Scanning Electron Microscopic Observations on Spicules, Gemmule Coats, and Micropyles of the Freshwater Sponges, *Eunapius ryuensis* (Sasaki)

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Abstract

Eunapius ryuensis was first collected in Korea by Sasaki in 1938. This species was recently collected from a pond in Okayama Prefecture, Japan and is here recorded for only the second time. The useful taxonomic characteristics of the spicules, gemmule coats, and micropyles of this species were studied by scanning electron microscopy and the findings are reported.

Gemmules with a rounded conical outline found freely and singly throughout the skeletal network. The pneumatic layer was well but unevenly developed. At the base of each gemmule, this layer consisted of one to several layers of low polygonal prismatic alveoli. There was a gradual increase in the number of layers of alveoli toward the micropyle. Some of gemmoscleres were attached to the whole surface of the gemmule tangentially, whereas others embedded in a pneumatic coat near an inner gemmular membrane tangentially. The micropyle was tubular and traversed the thick pneumatic layer. Megascleres were of two type ; amphioxea and amphystrongyla. Both types were entirely smooth. Microscleres were absent. Gemmoscleres were fusiform with some having sharp ends and others rounded ends. Only a small number recurved spines were noted on the surface.

Our findings corresponded to those of Sasaki with the exception of the diameter of the gemmules, the presence of a pneumatic layer at base of the gemmule and the number of spines on gemmoscleres.

In many respects, *E. ryuensis* resembles *E. coniferus*. The major distinction between the two species is the distribution of gemmoscleres, because the gemmoscleres of the latter are embedded in the pneumatic layer only near the micropyle. The characteristic structure of *E. ryuensis* resembles that of any species of *Eunapius*. Therefore, it is an important species when one study the systematic of *Eunapius*.

Introduction

To date, about 16 species of *Eunapius* have been reported worldwide [6, 9], but some of these species 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 is to study the common characteristics of individual sample by the same method.

We have been studying 12 Japanese species of freshwater sponges by scanning electron microscopy and have laid emphasis on the spicules, the structure of gemmule coat and micropyle as taxonomic characteristics.

Sasaki [8] first reported *E. ryuensis* as a new species and described it in detail using light microscopy. He collected this species from Lake Ryu, Tochomyon Munchongun Hamkyongnamdo in Korea (39°20′N, 127°20′E) in 1938. Our materials were collected from a pond in Japan and this is only the second recording of this species worldwide. Therefore, a comparison of this Japanese specimen with the descriptions of Sasaki and with some species of *Eunapius* which resemble *E. ryuensis* is important.

Materials and Methods

The sponge used in this SEM study was obtained from a small pond (local name: Shin-ike) in Okayama Prefecture (34°36′N, 133°50′E) on 8 February, 1991.

Spicules: In the test tube, spicules were free from the specimens by boiling in concentrated nitric acid and rinsing with distilled water followed by rinsing 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 dessicator and allowed to dry.

Gemmule coats and micropyles: Gemmules were fixed in 2% glutaraldehyde in 0.1M phosphate buffer (pH 7.3) and then fixed in 1% osmium tetroxide in the same buffer. After fixation, the specimens were 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 sections of the gemmules, some of the treated gemmules were cut in two through the micropyle 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 maximum diameter of gemmules and dimensions of each spicule, the IBAS analysis system (Zeiss) was used morphometrically.

Results

Habitat: *E. ryuensis* was collected from a small irrigation pond in a hilly area (about 30 meters above the sea) in the southern part of Okayama Prefecture. *Ephydatia fluviatilis*, *E. japonica*, *Radiospongilla cerebellata* and *Trochospongilla philottiana* were also observed in this pond. *E. fluviatilis* and *R. cerebellata* were found abundantly, but the others were not. The station where *E. ryuensis* was found was near a drainage canal in gentle running water. The sponge was attached to the surface of stones and concrete used for protection of the embankment at a depth of 0.1-0.3 meters.

Sponges: This sponge formed a thin encrusting layer. Its surface was rather smooth (Fig. 1). It was soft and fragile in consistency. Oscula were relatively small and existed at the center of each radial canal. Although ostia were numerous, they were so small that they were indiscernible to the naked eye. Primary fibers (vertical fibers) were composed of 5-7 megascleres in cross section and were interconnected in a disorderly manner by secondary fibers (transverse fibers) which were composed of 2-3

magascleres. As a whole, the skeleton exhibited an irregular network. The color of the living mature sponge grown in light was green, but it was white to light yellow in the shade. Some of sponges began to gemmulate at the beginning of August.

Megascleres : The megascleres were entirely smooth and slightly curved, and were classified to two types i.e., amphioxea and amphistrongyla (Fig. 2). The former were usually longer than the latter ; length range 186-320 μm (mean value 256 μm), width range 10.6-17.6 μm (mean value 14.2 μm).

Microscleres : Microscleres were absent.

Gemmoscleres : The gemmoscleres were feebly curved, fusiform, conical or rounded at both ends. Only a small number of recurved spines often were aggregated near the tip of the spicules (Fig. 3-5) ; length range 71-103 μm (mean value 88 μm), width range 3.6-6.2 μm (mean value 4.8 μm).

Gemmules : The gemmules were moderately abundant in mature sponges, scattered freely, singly, not in groups, throughout the skeletal network. They had a rounded conical outline and an almost flattened base (Fig. 6, 10, 11). The greatest diameter range was 314-413 μm (mean value 368 μm). The gemmule coat, which consisted of a pneumatic layer and outer and inner gemmular membranes was unevenly developed (Fig. 10, 11). The pneumatic layer consisted of stacks of low polygonal prismatic alveoli (Fig. 13, 14). The pneumatic layer at the base of most of the gemmules consisted of several layers of alveoli (Fig. 10, 15). That of a few gemmules consisted of only one layer of alveoli at the center of the base of the gemmules (Fig. 11). The layers of alveoli gradually increased in number toward the micropyle (Fig. 10, 11). Several pores interconnecting alveoli were present on the upper and lower walls of individual alveoli (Fig. 14). Gemmoscleres were arranged in two layers. One group was attached tangentially to the whole surface of the gemmule (Fig. 6, 7). The second group was embedded in the pneumatic layer rather near the inner gemmular membrane (Fig. 13, 15).

Micropyles : The micropyles were situated singly at the top of the gemmule (Fig. 8, 10, 11). They were rather long and tubular (Fig. 12), and traversed the thick pneumatic layer and protruded slightly from the outer gemmular membrane (Fig. 9).

Discussion

E. ryuensis was first reported and described in detail by Sasaki [8]. His description of the characteristics of this species is as follows. "Most of the gemmules scattered freely and singly throughout skeletal meshwork. A few gemmules are attached in groups, but, they never form a continuous gemmule coat. The megascleres can be classified into two types : amphioxea and amphistrongyla. Both types are entirely smooth and straight or slightly bent ; length range 180-340 μm (mean value 268.4 μm), width range 9-15 μm (mean value 11.14 μm). The gemmoscleres are fusiform and straight or slightly bent and have rounded or conical tip at both ends. They are covered with many microspines ; length range 60-150 μm (mean value 88.82 μm), width range 3-5.5 μm (mean value 4.42 μm). The gemmules have a conical or semicircle outline. Their diameter range 180-390 μm (mean value 326.1 μm). Their gemmule coat

is unevenly developed. The base of the gemmule coat is the thinnest and consists of outer and inner gemmular membranes without a pneumatic layer. The gemmule coat thickens gradually toward the micropyle at the top of the gemmule as the layers of alveoli gradually increase in number. The gemmoscleres are distributed tangentially on the surface of the gemmule and in the pneumatic layer. The micropyle is tubular and protrudes slightly from the outer gemmular membrane."

Our Japanese species differs from the Korean species in the following three respects. First, the Japanese gemmules are larger than the Korean. Secondly, the base of the Japanese gemmule coat includes a thin pneumatic layer, whereas, that of Korean species does not. Thirdly, the number of spines on Japanese gemmoscleres is smaller than that on Korean gemmoscleres. In other respects; i.e., the distribution of gemmules in the sponge, the distribution of gemmoscleres and the structure of pneumatic layer, etc., the Japanese species corresponds to the Korean.

In discussing the Korean species, Sasaki reported as follows. "The gemmoscleres resembled those of *Eunapiuis fragilis*, but the gemmules greatly differed from those of *E. fragilis*. In shape, the gemmules resembled those of *Eunapius coniferus*, but the gemmoscleres differed from those of *E. coniferus*, which were entirely smooth."

We agree with Sasaki regarding *E. ryuensis*'s distinction from *E. fragilis*. In the case of *E. coniferus*, however, adoption of the presence of spines on the gemmosclere may be questionable. Certainly, his opinion is correct regarding comparison with the *E. coniferus* species from Japan and Korea, because in both gemmoscleres are entirely smooth [5, 7, 8]. Annandale [1, 2] and Penney and Racek [6], however, reported that gemmoscleres of the Chinese species of *E. coniferus* were covered with spines. Therefore, we suggest adoption of the distribution of gemmoscleres as a characteristic for distinguishing between *E. ryuensis* and *E. coniferus*, because the gemmoscleres of the latter are embedded in the pneumatic layer only near the micropyle.

In many respects, the structures of *E. ryuensis* resemble those of most of the other species of *Eunapius*. Therefore, it is an important and useful species for the systematic study of *Eunapius*.

Acknowledgements

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Explanation of Figures

Fig. 1. *Eunapius ryuensis* was collected on 8 August, 1991. The sponge formed a thin crust over a stone. Its surface is smooth. The color of sponge is bright green. The oscula are inconspicuous in this figure, although they exist at the center of each radial canal. The ostia are indiscernible to the naked eye. The ruler is graduated in millimeters. × 1.3

Fig. 2. Megascleres. a. This spicule is entirely smooth and only slightly curved and fusiform and sharp at both ends (Amphioxea type). × 280. b. This spicule is also entirely smooth and only slightly curved and fusiform and blunt at both ends (Amphistrongyla type). × 280. c. An enlarged view of Fig. 2b showing the tip on one side. × 2,100.

Fig. 3-5. Gemmoscleres. a. These spicules are fusiform and slightly curved, and have some spines on their surface which are rather numerous near the tips of the spicules. × 480. b. Enlarged view of Fig. 3a-5a, respectively, showing recurved spines near the sharp or rounded tip. × 2,400.

Fig. 6. Side view of a gemmule with a rounded triangular outline. A micropyle is seen at the top of the gemmule. Many minute circular depressions are seen uniformly distributed on most of the surface of the gemmule. Many gemmoscleres are attached to whole surface of the gemmule tangentially. × 145.

Fig. 7. Oblique bottom view of a gemmule. Many minute circular depressions are seen on the surface of the gemmule. Several gemmoscleres are attached to the bottom surface of the gemmule tangentially. × 145.

Fig. 8 Apical view of a gemmule. Many gemmoscleres are attached to the surface of the gemmule except in the neighborhood of the micropyles. × 145.

Fig. 9. An enlarged view of Fig. 8 showing a micropyle protruding slightly from the outer gemmular membrane. Many minute circular depressions are seen on the surface in the neighborhood of the micropyle. × 580.

Fig. 10. A cross section of a gemmule cut through a micropyle. The gemmule coat is unevenly developed. It gradually becomes thick toward the micropyle because of an increase in the thickness of the pneumatic layers. The pneumatic layer of the basal gemmule coat consists of several layers of alveoli. × 145.

Fig. 11. A cross section of another gemmule cut through a micropyle. This gemmule coat is also unevenly developed and it is very thin at the center of the base where it consists of only one layer of alveoli. × 145.

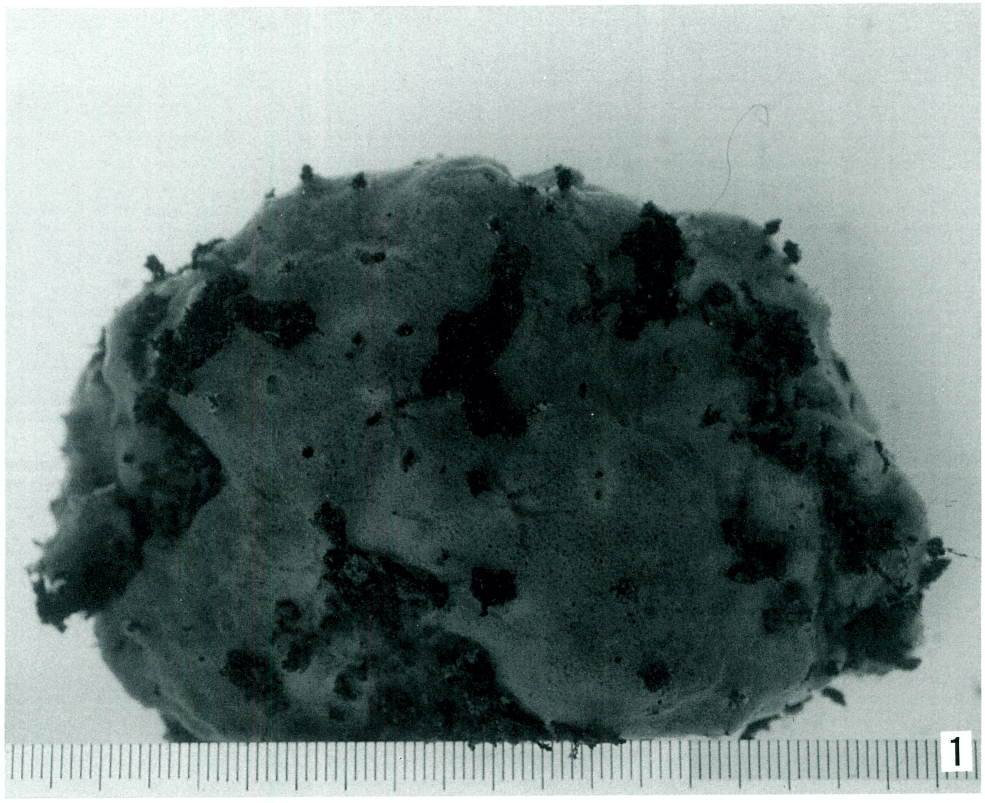
Fig. 12. An enlarged view of Fig. 11. The tubular micropyle slightly bends. Many sections of gemmoscleres are seen at the pneumatic layer near the inner gemmular membrane. × 580.

Fig. 13. A part of a cross section of side of a gemmule coat which consisting of an outer and an inner gemmular membrane and a pneumatic layer. The pneumatic layer consists of alveoli arranged in several tires. Some gemmoscleres are seen on the surface and others are near the inner

gemmular membrane. × 720.

Fig. 14. A part of a cross section of a gemmule coat which is rather near the micropyle. The pneumatic layer is thick and consists of alveoli stacked uniformly. Several pores which interconnect the alveoli are seen on the upper and lower wall of individual alveoli. Two sections of gemmoscleres are seen near the inner gemmular membrane. × 1,440.

Fig. 15. A part of a cross section of the base of a gemmule coat. Several sections of gemmoscleres are seen in the pneumatic layer. The alveoli are somewhat depressed by sectioning of the gemmule. × 720.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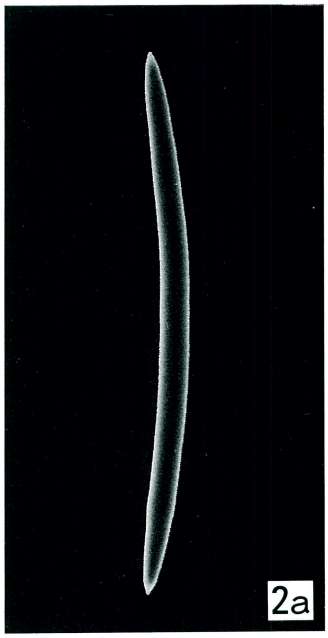
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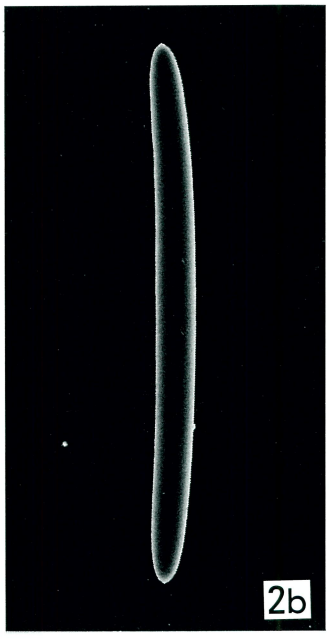
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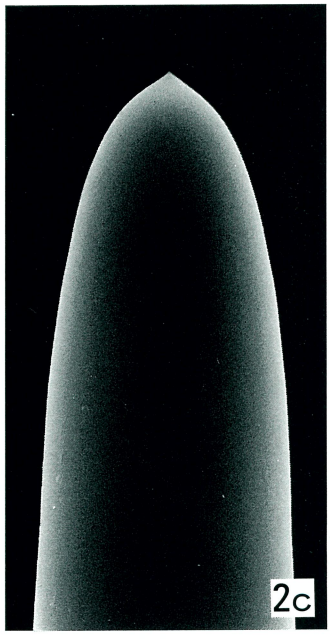
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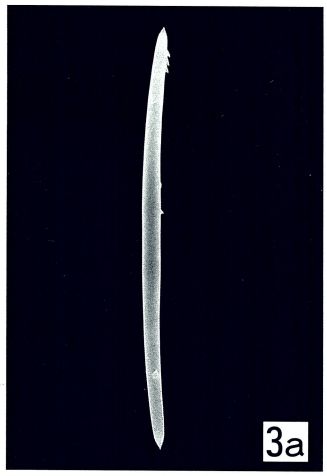
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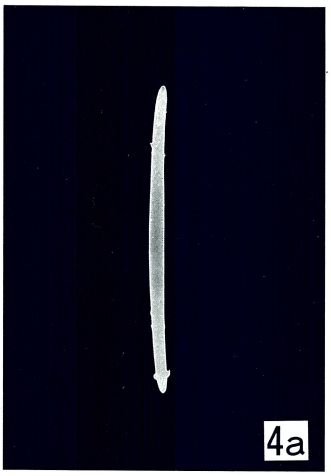
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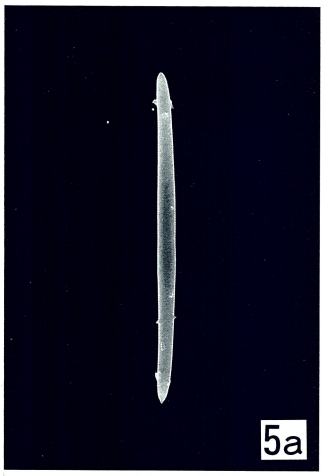
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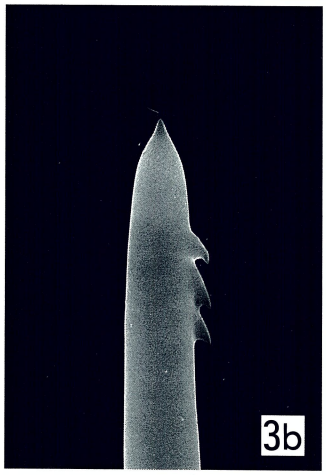
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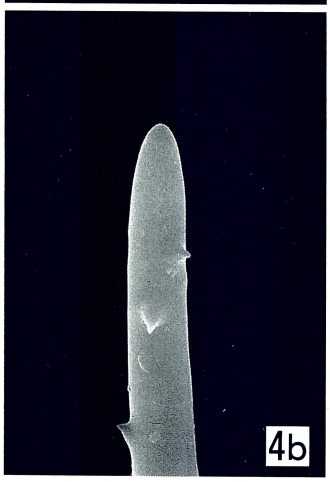
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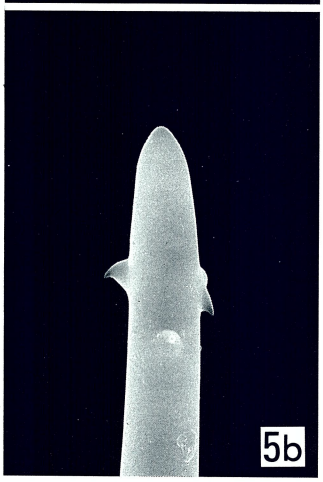
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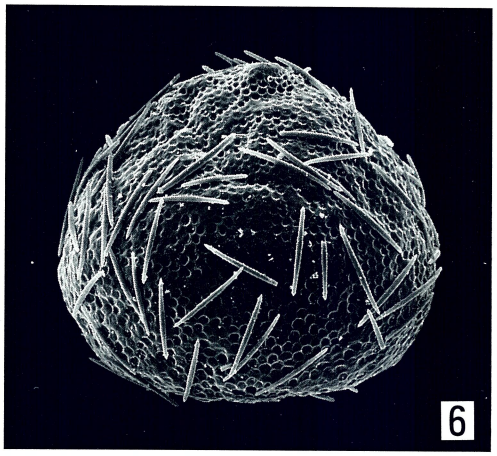
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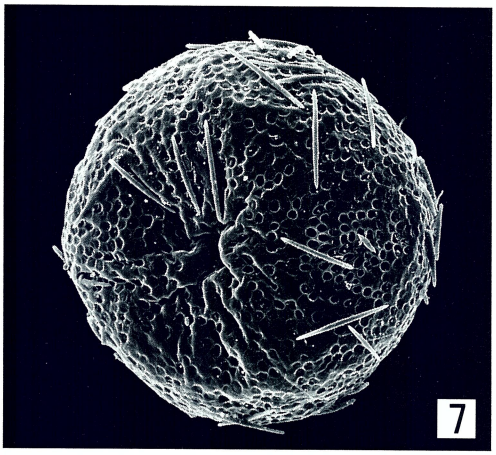
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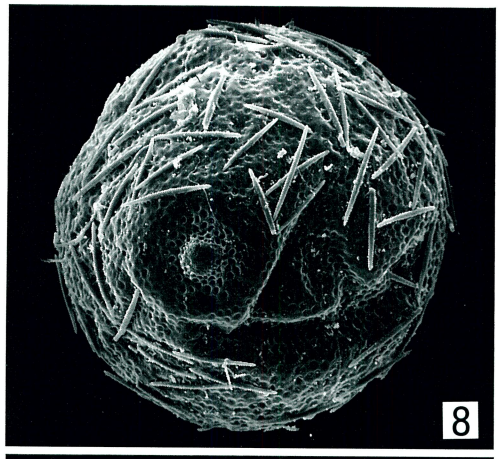
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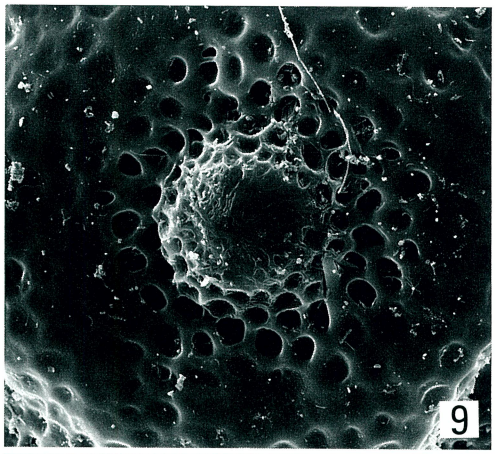
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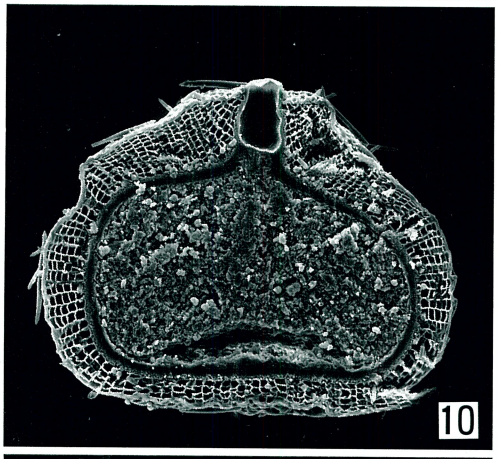
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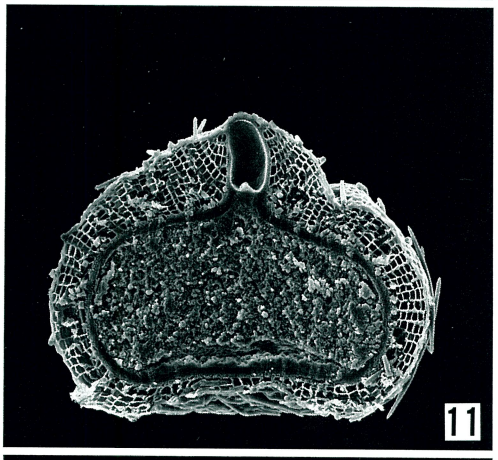
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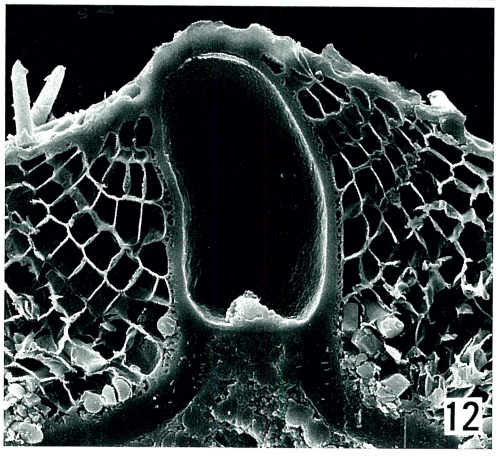
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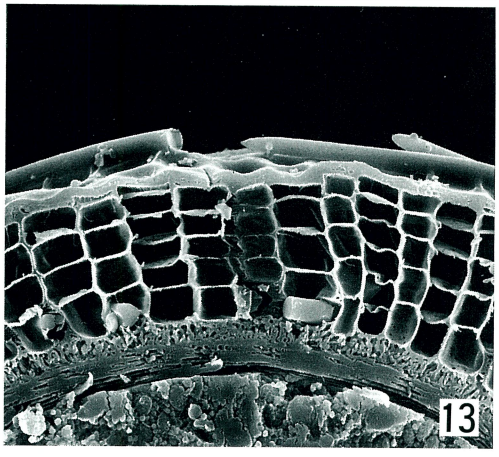
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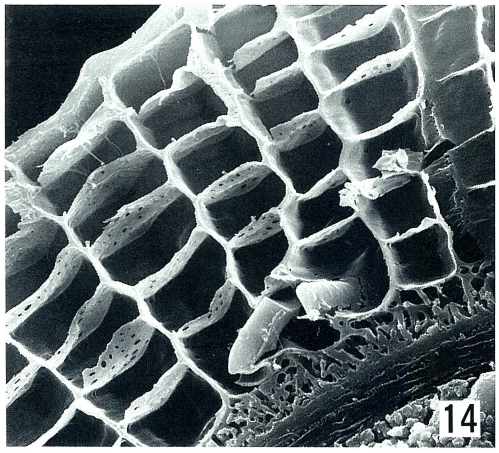

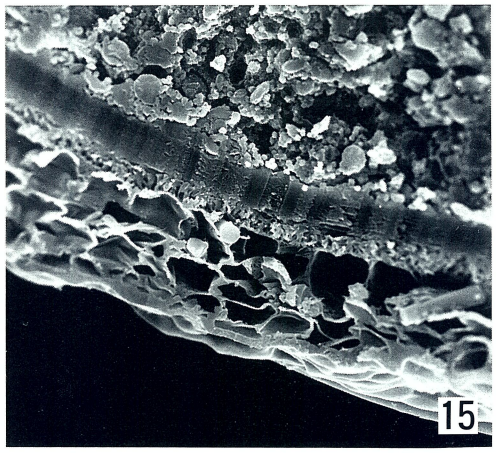

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