

## Resting spores of the freshwater diatoms *Acanthoceras* and *Urosolenia*

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### Abstract

Diatom resting spores are a widespread, but sometimes misconstrued component of siliceous microfossil assemblages. We illustrate and discuss resting spore morphology found in populations of *Acanthoceras* and *Urosolenia*, two widely distributed freshwater genera. Taxonomic status of these genera and the potential paleolimnologic interpretation of resting spores are discussed.

### Introduction

Resting spores are especially common in temperate, neritic, marine centric diatoms. In most taxa, they are an asexual stage in the diatom's life history and formed under conditions of nutrient stress. Recent articles by Hargraves & French (1983) and Garrison (1984) review the characteristics and ecological importance of resting spores. In short, resting spores are thought to function as a resistant stage during periods of environmental extreme, but may also function for grazing resistance and as a means of increasing species dispersal potential. While unable to survive years of dormancy, resting spores have greater viability than vegetative cells under conditions of darkness, temperature extreme, and desiccation. Excystment usually occurs when spores are resupplied with light and nutrients. Also known as statospores, statocysts, or *Dauersporen*, the terms resting spores or hypnospores are now most widely accepted.

Spores are characterized by two heavily silicified valves, termed primary and secondary, and may or may not have copulae or girdle bands

(Syvertsen, 1979). They have a higher chlorophyll content and faster sinking rates than vegetative cells (French & Hargraves, 1980), and are rich in storage products (Hargraves & French, 1983). Three types of resting spores have been described, based on the association of the spore to the mother cell; exogenous, semi-endogenous, and endogenous (Anonymous, 1975).

The occurrence of diatom resting spores in inland waters is limited to a few centric and pennate taxa (von Stosch & Fecher, 1979). The centric representatives belong to groups of taxa that have classically been placed in marine genera, including *Chaetoceros* Ehrenb., *Rhizosolenia* Brightwell, and *Attheya* West. *Chaetoceros* is widely distributed in inland saline lakes and brackish waters (Rushforth & Johansen, 1986). Its resting spores are commonly preserved in saline lacustrine sediments and have proven to be excellent paleolimnologic indicators of higher salinity levels (Fritz & Battarbee, 1988). In *Rhizosolenia* and *Attheya*, recently proposed nomenclatural changes have separated the marine and freshwater taxa. Freshwater *Rhizosolenia* have been transferred to the genus *Urosolenia* Round and Crawford (Round

*et al.*, 1990) and *Attheya zachariasii* J. Brun. was transferred to the monotypic genus *Acanthoceras* Honigmann (Simonsen, 1979). These changes have taken into account ecological, cytological, and frustular differences between freshwater and marine species. As these two freshwater genera also form resting spores (Huber-Pestalozzi, 1942), we felt that a better understanding of this aspect of their life history and a presentation of resting spore morphology might provide taxonomic assistance and information on their paleolimnologic interpretation.

### Materials and methods

Nearsurface plankton containing populations identified as *Urosolenia eriensis* (H. L. Smith) Round and Crawford (= *Rhizosolenia eriensis* H. L. Smith) and *Acanthoceras zachariasii* (Brun) Simonsen (= *Attheya zachariasii* J. Brun) was collected from East Lake Okoboji, Dickinson County, Iowa, USA, on 31 July 1991. East Lake Okoboji is a shallow (10 m), alkaline, hyper-eutrophic lake (Bachmann & Jones, 1974). A population identified as *Rhizosolenia longiseta* Zacharias producing resting spores was found in plankton collected on 02 July 1992 from East Pike Lake, Cook Co., Minnesota, USA. East Pike Lake is a shallow (14 m), circumneutral, softwater, moderately productive lake (Minnesota Department of Natural Resources, 1990).

In addition to whole plankton burn mounts, formalin-preserved samples were prepared for microscopy by oxidizing the organic content in fuming HNO<sub>3</sub> for one hour or overnight in cold 30% H<sub>2</sub>O<sub>2</sub>. Oxidation byproducts were removed with six successive rinses with distilled water, allowing six hours between rinses for settling. Cleaned diatoms were dried onto coverslips. Samples for light microscopy were mounted in Hyrax<sup>TM</sup> and the slides deposited in the Center for Great Lakes and Aquatic Sciences Diatom Herbarium (Dr E. F. Stoermer, University of Michigan). For scanning electron microscopy, pieces of coverslips with cleaned diatoms were attached to aluminum stubs, coated with 20 nm

AuPd, and viewed at an accelerating voltage of 25 kV in a JEOL JSM-T100 SEM.

### Results

#### *A note on taxonomy of Attheya/Acanthoceras and Urosolenia/Rhizosolenia*

Since there have been a number of nomenclatural changes involving taxa discussed here, and further changes apparently required, a brief discussion of historical nomenclature and current taxonomic status is in order.

Simonsen (1979) pointed out that the freshwater taxon commonly reported as *Attheya zachariasii* Brun differs morphologically from marine species of *Attheya*, and is better referred to as *Acanthoceras* Honigmann. *Acanthoceras* is based on *A. magdeburgense* Honigmann, an apparent later specific synonym of *Attheya zachariasii* Brun. *Acanthoceras zachariasii* (Brun) Simonsen is widely distributed and occasionally abundant but ephemeral, in alkaline, eutrophic, lakes, ponds, and rivers in North America and northern Europe (Beaver, 1981).

We should also point out that further nomenclatural changes affecting this taxon may be required. *Acanthoceras* Honigmann 1909 appears to be a later homonym of *Acanthoceras* Kützting 1842, a name applied to a red alga (Rhodophyta). A new generic name may eventually be required (P. Compère, pers. comm.).

Round & Crawford (Round *et al.*, 1990) erected the genus *Urosolenia* to include certain freshwater taxa formerly assigned to the primarily marine genus *Rhizosolenia* Brightwell. Round & Crawford based their new genus on *Rhizosolenia eriensis* H. L. Smith. *Urosolenia eriensis* (H. L. Smith) Round & Crawford is widely reported from an extreme diversity of habitats in Europe and North America. Another commonly recorded freshwater taxon, *Rhizosolenia longiseta* Zacharias, was not formally transferred to *Urosolenia* by Round *et al.* (1990) or, so far as we are aware, by subsequent authors. Being of the opinion that the taxon commonly reported in the literature as *Rhizosolenia longiseta* has the morphological char-

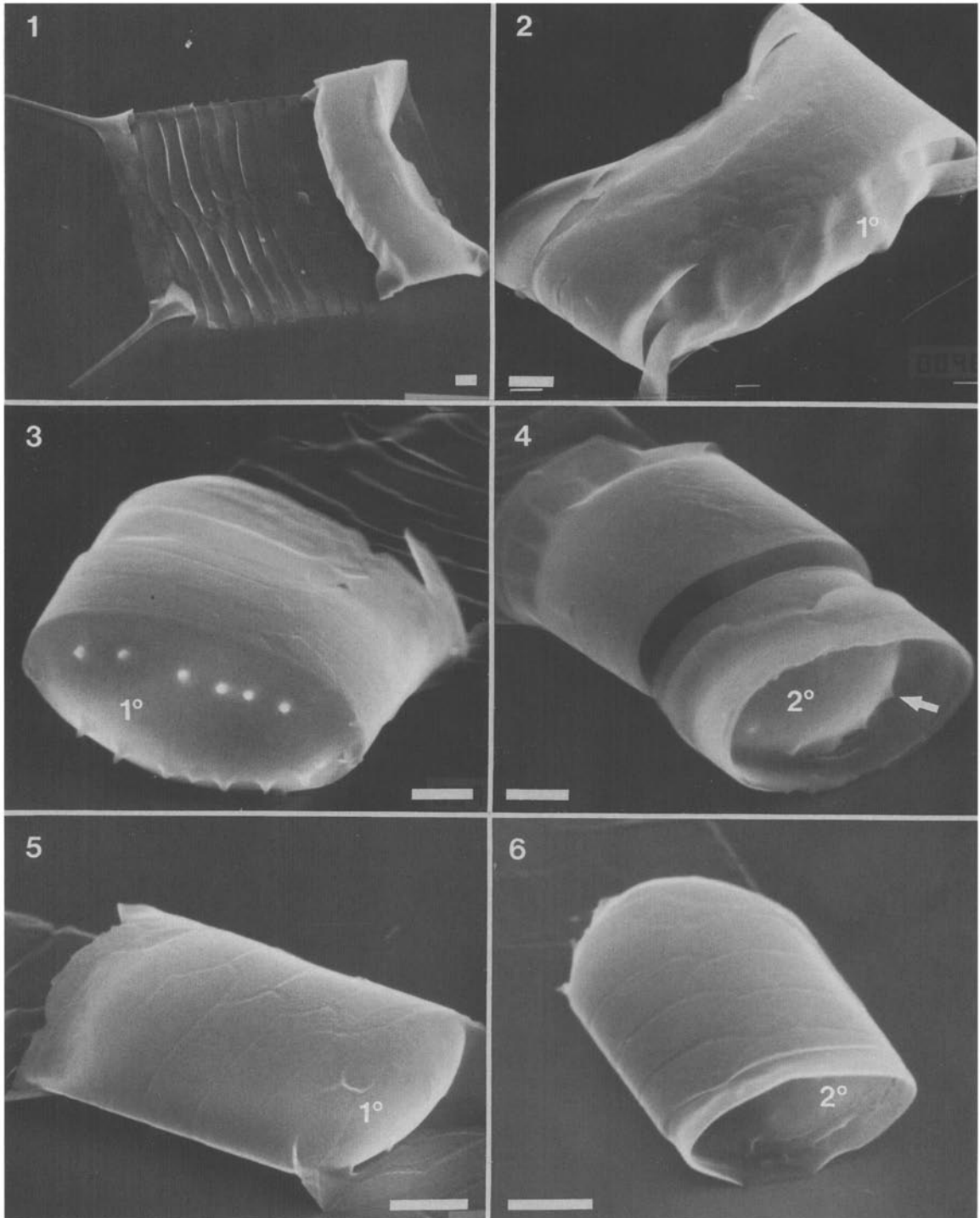


Plate I. Scanning electron micrographs of diatom resting spores. Scale bars = 2.0  $\mu\text{m}$ , 1° = primary spore valve, 2° = secondary spore valve. Fig. 1. Association of *Acanthoceras zachariasii* spore with mother frustule. Fig. 2. Primary valve face of *A. zachariasii* spore. Fig. 3. Primary valve face of *Urosolenia eriensis* spore with ring of short spines. Fig. 4. Secondary valve face of *U. eriensis* spore with small spines sometimes present (arrow). Fig. 5. Primary valve face of *U. longiseta* spore devoid of spines. Fig. 6. Secondary valve face of *U. longiseta* spore.

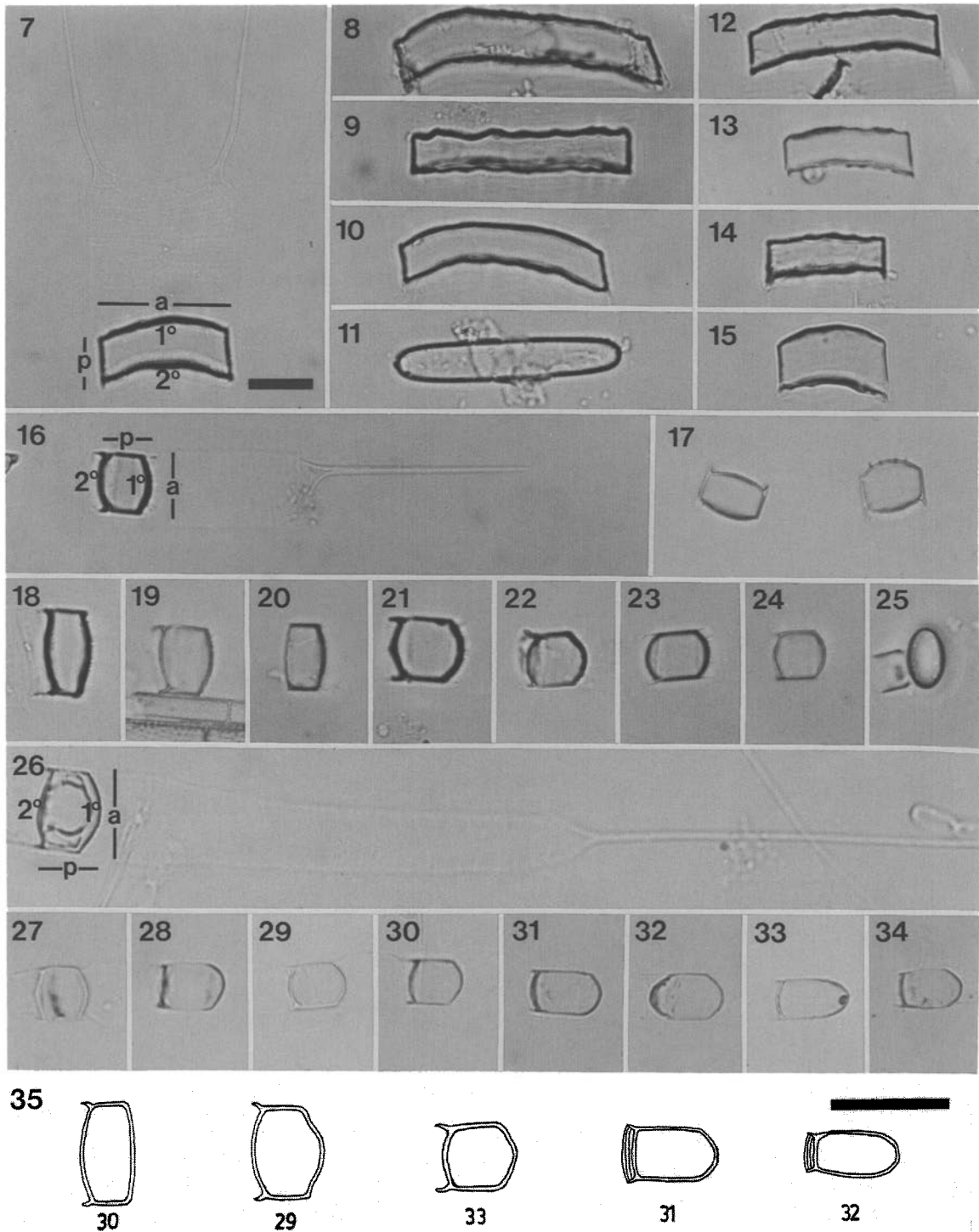


Plate II. Light micrographs and line drawings of diatom resting spores. Scale bars = 10  $\mu$ m. All light micrographs at same magnification (scale bar in Fig. 7). a = apical axis, p = perivalvar axis, 1° = primary spore valve, 2° = secondary spore valve. Figs 7-15. *Acanthoceras zachariasii* resting spores. Fig. 7. Girdle view of spore still associated with half of mother frustule. Figs. 8-10, 12-15. Girdle views of *Acanthoceras* spores showing size and morphological range. Fig. 11. Valve view. Figs. 16-25.

acteristics of *Urosolenia* and not those of *Rhizosolenia*, we propose the new combination:

*Urosolenia longiseta* (*Zacharias*) *comb. nov.*

*Rhizosolenia longiseta* *Zacharias, Forschungsber. Biol. Stat. Plön, Bd. I, S. 38, Fig. 7. 1893.*

In making this new combination, we recognize that the current taxonomy of this group is extremely uncertain, and liable to extensive further change. Specifically, *Rhizosolenia longiseta* may prove to be a later synonym of *Rhizosolenia gracilis* H. L. Smith. Generally, the extreme range of physical and chemical habitat types to which some of the taxa referable to *Urosolenia* have been reported (Beaver, 1981), leads to suspicion that either the taxonomy of this group has not been sufficiently resolved, misidentification is rampant, or both. In order to prevent further confusion in nomenclature, resolution of true taxonomic affinities in *Urosolenia* would require a monographic study. Unfortunately, this is a task beyond our present resources.

#### *Morphology of the Acanthoceras resting spore*

*Acanthoceras zachariasii* produced a single, heavily silicified, endogenous resting spore centrally located within the mother cell (Figs 1, 7). The transapically flattened resting spore was usually seen in girdle view with a convex primary valve and a concave secondary valve (Figs 1, 2). The primary valve had a furrowed or crenate valve face while the secondary valve was nearly smooth or only slightly furrowed. Spores ranged in size from 14.3 to 41.0  $\mu\text{m}$  in the apical axis, and 5.2 to 11.5  $\mu\text{m}$  in the perivalvar axis (Figs 7–15). In valve view, *Acanthoceras* spores were unorna-

mented and oval, resembling diatom girdle bands (Fig. 11). In our material, we were unable to discern any girdle bands in the *Acanthoceras* spore, and there was no evidence of pores or portulae in the spore wall (Figs 1, 2). Spore shape and lack of ornamentation are distinguishing characters in the light microscope.

#### *Morphology of the Urosolenia resting spore*

The *Urosolenia* specimens from East Lake Okobojo were identified as *U. eriensis* (Huber-Pestalozzi, 1942). *Urosolenia eriensis* produced one endogenous resting spore per mother cell (Fig. 16). A range of cell sizes were able to form spores, and correspondingly, spores also had a small range of sizes. Spores were usually seen in girdle view on prepared mounts and varied in size from 6.8 to 18.0  $\mu\text{m}$  in the apical axis and 6.4 to 12.5  $\mu\text{m}$  in the perivalvar axis (Figs 16–24). Spores with a smaller apical axis had a proportionately larger perivalvar axis. The single spore was centrally located within the mother cell and was formed of two heavily silicified valves (Figs 4, 16). The primary valve face was convexly domed and elliptical in valve outline with a variable number of small spines in a ring near the primary valve margin (Figs 3, 25). The secondary valve was concentrically undulate with a domed center and had a pronounced rim around the valve margin (Fig. 4). A few spines may or may not be present on the secondary valve (Fig. 4, arrow). Neither spore valve had any pores or portulae, and girdle bands were not evident in our *U. eriensis* spore material (Figs 3, 4) although girdle bands from the mother cell remained loosely attached to the spore.

*Urosolenia longiseta* also produced a single endogenous resting spore per mother cell (Fig. 26). Spores in this taxon varied in size from 5.0 to

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*Urosolenia eriensis* resting spores. Fig. 16. Girdle view of spore still associated with half of mother frustule. Figs. 17–24. Girdle views of spores showing size and morphological range. Fig. 25. Valve view. Figs. 26–34. *Urosolenia longiseta* resting spores. Fig. 26. Girdle view of spore still associated with half of mother frustule. Figs. 27–34. Girdle views of spores showing size and morphological range. Fig. 35. Line drawings of chrysophyte cyst, *Cysta microcarpa* Nygaard (redrawn from Nygaard, 1956, P1. XII, Figs. 29–33), illustrating similarity to *Urosolenia longiseta* resting spores.

14.2  $\mu\text{m}$  in apical length and 7.0 to 12.2  $\mu\text{m}$  in the perivalvar axis (Figs 26–34). The primary spore valve was convexly domed but had no spines (Fig. 5). The secondary spore valve was concentrically undulate with a convexly domed center and also lacked any evidence of spines (Fig. 6). A siliceous rim surrounded the secondary valve margin (Fig. 6), producing two horn-like projections in the light microscope (Fig. 30). Again, spores with a smaller perivalvar axis tended to have a larger apical axis. While spores of *U. longiseta* overlapped in size with *U. eriensis*, the somewhat heavier silicification and presence of spines on the primary valve face of *U. eriensis* separated the two taxa. Researchers must be cautioned that while spores can usually be treated as definitive taxonomic tools, in some instances the presence and development of characters such as spines may be variable and reflect environmental or genetic variability (Hargraves, 1979; Rushforth & Johansen, 1986).

## Discussion

Understanding the morphology of diatom spores is only the first step to interpreting this interesting variation in the diatom life cycle. Questions continue to be raised regarding the taxonomic, ecologic, and systematic importance of diatom resting spores. For taxonomic purposes, spores provide excellent diagnostic characters and are considered morphologically consistent, species-specific, conservative cell types (Syvertsen, 1979). With regard to plankton ecology, resting spore production by *Urosolenia* and *Acanthoceras* seems primitive and costly. Most freshwater planktonic diatoms have instead been reported to form resting cells. These cells are cytologically and physiologically distinguishable from the vegetative cell (Sicko-Goad *et al.*, 1989), and are able to survive lengthy time periods (> 10 years) incorporated within anoxic sediments. Resting cells possess similar physiological characteristics to resting spores (high chlorophyll and lipid content, low respiration), yet do not undergo any special mitoses to become dormant (Anderson, 1975; von

Stosch & Fecher, 1975; Hargraves & French, 1983).

Since very few freshwater diatoms produce resting spores, many scientists have not encountered them. In some cases resting spores have been taxonomically confusing (von Stosch & Fecher, 1979) and several fossil diatom genera probably represent only resting spores of genera whose vegetative frustules were unpreserved (Hargraves, 1986). Paleolimnologists are especially cautioned to differentiate between chrysophyte cysts and the minimally ornamented spores of *Urosolenia* and/or *Acanthoceras* preserved in sediment assemblages (Simola *et al.*, 1990). Chrysophyte cysts have become increasingly important tools for paleolimnologists as we gain detailed knowledge of their ecology, taxonomy, and environmental preferences. Nygaard (1956), in his classic work on Lake Gribso, was one of the first researchers to classify chrysophyte cysts. However, it appears that his *Cysta microcarpa* specimens (Nygaard, 1956, Pl. XII, Figs 29–33) are actually *Urosolenia longiseta* resting spores (Fig. 35, redrawn from Nygaard, 1956).

While poor sedimentary and preparatory preservation of the vegetative frustule of *Urosolenia* and *Acanthoceras* may occur (Yung *et al.*, 1988; Haworth, 1984; Simola *et al.*, 1990), the identification of fossil resting spores can provide environmental clues for paleolimnologists (Sandman *et al.*, 1990; Simola *et al.*, 1990). *Urosolenia eriensis*, *U. longiseta* and *Acanthoceras zachariasii* are considered circumneutral, euplanktonic diatoms. *Acanthoceras* occurs ephemerally in north temperate, alkaline, eutrophic habitats, while *Urosolenia* occurs seasonally in oligotrophic to eutrophic waters. Final resolution of taxonomic difficulties and ecological preferences within *Urosolenia* remains critical to interpreting this genus in sedimentary microfossil assemblages. We hope to study further the precise environmental variables inducing spore formation so that detailed ecological inferences can be made when resting spores are found in sediment samples. Presently, we feel that the formation of resting spores by *Urosolenia* and *Acanthoceras* may reflect periodic nitrogen limitation in the system. Nitrogen depletion is com-

mon during midsummer in East Lake Okoboji (Bachmann & Jones, 1974) and has been implicated as a trigger of resting spore formation in many marine diatoms (Hargraves & French, 1983).

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