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Description of *Epistylis riograndensis* n.sp. (Ciliophora: Peritrichia) found in an artificial lake in Southern Brazil

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Abstract

Epistylis riograndensis **n. sp.**, a freshwater peritrich hosting symbiotic algae in its cytoplasm, was collected from an artificial lake, in a Botanical garden in Southern Brazil. Its detailed morphology was investigated using live and silver-stained specimens. The colonial sessile *E. riograndensis* has elongate zooids measuring, on average, 162 µm in length and 45 µm in width. A single contractile vacuole located near the infundibulum and a C-shaped macronucleus located transversely in the adoral half of the cell were also observed. The oral infraciliature revealed in silver-stained specimens was typical of peritrich ciliates. Three infundibular polykineties consisting of 3 rows of kinetosomes were observed. Molecular analyses of 18s rDNA placed *E. riograndensis* among other *Epistylis* species in the Order Vorticellida.

Key words: peritrichs, morphology, freshwater, ciliates, 18s rDNA

Introduction

The genus *Epistylis* Ehrenberg, 1830 is commonly found in aquatic environments, including freshwater, marine, and terrestrial habitats. The approximately 100 described species (Lynn 2008) usually have elongated zooids, eversible peristomial lip, single turn of peristomial cilia, colonial habit, and absence of a spasmoneme inside the stalk. Species of *Epistylis* generally colonize non-living substrates, but some of them live as epibionts on aquatic invertebrates such as mollusks, crustaceans and insects (e.g. Sladecek 1986).

Species of *Epistylis* generally are colorless, with the record, so far, of only two species in the genus (*Epistylis chlorelligerum* and *Epistylis lalinensis*) harboring green algae in their cytoplasm. *Epistylis chlorelligerum* Shen, 1980 and *E. lalinensis* Qi *et al.* 2009 were originally described from freshwater environments in China. The former was found as epibiont on aquatic plants, and the latter was discovered colonizing the shell of a snail of the genus *Bellamya*. During a survey on freshwater peritrich ciliates in Southern Brazil we found a population of an undescribed species of *Epistylis* harboring green algae in its cytoplasm, which we named *Epistylis* **n. sp.** *riograndensis*. The present study provides a detailed description of *Epistylis riograndensis* n.sp. and compares it with other members of its genus, especially the two other known species of "green" *Epistylis*.

Material and methods

Morphological analyses. Seasonal sampling was carried out in an artificial lake in the Botanical Garden located in Porto Alegre municipality, the capital of Rio Grande do Sul state, Brazil. One Plexiglas® rectangular box (6 cm long X 3.5 cm wide) holding 12 cover-slips (22 X 22mm) was submerged in the lake for a period of seven days. At recovery time, traps were directly placed in a bottle filled with water from the site and taken to the laboratory. The cover-slips were analyzed using an Olympus CH30 microscope for the presence of peritrichs. Morphological characters used for peritrich identification, such as zooid length and width, basal stalk length and width, width of

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a wider peristomial lip (56 –78 µm; Wu *et al.* 2011), than the latter species (40–62 µm; Table 1). Oral polykinetids PK2 and PK3 of *E. chlorelligerum* consist of three rows of kinetosomes of different lengths (Wu *et al.* 2011), with rows 2 and 3 of PK3 extending beyond the adstomal end of PK1. These characteristics are not observed in *E. riograndesis* that presents rows of kinetosomes on both PK2 and PK3 of similar length (Figures 8, 9, and 11).

Living zooids of *E. riograndensis* and *E. lalinensis* are very similar in size (162 X 45 µm vs. 165 x 40 µm; Table 1, Qi *et al.* 2009). However, the pattern of kinetosomal rows in oral polykinetids of these two species is different. All oral polykinetids were composed of three rows of kinetosomes in both species, but row 1 of PK2 was shorter than the others in *E. lalinensis* (Qi *et al.* 2009), while all three rows of kinetosomes in PK2 were the same length in *E. riograndensis* (Figs 8 and 11).

The pattern of kinetosomal rows in oral polykineties has been used by several authors as a main character to distinguish species in the Subclass Peritrichia (e.g. Esteban & Fernandez-Galiano1989; Clamp 1990; Leitner & Foissner 1997; Clamp 2005; Sun *et al.* 2006). The number and relative lengths of rows and the points in which the rows terminate are very important for genus and species determination (Foissner & Brozek 1996; Sun *et al.* 2006; Gentekaki & Lynn 2010). In a study with different populations of *Carchesium polypinum* Gentekaki and Lynn (2010) reported a variation in the length of kinetosomal rows among isolates. This variation in morphology was in accordance with genetic differences found among populations, suggesting that these populations may represent different species. Therefore, we consider the results presented here as strongly supportive of a species-level distinction between *E. riograndensis* and *E. lalinensis*.

With respect to the molecular analyses we performed in this study, our results indicate that the *E. riograndensis* sequence is quite distinct from the other included *Epistylis* species, although it tends to strongly group with them when assessed with most phylogenetic methods (see Figure 12). Since *E. riograndensis* was collected from a Botanical Garden which receives plants from many parts of the world, molecular analyses comparing *E. riograndesis* and *E. lalinensis* species will help to evaluate their evolutionary relationships.

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