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European Journal of Protistology 50 (2014) 122–133

European Journal of PROTISTOLOGY

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Ciliates from eutrophized water in the northern Brazil and morphology of *Cristigera hammeri* Wilbert, 1986 (Ciliophora, Scuticociliatia)

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Received 25 September 2013; received in revised form 23 January 2014; accepted 27 January 2014
Available online 4 February 2014

Abstract

Ciliates occur in all major aquatic and soil environments worldwide and are important links in the microbial food webs, which, along with other free-living protists, are generally overlooked in biodiversity conservation programs. In Brazil, the northern region comprises the Brazilian Amazonia, an area widely known for its huge biodiversity. However, the diversity of ciliates in that region is still almost unknown. As result of the present study, a total of 21 species of ciliates, distributed among 15 genera, were inventoried from samples of eutrophized water collected in the city of Belém, capital of the state of Pará, one of the states which comprise the Brazilian Amazonia. In addition, a local population of the rare scuticociliate *Cristigera hammeri* is described from optical and electron microscopy observations, and its synonymy with *C. pleuronemoides* is rejected based on new evidence.

Keywords: Amazonia; Oligohymenophorea; Synonymy; Systematics; Taxonomy

Introduction

Ciliates have successfully colonized most of the world habitats, occurring in their majority as free-living forms in fresh and brackish water, marine environments, including deep-sea, polar and desertic regions, soils, associated to bromeliad tanks, mosses and other vegetation (e.g. Fenchel 1987; Foissner et al. 2002; Lynn 2008; Orsi et al. 2012; Petz et al. 2007). However, it is estimated that around 83–89% of the ciliate diversity remains unknown to science (Foissner and Hawksworth 2009); which, according to Cotterill et al. (2008), reflects the fact that protists in overall are scarcely considered in biodiversity conservation and management programs, even though the importance of their roles in ecosystems is thoroughly emphasized in the literature (e.g. Corliss 2004; Cotterill et al. 2008; Dopheide et al. 2009; Fenchel 1987; Foissner 2006).

The northern region of Brazil comprises most of the Brazilian Amazonia and that includes both areas of preserved tropical forest but also important urban centers. According to Albagli (2001) and Vieira et al. (2008), it holds a large diversity of species and populations. Recent compilations indicate the occurrence of at least 40,000 species of plants, 427 of mammals, 1294 of birds, 378 of reptiles, 427 of amphibians, around 3000 of fishes, and 2,500,000 of arthropods (Albagli 2001; Silva et al. 2005). In spite of that, the

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http://dx.doi.org/10.1016/j.ejop.2014.01.005

diversity of ciliates in the northern Brazil is still little known. Only some occasional reports exist (e.g. Rocha-Leite et al. 2009; Tavares-Dias et al. 2010), but relatively few inventories and alpha-taxonomic surveys were carried out (e.g. Foissner 1997; Paiva et al. 2012).

The present study provides a survey of ciliates found in eutrophized water collected from a septic tank in the city of Belém, capital of the state of Pará (PA), one of the states which comprise the Amazonian region in the north of Brazil. In addition, a novel population of the rare scuticociliate *Cristigera hammeri* Wilbert, 1986, is described based on optical and transmission electron microscopy. Until now, this species was known only from its type locality, namely Lake Ontario in Canada (Wilbert 1986). It was considered by Esteban and Olmo (1997) as junior synonym of *Cristigera pleuronemoides* Roux, 1899, type of *Cristigera* Roux, 1899. Esteban and Olmo (1997) also proposed the synonymy of *Cristigera penardi* Kahl, 1935 and *Paurotricha cyclidiformis* Dragesco and Dragesco-Kernéis, 1991 with *Cristigera pleuronemoides*. This synonymy is also discussed on the light of new data on *Cristigera hammeri*.

Material and Methods

The study area consisted of a small septic tank containing eutrophized water, located in the campus of the Universidade Federal do Pará, next to the entry of the Instituto de Ciências Biológicas (1° 28′ 23″ S; 48° 27′ 26″ W). The tank daily receives water input from rainfalls and the sewers, also accumulating organic matter from leaves and fruits that fall from the adjacent vegetation. Physico-chemical characteristics of the water therein were measured in February 2012 with an electronic multimeter, and were: conductivity = 400 μS/cm; dissolved oxygen concentration = 0.41 mg/l; pH = 5.82; salinity 0.19‰; temperature = 27.1 °C.

Samples were collected manually using glass flasks of about 500 ml, in September 2011, January, February, May, and October 2012. In the laboratory, aliquots of 15 ml were transferred directly from fresh samples, using glass volumetric pipettes, to Petri dishes, without concentrating the ciliates. The Petri dishes were then scanned under the stereomicroscope, in order to estimate the relative abundance of ciliate morphotypes and further isolate them for identification.

When necessary, simple limnetic cultures were made to increase the number of specimens used for microscopic techniques and facilitate their identification. Such cultures were made in Petri dishes with addition of water from the very samples plus crushed rice grains to promote the growth of bacteria, which serve as initial food source for the ciliates (Foissner et al. 2002; Paiva and Silva-Neto 2007).

The relative abundance of ciliates was estimated in the same day of sampling, using a subjective criterion based on the quantification of living specimens and its codification in scores (e.g. Mieszkowska et al. 2006). According to the approximate number of specimens observed by scanning the entire Petri dish, each containing the same water volume (15 ml), the codification in scores was: ● = rare (1–3 specimens); ●● = little frequent (4–9 specimens); ●●● = frequent (~10–20 specimens); ●●●● = abundant (>20 specimens). Such procedures, even though criticized for its supposed difficult replicability (Kent 2000), allow quick estimates for the construction of environmental quality indexes (e.g. Foissner 1992; Zelinka and Marvan 1961).

For identification, the ciliates were observed in vivo and after silver impregnation under bright field and phase contrast microscopy at magnifications of 100×, 200×, 400×, and 1000×. Silver-impregnation with protargol was performed according to the protocol of Wilbert (1975) and with silver nitrate following Klein (1926, 1958). Additionally, transmission electron microscopy preparations were made for *Cristigera hammeri* following Silva-Neto (1994). Illustration of the living specimen is a free-hand sketch. Diagramatic schemes of *C. hammeri* representing its kinetome were based on various photographs of protargol-impregnated specimens and assembled with aid of Adobe PhotoShop CS5 for the Windows 7 operational system. All measurements in Table 2 are in μm and were performed at 200× for living organisms and at 1000× for protargol-impregnated specimens. Descriptive statistics were calculated with the computer program GraphPad Prism 4 (Motulski 1999). The classification system and terminology adopted herein mostly follows Lynn (2008). The numbering system of the fragments of the somatic kineties is introduced in the present paper, namely, the somatic kineties SK2–SKn-1 are fragmented into five fragments, F1–F5, forming separate ciliary belts.

Results and Discussion

Composition of ciliate community

A total of 21 species, distributed in 15 genera, were found in the analyzed samples (Table 1). *Urocentrum turbo* was the most abundant species and the only one that was present in all samples, followed by *Cristigera hammeri*. *Caenomorpha medusula*, *Brachonella* sp., *Cristigera hammeri*, *Paramecium* sp., and *Tetmemena pustulata* were present in three of the samples, of which *Cristigera hammeri* was the most abundant.

Most of the ciliates found in the septic tank are known to occur in mesosaprobic and especially in polysaprobic environments. Of those, eight species are indicative of the polysaprobic zone (Foissner and Berger 1996; Kolkwitz and Marsson 1908, 1909; Sládeček 1973), which is characterized by low dissolved oxygen concentration and high dissolved organic matter (Bick 1963; Foissner and Berger 1996) (Table 1). Among the ciliates found, the armophoreans are adapted to life in anaerobiosis. Such organisms had their mitochondria transformed into hydrogenosomes along the course of their evolution (Fenchel and Finlay 1991a,b;

Table 1. Inventory and relative abundance of ciliates observed in the septic tank.

Species[a]	Class	September (2011)	January (2012)	February (2012)	May (2012)	October (2012)	Saprobity[b]
Brachonella elongata	AP	●●●	-	-	●●	●	p
Brachonella sp.	AP	●●●●	-	●	●●●	●	p
Caenomorpha medusula	AP	●●●●	-	●	●●●	●	p
Chilodonella caudata	PP	-	-	●●	●	-	?
Coleps hirtus	PS	-	-	●	-	-	a-b
Cristigera hammeri	OH	●●●●	-	●●●●	●●●●	●	?
Euplotes aediculatus	ST	-	●	●●	●●	●	a
Euplotes sp.	ST	-	●	-	-	-	
Loxodes striatus	KR	●●●●	-	-	-	●	p
Metopus sp. 1	AP	●	-	-	-	-	p
Metopus sp. 2	AP	-	-	-	●	-	p
Paramecium putrinum	OH	-	●	-	●●	-	p
Paramecium sp.	OH	-	●●	●●●	●●	●	?
Plagiopyla frontata	PY	●●	-	-	●	-	?
Plagiopyla sp.	PY	-	-	●	-	-	?
Saprodinium dentatum	PY	●	-	-	-	-	p
Spirostomum minus	HT	-	-	-	●●●	-	a–b
Spirostomum sp.	HT	-	-	-	●	-	?
Tetmemena pustulata	ST	●●●	●●	●●	-	-	b
Urocentrum turbo[c]	OH	●●●●	●●	●●●●	●●●	●●	a–b
Urosoma macrostyla	ST	-	●●●	●	●●	-	?

● – rare; ●● – little frequent; ●●● – frequent; ●●●● – abundant; - – absent; a – alpha-mesosaprobic; AP – Armophorea; b – beta-mesosapric; HT – Heterotrichea; KR – Karyorelictea; OH – Oligohymenophorea; p – polysaprobic; PP – Phyllopharyngea; PS – Prostomatea; PY – Plagiopylea; ST – Spirotrichea; ? – not determined.

[a] For authors and combining authors, see Foissner et al. (1995).

[b] Data from Foissner and Berger (1996).

[c] The typical morphotype, not the "*Urocentrum turbo*-like" found by Stoeck et al. (2007).

Lynn 2008) and are components of the sulfureta communities (Baas-Becking 1925; Dyer 1989; Fenchel 1987). Additionally, *Euplotes aediculatus*, *Loxodes striatus*, *Paramecium putrinum*, *Plagiopyla* spp., *Saprodinium dentatum*, *Spirostomum minus*, *Tetmemena pustulata*, and *Uriocentrum turbo* are also commonly found in stagnant waters of eutrophized environments (Foissner and Berger 1996). Remarkably, *E. aediculatus* is a considerably sensible indicator used for the assessment of heavy metal toxicity (Albergoni et al. 2000; Madoni 2000). The composition of the ciliate community in the studied septic tank is consistent with its water characteristics; which, even though measured only once, clearly indicate a hypoxic environment (Mortimer 1956). This reinforces the importance of ciliates as water quality indicators (Foissner and Berger 1996; Paiva and Silva-Neto 2004).

All species found in the present study are new occurrences for the state of Pará and, with exception of *Urosoma macrostyla* that was previously reported from soil samples from Manaus (Foissner 1997), are also new records for the Brazilian Amazonia. Some species found in the present survey have widespread geographic distributions, such as *Caenomorpha medusula*, *Coleps hirtus*, *Euplotes aediculatus*, *Loxodes striatus*, *Paramecium putrinum*, *Saprodinium dentatum*, *Spirostomum minus*, *Tetmemena pustulata*, and *Urocentrum turbo* (Foissner et al. 1991, 1992, 1994, 1995). The fact that they have not been reported previously in this region reflects how incipient is our current knowledge on ciliates and also on other free-living protists from the Brazilian Amazonia. Moreover, the findings of new populations of *Brachonella elongata*, previously known from the territory of the former Soviet Union (Jankowski 1964; Koval'chuk 1999), and *Cristigera hammeri* that was known only from its type location, namely Lake Ontario in Canada (Wilbert 1986), further emphasize the importance of taxonomic surveys on expanding the knowledge of biogeography of protists. Preliminary investigations on the diversity of ciliates from other environments in the state of Pará indicate that their occurrence and distribution is mostly heterogeneous (unpublished data), exhibiting variations consistent with the influence of geographic barriers in a microscopic scale, which facilitate the establishment of characteristic ciliate assemblages according to the microhabitat.

Characterization of *Cristigera hammeri* Wilbert, 1986

Subclass Scuticociliatia Small, 1967

Order Pleuronematida Fauré-Fremiet in Corliss, 1956

Family Cyclidiidae Ehrenberg, 1838

Genus *Cristigera* Roux, 1899

Improved diagnosis: Body 44–55 × 25–33 μm in vivo; oral apparatus about 38% of body length; 16–19 somatic kineties (SK); SK1 and SKn composed of three to four monokinetids posteriorly; SK2–SKn-1 divided in five longitudinal fragments (F); ventral gap between F4 of SK2

and SKn-1 measuring about 14 μm; one (rarely two) caudal cilia; one or two (rarely three) macronuclear nodules and one micronucleus.

Remarks: The diagnosis is based on the original description (Fig. 1d–f) and the population from Belém, Brazil (Figs 1a–c, 2–5). Type material was deposited by Prof. Dr. Norbert Wilbert in the Institut für landwirtschaftliche Zoologie und Bienenkunde der Universität Bonn (Wilbert pers. comm.), while voucher slides of *C. hammeri* from Belém are deposited in the collection of Laboratório de Protistologia, Dept. de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Morphology of the population from Belém, PA, Brazil (Table 2; Figs 1a–c, 2–5). Body doliform in outline, dorsoventrally flattened 3:1 and exhibiting the characteristic ventral groove (Figs 1a–c, 2, 3). Anterior region transversely truncated and without cilia; posterior region with an inconspicuous protuberance at its right margin (Fig. 3c). Pellicle inflexible. Cytoplasm green colored anteriorly, probably due to ingested algae, and hyaline below midbody. Single contractile vacuole located on right side of body, at approximately 30% of its length (Fig. 2b). Dorsal argyrome with columns of four relatively long rectangular cortical alveoli, delimited by ectoplasmic crests (Fig. 3f–g); ventral argyrome not observed due to insufficient impregnation. Cortex with an epiplasmic layer and a further bundle of cortical microtubules adjacent to it, probably of post-ciliar origin (Fig. 4f), which delimitates the epiplasm from the mitochondria immediately below. As shown by electron micrographs, chondriome formed by numerous relatively large mitochondria bearing dense tubular cristae. Mitochondria mostly located adjacent to cortex and associated to ectoplasmic crests (Figs 4, 5a, d). Rough endoplasmic reticulum placed below mitochondria (Fig. 4b). Cytoplasm with prokaryote endosymbionts and trichocyst extrusomes (Fig. 5b–d).

Oral apparatus associated to ventral groove, 15–20 μm long, with three membranelles composed of imperfectly cohered cilia (Figs 1, 2g–h). Paroral membrane hook-shaped, with about 20–22 μm long cilia, and located 4–6 μm far from anterior region. Scutica permanent, formed by three to five basal bodies, located immediately below paroral membrane or slightly left of its proximal end.

Somatic ciliature formed by 16–18 somatic kineties (SK1–SKn), composed of densely packed dikinetids at anterior region of body, followed by spaced monokinetids (Figs 1a–c, 2). SK1 and SKn composed anteriorly of 10–12 dikinetids plus one monokinetid, and three or four (three on average) spaced monokinetids posteriorly. SK1 interrupted at equatorial region of the body. SK2 to SKn split into five fragments (F1–F5) along antero-posterior body axis. First fragment (F1) extending from distal region to approximately proximal end of paroral and formed by 8–14 dikinetids followed by a monokinetid; F2 and F3 formed by two equatorial belts of monokinetids with basal bodies probably unciliated or bearing rudimentary cilia; F4 located below equator of body and composed of short lines of 4–6 monokinetids, shortest lines located in the dorsal region; F5 composed of isolated monokinetids, encircling posterior region of body. Some specimens present three cilia located next to the right margin of ventral groove, between F4 and F5 (Fig. 2f). Cilia of F1, F4, and F5 about 10 μm long. One (rarely two) ca. 26 μm long caudal cilium, arising next to posterior process and conspicuous in live specimens (Figs 2c, 3b).

Nuclear apparatus located at equatorial region of body and composed of two macronuclear nodules in 49 of 50 analyzed specimens; a single specimen with three nodules (Fig. 3d). Macronuclear nodules usually attached to each other, either along antero-posterior body axis or perpendicular to it; however, in some specimens placed about 15 μm away from each other and perpendicular to body length. Spherical to roughly ellipsoid or ovate, measuring 4–8 × 5–8 μm after protargol-impregnation, with typical karyomembrane and numerous chromatin condensations (Figs 3d–e, 5e–f). Micronucleus not observed.

Comparison with related species

Among the family Cyclidiidae Ehrenberg, 1838, the representatives of the genus *Cristigera* are characterized by a dorsoventrally flattened body, the peristomial region occupying approximately half of the body length, and by the presence of a ventral groove where the paroral membrane is placed. Somatic kineties are usually uniform and their kinetids are more densely packed anteriorly (Carey 1992; Curds et al. 1983; Lynn and Small 2002). The literature contains 15 valid nominal species of *Cristigera* (Jamadar and Choudhury, 1988; Kahl 1928, 1931, 1933; Madsen 1931; Penard 1922; Roux 1899; Wilbert 1986) that can be assigned to two morphological groups based on the organization of the somatic kineties; namely, species bearing continuous kineties along the antero-posterior body axis (e.g. *Cristigera media* Kahl, 1928; *Cristigera vestita* Kahl, 1928) and species with fragmented kineties, forming basal body belts (e.g. *Cristigera pleuronemoides*; *Cristigera hammeri*) (Esteban and Olmo 1997; Fan et al. 2011; Kahl 1928, 1931; Wilbert 1986). Variations of the latter kinetome organization are also found in *Paurotricha cyclidiformis* Dragesco and Dragesco-Kernéis, 1991 and *Gymnocyclidium nabranicum* Alekperov, 2009a,b, which belong to monotypic genera (Alekperov 2009a,b; Dragesco and Dragesco-Kernéis 1991).

The population of *Cristigera* characterized in the present study was identified as *C. hammeri* because it has fragmented somatic kineties and mostly two macronuclear nodules, thus overlapping with data from Wilbert (1986). Interestingly, he reported the presence of one or two nodules, unlike the Brazilian population of *C. hammeri*, where specimens with one macronuclear nodule were not observed. In addition, the Brazilian isolate had 16–18 somatic kineties, which approximates the original description (19, interpreted from Wilbert's drawings in Fig. 10, p. 391). It is worthy of note that in the improved diagnosis of *C. pleuronemoides*, Esteban and

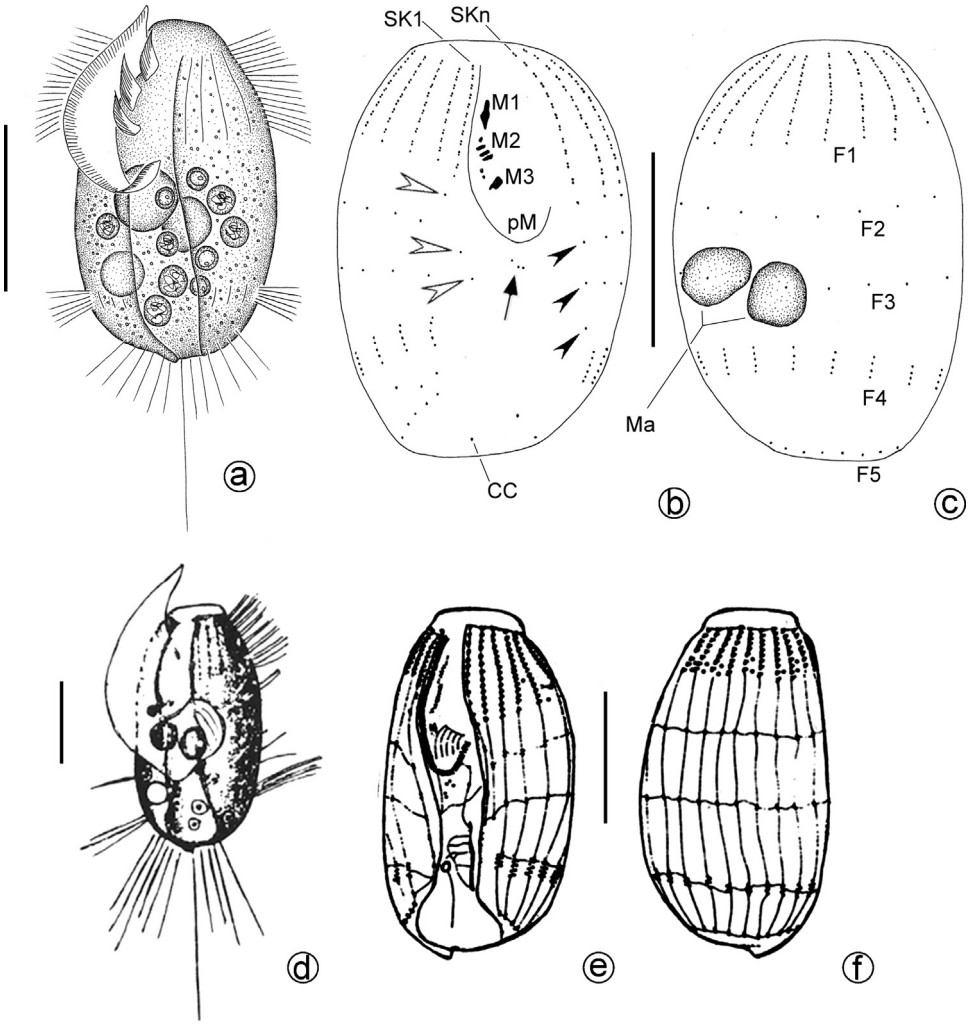

Fig. 1. Morphology of *Cristigera hammeri* in vivo (a, d), after protargol impregnation (b, c), and silver nitrate impregnation (e, f), showing the organization of the ciliature and the macronucleus (a–c, originals; d–e, from Wilbert 1986). **a, d**: Ventral side of live specimens. **b**: Ventral infraciliature; white arrowheads show the monokinetids from posterior region of somatic kinety 1; black arrowheads show the monokinetids from posterior region of somatic kinety n; arrow points to scutica. **c**: Dorsal infraciliature and macronucleus. **e**: Ventral argyrome. **f**: Dorsal argyrome. CC – caudal cilium; F1–5 – somatic kinety fragments 1 to 5; M1–3 – adoral membranelles 1 to 3; Ma – macronuclear nodules; pM – paroral membrane; SK1–*n* – somatic kineties. Scale bars = 20 μm.

Olmo (1995) wrote "19–23 kineties" (Esteban and Olmo 1997, p. 427 and Table 1) because they synonymized *C. hammeri*, *C. penardi*, and *Paurotricha cyclidiformis* with *C. pleuronemoides*; thus included the data from Roux (1899), Penard (1922), Dragesco and Dragesco-Kernéis (1991), and Wilbert (1986). Later in the same paper, Esteban and Olmo (1997) mentioned that "21 to 23 kineties form the somatic infraciliature" when referring to the Spanish population of *C. pleuronemoides* (Esteban and Olmo 1997, p. 428). We herein treat *C. hammeri* and *C. pleuronemoides* separately, as discussed below.

Unlike the type population, the micronucleus was not observed in specimens from Belém. The absence of micronucleus might be related to the occurrence of amicronucleate populations, which is a common phenomenon in *Tetrahymena* Furgason, 1940 (Lynn 2008; Quintela-Alonso et al. 2013), or, most likely, an artifact resulting from insufficient impregnation by the protargol. According to Wilbert (1986), the micronucleus of *C. hammeri* is located next to macronucleus and measures 2 μm in diameter.

To the present date, studies on the ultrastructure of *Cristigera* were performed only for the anaerobic species *Cristigera vestita* by Fenchel and Finlay (1991b). They found large, elongated hydrogenosomes located adjacent to epiplasm, like the mitochondria of *Cristigera hammeri.* Generally, the chondriome of *Cristigera hammeri* is similar to those of other scuticociliates of known ultrastructure (Lynn 2008). Hydrogenosomes were not found in *Cristigera hammeri*.

Among species of *Cristigera* exhibiting a fragmented somatic ciliature, only *Cristigera pleuronemoides* was studied with silver impregnation techniques (Esteban and Olmo 1997); thus new investigations are necessary in order to assess

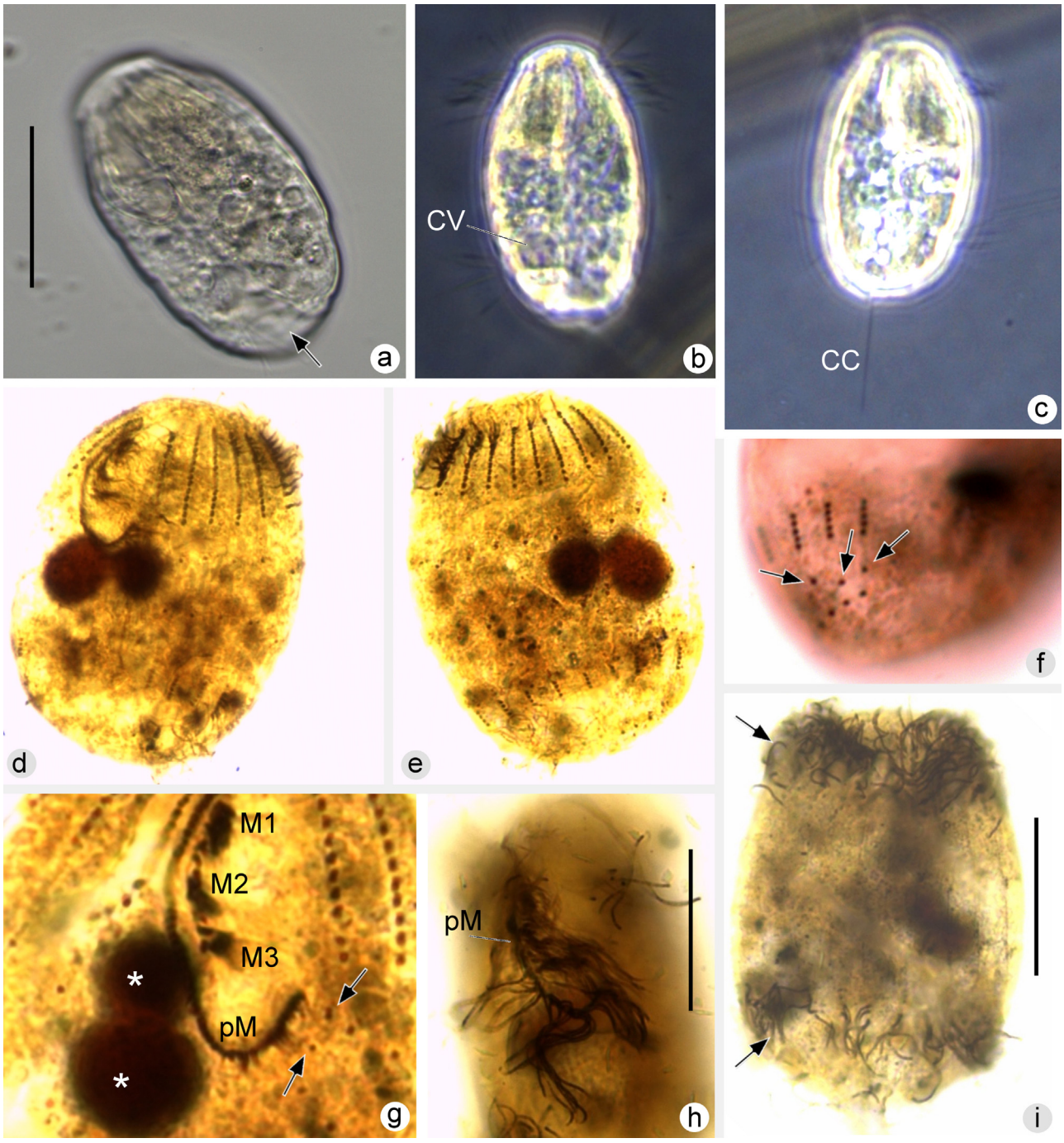

Fig. 2. Morphology of *Cristigera hammeri* from Brazil in vivo (a–c) and after protargol-impregnation (d–i). **a**: Posterior region of ventral gap (arrow). **b**: Contractile vacuole. **c**: Caudal cilium. **d, e**: Infraciliature and nuclear apparatus of same specimen in ventral and dorsal view, respectively. **f**: Kinetids located between the fragments F4 and F5 (arrows) of the somatic kineties. **g**: Detail of the oral apparatus. Arrows indicate the scutica, asterisks mark macronuclear nodules. **h**: Detail of the oral apparatus showing length of paroral and membranelles cilia. **i**: Detail of dorsal region showing length of somatic cilia. CC – caudal cilia; CV – contractile vacuole; M1–3 – adoral membranelles; pM – paroral membrane. Scale bars = 20 μm.

in detail the somatic ciliature of the other species assigned to the genus. Nevertheless, the present population of *Cristigera hammeri* differs from all known congeners in relation to the number of macronuclear nodules (two, rarely three vs. consistently one). *Cristigera hammeri* from Belém differs from *C. pleuronemoides* studied by Esteban and Olmo (1997) in the number of ventral kineties (16–18 vs. 21–23); the composition of SK1 behind the region with dikinetids (shortened at equatorial level, formed by three or four spaced monokinetids vs. long, formed by 6–10 dikinetids closely packed in a line);

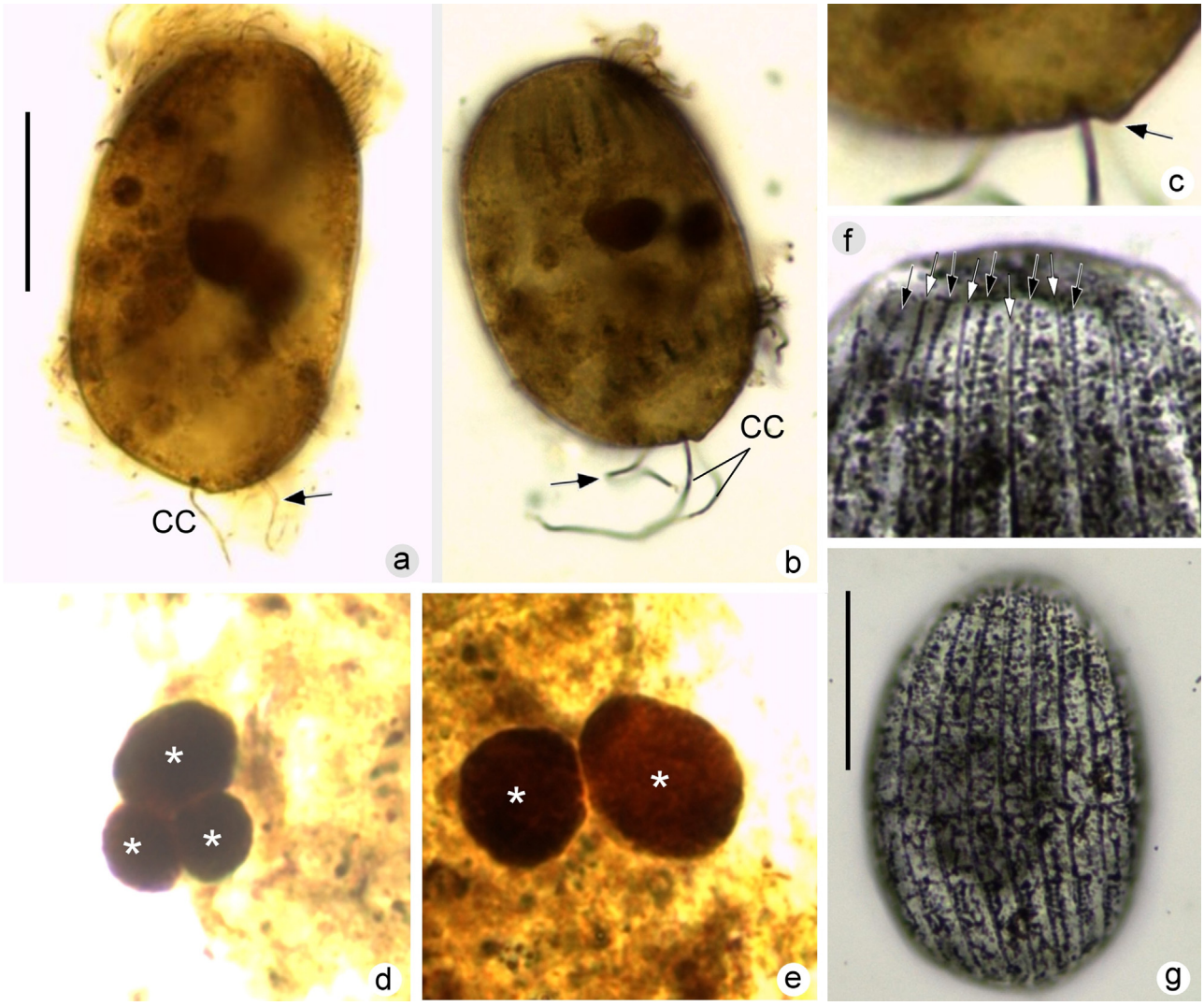

Fig. 3. Morphology of *Cristigera hammeri* from Brazil after protargol-impregnation (a–e) and dry silver nitrate-impregnation (f, g). **a, b**: Specimens showing one (a) and two (b) caudal cilia. Arrows point to cilia of somatic kinety fragment 5. **c**: Posterior protuberance. **d, e**: Specimens exhibiting three (d) and two (e) macronuclear nodules (asterisks). **f**: Detail of the anterior region of the dorsal argyrome. Black arrows indicate kineties, while white ones show lines of argyrome. **g**: Dorsal argyrome. CC – caudal cilium. Scale bars = 20 μm.

the composition of SKn behind the region with dikinetids (formed by three or four spaced monokinetids vs. formed by 6–10 dikinetids closely packed in a line); and the composition of basal body rows of F4, specially in the dorsal region of body (4–6 monokinetids vs. 5–10 dikinetids). It is worth mentioning that somatic kineties of *Cristigera pleuronemoides* are formed by dikinetids (Esteban and Olmo 1997). Likewise, Wilbert (1986) referred to the ciliature located below F1 of *C. hammeri* as basal body pairs, describing those in F4 as arranged in zig-zag. In the present study, the kinetids located behind F1 are monokinetids, with those of F4 forming straight lines.

In addition to the kinetome, the ventral argyrome documented by Wilbert (1986) for *C. hammeri* is seemingly simpler than that of *C. pleuronemoides*, because it apparently lacks the transverse commissures linking some kinetids of equatorial/posterior region of SK2 to the longitudinal silverline of SK1. In addition, *C. hammeri* has a simpler alveolar pattern in the ventral groove (cf. Fig. 5 in Esteban and Olmo 1997).

Cristigera hammeri from Brazil differs from *Paurotricha cyclidiformis* described by Dragesco and Dragesco-Kernéis (1991) in the number of macronuclear nodules (two, rarely three vs. one, respectively) and in the absence of fragments F2 and F3 in *P. cyclidiformis*. Instead of F2 and F3, *P. cyclidiformis* has one oblique row of kinetids, the ventral ciliary scarf. Remarkably and in agreement with *C. hammeri* from Belém, the somatic kineties of *P. cyclidiformis* are formed by dikinetids followed by one monokinetid in F1 (as interpreted from Fig. 8, p. 228 in Dragesco and Dragesco-Kernéis 1991) and by monokinetids in F4.

Finally, the Brazilian population of *Cristigera hammeri* differs from *Gymnocyclidium nabranicum* in the number of macronuclear nodules (two, rarely three vs. one), the

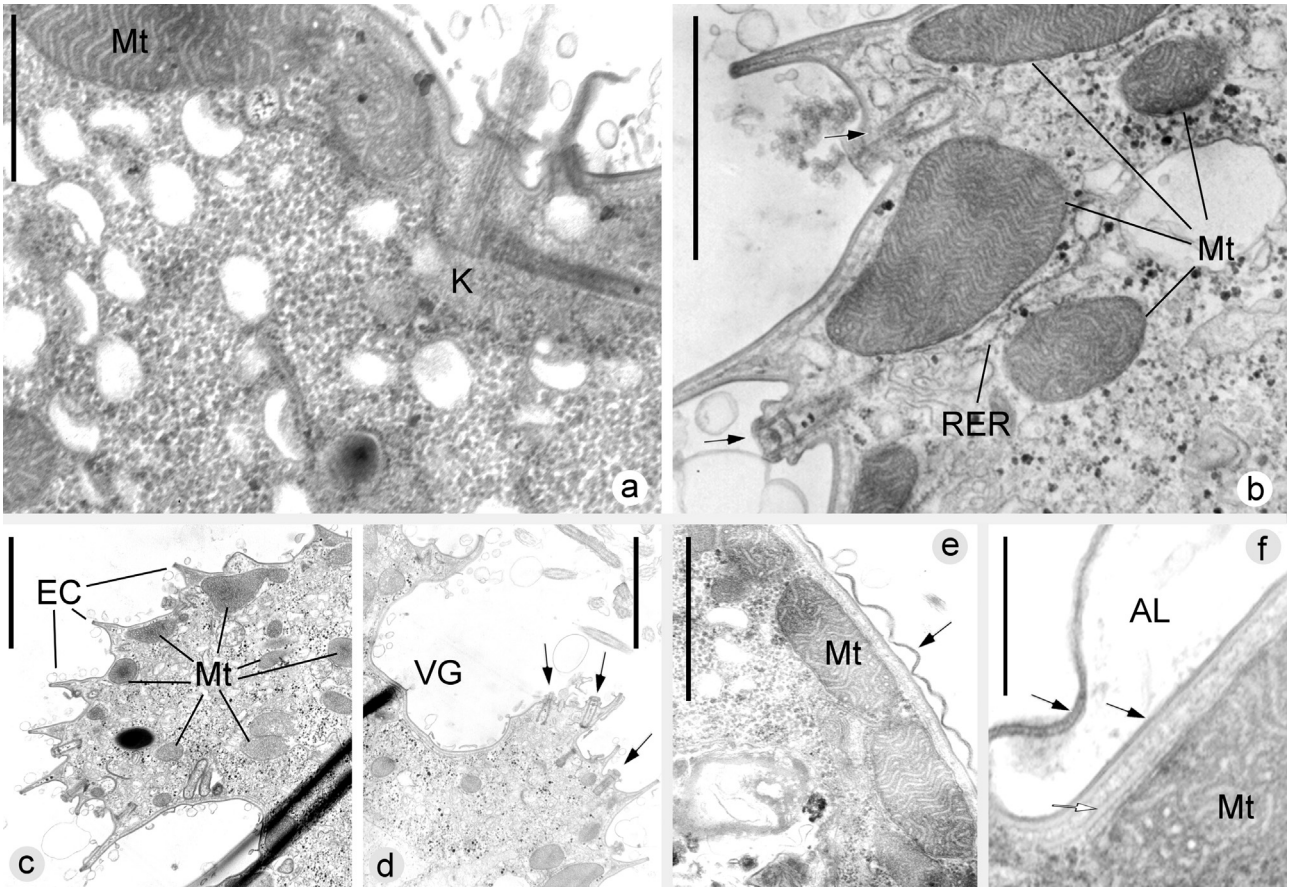

Fig. 4. Morphology of *Cristigera hammeri* from Brazil after transmission electron microscopy. **a**: Detail of a somatic kinetid. **b**: Region next to body surface, showing mitochondria. Arrows indicate basal bodies. **c**: Mitochondria located next to ectoplasmic crests. **d**: Basal bodies located close to ventral gap. **e**: Mitochondria adjacent to the cortex (arrow indicates the cell membrane). **f**: Detail of the cortex. Black arrows indicate sections of the cell membrane that border a cortical alveolum, while white arrow shows a ribbon of post-ciliary microtubles, located between a mitochondrion and the epiplasm. AL – cortical alveolum; EC – ectoplasmic crests; K – kinetid; Mt – mitochondrion; RER – rough endoplasmic reticulum; VG – ventral suture. Scale bars: a, f = 0.5 μm; b, e = 1 μm; c, d = 2 μm.

Table 2. Morphometric characterization of *Cristigera hammeri* from Belém, PA, Brazil.

Character[a]	Mean	*M*	SD	SE	CV (%)	Min	Max	*N*
Body length in vivo	48.5	50.0	3.5	0.9	7.3	44.0	55.0	15
Body width in vivo	29.2	30.0	3.2	0.8	11.0	25.0	33.0	15
Body length	47.0	45.5	4.4	0.6	9.4	40.0	56.0	50
Body width	31.1	32.0	4.1	0.6	9.4	24.0	42.0	50
Length of oral apparatus	17.9	18.0	1.2	0.3	6.8	15.0	20.0	20
Length of membranelles	9.5	10.0	0.9	0.2	10.0	8.0	11.0	20
Distance from apical pole to paroral	4.7	5.0	0.6	0.1	13.0	4.0	6.0	20
Number of dikinetids in anterior region of SK1	10.9	11.0	0.6	0.2	5.7	10	12	16
Number of dikinetids in anterior region of SKn	10.9	11.0	0.9	0.2	8.0	10	12	14
Number of monokinetids in posterior region of SK1	3.1	3.0	0.3	0.1	8.4	3	4	15
Number of monokinetids in posterior region of SKn	3.2	3.0	0.4	0.1	12.9	3	4	15
Number of somatic kineties	17.2	17.0	0.5	0.1	2.9	16	18	26
Ventral gap between F4 of SK1 and SKn	14.3	14.0	3.3	0.9	23.5	10.0	20.0	15
Number of macronuclear nodules	2.0	2.0	0.1	<0.1	7.0	2	3	50
Length of macronuclear nodules[b]	6.5	7.0	1.5	0.2	22.2	4.0	8.0	49
Width of macronuclear nodules[b]	6.6	6.5	0.9	0.1	13.5	5.0	8.0	49

[a] All measures are in μm. Data from protargol-impregnated specimens, unless indicated. CV – coefficient of variation; *M* – median; Max – maximum value observed; Mean – arithmetic mean; Min – minimum value observed; *N* – sample size; SD – standard deviation; SE – standard error.

[b] Measured in specimens with two macronuclear nodules.

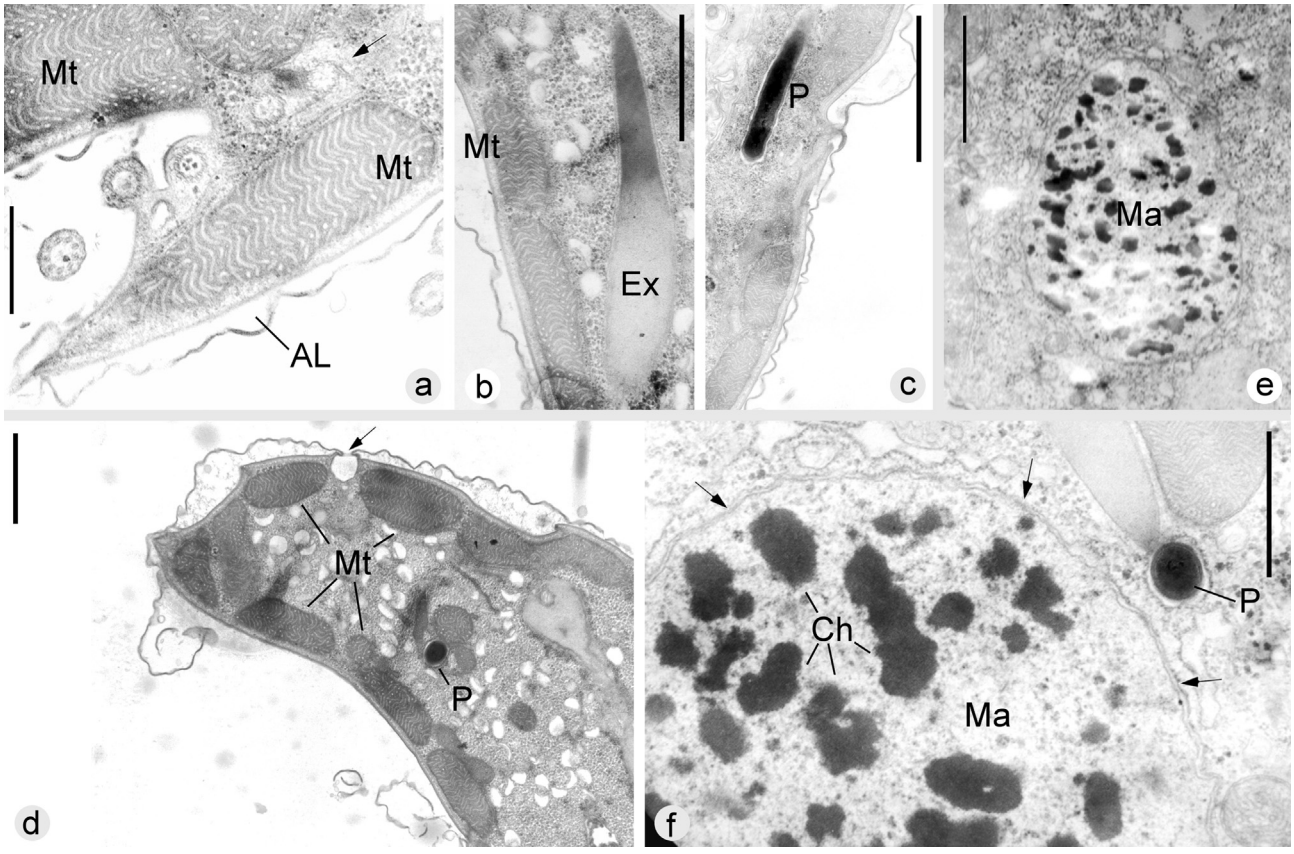

Fig. 5. Morphology of *Cristigera hammeri* from Brazil after transmission electron microscopy. **a**: Mitochondrion located in an ectoplasmic crest between somatic kineties (arrow). **b**: Section exhibiting long mitochondrion adjacent to cortex and an extrusome. **c**: Section showing a prokaryote endosymbiont. **d**: Arrangement of the mitochondria adjacent to the cortex. The arrow indicates a ciliary pit. **e, f**: Macronucleus. Arrows indicate karyomembrane. AL – cortical alveolum; Ch – chromatin condensations; Ex – extrusome (probably trichocyst); Ma – macronuclear nodule; Mt – mitochondrion; P – prokaryote. Scale bars: a = 0.5 μm; b, d–f = 1 μm; c = 2 μm.

extension of ventral gap between F4 from SK1 and SKn (about 8.5 μm vs. 10–20 μm), and in the absence of F2 and F3 in *G. nabranicum* (Alekperov 2009a,b). Based on the reduction of somatic kineties in *G. nabranicum*, Alekperov (2009a,b) established the family Gymnocyclidiidae, including also *Paurotricha cyclidiformis* and *Paracyclidium rhabdotectum* (Powers 1935) Grolière et al., 1980. The pattern of somatic kineties of *C. hammeri*, *C. pleuronemoides* (type species of *Cristigera*) and probably of *C. penardi* greatly resembles that of the Gymnocyclidiidae, thus suggesting that *Cristigera* is a potential member of that family. In that case, the species of *Cristigera* bearing continuous somatic kineties should be transferred to a genus allocated in the family Cyclidiidae.

Reconsideration of the synonymy of *Cristigera pleuronemoides* Roux, 1899

In their paper, Esteban and Olmo (1997) proposed the synonymy of *C. hammeri*, *C. penardi*, and *Paurotricha cyclidiformis* with *C. pleuronemoides* after a morphologic investigation of 40 specimens of the latter species, based on a population from the Guadarrama river (Madrid, Spain). Accordingly, the silver impregnation techniques do not unveil the whole ciliature in every specimen, hence inducing different interpretations by different authors when a relatively large number of specimens are not analyzed (Esteban and Olmo 1997). In the present study, it was possible to confirm the difficulty in impregnating some elements of the ciliature, such as the components of F2 and F3, as previously reported by Esteban and Olmo (1997). Even so, analysis of various specimens in our preparations allowed to verify the differences mentioned in the above section, which suggest the separation of both *C. pleuronemoides* and *C. hammeri* in two distinct morphological species, even though their discernment rely on relatively few features.

After examining the original drawings of *C. penardi* it can be noted that this species has a much less conspicuous ventral groove and relatively shorter paroral membrane, bearing shorter cilia than *C. pleuronemoides* (Kahl 1931; Penard 1922); suggesting that they could possibly be different species. However, proper evaluation of the synonymy of *C. penardi* with *C. pleuronemoides* needs reassessment of the former based on silver impregnation techniques.

The pattern of the somatic ciliature of *Paurotricha cyclidiformis* was not observed in any of the analyzed specimens in our study. The ventral ciliary scarf of *P. cyclidiformis* is an oblique row of kinetids that extends from the dorsal to the ventral side of the body and is typical of this genus and species, as described by Dragesco and Dragesco-Kernéis (1991). Although this pattern was reported by Esteban and Olmo (1997) in insufficiently impregnated specimens of *C. pleuronemoides*, this feature was not discussed in detail or illustrated in order to clarify the precedence of the kinetids that form the ventral ciliary scarf. Unfortunately, Dragesco and Dragesco-Kernéis (1991) found *P. cyclidiformis* only in protargol preparations and did not observe the species in vivo; therefore, the presence of the typical ventral groove of *Cristigera* could not be confirmed. In our opinion, further investigation is needed to support or disprove that *P. cyclidiformis* is a synonym of *C. pleuronemoides*.

Finally, the absence of the somatic kinety fragments F2 and F3 in *Gymnocyclidium nabranicum* was described by Alekperov (2009a,b) based on 10 specimens. Since, as already mentioned, the basal bodies in those fragments are sometimes difficult to impregnate with silver; thus, synonymy with *C. pleuronemoides* is probable. On the other hand, Alekperov (2009a,b) did not mention the presence of the typical ventral groove of *Cristigera* in the living cells of *Gymnocyclidium*. In conclusion, *G. nabranicum* must be investigated more extensively in vivo and after protargol-impregnation in order to verify whether F2 and F3 are actually lacking or their absence is the result of insufficient silver impregnation as discussed above.

Acknowledgements

This study is part of L.A.M. de Castro's bachelor dissertation, supervised by T. da S. Paiva. The authors are thankful to the colleagues from ICB-UFPA Cláudia Regina Batista de Souza, Edilene Oliveira da Silva and Rosildo Paiva for allowing the use of the equipment necessary for the development of the present study, to Fernanda Brasil dos Santos Lobo for her technical support, and to the editor and peer reviewers for their comments, criticism and suggestions. Financial support: DCR FAPESPA/CNPq (ICAAF 002/2013) and CNPq Universal 485974/2013-4.

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