Description and phylogenetic position of *Corlissina maricaensis* gen. nov., sp. nov. (Karyorelictea, Geleiidae), a novel interstitial ciliate from Brazil, with redefinition of the family Geleiidae

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*Corlissina maricaensis* gen. nov., sp. nov. was obtained from samples of sediment collected in a brackish lagoon of Maricá city, Rio de Janeiro state, Brazil. The morphological description was based on live observations, after protargol staining, and scanning electron microscopy. The novel species has a cylindrical body shape that is slightly contractile, 230–550 × 35–65 μm, a cytoplasm with many globular inclusions, one row of irregular cortical granules between each somatic kinety, approximately 40–62 somatic kineties, two globular macronuclei measuring 9–24 μm and one micronucleus of approximately 4–9 μm. A subapical oral cavity was approximately 20–80 × 9–25 μm, with an adoral zone on the left side of the buccal field, which was composed of 32–60 polykineties and a paroral at the right side that was composed of 40–57 short polykineties. The new genus is distinguished from other geleiids by a loop-shaped posterior end of the paroral ciliature, made up of two rows of short polykineties, and the oralization of the central superior kinety (K0), forming a row of dikinetids that borders the adoral zone internally, followed by several rows of monokinetids. In the phylogenetic analyses, the novel species was recovered as the sister group of *Parduczia orbis* with full support values based on 18S rRNA gene sequences. This work also indicates some problems in the definitions of the Geleiidae and proposes a new diagnosis for this karyorelictid family.

INTRODUCTION

The class Karyorelictea is represented by contractile ciliates of elongated body shape and reduced somatic ciliature that inhabit the marine interstitial (Puytorac, 1994; Hausmann & Hülsmann, 1996; Foissner, 1998; Lynn & Small, 2002; Jankowski, 2007; Lynn, 2008). These ciliates are considered the ancestral lineage of the Ciliophora because they retain basal characteristics, such as an indivisible macronucleus and the simplest oral ciliature pattern (Jankowski, 2007; Lynn, 2008; Gao & Katz, 2014). Despite its importance for evolutionary and taxonomic studies within the Ciliophora, the class Karyorelictea is still underrepresented and there are few studies on the morphology and phylogenetic relationship of karyorelictian species. In some internal groups, the lack of information has contributed to great taxonomic misinterpretations and nomenclatural errors, as seen in the order Protoheterotrichida, which included the families Geleiidae Kahl, 1933 and Aveliidae Dragesco, 1999.

Originally, these two groups were one family, Geleiidae Kahl, 1933, which included only the genus *Geleia* Kahl, 1933. Nouzarede (1977) described the genus *Avelia* (nom. nov. for *Avela* Nouzarede, 1975) and included it in the family Aveliidae. Subsequently, Foissner (1998) re-established the genus *Geleia*, which was considered by him a *nomen nudum* due to the lack of the type, and declared a type
species, namely *Geleia fossata* Kahl, 1933. In Dragesco (1999), the family Geleiidae appears assigned as Geleiidae Foissner, 1998, which appears to be a nomenclatural mistake, since Foissner did not redefine the family. Dragesco (1999), based on the paroral infraciliature pattern, excluded *Avelia* from the Geleiidae and included it in his own family Aveliidae Dragesco, 1999. The differences between these two genera were reported by Nouzaréde (1977); however, he was not able to identify them. In the same work, Dragesco (1999) established two new genera, i.e. *Gellertia*, which was assigned to the family Geleiidae, and *Parduczia*, which was assigned to the Aveliidae. Jankowski (2007) followed Dragesco's classification, in which the order Protoheterotrichida is represented by the two families Geleiidae and Aveliidae. In Lynn (2008), these two families appeared as synonyms, but no argument to justify this synonymization was given.

We present a detailed description of the morphology and the phylogenetic positions based on the 18S rRNA gene sequences of a new genus and a novel species of the Geleiidae named *Corlissina maricaensis* gen. nov., sp. nov., from the brackish Maricá lagoons, Rio de Janeiro State, Brazil. We have included descriptions of the morphological characters that were observed from live specimens, after protargol staining, and scanning electron microscopy. The systematic classification of the novel species is based on morphological comparisons and phylogenetic analyses, including other geleid species.

**METHODS**

**Morphology.** *Corlissina maricaensis* gen. nov., sp. nov. was collected in the interstice of the Maricá lagoon margin (22° 57’ 30” S 42° 48’ 46” W), Maricá, Rio de Janeiro, Brazil. The sampling location is a public lagoon; therefore, no specific permissions were required to collect the material that was necessary for the present study. No known endangered or protected species were involved in the present study. Samples were taken to the Laboratory of Protistology of the Federal University of Rio de Janeiro (UFRJ), where cultures were established in Petri dishes with filtered water and macerated rice grains (Foissner, 1992). Ciliates were isolated and placed on glass slides according to Foissner (1991) for observations in vivo under an optical microscope (Zeiss; Axios Imager A2) in differential interference contrast. For the infraciliature study, two protocols for protargol staining were followed, i.e. that of Dieckmann (1995) and that of Wilbert as modified by Foissner (1991). The specimens were fixed with a variation of Bouin’s fluid: 7.5 ml concentrated picric acid, 3 ml formaldehyde (37 %), 0.5 ml glacial acetic acid and 1 g NaCl diluted in 1 ml distilled water. Many other fixers were tested, but Bouin’s fluid showed the best results, despite having substantial material loss. The fixation could not last more than 5 min to facilitate the clarification process of the ciliate, as this group has many pigments (Dragesco, 1999). After fixation, specimens were washed with distilled water and prepared for staining according to the protocols mentioned above. Morphometric data were obtained from up to 45 individuals that were randomly selected. The software that was used in the biometric analysis was Axion Vision SE64. For scanning electron microscopy, ciliates were fixed in Bouin’s fluid, washed with distilled water and post-fixed with a mixture of 1 ml glutaraldehyde (9 %), 1 ml sodium cacodylate buffer (pH 6.5) and 1 ml osmium tetroxide (2 %). The material was then prepared according to Silva-Neto et al. (2012) and observed under a microscope (JEOL JSM-6510).

**DNA isolation, amplification, cloning and sequencing.** Genomic DNA of *C. maricaensis* was extracted using the Nucleo Spin Extract kit II (Macherey-Nagel), and the 18S rRNA gene was amplified using the universal eukaryotic primers Euk A (5’-AACCTGGTTGATCTCTGC-GCATG-3’) and Euk B (5’-TGATCCTTGCAGGTTACCTAC-3’) (Medlin et al., 1988).

The 25 µl PCR mixture contained 14.8 µl HPLC water, 2.5 µl 10 × DreamTag Green buffer, 0.2 µl DreamTag DNA polymerase (both from Thermo Scientific), 2.5 µl 10 µM dNTPs, 1.0 µl of each primer (10 µM stock concentration) and 3.0 µl extracted DNA. PCR amplifications were performed in a Nexus Mastercycler (Eppendorf). The amplification protocol was as follows: 5 min at 94 °C followed by 30 cycles of 94 °C for 60 s, 60 °C for 75 s and 72 °C for 90 s and a final extension at 72 °C for 10 min. PCR products were purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel) followed by the manufacturer’s protocol.

Purified PCR products of the expected size (~1700 bp) were ligated into the pGEM T-easy vector (Promega) and cloned into competent cells of *Escherichia coli* MJ109. After incubation, white colonies were selected for PCR with primers M13 forward (5’-TGTAAAACGACGGCCAGT-3’) and M13 reverse (5’-CAGGAAAACGCTATGAC-3’) (Messing, 1983). The protocol for this PCR was 3 min at 94 °C followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 90 s and a final extension at 72 °C for 5 min. Following amplification, PCR products from the M13 primers were visualized in an agarose gel, and two of those of the expected size (~1700 bp; clones 01 and 02) were selected for purification and direct sequencing at GATC Biotech (Konstanz, Germany). The sequences were assembled in contigs, analysed and edited with Geneious 7.1.7 and then compared with sequences in the NCBI database using the BLASTN algorithm.

**Phylogenetic analyses.** To determine the phylogenetic position of *C. maricaensis*, we downloaded a dataset from GenBank that was composed of 44 18S rRNA gene sequences of karyorelictids as well as six sequences of heterotrichs, which were used as the outgroup for all analyses. Alignments were achieved with the R-Coffee package (Wilm et al., 2008) using structural information. Primer sequences were removed, and the final alignment was cured with GBLOCKS server to clean ambiguously aligned positions (Castresana, 2000; http://molevol.cmima.csc.es/castresana/Gblocks_server.html).

A maximum-likelihood (ML) analysis was performed with RaxML-HPC2 implemented on the CIPRES portal (https://www.phylo.org) using default parameters and the evolutionary model GTR+GAMMA. Clade support was estimated by using 1000 bootstrap replicates. A Bayesian inference (BI) analysis was performed using the program MrBayes 3.2.3 (Ronquist & Huelsenbeck, 2003) also implemented on the CIPRES portal using the GTR+I+Γ substitution model (I=0.3940; Γ=0.5340). This model was the best fit to our data according to the program jModelTest 2 (Darriba et al., 2012). A Markov chain Monte Carlo analysis consisted of two independent trials with four chains each. Each chain was run for 10 000 000 generations and sampled every hundredth cycle. The first 250 000 generations were discarded as the burn-in. The BI tree consisted of the 50 % majority-rule consensus of the topologies that were collected after the burn-in, and clade support was calculated by the frequency of each clade in the sampled topologies. The neighbour-joining tree was inferred on MEGA software (Tamura et al., 2013) with the same evolutionary model used for BI analysis and 1000 bootstrap replicates. The similarity and absolute difference matrices were exported from Geneious. The phylogenetic trees obtained were visualized and edited using the FigTree software (http://tree.bio.ed.ac.uk/software/figtree/).
RESULTS

Family Geleidae Kahl, 1933

Corlissina gen. nov.

Diagnosis. Geleidae with a paroral ciliature composed of two rows of polykineties forming a loop at the posterior end. Adoral zone composed of dikinetids organized in short polykineties (two or three dikinetids innermost to the oral cavity) followed by a row of monokinetids. Oralization of the central somatic kinety (K0s) forming part of the adoral infraciliature. The nuclear apparatus follows the pattern of the Geleidae, i.e. two globular macronuclei that are linked by a single micronucleus.

Etymology. We propose the generic name Corlissina in honour of an extraordinary researcher and authority of the class Karyorelictia, Professor Dr John O. Corliss, who passed away in December 2014. Syllabification: Cor.lis.si’na.


Corlissina maricaensis sp. nov.

Diagnosis. Extended cells in vivo approximately 230–550 μm long and cylindrical body slightly contractile, exhibiting brown pigments in the cytoplasm, especially in the anterior region; cytoplasm with many globular inclusions; one row of very irregular cortical granules between each somatic kinety; approximately 40–62 somatic kineties that are separated by 2–10 μm; two globular macronuclei measuring approximately 9–24 μm and one micronucleus of approximately 4–9 μm. Subapical oral cavity of approximately 20–80 μm wide (Figs. 1a and 2a, b; Table 1); long and cylindrical body shape with tapered anterior and posterior ends (Figs. 1a–c, 2a, b and 3c, d). They are slightly contractile and move slowly on the bottom of Petri dishes through the sand grains; backward swims are usually seen. Cytoplasm filled by globular inclusions (Figs. 1d and 2f); small brown pigment granules throughout the cytoplasm, especially in the anterior region of the body (Fig. 1a, d, h); one row of very irregular cortical granules between each somatic kinety (Figs. 1e, f, 2c and 4f); two globular macronuclei measuring approximately 9–24 μm are separated by one micronucleus that is globular to distinctly ellipsoid, measuring approximately 4–9 μm (Figs. 1g, 2e and 4h; Table 1); in vivo, the nucleus is united (Fig. 1g) but, after protargol staining, it may be separated, especially after the Wilbert technique (Fig. 4h). We also observed some specimens with three macronuclei or a pair of two macronuclei and one micronucleus.

The surface of C. maricaensis is densely ciliated, presenting approximately 40–62 somatic kineties that are separated from each other by approximately 2–10 μm (Figs. 2c and 5a, b; Table 1). Somatic kineties are formed by oblique dikinetids, in which only the anterior kinetosome is ciliary (Figs. 2d and 4f). The inferior ventral central kinety (K0i), which forms the longitudinal axis of the ciliate, is interrupted before the oral field, near the paroral zone (Figs. 2c, 3a and 4e). On the left of the adoral zone, only three somatic kineties (K1, K2 and K3) are continuous until the anterior end of the cell, and the adjacent kineties are interrupted below and around the oral field (Figs. 2c, 3a and 4f). The superior ventral central kinety (K0s) extends through the adoral zone (Figs. 3e, f and 4d, e). The somatic dikinetids present argentophilic structures as post-ciliary fibres, forming a line parallel to the kineties (Fig. 4g).

The oral field is subapical, similar to species of Geleia (Figs. 1c and 2a, b); the distance from the anterior end to the oral field is approximately 14–66 μm; the dimensions of the oral field are approximately 16–88 × 8–30 μm (Figs. 1h, 2c and 5c; Table 1). Because of contraction of the body, the shape and size of the oral field change substantially after fixation (Figs. 3e and 4b). The paroral is constituted of two structures, one internal row that is formed by 40–57 short kineties (rounding the oral field perpendicularly, forming an arch) made up of 2 dikinetids, and one external row that is composed of 40–57 (rounding the oral field perpendicularly) formed by 2–3 dikinetids (Figs. 3e, f and 4e; Table 1). These two parallel rows are joined posteriorly, forming a loop (Figs. 3e, f and 4e). Some cells lost this loop after protargol staining, but the
Fig. 1. (a) General view of a typical specimen. (b) Cell posterior end. (c) Anterior end of the body. Filled arrowheads show interrupted kineties around the oral field; open arrowheads show kineties K1, K2 and K3. (d) Cytoplasmic inclusions. Filled arrowheads show cytoplasmic granules and cortical granules. (e) Macronuclei and micronucleus. (f) Details of the oral infraciliature. APK, Adoral polykineties; AZ, adoral zone; CG, cortical granule; Di, dinoflagellate in digestive vacuole; DK, dikinetids; Ma, macronucleus; Mi, micronucleus; Mk, monokinetids; NA, nuclear apparatus; OF, oral field; PZ, paroral zone; SK, somatic kineties. Bars, 50 μm (a), 25 μm (b, c, h) and 10 μm (e–g).

Fig. 2. (a, b) General representation of the body shape of C. maricaensis in vivo; (b) shows the ciliate contracted. (c) Cell posterior end showing the buccal infraciliature in vivo. (d) Details of the somatic kineties and cortical granulation after protargol staining. (e) Nuclear apparatus after protargol staining. (f) Cell posterior end showing cytoplasmic inclusions (white) and granules (black). AZ, Adoral zone; C, cilia; CG, cortical granulation; CI, cytoplasmic inclusions; CyG, cytoplasmic granules; Dk, dikinetids of somatic kinety; DV, digestive vacuole; Ma, macronucleus; Mi, micronucleus; NA, nuclear apparatus; OF, oral field; PZ, paroral zone; SK, somatic kineties. Bars, 50 μm (a, b) and 25 μm (c–f).
vast majority retained it. On the paroral right side, there is a ‘dense zone’ (sensu Dragesco, 1999), an arch that is composed of argentophilic fibres (Figs. 3f and 4e). The adoral zone is composed of 32–61 short polykineties that are formed by 1–3 internal dikinetids (a somatic kinety continuation) followed by 2–14 monokineties around the oral field (Figs. 1h, 3f and 4d, e; Table 1). We observed cells in conjugation and binary fission, usually coordinated, i.e. many conjugant cells at the same time.

Phylogenetic analyses. The partial 18S rRNA gene sequences (clones 01 and 02) of C. maricaensis are composed of 1,479 and 1,481 kb, respectively, with four nucleotide transitions between clones, probably resulting from Taq DNA polymerase errors. The sequence of clone 01 is composed of 27.2 % A, 20.2 % C, 29.3 % G and 23.3 % T, while the sequence of clone 02 is composed of 27.2 % A, 20.1 % C, 29.5 % G and 23.4 % T. In the phylogenetic analyses, the class Karyorelictea is recovered as a well-defined monophyletic group with full support values. When compared with other geleiids, C. maricaensis clustered with P. orbis with full support values for all analyses (Fig. 6). The Corlissina + Parduczia cluster was recovered as a sister group of Geleia (Fig. 6).

DISCUSSION

Comparison with related genera

When compared with other geleids, Corlissina is closer to the genus Geleia, in both the dimensions of the body as well as the organization of the oral ciliation; however, as shown in our phylogenetic analyses, Corlissina is more closely related to the genus Parduczia than to Geleia. Nevertheless, few morphological features recover this closeness. In addition to Corlissina, the genus Parduczia has the adoral intrabuccal kinety as an extension of the median ventral somatic kinety (K0s), also being made up of dikinetids (Dragesco, 1999). Furthermore, different from the genera Geleia and Avelia, which can have the paroral intrabuccal kinety as an extension of median ventral kinety (K0i), in Parduczia and Corlissina, the paroral intrabuccal kinety is independent of the somatic ciliation (Dragesco, 1999). However, the body dimensions and oral infraciliature organization of Parduczia and Corlissina are completely different. The genus Geleia shares some features with

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**Table 1. Morphometric data of C. maricaensis**

All data are based on live and protargol-impregnated specimens.

<table>
<thead>
<tr>
<th>Character</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>SEM</th>
<th>CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length, <em>in vivo</em> (µm)</td>
<td>232.27</td>
<td>535.86</td>
<td>373.82</td>
<td>362.89</td>
<td>92.55</td>
<td>1.03</td>
<td>85.65</td>
<td>20</td>
</tr>
<tr>
<td>Body length, protargol (µm)</td>
<td>143.38</td>
<td>542.90</td>
<td>295.63</td>
<td>295.82</td>
<td>80.08</td>
<td>1.00</td>
<td>64.12</td>
<td>38</td>
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<tr>
<td>Body width, protargol (µm)</td>
<td>33.11</td>
<td>68.61</td>
<td>52.63</td>
<td>54.20</td>
<td>9.73</td>
<td>0.97</td>
<td>0.94</td>
<td>20</td>
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<tr>
<td>Body width, <em>in vivo</em> (µm)</td>
<td>61.95</td>
<td>196.46</td>
<td>103.11</td>
<td>105.32</td>
<td>29.74</td>
<td>0.98</td>
<td>8.84</td>
<td>38</td>
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<tr>
<td>Somatic kineties (n)</td>
<td>40</td>
<td>62</td>
<td>49.85</td>
<td>50.00</td>
<td>5.86</td>
<td>1.00</td>
<td>0.34</td>
<td>45</td>
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<tr>
<td>Anterior end to oral field, distance (µm)</td>
<td>14.16</td>
<td>66.66</td>
<td>25.90</td>
<td>25.55</td>
<td>8.29</td>
<td>1.01</td>
<td>0.68</td>
<td>38</td>
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<td>Oral field, length (µm)</td>
<td>16.82</td>
<td>88.81</td>
<td>37.47</td>
<td>79.76</td>
<td>13.12</td>
<td>0.47</td>
<td>1.72</td>
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<td>Oral field, width (µm)</td>
<td>8.40</td>
<td>29.47</td>
<td>16.05</td>
<td>15.66</td>
<td>6.16</td>
<td>1.02</td>
<td>0.31</td>
<td>38</td>
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<td>Macronuclei (n)</td>
<td>2</td>
<td>4</td>
<td>2.11</td>
<td>2.00</td>
<td>0.40</td>
<td>0.03</td>
<td>0.01</td>
<td>45</td>
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<tr>
<td>Largest macronucleus, diameter (µm)</td>
<td>9.82</td>
<td>24.89</td>
<td>14.45</td>
<td>14.25</td>
<td>4.24</td>
<td>1.01</td>
<td>0.11</td>
<td>30</td>
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<td>Smallest macronucleus, diameter (µm)</td>
<td>8.99</td>
<td>22.27</td>
<td>12.73</td>
<td>12.82</td>
<td>2.85</td>
<td>0.99</td>
<td>0.08</td>
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<td>Micronuclei (n)</td>
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<td>1</td>
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<td>45</td>
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<td>Micronuclei, diameter (µm)</td>
<td>3.59</td>
<td>8.53</td>
<td>5.50</td>
<td>5.46</td>
<td>2.30</td>
<td>1.01</td>
<td>0.02</td>
<td>28</td>
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<td>Paroral polykineties (left side) (n)</td>
<td>40</td>
<td>57</td>
<td>51.58</td>
<td>54.00</td>
<td>7.04</td>
<td>0.96</td>
<td>0.49</td>
<td>5</td>
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<td>Adoral polykineties (right side) (n)</td>
<td>32</td>
<td>61</td>
<td>48.60</td>
<td>51.00</td>
<td>8.33</td>
<td>0.94</td>
<td>0.69</td>
<td>22</td>
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<tr>
<td>Adoral monokineties, number in longest row</td>
<td>9</td>
<td>14</td>
<td>10.96</td>
<td>10.50</td>
<td>1.91</td>
<td>1.04</td>
<td>0.03</td>
<td>10</td>
</tr>
<tr>
<td>Adoral monokinets, number in shorter row</td>
<td>2</td>
<td>5</td>
<td>3.17</td>
<td>3.00</td>
<td>0.95</td>
<td>1.06</td>
<td>0.01</td>
<td>10</td>
</tr>
<tr>
<td>Distance between somatic kineties (µm)</td>
<td>1.99</td>
<td>10.20</td>
<td>4.79</td>
<td>4.69</td>
<td>2.10</td>
<td>1.02</td>
<td>0.04</td>
<td>30</td>
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<tr>
<td>Cortical granules, number of rows between</td>
<td>1</td>
<td>1</td>
<td>1.00</td>
<td>1.00</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>45</td>
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<tr>
<td>two somatic kineties</td>
<td></td>
<td></td>
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Fig. 3. Schematic diagrams representing the ciliature organization of *C. maricaensis* after protargol staining. (a, b) General representation of two specimens after impregnation; (a) shows a more contracted cell. (c) Detail of the dorsal somatic ciliature of the anterior end. (d) Detail of the posterior dorsal somatic ciliature. (e, f) Detail of the anterior region of the body showing the oral infraciliature. Filled arrowheads in (e) show the superior ventral central kinety (K0s); open arrowheads show the inferior ventral central kinety (K0i). APK, Adoral polykineties; C, cilia; DK, dikinetids; DZ, dense zone; EPK, extrabuccal paroral kineties; ESPK, extrabuccal short polykineties; IPK, intrabuccal paroral kineties; ISPK, intrabuccal short polykineties; MK, monokinetids; NA, nuclear apparatus; OF, oral field; SK, somatic kineties; P, peristome; PL, paroral loop. Bars, 50 μm (a, b) and 25 μm (c–f).
Fig. 4. Dieckmann protargol staining of *C. maricaensis* (a–e) and Wilbert protargol staining (f–h). (a) General view of the ciliate. (b, c) Anterior ventral views to show the oral infraciliature. Filled arrowheads in (c) show the interrupted somatic kineties on the adoral left side; open arrowheads show kineties K1, K2 and K3.(d) Anterior dorsal end. (e) Details of paroral and adoral infraciliature. (f, g) Anterior ventral kineties, showing the interrupted kineties and cortical granules. (f) Open arrowheads show kineties K1, K2 and K3.(g) Somatic kineties with post-ciliary fibres forming a parallel row. (h) Macronuclei and micronuclei. APK, Adoral polykineties; AZ, adoral zone; CG, cortical granules; DZ, dense zone; DK, dikinetids; ESPK, extrabuccal short polykineties; IK, interrupted kinety; ISPK, intrabuccal paroral kineties; Ma, macronuclei; Mi, micronucleus; NA, nuclear apparatus; OF, oral field; PcF, post-ciliary fibres; PL, paroral loop; PZ, paroral zone. Bars, 50 μm (a), 25 μm (b–e) and 10 μm (f–h).
Corlissina, such as long adoral polykineties, which are, however, made up by dikinetids in Geleia and monokinetids (preceded by 1–2 internal dikinetids) in Corlissina.

Phylogenetic position of C. maricaensis

Among the representatives of the family Geleiidae, 18S rRNA gene sequences have been determined only from species of the genera Geleia and Parduczia. This study presents two 18S rRNA gene sequences and the phylogenetic position of C. maricaensis based on this gene. Because there are as yet no sequences of species of the genera Avelia and Gellertia available, we do not know the phylogenetic relationship between Corlissina and these two genera. Corlissina is morphologically more similar to Geleia (see discussion above); however, in molecular phylogenetic reconstructions, C. maricaensis clustered with Parduczia with full support. In previous phylogenetic studies, Parduczia was grouped with species of the genus Geleia (Xu et al., 2013, 2015), but the present study reveals the closest relationship of Parduczia with Corlissina. Nevertheless, to understand the relationships among the internal groups of the Geleiidae better, it is necessary to increase the number of available gene sequences, mainly of species of Avelia and Gellertia.

Problems with definitions in the Geleiidae and Corlissina as a new genus

The main features that distinguish the groups of the Protoheterotrichida are related to the pattern of the oral infraciliature (Fig. 7). The family Geleiidae, for example, according to the new definition of Dragesco (1999), is characterized by numerous adoral polykineties that consist of 5–15 dikinetids and a paroral formed by an internal buccal kinety (continuation of the central ventral kinety) and a row of oblique short polykineties. However, the oral infraciliature of members of some of the genera included in this family does not match the description of the Geleiidae. The genus Gellertia, for example, is characterized by an adoral infraciliature that is simplified, i.e. reduced to a single row of two dikinetids. The genus Parduczia also does not fit the definition of the family. This genus has an adoral ciliature that is very little differentiated, formed by a row of dikinetids and three prebuccal kineties (sensu Dragesco, 1999). In Avelia, the buccal infraciliature is made up of an adoral row that is formed by short polykineties of three dikinetids that are connected between them, also different from the pattern that is described for the family. The only genus that fits the description of the Geleiidae made by Dragesco (1999) is Geleia, which in fact has long adoral polykineties that...
are made up of dikinetids. In addition to this problem, some other characters included in the diagnosis of the Geleiidae do not correspond to all of the genera. We can cite as an example the paroral with an extension to below the oral field, becoming a median somatic kinety, which is described for Geleiidae and Geleia but is not seen in other genera. This character, despite being cited by Dragesco (1999), does not appear in his figures or protargol

**Fig. 6.** ML phylogenetic tree inferred from 18S rRNA gene sequence alignment. The new sequences and the phylogenetic position of *C. maricaensis* are shown in bold and indicated by an arrow. Support values at nodes are for the ML/BI/NJ trees, respectively; dashes indicate mismatch in branching pattern. Asterisks denote full support in all analyses. Accession numbers in the GenBank database are given before the taxon names. Bar, 4 substitutions per 100 nucleotide positions.
In view of these problems, a revision for the family Geleiiidae is required, with a detailed redefinition of the diagnostic characteristics of the group.

Based on this information about the oral infraciliature and molecular data, we conclude that Corlissina is, in fact, a new genus of the Geleiidae, presenting paroral and adoral ciliatures that are different from those of other genera. C. maricaensis has a paroral that is formed by an intrabucal kinety that is made up of dikinetids and another external row of short polykineties that are also formed by dikinetids. The two paroral kineties come together, forming a loop in the basal region of the oral field. The adoral region consists of polykineties that are formed by some dikinetids (continuation of the median ventral somatic kinety K0s) and several rows of monokinets. According to Dragesco (1999), the genus Geleia has adoral polykineties that are made up of dikinetids, although they almost always appear as monokinetids. These conclusions come from the work of Nouzarede (1977), who studied the ultrastructure of Geleia and concluded that the polykineties are made up of dikinetids (Dragesco, 1999). However, we have seen both in vivo and in protargol staining that Corlissina has polykineties formed by monokinets (with dikinetids limited to the innermost portion of the polykineties). Therefore, we kept the description as monokinetids.

In addition, Foissner (1998), in his chapter about the Karyorelictea, says that the main apomorphy of the Geleiidae is the presence of monokinets on both sides of the oral field, in contrast to what was later described by Dragesco (1999).

**Synonymization of Aveliidae and Geleiidae**

The family Geleiidae, which includes Geleia and Avelia, was separated into two by Dragesco (1999), i.e. Geleiidae (including Geleia and Gellertia) and Aveliidae (including Avelia and Parduzcia). These two families appear as synonymous in Lynn (2008), without any argument about the change. However, we agree with the two families’ synonymization. The only morphological diagnosis that Dragesco (1999) designates for Aveliidae is the presence of two paroral structures, i.e. one intrabucal kinety and a series of right polykineties. However, a very similar paroral organization is seen and described for the family Geleiidae, differing only by the polykineties, which are longer in Aveliidae, making the division of the two families unsustainable.

**New definition of Geleiidae**

The geleiids are very large ciliates (200–5000 μm) with a cylindrical body and an apical region that is slightly...
tapered, often in beak form. The oral field is subapical and small in relation to the body length. The geleiids are characterized mainly by a paroral organization, i.e., formed by one intrabuccal kinety that is made up of 1–2 dikinetids, and an extrabuccal row, which can either be made up of long polykineties (e.g. 15 in Avelia) or by only one kinety (as in Gellertia). The adoral zone is very diverse between the genera, as it can be formed by polykineties (e.g. Avelia, Corlissina and Geleia) or by only one row of dikinetids (Gellertia and Parduczia). The somatic and buccal kineties present many argentophilic fibres. The geleiids have different pigment granules in the cortex and cytoplasm and are, in most cases, quite contractile. The nuclear apparatus consists of two macronuclei and one micronucleus.

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