

ESCOLA DE CIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E EVOLUÇÃO DA BIODIVERSIDADE
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**AVALIAÇÃO TAXONÔMICA DA COMUNIDADE DE CILIADOS PERITRÍQUIOS
(CILIOPHORA:OLIGOHYMENOPHOREA: PERITRICHIA) EPIBIONTES DE
INVERTEBRADOS E DE PLANTAS EM ECOSISTEMAS LIMNICOS SUBTROPICAIS**

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Pontifícia Universidade Católica
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DISSERTAÇÃO DE MESTRADO

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RESUMO

Atualmente, grande parte dos ecossistemas do planeta está com sua diversidade biológica ameaçada. Vários fatores, - tanto na ordem biológico-ambiental, como no âmbito antrópico-, estão interagindo e ocasionando essa mudança na arquitetura de vida nos diferentes ambientes da Terra. Os ecossistemas aquáticos, em especial os dulceaquícolas, são pontos bastante sensíveis dentro dessa teia de degradação. Dessa forma, estudos focados na fauna e na flora residentes nesses locais são de fundamental importância para que planos de manejo e de conservação sejam propostos e executados. Eucariotos unicelulares, principalmente os pertencentes ao Filo Ciliophora, estão amplamente distribuídos em ecossistemas de água doce, podendo ser utilizados, devido a suas características biológico-fisiológicas (como a rápida resposta que esses organismos possuem às mínimas modificações sofridas nos habitats onde residem), como excelentes ferramentas em trabalhos de monitoramento aquático.

Ciliados peritríquios estão distribuídos nas diferentes variedades de ambientes de água doce, podendo constituir relações ectossimbióticas com os diversos organismos vivos aquáticos. Entretanto, estudos acerca desses organismos, desde o âmbito taxonômico, até das características de vida e ecológicas desses eucariotos são escassos na literatura específica. Assim, o objetivo central da presente pesquisa é trazer descrições detalhadas da morfologia de ciliados peritríquios (tanto *in vivo*, como em organismos fixados) epibiontes de invertebrados e de plantas de ecossistemas límnicos, provendo um maior subsídio a estudos futuros pertencentes às diversas áreas científicas, que possam utilizar essas informações básicas como instrumento.

Palavras-chave: Epibiose, *Vorticella*, *Pomacea canaliculata*, *Salvinia auriculata*, ambientes límnicos subtropicais.

ABSTRACT

Currently, the biological diversity of different ecosystems worldwide is jeopardized. Several factors, including anthropic impact, are interacting and causing a change in the architecture of life in different environments of the Earth. Aquatic ecosystems, especially freshwater habitats, are very sensitive points within this web of degradation. Thus, studies focused on the fauna and flora inhabiting these places are of fundamental importance for management and conservation plans to be proposed and implemented. Unicellular eukaryotes, mainly those belonging to the Phylum Ciliophora, are widely distributed in freshwater ecosystems and could be used as environmental indicators, due to their biological and physiological characteristics (such as the rapid response that these organisms possess to the minimal changes they suffer in the habitats where they live).

Peritrich ciliates are distributed in different varieties kinds of freshwater environments, being able to constitute ectosymbiotic relationships with diverse living aquatic organisms. However, studies of these epibiont organisms from the taxonomic scope, and with respect to their life cycle, and ecological characteristics are scarce in the specific literature. Thus, the central aim of the present research is to provide detailed descriptions of the morphology of peritrich ciliates (both *in vivo* and from fixed organisms), epibionts of invertebrates and plants in freshwater ecosystems, providing valuable information to future studies from different scientific areas, that could use this basic information as an instrument.

Keywords: Epibiose, *Vorticella*, *Pomacea canaliculata*, *Salvinia auriculata*, subtropical freshwater environments

APRESENTAÇÃO

A presente pesquisa, em linhas gerais, visa identificar a associação ectossimbiótica existente entre ciliados peritríquios e invertebrados ou plantas de ambientes límnicos do estado do Rio Grande do Sul. Atualmente, estudos específicos acerca das relações epibióticas que envolvem organismos residentes de ambientes aquáticos são escassos, especialmente os que abrangem a interação entre ciliados e a biota local. Dessa forma, trabalhos no âmbito taxonômico acerca dos organismos pertencentes a essa interação são imprescindíveis, podendo ser utilizados como ferramentas para estudos de manejo, bioindicação e qualidade dos ecossistemas aquáticos globais.

As informações obtidas ao longo desse estudo estão organizadas em 4 capítulos. O primeiro traz uma introdução geral acerca da epibiose e dos organismos envolvidos nessa relação (redigido nas regras propostas pelo Associação Brasileira de Normas Técnicas-ABNT), o segundo aborda um estudo sobre a relação epibiótica entre moluscos límnicos e duas espécies não descritas de *Vorticella*, num manuscrito intitulado “Description of two new species of the genus *Vorticella* (Ciliophora:Peritrichia) epibionts on *Pomacea canaliculata* (Mollusca:Ampullariidae:Gastropoda) in Southern Brazil” (artigo redigido nas normas da revista *The Journal of Eukaryotic Microbiology*); o terceiro, intitulado “A new epibiont peritrich ciliate from subtropical freshwater environment: *Vorticella ovalistriata* n.sp. (Ciliophora: Oligohymenophorea: Peritrichia)” (artigo redigido nas normas da revista *Zootaxa*); e o último que traz a conclusão geral e as perspectivas futuras a respeito da presente pesquisa (redigida nas normas da ABNT).

- Capítulo I-

INTRODUÇÃO GERAL

INTRODUÇÃO GERAL

Epibiose é um tipo de interação ectossimbiótica, caracterizada pela associação de um organismo vivo (chamado de epibionte), em, pelo menos, alguma fase de seu ciclo de vida, à superfície externa de outro (denominado de basibionte ou hospedeiro), que serve como substrato vivo dessa relação (Negreiros-Fransozo *et al.* 1995, Wahl & Mark 1999, Threlkeld *et al.* 1993, Utz & Coats 2005, Delgery *et al.* 2006, Fernandez-leboranz & Von Rintelen 2007, Utz 2007, Cabral *et al.* 2010, Corbi *et al.* 2016). Esse tipo de interação é dependente de locais úmidos, sendo amplamente distribuída em ambientes dulceaquícolas, marinhos e estuarinos, mas também há casos relatados em ambientes terrestres, como em florestas e outros ecossistemas onde sombra e umidade prevalecem (Wahl & Mark 1999, Zotz 2001, Utz & Coats 2005, Utz 2007).

Classicamente, a epibiose vem sendo entendida como uma variação da interação mutualística (Delgery *et al.* 2006, Corbi *et al.* 2016). Entretanto, atualmente, sabe-se da existência de um gradiente ao longo de várias interações biológicas, que culmina na existência de fases ao longo da história coevolutiva dos organismos envolvidos nesse processo. Dessa forma, a epibiose, de acordo com este modelo, transcorre, inicialmente, de um processo que pode ser deletério aos organismos envolvidos (geralmente para o basibionte, em função, principalmente, da competição com os epibiontes), até uma fase em que estes viverão harmonicamente, em vista da interação mutualística, - recorrente do processo coevolutivo efetuado historicamente (Barea-Arco *et al.* 2001, Delgery *et al.* 2006, Corbi *et al.* 2016).

Dentro do entendimento da ocorrência de epibiose num gradiente espaço-temporal, essa interação pode ser classificada de duas formas, de acordo com o grau de dependência existente entre epi e basibionte. A relação epibiótica é chamada de obrigatória, quando o organismo epibionte consegue sobreviver unicamente sobre o substrato vivo basibionte; caso o epibionte consiga sobreviver, também, sobre substratos não vivos a relação é denominada de não-obrigatória ou facultativa (Clamp 1973, Gilbert & Schröder 2003, Utz 2008). No caso das interações obrigatórias, a coevolução existente entre os componentes envolvidos é o mecanismo, provavelmente, responsável pela manutenção desse sistema biológico viável (Al-Dhaheeri & Willey 1996).

De acordo com a natureza do basibionte, o epibionte pode ser classificado de duas maneiras: epifítico, caso o basibionte seja uma alga ou planta (Wahl & Mark 1999, Zotz et al. 2001, Cigliano *et al.* 2007, Harder 2008); ou epizoóico, caso o substrato vivo seja um animal (Wahl & Mark 1999, Harder 2008). Musgos, samambaias, orquídeas e bromélias são basibiontes vegetais bastante recorrentes na literatura (Johansson 1974, Zotz *et al.* 2001, Zotz 2005); enquanto poríferos; cnidários; rotíferos; moluscos (gastrópodos, principalmente); crustáceos como copépodos (Calanoida, Cyclopoida e Haparticoida), cladóceros, isópodos e mysidáceos; larvas de insetos (Diptera, Trichoptera, Ephemeroptera e Odonata); peixes, girinos e tartarugas de ambientes límnicos são metazoários frequentemente relatados como basibiontes (Henebry & Ridgeway 1979; Baldock 1985, Cook 1988, Kankaala 1987, Daivis *et al.* 1994, Negreiros-Fransozo *et al.* 1995, Wahl & Mark 1999, Leborans & Tato-Porto 2002, Fernandez-Leborans 2004, Gong *et al.* 2005, Delgery *et al.* 2006, Dias *et al.* 2006, Utz & Coats 2005 e 2008, Fernandez-Leboranz & Von Rintelen 2007, Utz 2007, Vasconcelos *et al.* 2007, Dias *et al.* 2008, Cabral *et al.* 2010, Azevedo *et al.* 2013, Chatterjee *et al.* 2013, Sedlacek 2013, Corbi *et al.* 2016). Existem representantes de grupos variados vivendo como epibiontes sobre outros organismos vivos: procariontes (bactérias e cianobactérias); algas; ciliados (peritríquios, suctórios, conotriquídeos, heterotríquios); poríferos; cnidários (antozoários); platielmintos; nemertinos; rotíferos; nematódeos; moluscos (gastrópodos e bivalvíos); anelídeos; briozoários; foronídeos; branquiópodos; crustáceos (cirripédios, principalmente); equinodermos, hemicordados e urocordados (Green 1974, Holland & Hergenrader 1981, Negreiros-Fransozo *et al.* 1995, Poltz *et al.* 1998, Wahl & Mark 1999, Fernández-Leborans & Tato-Porto 2000, Barea-Arco *et al.* 2001, Williams & McDermott 2004, Utz & Coats 2005, Fernandez-Leboranz & Von Rintelen 2007, Utz 2007, Utz & Coats 2008, Cupul-Magaña *et al.* 2011, Corbi *et al.* 2016).

Sabe-se que os epibiontes respondem fortemente a pequenas variações no ambiente, sendo, por isso, utilizados em estudos de bioindicação e monitoramento nos diversos ecossistemas do planeta. Epibiontes unicelulares, como bactérias e ciliados, são ferramentas poderosas utilizadas nesse tipo de trabalho, pois além de responderem às variações locais, são componentes envolvidos no mecanismo conhecido como “alça microbiana” em cadeias tróficas aquáticas, possuem um rápido ciclo reprodutivo e são mantidos com facilidade em culturas laboratoriais (Burbanck 1967, Small 1973, Carins 1978).

Apesar de a epibiose ser considerada por muitos uma variante da relação comensal, existem relatos na literatura de efeitos deletérios causados, principalmente, ao basibionte em função dessa interação (Green 1974, Xu & Burns 1991 Utz & Coats 2005).

Em 1974, Green já observava mudanças diretas no comportamento e nas funções vitais do basibionte com relação ao epibionte que vive associado à sua superfície externa. O sistema observado, neste caso, incluía o cladóceros *Daphnia magna* que servia como substrato vivo para o rotífero *Brachionus rubens*, onde o rotífero competia diretamente com o cladóceros por alimento. Quando a colonização do crustáceo pelo rotífero mostrava-se elevada, a competição entre basibionte e epibionte tornava-se conspícua, demandando, conseqüentemente, muita energia do crustáceo para amenizar esse estresse, diminuindo significativamente a produção de ovos nas fêmeas de *D. magna*.

O trabalho de Utz e Coats (2005) mostrou que a colonização do copépodo calanóide *Acartia tonsa* pelos peritríquios *Epistylis* sp. e *Zoothamnium intermedium* na Chesapeake Bay (E.U.A.) não ocorria concomitantemente no seu hospedeiro (sugerindo uma competição espacial com exclusão de uma das espécies de epibiontes). *Epistylis* sp. provavelmente sofria exclusão competitiva devido ao poder de contração da colônia observado em *Zoothamnium intermedium*. Outro dado interessante obtido nesse estudo foi a presença de epibiontes somente em adultos e em copepoditos. Esse fato pode ser consequência direta dos seguintes fatores: a) diminuição da frequência de ecdises na fase de copepodito à fase de copépodo adulto; b) baixa exposição dos crustáceos na fase de copepodito a potenciais predadores.

Outro exemplo de competição entre epibiontes por espaço foi observado no estudo realizado por Ebert *et al.* 2001. Os peritríquios *Vorticella octava*, *Epistylis helenae*, junto com o euglenídeo *Colacium vesiculosum* competiam interespecificamente por espaço, tendo como basibionte uma espécie do gênero *Daphnia*. Em função de certa separação espacial, *E. helenae* e *C. vesiculosum* não competiam entre si. Entretanto, essas duas populações competiam significativamente com *V. octava* (que se mostrou um competidor inferior, provavelmente, devido à contratibilidade de seu pedúnculo basal) por espaço na carapaça do crustáceo.

De um modo geral, o epibionte parece ser o detentor maior de benefícios diretos da relação epibiótica. Atualmente, as mudanças mais comumente mencionadas na literatura, em relação ao basibionte são: vulnerabilidade a predadores em função do comprometimento da movimentação, diminuição do *fitness* reprodutivo, dificuldade na obtenção de alimentos e

oxigênio, competição por recursos entre basi e epibionte (Green 1974, Xu & Burns 1991 Utz & Coats 2005, Corbi *et al.* 2016).

A relação epibiótica também pode ser afetada por variáveis abióticas. A turbidez da água (por afetar a taxa de predação dos organismos), temperatura (por ocasionar variações sazonais de basi e epibiontes), taxas de oxigênio dissolvido (por basi e epibiontes reagirem de diferentes formas às variações temporais desse recurso), variação da salinidade (por ser deletéria dependendo da capacidade osmorregulatória dos organismos), exposição à radiação U.V. (que age em diferentes vias metabólicas dos organismos), são exemplos de fatores ambientais que determinam a distribuição espacial de epibiontes em seus basibiontes, e, também, dos basibiontes especificamente (Chiaveli *et al.* 1993, Periss & Labourn-Parry 1997, Lopez *et al.* 1998, Elloumi *et al.* 2006, Barea-Arco 2001, Utz & Coats 2005).

Dentre os diferentes grupos existentes em Ciliophora, a subclasse Peritrichia é uma das mais conhecidas, pois está representada em abundância nos diferentes ambientes aquáticos, sendo amplamente documentada em relações simbióticas com outros organismos (Baldock 1985, Utz & Coats 2005, Lynn 2008, Utz *et al.* 2014). Ciliados peritríquios são caracterizados pela presença de ciliatura somente na região oral, presente entre o lábio peristomial e a base da região infundibular, sendo a ciliatura somática restrita à região da banda trocal. São exemplos de ciliados peritríquios os gêneros *Vorticella*, *Epistylis*, *Rhabdostyla*, *Zoothamnium*, *Carchesium*, *Platycola*, entre outros (Lynn 2008). Baseado em análises moleculares, a subclasse Peritrichia está atualmente dividida em duas ordens (todas provenientes de Sessilida): Operculariida e Vorticellida (Utz *et al.* 2010), que abrigam os diferentes gêneros de peritríquios coloniais e solitários. Ciliados peritríquios, até pouco tempo, eram posicionados filogeneticamente próximos aos ciliados tigmotríquios devido à similaridade bucal entre ambos os grupos, e uma possível homologia existente entre a escópula de peitríquios e a área tigmotática dos tigmotríquios. Todavia, atualmente, em função de estudos morfológico-moleculares, a subclasse Peritrichia é reconhecida como grupo-irmão da subclasse Hymenostomatia (Utz & Eizirik 2007).

Muitos representantes de Peritrichia possuem relação epibiótica com outros organismos, sendo que os mais reportados na literatura são os gêneros *Epistylis*, *Vorticella*, *Rhabdosthyla*, *Zoothamnium*, *Carchesium* e *Opercularia* e *Epistylis* (Dias, Ávila & D'Agosto 2008, Utz 2007, Utz 2008, Rodrigues, Rogério & Oliveira, 2011, Azevedo *et al.* 2014, Corbi *et al.* 2016). Sabe-se que vários grupos vegetais e animais servem como

substrato para peritríquios epibiontes, ex. algas, pteridófitas, poríferos, cnidários, rotíferos, moluscos, anelídeos, tardígrados, crustáceos, larvas de insetos, peixes, girinos e tartarugas dulceaquícolas (Utz & Coats 2005, Fernandez-Leboranz & Von Rintelen 2007, Utz 2007, Cabral *et al.* 2010, Corbi *et al.* 2016) são hospedeiros geralmente citados na literatura.

Assim como o conhecimento das relações existentes entre peritríquios e outros organismos são importantes para o entendimento do ecossistema aquático; informações a respeito da vida vegetativa desses ciliados são indispensáveis para a caracterização dos espécimes e discussões sobre aspectos taxonômicos e ecológicos desses eucariotos. Medidas e formas do zoóide, dos macro e micronúcleos, da colônia, e de outras estruturas somáticas são fundamentais para determinação específica dos organismos. Estudos moleculares e da conformação da infraciliatura oral destes organismos são essenciais na descrição de espécies em Peritrichia (Utz 2007, Utz *et al.* 2010, Utz *et al.* 2014). Técnicas de impregnação em prata em peritríquios, que revelam a forma e a disposição da infraciliatura oral desses ciliados, são ferramentas poderosas para a distinção, definição e recharacterização de espécies em Peritrichia (Norf & Foissner 2009 e 2010). Dessa forma, estudos nesse âmbito, somados com análises moleculares são imprescindíveis para resolução taxonômica dos diferentes *taxa* contidos em Peritrichia (Norf & Foissner 2010).

Dessa forma, o objetivo do presente estudo é identificar a associação epibiótica que ocorre entre ciliados peritríquios e invertebrados e/ou plantas de ambientes dulceaquícolas subtropicais. Para isso, a caracterização morfológica desses organismos foi realizada, resultando numa maior acuidade taxonômica dos espécimes trabalhados. Estudos nesse âmbito são essenciais para uma redefinição taxonômica dentro de Peritrichia, bem como subsidiar trabalhos nas áreas de conservação e manejo de ambientes onde essa relação está representada.

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- Capítulo II -

Description of two new species of the genus *Vorticella*
(Ciliophora: Peritrichia) epibionts on *Pomacea canaliculata* (Mollusca: Ampullariidae:
Gastropoda) in Southern Brazil

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Description of two new species of the genus *Vorticella* (Ciliophora: Peritrichia) epibionts on *Pomacea canaliculata* (Mollusca: Ampullariidae: Gastropoda) in Southern Brazil

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Summary

Vorticella veloxiiforme **n.sp.** and *Vorticella ampullariiae* **nsp.** are freshwater ectosymbiotic peritrich attached to *Pomacea canaliculata* collected from Varzinha, in Rio Grande do Sul state, Brazil. The detailed morphology of both species was investigated using live and silver-stained specimens. *V. veloxiiforme* has a cup-shaped zooid measuring, on average, 57.5 μm in length and 42.5 μm in width, and a J-shaped macronucleus. *V. ampullariiae* has elongate zooid measuring, on average, 67.5 μm in length and 25 μm in width, presenting a C-shaped macronucleus, two contractile vacuoles and a lateral globular projection on the peristomial lip. The pattern of oral polykineties revealed in silver-stained specimens was typical of peritrich ciliates, with three rows of kinetosomes. Comparisons with similar species of *Vorticella* are provided.

Key words: *Vorticella*, epibiosis, morphology, *Pomacea canaliculata*

Introduction

Vorticella Linnaeus, 1767 is a peritrich genus that presents a bell-shaped zooid and a main stalk containing a spasmoneme. The genus is characterized by its solitary habit and its ability to contract, and is found in marine and freshwater habitats, as free-living or symbiotic with animals and plants (Warren 1986, Fernandez-Leboranz 2004, Sun 2015). Morphological identification of species morphological identification in *Vorticella* is based on somatic characters, such as shape, size and striation of the zooids, shape and size of the macronucleus, presence of endosymbionts, size of the basal stalk, among others. The arrangement of the oral polykinetids inside the infundibulum, revealed by the silver impregnation technique, is also an important taxonomic feature to separate different species.

Several species of *Vorticella* have been reported as epibionts on aquatic invertebrates such as crustaceans, mollusks, insect larvae, sponges, cnidarians, and annelids (Henebry & Ridgeway 1979, Baldock 1986, Cook *et al.* 1998, Fernandez-Leboranz 2004, Chatterjee *et al.* 2013, Sedlacek *et al.* 2013, Bielecka & Boehnke 2014), as well as on aquatic algae (Pratt & Rosen 1983, Nagasawa & Warren 1996).

Among aquatic invertebrates, crustaceans and mollusks are the most frequently hosts reported in the literature (Henebry & Ridgeway 1979, Bielecka & Boehnke 2014). The mollusk species *Pomacea canaliculata* Lamarck, 1822 is an ampullariidae gastropod native from South and Central Americas, that inhabits natural or artificial freshwater environments (Vega *et al.* 2006, Seixas *et al.* 2010) and has been frequently reported in the literature as host for peritrich epibionts (e.g. Utz 2007, Dias *et al.* 2010).

Although it has been recognized as host for peritrichs, thorough description of epibionts of *P. canaliculata* are still lacking. In the present work we describe two new

species of *Vorticella* (*V. ampullariiae* and *V. veloxiiforme*) found as epibionts on *P. canaliculata* from Southern Brazil. The description is based on morphological characters from live and stained specimens. We also provide a comparison with similar species of *Vorticella* described in the literature.

Material and Methods

Analysis of live specimens: Individuals of *Pomacea canaliculata* were collected by hand from Varzinha, Viamão Municipality, Southern Brazil (30° 19'7.07" S; 50°50'471W). The animals were taken to the laboratory where they were observed for the presence of epibionts. Specimens of *Vorticella* were removed from the gastropod shell using a histological needle, and placed in Petri dishes containing filtered water enriched with a wheat grass solution (Daggett and Nerad 1992), and cover-slips as a substrate for attachment. Separate cultures for each species were carefully maintained. After approximately 48 hours, the cover-slips were examined using an Olympus CH30 microscope for the presence of peritrich ciliates. Twenty-six individuals from each *Vorticella* species were measured using a calibrated ocular micrometer mounted to an optical microscope (Olympus CH30). Morphological characters such as length and width of the zooid, width and thickness of the peristomial lip, width of scopular region, length and width of basal stalk, were analyzed. Species identification was based on specialized literature (Kahl 1935, Warren 1986). Pictures of live individuals were taken using a digital camera mounted to an optical Olympus BX 50 microscope. Drawings were made based on live organisms and photomicrographs.

Protargol-stained specimens: Cover-slips containing *Vorticella* specimens were fixed using Bouin's fluid 5% (Coats & Heinbokel 1982), and the protargol staining procedure

(Zagon & Small 1970) was performed at least seven days after fixation. Cover-slips were run through the procedure and mounted on glass slides at the end of the process.

Morphological characters of the organisms such as length and width of the zooid, shape of macronucleus, length of the basal stalk were measured in, on average, 16 specimens of each *Vorticella* species using a calibrate ocular mounted to an optical microscope (Olympus CH30). The arrangement of the oral polykinetids, a morphological character used to differentiate peritrich species, was also observed. Pictures of stained specimens were taken with a digital camera mounted to an optical microscope (Olympus BX 50). Drawings were made based on photomicrographs.

Silver nitrate technique: To observe the silver line system of the *V. ampullariae* a modification of the dry silver nitrate technique (Foissner 1992) was applied to cultured individuals, with a slight modification. Cells were exposed to silver nitrate for 10 seconds, to the light for 7 seconds, and to the developer for 5 seconds. Cover-slips were mounted in slides using Permunt, and analyzed using an optical microscope Olympus BX 50.

SEM: Cover-slips containing the individual of the *V. ampullariae* (each species in separate sets of cover-slips) were washed in 0.2M phosphate buffer (three times/15 min. each), and then fixed in osmium tetroxide (OsO₄, w / v) for one hour. After that, the specimens were dehydrated in an alcohol series (30%, 50%, 70%, 90% for 10 minutes and 30 seconds). Samples were washed in 0.2 M phosphate buffer plus 1: 1 distilled water three times for 30 minutes each and dehydrated in acetone (90% for 20 minutes, and 100% twice for 10 and 20 seconds). Critical point was run in a BALZERS CPD 030 CRITICAL POINT DRYER. Specimens were coated with gold in a BALZERS SCD 050 SPUTTER COATER, and observed using a Field Emission Electron Microscope (FESEM), Inspect F50, FEI ®.

The silver nitrate technique and SEM were performed only with *V. apullariiae* because this species was easily maintained in laboratory in comparison with *V. veloxiiforme*. Sampling of new hosts with epibiont was difficult due to climate fluctuations throughout the year related to El Niño.

Results

Class Oligohymenophorea de Puytorac *et al.*, 1974

Subclass Peritrichia Stein, 1859

Family Vorticellidae Ehremberg, 1838

Genus *Vorticella* Linnaeus, 1767

***Vorticella veloxiiforme* n.sp.**

Diagnosis. Freshwater peritrich, epibiont on *P. canaliculata* with a cup-shaped zooid measuring 57.5 µm in length and 42.5 µm in width, on average, and presenting a striated pellicle. Macronucleus is J-shaped occupying 2/3 of the cell. Basal stalk presenting a strongly contracting myoneme, and green cytoplasmic granules. All infundibular polykinetids presented three rows of kinetosomes: PK2 and PK3 terminate at the same level adstomally. PK1 has one longer row of kinetosomes, terminating below the level of PK2 and PK3.

Type Locality. Varzinha, Viamão (30° 19'707"S; 50°50'471W), Rio Grande do Sul, Brazil.

Etymology. The specific epithet refers to the fast mode that the trophont contracts.

Deposition of slide. One slide with protargol-stained specimens was deposited in the Protist Collection of the Museum of Science and Technology of the Pontifícia Universidade Católica do Rio Grande do Sul, Brazil under the number **XXX**.

Morphology of live specimens. Individuals of *Vorticella veloxiiforme* presented a bell shaped zooid measuring on average 57.5 µm in length (Table 1) with striated pellicle, a thick peristomial lip, and a slightly elevated epistomial disk (Figs. 1A and 2A). A single contractile vacuole is found in the upper part of the zooid, close to the peristome (Figs. 1A and 2A). A J-shaped macronucleus lay in vertical position, occupying about 2/3 of the zooid (Figs. 1A, 2A and 2B). The stalk measured on average 77.5µm in length (Table 1). A myoneme was observed inside the stalk (Figs. 1A and 2A) and presented several green cytoplasmic granules throughout. Both the zooid and the stalk were very contractible.

Morphology of stained specimens. The infraciliature and macronucleus of *V. veloxiiforme* was easily revealed by the silver impregnation (Figs. 1B and 2D). Somatic myonemes extended from the peristomial lip to the scopula (Fig. 2E). The trochal band was located 3.8 µm above the scopula, on average (Table 2). The oral infraciliature was typical of peritrich ciliates, with all infundibular polykinetids presenting three rows of kinetosomes each (Figs. 1B and 2D). Rows of PK1 were different in length: the adstomial end of the row closer to P2 was longer than the other two that presented the same length (Figs. 1B and 2D). All rows of PK2 showed different lengths abostomally, but all terminated at the same level adstomally (Figs. 1B and 2D). PK3 consisted of three rows of equal length that terminated adstomally at the same level of PK1 and PK2 (Figs. 1B and 2D).

Class Oligohymenophorea de Puytorac *et al.*, 1974

Subclass Peritrichia Stein, 1859

Family Vorticellidae Ehremberg, 1838

Genus *Vorticella* Linnaeus, 1767

***Vorticella ampullariae* n.sp.**

Diagnosis. Freshwater peritrich with an elongate zooid measuring 67.5 μm on average, and epibiont on *Pomacea canaliculata*. A C-shaped macronucleus lies in the middle of the cell. Two contractile vacuoles are present. All infundibular polykinetids have three rows of kinetosomes each. Rows in PK1 and PK2 have different lengths adstomally; PK2 terminates at the adstomal curvature of PK1. PK3 terminates at the same level of PK1 adstomally, with the middle row being longer at the abstomal end.

Type Locality. Varzinha, Viamão (coordinates: 30° 19' 7.07"S; 50° 50' 47.1"W), Rio Grande do Sul, Brazil.

Etymology. The specific epithet refers to the family of the gastropod host.

Deposition of slide. One slide with protargol-stained specimens was deposited in the Protist Collection of the Museum of Science and Technology of the Pontifícia Universidade Católica do Rio Grande do Sul, Brazil under the number **XXX**.

Morphology of live specimens. Elongate zooids measuring on average 67.5 μm in length and 25 μm in width (Table 3), presenting a striated pellicle, narrow peristomial lip, and slightly elevated epistomial disk (Figs. 1C and 3A). Two contractile vacuoles were present: one close to the peristome and the other close to the scopula (Figs. 1C, 3A, 3B and 3C). A C-shape, elongate macronucleus was located vertically in the middle of the cell (Figs. 1C,

and 3C). The basal stalk measured 112.5 μm in length on average (Table 3). A myoneme with techoplasmic granules was observed inside the stalk (Fig. 3A).

Morphology of stained specimens. The infraciliature and the nuclear apparatus of *V. ampullariae* was easily revealed by silver impregnation (Figs. 1D and 3D). The trochal band composed by one row of kinetosomes was located 2 μm on average above the scopula (Table 4). All infundibular polykinetids presented three rows of kinetosomes (Figs. 1D and 3D). PK1 had three rows of different lengths in the adstomal end (Figs. 1D and 3E); PK2 also had rows of different lengths adstomally that terminate at the curvature of PK1 (Figs. 1D and 3E). PK3 presented rows of same length that terminate at the level of PK1 adstomally (Figs. 1D and 3F). Abstomally, rows of PK3 had different lengths with the middle row being longer than the other two (Figs. 1D and 3F). The micro and macronucleus are visible in zooid-stained specimens (Fig. 3F). Silver nitrate impregnation, and scanning electron micrographs revealed a pattern of horizontal silver lines in the pellicle, a diagnostic character of the genus *Vorticella* (Figs. 4C and 4D).

V. ampullariae SEM analyses revealed the presence of pores (about 55 distributed on pellicle) regularly organized on the pellicle as well as a horizontal pattern of silver lines (Figs. 4A, 4C and 4D). SEM pictures also revealed the trochal band (Fig. 4B).

Discussion

Vorticella is a genus of peritrich ciliates characterized by solitary zooids supported by a stalk bearing a contractile myoneme, and by the presence of a horizontal pattern of silver lines. Approximately 100 species in the genus have been described until now (Sun *et al.* 2015). From these about 37 were reported as epibionts on aquatic invertebrates and plants (Henebry & Ridgeway 1979, Pratt & Rosen 1983, Warren 1986, Baldock 1986, Negasawa

& Warren 1996, Cooks *et al.* 1998, Mayén-Estrada & Aladro-Lubel 2002, Dias *et al.* 2008, Babu 2013, Chatterjee *et al.* 2013).

The *Vorticella* species most frequently reported as epibionts on *Pomacea* are *V. campanula* Ehremerberg, 1830 and *V. microstoma* Ehremerberg, 1830 (Vega *et al.* 2006, Dias *et al.* 2008). *Vorticella campanula* has an elongated zooid measuring approximately 68 μm in length (Warren 1986), similar to *V. ampullariiae* that has a zooid measuring 67.5 μm on average. On the other hand, *V. campanula* has a J-shaped macronucleus and a wide peristomial lip (78 μm ; Warren 1986), while *V. ampullariiae* has a C-shaped macronucleus and a narrower peristomial lip (see Table 3). Also, *V. ampullariiae* presents a lateral globular projection on the peristomial lip, that it is not observed in *V. campanula*.

The majority of peritrich ciliates has one contractile vacuole located on the adoral side of the cell, but some exceptions are known. For example, *V. bivacuolata* Fukui & Morishita, 1961 and *V. dimorpha* Stiller, 1940 have two contractile vacuoles located close to the peristome and in the middle of the cell (Warren 1986). Likewise, *V. ampullariiae* has two contractile vacuoles: one located in the adoral portion of the cell, and the other between the middle and the aboral part of the zooid. The size of the zooid is similar in *V. bivacuolata* and *V. ampullariiae* (120 μm X 68 μm , and 87,5 μm X 32.5 μm , respectively), but the zooid of *V. ampullariiae* presents horizontal striation differently from *V. bivacuolata*. Although *V. dimorpha* possesses two contractile vacuoles, it differs from *V. ampullariiae* in the size of the zooid and in the position of the macronucleus (Warren 1986).

Vorticella microstoma and *V. veloxiiforme* present a similar zooid length (55 μm and 57 μm on average, respectively), but differ in the mean width of the zooid and the peristomial lip. The zooid of *V. veloxiiforme* is wider (42.5 μm on average) than the zooid

of *V. microstoma* (35 µm on average, Warren, 1986), as well as the peristomial lip (50 µm and 23 µm, respectively). The general morphology of the zooid of *V. pyriforme* Stiller, 1939, *V. cylindrica* Dons, 1915, and *V. fusca* Prechet, 1935 is similar to *V. veloxiiforme* (Warren 1986, Sun *et al.* 2006). However, a striated pellicle is present only in *V. veloxiiforme*, a character that distinguishes it from the other two. In addition, *V. pyriforme* presents a C-shaped macronucleus, while in *V. veloxiiforme* a J-shaped macronucleus can be easily observed. The pattern of the oral polykinetids observed in *V. fusca* is also different from that observed for *V. veloxiiforme*. In *V. fusca* the middle row of PK1 is longer than the other two (Sun *et al.* 2006), which is not observed in *V. veloxiiforme* (Figs. 1B and 2D). Also, PK3 in *V. fusca* presents three rows of kinetosomes of different lengths. In *V. veloxiiforme* rows in PK3 terminate at the same level adstomally (Figs. 1B and 2D). These differences are strong enough to support a species-level distinction between *V. veloxiiforme* and *V. fusca*.

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Tables

TABLE 1. Measurements of live specimens of *Vorticella veloxiiforme* attached to *Pomacea canaliculata* from Varzinha, Viamão, RS, Brazil. A total number of 26 zooids were measured for each character.

Morphological Character	Max (µm)	Min (µm)	Median(µm)	SD
Total lenght of the body	67.5	47.5	57.5	5.4
Lenght from the peristomial lip	50	20	42.5	7.6
Width of the body	50	30	42.5	4.9
Width of the body at midpoint	37.5	25	31.3	4.8
Width of peristomial lip	62.5	37.5	50	6.1
Thickness of peristomial lip	12.5	7.5	10	1.7
Width of scopula	10	7.5	7.5	0.5
Lenght of basal stalk	247.5	32.5	131.3	77.5
Width of basal stalk	2.5	2.5	2.5	0

TABLE 2. Measurements of stained specimens of *Vorticella veloxiiforme* attached to *Pomacea canaliculata* from Varzinha, Viamão, RS, Brazil. Numbers in parenthesis refer to the total number of individuals measured for each character.

Morphological Character	Max (µm)	Min (µm)	Median (µm)	SD
Total lenght of the body (16)	50	21	29.6	10.4
Width of the body (16)	37.5	15	21.6	6.8
Width of the body at midpoint (16)	50	20	31.1	12.5
Distance between trochal band and scopula (4)	5	2.5	3.8	1.4
Lenght of micronucleous (9)	7.2	2.5	5.2	1.6
Width of micronucleous (9)	5	2.5	2.3	0.9
Lenght of macronucleous (11)	32.5	12.5	21.7	11.8
Width of macronucleous at midpoint (10)	10	2	3.7	2.4

TABLE 3. Measurements of live specimens of *Vorticella ampullariiae* attached to *Pomacea canaliculata* from Varzinha, Viamão, RS, Brazil. A total number of 26 zooids were measured for each character.

Morphological Character	Max (µm)	Min (µm)	Median (µ)	SD
Total length of the body	87.5	55	67.5	8
Length from the peristomial lip	72.5	47.5	55	7
Width of the body	32.5	17.5	25	3.2
Width of the body at midpoint	32.5	22.5	30	4.9
Width of peristomial lip	37.5	25	30	3.6
Thickness of peristomial lip	12.5	2.5	5	2.1
Width of scopula	7.5	5	6.3	1.5
Length of basal stalk	207.5	17.5	112.5	66.1
Width of basal stalk	2.5	2.5	2.5	0

TABLE 4. Measurements of stained specimens of *Vorticella ampullariiae* attached to *Pomacea canaliculata* from Varzinha, Viamão, RS, Brazil. Numbers in parenthesis refer to the total number of individuals measured for each character.

Morphological Character	Max (µm)	Min (µm)	Median (µm)	SD
Total length of the body (34)	34	15	19.9	4
Width of the body (34)	20	11	14.2	2.6
Width of the body at midpoint (34)	25	16	20.3	3.7
Distance between trochal band and scopula (1)	2		2	-
Length of basal stalk (16)	37	4	17.3	10.1
Length of micronucleous (13)	3	1	2.2	0.6
Width of micronucleous (13)	3	1	2.2	0.6
Length of macronucleous (21)	25	11	16	3.6
Width of macronucleous at midpoint (17)	4	2	2.7	0.6

Number of silver lines from peristomial lip to trochal band (4)	54	40	34.8	18.6
Number of silver lines from trochal band to scopula (4)	14	7	10.8	2.6

Figure Legends

Figure 1: *Vorticella veloxiiforme* sp. n. and *Vorticella ampullariiae* sp.n. *in vivo* and protargol-stained specimens: **A.** Live zooid of the *V. veloxiiforme* showing the contractile vacuole (arrow) and J-shaped macronucleus (arrow head) (Bar 15 μ m). **B.** Protargol-stained zooid of *V. veloxiiforme* showing the oral polikinetids inside the infundibular region (PK1, PK2, PK3) (Bar 10 μ m). **C.** Live zooid of *V. ampullariiae* showing the position of the two contractile vacuoles (arrow) and C-shaped macronucleus (arrow head) (Bar 15 μ m). **D.** Protargol-stained zooid of the *V. ampullariiae* showing the oral polikinetids inside the infundibulum (PK1, PK2, PK3) (Bar 10 μ m).

Figure 2: *Vorticella veloxiiforme* sp. n. live and protargol-stained specimens: **A.** Live zooid showing the contractile vacuole, J-shaped macronucleus and spasmoneme into basal stalk (SM) (Bar 10 μ m). **B.** Live zooid showing position of the J- shaped macronucleus (MAC) (Bar 10 μ m). **C.** Protargol-stained zooid showing the J-shaped macronucleus (MAC) (Bar 10 μ m). **D.** Detail of a protargol-stained zooid showing the oral polykinetids (PK1, PK2, PK3) inside the infundibulum (Bar 10 μ m). **E.** Protargol-stained zooid showing the somatic myonemes (Bar 10 μ m).

Figure 3: *Vorticella ampullariiae* sp. n. live and protargol-stained specimens: **A.** Live zooid showing the position of the two contractile vacuoles and the myoneme (MY) inside the basal stalk (Bar 10 μ m). **B.** Live zooid showing the position of the aboral contractile vacuole (arrow) (Bar 10 μ m). **C.** Live zooid showing the position of the oral contractile vacuole and C-shaped macronucleus (MAC) (Bar 10 μ m). **D.** Protargol-stained zooid showing the oral polikinetids in the infundibular region (PK1, PK2, PK3) (Bar 5 μ m). **E.** Protargol-stained

zooid showing details of the oral polikinetids 1 and 2 (Bar 5 μm). **F.** Protargol- stained zooid showing the micro (MIC) and macronucleus (MAC) (Bar 5 μm).

Figure 4: *Vorticella ampullariae* sp. n. silver nitrate-stained species and SEM photomicrographs: **A.** Picture showing details of the pores on the pellicle (Bar 3 μm). **B.** SEM picture of the zooid showing the trochal band (arrow) (Bar 5 μm). **C.** Drawing from silver nitrate-stained zooid showing the silver lines on the pellicle (Bar 4 μm). **D.** Silver nitrate-stained zooids showing the silver lines on the pellicle (arrow) (Bar 4 μm).

Figures

Figure 1

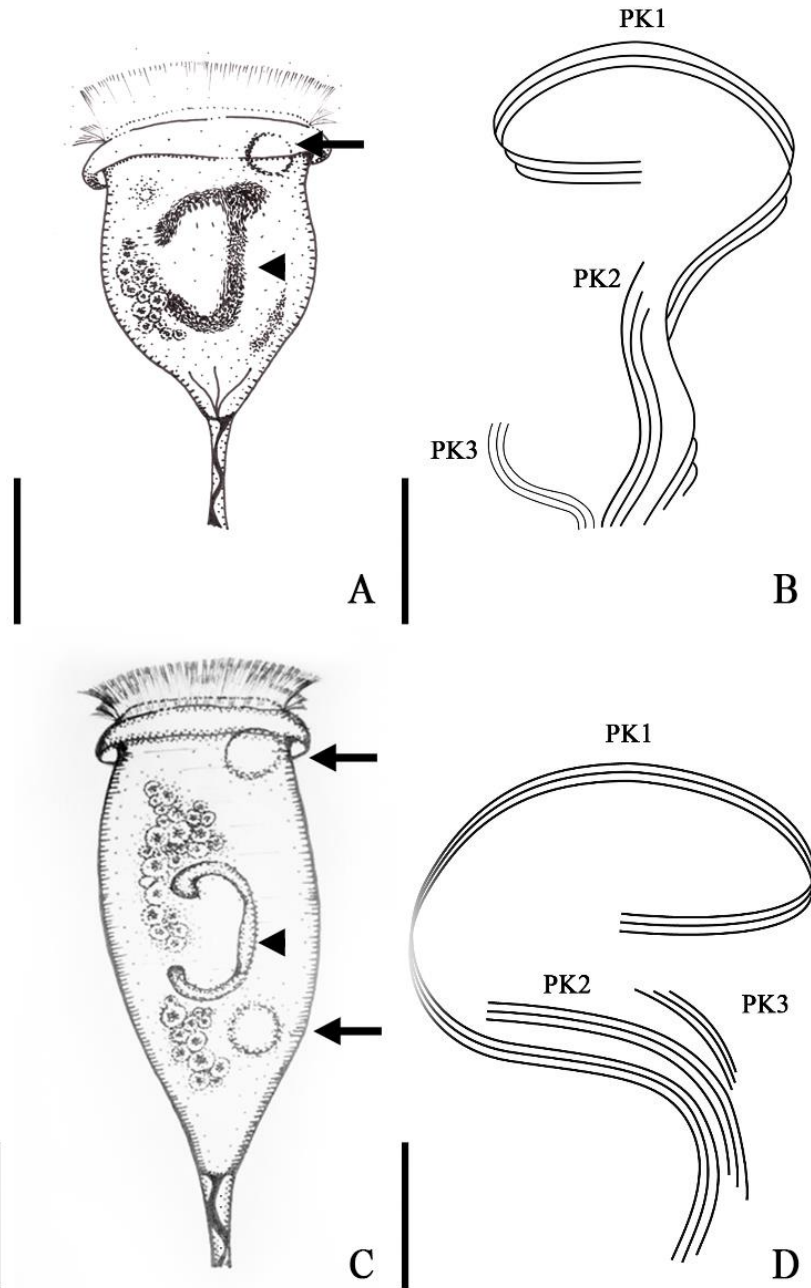


Figure 2

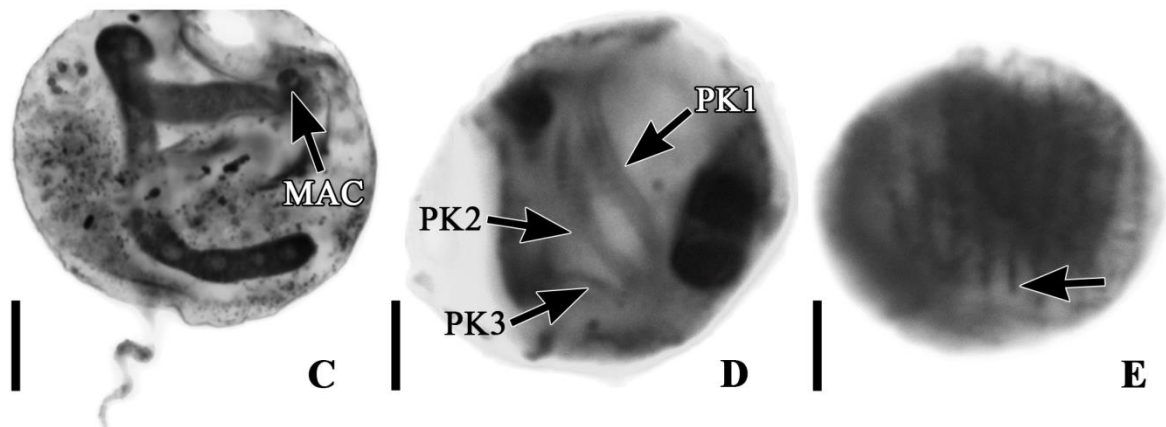
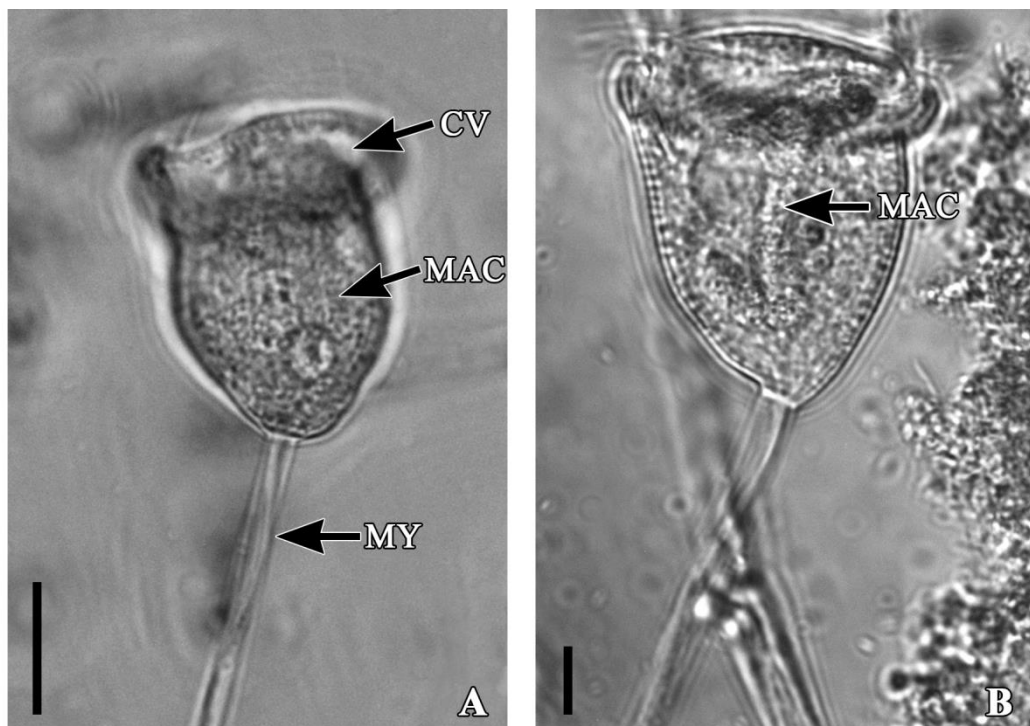


Figure 3

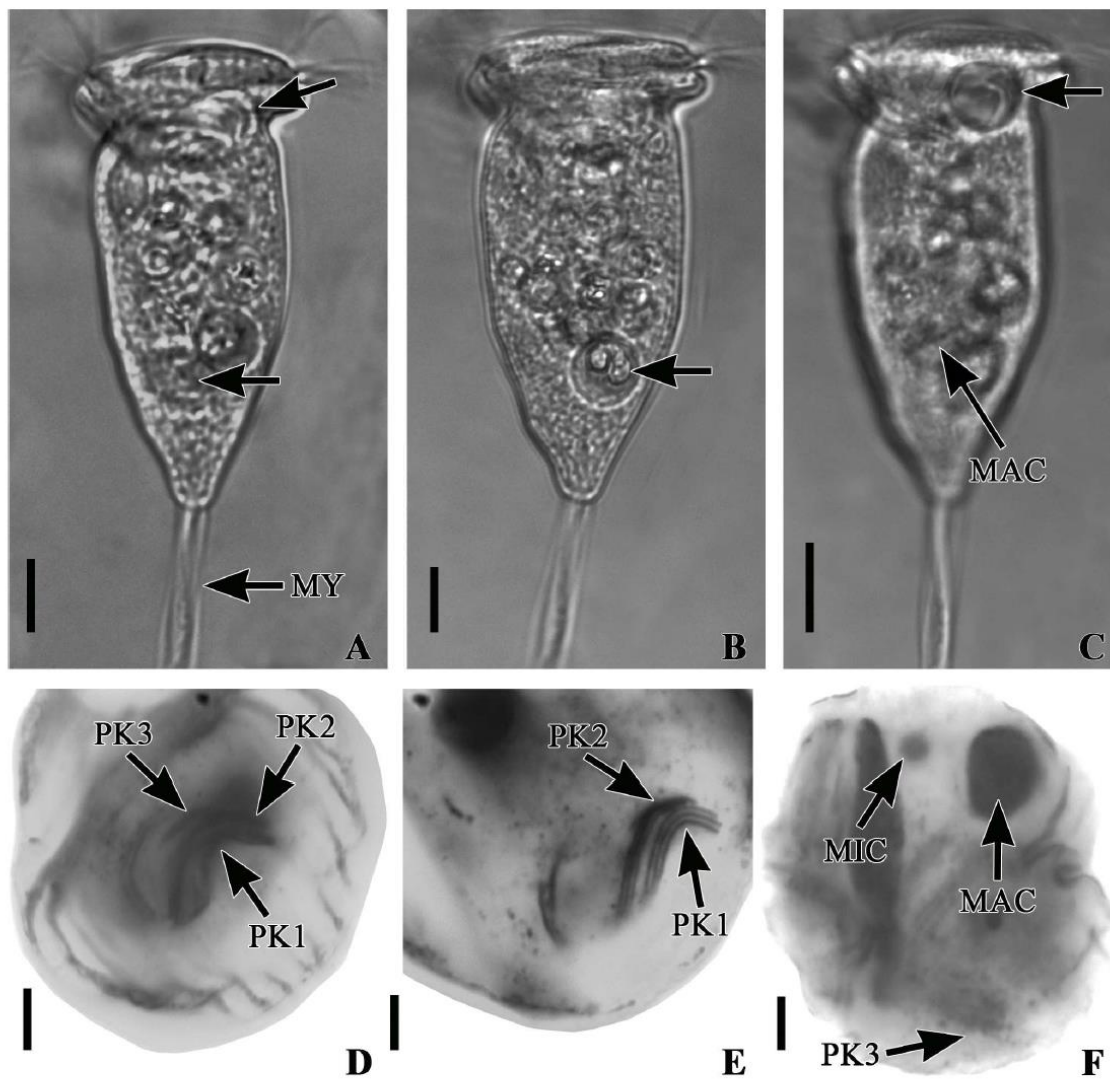
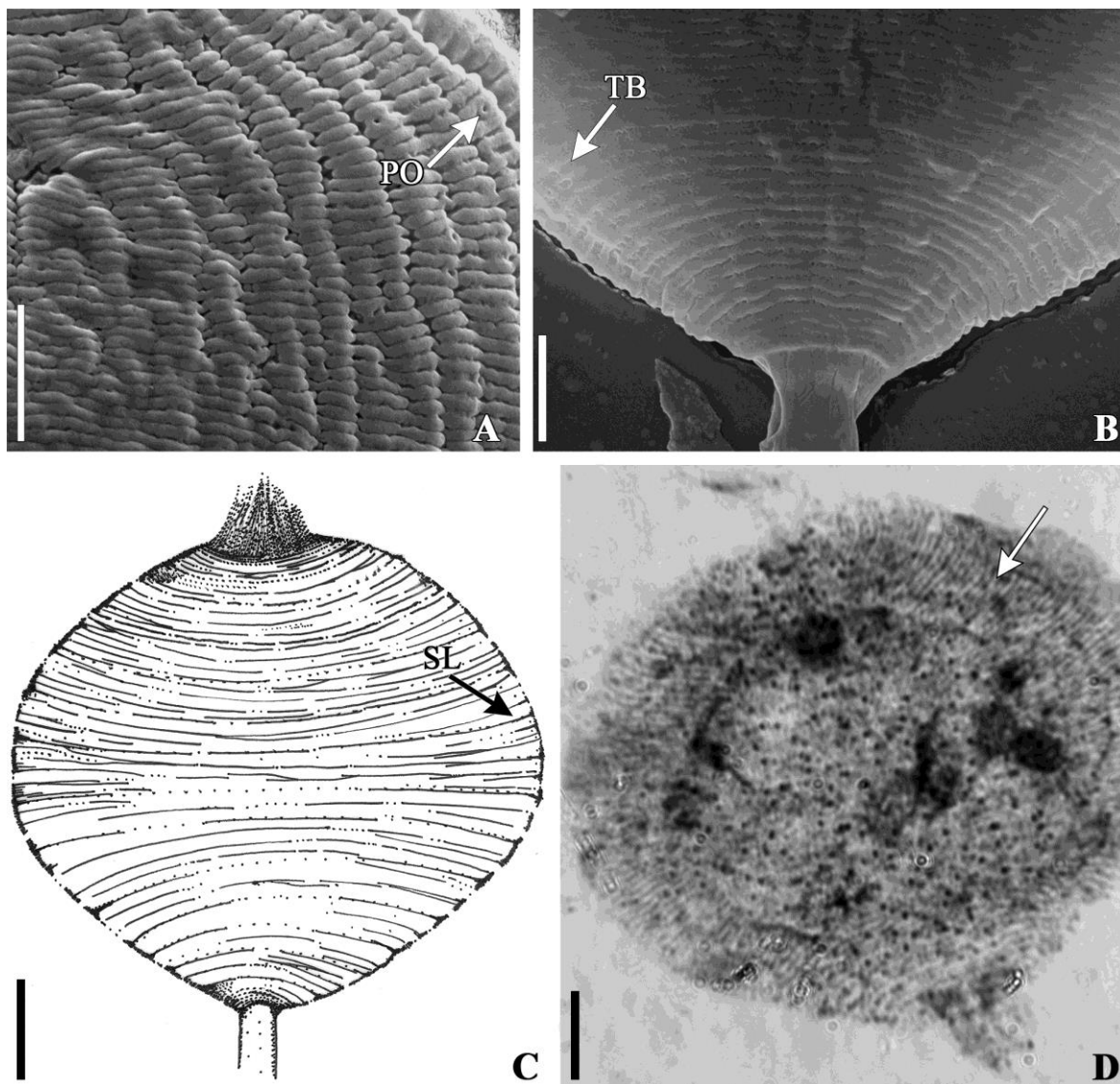


Figure 4



- Capítulo III-

A new epibiont peritrich ciliate from subtropical freshwater environment:
Vorticella ovalistriata n.sp. (Ciliophora: Oligohymenophorea: Peritrichia)

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A new epibiont peritrich ciliate from subtropical freshwater environment: *Vorticella ovalistriata* n.sp. (Ciliophora: Oligohymenophorea: Peritrichia)

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Abstract

The peritrich ciliate *Vorticella ovalistriata* **n.sp.** was found as epibiont on *Salvinia minima* in a swamp located in Ilha da Pintada, Porto Alegre municipality, Rio Grande do Sul state, Brazil. Live and protargol stained specimens were observed. Details of the external structure of the trophont were also revealed by SEM and by the dry silver nitrate technique. *V. ovalistriata* has a conical-shaped zooid, measuring 70 µm in length and 52.5 µm in width, on average and a J-shaped macronucleus. An oval projection of the peristomial lip could be observed in live specimens and is a peculiar morphological character of the species. The pattern of infundibular polykineties revealed by silver impregnation was typical of peritrich ciliates, composed by three oral polykinetids with three rows of kinetosomes each. PK1 is the longest polykinetid, PK2 terminates adstomally at the curvature of P1, and P3 has a shorter mid-row in the adstomal end. The silver line system is typical of the genus with horizontal lines. SEM analyses revealed the presence of membrane pores that are regularly observed in species of the genus *Vorticella*.

Keywords:

Peritrich ciliates, epibiosis, peristomial lip projection, SEM, *Salvinia minima*, swamp.

Introduction

Vorticella is a genus of peritrich ciliates that occurs worldwide in many different marine and freshwater environments (Warren 1986, Sun 2006, Sun *et al.* 2012). Currently, this genus comprises approximately 100 known species (Sun 2015). The trophont stage of *Vorticella* is solitary, with some species showing a pseudocolony organization. The zooids could present different shapes such as inverted bell-shaped, spherical, cylindrical, or conical. The stalk contains a central spasmoneme that effects a helical contraction. The taxonomy of this group has been problematic owing to the complex plasticity of shapes, sizes and contractility of the zooids, and also by the possible existence of cryptic species (Warren 1986, Vacchiano 1992, Sun 2006, Sun *et al.* 2012, Li 2013). Therefore, taxonomic-morphological revisions and molecular studies are necessary to elucidate the gaps that still exist within this taxonomic group of ciliates.

Specimens of *Vorticella* may have ectosymbiotic relationships with other organisms. This type of interaction is called epibiosis, and is characterized by the attachment of a living organism (called epibiont) to a living substrate (named basibiont or host) (Fernandez-Leboranz 2004, Dias *et al.* 2006, Fernandez-Leboranz *et al.* 2006., Utz 2007, Utz *et al.* 2014). There are many living organisms that are hosts for *Vorticella*, *e.g.* algae, seagrasses, macrophytes, mollusks, annelids, crustaceans (mysidaceans, cirripedians, cladocerans, copepods, amphipods, isopods, decapods), insect larvae (Henebry & Ridgeway 1979, Pratt & Rosen 1983., Kankaala & Eloranta 1987, Nagasawa & Warren 1996, Cook *et al.* 1998, Aladro-Lubel & Martínez-Murillo 1999, Fernandez-Leboranz 2004, Dias *et al.* 2006, Fernandez-Leboranz *et al.* 2006, Bielencka & Boehnke 2014), among others.

Salvinia minima is an abundant free-floating macrophyte in temperate, tropical and subtropical regions (Hoffmann *et al.* 2004, Prado *et al.* 2010, Fuentes *et al.* 2014). *S. minima* is native to Mexico, Central and South America and exhibits rapid vegetative growth, widely colonizing freshwater environments where it is introduced (Nichols *et al.* 2000, Sánchez-Galván *et al.* 2008, Tipping *et al.* 2009, Prado *et al.* 2010, Estrella-Gómez *et al.* 2012, Fuentes *et al.* 2014). Due to its high adaptive capacity, these plants could cause several problems for the human population, including modification of the environment, interference with recreation or commercial fishing and navigation of ships, among others (Nichols *et al.* 2000, Fuentes *et al.* 2014). Nevertheless, the growth of these macrophytes is largely controlled by the widespread herbivory of various invertebrates, such as lepidopteran, coleopteran, and dipteran (McIlraith 1989, Tipping *et al.* 2009). In addition, *S. minima* plays an important role in phytoremediation, accumulating heavy metals from the environment where it is inserted (*e.g.*, Pb, Cd and Cr), helping to control pollution and pH of aquatic environments (Nichols *et al.* 2000, Hoffmann *et al.* 2004, Sánchez-Galván *et al.* 2008, Prado *et al.* 2010, Estrella-Gómez *et al.* 2012, Fuentes *et al.* 2014, Ponce *et al.* 2014).

Thus, the aim of the present study was to characterize morphologically *Vorticella ovalistriata* n.sp. epibiont on *S. minima* collected in a swamp located in Ilha da Pintada, Rio Grande do Sul, Brazil. A complete species description based on *in vivo* observation and silver stained organisms as well as on scanning electron microscopy is provided.

Material and Methods

Collection and analysis of live specimens: Samples of *S. minima* were collected from a swamp located in Ilha da Pintada (30°1'47"S; 51°15'33"W), Rio Grande do Sul state, Southern Brazil. This habitat undergoes fluctuations of flood throughout the year, being the period from June to September characterized as the flood season (winter in the southern hemisphere). Environmental parameters, such as oxygenation and water temperature for example, as well as the biota present in the site are influenced by this temporal dynamics. The macrophytes sampled were taken to the laboratory where they were evaluated for the presence of peritrich ciliates. With the use of an optical microscope (Olympus CH30), specimens containing peritrich epibionts were acclimatized in cultures with dechlorinated water and glass cover-slips. Samples were kept at room temperature and enriched with a wheat grass solution (Daggett and Nerad 1992), on alternate days. Subsequently, 25 peritrich epibionts were measured, and characterized for morphological parameters of the zooid and basal stalk. Pictures (taken using a digital camera mounted to an optical Olympus BX 50 microscope), and schematic drawings were performed based on living specimens.

Morphology of stained specimens: After analysis of the living material, ciliates were submitted to the modified protargol technique (protocol A *in* Foissner 2013), aiming to reveal the oral infraciliature pattern and other somatic structures (*e.g.* macronucleus shape and somatic myonemes). The modifications were: ciliates were fixed in formalin 4% (at least 3 days) and then clarified in potassium permanganate 5%, without need for centrifugation and fixation with albumin-glycerol. The dry silver nitrate technique (Foissner 1992) was performed to reveal the silver line system. Photomicrographs were taken using an Olympus BX 50 microscope with a mounted digital camera attached to a computer. Line drawings of *in vivo* and stained specimens were based on photomicrographs and live observations.

Scanning electron microscopy: Ciliates from cultures were washed with phosphate buffer (0,2M) for three times for 15 minutes each and fixed in 1% (w/v) osmium tetroxide (OsO₄) for one hour. After this, the cover-slips with attached organisms were washed (0.2 M phosphate buffer plus 1: 1 distilled water) for 30 minutes (3 times each) and dehydrated in a graded ethanol series (30%, 50%, 70%, 90% for 10 minutes and 90% for 20 minutes) and 100% acetone two times for 10 and 20 seconds, respectively. Critical point was performed using a BALZERS CPD 030 CRITICAL POINT DRYER. Cover-slips were placed in stubs and specimens were coated with gold using BALZERS

SCD 050 SPUTTER COATER. Peritrichs were observed using a Field Emission Electron Microscope (FESEM), Inspect F50, FEI ®.

Results

Class Oligohymenophorea de Puytorac *et al.*, 1974

Subclass Peritrichia Stein, 1859

Family Vorticellidae Ehrenberg, 1838

Genus *Vorticella* Linnaeus, 1767

Vorticella ovalistriata n.sp.

Diagnosis. Freshwater peritrich, epibiont on *S. minima*. Zooid presenting conical shape, measuring 70 µm in length and 52.5 µm in width, on average, and green or transparent granules inside the zooid. Peristomial lip with one prominent flap, conferring a morphological asymmetry.

Macronucleus J-shaped in the central region of the cell. Pseudocolonies often observed. Infundibular polykinetids with three rows of kinetosomes each. Rows in PK1 equal in length; rows in PK2 equal in length, starting abnormally at 1/3 of PK1 and terminating at the curvature of PK1; PK3 with the middle row of kinetosomes short than the other two.

Type Locality. Swamp located in Ilha da Pintada, Municipality of Porto Alegre (30°1'47"S; 51°15'33"W), Rio Grande do Sul, Brazil.

Etymology. The specific epithet refers to the oval shape of the striated flap in the peristomial lip.

Deposition of slide. One slide with protargol-stained specimens was deposited in the Protist Collection of the Museum of Science and Technology of the Pontifícia Universidade Católica do Rio Grande do Sul, Brazil under the number **XXX**.

Morphology of live specimens. *V. ovalistriata* has a conical zooid measuring on average 70 µm in length and 52.5 µm in width (Figs. 1A, 1B and 1C, Table 1), one contractile vacuole close to the projection of the peristomial lip, a granulated cytoplasm (with green or transparent vesicles) (Figs. 1A, 1B and 1D), and a slightly everted epistomial disc (Fig. 1D). A J-shape, elongate macronucleus was located vertically in the middle of the cell (Fig. 2A). The contractile stalk (Figs. 1A and 1B) measured on average 92.5 in length and 2.5 µm in width (Table 1), with a considerable variation in length among the population.

Morphology of stained specimens. All infundibular polykinetids presented three rows of kinetosomes (Fig. 2B). PK1 was the longest and presented three rows of kinetosomes of equal length

(Fig 2B and 3A). PK2 presented three rows of kinetosomes equal in size, terminating at the curvature of PK1, adstomally (Fig. 2B and 3A). PK3 presents three rows of kinetosomes different in size with the middle row shorter than the other two. PK3 terminates adstomally at the middle of the curvature of PK1, being longer than PK2 (Fig. 2B and 3B). The presence of a J-shaped macronucleus that occupied a large portion of the cell body was observed (Figs. 2A).

The silver-nitrate technique revealed a horizontal pattern of silver lines, a morphological character of species in the genus *Vorticella* (Figure 4A and B). Scanning electron microscopy showed the presence of pellicular pores on the zooid membrane, a morphological character of peritrich ciliates (4C). The lip projection pointed out in live specimens can also be observed in SEM photomicrographs.

Discussion

Vorticella is a genus often found as epibiont in aquatic organisms. However, the association between *Vorticella* species with algae and aquatic plants is still poorly documented. *V. ovalistriata* presents a conical zooid, measuring 70 μm in length and 52.5 μm in width on average (see Table 1), a peristomial lip with a slightly everted epistomial disc, and J-shaped macronucleus. Despite this, *V. ovalistriata* has peculiar morphological characteristics, when compared with its congeners.

The conical zooid of *V. ovalistriata* presents slightly visible-striated pellicle (Figs. 1B and 1D), and a projection on the peristomial lip (Fig. 1D). Several species in the genus *Vorticella* are similar to *V. ovalistriata*, but some of them present round or tapered zooids (i.e., *V. anomala* Gourret & Roeser, 1886; *V. globosa* Ghosh, 1922; *V. globularia* Müller, 1773; *V. macrostyla* Schmarda, 1854), ornate zooids (i.e., *V. vernalis* Stokes, 1887; *V. verrucosa* Dons (1915), 1918), wavy or ornate peristomial lip (*V. cratera* Kent, 1881; *V. oboconica* (Dons, 1915) Kahl, 1935; *V. vesita* Stokes, 1883). However, none of these species has a distinctive conical zooid with projection on the peristomial lip as observed in *V. ovalistriata* (Warren 1986).

The projection in the peristomial lip shows a characteristic striation visible in SEM (Fig. 4C) and is more prominent in comparison with other species that present this characteristic, such as *V. picta* Ehrenberg, 1831 and *V. campanula* Ehrenberg, 1831. Ornaments, lobes, and flaps present in other species of *Vorticella* (i.e. *V. cratera*; *V. marginata* Stiler, 1931 and *V. vesita*), also, are very distinct (both in form and number) from the lip projection observed in *V. ovalistriata*.

The presence of a parallel pattern of silver lines (visible in silver nitrate preparations, Figs. 4A and 4B) in the pellicle, the striated projection of the peristomial lip, and the entrance of the cilia row inside the infundibulum are all easily observed from scanning electron microscopy (Figs. 4C, 4D and 4E). In addition to these morphological characters that could be observed with different techniques,

membrane pores (about 237 observed throughout the pellicle) distributed regularly over the cell body are also evident in SEM micrographs (Fig. 4D).

Regarding other *Vorticella* species, the shape of the zooid, the proportion of the peristomial lip relative to the width at the midpoint of the body cell, and the rectangular shape of the scopula of *V. ovalistriata* are similar to those observed in *V. alba* and *V. campanula* (Warren 1986). However, the dimensions of the peristomial lip and the zooid, shape and position of the macronucleus are distinct between *V. ovalistriata* and the other two species. The arrangement of the oral polykinetids of *V. campanula* are also different from the observed for *V. ovalistriata*. PK1 and PK3 of *V. campanula* merge adstomally forming six rows of kinetosomes of equal size (Foissner et al., 1992) while *V. ovalistriata* PK3 has a second row shorter than the others and terminates adstomally at the curvature of PK1. The shape and the position of the macronucleus are similar to observed in *V. sepulcreti* Foissner & Schiffmann, 1975 (Figs. 2A and 2B) (Warren 1986), but shape of the zooid, striations on the pellicle, shape and size of the peristomial lip differ greatly from *V. ovalistriata*.

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Tables

TABLE 1. Measurements of live specimens of *Vorticella ovalistriata* attached to *Salvinia auriculata* from Ilha da Pintada, Porto Alegre, RS, Brazil. A total number of 25 zooids were measured for each character

Morphological Character	Max (µm)	Min (µm)	Mean (µ)	SD
Total length of the body	92.5	70.0	37.5	11.6
Length from the peristomial lip	72.5	30.0	52.5	10.4
Width of the body	27.5	30.0	52.5	11.6
Width of the body at midpoint	72.5	15.0	42.5	14.1
Width of peristomial lip	80.0	35.0	50.0	12.5
Thickness of peristomial lip	10.0	2.5	5.0	1.8
Width of scopula	10.0	2.5	7.5	2.0
Length of basal stalk	260.0	17.5	92.5	77.8
Width of basal stalk	5.0	2.5	2.5	1.9

TABLE 2. Measurements of protargol stained specimens of *Vorticella ovalistriata* attached to *Salvinia auriculata* from Ilha da Pintada, Porto Alegre, RS, Brazil. Numbers in parenthesis refer to the total number of individuals measured for each character

Morphological Character	Max (µm)	Min (µm)	Mean (µm)	SD
Total length of the body (25)	47.5	22.5	32.5	6.0
Width of the body (25)	35.0	17.5	27.5	4.9

Width of the body at midpoint (25)	45.0	25.0	32.5	4.3
Length of basal stalk (25)	50.0	12.5	27.5	11.2
Length of macronucleus (21)	35.0	10.0	17.5	6.3
Width of macronucleus at midpoint (21)	7.5	2.5	2.5	1.0

Figure Legends

Figure 1: *Vorticella ovalistriata* sp. n. live specimens: **A.** Representation of a live zooid showing the contractile vacuole (CV), myoneme (MY) into the basal stalk and a projection on the peristomial lip (arrow) (Bar 20 μ m). **B.** Detail of a live zooid showing the position of the contractile vacuole (CV), infundibulum (INF) and myoneme (MY) inside the basal stalk (Bar 20 μ m). **C.** Detail of a live zooid attached to *Salvinia auriculada* (arrow) (Bar 30 μ m). **D.** Live zooid showing the elevation of the epistomial disk (ED) and the projection on the peristomial lip (arrow) (Bar 20 μ m).

Figure 2: *Vorticella ovalistriata* sp. n.: **A.** Drawing from a live zooid showing the J-shaped macronucleus (MAC), myoneme (MY) inside the basal stalk and a projection on the peristomial lip (arrow) (Bar 10 μ m). **B.** A detail of the oral polykinetids 1, 2, and 3 inside the infundibulum (Bar 5 μ m).

Figure 3: *Vorticella ovalistriata* sp. n. protargol stained specimens: **A.** Protargol-stained zooid showing oral polykinetids 1 and 2 (Bar 10 μ m). **B.** Protargol-stained zooid oral polykinetid 3 (Bar 10 μ m).

Figure 4: *Vorticella ovalistriata* sp. n. silver nitrate stained specimens, and Scanning Electron Microscopy (SEM) photomicrographs: **A.** Representation of a silver nitrate-stained zooid showing the silver lines on the pellicle (SL) (arrow) (Bar 10 μ m). **B.** Detail of a silver nitrate stained zooid showing the silver lines on the pellicle (arrow) (Bar 15 μ m). **C.** SEM photomicrograph of a zooid showing a detail of the striated projection on peristomial lip (arrow) (Bar 15 μ m). **D.** SEM photomicrograph of the zooid showing pellicular pores (arrow) (Bar 2 μ m). **E.** SEM photomicrograph of the zooid showing the entry of the row of cilia within the infundibular region (arrow) (Bar 10 μ m).

Figures

Figure 1

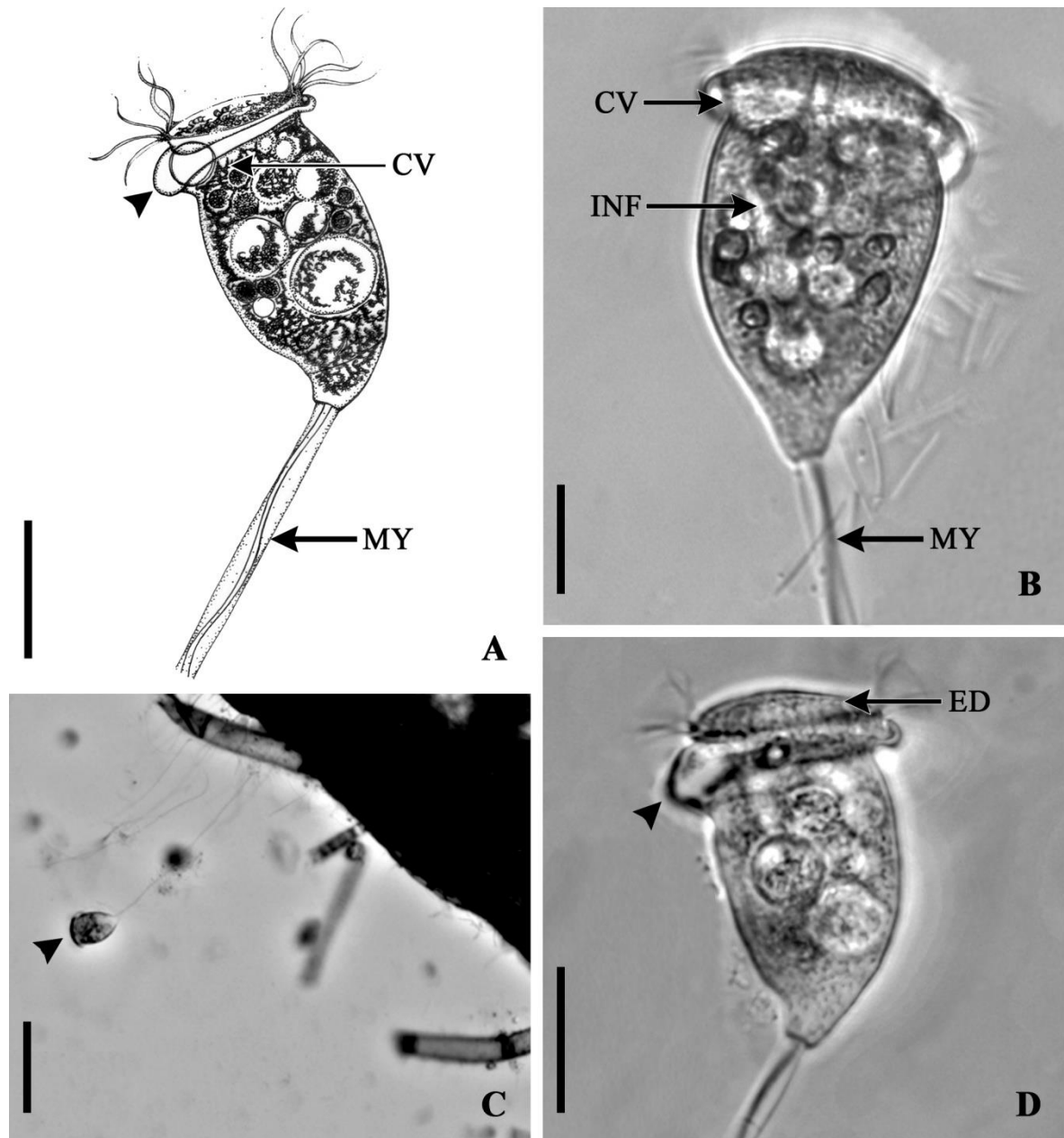


Figure 2

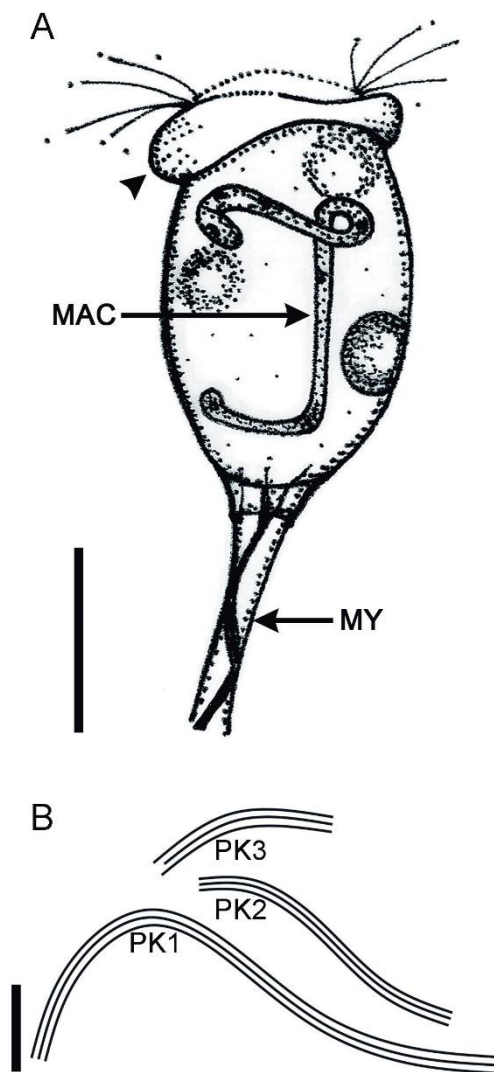


Figure 3

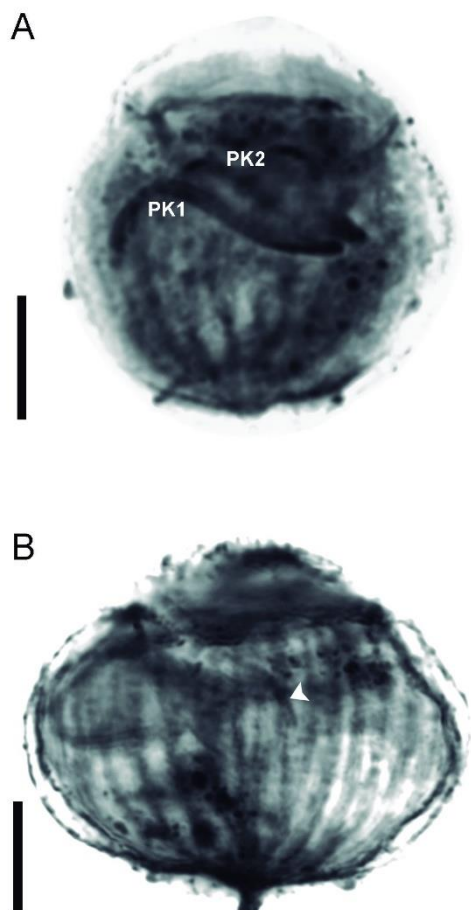
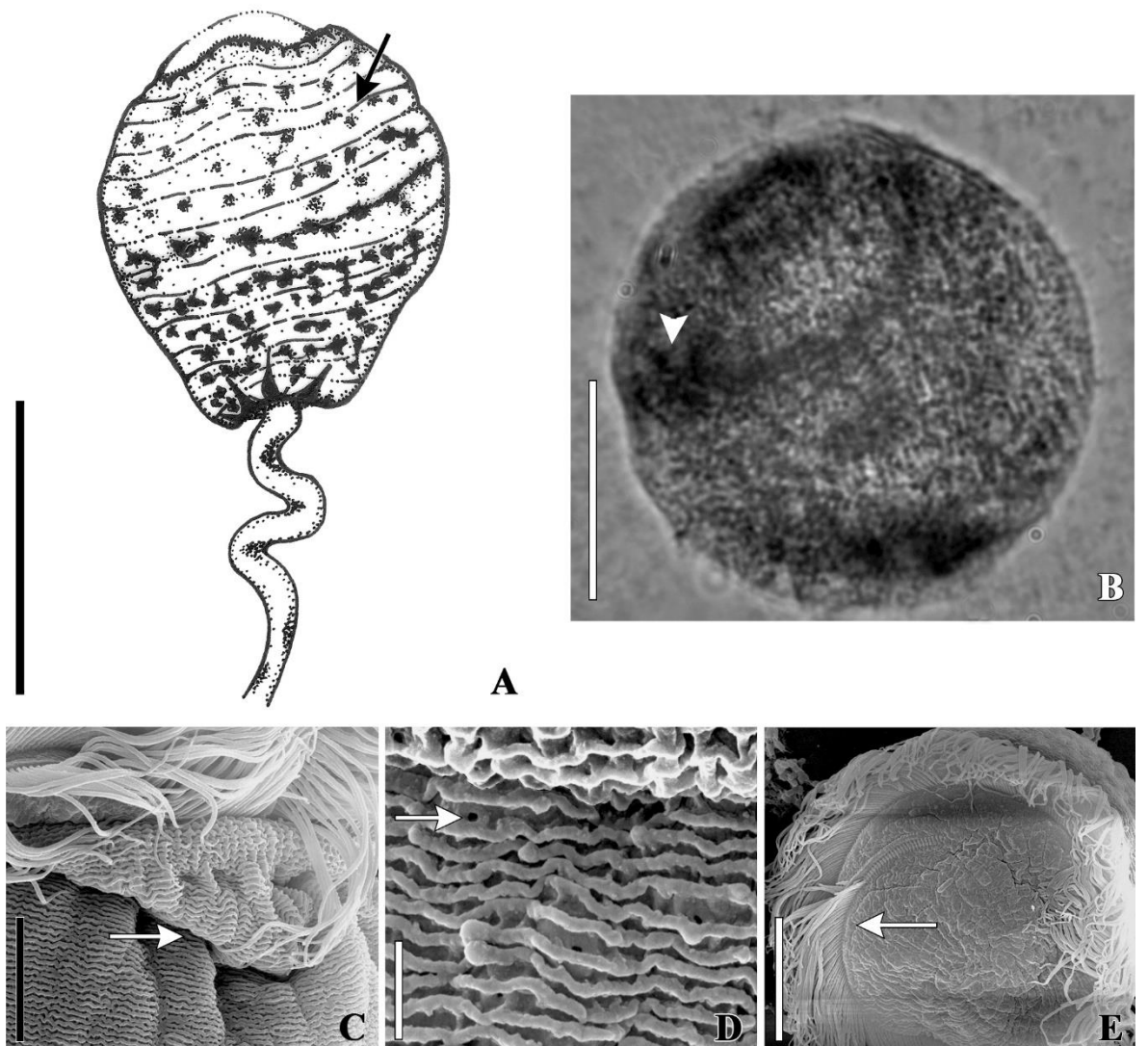


Figure 3



- Capítulo IV -

Considerações Finais e Perspectivas

CONSIDERAÇÕES FINAIS

O entendimento das relações existentes entre os diferentes seres vivos na natureza é fundamental para estudos na área da conservação biológica. Infelizmente, grande parte dos grupos de seres vivos ainda não foram compreendidos dentro desse contexto de redes complexas de interação. Muitas vezes, esse fato é consequência das dificuldades inerentes da biologia dos diversos organismos da Terra, e, também, da falta de dados preliminares básicos provindos de pesquisas anteriormente desenvolvidas. Por isso, estudos na área da Taxonomia e da Sistemática são imprescindíveis para pesquisas futuras no campo do manejo de espécies e estudos de impacto ambiental.

Elucidar a taxonomia de ciliados peritríquios com mais acuidade e aprofundamento nos permite uma compreensão mais clara dos ecossistemas aquáticos onde estes estão inseridos, bem como permite a utilização desses dados no âmbito de estudos maiores e mais focados. As relações ambientais de epibiose estabelecidas entre esses organismos unicelulares e outros seres vivos, em grande parte, são reflexo de inúmeras alterações sofridas no ambiente, que implicam em novos rearranjos e exploração de nichos feita para sobrevivência e realização de um *fitness* efetivo dos diferentes organismos envolvidos nesse sistema. Assim, a compreensão presente de um ambiente estudado é inerente do entendimento de outros tantos trabalhos focados nos diferentes setores ecológico-biológicos inseridos nessa teia de interações complexas.

Trazer à tona novas espécies de ciliados peritríquios que vivem em epibiose com outros organismos, descrevendo detalhadamente as mesmas é um instrumento poderoso a ser explorado posteriormente em estudos para compreensão da natureza. A taxonomia é uma área das Ciências Biológicas que traz um embasamento essencial para trabalhos, que em cadeia, serão desenvolvidos. A determinação taxonômica dos ciliados peritríquios epibiontes em organismos de ambientes aquáticos apresenta um apelo maior: desde muito tempo na literatura científica, sabe-se que ciliados são ótimos indicadores de qualidade ambiental, devido a capacidade que possuem de reagir às diferentes alterações sofridas nos ecossistemas. Além disso, ciliados apresentam um papel trófico bastante relevante dentro das teias alimentares, servindo como uma alça (tecnicamente conhecida como “*microbial loop*”) que faz interação entre níveis tróficos que na ausência deles não se relacionariam. Ciliados peritríquios que conseguem viver como epibiontes sobre outros organismos vivos

alcançam benefícios que podem facilitar sua permanência nos ambientes em questão (é inferido que um gradiente dentro dessa relação, assim como ocorre para muitas outras no ambiente, existe nessas interações ectossimbióticas, podendo estar intimamente ligado à historia coevolutiva dos organismos neste sistema inseridos). Dessa forma, estudos preliminares, como os desenvolvidos nessa pesquisa, são pontos-chave para trabalhos futuros, que vão desde o âmbito de interações nas redes tróficas, até pesquisas de manejo e conservação ecossistêmica.

PERSPECTIVAS FUTURAS

Em função da importância ecossistêmica que os ciliados peritríquios possuem, especialmente os que estão inseridos nas relações de epibiose com outros organismos, os seguintes aprofundamentos científicos, inerentes de pesquisas como a presente são propostos:

- estudos morfológicos cada vez mais detalhados, usando técnicas avançadas de microscopia e de impregnação em organismos fixados, propiciando cada vez mais diagnoses para esses organismos que apresentam uma enorme convergência em seu *Bauplan* corporal;

- trabalhos que se comprometam em compreender como as interações epibióticas entre organismos vivos e ciliados peritríquios acontecem, de modo a elucidar parâmetros coevolutivos, de historia de vida e de interações fisiológicas entre os indivíduos envolvidos;

- compreender se a relação epibiótica, em algum nível, entre ciliados peritríquios e organismos vivos traz algum empecilho ao desenvolvimento das atividades vitais de basibiontes (principalmente) e de epibiontes: interferência direta no *fitness* das espécies;

- pesquisar molecularmente os ciliados peritríquios, elucidando os padrões genéticos de relação dentro dos diferentes *taxa*, possibilitando uma maior acuidade de relação taxonômica dentro dos diferentes clados existentes em Peritrichia;

- aplicar estudos no âmbito da Taxonomia e da Sistemática de ciliados peritríquios epibiontes em outras áreas adjacentes (como na Ecologia, por exemplo), tornando mais dinâmico e efetivo o entendimento dos ecossistemas aquáticos do planeta.

ANEXO 1

Normas Periodic zootaxa (chapter III).

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2) The title should be concise and informative. The higher taxa containing the taxa dealt with in the paper should be indicated in parentheses: e.g. A taxonomic revision of the genus *Aus* (Order: family).

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words that are not present in the title. Abstract and key words are not needed in short correspondence.

5) The arrangement of the main text varies with different types of papers (a taxonomic revision, an analysis of characters and phylogeny, a catalogue etc.), but should usually start with an introduction and end with a list of references. References should be cited in the text as Smith (1999), Smith & Smith (2000) or Smith et al. (2001) (3 or more authors), or alternatively in a parenthesis (Smith 1999; Smith & Smith 2000; Smith et al. 2001). All literature cited in the text must be listed in the references in the following format (see a sample page here in PDF).

A) Journal paper:

Smith, A. (1999) Title of the paper. Title of the journal in full, volume number, page range.

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Smith, A. & Smith, B. (2000) Title of the Chapter. In: Smith, A, Smith, B. & Smith, C. (Eds), Title of Book. Publisher name and location, pp. x–y.

C) Book:

Smith, A., Smith, B. & Smith, C. (2001) Title of Book. Publisher name and location, xyz pp.

D) Internet resources

Author (2002) Title of website, database or other resources, Publisher name and location (if indicated), number of pages (if known). Available from: <http://xxx.xxx.xxx/> (Date of access).

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