Scanning Electron Microscopic Observations on Spicules, Gemmule Coats, and Micropyles of the Freshwater Sponge, *Eunapius sinensis* (Annandale)

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Abstract

In Japan, *Eunapius sinensis* was first collected from Lake Kawaguchi in Yamanashi Prefecture and Lake Ashino in Kanagawa Prefecture by Sasaki in 1935. This species was found in the Hyakken River in Okayama Prefecture in 1987, and is here recorded for the third time in Japan.

Based on the structure of its skeletons, this sponge is flat with spreading thin layers without any projecting branches. Megascleres were feebly curved and entirely smooth amphioxea, and were 192-295 μm in length and 8.1-15.3 μm in width. Microscleres were absent. Gemmoscleres were rather robust, usually slightly curved amphioxea, and ranged from being smooth to being covered with a few to many large and irregular spines. They were 37-110 μm in length, and 4.8-8.7μm in width. Malformation of the gemmoscleres was quite frequent.

Gemmules were somewhat depressed, subspherical and formed a pavement layer at the base of the sponge, which firmly adhered to bamboo for support. The pneumatic layer was more or less developed, of varying thickness, consisted of polygonial prismatic alveoli and formed a continuous coat over the gemmules. But the pneumatic layer at the base of the gemmule, which adhered to bamboo for support, lacked alveoli. Gemmoscleres were embedded tangentially in the pneumatic layer in great numbers. Micropyles were tubular. Porus tubes protruded slightly from the surface of the pneumatic layer.

Introduction

To date, about 16 species of *Eunapius* have been reported worldwide [5)6)7)]. Some of these species, however, seem to require reexamination to establish their specific status. Therefore, systematic study of *Eunapius* remains difficult, and a comparative study of *Eunapius* is needed. The first step of such a study is to determine the common characteristics of individual sample by the same method.

Using scanning electron microscopy, we have been studying 13 species of freshwater sponges, and especially the spicules, the structure of the gemmule coat and micropyles as taxonomic characteristics[1)2)3)4)].

Sasaki[7)] first reported the presence of *E. sinensis* in Japan and described it in detail using light microscopy. He collected this species from Lake Kawaguchi (35°30'N, 138° 45'E) in Yamanashi Prefecture and Lake Ashino (35°13′N, 139°01′E) in Kanagawa Prefecture in 1935. Our materials was collected from the Hyakken River in Okayama

Prefecture, and this is the third recording of this species in Japan.

Material and Methods

The sponge used in this study was obtained from the Hyakken River in Okayama Prefecture (34°37′N, 133°59′E) on November 28, 1987. The sponge had degenerated, leaving with gemmules and skeletons. A sponge sample was fixed with 70% ethanol for preservation.

Spicules: A 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. Next spicules were rinsed with distilled water and then with 95% ethanol. One drop of the specimen solution was pipetted from the test tube onto a cover glass mounted on an aluminum stub. The stub was placed in a desiccator and allowed to dry.

Gemmule coats and micropyles: 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: To determine the dimensions of the spicules, the IBAS analysis system (Zeiss) was used morphometrically.

Results

Habitat: *E. sinensis* was collected from the right bank of the Hyakken River near it's estuary. This river is a channel of the Asahi River and runs into Kojima Bay. The sponge adhered to the surface of bamboo which was situated at a depth of about 0.5 meters.

Sponges: The sponge had degenerated, leaving its skeletons and gemmules (Fig. 1). Accordingly, the color of the living sponge and characteristics of oscula and ostia cannot be cited in this report. Based on the structure of its skeletons, this sponge has spreading thin layers without any projecting branches. Primary fibers (vertical fibers) were composed of 3-5 megascleres in cross section and were interconnected in a disorderly manner by secondary fibers (transverse fibers) which were composed of 2-3 megascleres.

Megascleres: The megascleres were all entirely smooth and slightly curved amphioxea (Fig. 3), and were 192-295 μm in length (mean value 260 μm) and 8.1-15.3 μm in width (mean value 11.8 μm).

Microscleres: Microscleres were absent.

Gemmoscleres: The gemmoscleres were rather robust, usually slightly curved amphioxea, and ranged from being smooth to being covered with a few to many large irregular spines (Fig. 4,5). They were 37-110 μm in length (mean value 65 μm)and 4.8-8.7 μm in width (mean value 6.5 μm). Malformation was quite frequent.

Gemmules : The gemmules were moderately abundant, forming a pavement layer at the base of sponge (Fig. 2). Although most of the gemmules adhered to bamboo for support in a single layer, some were stacked on top of the single-layered gemmules (Fig. 6b). All gemmules were connected each other, forming a continuous gemmule coat. However, many interspaces existed among gemmules (Fig. 2). In the lower layer, all of the gemmules had a micropyle on the top of them (Fig. 6a). Some stacked gemmules had micropyles on the upper side of each gemmule (Fig. 6b). Individual gemmules had a rounded outline. The gemmule coat, which consisted of a pneumatic layer and outer and inner gemmular membranes, was unevenly developed (Fig. 6c). At the center of the base of individual gemmules adhering to bamboo for support, the pneumatic coat consisted of gemmoscleres and fibers without alveoli (Fig. 11,12). The layers of alveoli increased in number in the upper part of the gemmules (Fig. 6d). Several pores interconnecting alveoli were present on the upper and lower walls of individual alveoli (Fig. 10). Gemmoscleres were embedded tangentially in the whole pneumatic layer in great numbers (Fig. 6d).

Micropyles : The micropyles were rather short and tubular (Fig 8). Slightly bent porus tubes slightly protruded from the surface of the pneumatic layer (Fig. 7,8).

Discussion

Sasaki[7] reported that the gemmules of *E.sinensis* were of two types ; i.e., a fixed type having alveoli in the pneumatic layer and a free type having no alveoli in the pneumatic layer. The free gemmule type was not present in our sponge. It is unclear at present whether this difference was caused by differences in growth and habitat.

Penney and Racek[5] reported that *Eunapius stanleyi* was the same species as *E. sinensis* and that the distribution of *E. sinensis* was apparently restricted to Asia, ranging from mainland China north to Manchuria and the U.S.S.R. They wrote as follows :"The only criterion differentiating *S. stanleyi*, as described by Annandale, is the shape of the gemmoscleres in both species, being rather irregularly spiny in the former and almost entirely smooth in the latter. The structure, consistency, and skeletal component of both species are fully identical, and so are form, pneumatic coat, and armature of the gemmules. It is obvious that the slightly different gemmoscleres in both species are the result of ecomorphic adaptations, and that the retention of *S. stanleyi* as a separate species is unwarranted. Future detailed workers will have to decide whether it should be differentiated on a subspecific level."

Sasaki's reports[6),7] indicated that both species should not only be differentiated by the shape of gemmoscleres but also by the gemmule coats in both species. We, in this paper, treated *E. sinensis* as a different species from *E. stanleyi*. However, reexamination of the gemmule arrangements and gemmule coats in both species by the same method is needed. Therefore, at present, distribution of *E. sinensis* is considered to encompass Kiangsu and Cheking in China and Japan.

Explanation of figures

Fig. 1. *Eunapius sinensis* was collected on November 28, 1987. The sponge had almost degenerated, leaving skeletons and gemmules. Most of the gemmules were arranged in a single layer. × 2

Fig. 2. An enlarged view of Fig. 1. All gemmules connect with one another, but there are many interspaces among them. The micropyle of each gemmule can be seen at the top of the gemmule. × 23

Fig. 3. Magasclere. This megasclere is entirely smooth, only slightly curved, and fusiform and sharp at both ends (Amphioxea type). × 230

4. Gemmoscleres. Gemmoscleres of variable shape, ranging from smooth to having many spines. × 300

Fig. 5. Gemmosclere.

a. This spicule is robust, fusiform and slightly curved, and has three large spines on its surface. × 500

b-c. Two enlarged views of Fig. 5a. The tips of the spines taper off sharply and are slightly curved. × 2,200

d. A spine of another gemmosclere. This spine is trifurcated near the extremities. × 7,600

Fig. 6. Gemmule arrangement.

a. Upper view of gemmules in a single layer. Each micropyle is seen at the top of the gemmule. Many gemmoscleres are embedded in the surface of gemmules. × 50

b. Four gemmules are stacked on a lower layer of gemmules. Each of their micropyles is seen on the upper side of the gemmule. × 50

c. A cross section of gemmules arranged in a single layer made by cutting through two micropyles connected by the common gemmule coat. ×50

d. A cross section of two gemmules made by cutting through their micropyles. The gemmule coat is unevenly developed. The gemmule coat on the upper part of the gemmule is thicker than it is on the side and lower part. × 130

Fig. 7. Apical view of a micropyle. Several gemmoscleres surround a micropyle tangentially. × 500.

Fig. 8 A longitudinal section of a micropyle. The tubular micropyle slightly bends and protrudes slightly from the gemmule coat. × 500

Fig. 9. A part of a cross section of the thick pneumatic coat consists of alveoli arranged in many tiers. Some gemmoscleres are seen near the inner gemmular membrane and others are on the surface. × 1,300

Fig. 10. A part of a cross section of a gemmule coat consisting of an outer and inner gemmular membrane and a pneumatic layer. Several pores which interconnect the alveoli are seen on the upper and lower walls of individual alveoli. × 2,000.

Fig. 11. A part of a cross section of the base of gemmule coat. The middle part the pneumatic coat lacks alveoli. Many gemmoscleres are arranged tangentially. × 260

Fig. 12. An enlarged view of Fig. 11. Many thin fibers twine round gemmoscleres. × 1,300


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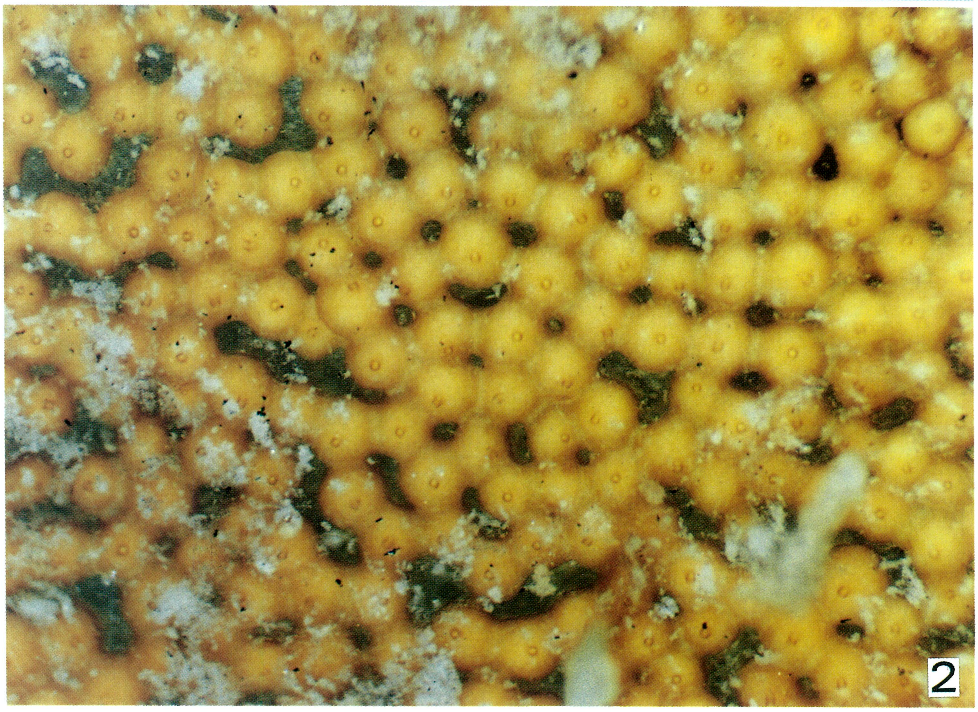
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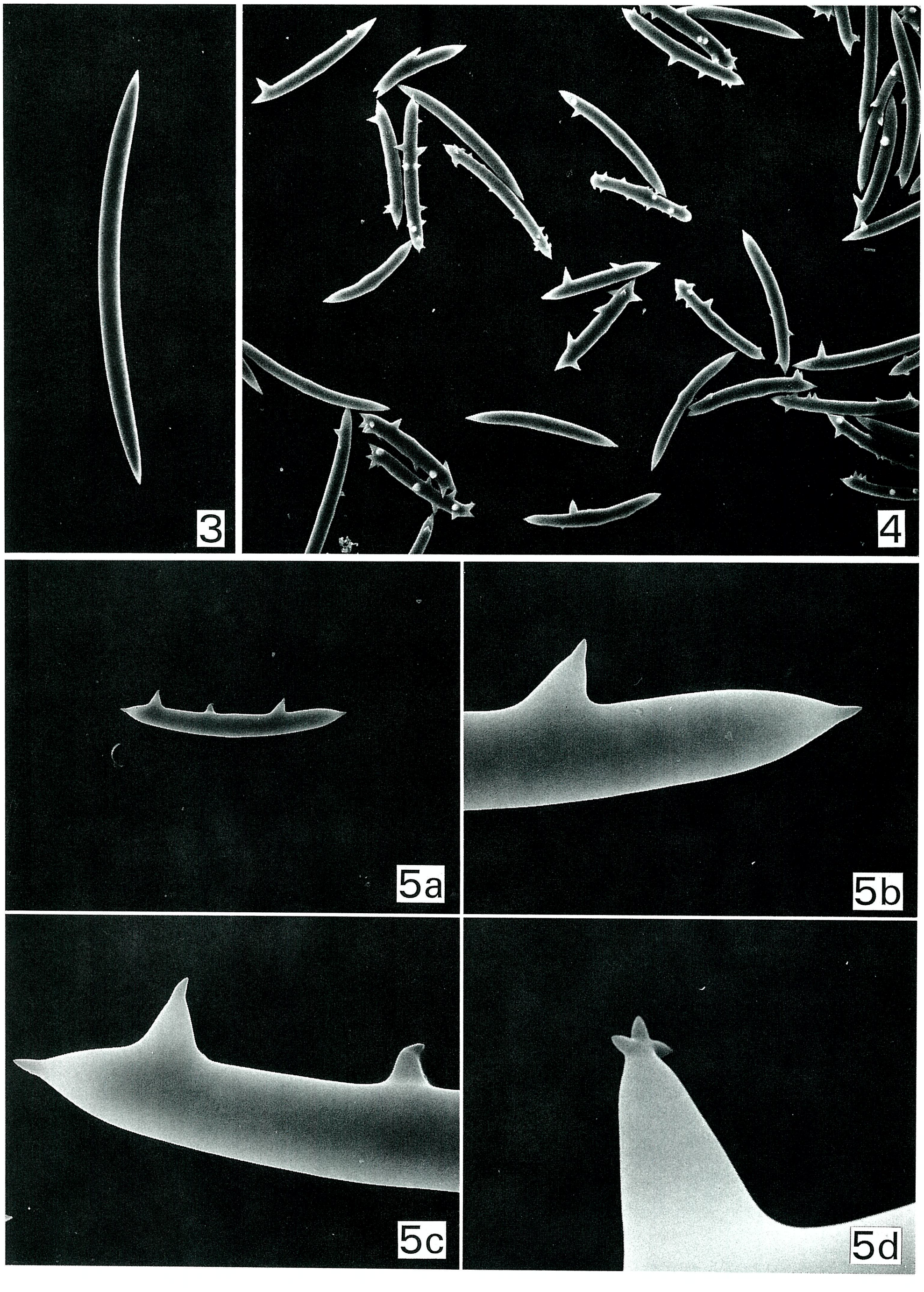
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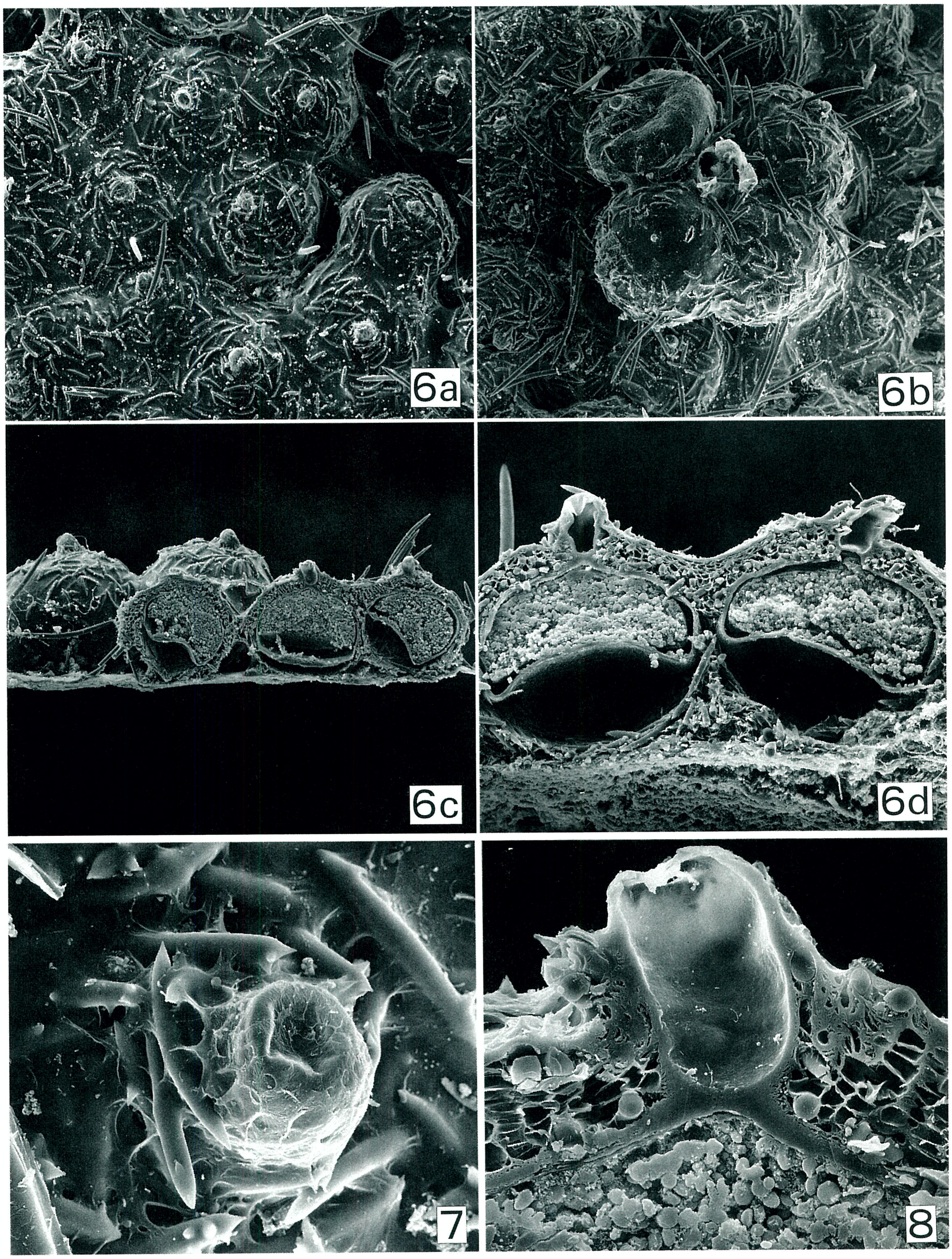
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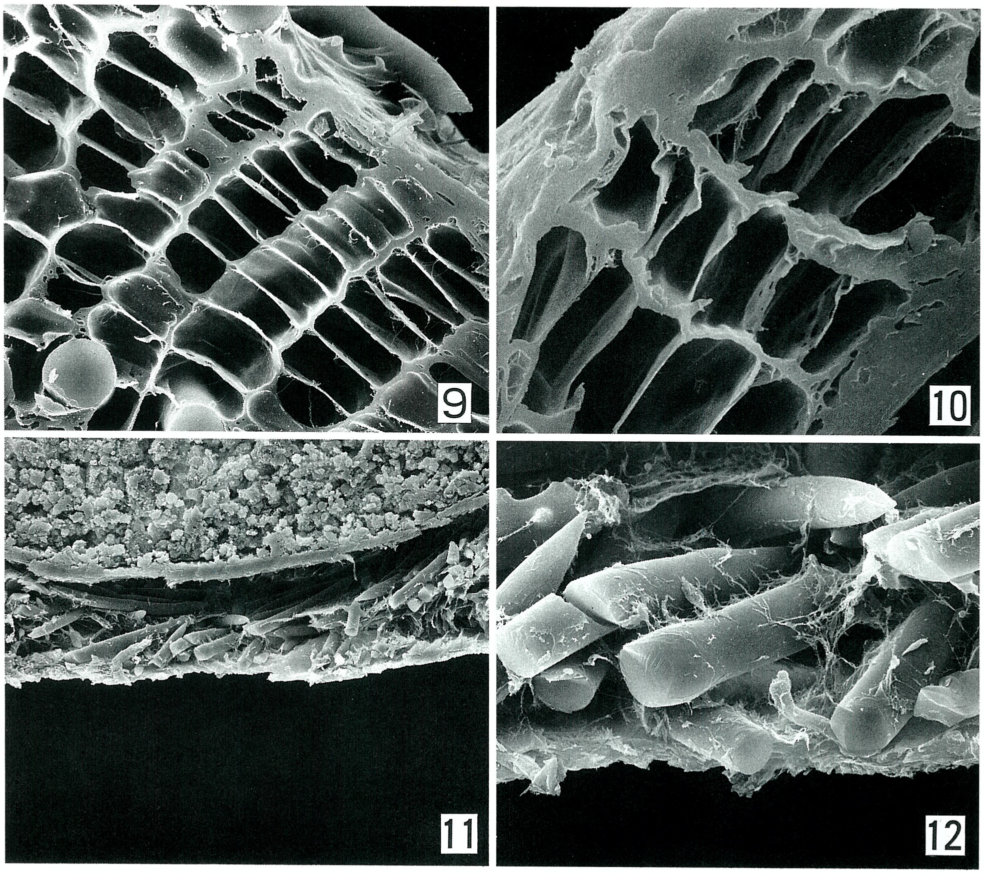

References

[1] Masuda, Y., Y. Takahashi and K. Satoh, 1981, Scanning electron microscopic observation on micropyles and spicules of the fresh-water sponges. Kawasaki Igakkai Shi Liberal Arts & Science Course, (7) : 65-77.

[2] Masuda, Y. and K. Satoh, 1984, Scanning electron microscopic observation on gemmule coats of freshwater sponges. Kawasaki Igakkai Shi Liberal Arts & Science Course, (10) : 56-66.

[3] Masuda, Y. and K. Satoh, 1989, Scanning electron microscopic observations on spicules, gemmule coats, and micropyles of the freshwater sponges, *Spongilla alba* Carter, *Eunapius coniferus* (Annandale) and *Trochospongilla latouchiana* Annandale. Kawasaki Igakkai Shi Liberal Arts & Science Course, (15) : 75-96.

[4] Masuda, Y. and K. Satoh, 1991, Scanning electron microscopic observations on spicules, gemmule coats, and micropyles of the freshwater sponges, *Eunapis ryuensis* (Sasaki), Kawasaki Igakkai Shi Liberal Arts & Science Course, (17) : 35-44.

[5] Penny, J.T. and A.A. Racek, 1968, Comprehensive revision of a world-wide collection of freshwater sponges (Porifera : Spongillidae) United States National Museum Bulletin, **272** : 1-184.

[6] Sasaki, N., 1970, Some fresh-water sponges from Korea. J. Shimonoseki Univ. Fish., **19**(1) : 35-49.

[7] Sasaki, N., 1973, Some fresh-water sponges collected from the middle part of Japan. J. Shimonoseki Univ. Fish., **21**(3) : 31-47.