Scanning Electron Microscopic Observations on Spicules, Gemmule Coats, and Micropyles of the Freshwater Sponges *Spongilla alba* Carter, *Eunapius coniferus* (Annandale) and *Trochospongilla latouchiana* Annandale

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Abstract

The useful taxonomic characters of spicules, gemmule coats, and micropyles of the freshwater sponges *Spongilla alba* Carter, *Eunapius coniferus* (Annandale), and *Trochospongilla latouchiana* Annandale were observed using scanning electron microscopy. All materials were collected from habitats in Japan. *T. latouchiana* is here recorded for the first time as occurring in Japan.

Scanning electron microscopy revealed clearly the structure of the taxonomic characters of the three species. The characteristic fine structures, some of which are difficult to discern with light microscopy, and which seemed to be useful taxonomic characters of intraspecific population were as follows. *S. alba:* 1. Megascleres had microspines on their surface. 2. The micropyle consisted of minute subspherical alveoli which resembled those of the pneumatic layer. *E. coniferus:* 1. Gemmoscleres were completely smooth. 2. The base of the gemmule coat consisted of an outer and inner gemmular membrane without a pneumatic layer. 3. The layers of alveoli, which were low hexagonal prisms, increased in number toward the micropyle. *T. latouchiana:* 1. Megascleres had spines on their surface. 2. The upper rotule of the gemmoscleres had many minute recurved spines on its margin and the lower rotule had a few minute spines on the margin. 3. The micropyle was slightly elevated, surrounded by a narrow collar, and situated at the center of a saucer-like structure of the outer gemmular membrane.

Introduction

Sasaki [56-63] observed in detail almost all of the Japanese freshwater sponges with light microscopy (LM) and demonstrated their taxonomic and morphological characteristics with clear figures. Harrison observed the gemmule coat of *Stratospongilla penneyi* [40] and the micropyles of *Heteromeyenia tubisserma* [41] with scanning electron microscopy (SEM). Rollins et al. [55] observed the spicules of five species of Minnesota sponges with SEM. Harrison [41] demonstrated that the application of SEM to systematic studies of freshwater sponges provided diagnostic capabilities not possible with LM. We also observed with SEM the taxonomic and morphological characteristics i. e., spicules, gemmule coats, and micropyles of freshwater sponges which were collected from western Japan [44-46]. Re-

cently, Poirrier et al. observed with SEM the microsclere structure in *Spongilla alba, S. cenota,* and *S. lacustris* and discussed the relationship among their materials [50].

The three species of this study are rare sponges in Japan. The method of preparing specimens for SEM of the micropyles and gemmule coat is easier and faster than in LM. We could also observe surface structures and ultrastructures clearly. The purpose of this study was to demonstrate the profiles of spicules, gemmule coats and micropyles of Japanese specimens of these three species. No SEM descriptions of the same three species from other countries have been reported except for the microscleres of *S. alba* [50]. Therefore, we compared our results with LM descriptions of the three species.

Materials and Methods

Sponges used in the SEM studies were obtained from the following localities. *S. alba:* Lake Hinuma (a brackish lake) in Ibaragi Prefecture (36°16′N, 140°30′E) on 26 March 1984. *E. coniferus:* a small pond (local name: Yokoo-ike) in Okayama Prefecture (34°41′N, 133°52′E) on 12 November 1988. *T. latouchiana:* the narrow channel to Lake Biwa in Shiga Prefecture (35°03′N, 135°56′E) on 21 January 1989.

Spicules: Spicules were freed from the sponge specimens by boiling in concentrated nitric acid and rinsing with distilled water followed by 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 buffe (pH 7.3) and then fixed in 1% osmium tetroxide in the same buffer. After the fixation, the specimens were dehydrated in an ethanol series, replaced with isoamyl acetate, and dried by a critical point drying method with a Hitachi HC-1. 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.

Results

Spongilla alba Carter, 1849

Spongilla alba: Carter, 1849, p. 83; 1881, p. 88 — Bowerbank, 1863, p. 463 — Gray, 1867, p. 553 — Potts, 1887, p. 193 — Weltner, 1895, p. 116 — Annandale, 1907a, p. 26; 1907b, p. 388; 1911, p. 76; 1912, p. 384; 1918, p. 211 — Gee, 1930b, p. 67; 1931a, p. 31; 1932a, p. 36 — Arndt, 1932, p. 550; 1936, p. 14 — Schröder, 1935, p. 105 — Penney, 1960, p. 12 — Penney and Racek, 1968, p. 16 — Racek, 1969, p. 271 — Poirrier, 1976, p. 203 — Poirrier et al., 1987, p. 302.

Spongilla lacustris var. *bengalensis:* Annandale, 1906, p. 56; 1907b, p. 389; 1915, p. 25 — Gee, 1931a, p. 33 — Penney and Racek, 1968, p. 16.

Spongilla alba var. *marina:* Annandale, 1907b, p. 389 — Gee, 1931a, p. 42 — Penney and Racek, 1968, p. 16.

Spongilla microsclerifera: Annandale, 1909b, p. 131; 1911, p. 53; 1918, p. 211 — Gee, 1931a, p. 43; 1931b, p. 69; 1932a, p. 41; 1932d, p. 507 — Penney, 1960, p. 25 — Penney and Racek, 1968,

p. 16.

Spongilla travancorica: Annandale, 1909a, p. 101; 1911, p. 81; 1912, p. 384; 1915, p. 26 — Gee, 1931a, p. 51 — Penney and Racek, 1968, p. 16.

Spongilla alba var. *bengalensis:* Annandale, 1911, p. 77; 1915, p. 26 — Gee, 1931a, p. 33 — Penney and Racek, 1968, p. 16.

Spongilla nana: Annandale, 1915, p. 32; 1918, p. 208 — Gee, 1930a, p. 88; 1931a, p. 44; 1932a, p. 41; 1932e, p. 299 — Penney, 1960, p. 25 — Penney and Racek, 1968, p. 16.

Spongilla alba var. *rhadinea:* Annandale, 1919a, p. 85 — Gee, 1931a, p. 47; 1932a, p. 36 — Penney and Racek, 1968, p. 16.

Spongilla wagneri: Potts, 1889, p. 7 — Weltner, 1895, p. 120 — Smith, 1922, p. 106 — Gee, 1931, p. 52 — Eshleman, 1950, p. 36 — Penney, 1960, p. 31 — Penney and Racek, 1968, p. 20 — Poirrier, 1976, p. 205 — Volkmer-Ribeiro, 1987, p. 225.

Megascleres: The megascleres were fusiform amphioxeas, slightly curved, sometimes angularly bent (Fig. 5a), and rather slender. Minute conical spines which were rather difficult to discern with LM (Fig. 1) existed on their surface (Fig 5b, c). It was very rare that a smooth one could be found with SEM.

Microscleres: The microscleres were slender, spindle shaped, and slightly curved (Fig. 7d). The tips of the spicules were blunt with of small recurved spines (Fig. 7e). They were covered throughout their lengths with two types of spines. One type, which was found everywhere except at tips of the spicules comprised complex spines which had a smooth pedicel with an apical burr (Fig. 7f). The other type, which was found near both ends of the spicules, comprised recurved spines (Fig. 7e).

Gemmoscleres: The gemmoscleres were stout, rather long, cilindrical, and feebly bent (Fig. 7a, b, c). They were covered with large recurved sharp spines which were often more numerous at the tips of the spicules.

Gemmules: The gemmules were usually large and spherical (Fig. 9a). The gemmule coat consisted of the outer gemmular membrane, a pneumatic layer, and the inner gemmular membrane (Fig. 9e). The outer gemmular membrane was very thin (Fig. 9f). The pneumatic layer, which consisted of very minute subspherical alveoli, was moderately thick. Gemmoscleres were embedded in this layer sparsely and at irregular angles (Fig. 9e). Their tips often projected beyond the outer gemmular membrane (Fig. 9a, b).

Micropyles: Each micropyle was situated singly and protruded slightly at the center of a saucer-like depression in the outer gemmular membrane where the gemmule coat was thin and the inner gemmular membrane protruded slightly (Fig. 9a, b, c). The micropyle was short and tubular and consisted of minute alveoli which resembled those of the neighboring pneumatic layer (Fig. 9d). The individual alveoli of the micropyle were interconnected by pores, as were those of the pneumatic layer.

Eunapius coniferus (Annandale, 1916)

Spongilla conifera: Annandale, 1916, p. 51; 1918, p. 203 — Gee and Wu, 1927, p. 8 — Gee, 1931a, p. 36; 1932a, p. 37; 1932c, p. 54 — Penney, 1960, p. 15 — Penney and Racek, 1968, p. 33 — Sasaki, 1969, p. 163.

Eunapius coniferus: Penney and Racek, 1968, p. 33.

Megascleres: The megascleres were rather short and fusiform amphioxeas and stout amphistrongyles, entirely smooth, and only slightly curved (Fig. 4a, b).

Microscleres: Microscleres were absent.

Gemmoscleres: The gemmoscleres were feebly curved, fusiform, conical at both ends, and entirely smooth (Fig. 4c).

Gemmules: The gemmules had a conical outline (Fig. 10a) and a flattened base (Fig. 10b, c). The gemmule coat was unevenly developed (Fig. 10c). The base of the gemmule coat consisted of an outer and inner gemmular membrane without a pneumatic layer (Fig. 10e). The outer gemmular membrane was thick and had many minute circular depressions distributed uniformly on its surface (Fig. 10a, b). At the upper part of the gemmule, the gemmule coat consisted of a pneumatic coat and outer and inner gemmular membranes. The layers of alveoli, which were a low hexagonal prisms (Fig. 10f), gradually increased in number toward the micropyle (Fig. 10c). Several pores interconnecting alveoli were present on the upper and lower wall of individual alveoli (Fig. 10f). A few gemmoscleres were embedded in the pneumatic layer near the micropyle, more or less tangentially.

Micropyles: Each micropyle was situated singly at the top of the gemmule. It was long and tubular, traversed the thick pneumatic layer, and protruded slightly from the outer gemmular membrane (Fig. 10a, c, d).

Trochospongilla latouchiana Annandale, 1907

Trochospongilla latouchiana: Annandale, 1907a, p. 21; 1908, p. 157; 1911, p. 115; 1918, p. 201 — Gee, 1926a, p. 110; 1926b, p. 181; 1927, p. 60; 1928, p. 225; 1929, p. 299; 1930b, p. 98; 1930c, p. 28; 1931a, p. 41; 1932a, p. 42, 1932b, p. 10; 1932c, p. 54; 1932d, p. 507; 1932a, p. 44 — Gee and Wu, 1927, p. 11 — Vorstman, 1927, p. 184; 1928, p. 116 — Rao, 1929, p. 269 — Arndt, 1932, p. 564; 1936, p. 10 — Schröder, 1935, p. 104 — Penney, 1960, p. 56 — Sasaki, 1967, p. 41 — Penney and Racek, 1968, p. 140 — Racek, 1969, p. 300.

Trochospongilla latouchiana subsp. *sinensis:* Annandale, 1919b, p. 457 — Gee and Wu, 1925, p. 226; 1927, p. 11 — Gee, 1926b, p. 181; 1931a, p. 49; 1932b, p. 13 — Penney and Racek, 1968, p. 140.

Trochospongilla latouchiana var. *pasigensis:* Gee, 1932a, p. 42; 1932b, p. 14; 1932d, p. 507 — Penney, 1960, p. 56 — Penney and Racek, 1968, p. 140.

Megascleres: The megascleres were almost straight amphioxeas, distinctly fusiform, and with small acute spines (Fig. 2) on their surface except at their tips (Fig. 6a, c). The number of spines on each spicule varied widely. In SEM, smooth megascleres were rare. Most of the spines curved inward from near their tips to approximately the middle one-third of the spicules (Fig. 6c). Most of the spines on the middle third of the surface of the spicules were direct conical spines (Fig. 6b).

Microscleres: Microscleres were absent.

Gemmoscleres: The gemmoscleres were minute birotulates with a slender, smooth shaft and terminating in circular rotules of unequal diameter (Fig. 3a, b; Fig. 8a, b). Upper rotules were smaller than lower rotules. Both rotules were considerably recurved at the

marginal portion (Fig. 8a, b). Minute spines, which were discernible only with difficulty under the light microscope (Fig. 3a), were seen on the margin (Fig. 8e, f). The number of spines on the upper rotules was far greater than on the lower ones (Fig. 8b).

Gemmules: The gemmules were small and subspherical but somewhat puckered at the portion where the micropyle was situated (Fig. 11a, b, c). The pneumatic layer was relatively thin and consisted of large, subspherical alveoli. Individual alveoli were interconnected by pores (Fig. 11d, f). Gemmoscleres were embedded in this coat in one layer.

Micropyles: Each micropyle was singly situated, slightly elevated, and surrounded by a narrow collar situated at the center of a saucer-like structure of the outer gemmular membrane (Fig. 11e).

Discussion

Spongilla alba Carter

1) Distribution

India: Carter, 1849; 1881 — Bowerbank, 1863 — Weltner, 1895 — Annandale, 1906; 1907a; 1907b; 1909a; 1911; 1912; 1915 — Gee, 1930a; 1930b; 1931a; 1932a; 1932e — Penney, 1960 — Penney and Racek, 1968 — Sasaki, 1973. **Philippine Islands:** Annandale, 1909b; 1918 — Gee, 1931a; 1931b; 1932d; 1933. **Perso-Afghan Frontier:** Annandale, 1919a — Gee, 1932a. **Thailand:** Gee, 1930a; 1932a; 1932e. **Indonesia:**Arndt, 1932 — Gee, 1932a. **Egypt:** Gee, 1932a — Penney, 1960 — Sasaki, 1973. **Madagascar:** Schröder, 1935 — Sasaki, 1973. **Australia:** Penney and Racek, 1968 — Racek, 1969 — Sasaki, 1973. **Brazil:** Weltner, 1985 — Penney, 1960. **South America:** Penney and Racek, 1968 — Sasaki, 1973. **U. S. A.:** Potts, 1889 — Gee, 1931a, 1932a — Eshleman, 1950 — Smith, 1922 — Penney, 1960 — Poirrier, 1976 — Poirrier et al., 1987. **Marina Islands:** Sasaki, 1973. **Japan:** Sasaki, 1973.

The *S. alba* is distributed in Asia, Africa, Australia, South America and Central America. Judged from this range, Lake Hinuma (36°16′N, 140°30′E) in japan seems to be the northern limit of this species. The habitats of *S. alba* are usually brackish areas. Lake Hinuma is also a brackish lake near the Pacific Ocean. Until recently, this lake was the only known habitat of this species in Japan. But, we were able to collect this species in Lake Shinji (35°31′N, 132°56′E) in Shimane Prefecture on 30 July 1989 (unpublished). This lake is also a brackish lake near the Sea of Japan.

2) Spicules

Carter [17, 18], Annandale [1, 5, 7], Gee [27, 30] and Penney and Racek [48] reported that the megascleres were completely smooth. Arndt [14] wrote as follows: (*sic*) "Die meisten von ihnen sind schwach rauh, doch kommen auch vereinzelte ganz glatte vor." Schröder [64] wrote as follows: (*sic*) "Nur vereinzelt beobachtete ich Nadeln mit wenigen feinen Dornen." Annandale [9] and Gee [35] reported that some megascleres of *S. nana* Annandale ,which was placed in the synonymy of *S. alba* [48], were sparsely and minutely spiny. Sasaki [63] reported that in LM, the megascleres were completely smooth or had very minute spines sparsely on their surface. He observed specimens from the same habitat (Lake Hinuma) as we did. As expected, our results corresponded to those of Sasaki. We add that in SEM, smooth megascleres were very few because even spicules bearing a few

spines on the surface could be discerned, and the spines of the megascleres were conical (Fig. 5b, c). Our observations on the microscleres were similar to those of Poirrier et al. [50] in respect to the spines on the surface (Fig. 7d, e, f).

3) Gemmule coat and micropyle

Annandale [3, 7], Gee [27] and Penney and Racek [48] reported that the micropyle was never tubular and often bore a shallow peripheral collar. Annandale [5, 7] reported that the micropyle of *S. travancorica,* which is a synonym of *S. alba,* was straight and conical. Sasaki [63] reported that the micropyle did not have a peripheral collar and that it was short tubule which consisted of many minute alveoli. Our observations confirmed those of Sasaki. In SEM, the micropyle consisted of minute subspherical alveoli which resembled those of the neighboring pneumatic layer (Fig. 9d). The individual alveoli of the micropyle were interconnected by pores like those of the pneumatic layer (Fig. 9d, f). These structures of the micropyle and gemmule coat resemble those of *Trochospongilla phillottiana* Annandale [44-46].

Eunapius coniferus (Annandale)

1) Distribution

China: Annandale, 1916; 1918 — Gee and Wu, 1927 — Gee, 1931a; 1932a; 1932c — Penney, 1960 — Penney and Racek, 1968 — Sasaki, 1969. **Japan:** Sasaki, 1969.

Annandale [10] first reported this species from the mouth of Moo-Too (Mu-Tu) creek, T'ai Hu, near Soochow, Kiangsu Province in China. Gee [33] reported this species from several places in Kiangsu Province, from a mountain reservoir near Peiping in Hopei Province, and from a pond near Tsingtao Univ. in Shantung Province. Sasaki [61] reported that he collected this species from a pond (33°37′N, 130°29′E) in Fukuoka Prefecture in Japan. Until recently, this pond was the only known habitat of this species in Japan. But recently we have been able to collect this species from several ponds in the southern part of Okayama Prefecture. Considering the range of this species in China, its range in Japan may be extended to the northern part of Japan.

2) Spicules

There have been only a few descriptions of the spicules and gemmules until the present [10, 11, 48, 61]. Our observations on the megascleres were similar to previous descriptions. Annandale [10] reported that gemmoscleres of Chinese species were relatively slender, sharply pointed at both ends, and irregular or sparsely spiny in outline. Penney and Racek [48] reported that they were feebly curved amphioxea, covered with microspines in an irregular manner. On the other hand, Sasaki [61] reported that in Japanese specimens, they were generally straight and smooth and their tips were rounded or blunt. We found that they were feebly curved, entirely smooth, and conical at both ends (Fig. 4c). Therefore, our observations resembled those of Sasaki. Free microscleres of this species were absent in our specimen as descriptions of Annandale [10], Penney and Racek [48] and Sasaki [63]. But Annandale [11] wrote as follows: (*sic*) "I have discovered a few free-microscleres in specimen since the original description was published. These microscleres are cylindrical, straight, blunt at the extremities and covered with short spines."

As these spicules resemble gemmoscleres from China, they may be immature gemmoscleres.

3) Gemmule coat and micropyle

The gemmule coat of this species is unique in structure and form. Annandale [10] and Penney and Racek [48] reported that the pneumatic layer of the gemmule coat developed unevenly and that the base and side of the gemmule were covered with a single layer of large polygonal alveoli and upper surface were covered with several many tires of such alveoli. However, Sasaki [61] reported that base side of Japanese gemmule had not the pneumatic layer. He discerned the presence of membrane which circumscribed to the outer gemmular membrane. Our result with SEM corresponded to those of Sasaki except for the presence of the membrane circumscribing to the outer gemmular membrane. Our result on gemmule coat was that the pneumatic layer consisted of alveolus which is a low hexagonal prism (Fig. 10f) and that several pores which interconnected alveoli, were present on the upper and lower wall of individual alveoli. This structure of the pneumatic coat resembles that of *Eunapius fragilis* (Leidy) [42, 43, 46].

Trochospongilla latouchiana Annandale

1) Distribution

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

Gee [34] reported that Nanking, in the southern part of Kiangsu, was the northern limit and the southern portion of Chekiang Province was the southern limit in China. We collected this species from Lake Biwa (35°03′N, 135°56′E) in Japan. This is the first record in Japan.

2) Spicules

Previous descriptions of megascleres of this spicule can be classified into large groups. One is that they are smooth [2, 7, 13, 27, 32, 34, 37, 38, 60, 67]. The other is that they are as a rule smooth [32, 48, 53]. Racek [53] wrote as follows: (*sic*) "The megascleres of Australian specimens are not always completely smooth as those recorded for the typical species from Asia and a great number possess incipient though clearly discernible spines. Even though such feebly spined megascleres have also been found by the author in the type slides examined, they are never as frequent as in the Australian material." Japanese megascleres had spines on their surface (Fig. 2, Fig. 6a, b, c), though the number of spines on each spicule varied widely. In SEM, smooth megascleres were rare.

Japanese gemmoscleres resembled those previously described except for the margins of

the rotules. All previous descriptions of rotules reported them as smooth and rounded. In contrast, our Japanese specimens had minute spines on their margins (Fig. 8a, b, c, e, f). Though it is impossible to discern the spines on the lower rotules in LM (Fig. 3b), the spines on the upper rotule are discernible even in LM (Fig. 3a). Therefore, it may be that only Japanese gemmoscleres have spines on the margins of the rotules.

3) Gemmule coat and micropyle

Annandale [7, 13] reported that the micropyle was situated on an eminence and was circular. Gee [34] wrote as follows: (*sic*) "The pore tube is simple and opens through the single layer of birotulates, sometimes with this layer arranged into craterlike structure around its opening." Penney and Racek [48] reported that the micropyle protruded into a conical opening and that it was a short porous tube. Sasaki [60] reported that the micropyle was a saucer-like stucture. Our results with SEM showed that the micropyle was elevated slightly and bore a narrow peripheral collar which was situated at the center of a saucer-like structure of the outer gemmular membrane (Fig. 11d, e). Therefore, the micropyle of Japanese specimens is never tubular. The varying descriptions of the micropyle of this species may be caused by the difference of observation methods. Our observations on the gemmule coat showed that the pnematic coat consisted of large subspherical alveoli and that individual alveoli were interconnected by pores (Fig. 11f). This structure resembles species of *Ephydatia* [19, 42, 43, 46].

Our results with SEM on spicules, gemmule coats and micropyles of these three species were in many respects different from those previously reported. It is not clear at present whether these differences were caused by the observation methods or the specimens.

We suggest that even if one can find some minor intraspecies morphological differences, one should not name new species thoughtlessly. SEM should be used for accurate observation of each specimen. The accumulation of such accurate descriptions will contribute to sponge systematics.

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Explanation of figures

Fig. 1. *Spongilla alba* Carter
LM photo of a part of a megasclere. Minute spines are seen on its surface except at the tip. ×700

Fig. 2. *Trochospongilla latouchiana* Annandale
LM photo of a part of a megasclere. Conical and recurved spines are seen on its surface. ×700

Fig. 3. *Trochospongilla latouchiana* Annandale
a. LM apical view of two gemmoscleres. The margins of the upper rotules are not smooth. ×800.
b. LM bottom view of two gemmoscleres. The margins of the lower rotules are nearly smooth. ×800

Fig. 4. *Eunapius coniferus* (Annandale)
a. Megasclere: This spicule is entirely smooth and only slightly curved, and has blunt tips at both ends (amphistrongyle). ×325
b. Megasclere: This spicule is entirely smooth and only slightly curved, and has sharp tips at both ends (an amphioxea). ×325
c. Gemmosclere: This spicule is entirely smooth and feebly curved, and has conical tips at both ends. ×675

Fig. 5. *Spongilla alba* Carter
a. Megasclere: This spicule is a fusiform amphioxea and bends slightly. ×190
b. Megasclere: An enlarged view of Fig. 5a showing many minute conical spines on its surface. ×900
c. Megasclere: An enlarged view of Fig. 5a showing many minute conical spines on its surface except at the tip. ×900

Fig 6. *Trochospongilla latouchiana* Annandale
a. Megasclere: This spicule is an almost straight amphioxea, is distinctly fusiform, and has small acute spines on its surface. ×190
b. Magasclere: An enlarged view of Fig. 6a showing the conical spines on its surface. ×900
c. Megasclere: An enlarged view of Fig. 6a showing the recurved spines on its surface except at the tip. ×900

Fig. 7. *Spongilla alba* Carter
a - c. Gemmosclere: These spicules are stout and feebly bent, and have recurved sharp spines which are often more numerous at the tips of the spicules. ×490
d. Microsclere: This spicule is slender, spindle shaped, and slightly curved. ×1,300
e. Microsclere: An enlarged view of Fig. 7d showing the blunt tip of the spicule which is composed of small spines. ×3,100
f. Microsclere: An enlarged view of Fig. 7d showing complex spines which have a smooth pedicel with an apical burr. ×3,100

Fig. 8. *Trochospongilla latouchiana* Annandale

a. Gemmosclere: These spicules are birotulates with a slender smooth shaft and terminal circular rotules of unequal diameter. ×1,800

b. Gemmosclere: Apical view of a spicule. Many minute spines are seen on the margin of the upper rotule. ×2,400

c. Gemmosclere: Side view of an upper rotule. Many minute spines are seen on the margin of the upper rotule. ×3,200

d. Gemmosclere: Side view of a lower rotule. The margin of the lower rotule is almost smooth. ×3,200

e. Gemmosclere: A part of the upper rotule and shaft of a spicule. Many recurved spines are seen on the margin of the upper rotule. ×8,900

f. Gemmosclere: Bottom view of a part of a lower rotule. A few microspines are seen on the margin of the lower rotule. ×4,900

Fig. 9. *Spongilla alba* Carter

a. Gemmule. A micropyle is seen at the center of the gemmule. ×90

b. An enlarged view of Fig. 9a showing an apical view of the micropyle which protrudes slightly. ×380

c. A cross section of a gemmule cut through the micropyle. ×90

d. A cross section of a micropyle cut vertically. The micropyle is short and tubular, and consists of minute alveoli which resemble those of the neighboring pneumatic layer. ×740

e. A part of the gemmule coat which consists of the outer gemmular membrane, a pneumatic layer, and the inner gemmular membrane. The pneumatic layer, where gemmoscleres are embedded rather sparsely and at irregular angles, consists of very minute subspherical alveoli. ×360

f. An enlarged view of Fig. 9e. The outer gemmular membrane is thin. The individual alveoli are interconnected by pores. Smooth area is the mark of a gemmosclere which was removed. ×3,600

Fig. 10. *Eunapius coniferus* (Annandale)

a. Side view of a gemmule which has a triangular outline. A micropyle is seen at the top of the gemmule. Many minute circular depressions are seen uniformly distributed on the surface of the gemmule. ×220

b. Oblique bottom view of a gemmule. Many minute circular depressions are seen uniformly distributed on the surface of the gemmule. ×220

c. A cross section of a gemmule cut through a tubular micropyle. The gemmule coat is unevenly developed. The base of the gemmule coat, which consists of an outer and inner gemmular membrane without a pneumatic layer, is far thinner than that near the micropyle. The gemmule coat becomes gradually thicker toward the micropyle because of the appearance of a pneumatic layer from the side to the top. Several cross sections of gemmoscleres are seen near the micropyle. ×220

d. Apical view of a micropyle which protrudes slightly from the outer gemmular membrane. ×740

e. A part of a cross section of the bottom gemmule coat. The inner gemmular mem-

brane is stratified. ×1,800

f. A part of a cross section of a gemmule coat which consists of an outer and inner gemmular membrane and a pneumatic layer. The pneumatic layer consists of alveoli arranged in several tiers. Several pores which interconnect the alveoli are seen on the upper and lower wall of individual alveoli. ×3,000

Fig. 11. *Trochospongilla latouchiana* Annandale

a. Gemmule. A micropyle is seen near the center of the gemmule. The arrangement of many upper rotules can be seen on the surface of gemmule except near the micropyle. ×220

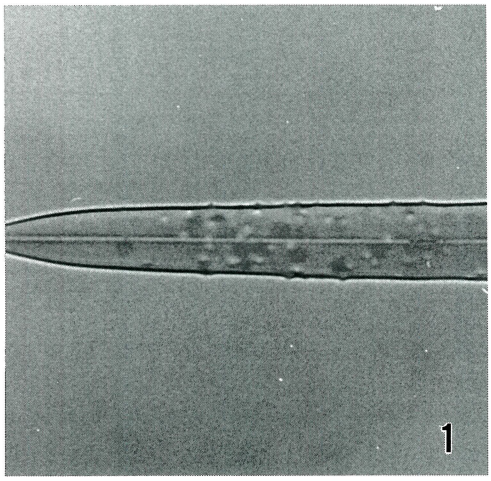
b. Side view of a gemmule. The outilne of the upper gemmule looks like that of a volcano. ×220

c. A cross section of a gemmule cut through the micropyle. Gemmoscleres are in a single layer in the pneumatic coat. ×220

d. An upper part of a cross section of gemmule cut through the micropyle. The micropyle is slightly elevated and is situated at the center of a saucer-like structure of the outer gemmular membrane. ×840

e. A cross section of a micropyle cut vertically. The micropyle bears a narrow peripheral collar and is situated at the center of a saucer-like structure of the outer gemmular membrane. ×1,800

f. A part of a cross section of the gemmule coat which consists of an outer and inner gemmular membrane and a pneumatic coat. The pneumatic coat consists of large subspherical alveoli. The individual alveoli are interconnected by pores. ×2,000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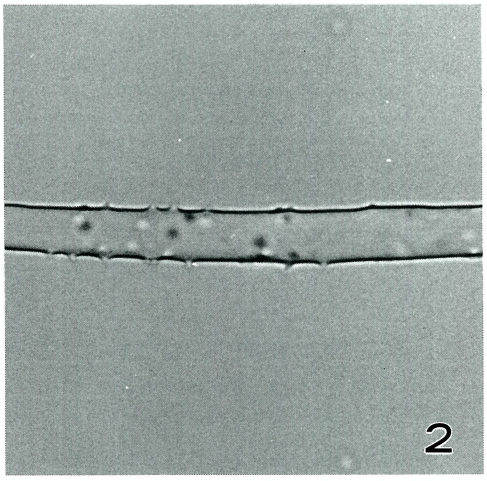
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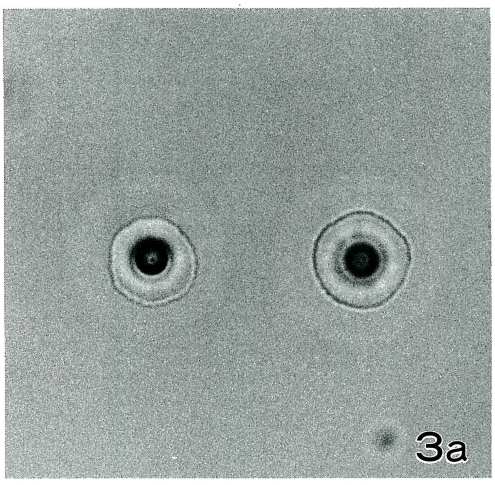
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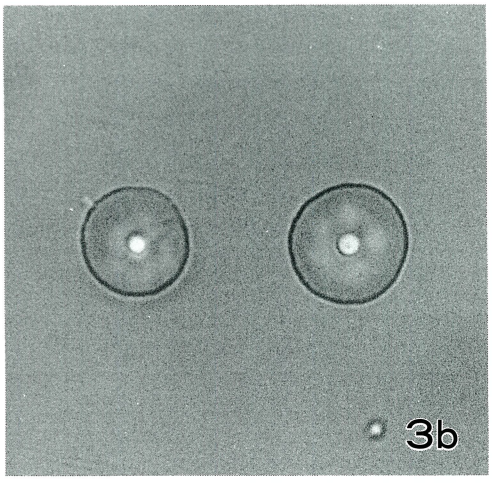
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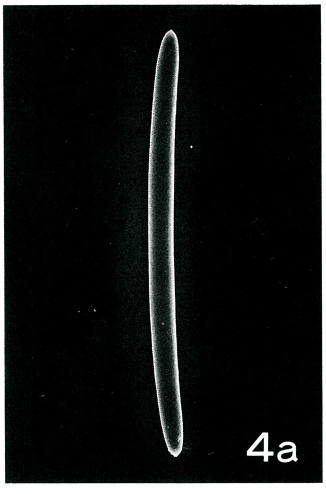
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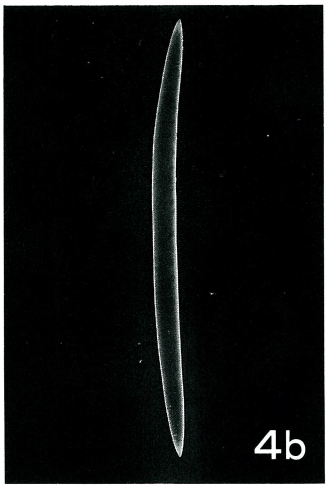
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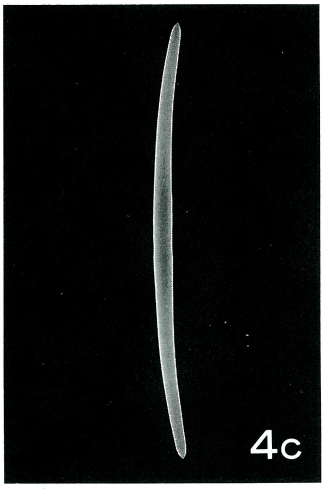
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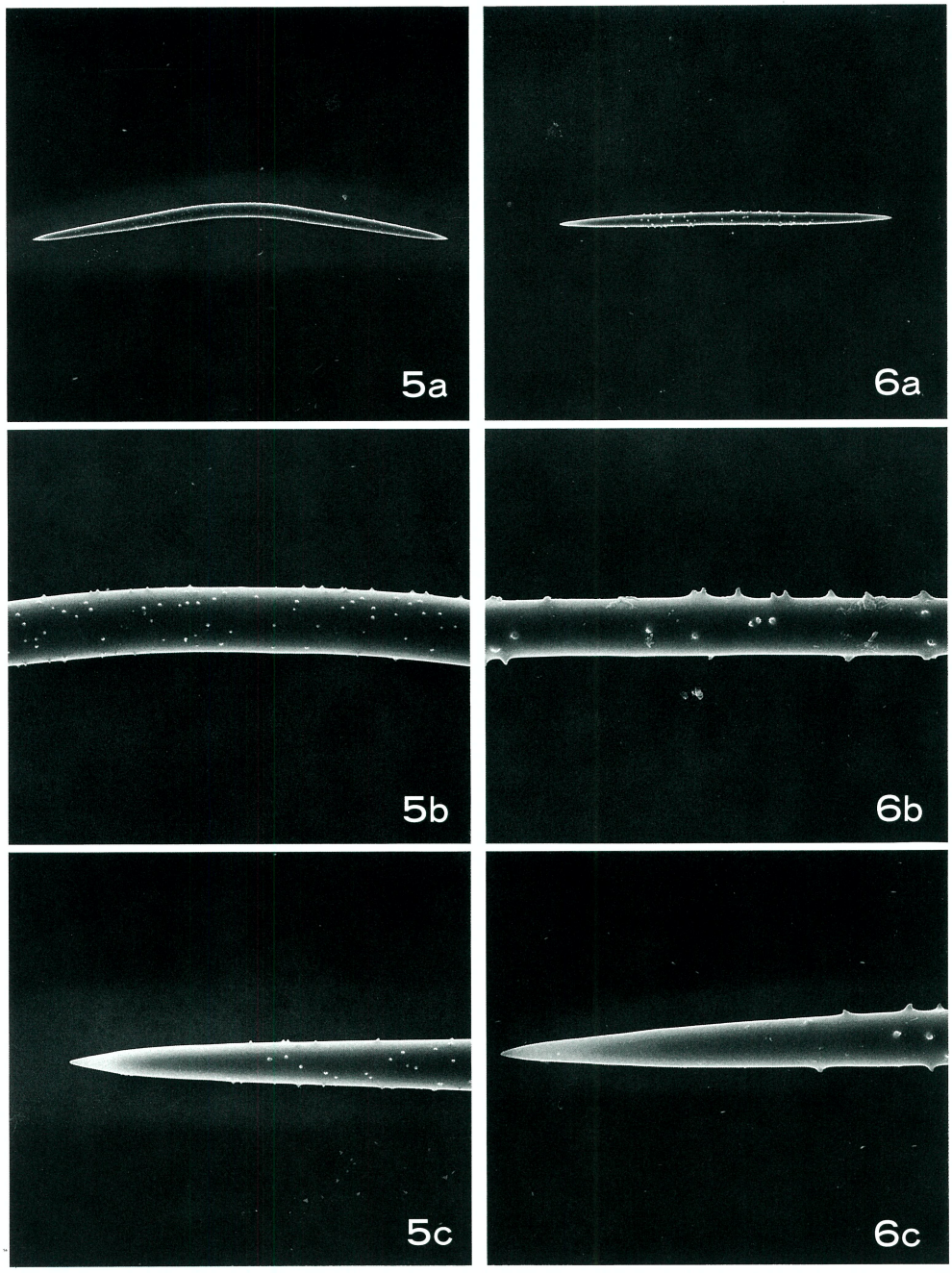
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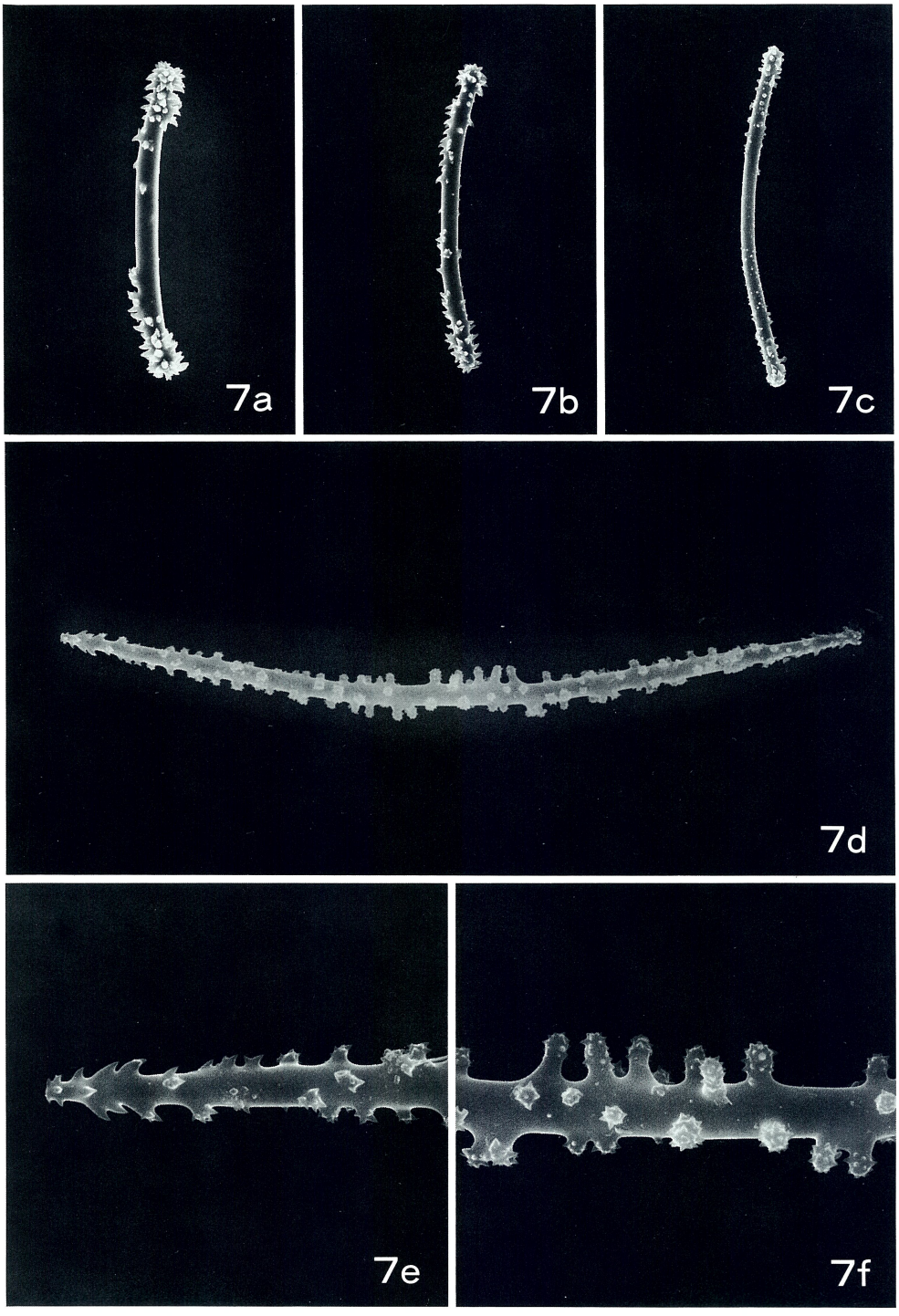
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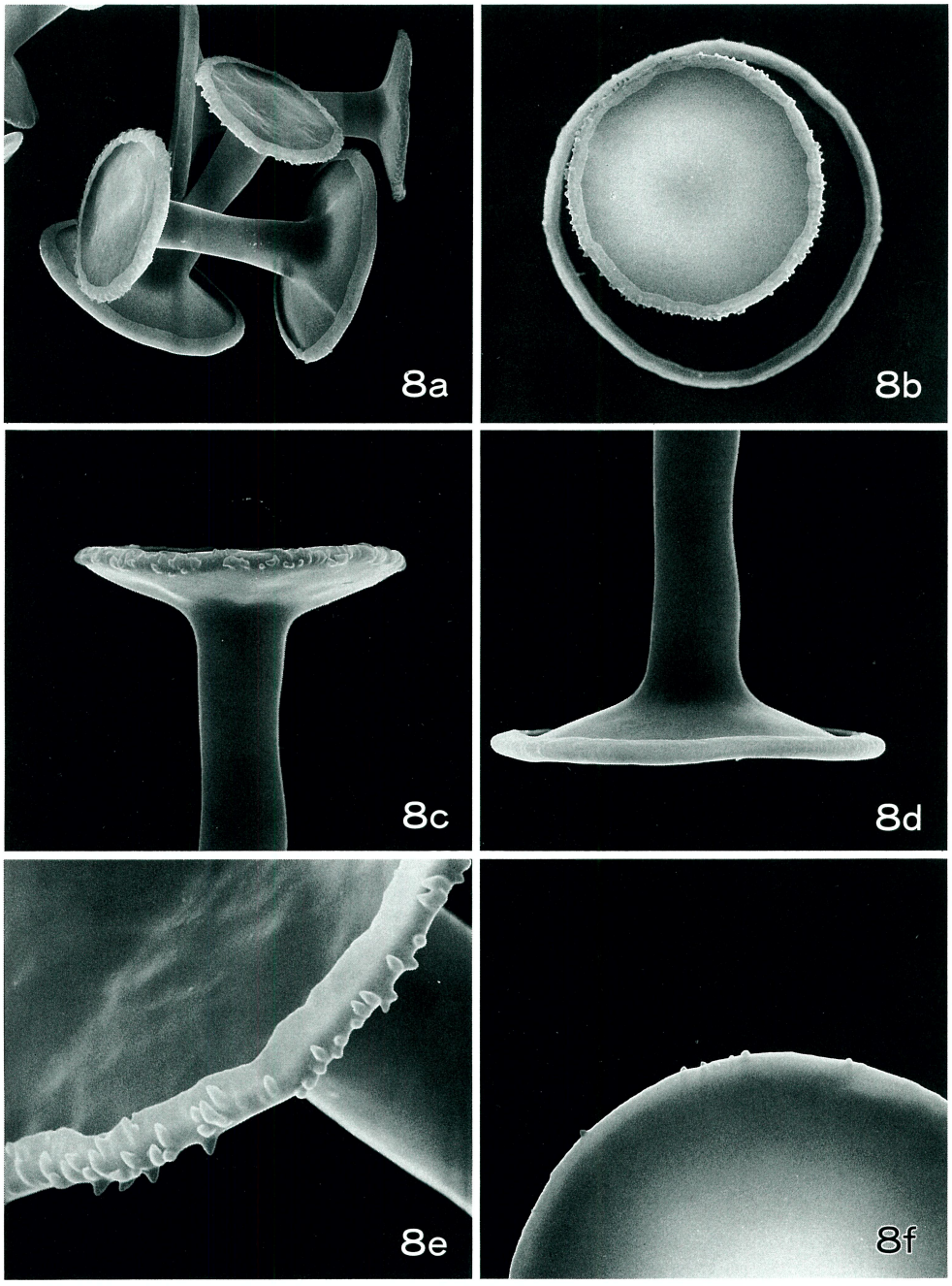
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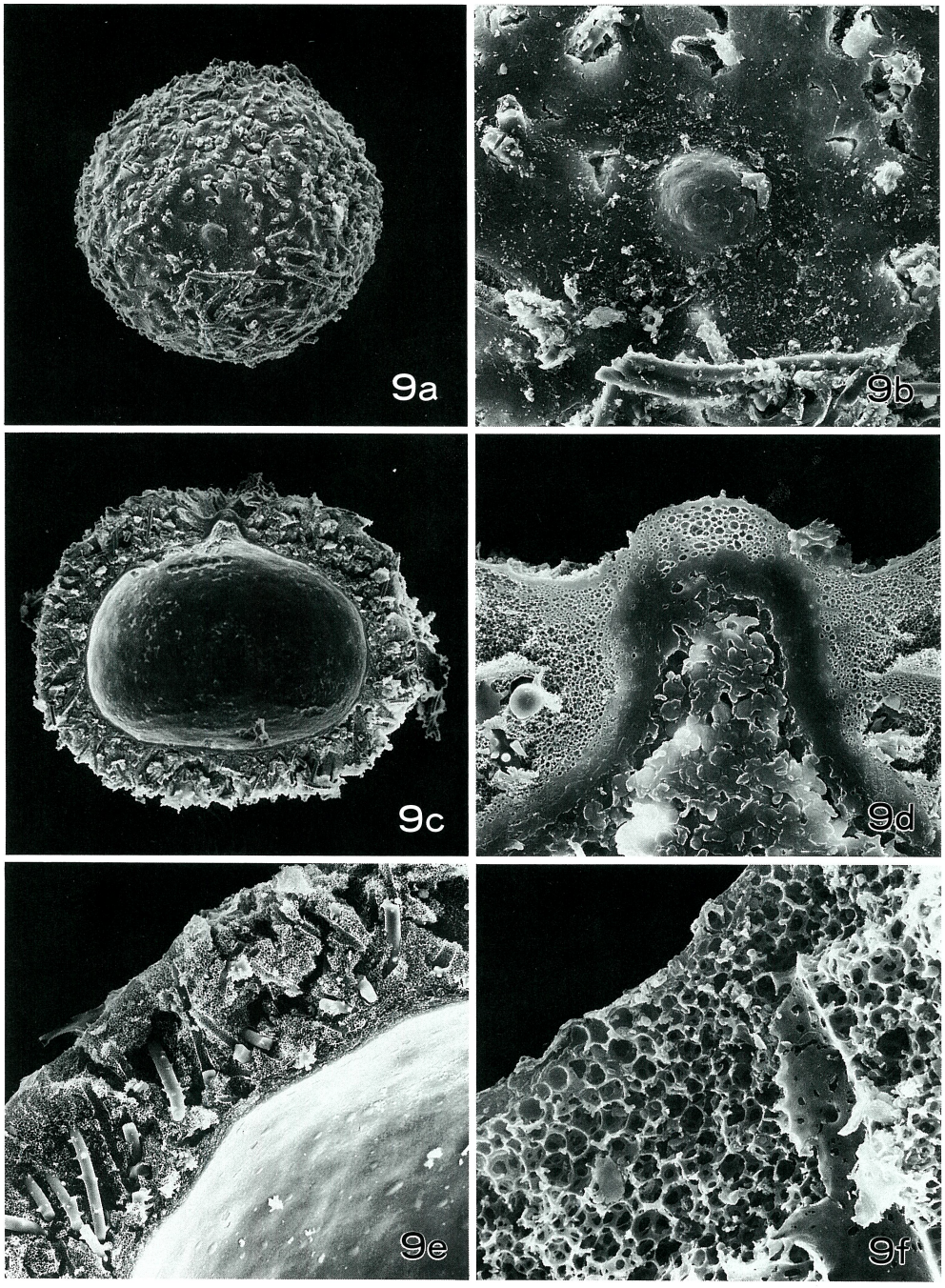
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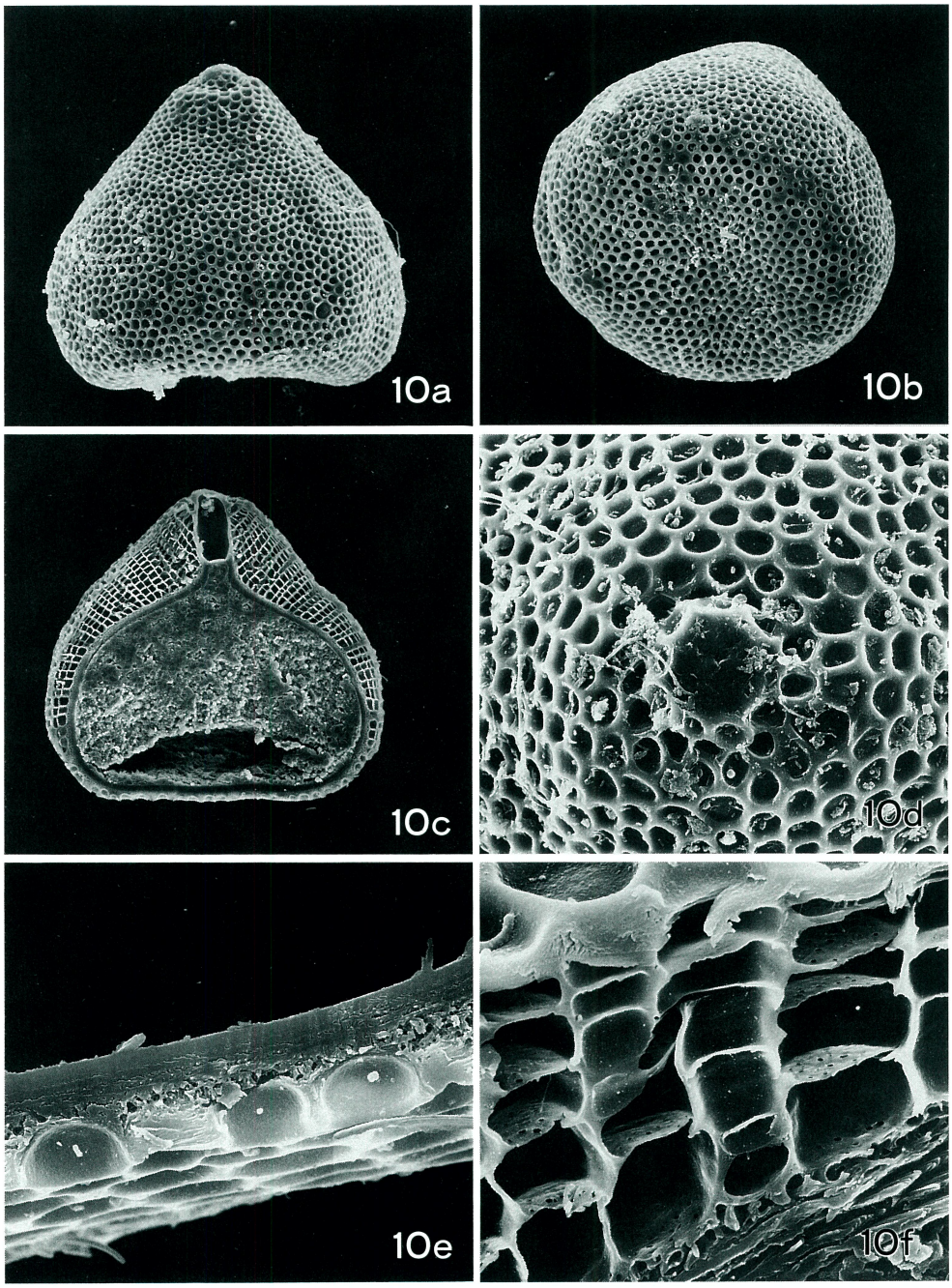
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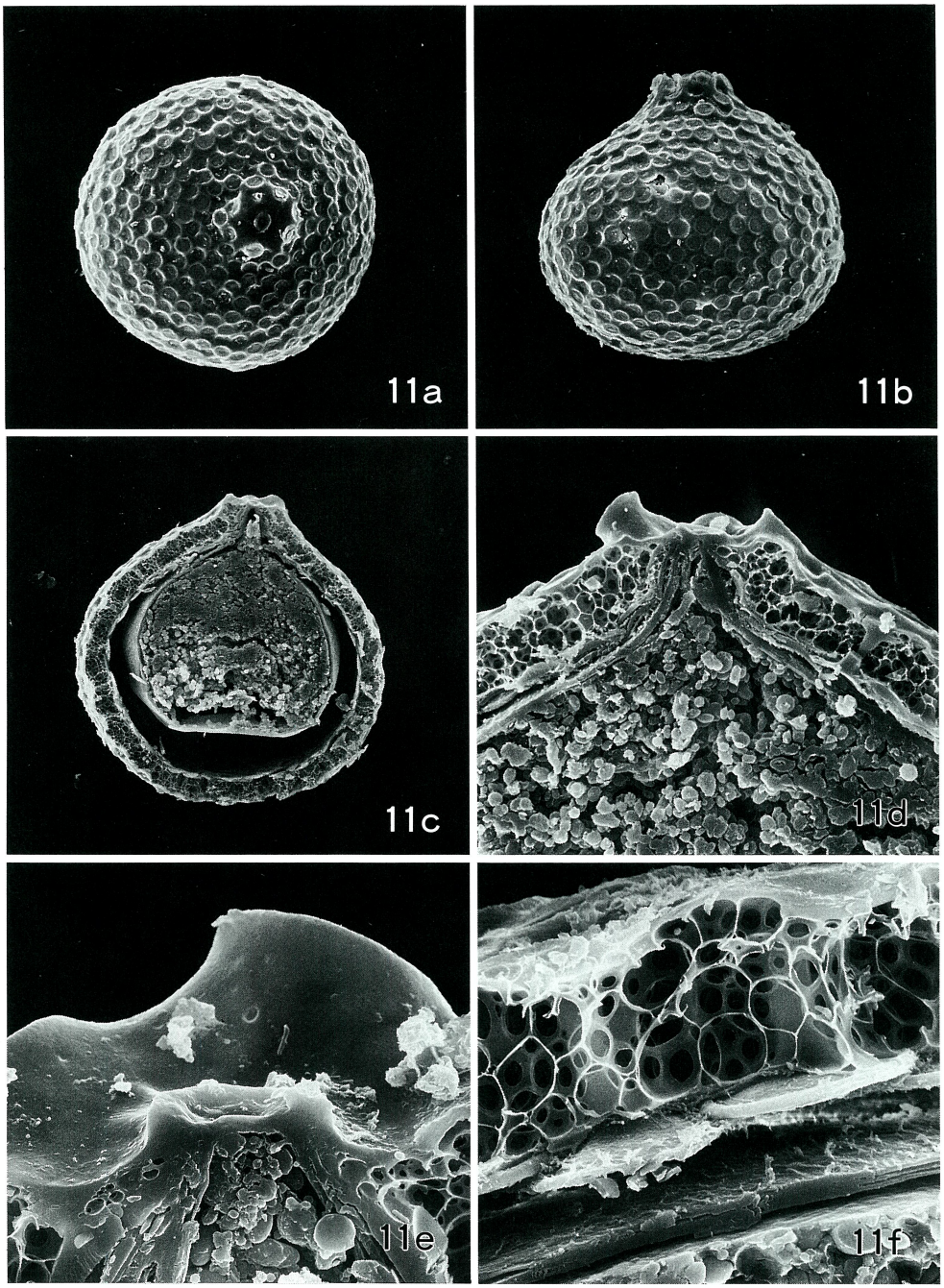
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