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

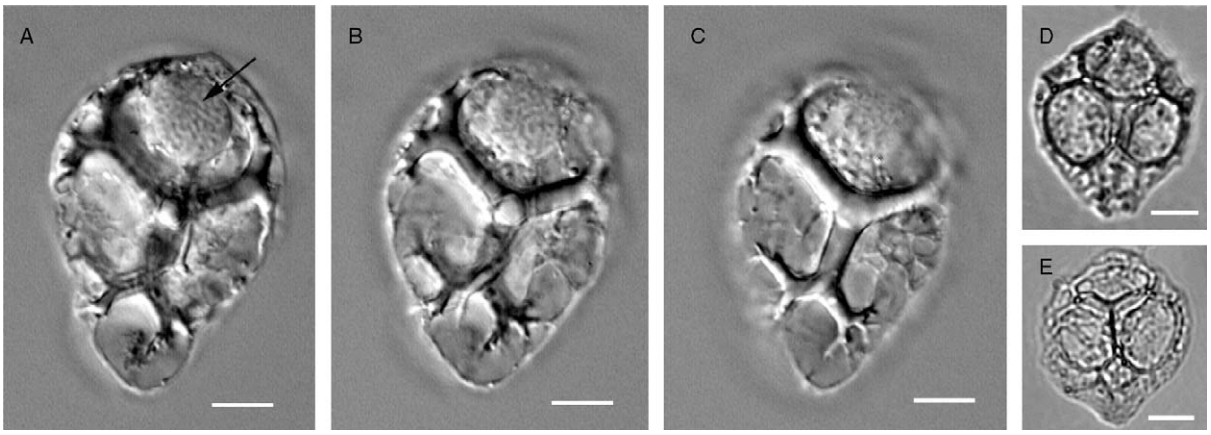

Figure 1. Light micrographs of *Ebria tripartita* isolated from plankton samples at English Bay, Vancouver and at the Bamfield Marine Sciences Centre. **A—C**. Differential interference contrast (DIC) micrographs showing the same cell (English Bay plankton) in different focal planes. Note the nucleus (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