Elongatocystis ecballocystiformis gen. et comb. nov., and some reflections on systematics of Oocystaceae (Trebouxiophyceae, Chlorophyta)

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Abstract: Three new strains of members of the family Oocystaceae collected in inland waters of Africa were studied microscopically and by molecular phylogeny. The new genus Elongatocystis was described, and the new combination Elongatocystis ecballocystiformis was proposed. The phylogenetic position of Oocystidium sp., and Quadricoccus ellipticus within the family was shown. The SSU rRNA phylogeny of Oocystaceae recovered a need for further studies to display the generic and species concept in this monophyletic group of green algae. The essential research steps were discussed.

Key Words: Elongatocystis gen. nov., molecular phylogeny, Oocystidium, Oocystis, Quadricoccus, SSU, taxonomy

Introduction

The family Oocystaceae is a natural lineage in the Trebouxiophyceae (Chlorophyta) proved by both ultrastructural and molecular criteria. The cell wall is multi–layered and contains crystalline cellulose fibres which are oriented in each layer perpendicular to that of the adjacent layers (Robinson & White 1972; Sachs et al. 1976; Quadir 1983). Molecular phylogenetic analyses revealed the monophyly of the family (Hepperle et al. 2000; Kriénitz et al. 2003; Pažoutová et al. 2010). However, the conception of genera and species within Oocystaceae remained obscure. In this study we analyse the phylogeny of three oocystacean isolates from African inland waters and discuss the systematic context of Oocystaceae. The new description Elongatocystis ecballocystiformis gen. et comb. nov. is given, and the phylogenetic position of Oocystidium sp., and Quadricoccus within this family is suggested.

Material and methods

Three new oocystacean strains were isolated by micropipettes directly from the water samples and grown in a modified Bourrelly medium (Hegewald et al. 1994, Kriénitz & Wirth 2006) in suspensions or on agar at room temperature under a 14 h : 10 h light–dark regime in the strain collection of the Leibniz–Institute of Freshwater Ecology and Inland Fisheries (IGB, Stechlin, Germany). Later, the strains were deposited at the Culture Collection of Algae and Protozoa (CCAP, Oban, UK). The designations and origin of the new strains are given in Table 1.

The morphology of algae was examined using a Nikon Eclipse E600 light microscope (LM) with differential interference contrast. Microphotographs (Figs 1–12) were taken with a Nikon Digitalcamera DS–Fi1, and Nikon software NIS–Elements D (Nikon Corporation, Tokyo, Japan).

The three new SSU rRNA gene sequences were compared with 15 other Oocystaceae, nine other members of Chlorellales, and (as outgroup) three prasinophytes (Fig. 13). These sequences were obtained from the GenBank (National Center for Biotechnology Information [NCBI] http://www.ncbi.nlm.nih.gov/). The accession numbers of sequences are given in Fig. 13. An alignment of 30 taxa with 1549 base positions was used for the phylogenetic analyses, introns were excluded. Four different methods were used for the tree reconstruction: maximum likelihood (ML), maximum parsimony (MP), distance (neighborjoining; NJ), and Bayesian analyses (MB) using PAUP* version 4.0b10.
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Table 1. Designation and origin of three new strains of Oocystaceae used in this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain designation at IGB</th>
<th>Strain designation at CCAP</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elongatocystis ecballocystiformis</td>
<td>KR 2000/14</td>
<td>CCAP 274/3</td>
<td>Rockpool at Belvedere River, Mpumalanga, South Africa</td>
</tr>
<tr>
<td>Oocystidium sp.</td>
<td>KR 2007/14</td>
<td>CCAP 222/49</td>
<td>River Zambesi near Livingstone, Zambia</td>
</tr>
<tr>
<td>Quadricoccus ellipticus</td>
<td>KR 2005/238</td>
<td>CCAP 286/1</td>
<td>Lake George near Katwe, Uganda</td>
</tr>
</tbody>
</table>

Results

Taxonomy

Elongatocystis KRIENTZ et C. BOCK gen. nov.


Cells green, solitary, elongated oval, at apices obtuse. Cell wall smooth, hyaline, thick and without polar thickenings. Facultatively with mucilaginous envelope. Chloroplasts 1–4 per cell, parietal, each containing a starch–covered pyrenoid. Propagation asexually by autosporulation (2, 4 or 8 autospores per mother cell). Genus differs from other genera of the family by the order of the nucleotides in SSU and ITS rDNA sequences.


Etymology: from latin: elongatus = elongated; cysta = cyst.

Epitype (designated here): Strain CCAP 274/3, cryoconserved at the Culture Collection of Algae and Protozoa, Oban, Scotland.

Morphology

Elongatocystis ecballocystiformis (Figs 1–6)

In a rockpool (Fig. 1) of the Belvedere River (South Africa) this alga established a mass development of vivid green colour and of jelly consistence. The cells were covered by a thick mucilaginous envelope (Fig. 2). In culture, no jelly was observed (Fig. 3). The vegetative cells were broad oval, without polar thickenings, 8–16 × 3–8 µm in size. The cells contained one or two parietal pyrenoid–bearing through–shaped chloroplasts which were often thickened in the central part looking like interconnections from one elongated part of the chloroplast to the other. Inside the cell numerous assimilate particles and oil droplets were contained. The mother cells produced two, four or eight autospores (Fig. 4). These autospores again propagated inside the mother cell forming large “grandmother cells” which contained several mother cells of different developmental stages (Fig. 5). Frequently, elongated, bented cells were
Figs 1–6. *Elongatocystis ECBallocystiformis* gen. et comb. nov., in field (Figs 1, 2) and culture (Figs 3–6): (1) sampling site, rockpool; (2) vegetative cells in field sample. The negative staining with Indian ink reveals the colourless mucilaginous envelope; (3) vegetative cells and young mother cells in culture. The cells are laying close together indicating the missing mucilaginous envelope; (4) dividing cell and mother cells with four and eight autospores, respectively. Black arrowheads indicate pyrenoids; (5) grandmother cell with several mother cells. The daughter cells contain large droplets of oil (white arrowheads); (6) elongated, bummerang–shaped cell with starch–grained chloroplasts and droplets of oil (white arrowheads). Scale bar 10 cm (Fig. 1), 10 mm (Figs 2–6).

Observed with divided nuclei and chloroplasts but not producing autospores (Fig. 6).

**Oocystidium sp. (Figs 7–9)**

In a water sample collected at the shoreline of the river Zambezi near the waterfront area at Livingstone (Zambia), shortly before the Victoria Falls, few specimens of *Oocystidium* were found. In wide mucilage mostly couples of broad oval cells attached to bipartited remnants of the mother cell wall were observed. Sometimes, within the wide mucilage solitary cells or arranged in pairs and tetrads were found (Fig. 7). Several cells were irregularly ovoid (Fig. 8) or elongated (Fig. 9). The vegetative cells were 5–6 × 3–5 µm, the mother cells 6–9 × 4–5 µm in size. The hyaline cell wall did not possess polar thickenings. The cells contained one or
Quadricoccus ellipticus Hortob. (Figs 10–12)

In the field sample from Lake George (Uganda), typical four–celled colonies were observed. The cells were arranged along the periphery of the bowl–shaped mother cell wall remnants. During isolation with a microcapillary it was taken care to catch such typical colony. In the successful fresh unialgal culture these typical Quadricoccus–shape was reflected, however, was lost during the years of maintainance under culture conditions. The ovoid or citriforme cells were not arranged on the edge of the cell wall remnants but were irregularly associated with multiformed, ruptured cell wall remnants (Fig. 10). Frequently, four cells were arranged in close affinity to empty mother cell walls (Figs 11 and 12). A joint mucilaginous envelope was very fine and surrounded narrowly the cells and colonies. The cells contained one or two girdle–like chloroplasts with pyrenoid. Cell size: autospores 5–6 × 4–5 µm, mother cells 7–12 × 6–9 µm.

Phylogeny

The topology of the phylogenetic tree (Fig. 13) was determined by the well supported main clade, the Oocystaceae. Eighteen strains were comprised under the monophy whole Oocystaceae including members of 13 different genera, 12 of them of coccoid and one (Planktonema) of filamentous morphology. The node that joins the Oocystaceae and the relationship to Chlorellaceae and a consortium of filamentous (Gloeotila and Catena) as well as bubbling (Marvania) chlorophytes was not well supported.

Elongatocystis ecballocystiformis established a sister to a lineage which contained Crucigeniella rectangularis (NAG.) Komárek and Makinoella tosaensis Okada. Quadricoccus ellipticus clustered in close relationship to Amphikrikos sp. whereas Oocystidium sp. established a sister to Ooploktella planocinvesa (Hindák) Pažoutová, Škaloud et Nemjová, and Oocystis sp.

Discussion

Oocystis ecballocystiformis Iyengar was found for the first time in a rockpool near the Jog Falls, Mysore Province, South India by Iyengar (1932).
Fig. 13. Phylogenetic analyses of SSU rRNA gene sequences of members of Oocystaceae with members of prasinophytes as outgroup. Number at the branches indicate Bayesian posterior probabilities (MB) and bootstrap support from maximum likelihood (ML, 100 replicates), maximum parsimony (MP, 1000 replicates) and neighbour joining (NJ, 1000 replicates). Hyphen indicate support below 50% for ML, MP, NJ and below 0.95 for MB.
This locus classicus is comparable to the habitat where we found the material in South Africa, a rockpool near the fast flowing pristine Belvedere River. The morphology of our strain CCAP 274/3 corresponds widely to the findings of Iyengar. The phylogenetic position of this alga near the coenobial genera Crucigeniella and Makinoella is distant from other Oocystis species. Therefore, we excluded this taxon from the genus Oocystis, described the new genus Elongatocystis and established a new combination Elongatocystis ecballocystiformis (see chapter Taxonomical measures).

The trend in developing large, colony–like “grandmother cells” by Elongatocystis reflect their phylogenetic position near large celled and colonial Oocystaceae such as Makinoella. The related Oocystis solitaria containing numerous chloroplasts could eventually after further studies considered as member of a new genus. Eremosphaera viridis De–Bary, the oocystacean member with the highest number of chloroplasts (about 50 per cell) is known for its fibrous cell wall rich in pectin and hemicellulose (De Boer et al. 1994). Stoyneva et al. (2006) reported on a new species of Eremosphaera, E. tanganyikae from Lake Tanganyika. Stoyneva et al. (2009) assumed based on ultrastructural similarities a close relationship of Oocystis, Eremosphaera and Neglectella.

The elongated filament–like cells of Elongatocystis ecballocystiformis (Fig. 6) found several times in cultures could be interpreted in two different ways: (i) as deformity or (ii) as link to the filamentous relatives of Oocystaceae such as Planctonema which evolved at the top of the oocystacean clade (Fig. 13). Microscopical studies revealed the facultative mucilaginous envelope and, interestingly, the layered character of apical cell wall thickenings bringing the cells within the filaments in a distant position (Skuja 1956; Bourrelly 1962; Heynig 1988). The position in the phylogenetic tree makes Planctonema a candidate to be included into Oocystaceae. The ultrastructural proof showing the fibrillar pattern of cell wall of Planctonema is unaccounted until now.

Our strain of Oocystidium sp. (CCAP 222/49) is clustering at the lower end of the tree as sister to Ooplanctella planoconvexa and Oocystis sp. Oocystidium is considered as a monotypic genus represented by Oocystidium ovale Korshikov. It is characterized by a wide mucilaginous envelope containing persistent bipartited mother cell wall remnants (Korshikov 1953; Hindák 1988). Our strain differs from the type species by smaller (~50%) and more elongated cells. However, we refrain from description of a new species before the taxonomical placement of Oocystidium, Oocystis and Oocystella is generally resolved. Microscopical findings showed Oocystidium–like extended mucilaginous envelope containing mother cell wall remnants also in several species of Oocystella such as O. oogama Hindák and O. parva (W. et G.S. West) Hindák (Hindák 1988).

We designated our strain CCAP 286/1 as Quadricoccus ellipticus because of its typical arrangement of four cells symmetrical on the edge of a bowl–shaped mother cell wall remnant when we isolated the strain and during its first weeks in culture. Later, this typical arrangement disappeared in culture. Probably, this symmetric position of the cells on a flat bowl is a phenotypic adaptation and supports the balance and buoyancy of the colony in the water column and is not essential under culture conditions. An other morphological criterion in Quadricoccus, the cell wall incrustation of the type species Quadricoccus verrucosus Fott is perhaps also a phenotypic adaptation. This makes it difficult to establish an authentic strain of the type species, because the incrustations disappear in culture. Also for other coccoid green algae the incrustation was assumed to be a phenotypic character such as for Dictyosphaerium granulatum Hindák and Raphidocelis div. spec. (C. Bock and L. Krienitz unpubl. results). The phylogenetic analyses showed the accommodation of Quadricoccus in Oocystaceae as a sister to Amphikrikos. Therefore, the former position of Quadricoccus within Dictyosphaerioideae according to Komárek & Fott (1983) is to revise.

Whereas no doubt exists about the natural delineation of the family (Pröschold & Leliaert 2007), the grouping inside the family on the level of genera and species is highly erratic. This is especially the case in regard of the type species of the genus Oocystis, O. naegelii A. Br. which does not possess pyrenoids. Because of uncertainties in the description of this species provided by Braun (1855), Řeháková (1969) suggested O. lacustris Chodat (which contains pyrenoids) as lectotype. However, Komárek & Fott (1983) rejected this suggestion and referred to the careful study of Skuja (1964) who re–examined the exsiccatea of the type species O. naegelii. He
found on the material from the boreal zone only one or two chloroplasts per cell which does not exhibited pyrenoids. The possession of pyrenoids in members of *Oocystis* seems to be of crucial interest. Lemmermann (1903) established the new genus *Oocystella* for *Oocystis*–like algae with pyrenoids based on the type *Oocystella natans* Lemm., which is only to differentiate from *O. laeustris* by the asteroid–like chloroplasts. Hindák (1988) came back to Lemmermann’s conception of *Oocystella* and transferred 12 pyrenoid–bearing species of *Oocystis* to *Oocystella*.

Resuming the phylogenetic positions of different *Oocystis* species throughout the whole clade of Oocystaceae, we are unable, to decide which is the real *Oocystis* lineage, because all the strains sequenced until now possess pyrenoids. In a first and most important step it would be essential to isolate and sequence material from the pyrenoid–less type species *O. naegelii*. Afterwards, a decision can be made about the taxonomical relevance of pyrenoids. In recent studies, controversial experiences were made regarding the taxonomical relevance of pyrenoids. In a second step, after recovering of the type species of *Oocystis in Oocystaceae* it can be decided about the question: What is the real *Oocystis*. Finally, in a third step, the circumscript of the remaining genera of Oocystaceae can be displayed.

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