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SHORT COMMUNICATION

Morphometric study of a Brazilian strain of *Carchesium polypinum* (Ciliophora: Peritrichia) attached to *Pomacea figulina* (Mollusca: Gastropoda), with notes on a high infestation

Roberto Júnio P. Dias[1, 3]; Adalgisa F. Cabral[1]; Isabel C. V. Siqueira-Castro[2]; Inácio D. da Silva-Neto[2] & Marta D'Agosto[1]

[1] *Laboratório de Protozoologia, Programa de Pós-graduação em Ciências Biológicas, Comportamento e Biologia Animal, Universidade Federal de Juiz de Fora. 36036-900 Juiz de Fora, Minas Gerais, Brazil.*
[2] *Laboratório de Protistologia, Departamento de Zoologia, Universidade Federal do Rio de Janeiro. 21941-590 Rio de Janeiro, Rio de Janeiro, Brazil.*
[3] *Corresponding author. E-mail: rjuniodias@hotmail.com*

ABSTRACT. During an ecological study of the epibiotic relationship between ciliate protists and *Pomacea figulina* (Spix, 1827) (Gastropoda, Ampullariidae), originating from an urban stream in southeast Brazil, a high infestation by the peritrich ciliate *Carchesium polypinum* (Linnaeus, 1758) Ehrenberg, 1830 (Ciliophora, Peritrichia) associated to the shell of one mollusc among 23 was observed. We provided a morphological and morphometric study of *C. polypinum* using observations of specimens *in vivo*, after protargol staining, and examined using scanning electron microscopy. The Brazilian-population of *C. polypinum* is characterized by: size of zooid *in vivo* 89 µm x 57 µm on average; colony regularly dichotomously branched with usually up to 40 zooids; macronucleus usually J-shaped; single contractile vacuole located in the upper third of body; myoneme not continuous throughout the colony; stalks contract despite the discontinuity of their individual myonemes; polykinety comprises three peniculi, each consisting of three kineties. The high infestation showed here could be related to the preference for eutrophic environments showed by *C. polypinum* and suggested that ciliate epibionts may be ecologically important in aquatic habitats.

KEY WORDS. Ampullariidae; epibiosis; gastropod; morphology; peritrichs.

The peritrich ciliate *Carchesium polypinum* (Linnaeus, 1758) Ehrenberg, 1830 is commonly found in freshwater ecosystems and is a good indicator of poor water quality (Wei *et al*. 2004). Ciliates of the genus *Carchesium* live in colonies containing zooids, contractile stalks and free-swimming telotrochs (Zagon 1971) and have high colonization rates in eutrophic ecosystems (Kusuoka & Watanabe 1989). They generally do not display host specificity and could be found on seaweeds, emerged and submersed macrophytes, and attached to different groups of aquatic invertebrates (Foissner *et al* 1992, Cook *et al*. 1998, Mayén-Estrada & Aladro-Lubel 2002), including the gastropod *Pomacea figulina* (Spix, 1827) (Dias *et al*. 2008). In addition to colonize living hosts, they also attach to inert substrates and have been recorded in diverse lotic systems associated to sediment (Kusuoka & Watanabe 1989, Sola *et al*. 1996, Madoni & Bassanini 1999, Madoni 2005, Madoni & Braghiroli 2007).

Prosobranch molluscs in the Ampullariidae family are widely distributed in subtropical and tropical regions, freshwater ecosystems, preferentially inhabiting still waters in lotic systems. Molluscs in the genus *Pomacea* also tolerate organic pollution (Thiengo 1995), and are promising indicators of water quality (Coler *et al*. 2005). This condition increases the ecological opportunity for colonization by peritrich ciliates which present a high preference for eutrophic environments.

During an ecological study on the epibiont ciliate community and prosobranch molluscs *Pomacea figulina* (Gastropoda: Ampullariidae) (Dias *et al*. 2008), collected in an urban stream, a high infestation of the peritrich ciliate *Carchesium polypinum* (Ciliophora, Peritirchia) associated to the shell of one mollusc, out of 23, was analyzed. The gastropod (5.0 x 4.6 cm) was collected from a sampling station (21°46'38.1"S, 43°24'0.4"W) in São Pedro stream, at an urban area of Juiz de Fora, Minas Gerais, southeast of Brazil. Water samples were collected and fixed with 10% formaldehyde (5 ml) to quantify the bacterial density (Hobbie *et al*. 1977), and to assess chlorophyll concentration (5 ml) (Apha 1992). Water temperature, conductivity, pH and dissolved oxygen data were measured with portable equipment. In the laboratory, the mollusc was scrubbed with a blader on Petri dishes

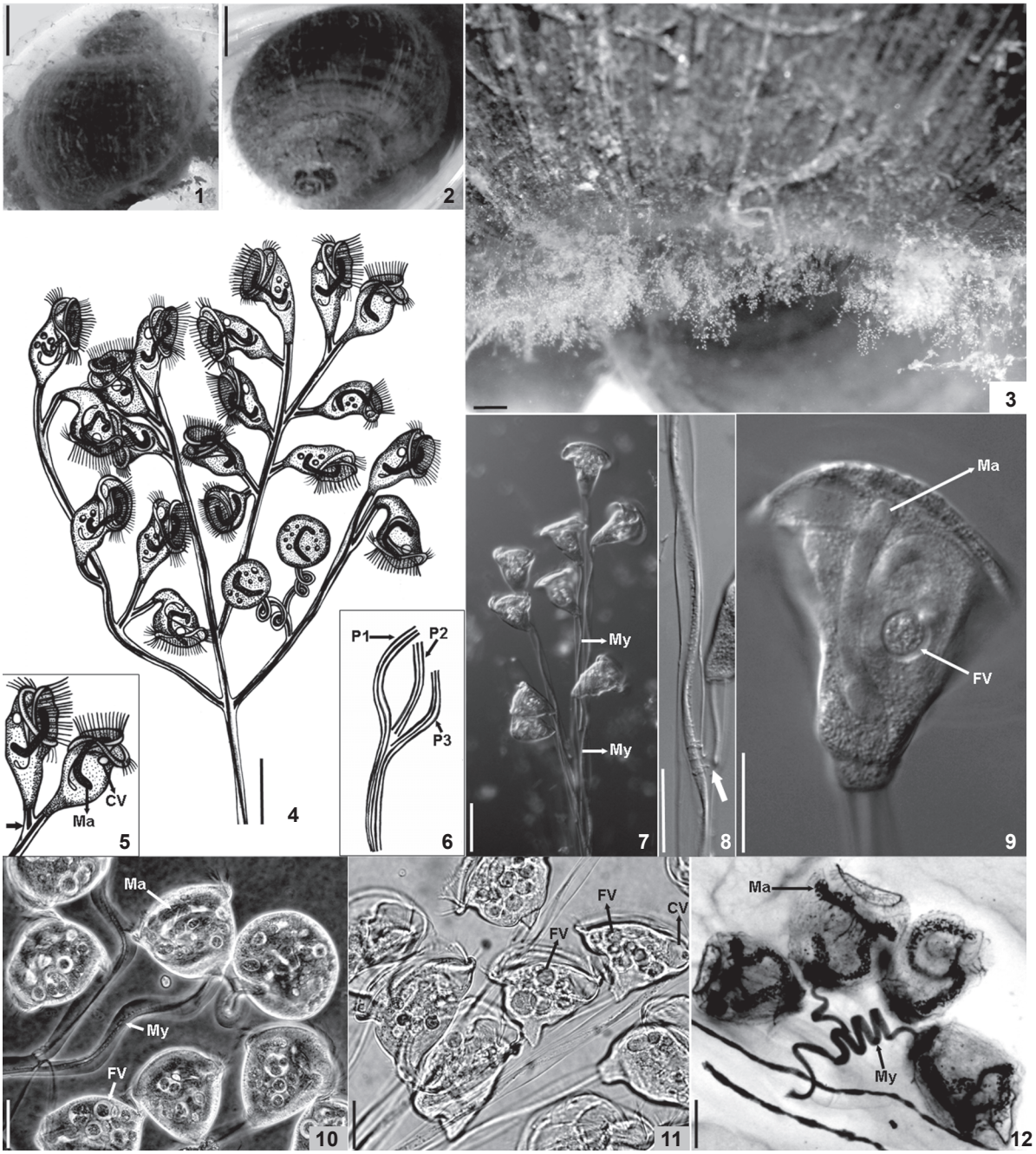
containing previously filtered water collected from the same place. The ciliates were observed *in vivo* through bright field and differential interference contrast microscopy, stained using the protargol technique (Dieckmann 1995), and prepared for scanning electron microscopy (Silva-Neto 1994). Examinations of *in vivo* and prepared slides were made with an Olympus BX51 bright field microscopy while the biometric analyses were performed using Image Pro-Plus 5.0 software. The ciliates were then identified according to Zagon & Small (1970), Esteban & Fernández-Galiano (1989), and Foissner *et al*. (1992). The mollusc was sent to the Malacology Sector, Instituto Oswaldo Cruz, and identified by Dr Silvana Thiengo.

Morphological analyses revealed that a single species of a colonial peritrich *Carchesium polypinum* colonized *P. figulina* collected in São Pedro stream (Figs 1-3). The overall morphology of the Brazilian strain of *C. polypinum* described in the present study is very similar to the species studied by Zagon (1970, 1971), Zagon & Small (1970), Curds *et al*. (1983), Esteban & Fernández-Galiano (1989), Foissner *et al*. (1992), so we considered both conspecific. We provided a morphometric characterization of the species based on 10 characters measured from living individuals and nine characters measured from protargol-stained specimens (Tab. I).

The colonies of *C. polypinum* are branched with inverted bell-shaped zooids arranged on the branches. The colonial assemblage has a main stalk that is joined to the substratum by a fixing disc. The colonies had usually up to 40 zooids. The zooids are found in symmetrical dichotomous colonies. Colonies range in length from 700 µm to 2 mm. Zooids *in vivo* from 76.9 to 110.6 µm in length, and between 35.6 and 83.6 µm in width. The size of impregnated zooids varied between 45.0-57.1 µm in length and 36.4-55.9 µm in width. The length of the zooid decreased by 42% when stained. Cytoplasm slightly greyish, usually containing several large bronwish food vacuoles (7-20 µm in diameter). A single contractile vacuole located in the upper third of body. Macronucleus J-shaped that usually descended to a region just aboral to the telotroch band. The macronucleus is large and occupies a significant proportion of the body volume. The micronucleus was located close to the J-shaped macronucleus. The peristomial disc *in vivo* is 50.1-107.8 µm wide and

Table I. Measurements (µm) of *C. polypinum* attached to *P. figulina* from São Pedro stream, southeast Brazil. A total number of 15 zooids were measured *in vivo* and 15 zooids after protargol staining. (Mean) Arithmetic mean, (SD) standard deviation, (CV) coefficient of variation in%.

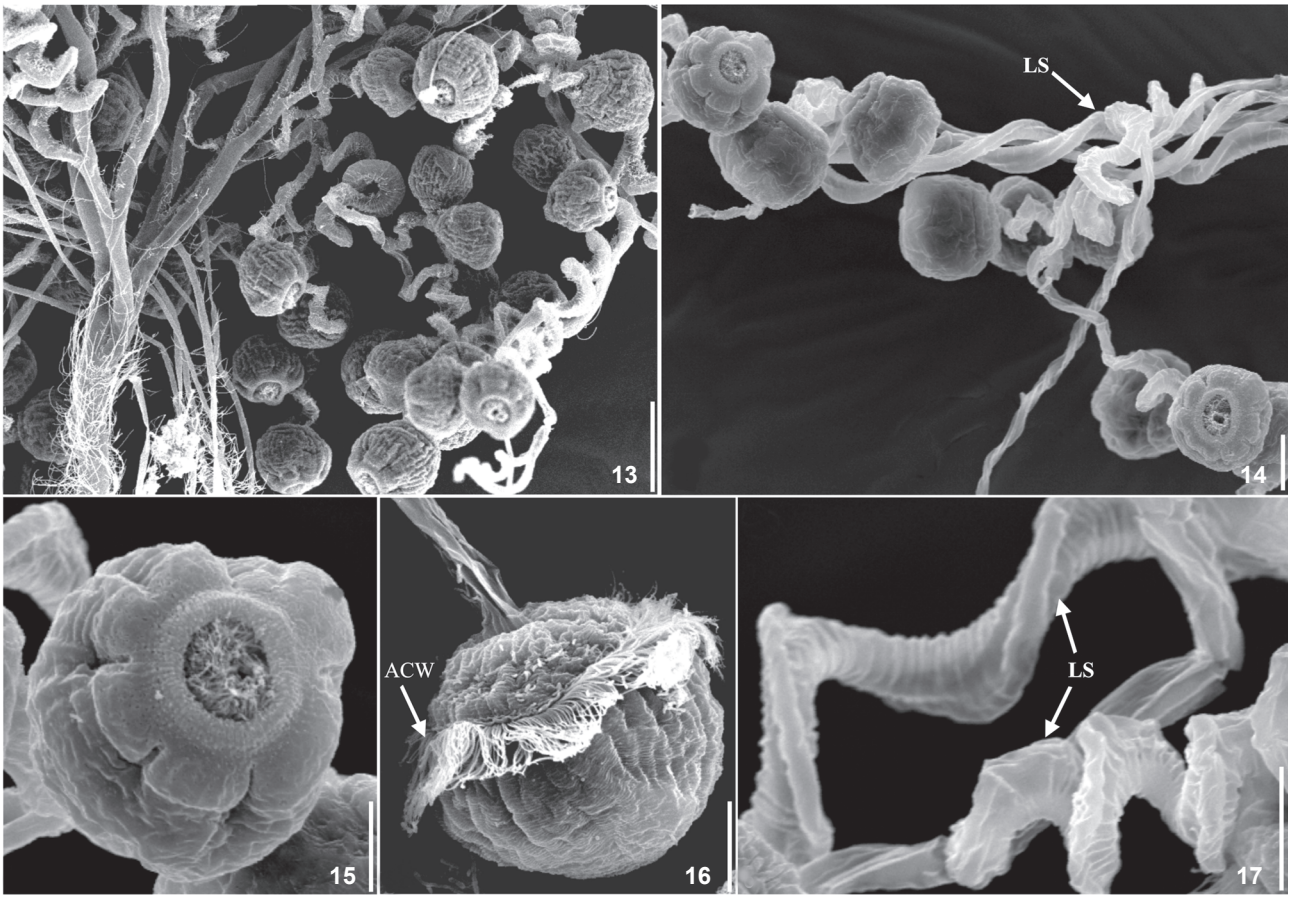
	Mean	SD	CV	Range
In vivo				
Total length of the body from epistomial disk to aboral end	88.9	14.7	16.5	76.9 – 110.6
Width of the body below the peristomial disk	57.1	14.2	24.9	35.6 – 83.6
Width of the body at midpoint between oral and aboral ends	37.1	8.2	22.2	25.9 – 51.3
Width of peristomial disk	70.8	17.0	24.0	50.1 – 107.8
Thickness of peristomial disk	8.7	4.0	46.3	5.0 – 20.8
Width of scopula	13.7	3.7	26.6	7.7 – 20.6
Width of basal stalk	12.7	3.7	29.0	7.0 – 19.0
Width of lateral stalk	13.0	3.1	24.1	10.8 – 17.4
Diameter of contractile vacuoles	17.2	3.9	22.6	10.3 – 25.0
Diameter of food vacuoles	13.3	5.4	40.4	6.7 – 26.0
Protargol				
Total length of the body from epistomial disk to aboral end	50.9	3.5	6.8	45.0 – 57.2
Width of the body at midpoint between oral and aboral ends	45.3	5.3	11.7	36.4 – 55.9
Distance between trochal band and scopula	12.6	2.1	16.8	9.2 – 17.6
Length of micronucleus	7.0	2.1	29.4	5.0 – 9.1
Width of micronucleus	8.1	0.2	2.3	7.9 – 8.3
Width of macronucleus at midpoint	4.6	1.3	29.2	2.9 – 7.4
Width of myoneme	2.9	1.0	34.8	1.2 – 4.9
Length of peristomial area	31.2	3.3	10.7	22.6 – 38.3
Width of peristomial area	22.2	3.4	15.5	14.9 – 27.2



Figures 1-12. Photomicrographic images of *C. polypinum* attached to the freshwater apple snail *P. figulina*. (1-3) Colonies of *C. polypinum* attached to *P. figulina* (high infestation). (4) Schematic drawing of the colony of *C. polypinum*. (5) Schematic drawing of two zooids showing macronuleus (Ma), contractile vacuoles (CV) and the non-continuous myonemes of the colony (arrow). (6) A diagrammatic drawing of the buccal morphology based on protargol silver preparations, showing oral polykinetids 1 (P1), oral polykinetids 2 (P2), and oral polykineitids 3 (P3). (7) Colony (*in vivo*) showing myonemes (My), differential interference contrast microscopy. (8) Detail of the non-continuous myonemes (arrow), differential interference contrast microscopy. (9) Zooid (*in vivo*) showing macronuleus (Ma) and food vacuoles (FV), differential interference contrast microscopy. (10-11) Colony (*in vivo*) showing macronuleus (Ma), myonemes (My), food vacuoles (FV) and contractile vacuoles (CV), phase contrast and bright field microscopy, respectively. (12) Protargol impregnation showing macronuleus (Ma) and myonemes (My). Bars: (1-2) 1 cm, (3) 1 mm, (4 and 7) 100 µm, (8-12) 50 µm.

5.0-20.8 µm thick. Located on the side opposite to the peristome is the lateral stalk, which in non-contracted specimens measured 10.8-17.4 µm in width. The lateral stalk surface had irregular folds as showed by scanning electron microscopy. The myoneme is not continuous throughout the colony. The stalks contract despite the discontinuity of their individual myonemes. Myoneme ranges from 1.2-4.9 µm in width, presenting fibers that extended anteriorly within the zooid, from the scopula to central part of the cell body. The myoneme stains heavily with silver, but the remainder of the stalk is transparent. As observed in other peritrichs, the infraciliature of the zooid of *C. polypinum* is formed by the oral infraciliature and the aboral ciliary wreath. The aboral ciliary wreath (trochal band) is constituted by a ridge of kinetosomes placed in two staggered rows surrounding the posterior end of the organism. The distance between the trochal band and the scopula is 9.2-17.6 µm. However, these numbers probably are smaller than the actual values because contraction is initiated by silver-impregnation. The oral infraciliature is well developed in this species. The oral apparatus is usual for peritrichs. The haplokinety and polykinety circle about one turn around the peristomial disc and make a further turn after plunging into the infundibulum. The polykinety comprises three peniculi (oral polykinetids) in the lower half of infundibulum, each consisting of three kineties. The oral polykinety 1 (P1) is paralleled at its point of separation by a second triple row of kinetossomes. The posterior ends of the three kineties of P1 terminate at slightly different levels. The oral polykinetid 2 (P2) appears to begin at a slight angle and from a single point presents a short distance from P1, turns slightly, and can then be clearly seen as three distinct ciliated rows of kinetossomes. P2 is interposed between oral polykinetids 1 and 3. The oral polykinetids 3 (P3), presents three short rows of kinetossomes, and appears approximately in the aboral one-third of the infundibulum (Figs 4-17, Tab. I).

The Brazilian strain of *C. polypinum* is similar to the population described by Foissner *et al.* (1992) in terms of body length



Figures 13-17. Scanning electron micrographs of the Brazilian strain of *C. polypinum* attached to *P. figulina*. (13) Colony of *C. polypinum*. (14) Zooids of *C. polypinum* showing the lateral stalk (LS). (15) Frontal view of *C. polypinum*. (16) Lateral view of *C. polypinum* showing the aboral ciliary wreath (ACW). (17) Lateral stalk (LS). Bars: (13) 100 µm, (14) 50 µm, (15-17) 25 µm.

(77-110 µm vs. 80-140 µm), number and position of contractile vacuoles, shape of macronucleus, number of zooids, and the oral infraciliature. As demonstrated by recent papers, the infraciliature revealed with silver impregnation is highly species-specific, especially the structure of infundibular polykineties in the oral apparatus, playing an essential role in the determination of species (Clamp 1990, Ji & Song 2004, Ji *et al.* 2005). Several published reports describe the morphology of the colony and zooids of *C. polypinum* (Kahl 1935, Lom 1964, Curds 1969, Zagon 1970, 1971, Zagon & Small 1970, Curds *et al.* 1983, Esteban & Fernández-Galiano 1989, Foissner *et al.* 1992), however, few morphometric characters were included in these studies. In the present study, we provide a characterization of the species based on new morphometric characters as used by Utz (2007).

Studies emphasizing genetic variation within-species are needed to determine whether *C. polypinum* collected from different sites around the world could be considered a single genetic unit. For example, Gentekaki & Lynn (2009) concluded that colonies of *C. polypinum* isolated from different locations in the Grand River basin in Southwestern Ontario, Canada, probably are not a single morphospecies as previously thought.

The only snail infested by the peritrich ciliate *Carchesium polypinum* (Linnaeus, 1758) Ehrenberg, 1830 (Ciliophora, Peritrichia) observed in this study was collected near to the entrance of domestic sewage in the stream suggesting that the organic pollution level could be the cause of this high infestation. Direct discharge of domestic sewage into the water causes an elevation in the concentration of phosphates and other nutrients, increasing the density of bacteria, the main food for peritrich ciliates (Primc 1988). The high infestation showed here could be related to the preference for eutrophic environments showed by *C. polypinum*. The autecological data recorded for *C. polypinum* in the present study and in previous studies as revised by Foissner *et al.* (1992) are presented in table II. This observed infestation demonstrates that ciliate epibionts may be ecologically important in aquatic habitats and their relative biomass should be taken into consideration when benthic ciliates, in a given habitat, are assessed.

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Table II. Autecological data recorded for *C. polypinum* attached to *P. figulina* from São Pedro stream, Southeast Brazil (present study) and in previous studies as revised by Foissner *et al.* (1992).

Parameters	Foissner *et al.* (1992)						Present study
Water temperature (°C)	0–25	4–8	3–32	5–16	3–19	0.1–11.2	25
Conductivity (µS/cm)	–	–	390–850	–	560–35530	142–688	89.7
pH	6.4–8.3	7.0–7.5	6.4–8.3	7.5–7.6	7.4–8.0	7.2–8.7	6.4
O2 (mg/l)	0.2–14	7–12	0.1–13	4.2–12	1.2–47	6.1–14.7	1.0
Chlorophyll (µg/l)	–	–	–	–	–	–	94.4
Bacterial density (cells/l)	10^6–10^7	$5x10^6$	44–$350x10^3$	–	–	315–29000	1.17×10^9

(–) Not informed.

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