Open access • Journal Article • DOI:10.1666/0094-8373(2000)026<0360:TAITNE>2.0.CO;2

Testate amoebae in the Neoproterozoic Era: evidence from vase-shaped microfossils in the Chuar Group, Grand Canyon — Source link

Susannah M. Porter, Andrew H. Knoll

Institutions: Harvard University

Published on: 01 Jun 2000 - Paleobiology (GeoScienceWorld)

Topics: Testate amoebae, Chuar Group, Arcellinida, Test (biology) and Population

Paleobiology, 26(3), 2000, pp. 360–385

Testate amoebae in the Neoproterozoic Era: evidence from vase-shaped microfossils in the Chuar Group, Grand Canyon

Susannah M. Porter and Andrew H. Knoll

Abstract.—Vase-shaped microfossils (VSMs) occur globally in Neoproterozoic rocks, but until now their biological relationships have remained problematic. Exceptionally preserved new populations from the uppermost Chuar Group, Grand Canyon, Arizona, display details of morphology and taphonomy that collectively point to affinities with the testate amoebae. The fossils are tear-shaped tests, ~20–300 μm long and ~10–200 μm wide, that are circular in transverse section, expand aborally toward a rounded or slightly pointed pole, and taper orally toward a ''neck'' that ends in a single aperture. Apertures may be circular, hexagonal, triangular, or crenulate, and may be rimmed by a distinct collar. Approximately 25% of the Chuar VSMs are curved, such that the oral and aboral poles do not lie opposite each other. Tests are preserved as mineralized casts and molds, commonly coated with organic debris or iron minerals, but they were originally composed of nonresistant organic matter. Approximately 1% have a ''honeycomb-patterned'' wall attributable to the original presence of mineralized scales whose bases were arranged regularly in the test wall. Scale-bearing testate amoebae, such as members of the Euglyphidae, are essentially identical to the honeycomb VSMs, and a close relationship between other Grand Canyon VSMs and additional testate amoebae, both lobose and filose, is likely. The VSM population therefore most likely represents a multispecies assemblage whose spatial association reflects a common habitat and/or taphonomic circumstances that favor test preservation. The assignment of these fossils to the testate amoebae strengthens the case for a major diversification of eukaryotic organisms by mid-Neoproterozoic times and, more significantly, provides the earliest morphological evidence for heterotrophic eukaryotes in marine ecosystems.

Susannah M. Porter and Andrew H. Knoll. Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, Massachusetts 02138. E-mail: sporter@oeb.harvard.edu

Accepted: 1 March 2000

Introduction

Molecular phylogenies indicate that the major clades of the Eucarya, including animals, fungi, green algae (and their descendants, land plants), stramenopiles, red algae, and alveolates (ciliates, dinoflagellates, and apicomplexans), diverged during a relatively brief interval of cladogenesis that substantially preceded the Cambrian diversification of crown-group metazoans (Gajadhar et al. 1991; Sogin 1991, 1994; Budin and Philippe 1998). The discoveries of fossilized red algae (Butterfield et al. 1990, Butterfield this issue) and multicellular stramenopiles (German 1990; Woods et al. 1998) in 1200–1000-Ma rocks place a minimum constraint on the timing of this divergence and imply the existence of other eukaryotic clades in Neoproterozoic oceans. Here we propose that vase-shaped microfossils (genera *Melanocyrillium* Bloeser 1985 and *Caraburina* Kraskov 1985) found widely in Neoproterozoic rocks have affinities with both filose and lobose testate amoebae. This interpretation contributes to the increasingly well documented view that diversification both among and within eukaryotic clades was well advanced by mid-Neoproterozoic time, and provides the earliest morphological evidence for heterotrophic eukaryotes.

Although abundant and globally distributed in Neoproterozoic rocks, vase-shaped microfossils (VSMs) have until now remained problematic. First discovered by Ewetz (1933) in phosphate nodules from the Visingsö Group, Sweden, these fossils have been variously interpreted as chitinozoans (Bloeser et al. 1977), algal cysts (Bloeser 1985), algal sporangia (Horodyski 1987, 1993), and heterotrophic, planktonic protists similar to tintinnids (Fairchild et al. 1978; Knoll and Vidal 1980; Knoll and Calder 1983). Schopf (1992) suggested that VSMs may be the cysts of testate amoebae, but did not provide detailed evidence to support this conclusion. Our interpretation of VSMs as testate amoebae is based

 0094-8373/00/2603-0004/$1.00

principally on newly discovered populations in diagenetic dolomite nodules from the uppermost Walcott Member of the Kwagunt Formation, Chuar Group, Grand Canyon. Both abundant and exceptionally well preserved, these fossils display hitherto unavailable (and taxonomically informative) details of morphology and test construction.

Geological Setting

The Chuar Group, a 1600-m-thick succession of predominantly siltstone and mudstone beds with subordinate sandstones and carbonates, is exposed over a ~150-km^2 area in the northeastern part of the Grand Canyon, cropping out in canyons formed by west-bank tributaries of the Colorado River and bounded to the east by the Butte Fault (Fig. 1A) (Ford and Breed 1973). The group has been divided into two formations and seven members, based principally on distinctive carbonate and sandstone marker beds (Fig. 1B) (Ford and Breed 1973).

The fossiliferous Awatubi and Walcott Members of the Kwagunt Formation are exposed in the Awatubi and Sixtymile Canyons, at the head of Carbon Canyon, and on the divide between Kwagunt and Nankoweap Canyons, where a complete section can be followed up Nankoweap Butte (Fig. 1A). Above a basal bed characterized by massive stromatolitic bioherms, the ~200–340-m-thick Awatubi Member consists predominantly of shales that yield filamentous bacteria (Horodyski 1993), possible eukaryotic filaments (Horodyski and Bloeser 1983), acritarchs (including abundant *Chuaria* [C. Downie in an appendix to Ford and Breed 1969; Ford and Breed 1973; Vidal and Ford 1985; Horodyski 1993]), and VSMs (Vidal and Ford 1985; Horodyski 1993). The overlying Walcott Member is ~250 m thick and is predominantly composed of black shales (maximum total organic carbon [TOC] = 9% [Palacas and Reynolds 1989; Cook 1991]) containing filamentous bacteria (Horodyski 1993), *Chuaria* (Walcott 1899; Ford and Breed 1973) and other acritarchs (C. Downie in Ford and Breed 1969; Vidal and Ford 1985; Horodyski 1993), and VSMs (Bloeser et al. 1977; Bloeser 1985; Vidal and Ford 1985; Horodyski 1993). The base of the Walcott Member is marked by a 3- to 10-m-thick dolomite unit comprising three distinct lithofacies (Cook 1991): a lower dolomitic wackestone with intraclasts and nodular pisolitic chert; a dolomite unit consisting of crinkled, folded, or broken microbial laminations, in part silicified (termed the ''Flaky Dolomite'' by Ford and Breed 1973); and a wavy- to horizontally laminated dolomicrite unit with cauliflower-like chert in its upper 30 cm.

Directly overlying the basal Walcott dolomite are *Chuaria*-bearing black shales (Walcott 1899; Ford and Breed 1973; this study) interbedded with thin dolomicrite to dolosiltite units containing mm-scale wavy laminae, scattered early diagenetic chert nodules, and local molar tooth(?) structures. The cherts preserve moderately well rounded, poorly sorted clasts of organically stained dolomicrite and dolosiltite, cemented with isopachous silica, and interlaminated organic-rich mats. VSMs are abundant in the mat horizons and also occur both within the clasts and as clasts themselves. Overlying the dolomite beds are ~130 m of black shales interbedded with thin pisolitic units. The lowermost pisolite, ~1.5 m thick, was described as a white ''oolite'' by Cook (1991) owing to its grain size and the color of its siliceous cement, but is perhaps better understood as an oncolite in light of the abundant cyanobacterial filaments found preserved within the coated grains (Schopf et al. 1973). Although VSMs are abundant in shales associated with this unit (Bloeser 1985), they are not observed in the oncolite itself (Schopf et al. 1973; this study). The overlying pisolites have a black, iron-rich siliceous cement and are present as thin beds or lenses (Cook 1991).

Two dolomite beds separated by 12 m of black shales form a bench approximately 130 m above the base of the Walcott Member (Cook 1991). The lower bed is 3.5 to 7 m thick and has variable lithological features, including wavy to broken algal laminations, ooids, and intraclasts (Cook 1991). The upper dolomite is a massive, coarsely crystalline to micritic, 9- to 12-m-thick unit in which stylolites, calcite-filled fractures, vugs, and breccias are common (Cook 1991). The dolomites are overlain by ~69 m of black organic-rich shales which, at Nankoweap Butte, are directly over-

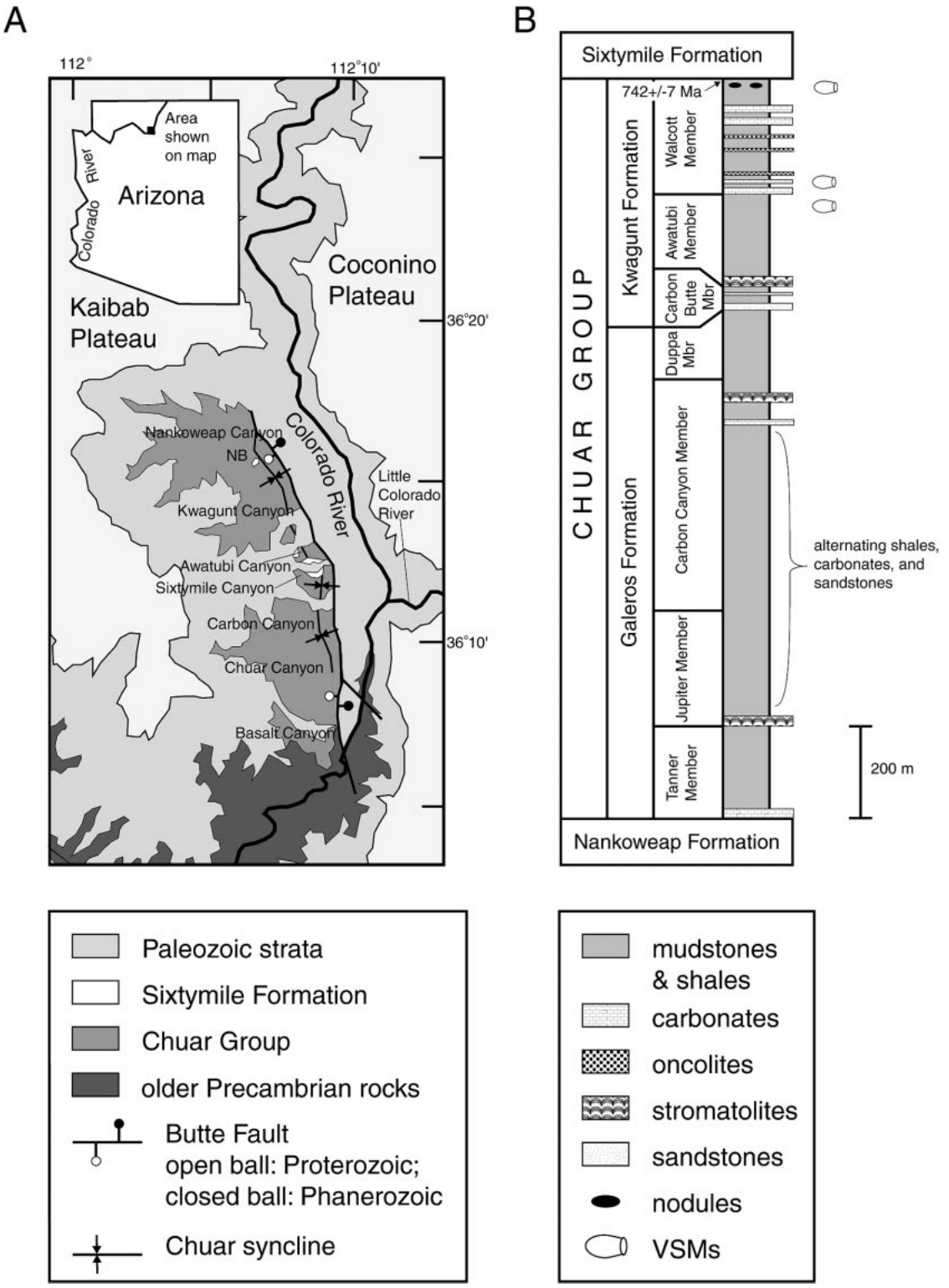

FIGURE 1. A, Geological map of the Chuar Group, northeastern Grand Canyon (modified from Link et al. 1993); NB = Nankoweap Butte. B, Generalized stratigraphic column of the Chuar Group, indicating horizons where VSMs have been found (modified from C. Dehler unpublished data). Radiometric date from Karlstrom et al. 2000.

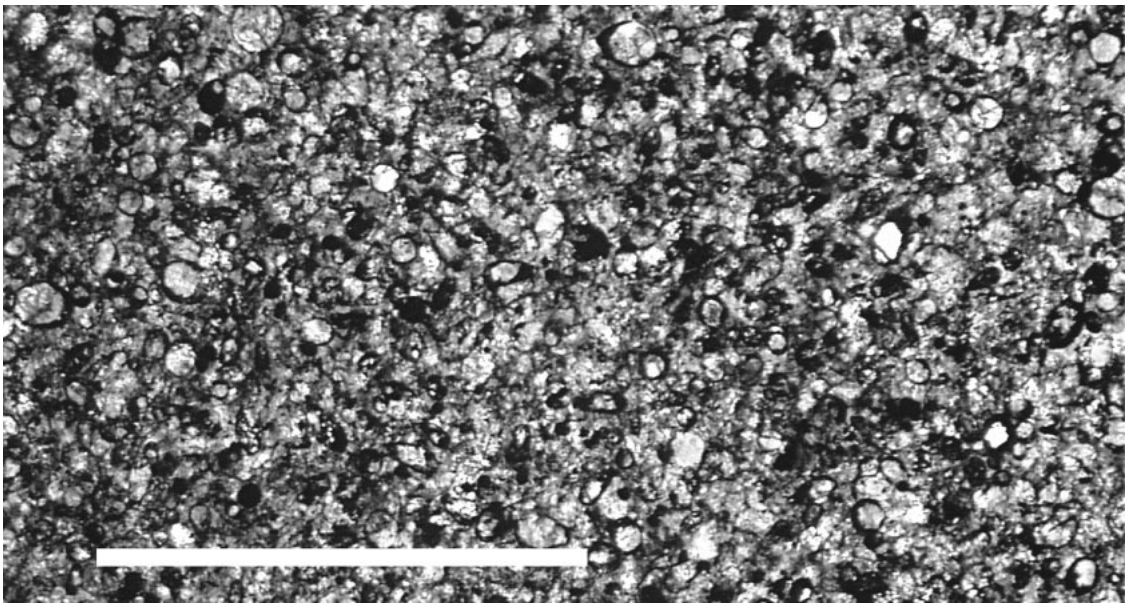

FIGURE 2. Thin-section of a carbonate nodule near the top of the Walcott Member, showing the abundance of VSMs. Scale bar, 1 mm. For this and all following images, sample or thin-section name and England Finder coordinates (where applicable) are given in parentheses; thin-section oriented such that the label is opposite the fixed corner. HUPC 62988 (AK10-53-13F–2B).

lain by the Sixtymile Formation. Approximately 15 m below this contact, the shales contain meter-scale early diagenetic dolomite nodules, which in some cases preserve precompacted shale laminations. An extraordinary abundance of VSMs (up to 4000/mm^3) is observed within these nodules (Fig. 2), but no VSMs or acritarchs have been found in surrounding shales. In Sixtymile Canyon, a 12-m-thick ''karsted,'' coarsely crystalline dolomite unit occurs near the top of the Walcott Member (Cook 1991; C. Dehler personal communication 1999).

Depositional Environment

Chuar Group sediments are thought to have been deposited in a quiet marine embayment connected to the global ocean (Ford 1990; Cook 1991; Dehler and Elrick 1998). Because of the high organic carbon content of the Walcott Member shales, Cook (1991) envisioned a silled basin comparable to the Black Sea, where restricted circulation generates anoxic bottom waters. Alternatively, high TOC might be explained by high productivity related to upwelling (Cook 1991).

A lacustrine environment has been suggested for Chuar Group deposition (Reynolds and Elston 1986), but several lines of evidence indicate that marine conditions must have predominated. Sedimentary structures and facies relationships are consistent with marine deposition (Dehler and Elrick 1998). Perhaps more compelling, Chuar sediments contain VSMs and more than a dozen acritarch taxa (Bloeser 1985; Vidal and Ford 1985) that occur elsewhere in marine successions (e.g., Vidal 1979; Knoll and Vidal 1980; Knoll et al. 1989, 1991). Indeed, the wide geographic distribution of these fossils by itself suggests a marine environment, simply because most sedimentary rocks are marine. In addition, carbon isotope data for the Chuar succession (Dehler et al. 1999) show high amplitude excursions similar in scale to those in the global curve constructed from marine rocks of similar age (Kaufman and Knoll 1995). Finally, Chuar rocks locally contain high concentrations of iron sulfides (Ford and Breed 1973). Walcott black shales collected in outcrop are somewhat weathered, but they still have a mean pyrite iron content of 1% by weight, with individual samples containing up to 3% (Y. Shen and D. Canfield personal communication

1999). Such pyrite abundances are commonly observed in marine sediments but are rare in lakes (Berner and Raiswell 1984; Canfield and Raiswell 1991).

The lowermost Awatubi sediments were deposited in shallow-subtidal to intertidal environments, as documented by a basal stromatolitic bioherm bed and the presence of mudcracks, salt casts, and intercalated sandstone units in overlying shales (Cook 1991; Horodyski 1993; C. Dehler personal communication 1999). Relatively deeper water conditions toward the end of Awatubi time are indicated by the absence of these features and rock types in the laminated, organic-rich shales located higher in the member. Thinly laminated black shales document subtidal deposition during most of Walcott time, with minor intervals of shallow-subtidal to supratidal deposition, recorded by the ''flaky dolomite'' and the ''oncolite'' beds, and massive to laminated and locally vuggy dolomites found near the base and in the upper part of the succession (C. Dehler personal communication 1999). The abundant VSMs found in the upper Walcott Member appear to have accumulated in a quiet subtidal marine environment characterized by high rates of organic carbon burial.

Age

The age of the uppermost VSM populations is sharply constrained by a U-Pb zircon date of 742 ± 7 Ma for an ash bed just above the fossiliferous dolomite nodules near the top of the Walcott Member at Nankoweap Butte (Dehler et al. 1999; Karlstrom et al. 2000). VSMs lower in the succession are, of course, older, but probably not dramatically so. Carbon isotopic profiles for the Awatubi and Walcott members support correlation with the upper Little Dal and Coates Lakes Groups in the Mackenzie Mountains Supergroup, N.W.T., and their Shaler Group equivalents on Victoria Island (Link et al. 1993; Kaufman and Knoll 1995; Dehler et al. 1999)—successions constrained to be younger than 778 Ma (Rainbird et al. 1996). Glaciogenic sediments in the Rapitan Group, which overlies the Mackenzie Mountains Supergroup, are thought to be Sturtian in age.

Vase-Shaped Microfossils in the Upper Walcott Dolomite Nodules

Morphology

Shape and Size.—Like previously reported VSMs, the new Walcott fossils are cup- to tear-shaped tests, circular in transverse section, that taper toward a single opening, often rimmed by a distinct collar, and flare out toward a rounded or slightly pointed aboral pole (Fig. 3). The population shows a wide variation in shape (Fig. 4; length/width ratios vary from 1.0/1 to 3.0/1) and in size (most individuals fall within 25 to 160 μm in length and 15 to 105 μm in maximum width, although VSMs from silicified dolosiltites lower in the Walcott Member can be up to 286 μm in length and 202 μm in maximum width).

Symmetry.—A majority of the Grand Canyon VSMs are radially symmetric, with the aperture directly opposite the aboral pole. Approximately 25%, however, are bilaterally symmetric, with a curved neck (Fig. 5) (Bloeser (1985), Vidal and Ford (1985), and Horodyski (1993) also noted curvature in their specimens from the Chuar Group). Both the general rigidity of the test (see below) and the absence of any wrinkling in the concave part of the neck indicate that this curvature is biological rather than the result of deformation. Curvature does not appear to correlate with any other aspect of morphology—VSMs of widely ranging shapes and sizes are curved.

Aperture and Operculum.—Bloeser (1985) observed distinctly shaped apertures in specimens released from shales in the lower Wal-

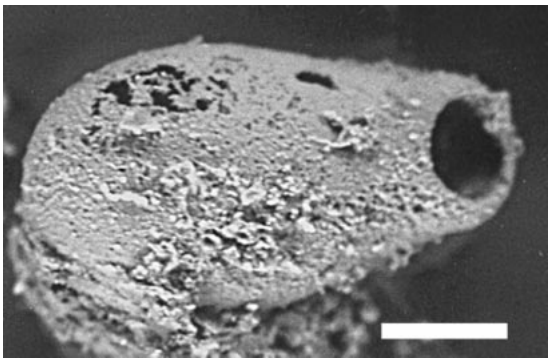

FIGURE 3. SEM image of a VSM, showing overall tear-shaped morphology and circular aperture. HUPC 62989 (AK10-53-13A). Scale bar, 25 μm.

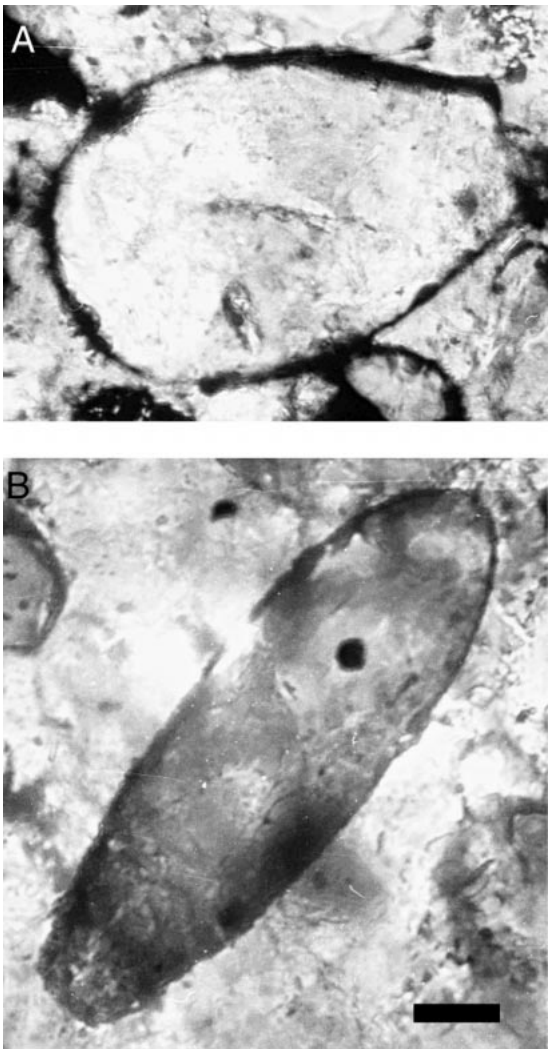

FIGURE 4. Stout and elongate individuals within the upper Walcott VSM population. A, HUPC 62988 (AK10-53-13F–2B; O-64/1). B, HUPC 62990 (AK10-53-13F–1; G-57/3). Scale bar, 20 μm for both.

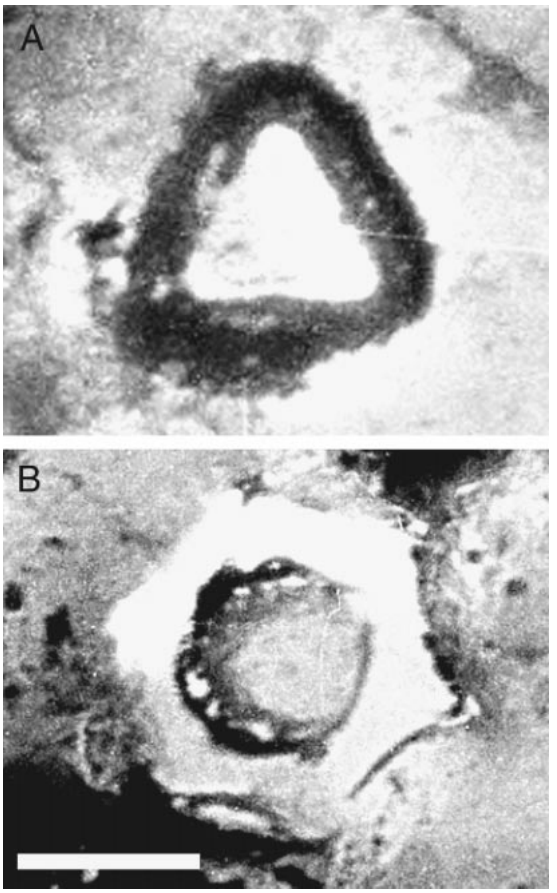

FIGURE 6. Triangular (A) and hexagonal (B) apertures. A, HUPC 62991 (AK10-53-13F–2A; S-61/1); scale bar, 15 μm. B, HUPC 62991 (AK10-53-13F–2A; T-65/1); scale bar, 25 μm.

cott Member. In fact, she used in part aperture margin shape as the basis for separating her genus *Melanocyrilllium* into three species: *M. hexodiadema* (hexagonal), *M. fimbriatum* (triangular), and *M. horodyskii* (circular). A similar

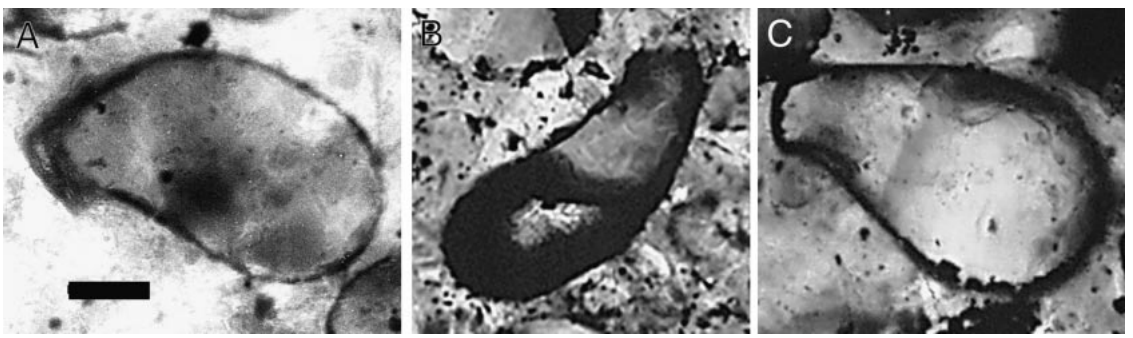

FIGURE 5. VSMs with curved necks. A, HUPC 62990 (AK10-53-13F–1; F-56/3). B, HUPC 62988 (AK10-53-13F–2B; H-64/1). C, HUPC 62988 (AK10-53-13F–2B; H-57/4). Scale bar, 30 μm for all.

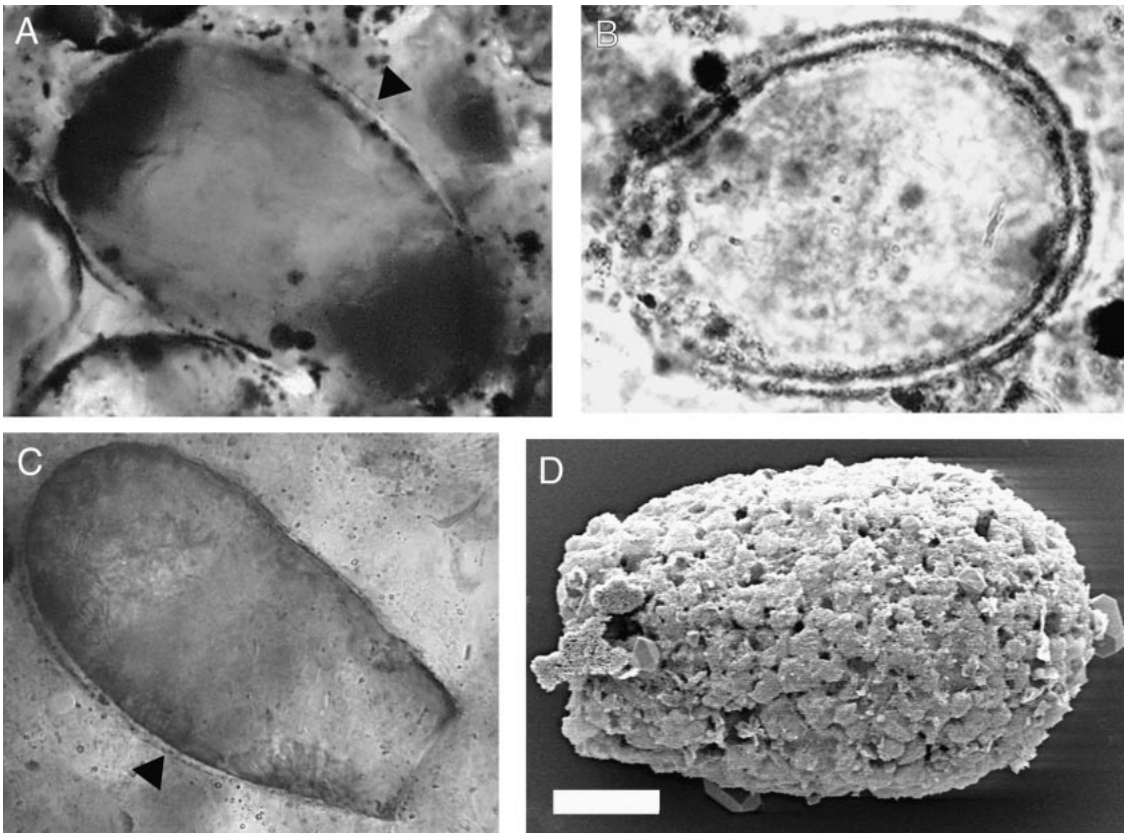

FIGURE 7. Preservation of the VSM wall. A, In dolomite nodules, the wall is preserved as a mineralized cast coated on one or both (shown here) sides with pyrite or iron oxide. HUPC 62988 (AK10-53-13F–2B; L62); scale bar, 30 μm. B, In silicified carbonates, the wall is sometimes preserved as a mineralized cast coated by organic material. HUPC 62992 (AK10-60-19-1; P-62/2); scale bar, 20 μm. C, A VSM from the same horizon studied by Bloeser (1985). Note mineralized cast coated by organic matter (arrow points to coated wall). HUPC 62993 (BL99; N-51); scale bar, 20 μm. D, Siliceous cast of a VSM from the uppermost Awatubi Member. Note agglutinated appearance of the wall. HUPC 62994 (AK10-53-3); scale bar, 25 μm.

diversity of aperture types can be found in our new population (Figs. 3, 6). Most apertures are within 5 and 40 μm in maximum width, although some can be up to 65 μm. Bloeser (1985) also interpreted small plugs in the apertures of a few specimens as opercula, but the irregular shapes of these features suggest that they are of sedimentary origin. None of the many thousands of Grand Canyon VSMs examined for this study possesses an operculum.

Internal Vesicles.—Horodyski (1987, 1993) noted numerous ~10-μm organic vesicles inside rare VSMs from Grand Canyon shales and interpreted them as a primary feature of the organism. We were unable to locate such specimens in Horodyski's collections but did examine color transparencies he prepared (from Horodyski 1993, images courtesy of B. Runnegar). The vesicles photographed by Horodyski are neither uniform in size nor regular in shape. Instead, they appear to be crystalline precipitates coated with organic matter. In the absence of specimens that unambiguously show the small vesicles to be both biogenic and part of the VSM life cycle, we have refrained from including this feature among our list of systematically informative characters.

Wall.—The new VSM populations are preserved as siliceous or calcareous casts coated internally and/or externally with a thin veneer of pyrite (based on EDX analysis) (Fig. 7A). Near nodule margins, the pyritic coating has been oxidized to iron oxide. Wall thickness is 1.0–3.0 μm (mean = 1.8 μm; SD = 0.6 μm;

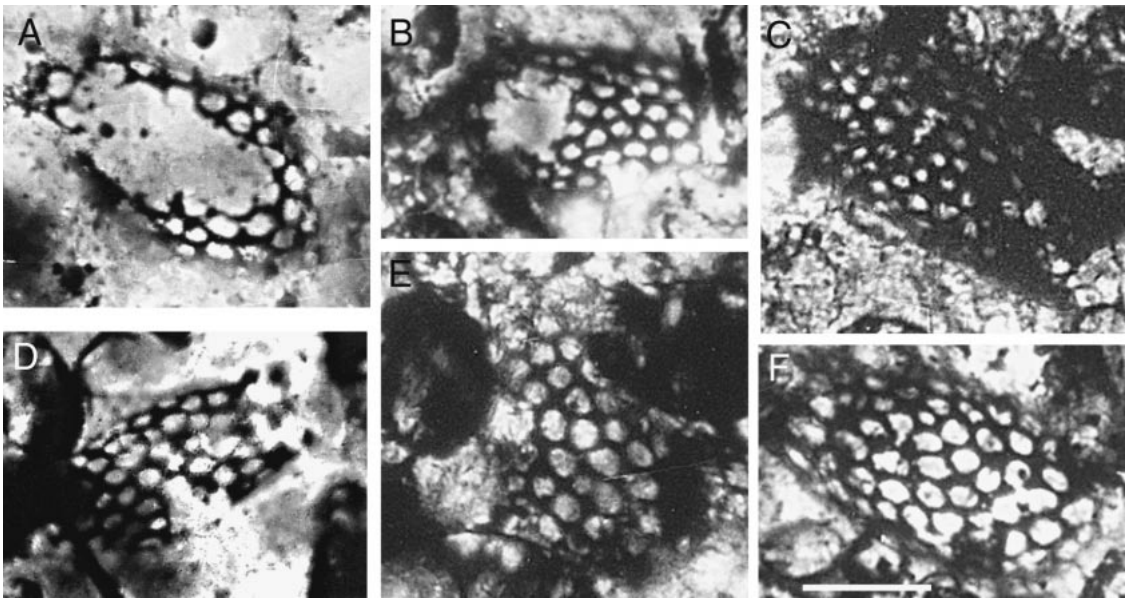

FIGURE 8. Honeycomb VSMs. Note constant size and regular arrangement of perforations within a single test. A, HUPC 62988 (AK10-53-13F–2B; N-42/1). B–F, HUPC 62990 (AK10-53-13F–1). B, (P-44/2). C, (R-47). D, (K-51). E, (L-61/4). F, (P-54/2). Scale bar, 50 μm for all.

$n = 71$). In some specimens the wall is broken but the test retains its shape, indicating rigidity at the time of deformation. The absence of crushed or flattened specimens suggests that the original wall was sturdy.

VSMs found in shales and cherts lower in the succession may be coated by a thin layer of organic matter (Fig. 7B). Bloeser (1985) reported organic-walled VSMs from Chuar shales, but in our preparations from the same horizons and, we believe, the specimens she illustrated, the fossils occur as siliceous casts coated with organic debris or iron precipitates. In thin-section, this is particularly evident (Fig. 7C). Thus, in all specimens that we have examined from dolomite concretions, bedded dolosiltite, and carbonaceous shales of the Chuar Group, the original test wall is preserved as a mineral cast, predominantly silica, with or without a coating of pyrite, iron oxide, or organic matter.

A small number of VSMs, found in shales from the top of the Awatubi Member, appear to be siliceous casts with what might be interpreted as an agglutinated surface texture (Fig. 7D). Clearly, test composition in VSMs differed substantially from that of most other microfossils in the Chuar Group.

"Honeycomb-Patterned" Wall.—The most distinctive character observed in the new Grand Canyon material is a ''honeycomb-patterned'' wall found in ~1% of VSMs from carbonate nodules (Fig. 8). The pattern consists of uniformly sized holes, where pyrite is absent, regularly distributed in a pyritized wall. The regular arrangement of the holes and the uniformity of their size (within a single test) suggest that this pattern reflects a biological trait rather than diagenetic degradation. The average diameter of holes from different tests ranges from 1.0 to 11.0 μm (mean = 5.2 μm, SD = 2.5 μm; $n = 99$), and is not significantly correlated with test length. Distributions of honeycomb test length and width are not significantly different ($p \ll 0.05$) from those of the larger population, and the range of test shapes is comparable.

Taphonomy

How can one account for the pattern of preservation observed in Chuar VSM populations? Noting the rigidity of test walls, Vidal (1994) proposed that they may originally have been mineralized in their entirety; however, the variable preservation of walls as silica, carbonate, or phosphate (see below) favors a diage-

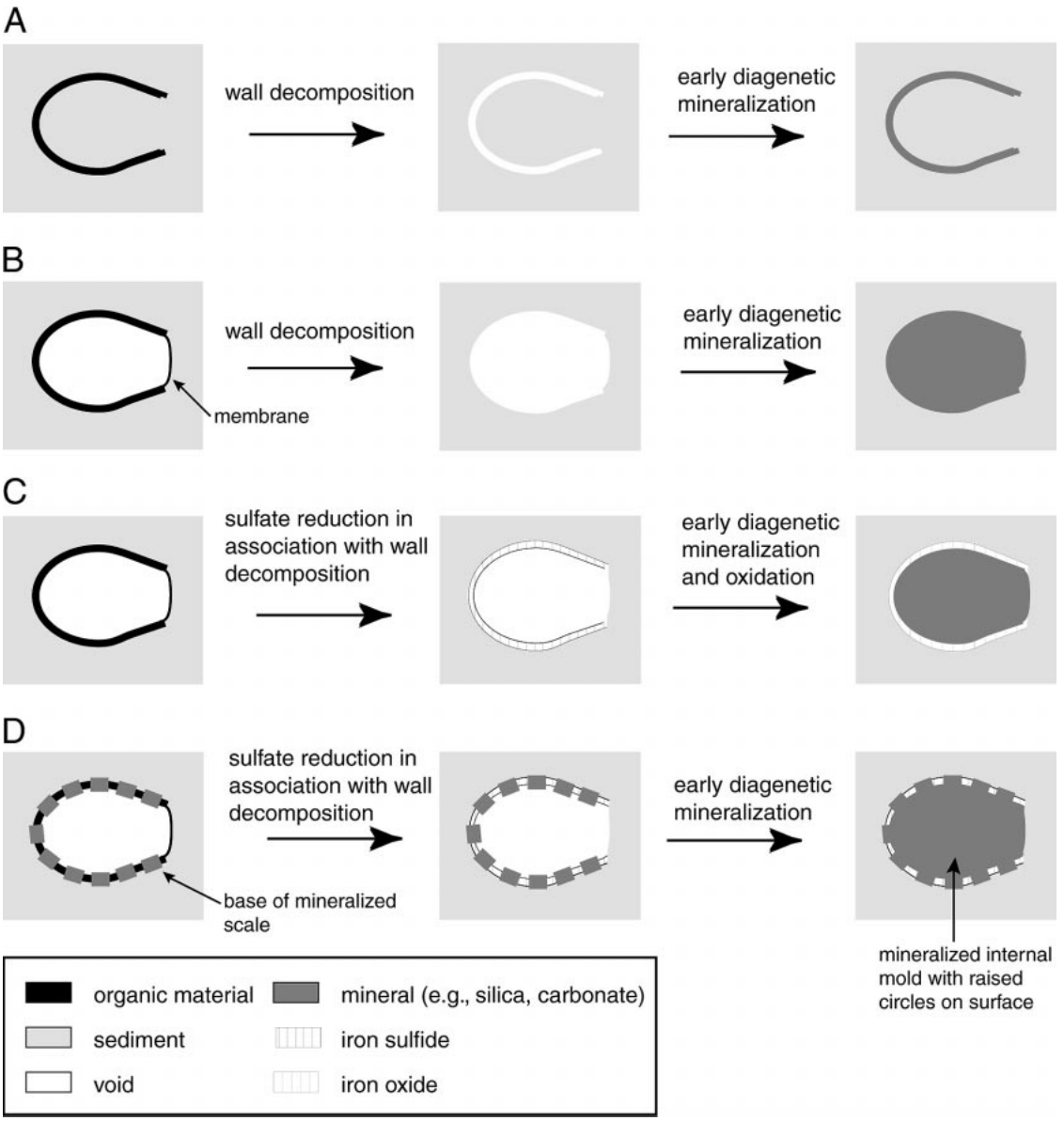

FIGURE 9. The observed variation in VSM test preservation can be explained in terms of a small number of well-established taphonomic processes (see text for discussion).

netic origin for most of the mineral content of preserved VSMs. Petrological observations bolster this view, revealing inwardly radiating crystal fans nucleated on the outer edge of test walls in both siliceous and phosphatic specimens (Knoll and Vidal 1980). Instead, the combination of three-dimensional preservation and pyritic coating suggests that test walls were originally composed of organic material that was mechanically strong but easily degradable and therefore not readily preserved. The honeycomb pattern further suggests that in some individuals the wall contained regularly distributed mineralized scales (as discussed below, preservation of the honeycomb pattern in VSMs from other localities shows that scales, rather than holes, were present in the test).

Given these features, VSM preservation can be explained as follows (Fig. 9):

1. The rigid tests were entombed in surface sediments made firm by penecontemporaneous lithification (nodules) or microbially produced extracellular polymeric molecules (Krumbein et al. 1994). Ensuing decay of wall constituents left a void that was filled by early diagenetic silica or carbonate, forming a mineralized cast (Fig. 9A). A mineralized internal mold could form if, at the time of deposition, the cell was still present within the test, preventing infilling of sediment (Fig. 9B).
2. In iron-rich sediments, sulfate reduction associated with decomposition of the wall would produce an iron sulfide coating (Fig. 9C) (Canfield and Raiswell 1991). VSMs coated with iron sulfide are usually found in siliciclastic sediments, consistent with the fact that these facies contain relatively high concentrations of iron. Iron oxide coatings would result from oxidation of the iron sulfide coat, as inferred from the restriction of iron oxide VSMs to the outer rind of the upper Walcott dolomite nodules.
3. If mineralized scales were embedded within the organic test, then during decomposition, iron sulfide precipitation would preferentially occur in association with the organic matrix rather than with the scales (Fig. 9D). In the absence of such precipitation, the honeycomb pattern would be difficult to discern. This helps to explain why honeycomb walls are readily identifiable in upper Walcott dolomite nodules but not in silicified carbonates lower in the succession. Furthermore, because honeycomb VSMs account for ~1% of upper Walcott specimens, they might be missed in the much smaller sample populations recovered from silicified carbonates and shales.

Summary

Combining morphological and taphonomic observations, we can summarize the systematically informative features of Grand Canyon VSMs. VSMs represent degradation-prone, organic-walled tests that vary widely in size and shape. The test walls are 1–3 μm thick and rigidly constructed. Apertures are small (not flaring), may be collared, and their margins may be circular, triangular, or hexagonal; there is no conclusive evidence for an operculum. Most tests are radially symmetric, but a significant proportion are curved. A small number are distinguished by a honeycomb-patterned wall that reflects the regular arrangement of mineralized scales in the test wall. The presence of internal vesicles has not been convincingly demonstrated. There exists some evidence for agglutinated VSMs.

VSMs from Other Localities

Morphology and Taphonomy

Although the Grand Canyon population is variable, the fossils are sufficiently similar in shape and size to warrant placement within a single morphological group, as Bloeser did in her taxonomy. Other aperturate microfossils reported from localities around the world (Tables 1, 2) are also indistinguishable in size and shape from the Grand Canyon population, and therefore we have included these fossils in our analysis of VSMs and consider their biological relationships to approximate those of the Grand Canyon fossils. Many other Proterozoic and Cambrian microfossils have shapes that are broadly tear- or vaselike; however, these have been excluded from the present analysis either because they do not resemble the Grand Canyon population or because they are not preserved or illustrated sufficiently well to allow confident attribution (Table 1).

Table 2 lists the localities and the salient characters of all confirmed (see Table 1) VSM populations, along with a summary of characters for the combined, global population of VSMs. (Only well-documented characters are included; features such as opercula and attached VSMs have been reported but not convincingly demonstrated.) Some features of the Grand Canyon populations are found in all other populations, including size range, overall morphology, and mode of preservation. (Note that reexamination of specimens reportedly composed of resistant organic matter [Knoll and Calder 1983] showed them to be mineralized casts coated with organic debris.) Other characters exhibited by the Grand Canyon population, in particular, neck curvature and honeycomb pattern, occur in some but not

TABLE 1. Localities where vasiform microfossils have been found.

I. Microfossils that are comparable in shape and size to the Grand Canyon VSMs and to each other, and thus are included in this analysis.
Visingsö Beds, Sweden (Ewetz 1933; Knoll and Vidal 1980)
Eleonore Bay Group, East Greenland (Vidal 1979; Green et al. 1988)
Jabal Rockham, Saudi Arabia (Binda and Bokhari 1980)
Ryssö Formation, Nordaustlandet (Knoll and Calder 1983)
Chatkaragai Suite, Tien Shan, Russia (Kraskov 1985; Yankaouskas 1989)
Togari Group, Tasmania (Saito et al. 1988)
Backlundtoppen Formation, Spitsbergen (Knoll et al. 1989)
Draken Conglomerate Formation, Spitsbergen (Knoll et al. 1991)
Elbobreen Formation, Spitsbergen (A. H. Knoll unpublished observations)
Pahrump Group, southeast California (Horodyski 1993)

II. Microfossils that do not resemble the Grand Canyon (and other VSM) populations, and the reason for their exclusion.
Xihaoping Formation, China (Duan 1985):300–800 μm in length
Doushantuo Formation, China (Duan and Cao 1989):lack of stable morphology in the population
Dengying Formation, China (Zhang and Li 1991; Zhang 1994):600–2400 μm in length
Tongying Formation, China (Duan et al. 1993):300–800 μm in length
Yuanjiaping section, China (Cao et al. 1995):no evidence for a wall, lack of stable morphology within the population

III. Microfossils that are not preserved or illustrated sufficiently to allow confident attribution.
Jacadigo Group, southwest Brazil (Fairchild et al. 1978)
Simla Slates, Satpuli, India (Nautiyal 1978)
Tanafjorden Group, Norway (Vidal and Siedlecka 1983)
Vindhyan Supergroup, India (Maithy and Babu 1988)
Tindir Group, northwest Canada (Allison and Awramik 1989)
Uinta Mountain Group, Utah (Link et al. 1993)
Upper Min'yar Formation, southern Urals (Maslov et al. 1994)
Vaishnodevi Limestone, Himalaya, India (Venkatachala and Kumar 1998)

all other populations. This may in part reflect lower specimen concentrations in other localities, as these characters are exhibited by a minority of the Grand Canyon VSMs. VSMs from the Chatkaragai Suite, Tien Shan, deserve special mention because they are preserved as mineralized molds that exhibit a clear honeycomb pattern as raised circles (Fig. 10) (Kraskov 1985; Yankaouskas 1989). Like the holes in pyritized honeycomb VSMs, to which they correspond, the circles are regularly arranged and uniform in diameter within a single individual. (VSM casts from the Eleonore Bay Group, East Greenland, also have holes, but their irregular size and arrangement indicate that they are diagenetic in origin [Vidal 1979].) The raised knobs on the Chatkaragai molds provide particularly strong evidence for the presence of regularly arranged scales (rather than holes) in VSM test walls. The voids left by the dissolution of the scales are represented in the mineralized cast by the raised knobs, and the surrounding matrix is cast as the depressions between those knobs (Kraskov 1985; Yankaouskas 1989) (Fig. 10). If there had been regularly distributed holes in the matrix rather than mineralized scales, casts with the opposite relief (which have not been observed) would be expected.

A few populations exhibit characters that are not observed in the Grand Canyon specimens. These include a larger length/width ratio of individual tests (5:1) and apertures that are crenulate in shape, both observed in the Tien Shan specimens (Kraskov 1985; Yankaouskas 1989; L. Kraskov personal communication 1999) (Fig. 10A).

Biostratigraphic Range

No other VSM populations are as sharply constrained by radiometric ages as the Chuar fossils, but all fall within the same broad stratigraphic window. VSMs have not been reported from paleontologically rich and well-studied late Mesoproterozoic or early Neoproterozoic successions such as the ~850-Ma Miroyedikha Formation or the >1000-Ma Lakhanda and Turukhansk Groups, in Siberia (German 1990; Sergeev et al. 1997). Nor have VSMs been discovered in rocks that postdate

TABLE 2. Characters of VSM populations from eleven localities; ''yes'' means at least some members of the population have the character, ''no'' means none do.

Locality	Size range; l/w ratios	Curvature	Aperture; operculum	Internal vesicles	Wall	Honeycomb	Attached forms	Mode of preservation	References
Chuar Group, Grand Canyon, Arizona	w: 17–202 μm l: 25–286 μm; l/w: 1.0/1 to 3.0/1	yes	hexagonal, triangular, and circular apertures; operculum reported but not conclusive	reported but not conclusive	1–3 μm thick, sturdy	yes	no	calcareous and siliceous casts and molds with coats of organic debris, iron sulfide, or iron oxide (some material reinterpreted)	Bloeser et al. 1977; Bloeser 1985; Vidal and Ford 1985; Horodyski 1993; this study
Visingsö Beds, Sweden	w: 25–62 μm l: 60–130 μm	no	aperture shape not noted; no operculum	yes	~2 μm thick	no	no	siliceous, calcareous, and phosphatic casts and molds	Ewetz 1933; Knoll and Vidal 1980
Eleonore Bay Group, East Greenland	l: 50–265 μm	no	circular aperture; no operculum	no	not noted	diagenetic holes	no	iron oxide coats	Vidal 1979; Green et al. 1988; S. Xiao and S. M. Porter unpublished data
Jabal Rockham, Saudi Arabia	w: 25–105 μm l: 35–160 μm	no	aperture shape not noted; operculum noted but not conclusive	no	not noted	no	noted but not conclusive	dolomite internal molds with iron oxide coats	Binda and Bokhari 1980
Ryssö Formation, Nordaustlandet	w: 16–119 μm l: 34–257 μm	no	hexagonal apertures; no operculum	no	not noted	no	no	calcareous and siliceous casts	Knoll and Calder 1983; this study
Chatkaragai Suite, Tien Shan, Russia	l/w: up to 5.0/1	yes	crenulate apertures; no operculum	no	not noted	yes	no	mineralized internal molds	Kraskov 1985; Yankauskas 1989; L. N. Kraskov personal communication 1999
Togari Group, Tasmania	w: 30–80 μm l: 40–120 μm	no	aperture shape not noted; operculum noted but not conclusive	no	not noted	no	noted but not conclusive	siliceous casts	Saito et al. 1988
Backlundtoppen Formation, Spitsbergen	not noted	no	hexagonal aperture; no operculum	no	not noted	no	no	mineralized internal molds	Knoll et al. 1989
Draken Conglomerate Formation, Spitsbergen	w: 30–105 μm l: 64–230 μm	no	aperture shape not noted; no operculum	no	not noted	no	no	siliceous internal molds	Knoll et al. 1991
Pahrump Group, southeast California	w: 50–150 μm l: 40–140 μm	no	aperture shape not noted; no operculum	no	not noted	no	no	siliceous cast	Horodyski 1993
Elbobreen Formation, Spitsbergen	not noted	yes	aperture shape not noted; no operculum	no	not noted	no	no	iron oxide coat	A. H. Knoll unpublished observations
Summary	w: 17–202 μm l: 25–286 μm l/w: 1.0–5.0/1	yes	hexagonal, triangular, circular, and crenulate apertures	yes	1–3 μm thick, sturdy	yes	no	mineralized casts and/or molds, coated with organic debris or iron compounds	

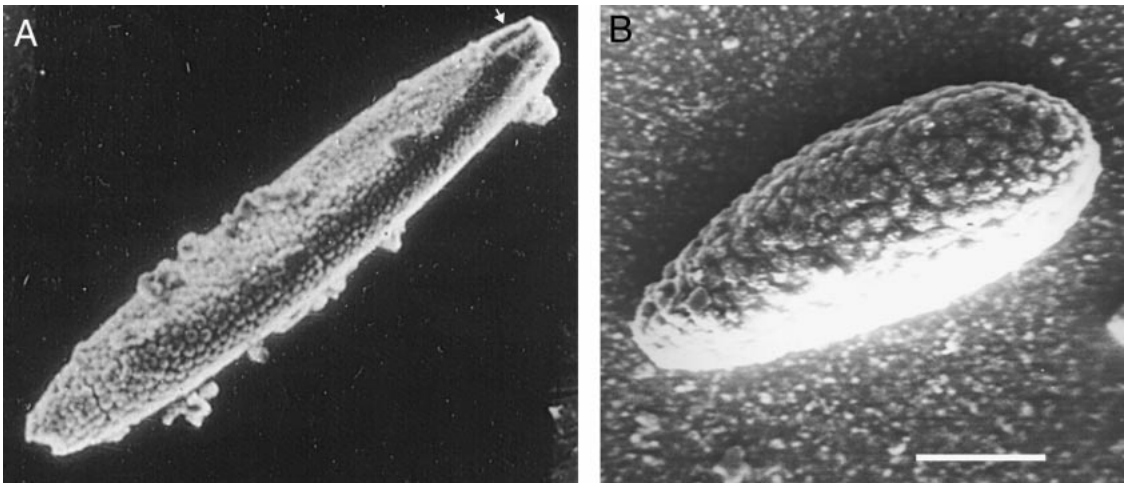

FIGURE 10. VSMs preserved as internal molds from the Chatkaragai Suite, Tien Shan. Note crenulate aperture (arrow) molded as indentations parallel to test axis (A), and raised knobs covering specimens in both A and B. Scale bar in A, 100 μm; in B, 40 μm. Courtesy of L. Kraskov.

Varanger or Marinoan glaciation—although their mode of preservation in shallow marine environments would have become increasingly difficult to achieve as bioturbating animals evolved.

VSMs are known from pre-Sturtian correlatives of the Chuar Group elsewhere in western North America (just below the tillite-bearing Kingston Peak Formation of the Pahrump Group [Horodyski 1993]), and they occur, as well, in Tasmanian rocks inferred to predate Sturtian glaciation (Saito et al. 1988; Calver 1998). In northern Europe and Greenland, Sturtian glaciogenic rocks have not been identified with confidence, but VSM populations in the Visingsö Beds, Sweden (Ewetz 1933; Knoll and Vidal 1980); the Upper Eleonore Bay Group, central East Greenland (Vidal 1979); and the upper Akademikerbreen Group, Spitsbergen, and its equivalents in Nordaustlandet (Knoll and Calder 1983; Knoll et al. 1989, 1991) all lie below Varanger tillites. The Visingsö Beds contain diverse acritarchs that permit biostratigraphic correlation with the upper Chuar Group (Vidal and Ford 1985).

Kennedy et al. (1998) proposed that lower Varanger tillites in northern Europe correlate with the Sturtian tillite in western North America, and that upper Varanger tillites correlate with the Marinoan tillite in Australia. If this view is correct, most or all VSMs fall within a relatively narrow stratigraphic interval just before the Sturtian ice age. Several considerations, however, call this correlation into question, not least, U-Pb dates of 660 ± 15 Ma on granites that intrude immediately sub-Varanger successions in the southern Urals (Semikhatov 1991). Sturtian glacial rocks are poorly dated but appear to be older than 700 Ma and younger than the 742 ± 7 Ma age of the upper Chuar Group; the most direct age constraint comes from U-Pb zircon dates of 723+16/ − 10 Ma recently reported from a tuff within the Ghubrah diamictite, Oman (Brasier et al. 2000).

Alternatively, the two Varanger tillites may document a Marinoan and a post-Marinoan glaciation (Kaufman et al. 1997; see also Vidal and Siedlecka 1983; Nystuen and Siedlecka 1988; Vidal and Moczydłowska 1995). The VSM-bearing Draken and Backlundtoppen Formations in Spitsbergen (which both underlie Varanger tillites [Knoll et al. 1989; 1991]) contain acritarch assemblages found in post-Sturtian but pre-Marinoan successions elsewhere (although the ranges of many acritarch taxa are long or not well-constrained [Walter et al. 2000]), and they overlie a $\delta^{13}C$ excursion interpreted as a biogeochemical proxy for Sturtian glaciation (Kaufman and Knoll 1995; Knoll 2000). Fossils that may be VSMs (see Table 1) have been reported from the demonstrably post-Sturtian Tindir Group, northwest Canada (Allison and Awramik 1989).

Thus, VSMs first appear in widely distributed basins shortly before the Sturtian ice age, probably not much before 800 Ma. Global first appearances coincide with a shift in the marine carbon isotopic record from a pattern of moderate secular variation (−1 to +4 permil) to one of pronounced fluctuation (extreme values of less than −4 and greater than 7 permil) that characterized the remainder of the Neoproterozoic Era. These fossils persist until the time of Sturtian glaciation (~723 Ma) and may persist until the Marinoan ice age (ca. 610–590 Ma); they have not, however, been found in uppermost Proterozoic successions.

Biological Affinities of VSMs

Bloeser et al. (1977) initially interpreted VSMs as chitinozoans on the basis of similarity in shape, but later rejected this idea because of morphological differences between the two groups, including the presence of appendages and basal horns on many chitinozoan tests and the capacity of these younger organisms to form chains and cocoons (Bloeser 1985). Additionally, unlike VSMs, chitinozoans are routinely preserved as resistant organic structures—hence their name. With this in mind, Bloeser (1985) tentatively concluded that VSMs are algal cysts (phylogenetic relationships unspecified), based on the presence of an operculum, their localized abundance, and the absence of intermediate growth stages. The presence of an operculum is not considered here to be characteristic of VSMs, however, and the other two features are not unique to algal cysts. Horodyski (1987, 1993) also suggested that VSMs might represent algal sporangia, but we cannot confirm the biological nature of the internal vesicles on which he based his interpretation. Morphological and ecological similarities between tintinnids and VSMs were noted by Fairchild et al. (1978), Knoll and Vidal (1980), and Knoll and Calder (1983), but tintinnid loricae differ in shape from VSM tests (often being most constricted at the aboral pole and flaring out toward the opening) and do not exhibit curvature or aperture shapes comparable to those of VSMs (Small and Lynn 1985; Tappan 1993). Gammacerane, a biomarker compound linked to ciliates, has been recovered from Chuar shales associated stratigraphically with VSMs (Summons et al. 1988), but there is no evidence that the gammacerane and the microfossils were produced by a single population. Other biomarkers and other fossils occur in the same unit.

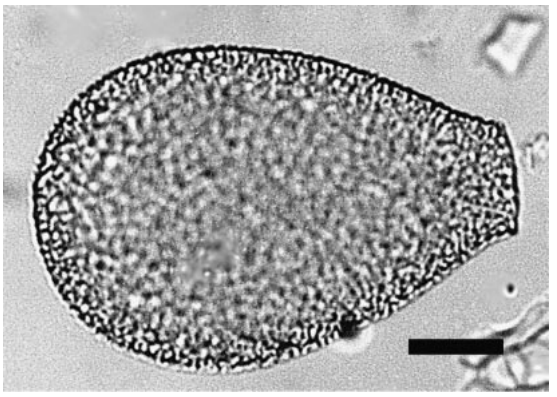

FIGURE 11. *Nebela bohemica,* a lobose testate amoeba, exhibiting the tear-shaped test characteristic of many testate amoebae. Scale bar, 20 μm. Courtesy of R. Meisterfeld.

Of the gamut of opinion previously expressed, Schopf's (1992) falls closest to the mark. The preserved vases are not cysts, but we believe that their systematic affinities do lie with the lobose and filose testate amoebae (Testacealobosea and Testaceafilosea, respectively). Several lines of evidence support this conclusion:

1. Only a limited number of protistan groups contain species that form vase-shaped tests or loricae; of these, the testate amoebae by far most closely approximate the morphologies observed in VSM populations. Like VSMs, most testate amoebae have a tear- or cup-shaped test with a relatively narrow aperture (Fig. 11). Indeed, the range of shapes exhibited by VSMs is matched by that of the testate amoebae. Short, wide VSMs, for example, are closely similar to members of the Difflugiidae (Testacealobosea), while pyriform VSMs are comparable to members of the Nebelidae (Testacealobosea [Ogden and Hedley 1980; Bovee 1985a,b]).
2. Although they can be as large as 600 μm (e.g., *Difflugia pyriformis* [Bovee 1985a]), testate amoebae most commonly range be-

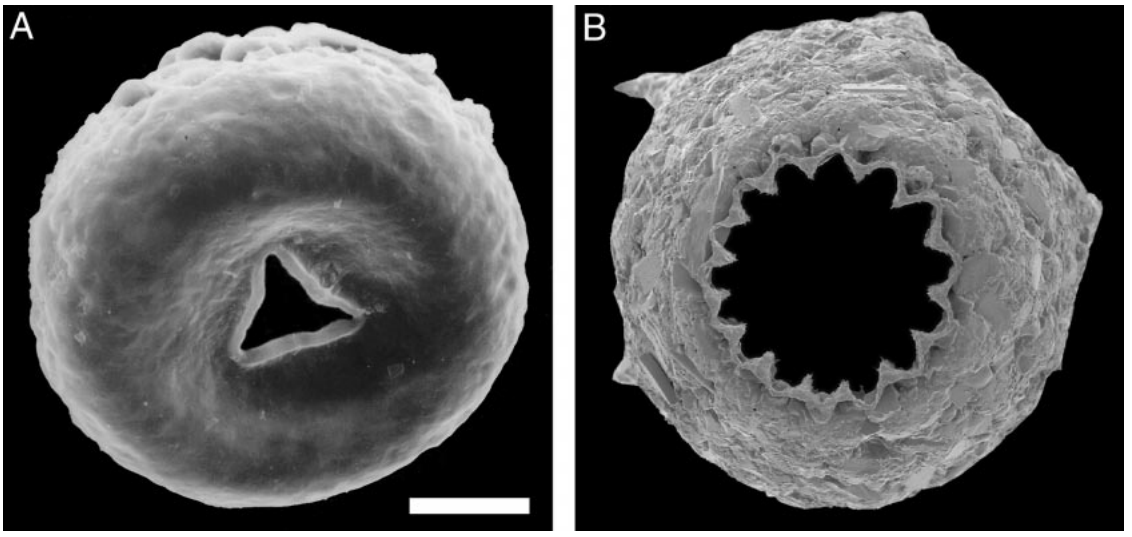

FIGURE 12. Triangular (A) and crenulate (B) apertures of testate amoebae. A, *Trigonopyxis arcula;* B, *Difflugia corona*. Scale bar, 20 μm for both. Courtesy of R. Meisterfeld.

tween 50 and 200 μm in length. Species smaller than 50 μm do exist and may be present in high abundances (R. Meisterfeld personal communication 1999; D. Patterson personal communication 1999). We have not observed an abundance of VSMs smaller than 50 μm, but the range of VSM sizes is comparable to that of the testate amoebae.

3. The range of aperture shapes observed in VSMs is also found in testate amoebae (Fig. 12). Hexagonal apertures comparable to those of VSMs are exhibited by some members of the Arcellidae (Testacealobosea [R. Meisterfeld personal communication 1999]), although the latter possesses tests that are wider and shorter than VSMs. *Trigonopyxis,* a lobose testate amoeba, has a triangular aperture like that found in many VSMs (Fig. 12A; Bovee 1985a), and many members of the Difflugiidae exhibit crenulate apertures like those found in VSMs from the Chatkaragai Suite (Bovee 1985a; Kraskov 1985; Yankaouskas 1989) (Fig. 12B). Many lobose and filose testate amoebae have circular apertures (Bovee 1985a,b).
4. Test curvature identical to that found in VSMs is exhibited by members of both the filose and lobose amoebae (for example, *Pomoriella* in the Paraquadrulidae [Testacealobosea], *Cyphoderia* in the Cyphoderiidae [Testaceafilosea], and many members of the Euglyphidae [Testaceafilosea] [Bovee 1985a,b]) (Fig. 13). In many species, curvature is a plastic character, occurring only when a physical barrier, such as a sand grain, obstructs test formation (R. Meisterfeld personal communication 2000).
5. The structure and composition of testate amoeban tests is comparable to that inferred for VSMs: the tests are rigid, with walls a few microns thick and composed of nonresistant (proteinaceous) organic material (Ogden and Hedley 1980). Iron and manganese are often incorporated into the test, promoting pyritization in anoxic bottom waters (Wolf 1995)—comparable to the mode of preservation observed in the new Grand Canyon population.
6. A number of testate amoebae have agglutinated tests, and others have tests in which internally synthesized scales of silica are arrayed in a regular pattern. For example, the tests of certain members of the Testaceafilosea (e.g., the Euglyphidae and the Cyphoderiidae) possess regularly arranged imbricated siliceous scales (Fig. 14) whose bases are embedded in an organic matrix (Bovee 1985a; Ogden 1991). The arrangement of these scale bases is similar to the pattern observed in honeycomb VSMs, and the range of scale sizes (~1 μm in di-

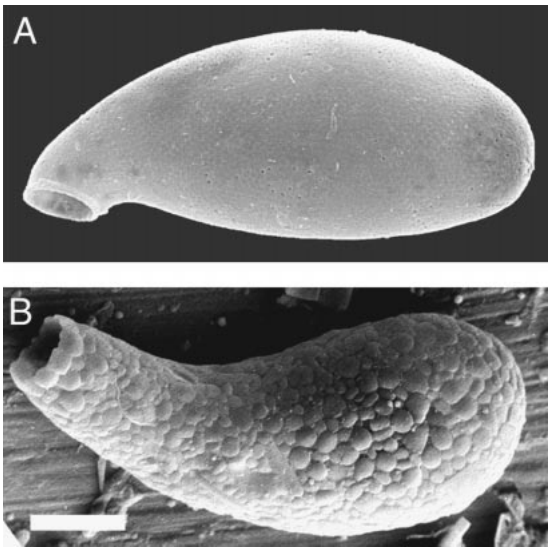

FIGURE 13. Testate amoebae with curved necks. A, *Cyphoderia ampulla,* and B, *Nebela retorta.* Scale bar in A, 20 μm; in B, 30 μm. Courtesy of R. Meisterfeld.

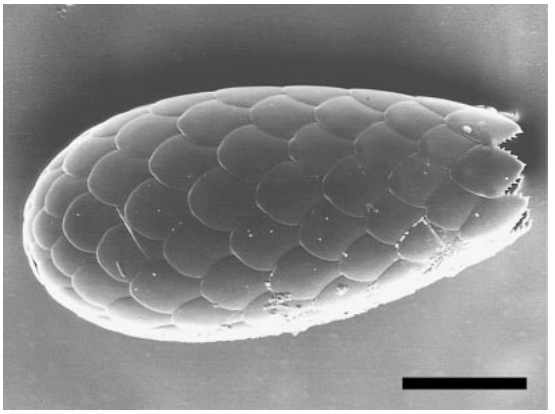

FIGURE 14. The test of *Euglypha tuberculata.* Note regularly arranged siliceous scales. Scale bar, 20 μm. Courtesy of R. Meisterfeld.

ameter in *Cyphoderia ampulla* [Bovee 1985a; D. Patterson personal communication 1999]; to >10 μm in *Spenoderia lenta* [Ogden and Hedley 1980]) matches that observed for the honeycomb VSMs. Species of *Nebela,* a lobose testate amoeba, also possess regularly arranged siliceous scales, which they acquire by consuming scale-producing filose testate amoebae, sequestering the scales of their prey, and then incorporating those scales into their own test (R. Meisterfeld personal communication 2000).

7. The tests of testate amoebas can become ''plugged'' with sedimentary material (Ogden and Hedley 1980; S. Bamforth personal communication 1999), consistent with the interpretation, favored here, that the ''opercula'' observed in some VSMs (Bloeser 1985) represent sedimentary debris.
8. While the biogenic origin of the internal vesicles observed by Horodyski (1993) is in doubt, it nevertheless should be noted that, during adverse conditions, testate amoebae can form a spheroidal internal cyst (Ogden and Hedley 1980).

In summary, living testate amoebae can account for the full range of morphological and taphonomic features observed in VSM populations from the Chuar Group and elsewhere. Indeed, a number of living testate amoebae would produce fossils indistinguishable from VSMs. Thus, testate amoebae provide strong candidates for VSM attribution. To determine whether the testate amoebae provide a *uniquely* close fit with VSMs, it is necessary to look at other protists that produce aperturate tests, cysts, or loricae (Table 3).

Clathrulina (Desmothoracida) possesses a comparably sized organic test, but the test is round, is covered with pores, lacks a differentiated aperture region, and attaches to the substrate by a stalk (Febvre-Chevalier 1985). *Trachelomonas* (Euglenozoa) possesses an aperturate test, but the apertural opening is very small, and the test is round to elliptical in shape, lacks a differentiated ''neck'' region, and exhibits neither curvature nor the variety of aperture shapes seen in VSMs. In addition, *Trachelomonas* is smaller than most VSMs (generally 20–50 μm), lacks mineralized scales, and is often ornamented with pores, spines, or warts (Leedale 1985; Graham and Wilcox 2000; D. Patterson personal communication 1999). Some stramenopiles, such as *Mallomonas* and *Mallomonopsis,* are covered with imbricated siliceous scales, but the tests are usually covered with long spines, lack a collar, do not display curvature, have apertures much smaller in diameter than those of VSMs, and commonly break apart upon death (Hibberd and Leedale 1985; D. Patterson personal communication 1999; P. Siver personal communication 1999). The tests of single-chambered fo-

TABLE 3. A comparison of VSMs with modern eukaryote candidates. A (+) indicates that at least some members of the taxon possess the character, a (−) indicates that none do.

VSM characters	Testate amoebae[c,f,g,h]	*Clathrulina*[c]	*Trachelomonas*[c,h,j]	*Mallomonas*[c,h,i]	Single-chambered Foraminifera[d,h]	Tintinnids[c,e]	Folliculunids[a,c]	Peritrichs[b,c]
Length commonly 25–160 μm	commonly 60–200 μm	~75 μm	20–50 μm	<100 μm	commonly ~200 μm	commonly 100–300 μm	200 to <800 μm	<100 μm
Sac- or tear-shaped	+	−	−	+	+	−	+	+
Nonresistant wall	+	?	−	+	+	?	?	+
Rigid wall	+	?	+	?	−	?	−	−
Curved neck	+	−	−	−	−	−	+	+
Aperture diameter mostly 5–40 μm	5–50 μm	pores are ~6–10 μm	<5 μm	~1 μm	~1–80 μm	20–100 μm	50–125 μm	10–50 μm
Apertural shapes	+	−	−	−	−	−	−	−
Collar	+	−	+	−	−	+	+	+
Honeycomb: non-organic scales	+	+	−	+	−	−	−	−
Other features that contradict an affinity with VSMs		with a stalk; no differentiated aperture	often with warts or spines on test	usually with long spines		collar often elaborate		with a stalk

[a] Fauré-Fremiet 1936; [b] Hamilton 1952; [c] Lee et al. 1985; [d] Loeblich and Tappan 1988; [e] Tappan 1993; [f] S. Bamforth, [g] R. Meisterfeld, [h] D. Patterson, [i] P. Siver, all personal communications 1999; [j] Graham and Wilcox 2000.

raminifera, such as *Allogromia* and *Lagynis,* are comparable in shape and size to Grand Canyon VSMs and are composed of a nonresistant organic material, but the tests are flexible, and none have a curved neck, a collar, comparable aperture shapes, or mineralized scales (Loeblich and Tappan 1988; D. Patterson personal communication 1999).

Many ciliates possess loricae; those groups most similar to VSMs are the tintinnids, the folliculinids, and the peritrichs. Tintinnid loricae are commonly larger, however, and are differently shaped, with a flaring aperture and no curvature. In addition, they often have elaborated collars (Small and Lynn 1985). The tests of some folliculinids are comparable in shape to VSMs and some are curved, but they are flexible (Fauré-Fremiet 1936), lack comparable aperture shapes, are without mineralized scales, and are, for the most part, much larger than VSMs (200 to >800 μm [Small and Lynn 1985]). The tests of the peritrichs *Vaginicola* and *Cothurnia* are similar in shape to VSMs, are composed of nonresistant organic matter, and may be curved. However, they are small (<100 μm) and flexible, lack mineralized scales, and attach to the substrate by a stalk (Hamilton 1952; Small and Lynn 1985; D. Patterson personal communication 1999).

No single character uniquely links VSMs to the testate amoebae; however, the *combination* of characters exhibited by these fossils is found only in the testate amoebae. We have particular confidence that specimens marked by honeycomb walls are related to scale-bearing testate amoebae, such as *Euglypha.* More broadly, the *range* of morphologies found in the upper Walcott dolomite nodules is matched by living species of both lobose and filose amoebae (for example, *Nebela, Cyphoderia,* and several genera in the Hyalosphenidae [Bovee 1985a,b]). We cannot rule out the possibility that some of the most generalized morphologies represent additional, unrelated diversity, such as single-chambered foraminifera, but testate amoebae appear to dominate the assemblage. By extension, we believe that morphologically comparable VSM populations found elsewhere are systematically comparable.

Evolutionary Implications

What Does This Assemblage Represent?

Given the preceding comments, we might interpret VSMs in several possible ways. Conceivably, they could represent an extinct protist group that independently acquired attributes similar to those of the testate amoebae. The strong similarity between the honeycomb VSMs and the scale-bearing testate amoebae, however, suggests that at least these VSMs represent testate amoebae, and it is not unreasonable to interpret the other VSMs as crown- or stem-group testate amoebae as well. If lobose and filose testate amoebae constitute a monophyletic group (a hypothesis defended by Cavalier-Smith [1993]), then at least some VSMs could represent a stem group related to the ancestors of both lobose and filose forms. If we use the taxonomy of modern testate amoebae as a guide, however, the morphological variation observed in the Chuar VSMs would suggest that the assemblage is composed not of one species, but a dozen or more that includes crown-group taxa (a diversity only hinted at in the current taxonomic classification of VSMs [Bloeser 1985]).

The most likely interpretation of these fossils, then, is that they are a multispecies assemblage that includes both lobose and filose testate amoebae, and, conceivably, other groups as well. This is consistent with the observed variability within the assemblage, and with the fact that diverse filose and lobose testate amoebae commonly occur together in modern environments (Bovee 1985b). Preservational association presumably would be the result of a common habitat and taphonomic selection for the test composition and/or structure.

Implications for the Evolution of Testate Amoebae

Several independent lines of evidence indicate that the Chuar Group is marine. Most modern testate amoebae, however, live in freshwater or terrestrial habitats. Thus, if our interpretations of both systematics and paleoenvironment are correct, then testate amoebae must once have been more conspicuous in marine environments than they are today.

This is not an unreasonable proposition, and it calls to mind animal groups such as onychophorans and chelicerate arthropods that have similar histories. Testate amoebae are not absent from the marine realm today; several groups, including members of both the Testaceafilosea (Golemansky 1974; Bovee 1985a; Sudzuki 1979) and the Testacealobosea (Sudzuki 1979; Bovee 1985b) inhabit marine environments such as tidal pools and beach sands. In molecular phylogenies of silica-secreting filose clades, the earliest branching species, *Paulinella chromatophora,* lives in fresh- and brackish-water conditions, but other *Paulinella* species are marine (R. Meisterfeld personal communication 1999).

Modern testate amoebae tend to live in habitats that are rich in organic matter (e.g., peat bogs, forest litter, humus [Bovee 1985a,b]), environments that would not have been represented to any great extent on land before the mid-Paleozoic radiation of land plants. Thus, like other heterotrophic groups that radiated on land, testate amoebae may have originated in marine environments and later expanded into terrestrial niches as land plants diversified. The narrow test aperture of testate amoebae is considered to be an adaptation for life in episodically dry terrestrial habitats (Hausmann and Hülsmann 1996); the Chuar fossils, however, imply that this is an exaptation that facilitated but did not originate with the invasion of nonmarine environments.

VSMs are preserved in association with high concentrations of organic matter in tidal-flat, lagoonal, and subtidal facies (Knoll and Vidal 1980; Knoll et al. 1991; this study). By analogy with modern testate amoeban ecology, it is reasonable to assume that the tidal-flat and lagoonal specimens lived in those environments. It is unclear, however, whether the VSMs from the subtidal facies are preserved in situ; there are examples of modern testate amoebae that live in deep-water conditions (Bovee 1985a,b), but the high concentration of VSMs in these facies suggests that they may have been transported. Indeed, transportation and winnowing of modern testate amoebae tests can result in concentrations comparable in magnitude to those observed for VSMs (R. Meisterfeld personal communication 1999).

Implications for Eukaryote Evolution

The Structure of the Eukaryotic Tree.—A widely accepted view of eukaryote evolution has been one in which a rapid crown radiation of the major taxa was preceded by a longer history during which more primitive taxa, such as microsporidia, diplomonads, slime molds, and trichomonads, gradually diverged (Sogin et al. 1986, 1989; Sogin 1991, 1994). That the earliest branching taxa lacked mitochondria, chloroplasts, and, in the case of diplomonads, even well-developed Golgi bodies supported their basal position in the tree, and suggested that the principal metabolic organelles were acquired gradually, long after the origin of the clade (Sogin et al. 1986, 1989). Recently, however, this view, derived from phylogenies constructed using small-subunit (SSU) rRNA sequences, has been questioned. Phylogenetic trees based on other gene sequences place some or all of these putatively basal taxa well within the crown-group eukaryotes (Baldauf and Doolittle 1997; Philippe and Adoutte 1998). Comparison of rates of sequence evolution between and within lineages indicates the reason for the incongruence: SSU rRNA sequences in many of the early-branching taxa have evolved at an unusually high rate (Philippe and Adoutte 1998; Stiller et al. 1998; Stiller and Hall 1999), suggesting that their basal position may be an artifact of long-branch attraction (Felsenstein 1978). Furthermore, the discovery of mitochondria-like genes in microsporidia (Germot et al. 1997; Hirt et al. 1997), diplomonads (Roger et al. 1998), and trichomonads (Bui et al. 1996; Germot et al. 1996) may indicate that mitochondria were secondarily lost in these taxa, invalidating their status as primitively amitochondriate groups. (Similar genes occur in the nuclear genome of the entamoebae, a group widely accepted as having lost its mitochondria [Clark and Roger 1995]). When corrections for long-branch attraction are made, however, the free-living, amitochondriate Pelobiontida (represented by the genus *Mastigamoeba*) remains below the enlarged crown of the eukaryotic rDNA tree (Stiller et al. 1998; Stiller and Hall 1999). *Giardia* and other diplomonads also retain their status as early

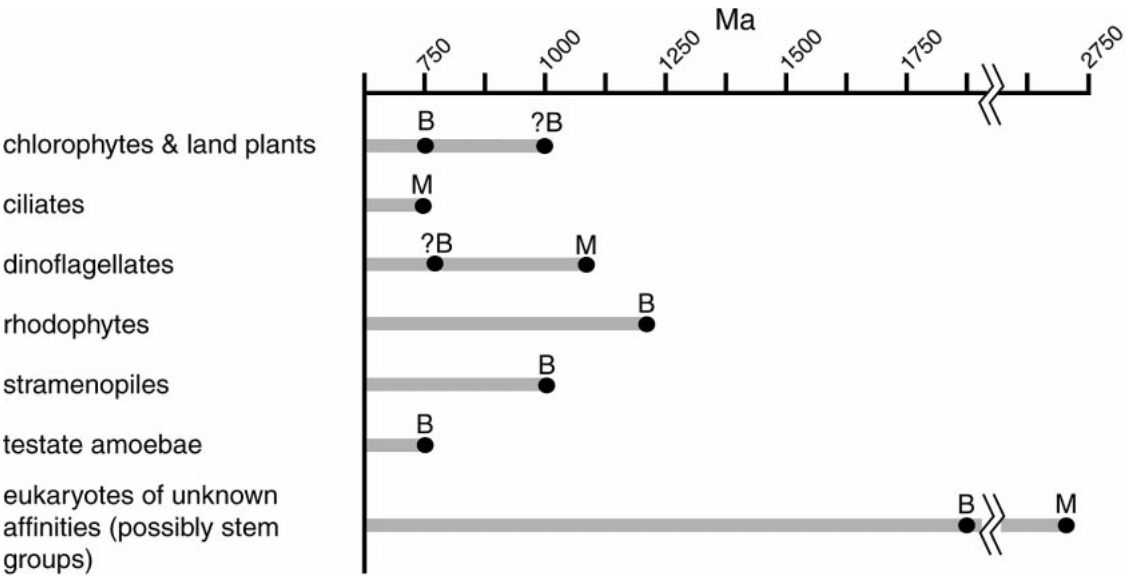

FIGURE 15. Summary of earliest eukaryotic fossil record (see text). B = body fossils (preserved morphology), M = molecular fossils (biomarkers). Sources of data noted in text.

branching in a number of other gene trees (Klenk et al. 1995; Baldauf and Doolittle 1997; Hashimoto et al. 1997; Stiller and Hall 1997; Hilario and Gogarten 1998). Therefore, a revised view of the eukaryotic tree still supports a rapid radiation, but with an evolving sense of the nature and character of subtending branches.

The Position of Testate Amoebae in the Eukaryotic Tree.—Testate amoebae are divided into two groups on the basis of pseudopod shape: the filose testate amoebae (Testaceafilosea) and the lobose testate amoebae (Testacealobosa). The relationship between these two groups is uncertain. Conventionally, they have been regarded as two distinct lineages that independently acquired tests (e.g., Schuster 1990), but the common possession of ramicristate mitochondria (mitochondria with tubular cristae that branch) suggests a connection between the two (Patterson 1994, 1999).

The relationships between the filose and lobose testate amoebae and other eukaryotes are also unresolved. On the basis of their ramicristate mitochondria, Patterson (1999) united all testate amoeba into a phylum-level clade that also includes other amoebans, acanthamoebans, plasmodial slime molds, leptomyxids, and *Gromia*; sequence comparisons of actin genes provide some support for this clade (Drouin et al. 1995; Bhattacharya and Weber 1997). Trees based on SSU rRNA indicate that the filose testate amoebae (represented by *Euglypha rotunda* and *Paulinella chromatophora*) are derived from sarcomonads (Cavalier-Smith and Chao 1996/7) and that this group is closely related to the Chlorarachniophyta (Philippe and Adoutte 1995; Cavalier-Smith and Chao 1996/7; Medlin et al. 1997). These analyses do not agree, however, on the relationship between this larger clade and the other eukaryotic lineages. Philippe and Adoutte (1995) place it in a sister relationship with the haptophytes, basal to a clade containing the Chlorobionta (green algae, plants), the Fungi, and the Metazoa. Analyses by Cavalier-Smith and Chao (1996/7) alternatively indicate that the clade is nested high within the tree, as a sister to the heterokonts, or that the clade is sister to a group that includes the Fungi, the Metazoa, the Chlorobionta, the Amoebozoa, and the Chromista. Medlin et al. (1997) concluded that the clade constitutes a sister group of the heterokonts and alveolates, and that together those clades are sister to the Chlorobionta.

Thus, the details of phylogenetic relationships are yet to be confirmed, but all phylogenies place the testate amoebae well within the ''crown'' of the eukaryotic tree.

Constraining the Timing of Major Events in Eu-

karyote Evolution.—(See Fig. 15.) The earliest morphological evidence for eukaryotes includes the spirally coiled megascopic alga *Grypania* from the ~1850-Ma (Hoffman 1987, personal communication 1999) Negaunee Iron-Formation, Michigan (Han and Runnegar 1992), and large (40–200 μm) spheromorphic acritarchs from the 1900–1800-Ma Chuanlinggou Formation, China (Zhang 1986). Steranes, biomarker compounds characteristic of eukaryotes, are found in >2700-Ma shales from the Fortescue Group, northwestern Australia (Brocks et al. 1999). Neither the fossils nor the biomarkers can be assigned to modern taxa, however, and some or all may represent extinct stem-group eukaryotes. They nevertheless suggest that the eukaryote clade had originated by 2700 Ma, and that some diversification had occurred by Paleoproterozoic times.

By the late Mesoproterozoic/early Neoproterozoic eras the divergence of modern eukaryotic clades had begun. This is documented by a growing inventory of fossils interpreted as crown eukaryotes, including bangiophyte red algae from the 1204 ± 22-Ma (Kah et al. 1999; L. Kah personal communication 1999) Hunting Formation in arctic Canada (Butterfield et al. 1990; Butterfield this issue) and the stramenopile *Paleovaucheria* from the >1000-Ma Lakhanda Group, eastern Siberia (German 1990; Woods et al. 1998). Dinosterane, a biomarker whose parent sterol is synthesized by dinoflagellates, has been reported from the ~1100-Ma Nonesuch Formation (Pratt et al. 1991), the ~800-Ma Bitter Springs Formation, and the ~570-Ma Pertatataka Formation (Summons and Walter 1990; Summons et al. 1992; Moldowan et al. 1996); and microfossils with polygonal excystment structures in the 780–750-Ma Wyniatt Formation, arctic Canada, are plausibly if not unambiguously interpreted as dinoflagellates (Butterfield and Rainbird 1998). Gammacerane in shales of the Chuar Group independently supports the presence of alveolates in Neoproterozoic ecosystems, in this case ciliates (Summons et al. 1988). *Proterocladus,* a *Cladophora*-like alga from the ca. 750–700-Ma Svanbergfjellet Formation of Spitsbergen suggests that green algal diversification was well advanced by the mid-Neoproterozoic (Butterfield et al. 1994); leiosphaerid acritarchs that go back to the beginning of the era may also be the phycomata of green phytoflagellates (Tappan 1980).

More generally, support for an early Neoproterozoic/late Mesoproterozoic eukaryote radiation comes from the dramatic increase in the diversity of acritarchs (Vidal and Knoll 1983; Knoll 1994; Xiao et al. 1997) and carbonaceous compression fossils (Hofmann 1994) at this time. Cellularly preserved remains like the 1200-Ma bangiophytes are rare, but acritarchs and carbonaceous compressions are suitably common to cast doubt on the interpretation of this record as a preservational artifact. Exceptionally well-preserved fossil assemblages have been found in Mesoproterozoic rocks, but none contains the diversity of eukaryotic morphological or biomarker fossils known from Neoproterozoic rocks. The diversification of multiple crown taxa at this time therefore reflects a real event, driven by intrinsic innovations (sexual reproduction is commonly invoked) and/or extrinsic causes (e.g., an increase in atmospheric oxygen or oceanic nitrate levels).

Heterotrophy in Early Eukaryotic Evolution.—From the summary in the previous paragraphs, it is evident that the Proterozoic fossil record of eukaryotes is dominated by photoautotrophic groups. Nonetheless, in the overall scheme of eukaryotic diversity, photosynthetic clades are far outnumbered by respiring and, to a lesser extent, fermenting taxa. Patterson (1999) recognized 71 phylum-level clades of eukaryotic organisms. Of these, 62 contain only heterotrophs, 6 contain mostly or exclusively photosynthetic organisms, and 3 include both autotrophic and heterotrophic subclades. Moreover, all algal protists acquired their chloroplasts via endosymbiosis (Gibbs 1992), so even the eukaryotic capacity for photosynthesis depends on the preexisting ability to swallow particles. Indeed, it is the physiological ability to ingest particles—born of a flexible membrane and cytoskeleton—that gave eukaryotes an ecological foothold in bacterial/archeal ecosystems; cell-ingesting predators added new dimensions to microbial ecosystems (Knoll and Bambach in press).

If eukaryotic heterotrophs existed more than 1200 million years ago (as inferred from the presence of red algae in rocks of this age), why are their earliest fossil representatives not found until ca. 800 Ma? Older heterotrophs may not have had preservable structures that were sufficiently diagnostic for us to recognize them—many heterotrophic clades have no fossil record, and, conceivably, some Mesoproterozoic or earliest Neoproterozoic acritarchs could be the remains of heterotrophic eukaryotes.

Alternatively, older heterotrophic protists may not have been diverse. A strong case can be made that global primary productivity was limited in Mesoproterozoic oceans and increased thereafter (Brasier and Lindsay 1998; Anbar and Knoll 1999). The first appearance of recognizable heterotrophs during the early to mid-Neoproterozoic Era coincides with an increase in acritarch diversity (Knoll 1994) as well as a major shift toward increased C-isotopic variability in the marine carbon cycle (Kaufman and Knoll 1995; documented locally in the Grand Canyon by Dehler et al. 1999). Thus, while VSMs are not a proxy for the origin of heterotrophy in eukaryotes, they may reflect a diversification of eukaryotic predators facilitated by increasing primary productivity—an event that may also have included other groups such as the microscopic ancestors of fungi and animals.

Conclusions

New morphological observations and taphonomic inferences derived from exceptionally preserved VSM populations in the upper Chuar Group, Grand Canyon, support the hypothesis that testate amoebae were widespread and relatively diverse constituents of mid- to late Neoproterozoic marine ecosystems. The fossils appear to represent a multispecies assemblage of filose and lobose testate amoebae; VSMs with a distinct honeycomb-patterned wall are nearly identical to scale-bearing testate amoebae, such as *Euglypha.* The presence of testate amoebae in marine rocks of this age more than doubles their stratigraphic range, which until now extended from the Recent to the Triassic (Cushman 1930; Bradley 1931; Frenguelli 1933; as discussed in Medioli et al. 1990a; Medioli et al. 1990b; Poinar et al. 1993; Waggoner 1996; Boeuf and Gilbert 1997), with questionable representatives found in Carboniferous rocks (Vasicek and Ruzicka 1957; as discussed in Medioli et al. 1990a; Wolf 1995).

The presence of testate amoebae in Neoproterozoic rocks supports the inference, drawn from molecular phylogenies in combination with the fossil record, that the major clades of eukaryotic organisms diverged from one another and began to diversify during late Mesoproterozoic/early Neoproterozoic time. It also confirms the existence of additional eukaryote clades in Neoproterozoic oceans. Most significantly, it provides the earliest morphological evidence for heterotrophic eukaryotes in marine ecosystems, thereby indicating that complex (multi-tiered) ecosystems were in place by Neoproterozoic time.

Acknowledgments

We thank the Grand Canyon research team, and in particular K. Karlstrom, C. Dehler, M. Timmons (all from the University of New Mexico), and A. Weil (University of Michigan) for many useful discussions about Chuar Group geology. C. Dehler is also thanked for sharing unpublished data and for many helpful comments on the manuscript. S. Bamforth (Tulane University), W. Foissner (University of Salzburg), V. Golemansky (Bulgarian Academy of Sciences), J. Lipps (University of California at Berkeley), R. Meisterfeld (Institute for Biology II, Aachen), F. Medioli and D. B. Scott (Dalhousie University), D. Patterson (University of Sydney), L. Rothschild (NASA/Ames Research Center), P. Siver (Connecticut College), M. Sogin and colleagues (Marine Biological Laboratories), and Anna Wasik (Nencki Institute of Experimental Biology, Warsaw) all provided useful advice and/or information on protistan biology. We also thank S. Awramik (University of California at Santa Barbara), T. Fairchild (University of São Paulo), and B. Runnegar (University of California at Los Angeles) for providing fossil material or photographs for comparative study, L. N. Kraskov (VSEGEI, Russia) for providing photographs for publication, D. Lange (Harvard University) for technical help, and S. Xiao

(Harvard University) for beneficial discussion. N. Butterfield (University of Cambridge) and R. Meisterfeld provided many valuable suggestions for improvement of the manuscript. Finally, we thank the National Park Service for permission to conduct research and collect samples within the Grand Canyon National Park. This work was supported by the National Science Foundation through grant EAR-9706496 to Knoll and a graduate student fellowship to Porter, and by the Conoco Corporation.

Literature Cited

Allison, C. W., and S. M. Awramik. 1989. Organic-walled microfossils from the earliest Cambrian or latest Proterozoic Tindir Group rocks, Northwest Canada. Precambrian Research 43:253–294.

Anbar, A. N., and A. H. Knoll. 1999. Trace metal limitation of primary production 1.85- 1.25 Ga. American Geophysical Union, 1999 fall meeting, Abstracts 80:F49.

Baldauf, S. L., and W. F. Doolittle. 1997. Origin and evolution of the slime molds (Mycetozoa). Proceedings of the National Academy of Sciences USA 94:12007–12012.

Bengtson, S., ed. 1994. Early life on Earth (Nobel Symposium No. 84). Columbia University Press, New York.

Berner, R. A., and R. Raiswell. 1984. C/S method for distinguishing freshwater from marine sedimentary rocks. Geology 12:365–368.

Bhattacharya, D., and K. Weber. 1997. The actin gene of the glaucocystophyte *Cyanophora paradoxa*: analysis of the coding region and introns, and an actin phylogeny of eukaryotes. Current Genetics 31:439–446.

Binda, P. L., and M. M. Bokhari. 1980. Chitinozoanlike microfossils in a late Precambrian dolostone from Saudi Arabia. Geology 8:70–71.

Bloeser, B. 1985. *Melanocyrillium,* a new genus of structurally complex Late Proterozoic microfossils from the Kwagunt Formation (Chuar Group), Grand Canyon, Arizona. Journal of Paleontology 59:741–765.

Bloeser, B., J. W. Schopf, R. J. Horodyski, and W. J. Breed. 1977. Chitinozoans from the Late Precambrian Chuar Group of the Grand Canyon, Arizona. Science 195:676–679.

Boeuf, O., and D. Gilbert. 1997. Presence de thécamoebiens du genre *Trinema,* au Pliocene supérieur, découverte a Chilhac (Haute-Loire, France). Comptes Rendus de l'Académie des Sciences, Série II, Sciences de la Terre et des Planets 325:623–627.

Bovee, E. C. 1985a. Class Lobosea Carpenter 1861. Pp. 158–211 *in* Lee et al. 1985.

———. 1985b. Class Filosea Leidy, 1879. Pp. 228–245 *in* Lee et al. 1985.

Bradley, W. H. 1931. Origin and microfossils of the oil shale of the Green River Formation of Colorado and Utah. U.S. Geological Survey Professional Paper 168.

Brasier, M. D., and J. F. Lindsay. 1998. A billion years of environmental stability and the emergence of eukaryotes; new data from northern Australia. Geology 26:555–558.

Brasier, M. D., G. McCarron, R. Tucker, J. Leather, P. Allen, and G. Shields. 2000. New U-Pb zircon dates for the Neoproterozoic Ghubrah glaciation and for the top of the Huqf Supergroup, Oman. Geology 28:175–178.

Brocks, J. J., G. A. Logan, R. Buick, and R. E. Summons. 1999. Archean molecular fossils and the early rise of eukaryotes. Science 285:1033–1036.

Budin, K., and H. Philippe. 1998. New insights into the phylogeny of eukaryotes based on ciliate Hsp70 sequences. Molecular Biology and Evolution 15:943–956.

Bui, E. T. N., P. J. Bradley, and P. J. Johnson. 1996. A common evolutionary origin for mitochondria and hydrogenosomes. Proceedings of the National Academy of Sciences USA 93: 9651–9656.

Butterfield, N. J. 2000. *Bangiomorpha pubescens* n. gen., n. sp.: implications for the evolution of sex, multicellularity and the Mesoproterozoic/Neoproterozoic radiation of eukaryotes. Paleobiology 26:386–404.

Butterfield, N. J., and R. H. Rainbird. 1998. Diverse organic-walled fossils, including 'possible dinoflagellates' from the early Neoproterozoic of arctic Canada. Geology 26:963–966.

Butterfield, N. J., A. H. Knoll, and K. Swett. 1990. A bangiophyte red alga from the Proterozoic of Arctic Canada. Science 250: 104–107.

———. 1994. Paleobiology of the Neoproterozoic Svanbergfjellet Formation, Spitsbergen. Fossils and Strata 34:1–84.

Calver, C. R. 1998. Isotope stratigraphy of the Neoproterozoic Togari Group, Tasmania. Australian Journal of Earth Sciences 45:865–874.

Canfield, D. E., and R. Raiswell. 1991. Pyrite formation and fossil preservation. *In* Taphonomy: releasing the data locked in the fossil record. P. A. Allison and D. E. G. Briggs, eds. Topics in Geobiology 9:337–387. Plenum, New York.

Cao, F., C. Duan, and L. Zhang. 1995. Discovery of Meishucunian vase-shaped microfossils in Ningqiang, Shaanxi and its significance. Geological Review 41:355–362, 2 plates.

Cavalier-Smith, T. 1993. Kingdom Protozoa and its 18 Phyla. Microbiological Reviews December: 953–994.

Cavalier-Smith, T., and E. E. Chao. 1996/7. Sarcomonad ribosomal RNA sequences, rhizopod phylogeny, and the origin of euglyphid amoebae. Archiv für Protistenkunde 147:227–236.

Clark, C. G., and A. J. Roger. 1995. Direct evidence for secondary loss of mitochondria in *Entamoeba histolytica.* Proceedings of the National Academy of Sciences USA 92:6518–6521.

Cook, D. A. 1991. Sedimentology and shale petrology of the Upper Proterozoic Walcott Member, Kwagunt Formation, Chuar Group, Grand Canyon, Arizona. M.S. thesis. Northern Arizona University, Flagstaff.

Cushman, J. A. 1930. The Foraminifera of the Choctawhatchee Formation of Florida. Florida State Geological Survey Bulletin 4:1–63.

Dehler, C. M., and M. E. Elrick. 1998. Implications for paleoenvironments and areal extent of the Chuar basin from facies analyses of the middle Chuar Group (Neoproterozoic) Grand Canyon. Geological Society of America Abstracts with Programs (Rocky Mountain Section) 30(6):7.

Dehler, C. M., D. J. DesMarais, S. Bowring, Z. Sharp, K. E. Karlstrom, and M. E. Elrick. 1999. Chuar Group (1.1–0.74 Ga), Grand Canyon: carbon-isotope systematics at the onset of Sturtian glaciation. Geological Society of America Abstracts with Programs 31:A487.

Drouin, G., M. Moniz de Sá, and M. Zuker. 1995. The *Giardia lamblia* actin gene and the phylogeny of eukaryotes. Journal of Molecular Evolution 41:841–849.

Duan, C. 1985. The earliest Cambrian vase-shaped microfossils of Fangxian County, Hubei Province. Bulletin of the Tianjin Institute of Geology and Mineral Resources 13:87–107.

Duan, C., and F. Cao. 1989. A new discovery of Precambrian vase-shaped microfossils in the eastern Yangtze Gorges of Hubei Province. Bulletin of the Tianjin Institute of Geology and Mineral Resources 21:139–145, 2 plates.

Duan, C., F. Cao, and L. Zhang. 1993. Vase-shaped microfossils

from top of the Tongying Formation in Xixiang, Shaanxi. Acta Micropaleontologica 10:397–408.

Ewetz, C. E. 1933. Einige neue Fossilfunde in der Visingsöformation. Geologiska Föreningens i Stockholm Förhandlingar 55:506–518.

Fairchild, T. R., A. P. Barbour, and N. L. E. Haralyi. 1978. Microfossils in the 'Eopaleozoic' Jacadigo Group at Urucum, Mato Grosso, southwest Brazil. Boletim IG 9:74–79. Instituto de Geociências, Universidade de São Paulo, São Paulo.

Fauré-Fremiet, E. 1936. The Folliculinidae (Infusoria Heterotricha) of the Breton Coast. Biological Bulletin (Woods Hole) 70: 353–360.

Febvre-Chevalier, C. 1985. Class Heliozoea Haeckel 1866. Pp. 302–321 *in* Lee et al. 1985.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Systematic Zoology 27:401–410.

Ford, T. D. 1990. Grand Canyon Supergroup: Nankoweap Formation, Chuar Group, and Sixtymile Formation. Pp. 49–70 *in* S. S. Beus and M. Morales, eds. Grand Canyon geology. Oxford University Press, New York.

Ford, T. D., and W. J. Breed. 1969. Preliminary geologic report of the Chuar Group, Grand Canyon, Arizona. Pp. 114–122 *in* Geology and natural history of the fifth field conference, Powell centennial river expedition, 1969. Four Corners Geological Society, Durango, Colo.

———. 1973. Late Precambrian Chuar Group, Grand Canyon, Arizona. Geological Society of America Bulletin 84:1243–1260.

Frenguelli, G. 1933. Tecamebiani e Diatomee nel Miocene del Neuquen (Patagonia Settentrionale). Bollettino della Società Geologica Italiana 52:33–43.

Gajadhar, A. A., W. C. Marquardt, R. Hall, J. Gunderson, E. V. Ariztia-Carmona, and M. L. Sogin. 1991. Ribosomal RNA sequences of *Sarcocystis muris, Theileria annulata* and *Cryptheconidium cohnii* reveal evolutionary relationships among apicomplexans, dinoflagellates, and ciliates. Molecular Biochemistry and Parasitology 45:147–154.

German, T. N. 1990. Organic world one billion year ago. Nauka, Leningrad.

Germot, A., H. Philippe, and H. Le Guyader. 1996. Presence of a mitochondrial-type 70-kDa heat shock protein in *Trichomonas vaginalis* suggests a very early mitochondrial endosymbiosis in eukaryotes. Proceedings of the National Academy of Sciences USA 93:14614–14617.

———. 1997. Evidence for loss of mitochondria in Microsporidia from a mitochondrial-type Hsp70 in *Nosema locustae*. Molecular Biochemistry and Parasitology 87:159–168.

Gibbs, S. 1992. The evolution of algal chloroplasts. Pp. 107–121 *in* R.A. Lewin, ed. Origins of plastids. Chapman and Hall, New York.

Golemansky, V. 1974. Psammonobiotidae fam. nov.—une nouvelle famille de thécamoebiens (Rhizopoda, Testacea) du psammal supralittoral des mers. Acta Protozoologica 13:137–141.

Graham, L. E., and L. W. Wilcox. 2000. Algae. Prentice-Hall, Upper Saddle River, N.J.

Green, J. W., A. H. Knoll, and K. Swett. 1988. Microfossils from oolites and pisolites of the upper Proterozoic Eleonore Bay Group, Central East Greenland. Journal of Paleontology 62: 835–852.

Hamilton, J. M. 1952. Studies on loricate Ciliophora. I. *Cothurnia variabilis* Kellicott. Transactions of the American Microscopical Society 71:561–568.

Han, T.-M., and B. Runnegar. 1992. Megascopic eukaryotic algae from the 2.1-billion-year-old Negaunee Iron-Formation, Michigan. Science 257:232–235.

Hashimoto, T., Y. Nakamura, T. Kamaishi, and M. Hasegawa. 1997. Early evolution of eukaryotes inferred from protein phylogenies of translation elongation factors 1α and 2. Archiv für Protistenkunde 148:287–295.

Hausmann, K., and N. Hülsmann. 1996. Protozoology, 2d ed. Thieme, Stuttgart.

Hibberd, D. J., and G. F. Leedale. 1985. Order 4. Chrysomonadida. Pp. 54–70 *in* Lee et al. 1985.

Hilario, E., and J. P. Gogarten. 1998. The prokaryote-to-eukaryote transition reflected in the evolution of the V/F/A-ATPase Catalytic and proteolipid subunits. Journal of Molecular Evolution 46:703–715.

Hirt, R. P., B. Healy, C. R. Vossbrinck, E. U. Canning, and T. M. Embley. 1997. A mitochondrial Hsp70 orthologue in *Vairomorpha necatrix*: molecular evidence that microsporidia once contained mitochondria. Current Biology 7:995–998.

Hoffman, P. H. 1987. Early Proterozoic foredeeps, foredeep magmatism, and Superior-type iron-formations of the Canadian Shield. Pp. 85–98 *in* A. Kröner, ed. Proterozoic lithospheric evolution. American Geophysical Union, Washington, D.C.

Hofmann, K. J. 1994. Proterozoic carbonaceous compressions (''metaphytes'' and ''worms''). *In* Bengtson 1994.

Horodyski, R. J. 1987. A new occurrence of the vase-shaped fossil *Melanocyrillium* and new data on this relatively complex Late Precambrian fossil. Geological Society of America Abstracts with Programs 19:707.

———. 1993. Paleontology of Proterozoic shales and mudstones: examples from the Belt Supergroup, Chuar Group and Pahrump Group, western USA. *In* B. Nagy, J. S. Leventhal, and R. F. Grant, eds. Metalliferous black shales and related ore deposits. Precambrian Research 61:241–278.

Horodyski, R. J., and B. Bloeser. 1983. Possible eukaryotic algal filaments from the Late Proterozoic Chuar Group, Grand Canyon, Arizona. Journal of Paleontology 57:321–326.

Kah, L. C., A. G. Sherman, G. M. Narbonne, A. H. Knoll, and A. J. Kaufman. 1999. $\delta^{13}C$ stratigraphy of the Proterozoic Bylot Supergroup, Baffin Island, Canada: implications for regional lithostratigraphic correlations. Canadian Journal of Earth Sciences, 36:313–332.

Karlstrom, K. E., S. A. Bowring, C. M. Dehler, A. H. Knoll, S. M. Porter, D. J. Des Marais, A. B. Weil, Z. D. Sharp, J. W. Geissman, M. B. Elrick, J. M. Timmons, L. J. Crossey, and K. L. Davidek. 2000. Chuar Group of the Grand Canyon: record of breakup of Rodinia, associated change in the global carbon cycle, and ecosystem expansion by 740 Ma. Geology 28:619–622.

Kaufman, A. J., and A. H. Knoll. 1995. Neoproterozoic variations in the C-isotopic composition of seawater: stratigraphic and biogeochemical implications. Precambrian Research 73: 27–49.

Kaufman, A. J., A. H. Knoll, and G. M. Narbonne. 1997. Isotopes, ice ages, and terminal Proterozoic earth history. Proceedings of the National Academy of Sciences USA 94:6600–6605.

Kennedy, M. J., B. Runnegar, A. R. Prave, K.-H. Hoffmann, and M. A. Arthur. 1998. Two or four Neoproterozoic glaciations? Geology 26:1059–1063.

Klenk, H.-P., W. Zillig, M. Lanzendörfer, B. Grampp, and P. Palm. 1995. Location of protist lineages in a phylogenetic tree inferred from sequences of DNA-dependent RNA polymerases. Archiv für Protistenkunde 145:221–230.

Knoll, A. H. 1994. Proterozoic and Early Cambrian protists: evidence for accelerating evolutionary tempo. Proceedings of the National Academy of Sciences USA 91:6743–6750.

———. 2000. Learning to tell Neoproterozoic time. Precambrian Research 100:3–20.

Knoll, A. H., and R. K. Bambach. In press. Directionality in the history of life: diffusion from a left wall or repeated scaling of the right? *In* D. H. Erwin and S. L. Wing, eds. Deep time:

Paleobiology's perspective. Paliobiology 26(Suppl. to No. 4) (in press).

Knoll, A. H., and S. Calder. 1983. Microbiotas of the late Precambrian Ryssö Formation, Nordaustlandet, Svalbard. Palaeontology 26:467–496.

Knoll, A. H., and G. Vidal. 1980. Late Proterozoic vase-shaped microfossils from the Visingsö Beds, Sweden. Geologiska Föreningens i Stockholm Förhandlingar 102:207–211.

Knoll, A. H., K. Swett, and E. Burkhardt. 1989. Paleoenvironmental distribution of microfossils and stromatolites in the Upper Proterozoic Backlundtoppen Formation, Spitsbergen. Journal of Paleontology 63:129–145.

Knoll, A. H., K. Swett, and J. Mark. 1991. Paleobiology of a Neoproterozoic tidal flat/lagoonal complex: the Draken Conglomerate Formation, Spitsbergen. Journal of Paleontology 65: 531–570.

Kraskov, L. N. 1985. Nakhodka problematichnikh organizmov v otlozheniykh chatkaragaikoii sviti (Talasskii khrebet). Pp. 149–52 *in* A. M. Obut and N. J. Tchernysheva, eds. Problematitika pozdnego dokembriya i paleozoya.

Krumbein, W. E., D. M. Paterson, and L. J. Stal, eds. 1994. Biostabilization of sediments. Oldenburg University Press, Oldenburg, Germany.

Lee, J. J., S. H. Hutner, and E. C. Bovee, eds. 1985. An illustrated guide to the Protozoa. Society of Protozoologists, Lawrence, Kans.

Leedale, G. F. 1985. Order 3. Euglenida Bütschli, 1884. Pp. 41–54 *in* Lee et al. 1985.

Link, P. K., N. Christie-Blick, W. J. Devlin, D. P. Elston, R. J. Horodyski, M. Levy, J. M. G. Miller, R. C. Pearson, A. Prave, J. H. Stewart, D. Winston, L. A. Wright, C. T. Wrucke. 1993. Middle and Late Proterozoic stratified rocks of the western U.S. Cordillera, Colorado Plateau, and Basin and Range province. Pp. 463–595 *in* J. C. Reed Jr., M. E. Bickford, R. S. Houston, P. K. Link, D. W. Rankin, P. K. Sims, and W. R. Van Schmus, eds. The geology of North America. Geological Society of America, Boulder, Colo.

Loeblich, J., A. R., and H. Tappan. 1988. Foraminiferal genera and their classification. Van Nostrand Reinhold, New York.

Maithy, P. K., and R. Babu. 1988. Chitinozoa-like remains from Vindhyan Supergroup of Son Valley. Palaeobotanist 37:77–80.

Maslov, A. V., Z. M. Abduazimova, L. A. Karsten, and V. N. Puchkov. 1994. Pervye nakhodki *Mellanoserillium* v etalonnykh rasresakh rifeya na Yuzhnon Urale. [First occurrence of *Melanocyrillium* in the Riphean type sections of the Southern Urals.] Pp. 90–91 *in* Sostoyanie, problemy i zadachi geologicheskogo kartirovaniya oblastei rasvitiya dokembriya na territorii Rossi. [State, problems, and goals of the geological mapping of the Precambrian areas in Russia]. Vserossiiskii Geologicheskii Institut, St. Petersburg. [In Russian.]

Medioli, F. S., D. B. Scott, E. S. Collins, and F. M. G. McCarthy. 1990a. Fossil thecamoebians present status and prospects for the future. Pp. 813–839 *in* C. Hemleben et al., eds. Paleoecology, biostratigraphy, paleoceanography and taxonomy of agglutinated foraminifera. Kluwer Academic, Dordrecht, Netherlands.

Medioli, F. S., D. B. Scott, E. S. Collins, and J. H. Wall. 1990b. Thecamoebians from the Early Cretaceous deposits of Ruby Creek, Alberta (Canada). Pp. 793–812 *in* C. Hemleben, M. A. Kaminski, W. kuhnt, and D. B. Scott, eds. Paleoecology, biostratigraphy, paleoceanography and taxonomy of agglutinated foraminifera. Kluwer Academic, Dordrecht, Netherlands.

Medlin, L. K., W. H. C. F. Kooistra, D. Potter, G. W. Saunders, and R. A. Anderson. 1997. Phylogenetic relationships of the 'golden algae' (haptophytes, heterokont chromophytes) and their plastids. Plant Systematics and Evolution 11(Suppl.): 187–219.

Moldowan, J. M., J. Dahl, S. R. Jacobson, B. R. Huizinga, F. J. Fago, R. Shetty, D. S. Watt, K. E. Peters. 1996. Chemostratigraphic reconstruction of biofacies: molecular evidence linking cyst-forming dinoflagellates with pre-Triassic ancestors. Geology 24:159–162.

Nautiyal, A. C. 1978. Discovery of cyanophycean remains and chitinozoans from the late Precambrian argillaceous sequence of Satpuli, Garhwal Himalaya, India. Current Science 47:222–226.

Nystuen, J. P., and A. Siedlecka. 1988. The 'Sparagmites' of Norway. Pp. 237–252 in J. A. Winchester, ed. Late Proterozoic stratigraphy of the Atlantic regions. Blackie, Glasgow.

Ogden, C. G. 1991. Ultrastructure of the vegetative organisation and initial stages of silica plate deposition in the soil testate amoeba *Corythion dubium*. Protoplasma 163:136–144.

Ogden, C. G., and R. H. Hedley. 1980. An atlas of freshwater testate amoebae. British Museum (Natural History), London.

Palacas, J. G., and M. R. Reynolds. 1989. Preliminary petroleum source rock assessment of Upper Proterozoic Chuar Group, Grand Canyon, Arizona. American Association of Petroleum Geologists Bulletin 73:397.

Patterson, D. J. 1994. Protozoa: Evolution and Systematics. Pp. 1–14 *in* K. Hausmann and N. Hülsmann, eds. Progress in protozoology. Proceedings of the IX international congress of protozoology, Berlin 1993. Gustav Fischer, New York.

———. 1999. The diversity of eukaryotes. American Naturalist 154(Suppl.):S96–S124.

Philippe, H., and A. Adoutte. 1995. How reliable is our current view of eukaryotic phylogeny? European Journal of Protistology 31:17–33.

———. 1998. The molecular phylogeny of eukaryota: solid facts and uncertainties. Pp. 25–56 *in* G. H. Coombs, K. Vickerman FRS, M. A. Sleigh, and A. Warren, eds. Evolutionary relationships among protozoa. Chapman and Hall, London.

Poinar, G. O., Jr., B. M. Waggoner, and U.-C. Bauer. 1993. Terrestrial soft-bodied protists and other microorganisms in Triassic amber. Science 259:222–224.

Pratt, L. M., R. E. Summons, and G. B. Hieshima. 1991. Sterane and triterpane biomarkers in the Precambrian Nonesuch Formation, North American Midcontinent Rift. Geochimica et Cosmochimica Acta 55:911–916.

Rainbird, R. H., C. W. Jefferson, and G. M. Young. 1996. The early Neoproterozoic sedimentary Succession B of northwestern Laurentia: correlations and paleogeographic significance. Geological Society of America Bulletin 108:454–470.

Reynolds, M. W., and D. P. Elston. 1986. Stratigraphy and sedimentation of part of the Proterozoic Chuar Group, Grand Canyon, Arizona. Geological Society of America Abstracts with Programs (Rocky Mountain Section) 18:405.

Roger, A. J., S. G. Svard, J. Tovar, C. G. Clark, M. W. Smith, F. D. Gillin, and M. L. Sogin. 1998. A mitochondrial-like chaperonin 60 gene in *Giardia lamblia*: evidence that diplomonads once harbored an endosymbiont related to the progenitor of mitochondria. Proceedings of the National Academy of Sciences USA 95:229–234.

Saito, Y., T. Tiba, and S. Matsubara. 1988. Precambrian and Cambrian cherts in Northwestern Tasmania. Bulletin of the National Science Museum, Tokyo C 14:59–70.

Schopf, J. W. 1992. Evolution of the Proterozoic biosphere: benchmarks, tempo, and mode. Pp. 585–600 *in* J. W. Schopf and C. Klein, eds. The Proterozoic biosphere: a multidisciplinary study. Cambridge University Press, Cambridge.

Schopf, J. W., T. D. Ford, and W. J. Breed. 1973. Microorganisms from the Late Precambrian of the Grand Canyon, Arizona. Science 179:1319–1321.

Schuster, F. L. 1990. Phylum Rhizopoda. Pp. 3–18 *in* L. Margulis, J. O. Corliss, M. Melkonian, D. J. Chapman, eds. Handbook of the Protoctista. Jones and Bartlett, Boston.

Semikhatov, M. A. 1991. General problems of Proterozoic stra-

tigraphy in the USSR. Soviet Scientific Reviews, Section G, Geology Reviews 1:1–192.
Sergeev, V. N., A. H. Knoll, and P. Yu. Petrov. 1997. Paleobiology of the Mesoproterozoic-Neoproterozoic transition: the Sukhaya Tunguska Formation, Turukhansk Uplift, Siberia. Precambrian Research 85:201–229.
Small, E. B., and D. H. Lynn. 1985. Phylum Ciliophora. Pp. 393–575 *in* Lee et al. 1985.
Sogin, M. L. 1991. Early evolution and the origin of eukaryotes. Current Opinion in Genetics and Development 1:457–463.
———. 1994. The origin of eukaryotes and evolution into major kingdoms. Pp. 181–192 *in* Bengtson 1994..
Sogin, M. L., H. J. Elwood, and J. H. Gunderson. 1986. Evolutionary diversity of eukaryotic small-subunit rRNA genes. Proceedings of the National Academy of Sciences USA 83: 1383–1387.
Sogin, M. L., J. H. Gunderson, H. J. Elwood, R. A. Alonso, and D. A. Peattie. 1989. Phylogenetic meaning of the kingdom concept: an unusual ribosomal RNA from *Giardia lamblia*. Science 243:75–77.
Stiller, J. W., and B. D. Hall. 1997. The origin of red algae: implications for plastid evolution. Proceedings of the National Academy of Sciences USA 94:4520–4525.
———. 1999. Long-branch attraction and the rDNA model of early eukaryotic evolution. Molecular Biology and Evolution 16:1270–1279.
Stiller, J. W., E. C. S. Duffield, and B. D. Hall. 1998. Amitochondriate amoebae and the evolution of DNA-dependent RNA polymerase II. Proceedings of the National Academy of Sciences USA 95:11769–11774.
Sudzuki, M. 1979. Marine Testacea of Japan. Sesoko Marine Science Laboratory Technical Report 6:51–61.
Summons, R. E., and M. R. Walter. 1990. Molecular fossils and microfossils of prokaryotes and protists from Proterozoic sediments. American Journal of Science 290-A:212–244.
Summons, R. E., S. C. Brassell, G. Eglinton, E. Evans, R. J. Horodyski, N. Robinson, and D. M. Ward. 1988. Distinctive hydrocarbon biomarkers from fossiliferous sediment of the Late Proterozoic Walcott Member, Chuar Group, Grand Canyon, Arizona. Geochimica et Cosmochimica Acta 52:2625–2637.
Summons, R. E., J. Thomas, J. R. Maxwell, and C. J. Boreham. 1992. Secular and environmental constraints on the occurrence of dinosterane in sediments. Geochimica et Cosmochimica Acta 56:2437–2444.
Tappan, H. 1980. Paleobiology of plant protists. W. H. Freeman, San Francisco.
———. 1993. Tintinnids. Pp. 285–303 *in* J. H. Lipps, ed. Fossil prokaryotes and protists. Blackwell Scientific, Boston.
Vasicek, M., and B. Ruzicka. 1957. Namurian Techamoebina from the Ostrava-Karvina coal district. Sbornik Naradniho Musea v Praze, Rada B, Prirodni Vedy. Acta Musei Nationalis Pragae, Series B, Historia Naturalis 13:333–340.
Venkatachala, B. S., and A. Kumar. 1998. Fossil microbiota from the Vaishnodevi Limestone, Himalayan Foothills, Jammu: age and palaeoenvironmental implications. Journal of the Geological Society of India 52:529–536.
Vidal, G. 1979. Acritarchs from the Upper Proterozoic and Lower Cambrian of East Greenland. Grønlands Geologiske Undersøgelse Bulletin 134:1–55.
———. 1994. Early Ecosystems: limitations imposed by the fossil record. Pp. 298–311 *in* Bengtson 1994.
Vidal, G., and T. D. Ford. 1985. Microbiotas from the Late Proterozoic Chuar Group (Northern Arizona) and Uinta Mountain Group (Utah) and their chronostratigraphic implications. Precambrian Research 28:349–389.
Vidal, G., and A. H. Knoll. 1983. Proterozoic plankton. Geological Society of America Memoir 161:265–277.
Vidal, G., and M. Moczydłowska. 1995. The Neoproterozoic of Baltica—stratigraphy, palaeobiology and general geological evolution. Precambrian Research 73:197–216.
Vidal, G., and A. Siedlecka. 1983. Planktonic, acid-resistant microfossils from the Upper Proterozoic strata of the Barents Sea Region of Varanger Peninsula, East Finnmark, Northern Norway. Norges Geologiske Undersøkelse 382:45–79.
Waggoner, B. M. 1996. Bacteria and protists from Middle Cretaceous amber of Ellsworth County, Kansas. PaleoBios 17:20–26.
Walcott, C. D. 1899. Precambrian fossiliferous formations. Geological Society of America Bulletin 10:199–244.
Walter, M. R., J. J. Veevers, C. R. Calver, P. Gorgan, and A. C. Hill. 2000. Dating the 850–544 Ma Neoproterozoic interval by isotopes of strontium, carbon and sulfur in seawater, some interpretative models. Precambrian Research 100:371–433.
Wolf, M. 1995. Verkieste Amöben in Steinkohlen aus dem Ruhrgebiet—erster Nachweis von Arcella Ehrenberg im Paläozoikum. Paläontologische Zeitschrift 69:1–6.
Woods, K. N., A. H. Knoll, and T. N. German. 1998. Xanthophyte algae from the Mesoproterozoic/Neoproterozoic transition: confirmation and evolutionary implications. Geological Society of America Abstracts with Programs 30:A232.
Xiao, S., A. H. Knoll, A. J. Kaufman, L. Yin, and Y. Zhang. 1997. Neoproterozoic fossils in Mesoproterozoic rocks? Chemostratigraphic resolution of a biostratigraphic conundrum from the North China Platform. Precambrian Research 84:197–220.
Yankaouskas, T. V., ed. 1989. Mikrofossilii dokembriia SSSR. Nauka, Leningrad.
Zhang, L. 1994. A new progress in research on vase-shaped microfossils from the Dengying Formation of Sinian in Southern Shaanxi Province. Acta Geologica Gansu 3:1–8.
Zhang, L., and Y. Li. 1991. The Late Sinian vasiform microfossils of Ningqiang, Shaanxi Province. Bulletin of the Xi'an Institute of Geological and Mineralogical Research, Chinese Academy of Geological Sciences 31:77–86.
Zhang, Z. 1986. Clastic facies microfossils from the Chuanlinggou Formation (1800 Ma) near Jixian, North China. Journal of Micropaleontology 5:9–16.