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European Journal of Protistology 50 (2014) 40–46

European Journal of PROTISTOLOGY

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New SSU-rDNA sequences for eleven colpodeans (Ciliophora, Colpodea) and description of *Apocyrtolophosis* nov. gen

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Received 29 July 2013; received in revised form 16 September 2013; accepted 27 September 2013
Available online 6 October 2013

Abstract

Using 11 new SSU-rDNA sequences, we analyze relationships within the class Colpodea, especially of some uncommon taxa, such as *Kalometopia duplicata*, *Cyrtolophosis minor*, and *Jaroschia sumptuosa*. The sequences do not change the basic structure of the molecular Colpodea tree, i.e., all belong to one of the four molecular clades recognized by Foissner et al. (2011): Colpodida, Cyrtolophosidida, Bursariomorphida, and Platyophryida. The addition of three *Colpoda* sequences strengthens the observation that species of this genus are distributed over the whole molecular Colpodea tree. Very likely, this is caused by a fast radiation of *Colpoda*, several species of which then evolved independently, forming new genera and families. *Cyrtolophosis minor*, which belongs to the molecular *Pseudocyrtolophosis* clade, is referred to a new genus, *Apocyrtolophosis* nov. gen., characterized by a comparably large, deltoid oral opening, an unciliated posterior region, and the absence of an oblique kinety in the left oral polykinetid. *Bryometopus triquetrus* does not erase the paraphyly of its genus. *Platyophrya vorax*, *P. spumacola*, and *P. bromelicola* form a highly supported clade in the order Platyophryida. *Platyophryides* and *Ottowphrya* are close genetically but differ in the silverline pattern (colpodid vs. platyophryid).

Keywords: *Colpoda*; *Cyrtolophosis minor*; *Jaroschia sumptuosa*; Kalometopia duplicata; Soil ciliates; Tank bromeliad ciliates

Introduction

The Colpodea Small and Lynn, 1985 is a middle-sized ciliate class comprising about 65 genera and 200 species, most living in terrestrial habitats (Bourland et al. 2011; Dunthorn et al. 2009; Foissner 1993, 2003, 2010; Foissner and Stoeck 2009; Foissner et al. 2002). However, this is only part of the total diversity. In a fourthcoming monograph on neotropic soil ciliates, we describe four new genera and 10 new species. Further, Foissner has another 20 undescribed species, waiting to be published.

Foissner et al. (2011) revised the classification of Foissner (1993), showing four well supported molecular clades (orders) in the class: colpodids, cyrtolophosidids, bursariomorphids, and platyophryids. The time-honored genus *Colpoda* proved to be non-monophyletic in small subunit ribosomal DNA (SSU-rDNA) polygenies, producing curious, sometimes highly supported clades composed of two or more genera that have little in common morphologically,

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0932-4739/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.ejop.2013.09.003

for instance, *Colpoda steinii* and *Bromeliothrix metopoides*. Foissner et al. (2011) suggested a fast radiation of *Colpoda*, several species of which then evolved independently, forming new genera and families. This model is supported by the present study, which shows the SSU-rDNA sequences of three additional *Colpoda* species and from some rare colpodids, especially, *Kalometopia* and *Jaroschia*, the latter being one of the most complex colpodids described (Foissner 1993).

Material and Methods

Taxon sampling, identification, and terminology

Eleven Colpodea were sampled for this study (Table 1), using ordinary methods for limnetic species and the non-flooded Petri dish method for species from terrestrial habitats (Foissner et al. 2002). Identifications used live observation and silver impregnation (Foissner 1991). Other colpodean sequences and two outgroups are from GenBank (Fig. 1). Terminology follows Foissner (1993) and Lynn (2008).

Table 1. Taxon sampling for sequence analyses. For author names and descriptions, see Foissner (1993).

Taxon	Sampling site
Apocyrtolophosis minor	Tank bromeliad, Jamaica
Bryometopus triquetrus	Litter and soil from the botanical garden in Rio de Janeiro, Brazil
Colpoda ecaudata[a]	Tree hole mud from the campus of the University of the West Indies, Jamaica
Colpoda elliotti	Field soil from the village of Obersiebenbrunn, Lower Austria
Colpoda lucida[b]	As *Bryometopus triquetrus*
Jaroschia sumptuosa	Mud of bamboo stumps in the botanical garden of the town of Castelton, Jamaica
Kalometopia duplicata	Sagebrush steppe soil, Idaho, town of Boise, USA
Platyophrya spumacola hexasticha	As *Colpoda elliotti*
Platyophryides n. sp.	Bamboo stump mud, Jamaica
Repoma cavicola	As *Colpoda ecaudata*
Woodruffides metabolicus	Rice field soil from the surrounding of the Lake Biwa museum, Japan

[a] Some specimens of this species often look like small *C. maupasi*, which also occurred in the sample. Thus, we cannot entirely exclude that some entered the sample and the sequence is from *C. maupasi*.

[b] The specimens of this population had not only the distinct extrusome fringe typical for the species but also a conspicuous postoral sac.

Amplification, sequencing, and genealogical analyses

Genomic DNA was extracted, and the nuclear SSU-rDNA locus was amplified and sequenced according to Foissner and Stoeck (2009). Vector and primer nucleotides were trimmed off. Sequences were added to the alignment of Foissner et al. (2011) and edited by eye in MacClade v4.05 (Maddison and Maddison 2005).

The GTR-I-Γ evolutionary model for all alignments was the best fitted model selected by AIC as implemented in jModelTest v2.1.3 (Darriba et al. 2012; Guindon and Gascuel 2003; Posada 2008). Maximum likelihood (ML) analyses were carried out in RaxML-HPC v7.2.5 (Stamatakis 2006; Stamatakis et al. 2008). Node support came from a majority rule consensus tree of 1000 multiparametric bootstrap replicates. Bayesian analyses (BI) were carried out in MrBayes v3.2.1 (Huelsenbeck and Ronquist 2003). Posterior probability was estimated using four chains running 20 million generations sampling every 1000 generations. The first 25% of sampled trees were considered burn-in trees and were discarded prior to constructing a 50% majority rule consensus trees. Trees were visualized with FigTree v1.3.1 (Rambaut 2006). For the ML bootstraps, we will consider values <0.70 as low, 0.70–0.94 as moderate, and ≥0.95 as high, following Hillis and Bull (1993). For the MrBayes and posterior probabilities, we will consider values <0.94 as low, and ≥0.95 as high.

Results and Discussion

The following text should be studied in connection with Foissner (1993), where the species concerned are reviewed, and with Foissner et al. (2011), who discuss the classification of the Colpodea. Accordingly, author names and dates of species descriptions are mentioned only when they are not contained in Foissner (1993) or when they are necessary for nomenclatural or taxonomic reasons.

Phylogeny (Figs 1, 2)

1. The new sequences do not change the basic structure of the molecular phylogeny of the Colpodea tree, i.e., they belong to one of the four molecular orders recognized by Foissner et al. (2011): Colpodida, Cyrtolophosidida, Bursariomorphida, and Platyophryida (Fig. 1).
2. We added six new sequences to the order Colpodida, for which we present two molecular trees: the "class (Colpodea) tree" (Fig. 1) and the "colpodid tree" that contains only colpodids s.str. (Fig. 2). First, we discuss the sequences from two rare and highly complex species, viz., *Kalometopia duplicata* and *Jaroschia sumptuosa*. Unfortunately, their classification remains ambiguous.

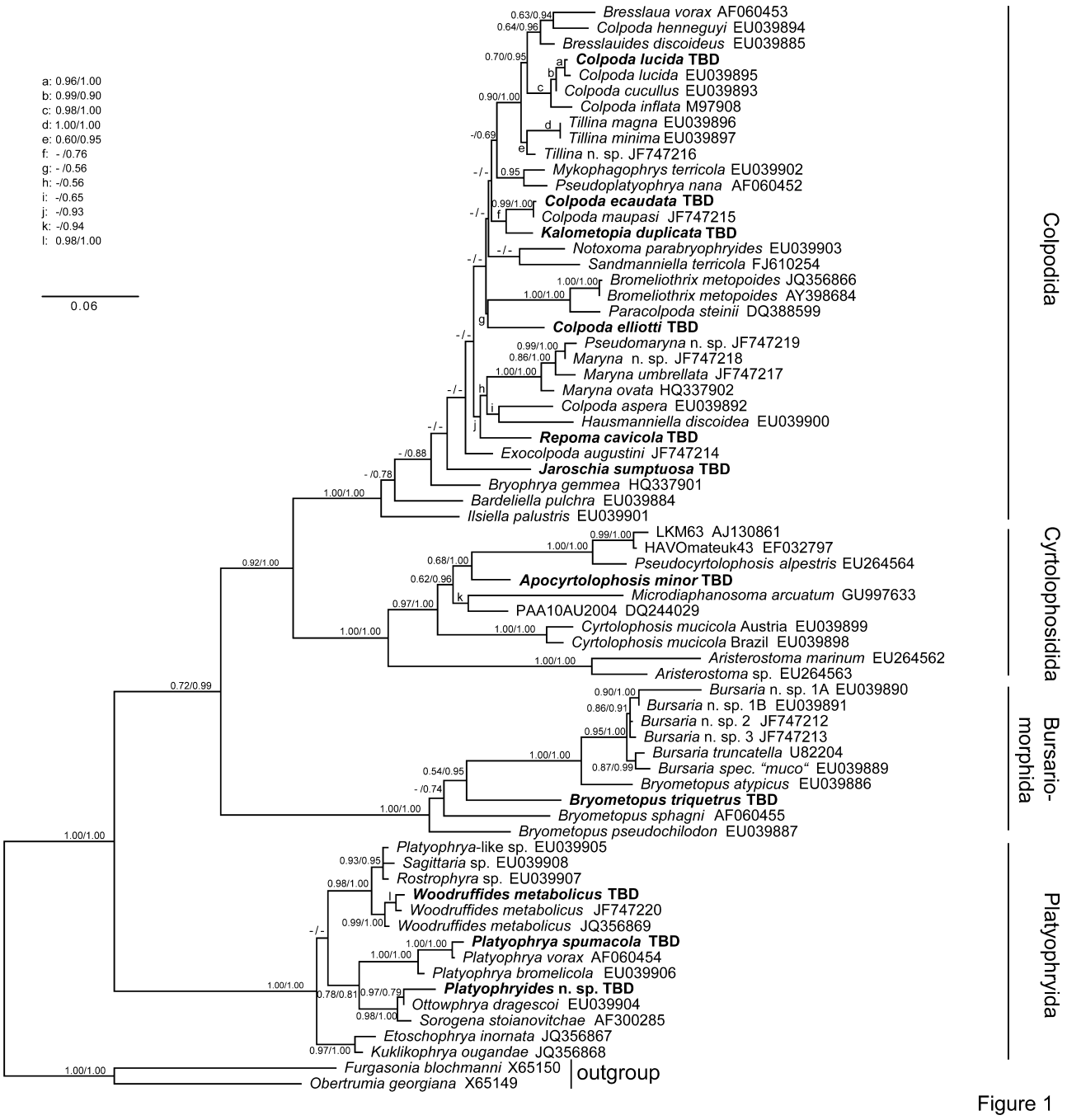


Fig. 1. The new Colpodea tree based on SSU-rDNA sequences. The most likely (ML) tree is shown. Node support: ML bootstrap/MrBayes posterior probability. Values ≤0.50 are shown as "-". New sequences are bolded.

Morphologically, *Kalometopia* belongs to the colpodids s.str., specifically to the Hausmanniellidae, because it has a colpodid general organization, a colpodid silverline pattern, and a hausmanniellid oral apparatus (Foissner 1993). However, the molecules do not provide support for or against such a relationship because node support values are low in both trees (Figs 1, 2).

Jaroschia sumptuosa has highly complex oral structures. Foissner (1993) classifies it into the Bryometopida due to the oral structures and the tightly-meshed silverline pattern. However, the SSU-rDNA sequence shows that it might belong to the bryophryid evolutionary branch (Fig. 1), but bootstrap values are poor, and thus there is no support. Morphologically, a relationship with the bryophryids is likely. Foissner (1993) considers *Jaroschia* as the representative of a distinct family, the Jaroschidae. This seems appropriate because *Bryophrya*, *Bardeliella*, and *Ilsiella*

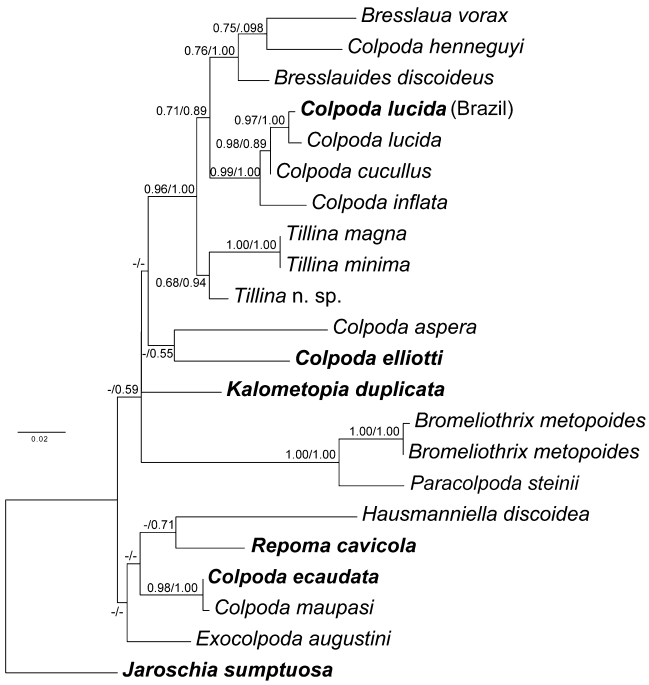


Fig. 2. SSU-rDNA tree of classical colpodas. The most likely (ML) tree is shown. Node support and node labels as in Fig. 1. New sequences are bolded.

have the same rank (Bourland et al. 2011; Foissner 1993).

The suborder Colpodina Foissner, 1978 shows the bewildering pattern discussed by Foissner et al. (2011), that is, species of the genus *Colpoda*, as defined by Kahl (1931) and Foissner (1993), are found throughout the order, often forming curious clades comprising two or more seemingly unrelated genera. Six such clades are recognizable in the Colpodea tree (Fig. 1): the moderately supported (0.64/0.96) *Bresslaua*, *Colpoda*, *Bresslauides* clade; the well supported (0.90/1.00) *Colpoda*, *Tillina* clade which is corroborated by distinct morphological apomorphies (Foissner et al. 2011); the poorly supported (<0.50) *Colpoda*, *Kalometopia* clade which is morphologically not unrealistic (Foissner 1993); the poorly supported *Bromeliothrix*, *Paracolpoda*, *Colpoda* clade which is rather unlikely because *Bromeliothrix* has several unique morphological, ontogenetical, and ecological features suggesting family rank (Foissner 2010; Weisse et al. 2013); the poorly supported *Pseudomaryna*, *Maryna*, *Colpoda*, *Hausmanniella*, *Repoma* (for *Colpoda cavicola*, see Novotny 1971 and Foissner 1993) clade, uniting species from three families (Foissner 1993); and the poorly supported *Exocolpoda augustini* clade.

The colpodid tree shows slightly different clades (Fig. 2): the moderately to well supported (0.76/1.00) *Bresslaua*, *Colpoda*, *Bresslauides* clade; the highly supported (0.96/1.00) *Colpoda*, *Tillina* clade; the poorly supported *Colpoda aspera* and *C. elliotti* clade although both are highly similar morphologically (Foissner 1993), providing some support for the *C. aspera* and *Hausmanniella* clade in the Colpodea tree (Fig. 1); the highly supported (1.00/1.00) *Bromeliothrix*, *Paracolpoda* clade (see above); and the poorly supported *Hausmanniella*, *Repoma*, *Colpoda*, *Exocolpoda* clade.

Although the bootstrap values are frequently low, it is unlikely that the classical *Colpoda* species will ever unite to a single clade because they are distributed over the whole Colpodea tree. This is recognizable also in earlier trees and motivated Foissner et al. (2011) and Dunthorn et al. (2012) to suggest a fast radiation of *Colpoda* that produced several new genera and families.

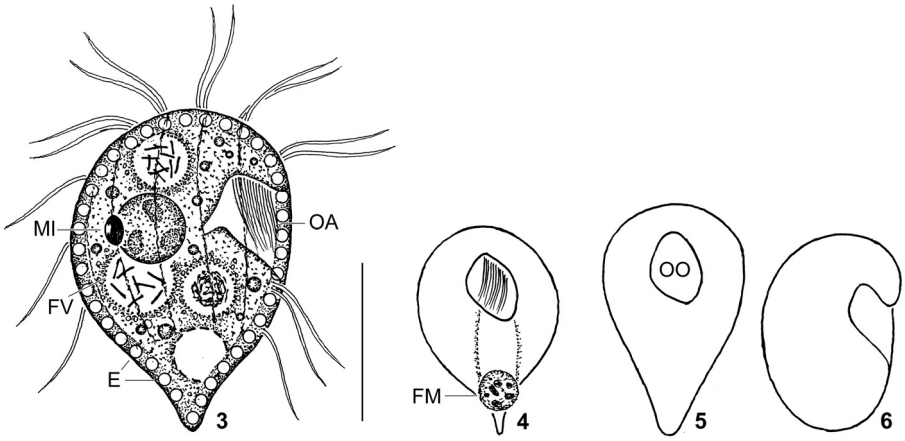
3. Within the order Cyrtolophosidida, we could sequence *Cyrtolophosis minor*, a species deviating from the congeners by the large, deltoid oral opening and the posteriorly reduced ciliature (Foissner 1993; Figs 3–10). A new genus is established for this species (see below) which is supported by the sequence making a rather well supported clade (0.68/1.00) with *Pseudocyrtolophosis*. Further details, see description below.
4. One sequence has been added to the Bursariomorphida, viz., from *Bryometopus triquetrus*, deviating from the congeners mainly by the triangular (vs. bow-shaped) oral opening. The sequence does not erase the non-monophyly of the genus.
5. Three new sequences have been added to the Platyophryida. The Japanese *Woodruffiides metabolicus* makes a strongly supported clade (0.99/1.00) with the two North American populations. *Platyophrya spumacola* makes a strongly supported clade (1.00/1.00) with *P. vorax* and *P. bromelicola* Foissner and Wolf, 2009, the latter being less related to *P. vorax* and *P. spumacola*, which matches the morphological data in that only *P. bromelicola* produces macrostomes (Foissner and Wolf 2009). The genus *Platyophryides* is genetically close to *Ottowphrya* Foissner et al., 2002, from which it differs by the colpodid (vs. platyophryid) silverline pattern (Foissner et al. 2002).

Apocyrtolophosis nov. gen.

Diagnosis: Very small, unflattened Cyrtolophosidida with unciliated posterior third and comparatively large, deltoid oral opening.

Type species: *Cyrtolophosis minor* Vuxanovici, 1963.

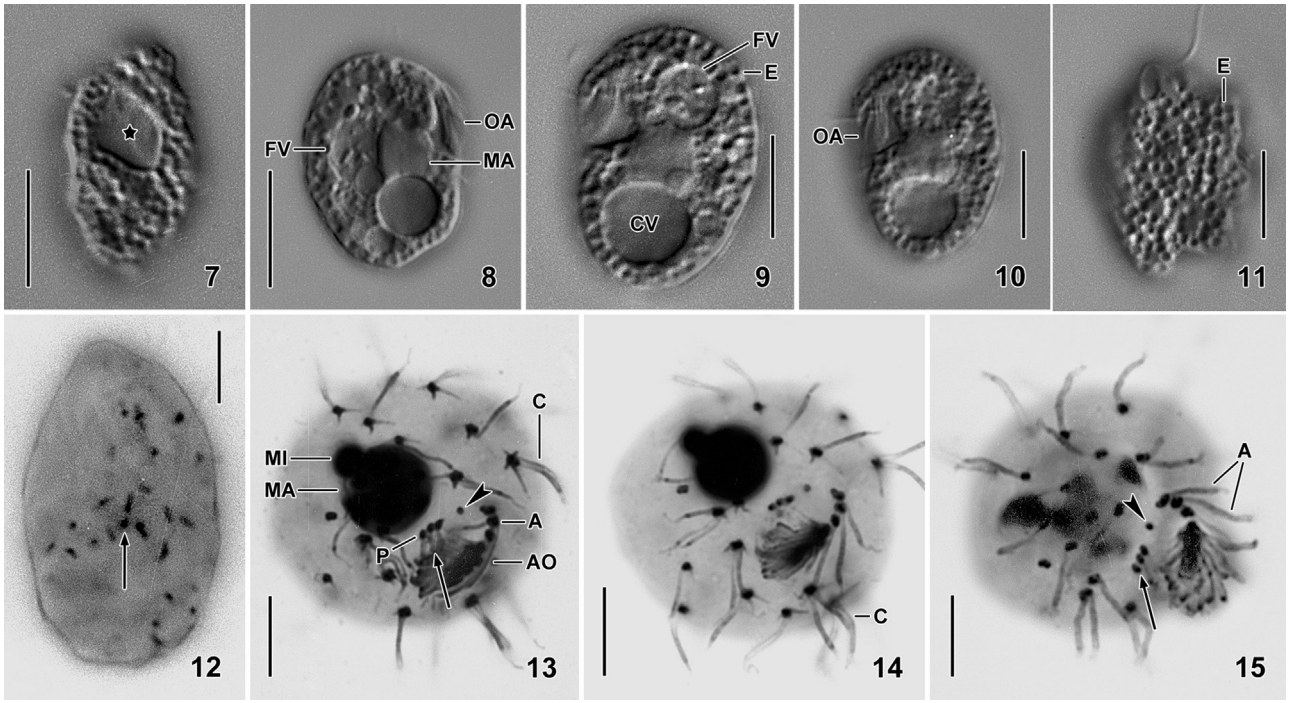
Etymology: *Apocyrtolophosis* is a composite of the Greek prefix *apo* (derived from) and the genus-group name *Cyrtolophosis* (curved, wearing a crest; Stokes 1885).



Figs 3–6. *Apocyrtolophosis minor*, Brazilian specimens from life. **3:** Right side overview of a representative specimen, length 20 μm. **4–6:** Variability of body shape. Most specimens have an acute posterior end (3–5), rarely it is broadly rounded (6). When the fecal mass leaves the body, a canal-like structure appears and extends to the buccal vertex. E – extrusome fringe, FM – fecal mass, FV – food vacuole, MI – micronucleus, OA – oral apparatus, OO – oral opening. Scale bars: 10 μm.

Species assignable: *Apocyrtolophosis minor* (Vuxanovici, 1963) nov. comb.

Remarks: Both, the morphological and molecular data suggest to classify this species into a distinct genus. It differs from *Cyrtolophosis*, *Pseudocyrtolophosis*, and *Plesiocaryon* Foissner et al., 2002 mainly by the lack of an oblique kinety anterior of the anteriormost adoral organelle. It also differs from *Cyrtolophosis* by the unciliated (vs. ciliated) posterior area. *Aristerostoma* possibly lacks an oblique kinety (Dunthorn et al. 2009) and the SSU-rDNA differs considerably (Fig. 1).



Figs 7–15. *Apocyrtolophosis minor*, Brazilian specimens from life (7–11), after methyl green-pyronin staining (12), and in silver carbonate preparations (13–15). **7:** Ventral view, showing the comparatively large, deltoid oral opening (asterisk). **8–10:** Lateral views in optical sections, showing the deep oral cavity and the extrusome fringe. **11:** Surface view, showing the globular, about 1 μm-sized extrusomes, likely mucocysts. **12:** Arrow marks (partially?) exploded mucocysts. **13–15:** Ventral views of oral and somatic ciliary pattern. The arrows mark the ciliated (13) posterior fragment of the paroral membrane. The arrowheads denote a single, likely unciliated kinetid between anterior and posterior fragment of the paroral membrane. A – anterior fragment of paroral membrane, AO – four adoral organelles, C – paired cilia, E – extrusomes, FV – food vacuoles, MA – macronucleus, MI – micronucleus, OA – oral apparatus, P – posterior fragment of paroral membrane. Scale bars 5 μm (12) and 10 μm (7–11, 13–15).

Supplementary observations on a Brazilian population of *A. minor*

An Australian population of this species has been described by Foissner (1993). Thus, we mention only new and/or interesting confirmatory observations from the Brazilian population, which was found in surface soil of the Anavilhanas archipelago in the Rio Negro, about 40 km west of the town of Manaus. Note that the molecular investigation was done on a different population, i.e. from Jamaican tank bromeliads (Table 1).

1. The body size is 15–25 × 12–18 μm, usually it is about 20 × 15 μm. Most Brazilian specimens have an acute posterior end (Figs 3–5) while the Romanian and Australian cells are rounded (Foissner 1993). Thus, we suspected that this could be a new species. However, the acute end often disappears when the cells are on the microscope slide for a while or when they are slightly squeezed by the coverslip (Figs 7–9). Further, we re-investigated the preparations from the Australian population. This showed two acute cells among 50 investigated. Finally, we noticed the common occurrence of acute and rounded cells in tank bromeliads. Thus, all these populations are very likely conspecific.
2. The contractile vacuole is in the rear body end. We could not observe short canals connecting the adventive vacuoles with the main vacuole (Vuxanovici 1963). The cytopyge is also in the posterior end. When the fecal mass leaves the cell, a canal-like structure extends to the oral vertex (Fig. 4).
3. *Apocyrtolophosis minor* has extrusomes about 1 μm across, providing the cortex with a honeycomb pattern (Figs 3, 7–11). When stained with methyl green-pyronin, they become broadly ellipsoidal and about 1.5 μm long (Fig. 12).
4. Movement conspicuous, i.e., irregularly dancing; never rests.
5. The somatic and oral ciliary pattern is as described for the Australian population (Figs 13–15). The somatic cilia are about 8 μm long and paired in the anterior body half; more posteriorly only single cilia occur, most likely originating from monokinetids, as in *Plesiocaryon* spp. (Diaz et al. 2000; Foissner et al. 2002).
6. The oral opening is deltoid and comparatively large, i.e., about 8 μm long and wide and 6–9 μm deep (Figs 3–7, 9). The adoral cilia are about 6 μm long, the paroral 3 μm.
7. *Apocyrtolophosis* has been recorded from Romania (Vuxanovici 1963), Australia (Foissner 1993), Central America (Foissner unpubl., rather common in tank bromeliads), and South America (this study). Not yet reported from Africa and Central Europe, indicating that it is not cosmopolitan.

Acknowledgements

Supported by the Austrian and German Science Funds (FWF grants P20360-B17, P22846-B17, DFG grant DU1319/1–1). The technical assistance of Mag. Barbara Harl, Robert Schörghofer, Andreas Zankl, and Hans-Werner Breiner is gratefully acknowledged.

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