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FRESHWATER SILICA-SCALED HETEROTROPHIC PROTISTA: HELIOZOA, THAUMATOMONAD FLAGELLATES, AMOEBAE, AND BICOSOECIDS, FROM THE LAKE ITASCA REGION, MINNESOTA

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Forty-nine plankton samples were collected from the Lake Itasca Region, Minnesota over a period sporadically covering the summers of 1980, 1981 and 1987. A total of 22 freshwater heterotrophic siliceous-scaled species were observed: 18 heliozoa, two thaumatomonad flagellates, one bicosoecid, and one testate amoeba. Scale identifications were based on transmission electron microscopy. New records for North America include two heliozoans and one thaumatomonad flagellate. Five heliozoa taxa and one thaumatomonad flagellate are new records for the U.S. Wujek DE. Freshwater silica-scaled heterotrophic protista: heliozoa, thaumatomonad flagellates, amoebae, and bicosoecids, from the Lake Itasca Region, Minnesota. *Minnesota Academy of Science Journal*. 2015; **78(2):**1-14.
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INTRODUCTION

The protist groups of golden brown algae (Chrysophyceae and Synurophyceae), heliozoa (Heliozoa, Filosea), thaumatomonads (Cercozoa, Thaumatomonadida), bicosoecids (Protozoa, Bicosoecidida), and testate amoebae (Rhizopoda, Himatismenida) are characterized by an exogenous siliceous or calcified envelope composed of scales and/or bristles or loricae. These organisms have long been recognized as important indicators of environmental conditions. The silica-scaled chrysophytes are second in importance only after diatoms as indicators of the past ecology of lacustrine environments[1,2]. For example, the effects of acid rain were demonstrated by examining present and past sediments for scaled chrysophytes to demonstrate changes in the environment over time.

Identification of scale-bearing protists is based upon the morphology of scales, which are often preserved in sediments[1]. Light microscopy-based identifications are of limited value, as electron microscopy (EM) usually is necessary to distinguish sufficient morphology for species identification in the scaled chrysophyte groups[3] and now have become the tool for other scaled protists.

North American heterotrophic protist studies using electron microscopy in general have lagged behind those for Europe and Asia. The U.S. Great Lakes and inland water's heterotrophic freshwater protistan taxa, in particular the silica-scaled heliozoa and scaled flagellates, are poorly documented using electron microscopy (EM) with only a single species being reported [4]. On the contrary, more extensive Canadian studies by Nicholls and coworkers[5-8] have been reported. In contrast, the North American silica-scaled chrysophyte protist group (Chrysophyceae and Synurophyceae) have been widely published[3,9-15], including the Lake Itasca Region[11-15].

Heliozoans are cosmopolitan, free-living aquatic protozoans. Species are found in marine, brackish and fresh waters. Some species have endosymbiotic algae, but most obtain their nutrition by phagocytosis, usually using characteristically long axopodia and short pseudopodia to capture their food. For recent reviews of the heliozoa see Febvre-Chevalier[16], Lee *et al.*[17], Page and Siemensma[18] and Patterson and

Hedley[19]. An assessment of the centrohelid group and their worldwide distribution has been compiled by Mikrjukov[20].

In this study, the silica-scaled heterotrophic protists from samples collected during the summer months of 1977, 1980 and 1987 from 32 locations in the Lake Itasca Region, Minnesota, were observed using transmission electron microscopy. All the sampling sites have a substantial and published silica-scaled phytoplankton record[11-15].

MATERIALS AND METHODS

Forty-nine phytoplankton samples were collected from 32 sampling sites with 20 µm mesh plankton net from the Lake Itasca Region, Minnesota during the summer months of 1980, 1981 and 1987 (Table 1). Samples were preserved with acid Lugol's in plastic screw-cap vials. Samples for transmission electron microscopy (TEM) were sub-sampled onto Formvar-coated, carbon stabilized, 3 mm copper grids. After air drying, they were examined with either a Philips EM300 or JEOL CM-10 TEM. All identifications were based on TEM. The heliozoan classification used is according to Mikrjukov[20].

Table 1. Lake Itasca Region sampling sites and dates containing heterotrophic silica-scaled protistans.

Sampling Site	Date		
	1980	1981	1987
Arco Lake	x		
Beaver Pond, Hwy 4	x	x	
Bohall Lake, north end	x		x
Bohall Pond, south end			x
Chamber Creek			x
Dahlberg Lake	x		
Darling Pond	x	x	
Deer Park Lake	x		
Deming Pond, north	x	x	x
Deming Pond, south	x	x	x
Elk Creek	x	x	x
Hay Creek	x	x	
Lasalle Creek	x		x
Lake Itasca, north arm		x	
Lake Itasca, east arm		x	x
Lake Itasca, west arm		x	x
Long Lake	x	x	
Mary Lake		x	
Mississippi River culvert		x	x
Nicollet Lake			x
Pickerel Lake	x		
Squaw Lake	x		
Twin Lake, east	x		
Twin Lake, west	x		
Two Spot Trail, north pond #1			x
Two Spot Trail, north pond #2			x
Two Spot Trail, south pond #1			x
Two Spot Trail, south pond #2			x
Upper Rice River	x	x	
Wild Rice River	x	x	
Wilderness Drive, south pond #1			x
Wilderness Drive, pond #2			x

RESULTS

Twenty two heterotrophic freshwater silica-scaled protists were observed during this investigation (Table 2): 18 heliozoa taxa, two thaumatomonad flagellates, and one taxon each of a bicosoecid and rhizopod amoeba (Plates 1-23). Of the eighteen heliozoa observed, two are newly reported for North America (Plates. 11, 19) and six for the U.S. (Plates. 5, 7, 8, 15, 16, 17). The heliozoans observed all have a cosmopolitan distribution[20,21]. *Raphidocystis tubifera* was the most widely observed heliozoan in this study with the genus *Acanthocystis* having the greatest number of species (Table 2). Included in my observations was the first heliozoan characterized using electron microscopy, *Raineriophrys erinaceoides* (Plates 12-13), a species whose original description was as an *Acanthocystis* taxon from Denmark[22]. Thaumatomonad flagellates observed for the first time in North America was *Gyromitus limax*, and for the U.S., *Thaumatomastix triangulata* (Plate 21). All of taxa in the study are new records for Minnesota.

Table 2. Checklist of silica-scaled heliozoa, thaumatomonad flagellates, amoeba, and bicosoecids from the Lake Itasca Region, Minnesota, 1977, 1980, 1987.

Taxon	Location
Centrohelida	
Acanthocystis bicornis Dürrschmidt	Deming Lake
Acanthocystis cornuta Dürrschmidt*	Nicolett Lake
Acanthocystis nichollsi Siemensma & Roijackers	Pickerel Lake
Acanthocystis penardi Wailes*	Hay Creek, Wild Rice River
Acanthocystis polymorpha Dürrschmidt*	Deming Lake ponds, Wild Rice River
Acanthocystis turfacea Carter	Arco Lake
Choanocystis aculeata (Hertwig & Lesser) Siemensma & Roijackers	Squaw Lake
Pterocystis tropica (Dürrschmidt) Siemensma**	Long Lake, Wild Rice River
Raineriophrys erinaceoides (Petersen & Hansen) Mikrjukov	Hay Creek, E. Twin Lake, Two Spot ponds, Wild Rice Lake
Raphidiocystis tubifera Penard	Fish Hook Lake, Pickerel Lake, S. Deming Pond, Squaw Lake, Pickerel Lake
Raphidiophrys elegans Hertwig & Lesser	E. Twin Lakes, Upper Rice Lake
Polyplacocystis marginata (Siemensma) Mikrjukov	N. Deming Pond
Pompholyxophrys ovuligera Penard*	Dahlberg Lake, Long Lake
Pompholyxophrys punicea Archer*	Wild Rice River
Pompholyxophrys stellata Dürrschmidt and Nicholls*	Deming Lake, Hay Creek, Pickerel Lake
Pinaciophora fluviatilis Greeff	Lake Itasca, all arms
Rabdiophrys anulifera Rainer emend. Siemensma	E. Twin Lake, Upper Rice Lake
Rabdiophrys monopora (Thomsen) Roijackers & Siemensma**	Pickerel Lake
Thaumatomonadida	
Thaumatomastix triangulata (Balanov) Beech & Moestrup*	Dahlberg Lake
Gyromitus limax Belcher & Swale**	S. Deming Pond
Himatismenida	
Cochliopodium bilimbosum Auerbach	Hay Creek, Pickerel Lake
Bicosoecida	
Cyathobodo crucifera Swale & Belcher	Deming ponds

*New record for the United States
**New record for North America (see Table 1 for dates)

DISCUSSION

Heliozoa

Heliozoa are defines as predatory organisms, distinguished by their spherical body that is characterized with fine radiating cytoplasmic projections - the axopodia. These cytoplasmic projections participate in the capture of prey, movement of cells, and adhesion to various substrates. Long considered a natural taxonomic group, recent molecular data indicate they are rather a polyphyletic assemblage of protists[23,24].

Order **Centroheliozoa** (Centrohelids)

Family **Raphidiophryidae**

All taxa have external scales surrounded by a hollow marginal rim. Spine scales, if present, are always symmetrical and either tubular, trumpet or funnel-shaped.

Polyplacocystis marginata (Siemensma) Mikrjukov Figure 1, Plate 1.

Polyplacocystis marginata, originally described as *Raphidiophrys marginata* Siemensma[21], it was later transferred by Mikrjukov[25] to his newly described genus *Polyplacocystis*. The genus contains five species.

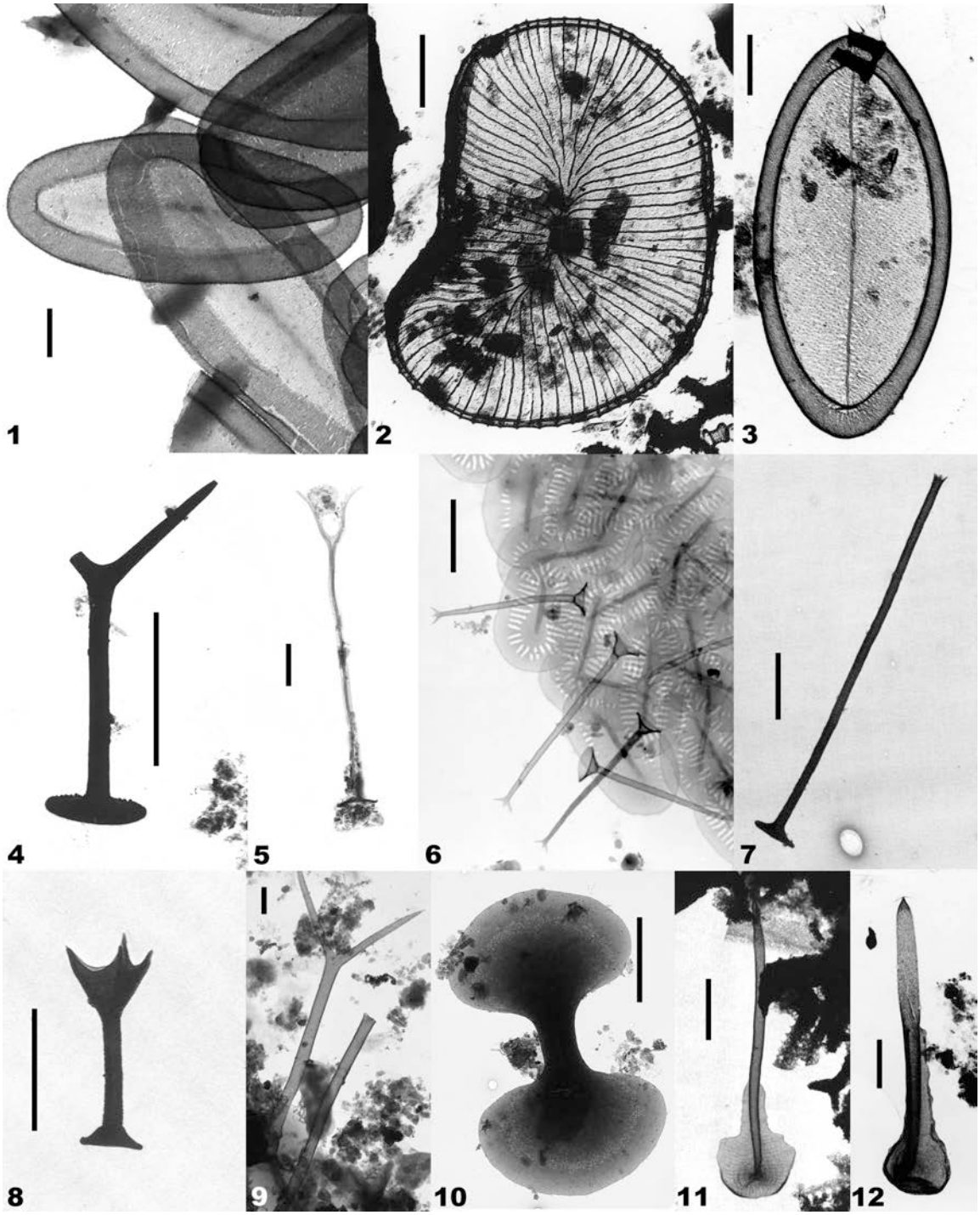

Figure 1. Plates 1-12. Isolated surface scales. Plate 1. *Polyplacocystis marginata*, body scale. Plate 2. *Raphidiophrys elegans*, body scale. Plate 3. *Raphidiocystis tubifera*, plate scale. Plates 4-9. *Acanthocystis*. 4. *A. bicornis*, spine scale. 5. *A. cornuta* spine scale. 6. *A. nichollsi*, body scales. 7. *A. penardi*, spine scale. 8. *A. polymorpha*, spine scale. 9. *A. turfacea*, spine scale. Plate 10. *Choanocystis aculeata*, a whole plate scale from cell surface. Plate 11. *Pterocystis tropica*, spine scale. Plate 12. *Raineriophrys erinaceoides*, spine scale. Scale bars = 1 µm.

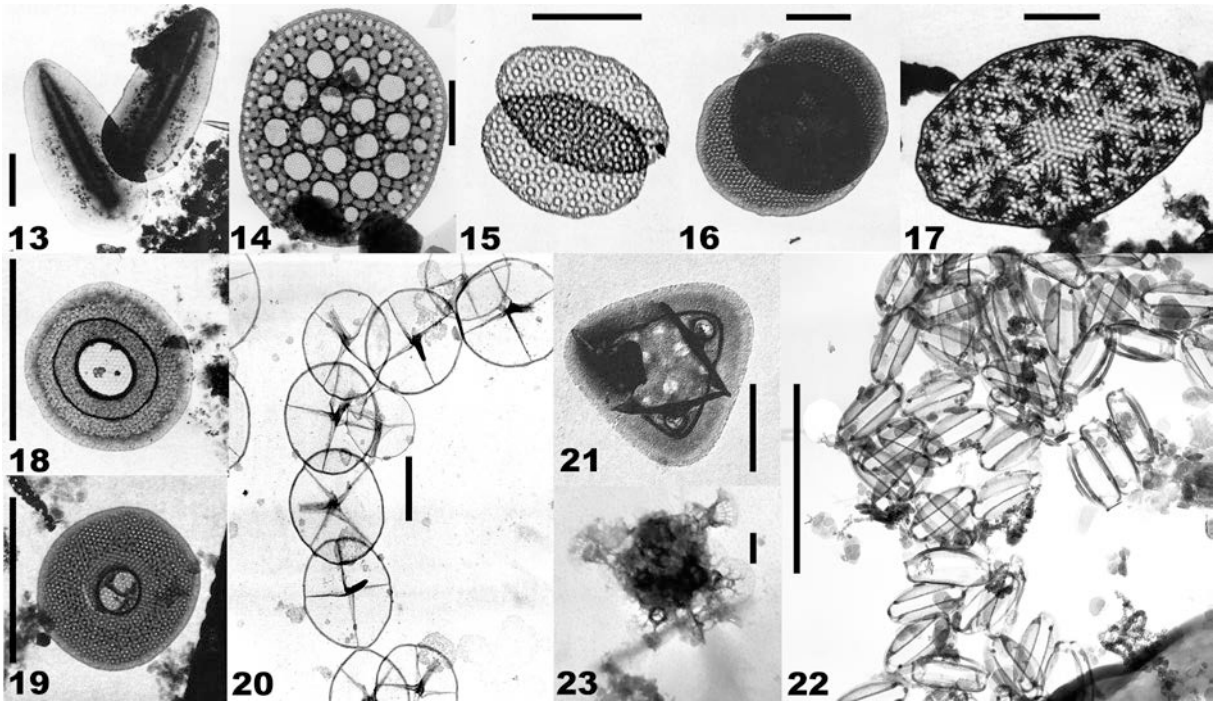

Figure 2. Plates 13-22. Isolated surface scales. Plate 13. *Raineriophrys erinaceoides*, plate scales. Plate 14. *Pinaciophora fluviatilis*, body scale. Plates 15-17. *Pompholyxophrys*. 15. *P. ovuligera*, two body scales. 16. *Pompholyxophrys punicea*, two body scales. 17. *P. stellata*, body scale. Plates 18-19. *Rabdiophrys*. 18. *R. anulifera*, body scale. 19. *R. monospora*, body scale. Plate 20. *Cyathobodo crucifera*, spine scales. Plate 21. *Thaumatomastix triangulata*, body scale. Plate 22. *Gyromitus limax*. Plate 23. *Cochliopodium bilimbosum*, body scales. Scale bars = 1 μm.

Cells are covered with one or several types of spindle form or flat siliceous scales. The flat scales are placed mainly in a tangential direction and have circular or smooth texture of the upper surface. Radial elements in the periplast are absent. As *Raphidiophrys marginata*, it was reported by Nicholls and Dürrschmidt[8] from Canada, Chile, New Zealand, and Sri Lanka, by Roijackers and Siemensma[26] from The Netherlands, and by Wujek[27] from Louisiana and Texas. Most recently Wujek and Ogundipe[28] reported it from Africa. I observed it in only one sample (Table 2).

Raphidiophrys elegans Hertwig & Lesser emend. Penard (Plate 2)

Rainer[29] described both colonial and solitary forms of this species, but I observed only solitary forms. EM images of the scales of this species were first published by Takahashi[30], but not identified as belonging to this taxon until the work of Siemensma and Roijackers[31]. Scales were observed only from two samples (Table 2). Although previously reported from Canada[8] (as *R. obiculatus*) and the southern U.S.[32], Minnesota is its most northern U.S. occurrence.

Raphidocystis tubifera Penard Plate 3.

Since its original description from Switzerland[33], electron microscopic reports include: Rees and co-workers[34] from England and Canada; Siemensma[31] from The Netherlands; Nicholls and Dürrschmidt[8] from Chile, New Zealand, and Malaysia; Croome[35] from Australia; Finlay and co-workers[36] in England; Mikrjukov[37-39] from Russia and Estonia; Vigna and Alberio[40] from Argentina; and by Wujek for the U.S. (three Gulf Coast states: 27; Indiana: 41). This species was the most widely observed heliozoan in this study, being observed in five samples (Table 2).

Family **Acanthocystidae**

The periplast is differentiated on basis of two scale types: external plate/tangential scales with a well-developed central sternum; these scales also are surrounded by a hollow marginal rim; spine scales are funnel shaped or possess a base-plate well developed wings or nodules. The genus *Acanthocystis* contains unicellular, free-floating planktonic or benthonic living organisms which are common in freshwater but also found more rarely in marine habitats. They are covered all over with siliceous scales of mainly 2 types, which form a more or less flexible armor (periplast). Other generic characteristics are the absence of a distinct covering of gelatin or mucus, the possession of a centroplast at the very centre of the cell from which the granulated axopodia radiate and, therefore, an eccentrically placed nucleus[42]. The morphology of the scales appears to be constant and is thus often used to distinguish the species, in addition to the general morphology of the cell itself. The fine structure of the scales is, for the most part, beyond the resolution of the light microscope, so that classification based on this feature was necessarily somewhat vague. A further difficulty in the taxonomy of the genus *Acanthocystis* is that the internal cell structure is largely unknown, except for *A. turfacea*[42]. The genus *Acanthocystis* contains more species than any of the other centrohelid genera. The genus, erected by Carter[43], is widespread with members occurring in both freshwater and marine habitats. Cells tend to be round covered by siliceous spine scales with a layer of overlapping siliceous body scales.

Acanthocystis bicornis Dürrschmidt Plate 4

A. bicornis is easily distinguished from all other species of *Acanthocystis* by the distinctly bifurcate tips of the small spine scales in addition to teeth on each apical branch. Nicholls[5] reports Canadian specimens of *A. turfacea* with "tiny teeth on the bifurcate apices of the long spines". However, the forks of the small spine scales are smooth and pointed. *A. cornuta* also has teeth along the inner edge of the branches of the apical furca by differs in that there are always three or more teeth present, and in that it has only one spine scale type. A survey of all scales hitherto examined shows that there are only minor variations in the spine scale structure among specimens from different sources. *A. bicornis* has been found in a small swamp in southern Chile, a small shallow pond near in E. Sri Lanka and Canada[44] and Russia[20,45]. Its occurrence in the Deming ponds (Table 2) is the first report for the United States.

Acanthocystis cornuta Dürrschmidt Plate 5.

A. cornuta most closely resembles *A. radiosa* Roskin and *A. pectinata* Penard, the only previously known *Acanthocystis* species with a single type of forked spine scales, and *A. bicornis* (this paper), from which it differs (1) in having only one type of spine scale, (2) in having more than 2 teeth along the inner edge of the apical furca, and (3) in the faint slit-pattern of the plate scales. Previous EM reports include Chile, New Zealand, Malaysia and Sri Lanka[44] and the Carolinas[32].

Acanthocystis nichollsi Siemensma & Roijackers Plate 6.

The only other report of this taxon for North America was as *Acanthocystis pectinata* from Canada[5] and the U.S. (Florida: 46). I observed it from **six** Minnesota locations (Table 2).

Acanthocystis penardi Wailes Plate 7.

Since its original description from British Columbia based on light microscopy[47], EM reports indicate it has a world-wide distribution[20]. In Minnesota, I observed it from two locations (Table 2), reaffirming it as a freshwater species, and its first report from the United States.

Acanthocystis polymorpha Dürrschmidt Plate 8.

Its only other North American report is from Canada[5], with other reports that include South America and Africa[20]. Its presence in Deming Lake ponds and the Wild Rice River represents it first report for the U.S. (Table 2).

Acanthocystis turfacea Carter Plate 9.

Carter[43] based the genus description of *Acanthocystis* on this species. *A. turfacea* is easily recognized by light microscopy and has been recorded worldwide. EM observations of this species are primarily from freshwater, but include brackish and marine waters. They include Chile[44], Canada[5], Australia[49], Antarctica[50], Russia[20,45] and the U.S. (Florida[46]). Considering it is one of the most widely distributed heliozoan species[20,21], it is surprising that I observed it only in one sample (Table 2).

Choanocystis aculeata (Hertwig & Lesser) Siemensma & Roijackers Plate 10.

Originally described as a species the genus *Acanthocystis*[51], it was transferred to *Choanocystis* by Roijackers and Siemensma[26] when they reported it from The Netherlands and recognized it as synonymous with *Acanthocystis serrata*[5]. Two types of scales are present: plate and spine. The spine scales are characterized with spicules attached more or less eccentrically in the base of the grove or incision of the heart-shaped base-plate. Previously reported from the Gulf States[27], I observed it only in one sample (Table 2).

Pterocystis taxa possess tangential plate scales and radial spine scales. The spine scales are bilaterally symmetrical, with a cylindrical shaft and membranous base which merge into two lateral membranous wings that extend for some distance along the shaft.

Pterocystis tropica (Dürrschmidt) Siemensma Plate 11.

Described from Sri Lanka as a taxon in the genus *Acanthocystis*[44], it was later transferred to *Pterocystis* by Siemensma[21]. This is the first report of this taxon for North America (Table 2) and its first from a non-tropical habitat.

Raineriophrys is a genus described by Mikrjukov[20] by separating eight species from the genus *Pterocystis*. Also possessing two types of scales, the spine scales form an external jacket that consists of a distinctly developed shaft with lateral wings, extending along the shaft some distance, with a basal wing that lies perpendicular with the shaft and forms the spine base.

Raineriophrys erinaceoides (Petersen and Hansen) Mikrjukov Plates 12-13.

The first heliozoan described by electron microscopy[22] (as *Acanthocystis erinaceoides*), all newly described taxa since have involved EM. It has been reported worldwide: Germany[42], Canada[5], Chile[48], Australia[49], Russia[45], and the U.S.[41] (Indiana). It was tied for the second most observed species in this study (Table 2). Both spine (Plate 12) and plate scales (Plate 13) are illustrated.

Rhizopoda

Order **Rotosphaerida** (Rotosphaerids)

Family **Pompholyxophryidae** (Filose amoebae; heliozoa-like amoebae)

Numerous, but variable round, biconvex, perforated, overlapping scales cover the cells of *Pinaciophora*. Like all filose amoeba, this genus lacks axonemes and extrusomes observed in the centrohelid heliozoa.

Pinaciophora fluviatilis Greeff Plate 14.

First reports of this organism for the U.S., under the name of *Potamodiscus kalbei* Gerloff, were from Alaska[52], Mississippi and Ohio[53]. Gaardner *et al*.[53] showed that it was incorrectly described as a centric diatom. It has since been reported from the U.S. – Lake Erie[4], New York[54], the Gulf States[27], and the Carolinas[32]. Other worldwide EM reports include both marine and freshwater habitats[4]. I observed it from only the Lake Itasca sites (Table 2).

The genus *Pompholyxophrys* has been described as a nucleariid amoebae[54]; Siemensma[21] classifies them as heliozoa belonging to the Super Class Rhizopoda, Class Filosea. Other references for this genus are Nicholls and Dürrschmidt[8] and Mikrjukov[20]. Seven species are recognized[21].

Pompholyxophrys ovuligera Penard Plate 15.

On the basis of EM, Takahashi[30] illustrated scales of this taxon as fig. 67 from Japan. Later EM reports

include Nicholls and Dürrschmidt[8] from Canada, Chile, New Zealand, and Sri Lanka, Croome[49] from Australia, Roijackers and Siemensma[26] from Holland and Sweden, and Wujek[32] from the Carolinas. It was observed in three samples (Table 2).

Pompholyxophrys punicea Archer Plate 16.

Observed in samples from the Wild Rice River (Table 2), it is most common in freshwater and wet *Sphagnum*, especially in the summer. Scales of this taxon represent the first record for the U.S. (Table 2).

Pompholyxophrys stellata Dürrschmidt and Nicholls Plate 17.

Previously reported for North American from Florida[46], isolated scales were observed from the Hay Creek samples (Table 2).

The cells of *Raphidiophrys* are coated with curved, spindle-shaped scales. Eighteen species are recognized[56,57].

Rabdiophrys anulifera Rainer emend. Siemensma Plate 18.

First described by Rainer[26] using LM, Siemensma[21] later emended its description using EM. Other than Siemensma's report of it from The Netherlands, its only other reports documented with EM are Nicholls[6] from Canada as *Pinaciophora pinea* and Florida[46]. All reports are, from freshwater localities. This is the third record of it for North America. I observed it from two locations (Table 2).

Rabdiophrys monopora (Thomsen) Roijackers & Siemensma Plate 19.

Originally described in the genus *Pinaciophora* (as *P. monopora* Thompson) from Denmark, Roijackers and Siemensma[26] in their revision of the genus *Pinaciophora* transferred the species based on their Holland samples. A plate scale, but no spine scales from a cell of *R. monopora* were observed from the Hay Creek and Pickerel Lake samples (Table 2) and represent the first report of this species for North America.

Protozoa

Order Bicosoecida (Bicosoecids)

Cells bearing two flagella that insert anterio-laterally. One flagellum attaches to the substrate or lorica either directly or indirectly (via a thread of mucus) to the substrate. The anterior flagellum creates currents of water from which particles are ingested at a discrete ingestion area. Fresh-water and marine taxa have been described.

Family **Pseudodendromonadidae**

Heterotrophic flagellates with two flagella and discrete ingestion area formed by loop of two microtubular roots. Cells may be solitary and motile, attached by a stalk or colonial and attached. They or may not have surface scales. Only two genera are in the family.

Cyathobodo crucifera Swale and Belcher Fig. 20

Described from England[58], scales of this taxon were illustrated, but not identified, in a figure reporting *Chrysochromulina parvula* Lackey for the United States[59]. It has since been reported twice from the U.S.[41,60]. The genus is placed by Hibberd[61] within the order Pseudodendromonadida, together with the genus *Pseudodendromonas*. The Pseudodendromonadida are members of the class Cyathobodonea within the phylum Opalozoa[62]. Karpov[63] recently recognized still another order, the Bicosoecida, into which he placed *Cyathobodo* and *Pseudodendromonas* into his newly recognized family Pseudodendromonadidae. This is the first report of this taxon for the Great Lakes area (Table 2).

Cercozoa

Order **Thaumatomonadida**

Family **Thaumatomonadidae** (Thaumatomonad flagellates)

Cells of members of this family are typically biflagellated swimming or gliding cells capable of producing thin pseudopodia from one (ventral) face of the body which may be grooved. Mostly medium

sized (15 - 50 µm). All species studied to date by electron microscopy have surface silica scales of two forms arranged in a single layer: oval-triangular body scales and spine scales. The family Thaumatomonadidae is now restricted to five genera: *Thaumatomonas, Allas, Reckertia*, *Gyromitus*, and *Thaumatomastix*[64].

Thaumatomastix triangulata (Balanov) Beech & Moestrup Plate 21

Scales from a colorless free-living flagellate belonging to the Class Thaumatomonadida were observed in the Hay Creek sample (Plate 20; Table 2) representing its first report from the U.S. *Thaumatomastix triangulata* was originally described and placed in the algal genus *Chrysosphaerella*[65]. Later Beech and Moestrup[66] recognized the species was not a photosynthetic chrysophyte alga and transferred it to the testate amoeba *Thaumatomastix*. Recently Nicholls[67] when reporting its first occurrence in North America from Ontario, Canada challenged the supposition of Howe *et al.*[64] that Beech and Moestrup[66] "were wrong" and that Balanov[65] made "errors in interpreting his light microscope observation."

The thaumatomonads are biflagellate swimming or gliding marine or freshwater cells capable of producing slender pseudopodia from one (ventral) face of the normally grooved body. Medium in size (15-50 µm), all thaumatomonad cells studied to date by electron microscopy have siliceous surface scales. Patterson and Zölffel[68] recognized five genera. However, based on molecular data, seven genera currently placed in the family Thaumatomastigaceae by Howe *at al.*[64]. The genus *Thaumatomastix* appears to be an unwieldy, artificial conglomeration of forms that produce scales of two morphologies: triangular or elliptical symmetry, and the presence or absence of spine scales. The scales are observable only using electron microscopy. The genus *Thaumatomastix* differs from the closely related genus *Thaumatomonas* by the presence of flagellar scales and a longer anterior flagellum. The distinctions between the two genera are not clearly defined, and the validity of each genus needs to be confirmed[68]. Molecular studies may resolve this taxonomic problem, especially their relationship to the closely allied thaumatomonad genera *Thaumatomonas* and *Allas*[69]. Indeed, Wyleczich *et al.*[69] has shown only small sequenced differences between them and other genera in the family, primarily in their biology, scale structure, and cell shape.

Gyromitus limax Belcher & Swale Plate 22

Scales of another taxon, *G. limax*, belonging to the Family Thaumatomonadidae described from its original location, England[70], with subsequent reports of *G. limax* from Finland[71], Swiss soil[72] and Nigeria[73], were observed in the S. Deming Pond (Table 2) and represent its first record for North America. Nicholls[74] using X-ray emission spectra on another species, *G. disomatus*, has shown that the scales are made of silica but are not calcified, and hence do not represent coccoliths. Howe *et al.*[64] however in a somewhat different classification recently split *Gyromitus* from this family and placed it along with a new genus, *Peregrinia* subsequently placing both genera in a new family, Peregriniidae.

Rhizopoda

Order **Himatismenida**

Family **Cochliopodiidae** (Testate/rhizopod amoebae)

Amoebae partially enclosed within a flexible cuticle or wall (tectum) usually covered with microscales. The covering is open along the region of attachment to the substratum; with no well defined aperture.

Cochliopodium bilimbosum Auerbach Plate 23

Species of the genus *Cochliopodium* are rhizopod or testate amoebae with one or more pseudopodia and without a firm shell[19]. They have been reported, frequently in large numbers, from a broad range of habitats ranging from marine and fresh waters, activated sludge plants, percolating filters, small streams and ponds, sphagnum swamps, soil, fecal material and even from cooling towers [see Wujek[8], and literature therein]. It is perhaps surprising,

therefore, to find that species this genus have not formed the subject of any major investigation.

Cochliopodium was described by Hertwig and Lesser[51] and now comprises approximately 17 species. Cells in the genus are comprised of lens-shaped lobose amoebae bearing a tectum – a monolayer of scales covering the cell. The taxonomic position of the genus has been the subject of dispute. It has been variously described as a testate amoeba (family Testacea) with a thin and flexible test[75,76] and as a naked amoeba with a stiffened pellicle[77]. The most recent treatment places the genus in the Euamoebae[19] and the Class Lobosea, Phylum Rhizopoda[78]. This group as now constituted includes the rhizoid amoebae which have one or more broad pseudopodia, and a firm shell. In addition, cells of *Cochliopodium* when observed with phase microscopy possess a regular pattern on their surface (scales) that appear as a series of radiating dots and internal crystals.

Only isolated scales were observed in the TEM preparations (Plate 2**1**), but the structure of these scales clearly identified them as belonging to a species of *Cochliopodium*, *C. bilimbosum* (Auerbach) Leidy. Each scale consists of a flat circular base plate from which arises perpendicularly a lattice-like column surmounted by a funnel-shaped capital. The scales lie directly on the plasma membrane[79]. No cells were observed using light microscopy. Electron microscopy is now required for species identification. The species is widespread in Europe and North America[80]. This report is the third EM observation of this taxon for North America and the first report for Minnesota.

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