Wisconsin Freshwater Sponge Species Documented by Scanning Electron Microscopy

Janis Annesley and Joan Jass[a]
Milwaukee Public Museum
Milwaukee, Wisconsin 53233 USA
and
Dreux Watermolen
Wisconsin Department of Natural Resources
Madison, Wisconsin 53707 USA

ABSTRACT

The majority of the presently recognized species of Nearctic freshwater sponges have been collected and reported from the State of Wisconsin. Important early contributions to freshwater sponge research were made by Minna E. Jewell and James R. Neidhoefer, both of whom deposited voucher specimens in the Milwaukee Public Museum. These authors included in their publications figures illustrating species diagnostic traits, based on examination of specimens with the light microscope. We re-examined their historically significant voucher specimens of Wisconsin sponge species using scanning electron microscopy and here provide a reference atlas of diagnostic criteria for these species. The material we have examined does not support a valid Wisconsin record for *Spongilla aspinosa*.

INTRODUCTION

Smith (1921a and 1921b) compiled a list of 12 sponge species for Wisconsin based on records from the literature as well as specimens in several collections. Jewell (1935) found 10 sponge species in 103 lakes and 15 streams, including a species not previously known from the state, which brought the Wisconsin total to thirteen. Jewell donated a number of specimens to the Milwaukee Public Museum (MPM) and also deposited vouchers at the National Museum of Natural History (NMNH, Smithsonian Institution).

Neidhoefer (1938) reported one additional species from Wisconsin, finding 12 of the 14 sponges that had been reported from the state. He provided a key to species, described taxonomic characteristics, included photographs of dried specimens and microscopic preparations of spicules, and gave a limited amount of habitat data for each species. Frost and his colleagues investigated sponge ecology in northern Wisconsin lakes throughout the 1990s (e.g., Frost and Elias 1990, Frost et al. 1997). Colby et al. (1999) returned to 18 of the lakes Jewell (1935) surveyed to evaluate the long-term stability of sponge distribution patterns. These investigators analyzed Jewell's original dataset statistically and used the results to predict sponge distribution in a new set of lakes surveyed in 1996.

Scanning electron microscopy (SEM) has been used to study many aspects of sponge biology, providing diagnostic advantages not available with light microscopy. We applied this SEM technique to the examination of diagnostic criteria of Wisconsin freshwater sponges, focusing on Jewell and Neidhoefer voucher specimens where possible.

METHODS AND MATERIALS

A Zeiss EVO 40 scanning electron microscope was used to examine stub-mounted whole gemmules and spicule preparations from freshwater sponge specimens. Nitric acid digestion of tissues and gemmules prepared the spicules for scanning. Comparison of the resulting scans to the species diagnostic traits as described in the literature was used to

[a]Corresponding author; E-mail: jass@mpm.edu

confirm determinations of the scanned material. To increase the usefulness of this work for making interspecific comparisons among all sponges reported from the state, we also obtained sponge tissue on loan from specimens in other institutions in order to produce representative images for those species absent from the MPM collection.

RESULTS AND DISCUSSION

Wisconsin sponges are treated below alphabetically by species under each family, as given by the World Porifera Database (http://www.vliz.be/vmdcdata/porifera/). In order to facilitate use of the older Wisconsin literature for future studies, synonyms used by earlier authors are initially cited for the sponge species discussed here. The descriptive summaries of gemmule and spicule traits are based largely on the Penney and Racek (1968) compilation for freshwater sponge species worldwide. In cases where subsequent researchers (e.g., Reiswig and Ricciardi 1993) have revealed errors in Penney and Racek (1968), that differing information is cited. For comparison with our results, Tables 1 and 2 give ranges for gemmule and spicule measurements reported by Penney and Racek (1968) and Ricciardi and Reiswig (1993). A high degree of ecomorphic variation in certain sponge traits has been noted by many researchers, but our focus was confined to the examination of voucher specimens, and we have not attempted an analysis of this intraspecific variation.

Species are represented by at least two SEM images of voucher specimens, one of the whole gemmule and one of the spicules. The different spicule types are indicated by the following letters: g -- gemmosclere (g1, g2 if of two types), M -- megasclere, m -- microsclere. If more than one type of gemmosclere occurs, they are designated as g1 and g2. Other abbreviations used are: f -- foramen and r -- rotule.

Family Metaniidae Volkmer-Ribeiro 1986

Corvomeyenia everetti (Mills 1884) [=*Ephydatia e.* Jewell 1935, 1939; Neidhoefer 1940]

Material examined: MPM Radiate #1715 *Ephydatia everetti*.

Gemmule (Fig. 1a) diameter falls below the range given by Penney and Racek (1968). The foramen has a short collar. Gemmoscleres are birotulate, of two size classes (Penney and Racek 1968). In contrast, Ricciardi and Reiswig (1993) reported a single size class. Longer gemmoscleres protrude through the outer surface of the gemmule. They have straight to slightly curved smooth shafts with rotules bearing 5-7 curved hooks (Fig. 1b). Megascleres are fusiform, slender, slightly curved and entirely smooth. Microscleres are birotulate, ending on both sides in rotules with 5-8 distinctly recurved spines.

Table 1. Ranges of gemmule diameters (μm) from Penney and Racek (1968) and Ricciardi and Reiswig (1993) for sponge species found in Wisconsin.

Species	Penney and Racek	Ricciardi and Reiswig
Corvomeyenia everetti	480-530	710-902
Anheteromeyenia argyrosperma	400-450	558-686
Anheteromeyenia ryderi	320-350	300-400
Duosclera mackayi	180-260[a]	263-841
Ephdatia fluviatilis	350-450	400-600
Ephydatia muelleri	350-450	300-400
Eunapius fragilis	180-290[a]	None given
Heteromeyenia baileyi	450-480	Less than 500
Heteromeyenia tentasperma	420-450	Not studied
Heteromeyenia tubisperma	500-550	534-661
Radiospongilla crateriformis	370-450	261-520
Spongilla lacustris	500-800	290-842
Trochospongilla pennsylvanica	190-390	310-396

[a] Inner layer diameter

Family Spongillidae Gray 1867

Anheteromeyenia argyrosperma (Potts 1880) [=*Heteromeyenia a.* Jewell 1935, 1939; Neidhoefer 1940]

Material examined: MPM Radiate #1726 (alc) *Heteromeyenia argyrosperma* Potts.

The gemmule (Fig. 1c) has many megascleres attached externally and gemmoscleres, which are embedded radially, protrude well beyond its outer layer. Ricciardi and Reiswig (1993) reported the foramen as a simple pore. Gemmoscleres (Fig. 1d) are birotulate of two groups of sizes. Ricciardi and Reiswig (1993) reported the shorter ones as being more densely spined. Both types have spines on the shafts and several claw-like hooks on both ends. Megascleres are subfusiform to cylindrical, and sparsely covered with small spines the entire length. Microscleres are absent.

Anheteromeyenia ryderi (Potts 1882) [=*Heteromeyenia r.* Jewell 1935, 1939; Neidhoefer 1940. Synonymized under genus *Racekiela* by World Porifera Database.]

Material examined: MPM Radiate #1724 (dry) *Heteromeyenia ryderi*.

The gemmoscleres are embedded into the gemmule radially, with the longer class protruding from its outer layer (Fig. 1e). Megascleres are somewhat variable in shape, usually slender and fusiform, with spines except at the ends (Fig. 1f, 1g). Gemmoscleres (Fig. 1f, 1g) are of two distinctly different shapes and sizes, though both are birotulate. The shorter have a few spines on a narrow cylindrical shaft and flattened rotules with a large number of small teeth. The longer have large curved spines on a cylindrical shaft and curved claw-like hooks on the ends. Rotules of the two types can be seen on the gemmule surface (Fig. 1e), as the gemmoscleres are embedded in the outer layer of the gemmule. Microscleres are absent.

Duosclera mackayi (Carter 1885) [=*Spongilla igloviformis* Jewell 1935, 1939; Neidhoefer 1940]

Material examined: MPM Radiate #1728 (dry) *Spongilla igloviformis*.

The positioning of gemmoscleres embedded into the gemmule (Fig. 2a) is described as tangential by Penney and Racek (1968) but radial by Reiswig and Ricciardi (1993). The foraminal region clear of gemmoscleres has a surface pattern created by polygonal spongin (Reiswig and Ricciardi 1993). The foramen itself is short with a small collar; Reiswig and Ricciardi (1993) corrected an inaccurate description of it as being long and tubular (Penney and Racek 1968). Both megascleres and gemmoscleres (Fig. 2b) are stout, slightly curved, and covered with spines. The only difference between megascleres and gemmoscleres is one of size. Microscleres are absent.

Table 2. Spicule lengths (μm) from Penney and Racek (1968) and Ricciardi and Reiswig (1993) for sponge species found in Wisconsin. M = megasclere, m = microsclere, g = gemmosclere.

Species	Penney and Racek			Ricciardi and Reiswig		
	M	m	g	M	m	g
Corvospongilla everetti	195-285	16-19	60-72, 42-57	143-260	14-26	33-78
Anheteromeyenia argyrosperma	240-280	Absent	110-125, 65-80	250-304	Absent	114-116, 65-89
Anheteromeyenia ryderi	190-220	Absent	50-75, 30-40	194-253	Absent	46-64, 28-41
Duosclera mackayi	190-265	Absent	68-120	177-302	Absent	79-267
Ephydatia fluviatilis	210-400	Absent	26-30	253-439	Absent	20-26
Ephydatia muelleri	200-350	Absent	12-20	171-311	Absent	8-28
Eunapius fragilis	180-270	Absent	75-140	165-261	Absent	32-121
Heteromeyenia baileyi	255-315	75-85	80-85, 50-60	216-320	53-83	49-86, 38-51
Heteromeyenia tentasperma	260-280	75-80	65-72, 50-55	Not studied		
Heteromeyenia tubisperma	190-230	85-90	60-70, 40-48	238-337	73-118	33-62
Radiospongilla crateriformis	240-300	Absent	60-75	254-298	Absent	60-80
Spongilla lacustris	200-350	70-130	80-130	158-362	32-94	18-58
Trochospongilla pennsylvanica	140-280	Absent	9-11	100-432	Absent	11-41

Ephydatia fluviatilis (Linnaeus 1758) [=*E. fluviatilis* (Auctorum) Neidhoefer 1940]
Material examined: From the National Museum of Natural History (formerly United States National Museum) USNM #39291. Gist Gee Freshwater Sponge Collection, China; Shandong; Jinan.

The shape of the gemmule is spherical, excepted when deflated by drying (Fig. 2c). Attached to the gemmule is one of the megascleres, which are smooth and fusiform. Gemmosclere rotules are arranged radially and exposed on the surface of the gemmule. The foramen is very slightly elevated with a minute collar. Gemmoscleres (Fig. 2d) are birotulate and have smooth slender shafts. The rotules are equal, flat and irregularly but not deeply incised, with not less than 20 teeth. The numerous rotule teeth and a greater length of the gemmosclere in proportion to rotule diameter (Ricciardi and Reiswig 1993) are traits distinguishing this species from *E. muelleri*. Microscleres are absent.

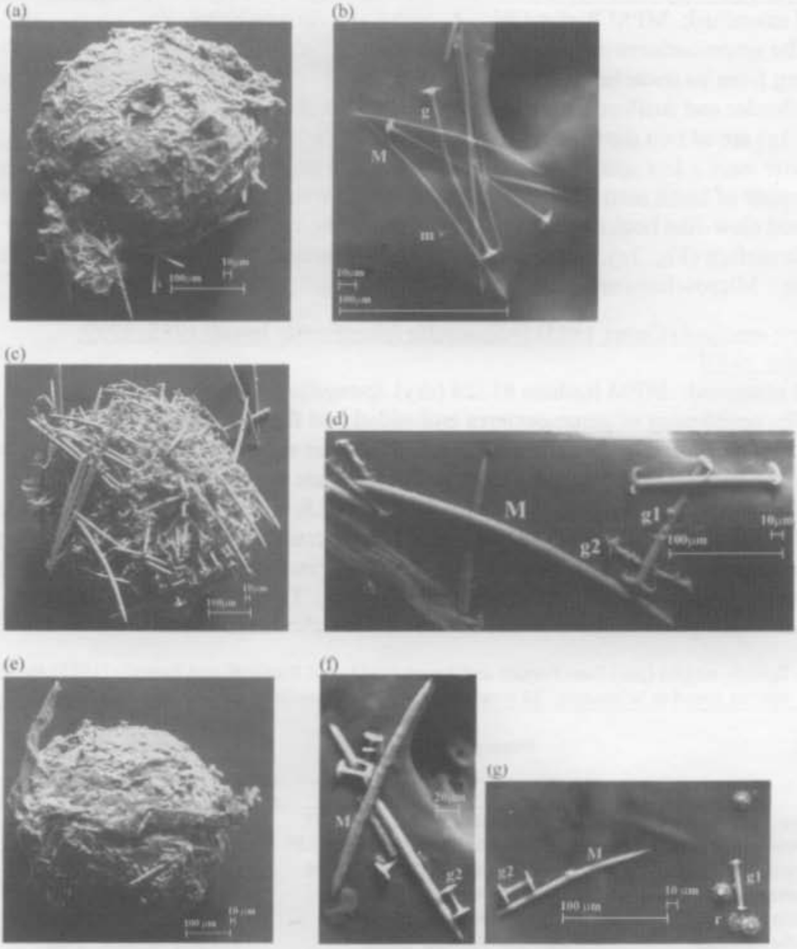

Figure 1. Scanning electron micrographs of Wisconsin sponge species: (a-b) *Corvomeyenia everetti*; (c-d) *Anheteromeyenia argyrosperma*; (e-g) *Anheteromeyenia ryderi*. f = foramen, g = gemmosclere, m = microsclere, M = megasclere, r = rotule, g1 and g2 = two classes of gemmoscleres.

Ephydatia muelleri (Lieberkühn 1855) [=*E. mulleri* Jewell 1935, 1939; Neidhoefer 1940]

Material examined: MPM Radiate #1735 (dry) *Ephydatia mulleri* (Lieberkuhn).

The shape of the gemmule is spherical, excepted when deflated by drying (Fig. 2e). The birotulate gemmoscleres are embedded radially and their rotules are thus exposed on the surface of the gemmule. The gemmosclere has less than 12 teeth (Fig. 2f) which distinguishes it from *E. fluviatilis*. The fusiform megascleres have small spines along their length (Fig. 2g), another characteristic which distinguishes this species from *E. fluviatilis*. Microscleres are absent.

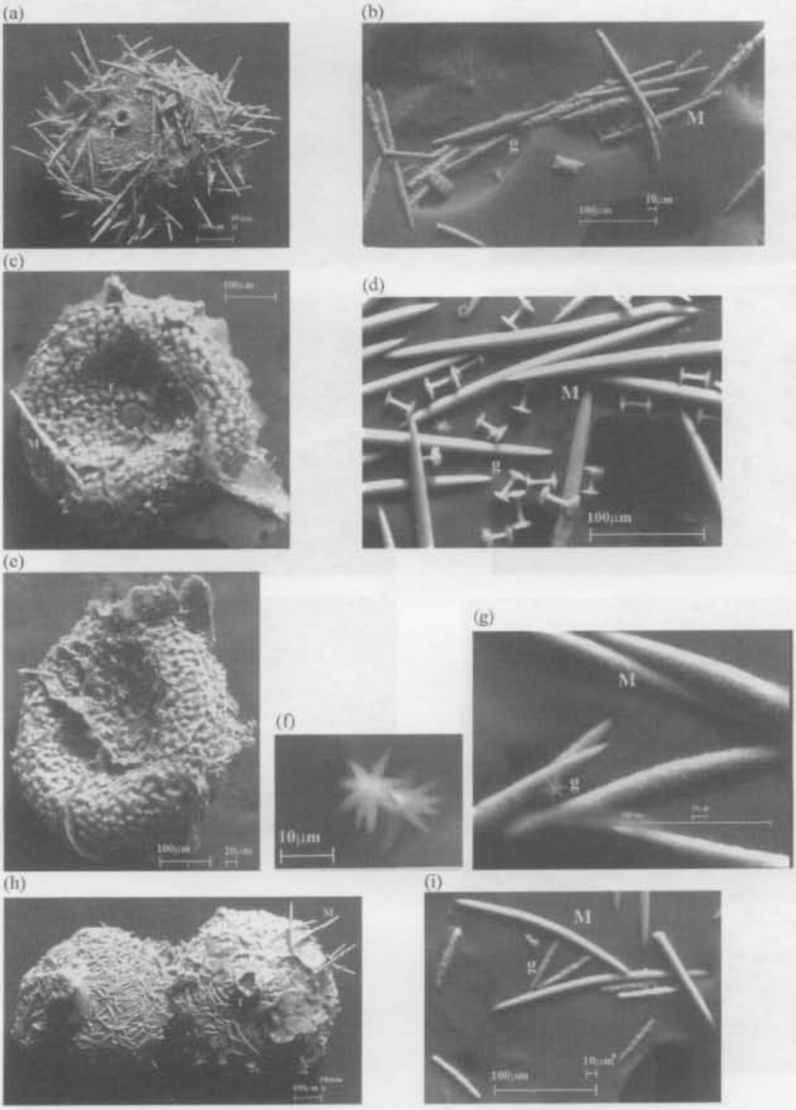

Figure 2. Scanning electron micrographs of Wisconsin sponge species: (a-b) *Duosclera mackayi*; (c-d) *Ephydatia fluviatilis*; (e-g) *Ephydatia muelleri*; (h-i) *Eunapius fragilis*. f = foramen, g = gemmosclere, M = megasclere.

Eunapius fragilis (Leidy 1851) [=Spongilla f. Muttkowski 1918; Jewell 1935, 1939; Neidhoefer 1940]

Material examined: MPM Radiate #1733 (dry) *Spongilla fragilis*.

Gemmules may occur in clusters (Fig. 2h). Gemmoscleres are attached tangentially to the outer pneumatic layer that forms a coat over the gemmules. Megascleres are fusiform and entirely smooth. The foramen is tubular in shape. Conspicuous spines densely cover the gemmoscleres (Fig. 2i). Microscleres are absent.

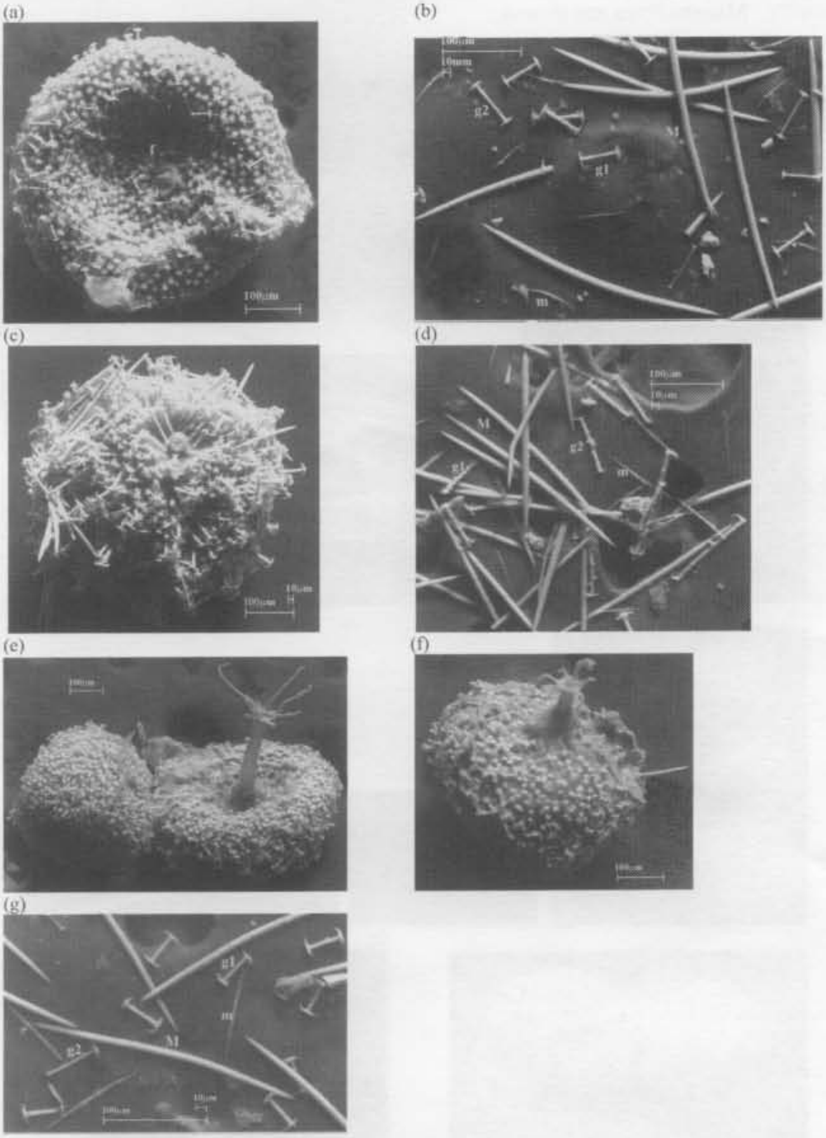

Figure 3. Scanning electron micrographs of Wisconsin sponge species: (a-b) *Heteromeyenia baileyi*; (c-d) *Heteromeyenia tentasperma*; (e-g) *Heteromeyenia tubisperma*. f = foramen, m = microsclere, M = megasclere, g1 and g2 = two classes of gemmoscleres.

Heteromeyenia baileyi (Bowerbank 1863) [*H. repens* Jewell 1935, 1939; Neidhoefer 1940]

Material examined: From USNM #39793. *Heteromeyenia baileyi* var. *repens* (Potts). Gist Gee Freshwater Sponge Collection, Germany.

The shape of the gemmule is spherical, excepted when deflated by drying (Fig. 3a). The foramen is slightly raised. The rotules of the gemmoscleres appear on the gemmule surface, as gemmoscleres are embedded in the pneumatic layer and arranged radially. The gemmoscleres (Fig. 3b) are birotulates of two groups. The smaller ones (g1) are more abundant, but similar in shape to the others. There are fewer of the longer type (g2), and they have straight cylindrical shafts with conical spines and umbonate rotules with deeply incised recurved teeth. Ricciardi and Reiswig (1993) described the smaller ones as having rotules which are flat and serrated. Megascleres are slender and gradually tapered, with sharp pointed tips. Microscleres are more delicate than the megascleres, have sharp pointed tips, and are slightly curved.

Heteromeyenia latitenta (Potts 1881) [=*Carterius latitentia* Neidhoefer 1940]

Heteromeyenia latitenta is not included here because the Wisconsin record apparently is based solely on a single gemmule from the bottom of Lake Mendota in Dane County (Smith 1921), documented by a microslide at the USNM (#30699).

Heteromeyenia tentasperma (Potts 1880) [=*Carterius tenosperma* Neidhoefer 1938, 1940]

Material examined: MPM Radiate #1739 (alc) *Carterius tenosperma* (sic).

Gemmoscleres protrude well beyond the outer layers of the gemmule (Fig. 3c). The foramen is distinct, but the characteristic tendril-like projections are missing. The gemmoscleres (Fig. 3d) are birotulate and of two class sizes. They have thick shafts with few spines. The rotules are equal and made up of several lateral spines. Megascleres are slender, straight to slightly curved fusiform, and have few microspines. Microscleres are very slender and evenly covered with microspines.

Heteromeyenia tubisperma (Potts 1881) [=*Carterius t.* Jewell 1935, 1939; Neidhoefer 1940]

Material examined: From USNM #39212, Gist Gee Collection and from USNM #40297.

The gemmule has a long tubular foramen with attached tendrils. The tubule (Fig. 3e, USNM #39212) may reach a length of 0.5 times the diameter of the gemmule (260µm/520µm). The tubule may also be somewhat shorter in proportion to gemmule diameter (Fig. 3f, USNM #40297). Gemmoscleres (Fig. 3g) are birotulate of two groups by length, with stout cylindrical shafts with a small number of acute spines. Both rotules are of equal diameter, and are made up of recurved teeth. Megascleres are slender and fusiform. Microscleres are slender and covered with microspines which are distinctly longer in the mid-section of the microsclere.

Radiospongilla crateriformis (Potts 1882) [=*Ephydatia c.* Neidhoefer 1940]

Material examined: From USNM #38684 Penney Freshwater Sponge Collection, collected July 5 1957, Farm Ponds, Auburn Univ., Trib. Tallapoosa R., Lee Co. Ala, by J.S. Dendy.

The Smith (1921) reference to this species in Wisconsin said that it was represented in the Conklin Collection from Douglas County, which was destroyed by fire. Neidhoefer (1940) offered no original documentation for the state, but instead quoted Potts (1887) who did not specifically mention Wisconsin. Frost et al. (2001) listed Wisconsin.

Slight cratering on the gemmule surface (Fig. 4a) identifies the foraminal area.

Gemmoscleres are slender, slightly curved with variable small conical spines, and have slightly recurved spines on the ends, making up pseudorotules (Fig. 4b). Megascleres are slender, fusiform, sharply pointed, and sparsely microspined except at the tips. Microscleres are absent.

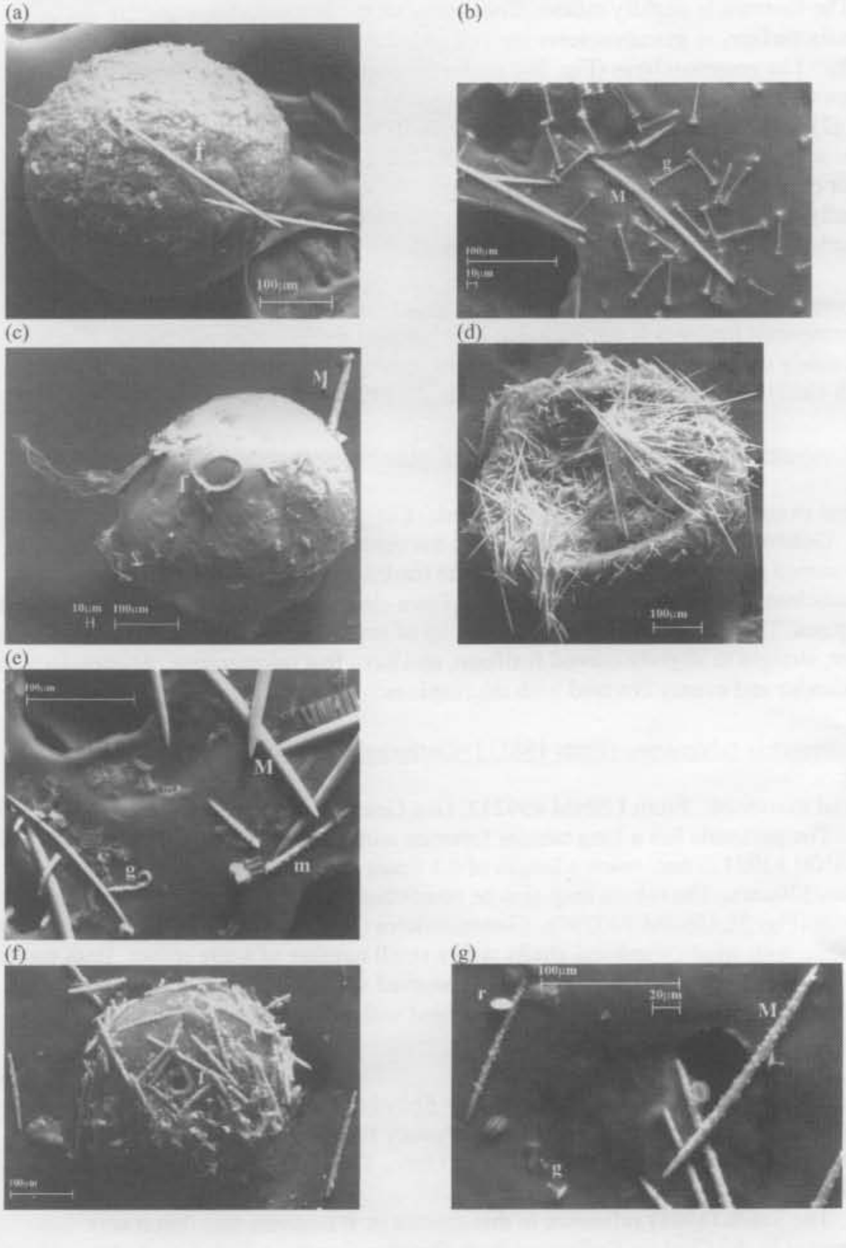

Figure 4. Scanning electron micrographs of Wisconsin sponge species: (a-b) *Radiospongilla crateriformis*; (c-e) *Spongilla lacustris*; (f-g) *Trochospongilla pennsylvanica*. f = foramen, g = gemmosclere, m = microsclere, M = megasclere, r = rotule.

Spongilla aspinosa Potts 1880

This species is known for the scarcity of its gemmules, a factor no doubt contributing to the difficulty of determining the true extent of its range in North America. Defining traits of Potts' (1880 and 1887) species were having gemmules clustered and covered with microscleres (Volkmer-Ribeiro and Traveset 1987).

Jewell (1935) listed an "atypical *Spongilla lacustris*" from a number of Wisconsin lakes and compared this to *Spongilla aspinosa*. Jewell (1935) used Potts' specimen material from the Smithsonian for her Figure 26, and concluded that his *S. aspinosa* and the atypical *S. lacustris* she had collected were separate entities. The NMNH's online invertebrate zoology database (www.nmnh.si.edu/iz/) includes 19 records of Wisconsin sponge specimens with identifications by Jewell as *Spongilla aspinosa*. Eighteen of these records are Jewell-collected specimens from the same lakes that she (1935) had given as the sources for her "atypical *S. lacustris*." Based on our examination of the specimen material we received from the NMNH for this study, we conclude that Jewell's original assessment of this material as "atypical *S. lacustris*" (Jewell 1935) was probably correct and that it therefore does not support a valid Wisconsin record for this species. We have found no other valid record for this species from Wisconsin.

Spongilla lacustris Linnaeus 1758 [=S.l. Neidhoefer 1940]

Material examined: MPM Radiate #1711 and MPM Radiate #2613 *Spongilla lacustris*.

The thin-walled gemmule (Fig. 4c) has few if any gemmoscleres (Frost et al. 2001). The foramen is simple with a small collar. This species also has thick-walled gemmules (Fig. 4d), embedded with numerous gemmoscleres and overlain with megascleres. Spicule types (Fig. 4e) include spine-covered, curved gemmoscleres, fusiform and entirely smooth megascleres, and the slightly curved, spine-covered microscleres. *S. lacustris* is the only Wisconsin species with a gemmule that can be without gemmoscleres or microscleres embedded or surrounding the gemmule.

Trochospongilla pennsylvanica (Potts 1883) [=Tubella p. Jewell 1935, 1939; Neidhoefer 1940]

Material examined: MPM Radiate #1740 (dry) *Tubella pennsylvanica*..

Many megascleres overlie the gemmule surface (Fig. 4f). The small distal ends of rotules are embedded and spaced on the surface of the gemmule. The foramen is a short tube. The gemmoscleres are birotulate, with rotules of very different size diameters, and the megascleres are slightly curved and coarsely spined (Fig. 4g). Microscleres are absent.

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