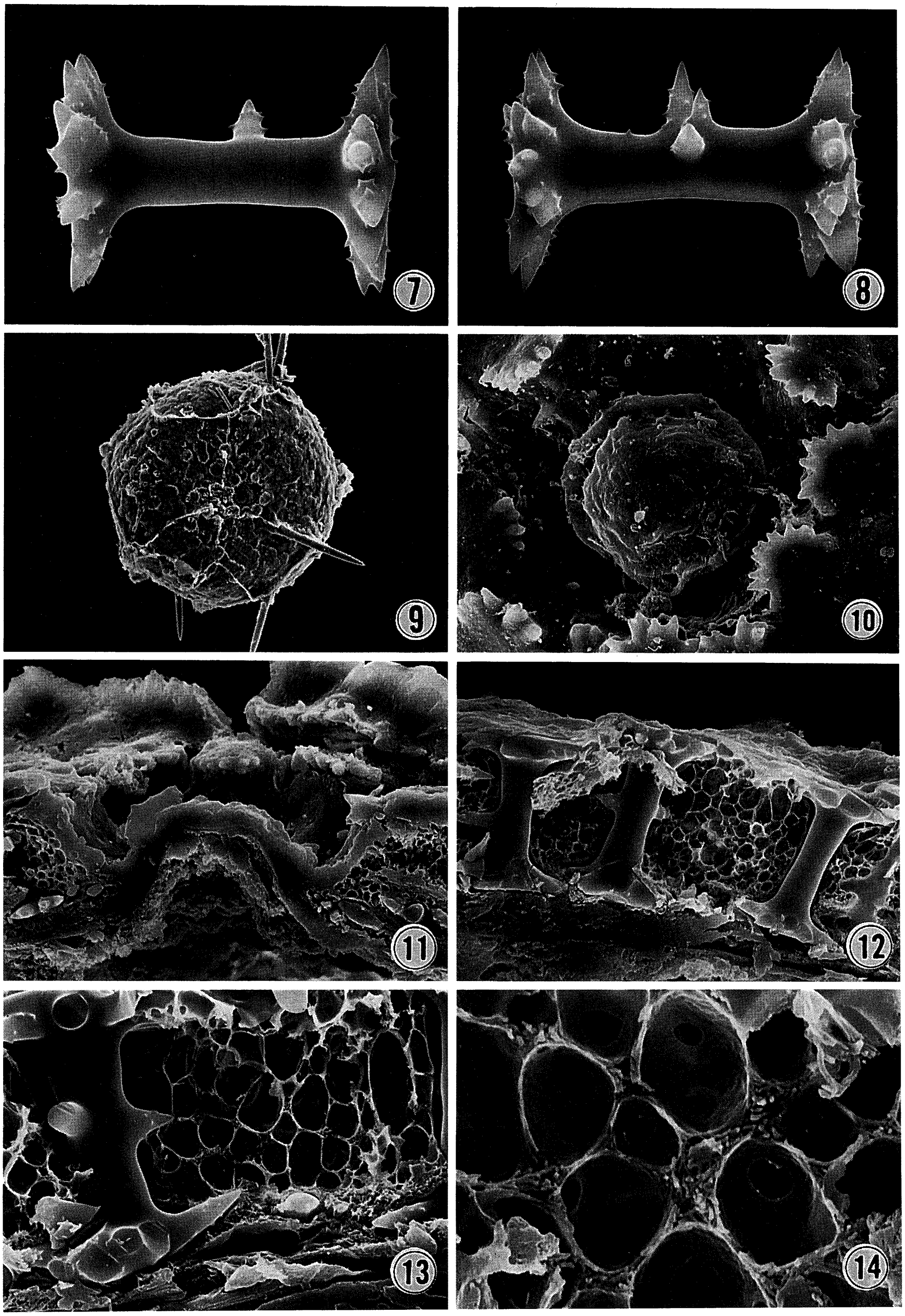
Poirrier suggested that the 'robusta' morph was the result of a morphological response to the presence of chloride salts and high alkalinity.[9] Lake Sampaloc, where our sponge was collected, showed high alkalinity, but a very low concentration of chloride (5.6mg/1). In Japan, a typical *E. fluviatilis* inhabits the estuary of river which flow into a brackish lake, the water of which contains a high concentration of chloride.[15]

As noted above, there are still taxonomical problems to be overcome to detemine the specific status of our sponge clearly. Until additional data on water quality and the detailed results of observations of the *Ephydatia* group worldwide have been obtained, it is advisable to retain their taxonomic status according to Penney and Racek's monograph. Therefore, it is proposed that our sponge from Lake Sampaloc be treated as *Ephydatia fluviatilis* (L.) and that we should record and publish detailed finding with regard to the above problems hereafter.

Regarding Philippine freshwater sponges, Annandale first reported one genus and three species.[1-2] Then, Gee reported 4 genera and 14 species containing Annandale's species.[3-6] According to Penney and Racek's monograph, there are 7 genera and 14 species. Racek (1969) changed *Spongillia philippinensis* to *Radiospongilla philippinensis*.[8] In addition, according to de Drago's scheme, *Ephydatia fortis* may be changed to *Ephydatia fluviatilis ramsay*. However, as already mentioned above, we suggest that Philippine freshwater sponges be classified into 7 genera and 15 species, as shown in Table 2, until additional data on water quality and the detailed results of observations of the *Ephydatia* group worldwide can be obtained.

A part of this study was carried out by the JSPS Cooperation Programs with Southeast Asia Countries under the Core University System.

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Explanation of Figure 3-9

Fig. 3 The megasclere is a fusiform amphioxea and entirely smooth and only slightly curved. ×180

Fig. 4 The megasclere is a fusiform amphioxea with some minute spines. ×180

Fig. 5 An enlarged view of Fig. 4. Some spines were porifurcated. ×2,800

Fig. 6 Upper view of a gemmosclere. The rotules were incised irregularly but not too deeply. ×1,400

Fig. 7 Side view of a gemmosclere. The gemmosclere has rotules of equal diameter. One large spine which has a minute spine on it is seen at the middle portion. ×1,400

Fig. 8 Side view of another gemmosclere. Three large spines are seen at the middle portion. ×1,400

Fig. 9 A gemmule. A micropyle is seen at the center of the gemmule. ×70

Fig. 10 An enlarged view of Fig 6 showing an apical view of a micropyle which is slightly protruding and bears narrow peripheral color. ×560

Fig. 11 A cross section of the micropyle vertically. The micropyle is situated at the center of the saucer-like structure of the outer gemmular layer and slightly protruding from it. ×560

Fig. 12 A part of a cross section of the gemmule coat which consists of outer and inner gemmular layers and a pneumatic layer. Gemmoscleres were embedded in the gemmule coat in one layer and strictly radial, resting with one rotule on the inner gemmular layer, and the other reaching the outer layer. ×700

Fig. 13 A part of a cross section of the inner gemmular layer and pneumaic layer. Reticulated fibers are seen near the inner gemmular layer and a lower rotule of a gemmosclere. ×1,400

Fig. 14 A part of a cross section of the pneumatic layer which consists of subspherical alveoli. Individual alveoli are interconnected by some pores. Fibers are seen in the interstices surrounded by alveoli. ×5,600

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