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# Ultrastructural Features of Spicules of Five Species of Minnesota Sponges

LOUISE A. ROLLINS\* and LYNN C. HYLAND\*\*

**ABSTRACT**—Scanning electron microscopy of sponge spicules reveals minute spines and microspines on some types of spicules that cannot be observed by light microscopy. These are species-specific characteristics that have not yet been reported. Light microscopic features that characterize each species are reviewed, and a description is presented of features revealed only by SEM.

Fresh-water sponges have long been of interest to invertebrate biologists. In spite of their simple organization and lack of specialized tissues, they survive desiccation and the temperature extremes of our Minnesota climate and grow abundantly in suitable lakes, ponds and streams. Perhaps one reason why fresh-water sponges are not studied more frequently is that the different species are difficult to distinguish. With a few exceptions, they have no specifically recognizable growth forms. One must examine their skeletal elements, both skeletal spicules and the spicules of the overwintering bodies (gemmules) to identify them. Most taxonomic keys for fresh-water sponges include line drawings of representative spicules (Pennak, 1953; Eddy and Hodson, 1950; Edmondson's Ward and Whipple, 1959). The drawings are helpful; but sometimes the dimensions and surface detail (both critical for identification) are distorted. Since the publication of these taxonomic keys, the scanning electron microscope (SEM) has become widely used as a tool for examining the surface detail of very small objects. We chose to examine sponge spicules by SEM in the hope that species-specific details would be revealed.

The familiar light-microscopic features of the sponge spicules were revealed by SEM as well as some new details that cannot be observed except by SEM. For an understanding of the following account of these features it is important to remember that there are two types of skeletal spicules (large = megascleres and small = microscleres) and gemmule spicules (gemmoscleres) found only in the overwintering bodies. Some types of gemmule spicules are shaped like tiny axels with two wheels (birotulate spicules). The most recent revision of the classification of freshwater sponges (Penny and Racek, 1968) has placed some of our familiar Minnesota species into new genera. The new generic names are listed below with the old genera in parentheses.

A number of sponges representing a variety of growth forms were collected from ponds and streams in Itasca State Park, Clearwater County, Minnesota. Preparation of spicules has been described (Rollins, 1972). Briefly, a small portion of each sponge was placed in a test tube and boiled in nitric acid for about five minutes. The spicules were centrifuged, washed several times in water, then in distilled water, and suspended in 95 percent ethanol. After examination with the light microscope, samples of spicules from each species were selected for scanning electron microscopy. For SEM, several

drops of 95 percent ethanol containing spicules were placed on standard aluminum specimen stubs and ignited to concentrate the spicules. The spicules were shadowed with gold and carbon and examined at 20 kv in a Cambridge Stereoscan scanning electron microscope. About 200 examples of each type of spicule were examined.

## Interpretation and identification of illustrations

### *Trochospongilla* (= *Tubella*) *pennsylvanica* (Fig. 1).

By light microscopy, megascleres are slender, sharply pointed and invariably spined, about 140-180  $\mu\text{m}$  long. The gemmoscleres resemble miniature umbrellas; one end is distinctively larger than the other. The shaft of each gemmosclere is about 10  $\mu\text{m}$  long. The larger and smaller ends are, respectively, about 16  $\mu\text{m}$  and 6  $\mu\text{m}$  in diameter. SEM shows that the spines on the megascleres (Fig. 1-a, d, and f), except those at the tips, are divided into three parts. Spines

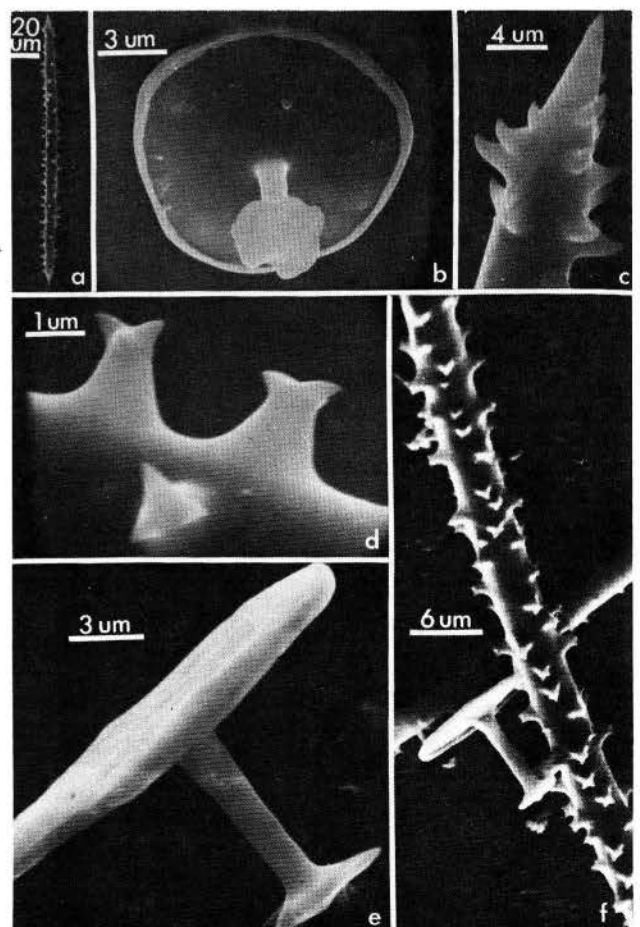


Fig. 1. Spicules of *Trochospongilla pennsylvanica*. a. Megasclere. b. Gemmosclere. c. Enlarged tip of megasclere. d. Enlarged lateral surface of megasclere showing tripartite spines. e. Gemmosclere. f. Gemmosclere and portion of megascleres.

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at the tips are sharply curved (Fig. 1-c). The gemmoscleres are smooth, lacking surface ornamentation (Fig. 1-b, c, and f). *Ephydatia* (= *Meyenia*) *mulleri* (Fig. 2).

With the light microscope, megascleres appear to be rather stout, slightly curved, and with small spines except at the tips. They are about 200  $\mu\text{m}$  long. Gemmoscleres are birotulate, with deeply incised rotules of equal diameter. The shaft of the gemmoscleres is about 12  $\mu\text{m}$  in length, the diameter of the rotules is about 14  $\mu\text{m}$ . SEM shows the spines of the megascleres lacking in further surface ornamentation (Fig. 2-a and b). The birotulate gemmoscleres are highly distinctive. Each rotule is deeply incised into 10-15 rays (Fig. 2-c). The tip of each ray has a variable number of microspines (Fig. 2-c and d). Figures 2-c and 2-f show the orientation of the gemmoscleres in the surface of an intact gemmule.

*Anheteromeyenia* (= *Heteromeyenia*) *argyrosperma* (Fig. 3).

By light microscopy, megascleres are straight, slender, and sparsely covered with small spines. Their length is about 275  $\mu\text{m}$ . The birotulate gemmoscleres are of two distinct lengths but similar morphology. Both have a number of large tooth-like projections along the shaft. These projections are more numerous in the shorter of the two types. The rotules are deeply incised into a variable number of rays (usually 4-8) with hooked or curved ends. The rays are more numerous in the shorter of the two types. The lengths of

the longer and shorter class of gemmoscleres are, respectively, about 110  $\mu\text{m}$  and 70  $\mu\text{m}$ . SEM shows the spines on the megascleres to be slightly curved, lacking surface detail (Fig. 3-a and b). The morphology of the gemmoscleres is clearly shown (Fig. 3-c, d, and e). Both shafts and projections are entirely smooth.

*Eunapius* (= *Spongilla*) *fragilis* (Fig. 4).

When examined with a light microscope, megascleres of this species are rather straight, stout, and entirely smooth. They range in length from 200-260  $\mu\text{m}$ . Gemmoscleres are straight or slightly curved with pointed or curved ends and conspicuous spines throughout the length of the spicule. Gemmoscleres range in length from 75-90  $\mu\text{m}$ . SEM clearly shows the above features (Fig. 4-a, b, c, and d). In addition, gemmoscleres appear to have a few microspines on some of their larger spines (Fig. 4-d).

*Spongilla lacustris* (Fig. 5).

A light microscope examination of skeletal spicules of *Spongilla lacustris* shows slender, smooth megascleres, about 275  $\mu\text{m}$  in length. Microscleres are slender, straight, and abundantly spined. Microscleres are about 50  $\mu\text{m}$  in length. Gemmoscleres, about 50  $\mu\text{m}$  long, are curved and stout, with pronounced spines. SEM shows megascleres to be entirely smooth (Fig. 5-a). Spines on the gemmoscleres are slightly curved (Fig. 5-b). Spines on the microscleres are more numerous than on the gemmoscleres (Fig. 5-c and d), and the spines have numerous microspines (Fig. 5-d).

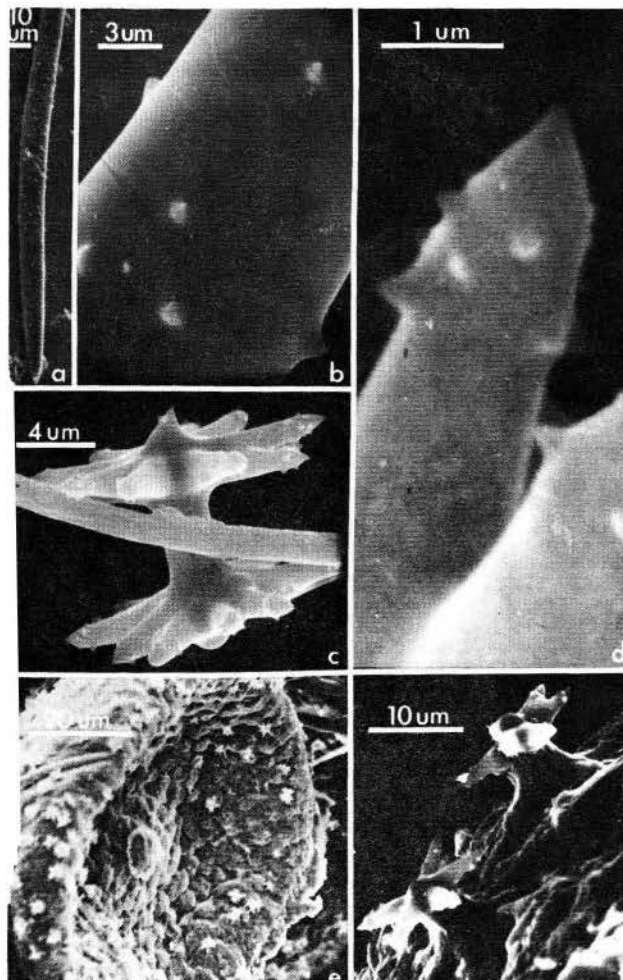


Fig. 2. Spicules and intact gemmule of *Ephydatia mulleri*. a, Megasclere. b, Enlarged lateral surface of megasclere. c, Birotulate gemmosclere. d, Enlargement of two rays of gemmosclere showing microspines at tips. e, Intact gemmule with gemmoscleres protruding through surface coat. f, Enlargement of surface of gemmule with gemmosclere protruding.

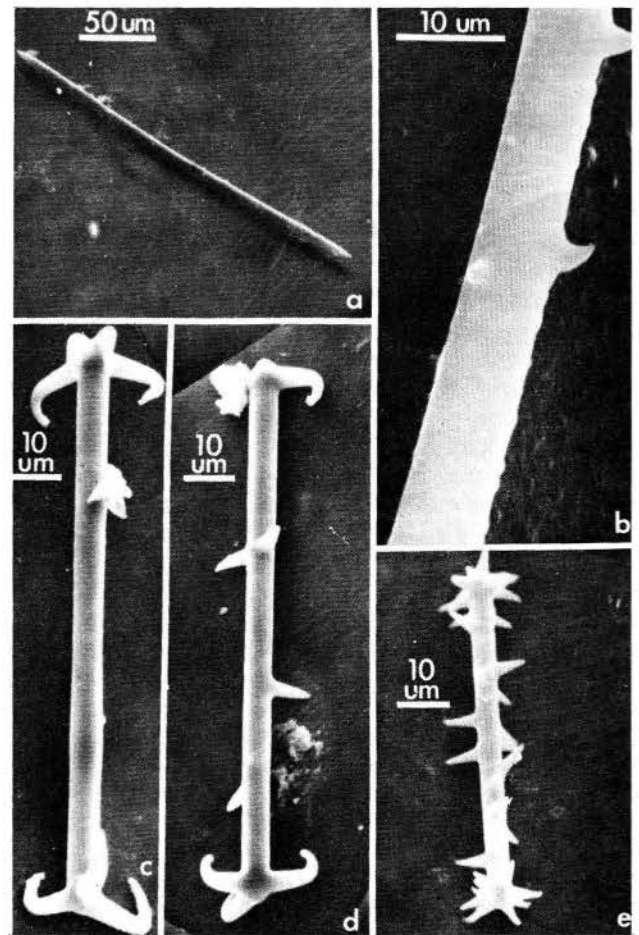


Fig. 3. Spicules of *Anheteromeyenia argyrosperma*. a, Megasclere. b, Enlargement of portion of megasclere showing curved spines. c-e, Birotulate gemmoscleres showing the two distinct morphological types.

#### About the 3-dimension array

Freshwater sponge spicules lend themselves to examination by scanning electron microscopy. With this instrument a three-dimensional array of rays, spines, and microspines is revealed. The enlarged views of characteristic features of five species of Minnesota sponges published here may aid new investigators in identification of the species they have selected. In addition, new features revealed only by SEM may be useful to taxonomists.

As we view the spicule morphology, several questions of biological importance occur to us. For example, it is well known that spicule morphology varies somewhat with environmental conditions. Jewell (1935) showed that in conditions of low silica, spicules of *Spongilla lacustris* were attenuated, although spines on microscleres were still present. Would the microspines we have described disappear in waters of low silica content? Could sub-species be distinguished by subtle differences in ultrastructural features?

When the morphological variability of sponge spicules is better understood, they may be useful as indicators of water conditions in past environments. Sponges occur in a variety of freshwater habitats. Some species are cosmopolitan (e.g. *Spongilla lacustris*), but many species are limited by increasing calcium and pH (Jewell; 1935, 1939). Like diatoms which are widely used as environmental indicators, sponge spicules are preserved in the sediments of lakes and ponds. A paleoecologist examining sediments is confronted with a confusing array of spicules or spicule parts. If a catalogue of the ultrastructural features of sponge spicules were available, investigators using SEM in addition to light microscopy might more easily identify species when only a few spicules or spicule parts were preserved.

We see in these sponges the least complicated of multicellular animals, a surprising degree of ultrastructural complexity in the skeletal elements. The condition is aesthetically pleasing as well as it is scientifically curious.

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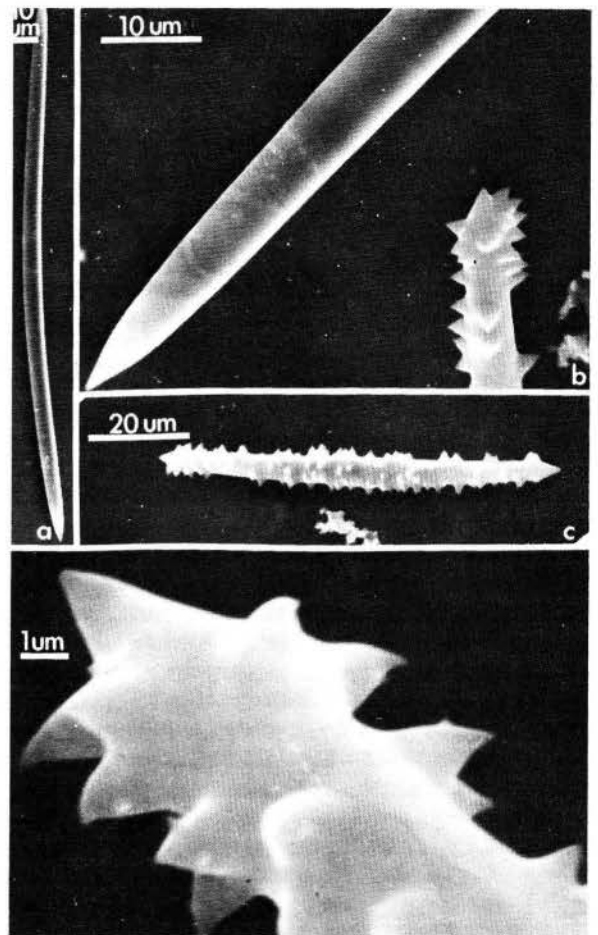


Fig. 4. Spicules of *Eunapius fragilis*. a. Megasclere. b. Enlargement of portion of megasclere and gemmosclere showing lack of ornamentation on megasclere. c. Gemmosclere. d. Enlargement of gemmosclere showing minute microspines on some of the larger spines.

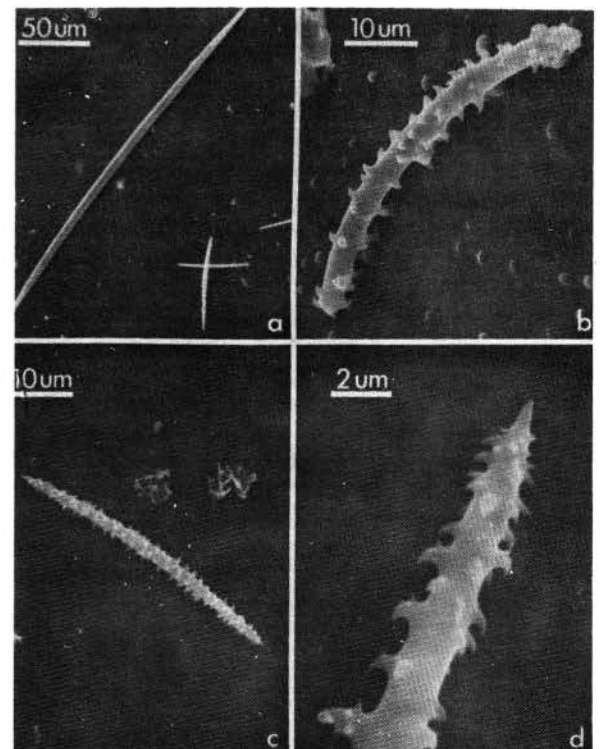


Fig. 5. Spicules of *Spongilla lacustris*. a. Megasclere and two microscleres. b. Gemmosclere. c. Microsclere. d. Enlargement of microsclere showing microspines on some of the larger spines.