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New record and morphology of *Fragmocirrus espeletiae* Foissner, 2000 (Alveolata, Ciliophora, Stichotrichia) from soils of the Atlantic Forest in Rio de Janeiro, Brazil

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Abstract. The first record and morphologic characterization of a Brazilian population of the rare ciliate *Fragmocirrus espeletiae* are provided based on specimens obtained from samples of leaf litter and soil collected from an Atlantic Forest fragment located near the town of Nova Friburgo, RJ. The specimens of *F. espeletiae* from the Atlantic Forest are conspicuously small and their meristic characters related to the ciliature have generally smaller average values in comparison to the Venezuelan type population. However, most of the comparable morphometric data overlap with the original description, thus both populations are considered morphologically conspecific.

Keywords: Kahliellidae, morphometry, South America, Spirotrichea, taxonomy

Resumo. Novo registro e morfologia de *Fragmocirrus espeletiae* Foissner, 2000 (Alveolata, Ciliophora, Stichotrichia) presente em solos da Mata Atlântica no Rio de Janeiro, Brasil. O primeiro registro e a caracterização morfológica de uma população brasileira do raro ciliado *Fragmocirrus espeletiae* são providos com base em espécimes obtidos de amostras de folhiço e solo coletadas de um fragmento da Mata Atlântica localizado próximo à cidade de Nova Friburgo, RJ. Os espécimes de *F. espeletiae* da Mata Atlântica são notavelmente menores e seus caracteres merísticos relacionados à ciliatura apresentam valores médios geralmente reduzidos em comparação com a população-tipo venezuelana. Entretanto, a maioria dos dados morfométricos comparáveis sobrepõe-se aos da descrição original, sendo então as duas populações consideradas morfologicamente conspecíficas.

Palavras-chave: Kahliellidae, morfometria, América do Sul, Spirotrichea, taxonomia

Fragmocirrus Foissner, 2000 is a rare genus of stichotrich ciliate presenting a variable ventral ciliature (Foissner, 2000), in which pattern resembles that of *Nudiamphisiella interrupta* Foissner, Agatha & Berger, 2002, however exhibiting extra marginal cirral rows formed as in *Parakahliella* Berger, Foissner & Adam, 1985, and also transverse cirri (Berger *et al.*, 1985; Berger & Foissner, 1989; Foissner, 2000; Foissner *et al.*, 2002; Paiva & Silva-Neto, 2009). Currently, there are two nominal species assigned to genus *Fragmocirrus*, namely the type species *F. espeletiae* Foissner, 2000, and *F. terricola* (Buitkamp, 1977) (Foissner, 2000). The former was described from leaves of dead *Espeletia* trunks collected from Cordillera de Mérida, Venezuela (Foissner, 2000) and the latter from a pasture near Bonn, Germany (Buitkamp, 1977). We present herein the first record and morphologic characterization of a population of *F. espeletiae* from soil samples of an Atlantic Forest fragment in Rio de Janeiro, Brazil.

Samples of leaf litter and soil from the Atlantic Forest were collected near the town of Nova Friburgo-RJ, Brazil (22°23'21"S; 42°20'20"W) in August of 2008 and brought to the laboratory, where they were rewetted by the "non-flooded" Petri dish technique (see Foissner *et al.*, 2002). The water from the soil infusion containing ciliates was collected and transferred to additional Petri dishes, where cultures were made with addition of mineral water from Petrópolis (RJ) and crushed rice grains. The organisms were fixed with alcoholic Bouin's fluid and impregnated with protargol, following the protocol described by Dieckmann (1995). Unfortunately, individuals of *F. espeletiae* could not be examined *in vivo*, thus only data from specimens in protargol-impregnation slides are provided.

Measurements present in Tab.1 are in µm, and were made under 1,000× magnification (oil immersion). Diagramatic representations of *F. espeletiae* showing the ciliature pattern were made with aid of computer image edition software and were based on photographs of actual protargol-impregnated specimens. Descriptive statistics were calculated in the computer software GraphPad Prism 4 (Motulsky, 1999). Permanent voucher protargol-impregnation slides of *F. espeletiae* and of other ciliates obtained during our survey were deposited in the collection of Laboratório de Protistologia, UFRJ.

Morphology of Fragmocirrus espeletiae from Atlantic Forest (Figs. 1–11)

Specimens of *F. espeletiae* found in samples of soil from the Atlantic Forest are dorsoventrally flattened and exhibit body outline elliptical, usually narrowed at anterior and posterior ends, with the left margin slight curved inwards. The adoral zone (AZM) is shaped as a question mark, occupies ~ 37% of body length and is formed by on average 29 membranelles (Fig.1; Tab.1), presenting a DE-value (see Berger, 2006) of ~ 0.2. Right of the AZM, the paroral membrane optically intersects the endoral at about midline, exhibiting the typical "*Oxytricha*-pattern" (Berger & Foissner, 1997) (Fig.1).

The fronto-ventral ciliature is formed by invariably three strong frontal cirri, plus 2–4 buccal and 2–5 fronto-ventral cirri. The buccal cirri are located behind the middle frontal cirrus, and the fronto-ventral behind the right frontal cirrus. A conspicuously gapped amphisiellid cirral row is present. It is formed by an anterior piece composed of ~ 12 cirri, which stands right of the fronto-ventral cirri, and a posterior piece formed of ~ 11 cirri, which occupies the middle region of the ventral surface. Rarely, an extra cirral row is present behind the anterior piece, to the right of the posterior one (Fig.5). Additionally 1-4 transverse cirri are present, arranged in a cluster near the posterior end of the ventral surface.

The marginal cirri are organized as two long rows, viz. the outer right and inner left marginal rows; plus two short additional ones, viz. the inner right and outer left marginal rows. Remarkably, the inner right marginal row is present in most studied specimens (Figs.1, 4), but only three individuals out of 25 bore an outer marginal row, which in the specimen shown in Fig.6, is subdivided in two segments. On the dorsal surface there are always three long kineties plus an additional fourth, dorsomarginal kinety (Figs.2, 6). Remarkably, the leftmost kinety is always shortened anteriorly (Fig.7). In some specimens, kinety 3 is strongly bent rightwards,

so that its posterior region appears continuous with the dorsomarginal kinety (Fig.8). Two to three caudal cirri are present at the posterior end of the dorsal surface (Figs.9, 10).

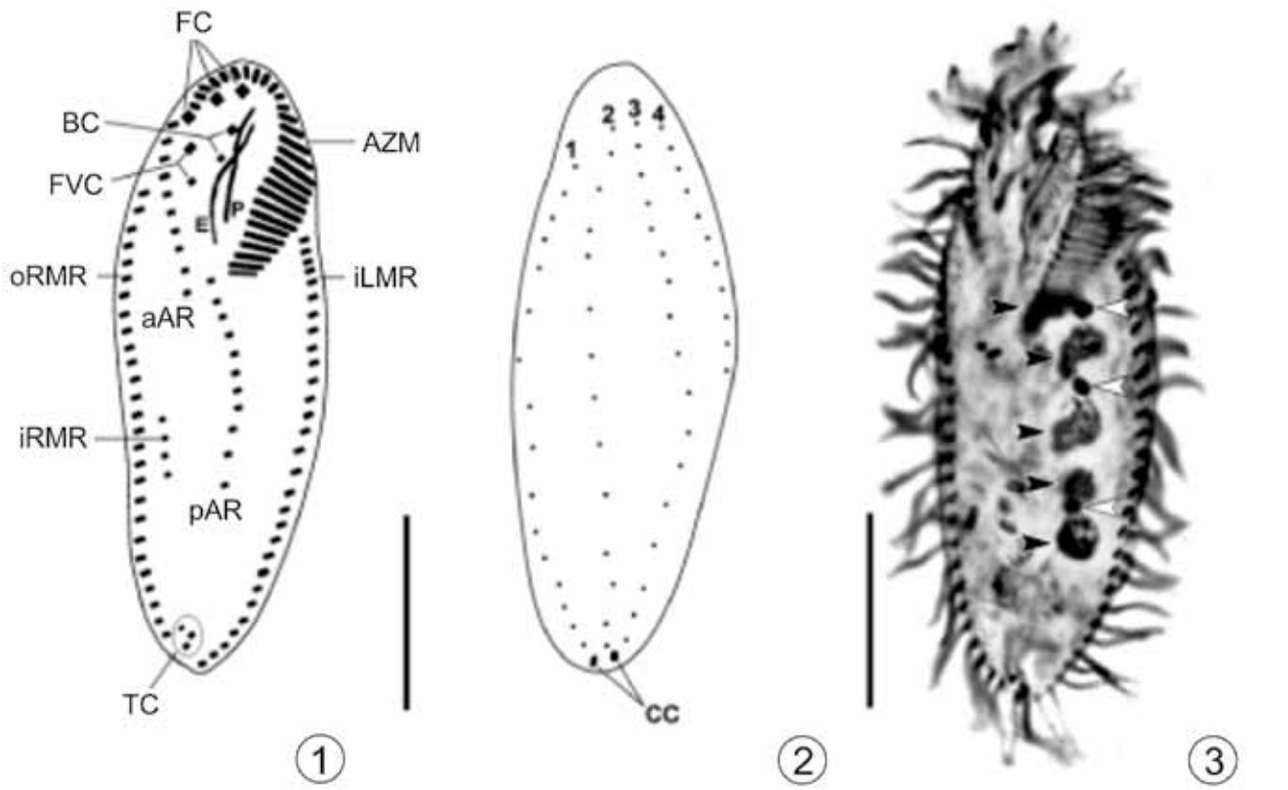
The macronucleus is formed by 4-7 nodules which vary from roughly ovoid or ellipsoid to irregular forms. They are located along the left side of the organisms, and the anterior most nodule measures ~ 7.5 x 5.5 µm. One to eight spheroid micronuclei of ~ 2 µm in cross-section are present at the vicinity of the macronuclear figure (Figs.3, 11).

Table 1. Morphometric characterization (µm) of the Atlantic Forest population of *Fragmocirrus espeletiae*. CV – coefficient of variance; M – median; Max – maximum; Mean – arithmetic mean; Min – minimum; N – sample size; SD – standard deviation; SE – standard error.

Character	Mean	M	SD	SE	CV(%)	Min	Max	N
Body length	73.2	73.0	6.9	1.4	9.4	64.0	85.0	25
Body width	26.8	27.0	3.9	0.8	14.6	21.0	35.0	25
Distance from anterior end of the body to proximal end of the adoral zone	27.1	28.0	3.0	0.6	11.0	22.0	32.0	25
Number of adoral membranelles	29.0	29.0	2.2	0.5	7.7	24	32	24
Length of paroral membrane	16.0	15.0	3.0	0.6	18.5	13.0	23.0	23
Length of endoral membrane	16.5	15.5	3.1	0.7	18.7	13.0	23.0	22
Distance from anterior end of body to paroral membrane	4.2	4.0	0.8	0.2	20.0	3.0	6.0	23
Distance from anterior end of body to endoral membrane	5.5	6.0	1.0	0.2	17.4	3.0	7.0	22
Number buccal cirri	2.3	2.0	0.6	0.1	24.0	1	3	25
Number of fronto-ventral cirri	2.5	2.0	0.6	0.1	23.6	2	4	25
Number of ventral cirral rows	5.1	5.0	0.3	0.1	5.5	5	6	25
Number of cirri in anterior piece of amphisiellid row	11.5	11.5	1.9	0.4	16.8	8	15	22
Number of cirri in posterior piece of amphisiellid row	11.0	11.0	1.4	0.3	12.7	9	15	24
Distance from anterior end of anterior piece of amphisiellid cirral row to anterior end of body	25.1	25.0	3.6	0.8	14.1	21.0	39.0	22

Table 1. Continuação

Distance from posterior end of posterior piece of amphisiellid cirral row to posterior end of body	44.8	44.5	5.0	1.0	11.2	35.0	55.0	24
Lateral distance between anterior and posterior pieces of amphisiellid cirral row	3.6	3.0	0.9	0.2	23.8	2.0	5.0	22
Number of transverse cirri	2.7	3.0	0.6	0.1	23.4	1	4	25
Number of left marginal cirral rows	1.1	1.0	0.3	0.1	29.6	1	2	25
Number of right marginal cirral rows	1.8	2.0	0.4	0.1	20.8	1	2	24
Number of cirri in inner left marginal row	27.3	27.0	2.4	0.5	8.6	22	31	25
Number of cirri in outer left marginal row	10.3	10.0	1.5	0.9	14.8	9	12	3
Number of cirri in inner right marginal row	6.0	6.0	3.1	0.7	51.9	1	15	20
Number of cirri in outer right marginal row	31.4	31.0	3.7	0.8	11.8	25	39	24
Number of bristles in dorsal kinety 1	15.6	15.0	2.3	1.0	14.8	13	18	5
Number of bristles in dorsal kinety 2	13.8	13.0	2.6	1.2	18.8	11	17	5
Number of bristles in dorsal kinety 3	18.2	20.0	3.0	1.4	16.7	14	21	5
Number of bristles in dorsal kinety 4	10.6	11.0	2.1	0.9	19.6	8	13	5
Number of caudal cirri	2.2	2.0	0.4	0.1	19.5	2	3	21
Number of macronuclear nodules	5.7	6.0	1.0	0.2	17.1	4	7	25
Length of anterior most macronuclear nodule	7.5	6.0	3.1	0.6	41.4	4.0	18.0	25
Width of anterior most macronuclear nodule	5.5	5.0	1.7	0.3	30.1	2.0	10.0	25
Number of micronuclei	4.0	4.0	1.6	0.3	38.5	1	8	22
Size of micronuclei in cross-section	2.2	2.0	0.4	0.1	18.1	2.0	3.0	22



Figures 1-3. Morphology of *Fragmocirrus espeletiae* after protargol-impregnation. **1.** Diagramatic representation of the ventral region; **2.** Diagramatic representation of the dorsal region with dorsal kineties numbered in arabics; **3.** Specimen showing the macronuclear nodules (black arrowheads) and micronuclei (white arrowheads). AZM – adoral zone; aAR – anterior piece of the amphisiellid cirral row; BC – buccal cirri; CC – caudal cirri; E – endoral membrane; FC – frontal cirri; FVC – fronto-ventral cirri; iLMR – inner left marginal cirral row; iRMR – inner right marginal cirral row; oRMR – outer right marginal row; P – paroral membrane; pAR – posterior piece of the amphisiellid cirral row. Scale bars = 20 µm.

Figures 4-11. Morphology of *Fragmocirrus espeletiae* after protargol-impregnation. **4.** Ventral side. Arrowhead points to the inner right marginal cirral row; **5.** Ventral side of specimen with a surplus ventral row (white arrows) behind the anterior piece and to the right of the posterior piece of the amphisiellid cirral row (black arrows); **6.** Ventral side of specimen with a bipartite outer left marginal row (arrowheads); **7.** Dorsal side of specimen with the dorsal kineties numbered in arabics. Arrowheads mark a conspicuous gap between the posterior end of the dorsomarginal kinety 4 and the middle region of the dorsal kinety 3; **8.** Dorsal side of specimen with the dorsal kineties numbered in arabics. Arrowheads mark an inconspicuous gap between the posterior end of the dorsomarginal kinety 4 and the middle region of the dorsal kinety 3, which is strongly bent rightwards; **9.** Posterior region of dorsal side of specimen with three caudal cirri (arrowheads); **10.** Posterior region of dorsal side of specimen with two caudal cirri (arrowheads); **11.** Specimen with irregular shaped macronuclear nodules (black arrowheads). The white arrowhead shows a micronucleus. Scale bars = 20µm.

Discussion

According to Foissner (2000), the type population of *F. espeletiae* differs from *F. terricola* in the number of adoral membranelles (38 vs. 28), dorsal kineties (4 vs. 5), left marginal rows (1–2 vs. 3) and macronuclear nodules (4 vs. 8) (Buitkamp, 1977; Foissner, 2000). The new data from the Atlantic Forest population indicate the differences between both species are rather subtle, as its number of adoral membranelles is closer to that of *F. terricola* (29 vs. 28) than to the type population, and in addition, its average number of macronuclear nodules (6) is intermediate between the Venezuelan population of *F. espeletiae* and *F. terricola*. However, the presence of invariably four dorsal kineties, and of 1-2 left marginal rows instead of three justify the assignment of the Atlantic Forrest population in *F. espeletiae* (Foissner, 2000). Recently, Berger (2011) transferred *F. terricola* back to genus *Parakahliella* because he supposed the small cirri left of the posterior end of the right marginal row in this species were not actual transverse cirri. According to Berger (2011), such cirri could be interpreted as remains of the parental ciliature. Investigation of morphogenesis in *F. terricola* is necessary to clarify this subject.

When further compared to the Venezuelan type population, the present specimens of *F. espeletiae* are conspicuously smaller. The specimens of the Atlantic Forest measured on average 73.2 x 26.8µm (vs. 155.9 x 64.5µm in the type population) and had the distance from anterior end of the body to proximal end of the AZM measuring on average 27.1µm (vs. 53.9µm in the type population). Hence, both populations have rather similar body length/width (2.73 vs. 2.42) and body length/AZM length (2.71 vs. 2.89) ratios.

Even though different amount of shrinkage from the type population can be expected due to differences in protargol-impregnation protocols used by Foissner (2000) and the present study, the obtained meristic characters related to the ciliature (Tab.1) have generally reduced average values than reported by Foissner (2000, p.64), being thus congruent with the smaller body size observed. Most of the comparable morphometric data, however, overlap with the original description and we did not find any invariable morphologic character that could justify the erection of a new species name for the present population (see Foissner, 1998). Hence, within a morphospecies context, we consider the Atlantic Forest population of *F. espeletiae* conspecific with the Venezuelan population.

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