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A first account of the heterotrophic eukaryote *Rabdiophrys* Rainer from the fossil record and description of a new species from an ancient Eocene Arctic freshwater lake

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Abstract

Rotosphaerids are unicellular, heterotrophic, eukayotic protists that have filopodia, an exterior covering consisting of highly ornamented siliceous scales, and are classified in the Rotosphaerida within the opistokont lineage. Given their appearance as relatively large spherical cells with protruding filopodia and a silica scale covering, they are often mistaken for centrohelid heliozoans. Even though these organisms are widely distributed in both marine and freshwater environments, many species are rarely reported, and none have been reported from the fossil record. We report extensive remains of a new species of *Rabdiophrys*, *R. giraffensis*, from an ancient waterbody that was situated near the Arctic Circle in northern Canada during the Eocene. The new species has both plate and spine scales that are similar in morphology, but significantly larger than its closest modern congeners, *R. monopora* and *R. anulifera*. The waterbody in which the new species grew and thrived is inferred to have been a moderately deep, circumneutral pond, with moderate concentrations of nutrients and dissolved humic material.

Keywords: Eocene; Giraffe Pipe; Opisthokonta; *Rabdiophrys*; Rotosphaerida; Silica scales

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Introduction

Rotosphaerids are a group of free living, non-flagellated, heterotrophic protists originally placed in the order Rotosphaerida Rainer 1968 (Lee et al., 1985; Nicholls, 2013; Patterson, 1985). These unicellular organisms are spherical-shaped, bear filose pseudopodia, or filopodia, and are covered with overlapping and species-specific siliceous scales (Nicholls, 2013; Rainer, 1968; Thomsen, 1978). Unlike centrohelid heliozoans from which they resemble under light microscopy, rotosphaerids lack an axoplast, the centrally-positioned structure from which the axopods radiate outward from the cell (Nicholls, 2013; Roijackers and Siememsma, 1988). Further, unlike centrohelid heliozoans, rotosphaerids lack extrusomes and possess flattened mitochondrial cristae (Nicholls, 2013; Patterson, 1985; Wujek and O'Kelly, 1991).

The position of the Rotosphaerida within the eukaryote tree of life, and its relationship to true heliozoans, has been slow to materialize. Because rotosphaerids produce spherical cells surrounded by siliceous scales and with stiff radiating filose pseudopodia, they were classically placed with

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heliozoans (Patterson, 1985). However, Patterson showed that ultrastructural features of *Nuclearia*) (Patterson, 1983, 1984), a naked amoeboid organism with filose pseudopodia, and *Pompholyxophrys* (Patterson, 1985), a spherical amoeboid organism covered with siliceous scales (perles) and radiating filose pseudopodia, differed significantly from heliozoans and rhizopod amoebae. Both *Nuclearia* and *Pompholyxophrys* possess mitochondria with flattened cristae, pseudopodia that are not associated with microtubules, and further lack extrusomes and an axoplast. This prompted Patterson (1985) to conclude that *Nuclearia* and *Pompholyxophrys* were closely related, a conclusion also made by Page (1987) who classified *Nuclearia* in the family Nucleariidae, and *Pompholyxophrys* in the family Pomphalyxophryidae, both within the order Cristidiscoidida (=Rotosphaerida Rainer, 1968). Patterson (1985) further suggested that the scale-bearing amoeboid genus *Pinaciophora*, which also possessed radiating filose pseudopodia that lack extrusomes, was allied with these organisms

Recently, molecular data has been used to better determine the position of the Rotosphaerida within the eukaryote tree of life. Based on gene sequences, this group of organisms belongs to the Nucletmycea (Holomycota), a robust clade within the Opisthokonts that also contains the fungi (Adl et al., 2019; Galindo et al., 2019; Liu et al., 2009; Zettler et al., 2001). Using data generated from single-celled genomic and transcriptomic techniques, and including six additional species, Galindo et al. (2019) uncovered the nucleariid amoeba as a monophyletic clade within the Nucletmycea, and further showed the naked species formed a sister clade to the one containing species covered with siliceous components.

Rainer (1968) originally placed five genera into the Rotosphaerida, *Lithocolla*, *Pinaciocystis*, *Pinaciophora*, *Pompholyxophrys* and *Rabdiophrys*. Subsequent authors (e.g. Croome, 1987; Mikrjukov, 1999; Nicholls, 2013; Roijackers and Siemensma, 1988; Thomsen, 1978, 1979) described new species, mostly assigned to *Pinaciophora*, proposed revisions to some genera, and erected a new genus. The generic revisions largely centered on the type and structure of scales. All taxa of rotosphaerids produce flat, siliceous plate scales with species-specific morphological characters. Scale designs are also distinctive for the genera. Some species produce a second type of scale, the spine scale, which consists of an elongated, slender shaft extending from a small flattened base. Spine scales are arranged on the outside of the covering of plate scales with the shafts radiating out from the cell. Some species of *Pinaciophora*, the largest genus in the order, were originally described as producing only plate scales, while other species were able to produce both scale types. However, Roijackers and Siemensma (1988) believed formation of spine scales was a genus-level trait, and moved all of the *Pinaciophora* species that produced spine scales into the genus *Rabdiophrys*, which included only taxa with both scale types. Nicholls (2013) questioned this transfer on the basis of the structures of the plate scales, noting that the morphology of plate scales produced by *Rabdiophrys* species was significantly different from those of the *Pinaciophora* species transferred by Roijackers and Siemensma (1988). To rectify this situation, Nicholls (2013) proposed a new genus, *Thomseniophora*, for species that form both *Pinaciophora*-like plate scales and spine scales.

Although Galindo et al. (2019) clearly showed the position of the genera *Lithocolla* and *Pompholyxophrys* within the nucleariids, they caution that molecular data is still lacking for the Rotosphaerida genera *Pinaciophora* (including *Thomseniophora*) and *Rabdiophrys*. Given that the genus *Pinaciophora* contains species with radiating pseudopodia that lack extrusomes (Patterson, 1985), it seems likely that their position within the nucleariids will be confirmed once molecular data becomes available.

Rotosphaerid species are found in freshwater and marine environments, either floating in the open water or associated with surface sediments (Esteban et al., 2007; Nicholls, 2013; Roijackers and Siemensma, 1988; Thomsen, 1978). The filose pseudopods aid in capturing food items, including bacteria, algal cells and organic detritus. Despite their apparent cosmopolitan distribution, the reality is that very little is known about the ecology of the group as a whole, and many of the species are rarely reported.

Upon cell death, the siliceous scale remains of rotosphaerids can accumulate in sediments, and potentially become part of the fossil record. Coupled with the fact that the scale morphologies are species-specific, Smol (1995) and Estaben et al. (2007) suggested that scale remains are potentially useful indicators of environmental change. However, in order for an organism to be a useful bioindicator, an understanding of the ecological conditions under which the individual species grew and thrived is required. Because of the scant number of records for most rotosphaerid species, the majority of which are not accompanied with environmental data, the use of this group of organisms as bioindicators is currently minimal. In addition to the few reports of rotosphaerid taxa in contemporary aquatic environments, to our knowledge there are no reports of these organisms in the fossil record, limiting a full understanding of the evolutionary history of the group.

As part of a large-scale effort to characterize an extensive array of microfossil remains from an ancient freshwater Eocene fossil locality, we uncovered numerous remains of a rotosphaerid species. The objectives of this communication are to describe the fossil specimens as a new species of *Rabdiophrys*, and to report on co-occurring organisms and potential environmental conditions under which the fossil species grew.

Materials and methods

Site and core description

Detailed descriptions of the Giraffe Pipe locality are given in Siver et al. (2015), Wolfe et al. (2017), and references therein. Briefly, the Giraffe Pipe locality (64°44′ N, 109°45′ W) is located within a crater formed during emplacement of a kimberlite diatreme into the Slave Craton, Northwest Territories, Canada. The emplacement occurred approximately 47.8 million years ago during the early to middle Eocene (Siver and Wolfe, 2005; Wolfe et al., 2006), between the Ypresian (56–47.8 Ma) and Lutetian (47.8–41.2 Ma). After emplacement, the diatreme crater harbored an aquatic environment that remained for what has been estimated to be thousands of years before transitioning to a terrestrial environment. The sediments containing the remains of the aquatic and terrestrial ecosystems were later capped by Neogene glacial deposits (Siver and Wolfe, 2005; Wolfe et al., 2006).

A 163 m long core, drilled at a 47° angle, was recovered from the Giraffe maar in 1999 by BHP Billiton Inc. (Siver and Wolfe, 2009). The lower 113 m of the core contains 68 m of lacustrine sediments, overlain with 45 m of peaty and terrestrial remains (Fig. 1A). The lake and terrestrial sediments underwent little to no post-deposition alteration, yielding well preserved material containing abundant fossils.

Laboratory methods

We examined 175 samples distributed over the lacustrine section of the core for microfossil remains. Sixty of the samples contained specimens of *Rabdiophrys* examined as part of this study (Fig. 1B), the majority of which were from a ~17 m section composed of massive brown mudstones (Fig. 1C). Mudstone fragments (0.5–1.0 g) from each sample were oxidized using 30% H_2O_2 under low heat for a minimum of 1–3 h, rinsed multiple times with distilled water, and the resulting slurries stored in glass vials at ~4 °C. This mild oxidation procedure results in separation of numerous siliceous microfossils from the mudstone. Aliquots of each slurry were used to prepare samples for observation with scanning electron microscopy (SEM) and light microscopy (LM). For SEM, an aliquot was diluted and air dried onto a piece of heavy duty aluminum foil, trimmed, and attached to an aluminum SEM stub with Apiezon® wax. Samples were coated with a mixture of gold and palladium for 2 min with a Polaron Model E sputter coater, and examined with a Leo (Zeiss) 982 FESEM or a FEI Nova NanoSEM 450 FESEM. For LM, aliquots of each sample slurry were air dried onto coverslips, mounted onto glass slides using Naphrax, and examined with a Leica DMR light microscope coupled with a Zeiss Axiocam 503 digital camera. Because it was not possible to uncover whole intact cells from the rock material, we were unable to view scales in position on the cell surface. In order to best describe the plate scales of the fossil species, we refer to the two sides as inner and outer surfaces based on observations made of modern species.

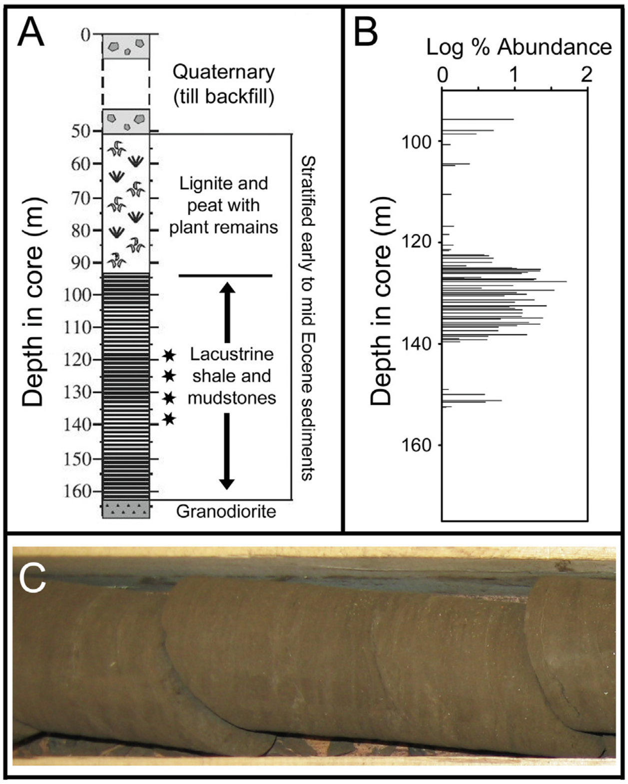

Fig. 1. (A) Stratigraphic diagram of the 163 m Giraffe Pipe core, noting the distribution of lacustrine shales and mudstones, terrestrial plant remains, and overlying Quaternary glacial sediments. The black stars indicate the portion of the core rich in remains of *Rabdiophrys giraffensis*. (B) Remains of *Rabdiophrys giraffensis* plate and spine scales within the lacustrine section of the core. (C) Example of the massive brown mudstone containing the largest concentrations of *Rabdiophrys giraffensis* fossil remains.

As part of a larger ongoing study, a minimum of 300 microfossils were identified and counted for each of the 175 samples using the prepared glass slides. Counts were done for each sample after a thorough examination was completed with SEM. For the current study, the percentages of *Rabdiophrys* plate and spine scales were calculated for each sample and are expressed in Fig. 1B as $\log_{10}$ values. Thus, the $\log_{10}$ values can range from 0 (no *Rabdiophrys* scales found in a sample) to 2 (*Rabdiophrys* scales accounted for all of the microfossils uncovered in a sample). Measurements of plate and spine scales were made directly from SEM images, or LM images using the Zeiss camera software package. SigmaPlot ver 12.5 was used for statistical analyses.

Results

Systematics

Amorphea Adl et al. 2012
Obazoa Brown et al. 2013
Opisthokonta Cavalier-Smith 1987
Nucletmycea Brown et al. 2009 [syn. Holomycota Liu et al. 2009]
Rotosphaerida Rainer 1968 [syn. Cristidiscoidida Page 1987]
Pompholyxophryidae Page 1987
Genus *Rabdiophrys* Rainer 1968
Rabdiophrys giraffensis sp. nov. P.A. Siver & A. Skogstad
Figs. 2–4.

Diagnosis: Plate scales are circular-shaped, and consist of two concentric layers fused along the peripheral margin (Figs. 2–3). Plate scales range in diameter from 2.7 to 4.2 µm, with a mean of 3.7 µm (Fig. 5C). The bottom, or inner layer, which aligns and faces the cell membrane, is flat and perforated with numerous closely-spaced pores arranged in a precise hexagonal pattern (Fig. 3B, D). The top, or outer layer, is fused with the bottom layer at the margin, then rises at a shallow incline towards the center of the scale, and connects to the rim of a wide, shallow and centrally positioned cylinder (Fig. 3A, C, E). The outer layer is also perforated with closely spaced pores, although they are not as precisely aligned as on the inner surface (Fig. 3A, C, E). Small papillae are randomly spaced on the outer surface.

The central cylinder, referred to as the central “hole,” ranges in diameter from 1 to 1.5 µm, with a mean of 1.3 µm (Fig. 5B). The height of the central cylinder is small, estimated at ~0.1 µm, and remains of material are observed along the walls with SEM (Fig. 3B, C, E). The incline results in a slight separation and space between the top and bottom layers, which is widest along the perimeter of the central cylinder. Material, presumably siliceous in nature, is deposited within the inner space, and best detected with DIC or phase optics (Fig. 2). The distribution of material often results in what is seen as a concentric ring positioned between the plate margin and central cylinder (arrows, Fig. 2). The uneven distribution of this material, and resulting concentric ring, is observed on most specimens, but can be less obvious on small plate scales.

Spines scales consist of a slender shaft attached to a small circular base plate (Fig. 4). The shaft and base plate range in length from 5.4 to 17.9 µm and 1–1.8 µm, with mean values of 11.3 µm and 1.3 µm, respectively (Fig. 5A). The shaft is

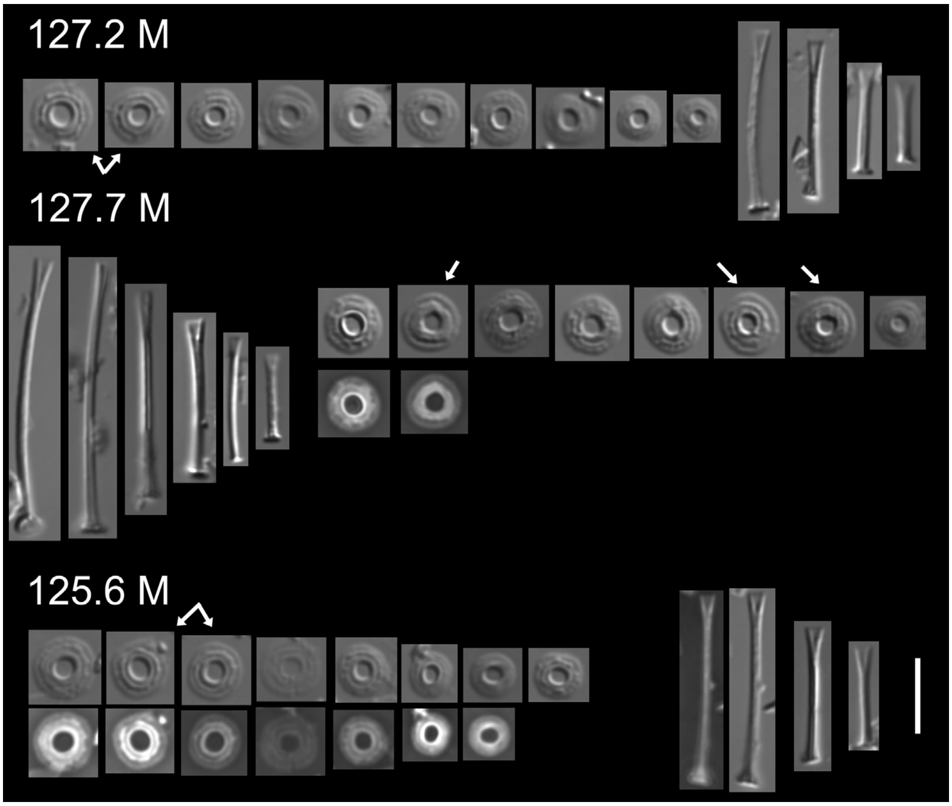

Fig. 2. Light microscopy images of *Rabdiophrys giraffensis* plate and spine scales from three depths in the Giraffe Pipe core. Top rows of plate scales for each depth were taken with DIC optics and the bottom row with phase contrast of the same specimens. Arrows depict examples of specimens within each depth sequence where concentric rings of material between the two scale surfaces are clearly observed. Scale bar = 5 µm.

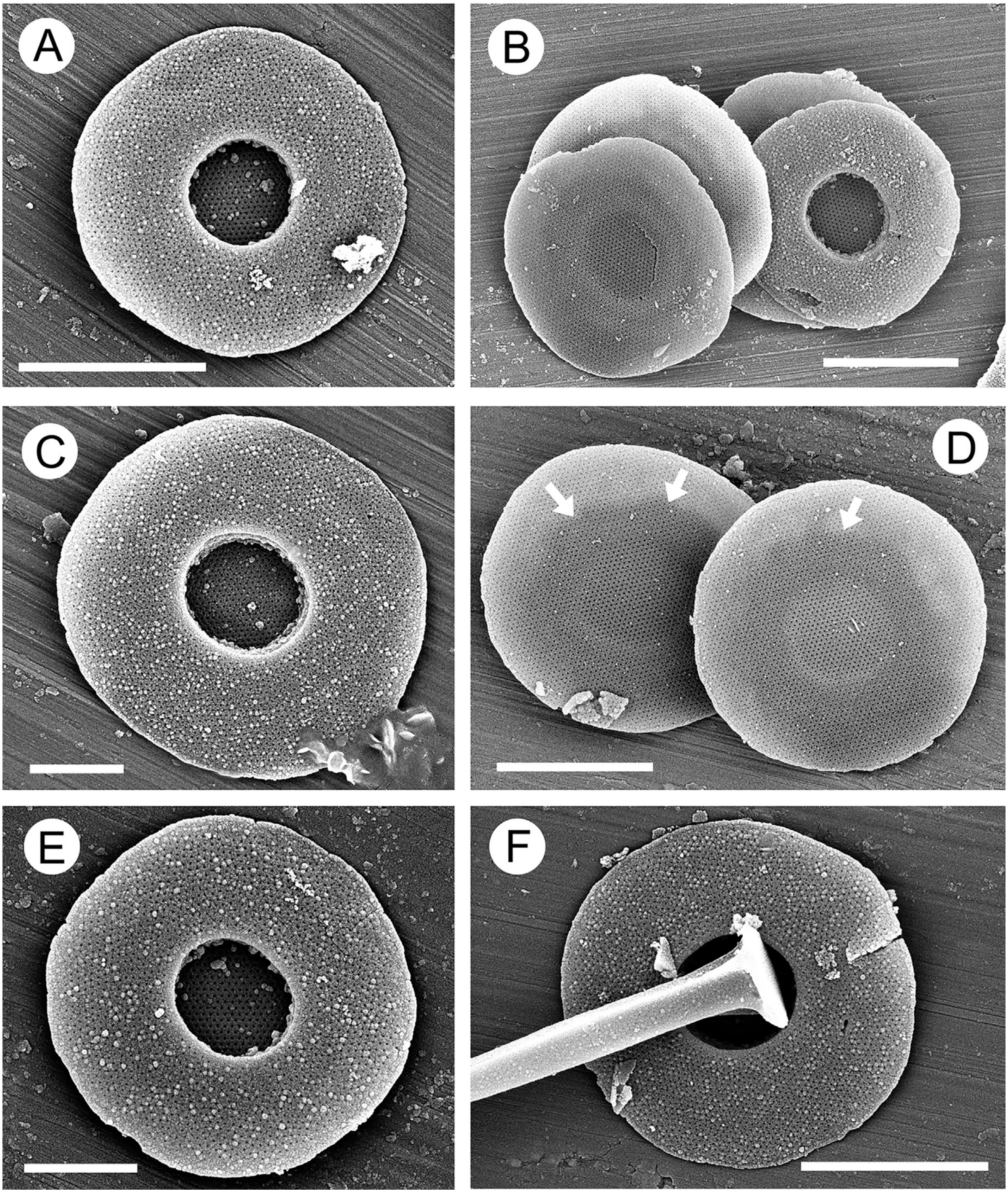

Fig. 3. Scanning electron micrographs of *Rabdiophrys giraffensis* plate scales from the Giraffe Pipe core. Views of the top (A, C, E–F) and bottom (B, D) of scales are given. Arrows in (D) indicate the position of the concentric ring of material observed with LM. The potential association of a plate and spine scale is shown in (F). Scale bars are 1 µm (C and E) and 2 µm (A–B, C, F).

4-ribbed where it attaches to the base plate, becoming flatten and expanded with two small apical points, giving the appearance of a fish tail (Fig. 4A-B). The shaft is straight, often becoming slightly curved on longer specimens (Fig. 4D). The bottom of the base plate contains linear rows of pores, spaced approximately every 75 nm (Fig. 4F).

Etymology: The species epithet, *giraffensis*, refers to the Giraffe Pipe core from which the organism was described.

Type specimen: Portion of a single gathering of cells on glass slide marked “GP 18-3-110”, “D” deposited at the Canadian Museum of Nature, CANA # 129304. Images

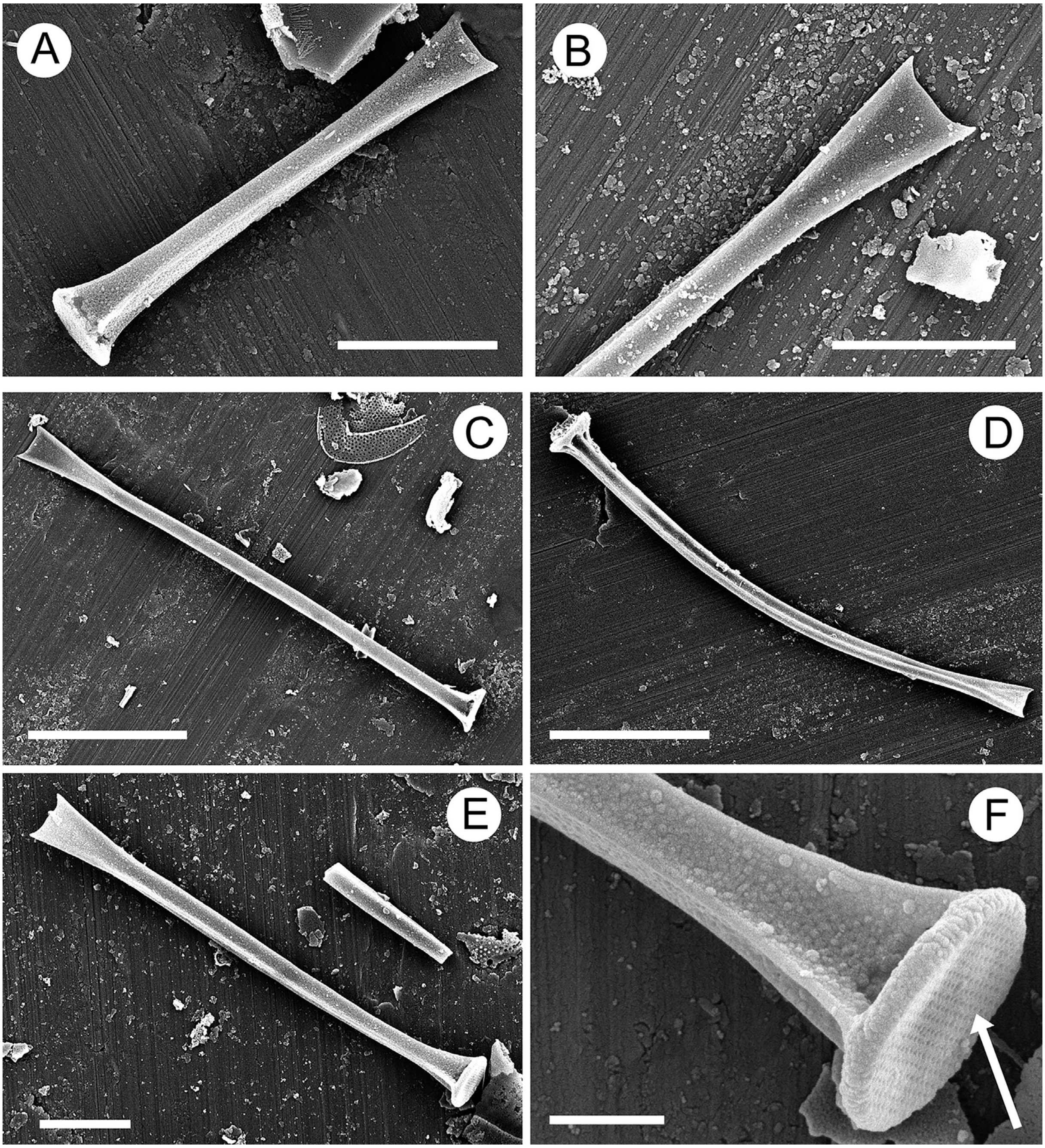

Fig. 4. Scanning electron micrographs of *Rabdiophrys giraffensis* spine scales from the Giraffe Pipe core. Short and medium-length spines with straight shafts (A, C, E), and a longer curved spine (D) are illustrated. Close-ups of the fishtail-shaped spine apex (B) and small circular base (F) are also illustrated. Note the evenly-spaced rows of pores on the bottom of the spine base (arrow on F). Scale bars are 500 nm (F), 2 μm (A-B, E), and 5 μm (C-D).

from this gathering are given in Fig. 2 (middle row) and Fig. 3D and E.

Type material: Mudstone collected by P.A. Siver from a depth of 127.7 m in the Giraffe Pipe core and deposited at the Canadian Museum of Nature, CANA # 129304. This section of the core is referred to as 18-3-110.

Distributional records: Although the results of an extensive study of the Giraffe core with over 100 different microfossil taxa is planned for a subsequent publication, details for *Rabdiophrys giraffensis* are presented here. Remains of plate and spine scales were uncovered in 60 of the 175 samples examined in the extensive Giraffe core (Fig. 1B). The vast majority of samples containing *R. giraffensis* specimens were from depths in the core between ~139 and 122 m (Fig. 1B), where it was co-dominant with the diatom *Aulacoseira giraffensis*, and the synurophytes *Mallomonas*

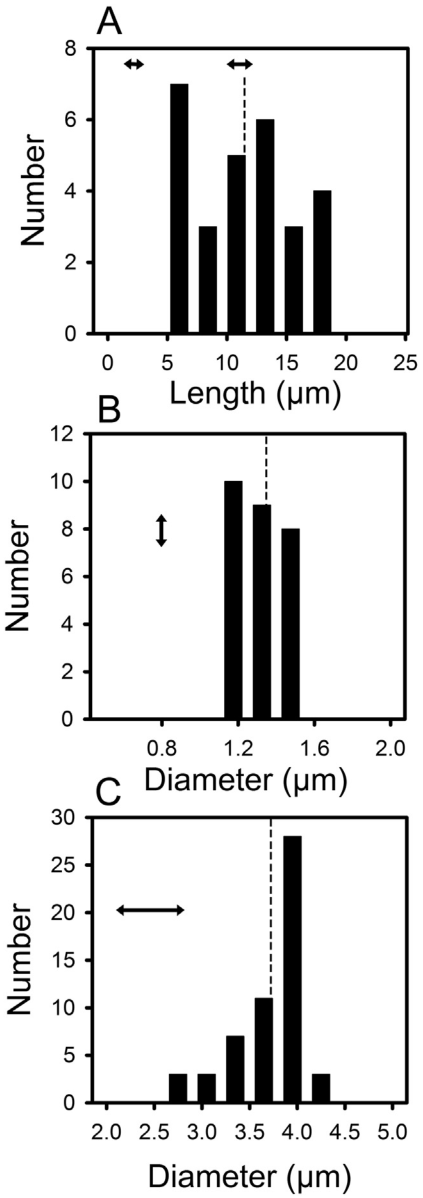

Fig. 5. Morphometric measurements of spine length (A), the diameter of the central hole on plate scales (B), and the diameter of plate scales of *Rabdiophrys giraffensis* specimens. The dotted lines indicate mean values for each set of measurements. The double black arrows depict the size ranges of spine (A) and plate (C) scales, and indicate the average diameter of the central hole on plate scales (B) for the modern species, *R. monopora* and *R. anulifera*.

insignis and *Synura recurvata*. Within this ~17 m zone, consisting of massive brown mudstone (Fig. 1C), *R. giraffensis* was found in 41 of the 43 samples examined and accounted for between 1% and 50% of the microfossils uncovered, with a mean of 11%. *Rabdiophrys giraffensis* was also found in six of the nine samples investigated between 149 and 152.5 m, but only accounted for 1.4% of the microfossils, and in a few scattered samples in the top portion of the core.

Additional observations: Measurements included in this study came from 19 of the 60 samples containing scales. In all of these samples, plate scales with a diameter of at least 3 µm and spines scales longer than 11 µm were found. Example specimens from three strata examined with LM are displayed in Fig. 2.

Discussion

There is no doubt that the fossil specimens reported in this study from the Giraffe Pipe locality represent the genus *Rabdiophrys*. The characteristics of both the plate and spine scales precisely fit into this genus, and our findings further indicate that scale morphology has been relatively stable since at least the early Eocene. Evolutionary stasis with respect to siliceous structures dating from the Eocene or older has been observed in other protist groups, including synurophyte scales (Siver and Wolfe, 2009), diatom frustules (Wolfe and Siver, 2009), and euglyphid testate amoebae (Barber et al., 2013). In the case of the fossil specimens from the Giraffe Pipe locality, both plate and spine fossil scales were easily assigned to *Rabdiophrys*. To our knowledge, this is the oldest known fossil record for any rotosphaerid species, and assures that this protistian lineage is a minimum of 48 million years old. Our findings offer a valuable geologic milepost for use in calibrating molecular phylogenies to the fossil record, specifically for the Rotosphaerida, and more generally for the Opisthokonta.

Rabdiophrys giraffensis shares similar characteristics with, and is most closely related to, two contemporary species, *R. anulifera* Rainer 1968 and *R. monopora* (Thomsen, 1978) Roijackers & Siemensma 1988 (Roijackers and Siemensma, 1988; Thomsen, 1978). All three species have plate scales that are circular-shaped, perforated with small pores, with a large central “hole,” and ribbed spine scales with a small circular base and a flattened and two-pointed apex. *Rabdiophrys anulifera* is distinguished from *R. monopora* on the basis of the length of the spine scales (Roijackers and Siemensma, 1988; Siemensma, 2021; Thomsen, 1978). Specimens of *R. monopora* have small spines that are approximately 1.5 µm in length, whereas spine scales of *R. anulifera* are both small (1.2–3.4 µm) and long (10–12 µm) (Fig. 5A). In addition, spine scales of *R. monopora* have a large hole on the base of the shaft (Thomsen, 1978), a feature also noted by Siemensma (Fig. m; 2021) for small spines scales of *R. anulifera*. Other than differences in spine length, the size of plate scales and the corresponding diameter of the central hole are similar for both contemporary species.

Given the morphometric criteria used to separate *R. monopora* from *R. anulifera*, *R. giraffensis* warrants description as a new species. Plate scales of the fossil species are significantly larger (diameter: 2.7–4.2 µm) than those of either of the modern species (Fig. 5C), and the central hole is 50% larger (Fig. 5B). Spine scales of *R. giraffensis* are also significantly longer than those of *R. anulifera*, and there is no overlap in length of spines with specimens of *R. monopora* (Fig. 5A). In addition, spines scales of *R. giraffensis* specimens lack the hole near the base of the shaft found on short spines of both modern species. Other than the significant differences in scale size, and lack of a hole on the spine shaft of *R. giraffensis*, morphological features are similar for all three taxa.

Roijackers and Siemensma (1988) estimated the diameter of *Rabdiophrys* cells to be between 25 and 40 µm. Despite differences in the siliceous components, we are not able to determine the cell size of *R. giraffensis* specimens. Some samples of mudstone contained extraordinarily large concentrations of scales that clearly represented the remains of large numbers of cells. However, we were not able to uncover what we believed to be the remains of individual cells through an examination of rock fragments with SEM. Cell size of eukaryotic protists has been reported as being inversely related to temperature, with smaller cells forming under higher temperatures (Atkinson et al., 2003; Montagnes and Franklin, 2001). It is not known if a similar rule applies to cell wall components, including scales made of silica. For example, if a lower (higher) temperature triggers development of larger cells, would the silica scales covering the cells also be larger (smaller)? There is evidence that the size of siliceous scales for species within the synurophyte genus *Mallomonas* is correlated with cell size (Siver, 1991; Siver et al., 2015). In this case, the cell coverings of many larger-celled *Mallomonas* species consist of larger scales. If this rule applies to rotosphaerids, then cells of *R. giraffensis* would likely have been larger than those of both contemporary species.

When observed with light microscopy, *Rabdiophrys* plate scales have a classic appearance given their circular outline and what has been described as a large centrally placed “hole.” However, in the case of *R. giraffensis*, the hole is not an actual perforation in the base of the plate. Rather, based on SEM observations, the bottom of the hole is covered with a continuation of the perforated base plate. Siemensma (1981) also noted the perforated covering using transmission electron microscopy (TEM), and the same structure is observed with TEM for plate scales of *R. monopora* (Thomsen, 1978). A surprise finding was the linear array of pores found on the bottom of the base of the spine scales on *R. giraffensis* specimens (Fig. 4F). The function of this linear array of pores is not known. One idea is that the base of the spine fits into the central hole on the plate scale, and that the pores are perhaps involved in cementing the spine in place. The similar diameters of the central hole of the plate scale and the base plate of the spine, both with mean diameters of 1.3 µm, provides indirect evidence to support this idea. However, to our knowledge, this concept is not mentioned in the literature for *Rabdiophrys*, and there are no images to support such an arrangement of the two scale types.

There is no question that the waterbody represented in the Giraffe core was freshwater, given findings of freshwater synurophytes and chrysophytes (Siver et al., 2015; Siver and Wolfe, 2009), diatoms (Siver et al., 2010; Wolfe and Siver, 2009), testate amoebae (Barber et al., 2013) and sponges (Pisera et al., 2013) within the core, including throughout the primary zone containing *R. giraffensis*. This observation based on Eocene fossil material supports the conclusion made by Galindo et al. (2019) that nucleariids originated in a freshwater environment. However, the ecological conditions that best support growth of contemporary rotosphaerids, including *Rabdiophrys* species, are virtually unknown. Siemensma (1981, 2021) reported finding *R. monopora*, originally described from samples collected from freshwater Lake Esron in Denmark (Thomsen, 1978), in a wide range of eutrophic waterbodies. Otherwise, details on ecological preferences are largely lacking.

In our study, *R. giraffensis* was found, often in high concentrations, throughout a 17 m section of the core, representing hundreds to thousands of years (Siver et al., 2019), and co-dominated with the diatom, *Aulacoseira giraffensis*, and two synurophytes, *Mallomonas insignis* and *Synura recurvata*. Although a complete reconstruction of the ecological conditions is not yet available for the Giraffe waterbody, a few general comments are noteworthy. The 17 m core section contains numerous long filaments of *A. giraffensis*, indicating remains of substantial populations of this fossil taxon. Most *Aulacoseira* species that form long filaments are more apt to develop as part of the planktic community in lakes or ponds with at least a moderate depth (Siver, 2021). Waterbodies rich in synurophytes are typically small, softwater, and slightly acidic lakes and ponds, with moderate dissolved humic material and nutrient concentrations (Siver, 2015). Although *S. recurvata* is an extinct species, it is most closely related to the contemporary species, *Synura uvella*. *Synura uvella* has been reported in small lakes and ponds with moderate dissolved humic content, often mesotrophic to eutrophic in nature, and that are slightly acidic to slightly alkaline (e.g. Kristiansen, 1975; Roijackers and Kessels, 1986; Wujek et al., 1975). Charles and Smol (1988) reported an average weighted mean pH of 6.8 for *S. uvella* for a suite of lakes in the Adirondack mountains of New York, U.S.A. Ecological records for *Mallomonas insignis* are few, but most suggest this taxon also favors smaller, shallow waterbodies with mesotrophic to eutrophic conditions, and slightly acidic to alkaline pH (Cronberg and Kristiansen, 1980; Kim, 1997; Péterfi and Momeu, 1976). In summary, we envision *R. giraffensis* having thrived in a moderately deep and circumneutral pond, with moderate dissolved humic content, and mesotrophic to eutrophic nutrient levels.

CRediT authorship contribution statement

Peter A. Siver: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Visualization, Project administration, Funding acquisition. **Asbjørn Skogstad:** Conceptualization, Formal analysis, Investigation.

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