Protist, Vol. 157, 279—290, May 2006
http://www.elsevier.de/protis
Published online date 26 May 2006

Protist

ORIGINAL PAPER

Ebriid Phylogeny and the Expansion of the Cercozoa

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Submitted November 30, 2005; Accepted March 4, 2006
Monitoring Editor: Hervé Philippe

***Ebria tripartita* is a phagotrophic flagellate present in marine coastal plankton communities worldwide. This is one of two (possibly four) described extant species in the Ebridea, an enigmatic group of eukaryotes with an unclear phylogenetic position. Ebriids have never been cultured, are usually encountered in low abundance and have a peculiar combination of ultrastructural characters including a large nucleus with permanently condensed chromosomes and an internal skeleton composed of siliceous rods. Consequently, the taxonomic history of the group has been tumultuous and has included a variety of affiliations, such as silicoflagellates, dinoflagellates, 'radiolarians' and 'neomonads'. Today, the Ebridea is treated as a eukaryotic taxon *incertae sedis* because no morphological or molecular features have been recognized that definitively relate ebriids with any other eukaryotic lineage. We conducted phylogenetic analyses of small subunit rDNA sequences from two multi-specimen isolations of *Ebria tripartita*. The closest relatives to the sequences from *Ebria tripartita* are environmental sequences from a submarine caldera floor. This newly recognized *Ebria* clade was most closely related to sequences from described species of *Cryothecomonas* and *Protaspis*. These molecular phylogenetic relationships were consistent with current ultrastructural data from all three genera, leading to a robust placement of ebriids within the Cercozoa.**

Key words: Cercozoa; *Ebria tripartita*; ebridians; ebriids; phylogenetic analysis; SSU rDNA.

Introduction

Ebria tripartita (Schumann) Lemmermann, 1899 (Basionym: *Dictyocha tripartita* Schumann, 1867) is present in coastal plankton communities worldwide (e.g. Bérard-Therriault et al. 1999; Campbell 1973; Drebes 1974; Horner 2002; Ikävalko 1998; Konovalova et al. 1989; Throndsen 1997; Throndsen et al. 2003; Tong et al. 1998; Vørs 1992), but usually in low cell concentrations (Fig. 1). *Ebria* belongs to the ebriids (syn. ebridians), a small group of marine flagellates with a long fossil record, starting in the Cretaceous and being most diverse in the Miocene (Deflandre 1952; Loeblich et al. 1968; Tappan 1980). Although there have been reports of other species, only two extant species of ebriids are known for certain, *E. tripartita* and *Hermesium adriaticum* Zacharias 1906 (Hargraves 2002). The former occurs in cold to warm temperate regions and the latter in warmer waters. Ebriids are characterized by having two unequal flagella inserted subapically, a nucleus with permanently condensed chromosomes during interphase, naked cells with no external cell wall and an internal, solid, siliceous skeleton composed of

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doi:10.1016/j.protis.2006.03.002

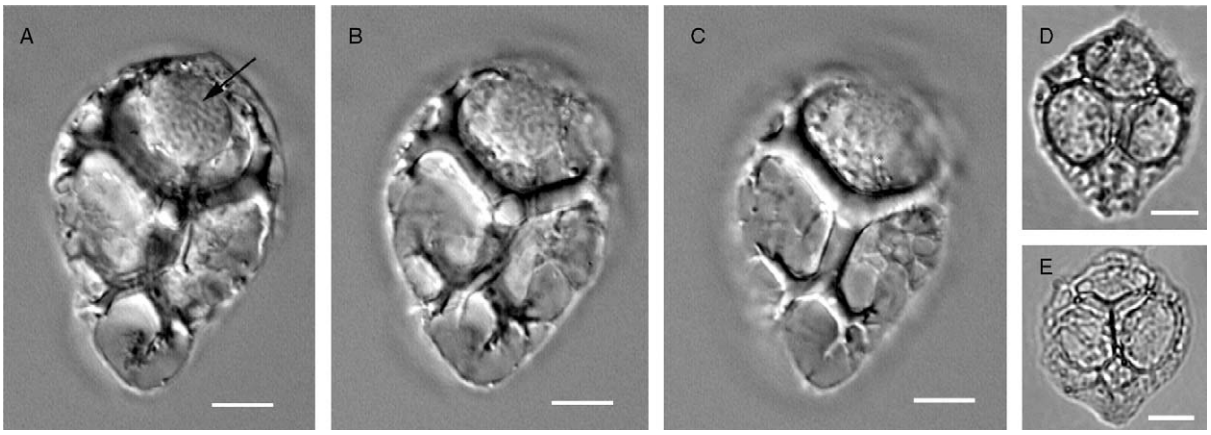

Figure 1. Light micrographs of *Ebria tripartita* isolated from plankton samples at English Bay, Vancouver and at the Bamfield Marine Sciences Centre. **A—C**. Differential interference contrast (DIC) micrographs showing the same cell (English Bay plankton) in different focal planes. Note the nucleus (arrow) with its granular appearance. **D—E**. Bright field micrographs showing two specimens from the Bamfield samples, which were used for DNA extraction. Bars = 10 μm.

branching or fenestrated rods, which is the best synapomorphy for the group (Hargraves 2002; Patterson 1999). *Ebria* cells are phagotrophic and range from 25 to 40 μm in length. Sexual reproduction is unknown. The name of the taxon comes from the Latin word *ebrius*, which means 'drunken' and refers to their distinctive swimming mode.

Ebriids are of ecological interest because they are herbivorous grazers that occasionally reach high cell concentrations (Hargraves and Miller 1974). *Ebria tripartita* feeds on phytoplankton, especially on diatoms like *Skeletonema* and *Thalassiosira*, but also on dinoflagellates (Hargraves 2002; Taylor 1990). The details of the feeding process are still unknown and the involvement of pseudopodia has not been definitively documented (Hargraves 2002; Taylor 1990). However, the capacity to produce pseudopodia has been indicated in the literature (Patterson 1999). Moreover, a species that has the ability to engulf diatom cells like *Thalassiosira* needs an ingestion mechanism and specialized cell structures. Although a discrete mouth would be one option, there is no ultrastructural data that support this possibility. Therefore, the involvement of pseudopodia in feeding is much more likely, which is consistent with the observation that *Ebria* is able to fold chains of *Skeletonema* during the engulfment process (Taylor 1990), a feeding mode that is reminiscent of pallium feeding in dinoflagellates (Gaines and Taylor 1984; Jacobson and Anderson 1986). Despite the ecological significance of the group, research on *E. tripartita* is rare and nearly restricted to taxonomic and stratigraphic accounts. A few reasons for this lack of knowledge are the inability to cultivate *Ebria* and the relatively low concentrations in which the cells are usually encountered.

The unusual combination of morphological characters found in ebriids has resulted in a long and muddled taxonomic history. Different generations of biologists have tentatively classified ebriids in over eight different groups of eukaryotes, and sometimes ebriids are placed in a sisterless group of their own. Moreover, like dinoflagellates and euglenids, ebriids have been the taxonomic victims of ambiregnal classification; several taxon names have been published in parallel, some conforming to the Botanical Code of Nomenclature and others conforming to the Zoological Code. Gemeinhardt (1930) placed ebriids in the class Silicoflagellatae as family Ebriaceae. Hovasse (1932, 1934) discussed them with the silicoflagellates or as a possible link between dinoflagellates and 'radiolarians'. Loeblich et al. (1968) listed ebriids in their "annotated index of fossil and recent silicoflagellates and ebridians …," and 1 year later, Loeblich and Loeblich (1969) classified them as a class within the Pyrrhophyta (dinoflagellates). Ebriids have also been regarded as (i) botanical class Ebriophyceae (Silva 1980) or order Ebriales in the class Dinophyceae (Sournia 1986), (ii) the zoological order Ebriida in the phylum Sarcomastigophora (Lee et al. 1985), (iii) class Ebridea in the phylum Opalozoa (Cavalier-Smith 1993) and (iv) class Ebridea in the phylum Neomonada (Cavalier-Smith 1996/97, 1998a, b). In the 'Handbook of

Protoctista', Taylor (1990) described ebriids as eukaryotic taxon *incertae sedis* (Margulis et al. 1990). However, in the 'Classification of the Marine Phytoplankton of the World from Class to Genus', the Ebriales were placed back within the Dinophyceae (Chrétiennot-Dinet et al. 1993).

This indecision prompted Patterson (1994, 1999) to list ebriids as taxon *sedis mutabilis*: 'taxon with clear identity and for which relatedness is evident but not to the resolution of sister group'. Accordingly, the family Ebriidae can also be found in the chapter "Residual free-living and predatory heterotrophic flagellates" in the 'Illustrated Guide to the Protozoa' (Patterson et al. 2002 in Lee et al. 2002). Cavalier-Smith (2000) placed the class Ebidae *incertae sedis* within Protozoa; and an ultrastructural study of ebriids led Hargraves (2002) to conclude that, at present, ebriids are most appropriately placed as *incertae sedis* within the Eukaryota. This view is followed in the most recent higher-level classification of eukaryotes (Adl et al. 2005). In retrospect, the taxonomic history of *E. tripartita* is severely convoluted because resolving the affinities of ebriids with other eukaryotes has been intractable with current morphological data. The presence of phagotrophic biflagellates with an internal siliceous skeleton and permanently condensed chromosomes is an evolutionary enigma with the tantalizing potential to provide insight into the origins of other derived planktonic eukaryotes, such as polycistine 'radiolarians', phaeodarean 'radiolarians', silicoflagellates and actiniscid dinoflagellates (Bursa 1969; Hansen 1993; Simakova and Konovalova 1995; Taylor and Cattell 1969). Thus, it seemed possible that ebriids could be members of any one of three major eukaryotic groups: rhizarians, alveolates or stramenopiles. This set of circumstances motivated us to explore the phylogeny of *E. tripartita* with molecular sequence data.

Results and Discussion

The Phylogeny of Ebriids

We generated two different SSU rDNA sequences from two different multi-specimen samples of *E. tripartita* collected on different days in June of 2005 at the same site at the docks of the Bamfield Marine Sciences Centre, Vancouver Island (BC, Canada). The two sequences differed at only three nucleotide positions. BLAST results indicated a high similarity of our two SSU rDNA sequences from *E. tripartita* with sequences from taxa in the emerging group Cercozoa. These results were confirmed with phylogenetic analyses of a global alignment of 61 taxa representing the bulk of eukaryotic diversity (1128 unambiguous sites). The statistical support for the cercozoan clade consisting of *Ebria*, *Chlorarachnion*, *Heteromita*, *Cryothecomonas* and *Protaspis*, was very high (bootstraps of 97—100 in maximum likelihood distance analyses and posterior probabilities of 1.00), which is consistent with previous results (see Fig. 2 in Keeling and Leander 2003). Moreover, the sequences from *Ebria* contained a signature deletion for the Cercozoa in the single-stranded loop at the end of helix 37 of V6 (according to Neefs et al. 1993; Cavalier-Smith and Chao 2003b), namely the position after nucleotide 1255 (5′… cagattga_agatcttt…3′) of *Ebria* sequence DQ303922. These data demonstrated that ebriids were not members of dinoflagellate alveolates, silicoflagellate stramenopiles or polycistine rhizarians, but the data did not rule out a close relationship with other planktonic cercozoans with internal siliceous skeletons, namely phaeodareans. Therefore, in order to help pinpoint the position of *E. tripartita* within the Cercozoa, we focused our attention on two multiple sequence alignments: a 63-taxon alignment consisting of ingroup cercozoans and related environmental sequences (987 unambiguous sites) and a 34-taxon cercozoan alignment excluding the shorter environmental sequences (1305 unambiguous sites). Alignments were submitted to TREEBASE (accession number SN2730). Highly divergent outgroup sequences from polycistines and foraminiferans were excluded from the alignments in order to include the maximum number of sites in our analyses. Our inferred phylogenies are shown in Figures 2 and 3.

The diversity of cercozoan flagellates is known mostly from environmental SSU rDNA sequences from uncultured organisms with uncharacterized cellular properties (Amaral Zettler et al. 2002; Bass and Cavalier-Smith 2004; Dawson and Pace 2002; López-Garcia et al. 2001; Massana et al. 2002, 2004; Moon-van der Staay et al. 2001; Stoeck and Epstein 2003; Stoeck et al. 2003) (Fig. 2). The cell structure of a few species, however, has been relatively well described with LM and EM, such as *Massisteria marina*, *Heteromita globosa*, *Cercomonas* spp, *Protaspis grandis*, *Pseudopirsonia* (as *Pirsonia*), *Cryothecomonas* spp. and *Thaumatomastix* spp. (Beech and Moestrup 1986; Drebes et al. 1996; Hoppenrath and Leander 2006; Karpov 1997; Kühn et al. 1996; Larsen and

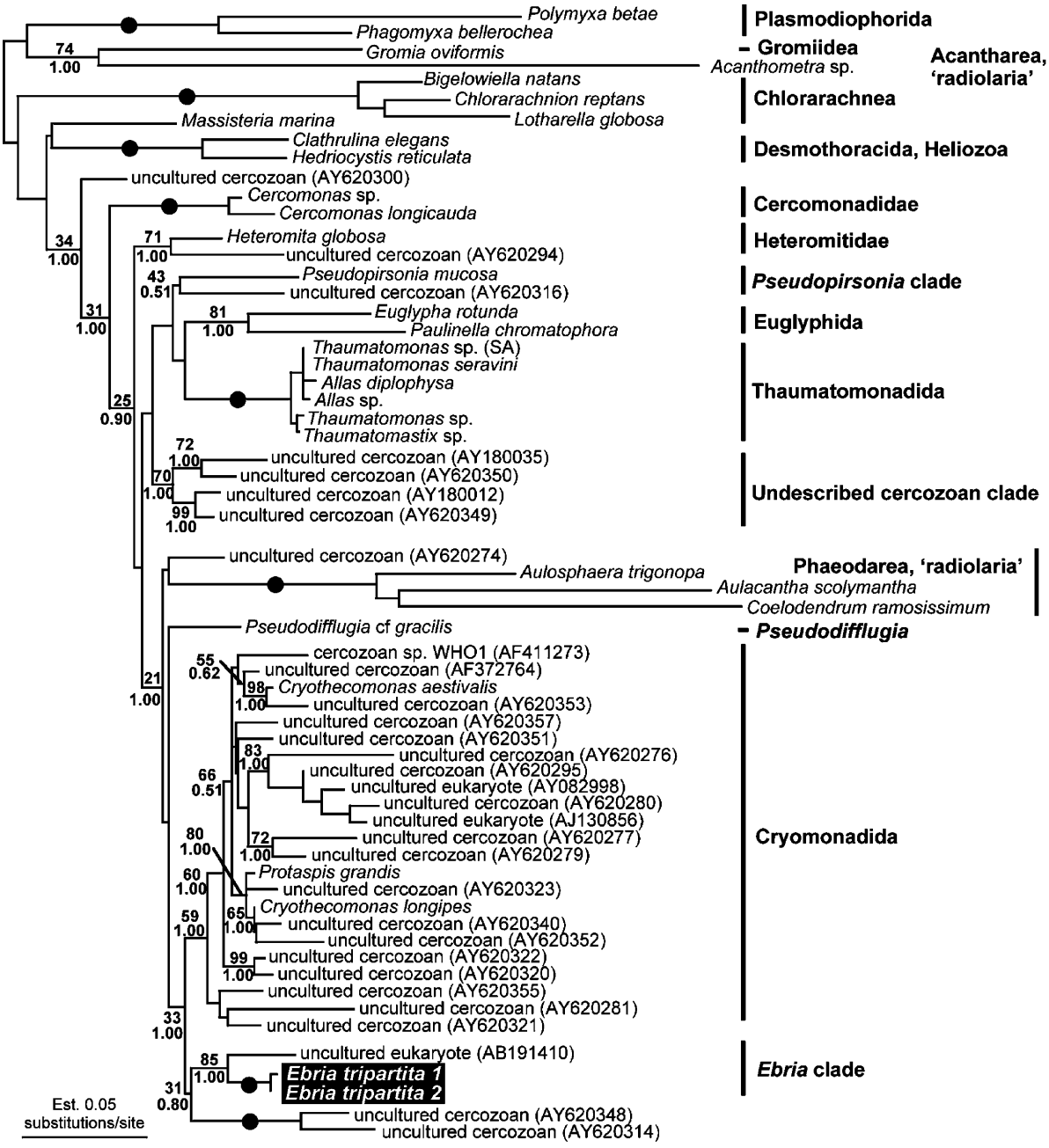

Figure 2. Gamma-corrected maximum likelihood tree ($-\ln L = 9793$, $\propto = 0.31$, 8 rate categories) inferred using the GTR model of substitution on an alignment of 63 SSU rDNA sequences and 987 unambiguous sites—the 63-taxon alignment. Numbers at the branches denote gamma-corrected bootstrap percentages of 100 replicates using weighted neighbor-joining (top) and Bayesian posterior probabilities—GTR (bottom). Black dots on branches denote bootstrap percentages and posterior probabilities greater than 95%.

Patterson 1990; MacDonald et al. 1977; Mignot and Brugerolle 1975; Mylnikov 1986; Mylnikov and Karpov 2004; Patterson and Fenchel 1990; Schnepf and Kühn 2000; Schuster and Pollak 1978; Thomsen et al. 1991). These species serve as organismal anchors that allow us to make inferences about the cellular origins of certain environmental sequences and whether the apparent diversity of cercozoan SSU rDNA sequences actually reflects an underlying diversity of cellular characteristics.

The sequences from *E. tripartita* provide a new reference taxon for understanding cercozoan

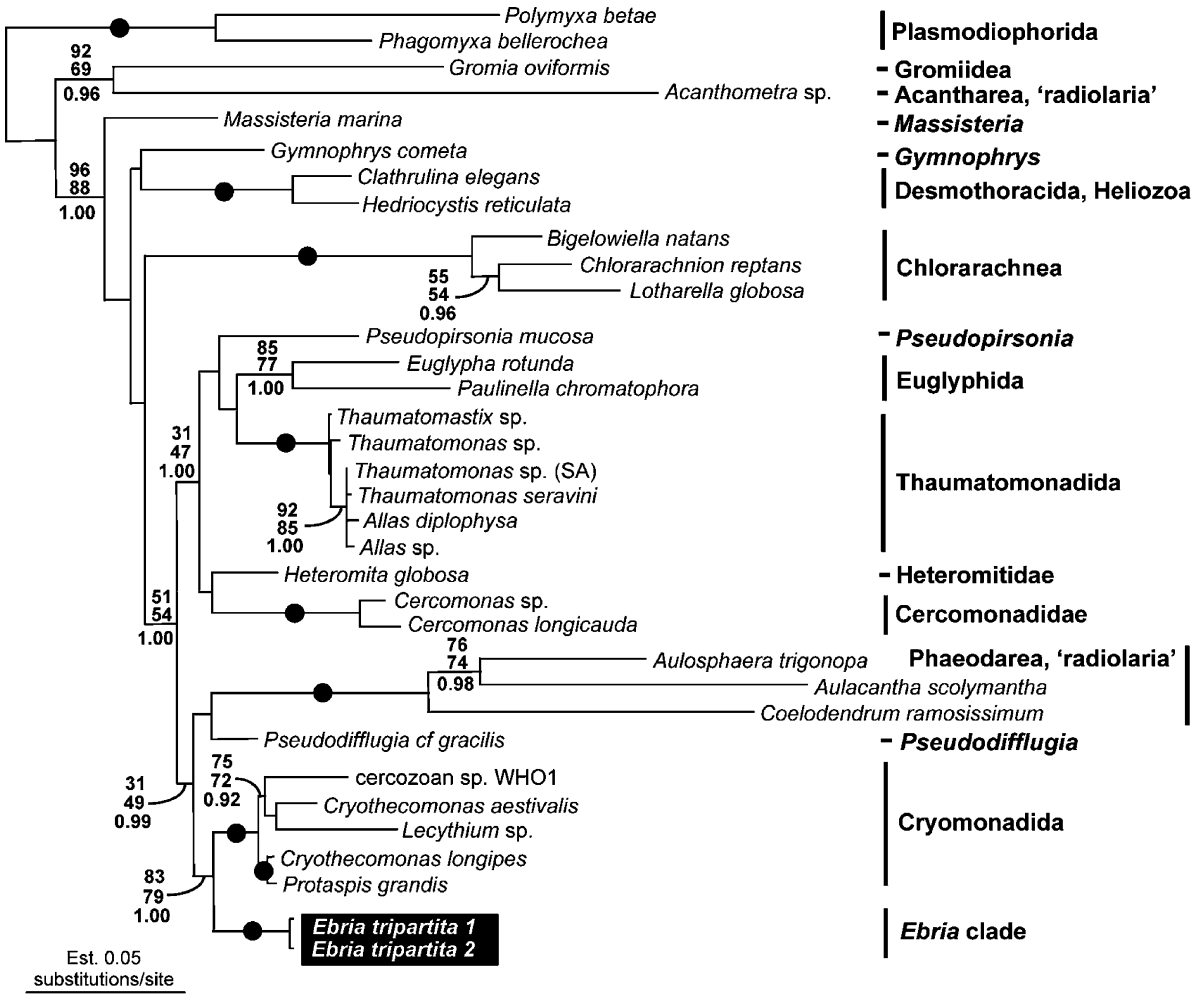

Figure 3. Gamma-corrected maximum likelihood tree ($-\ln L = 9805$, $\propto = 0.38$, 8 rate categories) inferred using the GTR model of substitution on an alignment of 34 SSU rDNA sequences and 1305 unambiguous sites—the 34-taxon alignment, excluding the shorter environmental sequences. Numbers at the branches denote bootstrap percentages using maximum likelihood—HKY (top), bootstrap percentages using weighted neighbor-joining (middle) and Bayesian posterior probabilities—GTR (bottom). Black dots on branches denote bootstrap percentages and posterior probabilities greater than 95%.

diversity. The lineage that branched closest to *E. tripartita* was an environmental sequence from anoxic sediment around fumaroles on a submarine caldera: AB191410 (TAGIRI-2 from Takishita et al. 2005) (Fig. 2). A second but significantly shorter environmental sequence (AB191409) branched closely to the aforementioned environmental sequence in separate analyses using fewer included sites (not shown). We infer that these two environmental sequences came from close relatives of *E. tripartita* and, thus, refer to the group as the '*Ebria* clade' (Figs 2 and 3). Two other environmental sequences (AY620348 and AY620314) branched more distantly with the *Ebria* clade, albeit with very weak support. These sequences came from benthic samples: sand/mud from Wreck Beach, Vancouver, Canada, and muddy sand from Portsmouth waterfront, UK ('novel clade 4' in Bass and Cavalier-Smith 2004). It is plausible that these environmental sequences came from *Hermesium adriaticum* or another as yet uncharacterized ebriid. Unexpectedly, all of the environmental sequences that branched nearest to *Ebria* were derived from benthic samples. Although life-cycle stages of ebriids are not known, one could speculate that they may produce resting cysts.

In our analyses, the *Ebria* clade was most closely related to the 'cryomonad clade', a large group of environmental sequences that includes

described species of *Cryothecomonas* and *Protaspis* (Figs 2 and 3). Although initially not obvious, this putative relationship is also supported by comparative ultrastructural data. Ebriids, *Protaspis grandis*, *Cryothecomonas aestivalis* and *C. longipes* are all morphologically characterized by having two unequal flagella, a nucleus with a conspicuous nucleolus and condensed chromosomes during interphase, tubular mitochondrial cristae (at least in *Hermesium*) and a pseudopodial-based mode of phagotrophy (Drebes et al. 1996; Hargraves 2002; Hoppenrath and Leander 2006; Schnepf and Kühn 2000; Thomsen et al. 1991). Indeed, the nuclear characteristics in ebriids led previous biologists to consider a close relationship to dinoflagellates and euglenids, which also have permanently condensed chromosomes. Nonetheless, although ebriids are usually described as naked cells without an external cell wall, a fine layer of fibrillar material lies outside the plasma membrane (Hargraves 2002). This periplast structure could be homologous to the fibrillar, multilayered walls of the cercozoan genera *Cryothecomonas* and *Protaspis* (Hoppenrath and Leander, 2006; Schnepf and Kühn 2000; Thomsen et al. 1991).

Molecular phylogenetic data indicate that *Lecythium* sp. is a close relative of *C. aestivalis*, and more distantly related to *C. longipes* (Fig. 3). It is currently not clear how this freshwater filose, testate amoeba (Nikolaev et al. 2003) relates to the rest of the cryomonad clade, because, to the best of our knowledge, no ultrastructural data of *Lecythium* are available.

Our analyses suggested that the *Ebria* and cryomonad clades together are most closely affiliated with phaeodareans and *Pseudodifflugia* (Figs 2 and 3), which is consistent with previous analyses of SSU rDNA (Nikolaev et al. 2004; Takishita et al. 2005). Although not the most parsimonious explanation, this raises the interesting possibility that the internal siliceous skeletons of ebriids and phaeodareans are homologous (i.e. arose from a common ancestor with an internal siliceous skeleton) and were subsequently lost in the cryomonad lineage and in *Pseudodifflugia*. However, in the context of recent phylogenetic analyses showing that radiolarians are polyphyletic and that internal siliceous skeletons have evolved several times independently (Nikolaev et al. 2004), it seems most likely that ebriids and phaeodareans evolved siliceous internal skeletons independently as well, perhaps from a common ancestor with the propensity to form solid silica. This view is consistent with the fact that thaumatomonads are capable of manufacturing siliceous scales and euglyphid amoebae have a siliceous test (Beech and Moestrup 1986; Patterson and Zölffel 1991; Cavalier-Smith and Chao 1996/97; Patterson et al. 2002).

Expansion of the Cercozoa

The identity and composition of the Cercozoa is rapidly developing. The first indication of a novel assemblage of filose and reticulose amoebae and nondescript heterotrophic flagellates with tubular mitochondrial cristae was evident in phylogenetic analyses published by Bhattacharya et al. (1995). Shortly thereafter, the phylum Cercozoa was erected (Cavalier-Smith 1998a, b) on the basis of molecular phylogenetic data alone. No morphological feature characterizes the whole phylum and the taxonomic diagnosis is unusually broad (Cavalier-Smith 1998a, p. 232): "unicellular phagotrophic heterotrophs or else photosynthetic algae with green chloroplasts and nucleomorphs within a periplastid membrane located inside a fourth smooth membrane; typically free-living aerobes having peroxisomes and mitochondria with tubular (or very rarely flat or vesicular) cristae; flagellates with two usually anisokont cilia or single cilium or non-flagellates (usually rhizopods) with a test and/or filose or reticulose pseudopodia or with a green plastid and nucleomorph; cilia without lateral flanges, paraxial rods, transition helix or tubular hairs; cortical alveoli and axopodia absent; heterotrophs have a flexible cell surface without a rigid dense protein layer inside or outside the plasma membrane; distinct cytopharynx absent; silica scales sometimes present *but internal silica skeleton absent*; extrusomes, if present, isodiammetric or a complex Stachel; often with walled cysts".

In the following years, the composition of the Cercozoa has continuously expanded, and its phylogeny and classification have been periodically updated (Cavalier-Smith and Chao 2003a; Bass and Cavalier-Smith 2004). Actin phylogenies provided first molecular evidence that the Cercozoa is closely related to the Foraminifera, an inference that was subsequently reinforced with data from polyubiquitin and other protein genes (Keeling 2001; Cavalier-Smith and Chao 2003a, b; Bass et al. 2005). This relationship is consistent with phylogenetic analyses of SSU rRNA gene sequences and multiple protein genes, which also demonstrated that the putative cercozoan *Gromia oviformis* is closely related to foraminiferans (Berney and Pawlowski 2003; Longet et al. 2003,

2004). Actin and SSU rDNA sequences also suggest that all members of the polyphyletic 'radiolaria' appear to be closely related to the cercozoan—foraminiferan clade: polycistines and acantharians form a clade that branches as the closest sister group to cercozoan-foraminiferan clade, while phaeodareans branch within the Cercozoa (Nikolaev et al. 2004; Polet et al. 2004) (Figs 2 and 3). Moreover, a unique insertion of one or two amino acids at the monomer-monomer junctions of the polyubiquitin tract turns out to be a molecular synapomorphy for members of both the Cercozoa and Foraminifera (Archibald et al. 2003; Bass et al. 2005). These data, for instance, can be used to identify different cercozoan subclades and have helped confirm that plasmodiophorids are members of the Cercozoa (Archibald and Keeling 2004; Bass et al. 2005). Obtaining these data for ebriids would be highly desirable. Although we were able to obtain sequences for half of the ubiquitin gene from *E. tripartita*, our repeated attempts to sequence across the monomer—monomer junction of the polyubiquitin tract were unsuccessful.

Nonetheless, the recently established inclusion of phaeodareans and now ebriids within the Cercozoa requires that the diagnosis of the group (Cavalier-Smith 1998a, p. 232) be amended to accommodate taxa with siliceous endoskeletons. This can be accomplished by supplanting the current phrase 'silica scales sometimes present but internal silica skeleton absent' with 'silica scales and endoskeletons sometimes present'. Unfortunately, this relatively straightforward change makes the diagnosis for this extremely broad group of eukaryotes even broader and even more difficult to define on morphological grounds alone; a situation reflected in the most recently published emended diagnosis of the Cercozoa (Adl et al. 2005, p. 416): "Diverse clade lacking distinctive morphological or behavioural characters …"

Methods

Collection of Organisms: For three consecutive days, near surface plankton samples were collected in the morning with a small net (mesh-size 20 μm) at the same site at the docks of the Bamfield Marine Sciences Centre, Vancouver Island (BC, Canada), in June 2005. Immediately after sampling, single cells from *Ebria tripartita* were identified (Fig. 1) and isolated from the mixed plankton sample by micropipetting.

Microscopy: Individual cells were placed on a slide and viewed with a Leica DMIL inverted microscope connected to a PixeLink Megapixel color digital camera and a Zeiss Axioplan 2 imaging microscope connected to a Leica DC500 color digital camera.

DNA extraction, PCR amplification, alignment and phylogenetic analysis: On two separate days, individually isolated cells were washed three times in filtered (eukaryote-free) seawater. Two different samples consisting of 75 and 62 cells respectively were prepared for DNA extraction as follows. Collected cells were placed directly into 400 μl CTAB extraction buffer (1.12 g Tris, 8.18 g NaCl, 0.74 g EDTA, 2 g CTAB, 2 g Polyvinylpyrolidone, 0.2 ml 2-mercaptoethanol in 100 ml water) in 1.5 ml Eppendorf tube. The tube was placed in a heat-block and incubated at 63 °C for 20 min with several vigorous shakes in between. After separation with chloroform:isoamyl alcohol (24:1), the aqueous phase was precipitated in 70% ethanol. The dry DNA pellets were stored in the freezer and transported to the University of British Columbia on ice. Distilled water was added to each sample and the small subunit (SSU) rRNA gene was amplified using PCR primers and a thermocycling protocol described previously (Leander et al. 2003). PCR products corresponding to the expected size were gel isolated and cloned into the pCR 2.1 vector using the TOPO TA cloning kit (Invitrogen, Frederick, Maryland, USA). At least eight clones from each product were screened for size and sequenced with ABI big-dye reaction mix and a vector primer. The genetic identity of the cloned sequences was established by BLAST analysis. Two new sequences from *E. tripartita* were completely sequenced using both vector primers and two internal primers (525F, 5′-AAGTCTGGTGCCAGCAGCC-3′; 1250R, 5′-TAACGGAATTAACCAGACA-3′) oriented in both directions (GenBank accession codes: DQ303922—DQ303923).

The ebriid SSU rDNA sequences were aligned with other eukaryotic sequences using MacClade 4 (Maddison and Maddison, 2000), giving rise to three multiple sequence alignments: (1) a global eukaryotic 61-taxon alignment (1140 unambiguous sites; not shown), (2) a 63-taxon alignment consisting of cercozoans and related environmental sequences (987 unambiguous sites) and (3) a 34-taxon cercozoan alignment excluding the shorter environmental sequences (1305 unambiguous sites). Maximum likelihood (ML), ML-distance and Bayesian methods under different DNA

substitution models were performed. All gaps were excluded from the alignments prior to phylogenetic analysis. The alpha shape parameters were estimated from the data using HKY and a gamma distribution with invariable sites and eight rate categories (63-taxon alignment: $\propto = 0.31$, Ti/Tv = 1.45, fraction of invariable sites = 0.02; and the 34-taxon alignment: $\propto = 0.38$, Ti/Tv = 1.49, fraction of invariable sites = 0.08). Gamma-corrected ML trees (analyzed using the parameters listed above) were constructed with PAUP* 4.0 using the general time reversible (GTR) model for base substitutions (Posada and Crandall 1998; Swofford 1999). Gamma-corrected ML tree topologies found with HKY and GTR were identical. ML bootstrap analyses were performed in PAUP* 4.0 (Swofford 1999) on one hundred re-sampled data sets under an HKY model using the alpha shape parameter and transition/transversion ratio (Ti/Tv) estimated from the original data set.

ML distances for the SSU rDNA data set were calculated with TREE-PUZZLE 5.0 using the HKY substitution matrix (Strimmer and Von Haeseler 1996). Distance trees were constructed with weighted neighbor joining (WNJ) using Weighbor (Bruno et al. 2000). One hundred bootstrap data sets were generated with SEQBOOT (Felsenstein 1993). Respective distances were calculated with the shell script 'puzzleboot' (M. Holder and A. Roger, www.tree-puzzle.de) using the alpha shape parameter and transition/transversion ratios estimated from the original data-set and analyzed with Weighbor.

We also examined the 63- and 34-taxon data sets with Bayesian analysis using the program MrBayes 3.04 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The program was set to operate with GTR, a gamma distribution and four MCMC chains starting from a random tree (default temperature = 0.2). A total of 2,000,000 generations were calculated with trees sampled every 100 generations and with a prior burn-in of 200,000 generations (2000 sampled trees were discarded). A majority rule consensus tree was constructed from 16,000 post-burn-in trees with PAUP* 4.0. Posterior probabilities correspond to the frequency at which a given node is found in the post-burn-in trees.

GenBank accession codes: (AF06324) *Acanthometra* sp., (AF411262) *Allas diplophysa*, (AF411263) *Allas* sp., (AY266294) *Aulacantha scolymantha*, (AY266292) *Aulosphaera trigonopa*, (AF054832) *Bigelowiella natans*, (AF101052) *Cercomonas longicauda*, (U42448) *Cercomonas* sp., (AF411273) Cercozoa sp. WHO1, (U03477) *Chlorarachnion reptans*, (AY305009) *Clathrulina elegans*, (AY266293) *Coelodendrum ramosissimum*, (AF290541) *Cryothecomonas aestivalis*, (AF290540) *Cryothecomonas longipes*, (DQ303922) *Ebria tripartita* 1, (DQ303923) *Ebria tripartita* 2, (AJ418784) *Euglypha rotunda*, (AJ457813) *Gromia oviformis*, (AJ514866) *Gymnophrys cometa*, (AY305010) *Hedriocystis reticulata*, (U42447) *Heteromita globosa*, (AJ514867) *Lecythium* sp., (AF076169) *Lotharella globosa*, (AF174372) *Massisteria marina*, (X81811) *Paulinella chromatophora*, (AF310903) *Phagomyxa bellerochea*, (AF310902) *Polymyxa betae*, (DQ303924) *Protaspis grandis*, (AJ418794) *Pseudodifflugia* cf *gracilis*, (AJ561116) *Pseudopirsonia mucosa*, (AF411261) *Thaumatomastix* sp., (AF411259) *Thaumatomonas seravini*, (U42446) *Thaumatomonas* sp., (AF411260) *Thaumatomonas* sp. (SA), (AF372764) uncultured cercozoan, (AY180012) uncultured cercozoan, (AY180035) uncultured cercozoan, (AY620274) uncultured cercozoan, (AY620276) uncultured cercozoan, (AY620277) uncultured cercozoan, (AY620279—AY620281) uncultured cercozoan, (AY620294) uncultured cercozoan, (AY620295) uncultured cercozoan, (AY620300) uncultured cercozoan, (AY620314) uncultured cercozoan, (AY620316) uncultured cercozoan, (AY620320, AY620321, AY620322, AY620323) uncultured cercozoan, (AY620340) uncultured cercozoan, (AY620348—AY620353) uncultured cercozoan, (AY620355) uncultured cercozoan, (AY620357) uncultured cercozoan, (AB191410) uncultured eukaryote TAGIRI-2, (AJ130856) uncultured eukaryote, (AY082998) uncultured eukaryote.

Acknowledgements

This work was supported by a grant to M. H. from the Deutsche Forschungsgemeinschaft (Grant Ho3267/1-1) and by grants to B.S.L. from the National Science and Engineering Research Council of Canada (NSERC 283091-04) and the Canadian Institute for Advanced Research.

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