Morphology and divisional morphogenesis of the brackish water ciliate *Novistrombidium rufinoi* sp. nov. (Ciliophora: Oligotrichia) from Brazil

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Abstract. The strombidiid ciliate *Novistrombidium rufinoi* sp. nov. is described from observations on live and protargol-impregnated specimens. The organisms were isolated from samples of slightly brackish water from Cabiúnas Lagoon, located in Parque Nacional da Restinga de Jurubatiba, an environment conservation area in the northern region of the state of Rio de Janeiro, Brazil. The new species measures ~40 x 35 µm in vivo and differs from congeners by having a 7–11 µm long, adherent, non-retractile tail that lacks cilia and a conspicuously spring-shaped adoral zone which has two thigmotactic membranelles. Stomatogenesis in the opisthe is hypoapokinetal and parental oral apparatus is retained in the proter.

Key words: Oligotrichida, restinga, Spirotrichea, Strombidiidae, taxonomy.

INTRODUCTION

Oligotrichous ciliates occur mostly in marine and freshwater plankton communities. They often display ovoid or obconical shape, with relatively reduced somatic ciliature, strong adoral membranelles used for swimming and food uptake, and, in some cases, have special thigmotactic membranelles that may allow temporary attachment to substrata (Kahl, 1932; Maeda & Carey, 1985; Maeda, 1986; Lynn & Small, 2002; Lynn, 2008). In general, oligotrichs, as well as other ciliates, are important elements in microbial communities because they feed on bacteria and other protists (Fenchel, 1987; Corliss, 2002). In spite of their important role on aquatic ecosystems, ciliates are overlooked in biodiversity conservation oriented studies most of the times (Corliss, 2004; Cotterill et al., 2008; Cotterill et al., 2013). In the present study, we describe a new strombidiid oligotrich, *Novistrombidium rufinoi* sp. nov., discovered in water samples from Cabiúnas Lagoon, located in Parque Nacional da Restinga de Jurubatiba, which is an environmental conservation area in the northern region of the state of Rio de
Janeiro, Brazil.

**MATERIAL AND METHODS**

In July of 2003, plankton samples from Cabiúnas Lagoon (see Petrúcio, 1998; Paiva & Silva-Neto, 2004, 2005) were collected with a 10 µm mesh net. Water samples with sediment from the bottom were obtained manually at a depth of about 1 m, using hermetic flasks. In the laboratory, samples were split into Petri dishes where ordinary limnetic cultures were made as described in Paiva & Silva-Neto (2007).

Identification was performed by observing free-swimming and protargol-impregnated specimens (Dieckmann, 1995), under bright field and phase contrast microscopy at 200–1,000’ magnifications. Illustrations are based on photographs, notes and sketches made during our observations, and on protargol-impregnated specimens. Unless specified, ciliates were illustrated with anterior end of body oriented to top of the page. All measurements in Table 1 are in µm and were made from protargol-impregnated specimens at 1,000’ with a high power oil immersion objective. Terminology adopted in this study mainly follows Agatha (2004a,b) and systematics is according to Lynn (2008).

**RESULTS**

Subphylum Intramacronucleata Lynn, 1996
Class Spirotrichea Büttschli, 1889
Subclass Oligotrichia Büttschli, 1887/1889
Order Strombidiida Petz and Foissner, 1992
Family Strombidiidae Fauré-Fremiet, 1970
Genus Novistrombidium Song and Bradbury, 1998

**Novistrombidium rufinoi** sp. nov. (Table 1; Figures 1a–e, 2a–k, 3a–i, 4a–f, 5a–i)

**Diagnosis.** Brackish water *Novistrombidium* measuring ~40 µm x 35 µm in vivo; body outline roughly ovoid, only slightly dorsoventrally flattened. Adoral zone ends at top of an apical protrusion, with ~12 ventral, two thigmotactic and ~38 anterior membranelles. Girdle kinety with ~48 dikinetids, commences above anterior end of ventral kinety, descending spirally around dorsal side, terminating near posterior end of ventral kinety; ventral kinety with ~19 kinetids, slightly right of thigmotactic membranelles, bends towards posterior end of body. Rod-shaped extrusomes with attachment sites slightly above girdle and along sides of ventral kineties. Adherent tail present, lacking kinetids, non-retractile, flexible at base. Macronucleus roughly globular.

**Species name.** T. da S. Paiva proposed the epithet “rufinoi” in memory of his great-grandfather, Mr. Francisco Rufino da Silva Filho (May 29, 1902 – July 09, 1982).

**Type locality.** Cabiúnas Lagoon, Macaé, RJ (22º 17’ 44.9” S; 41º 41’ 23.7” W). Water characteristics at the surface: conductivity = 3.810 µS/cm; dissolved [O2] = 7.9 mg/L; salinity = 2.1 ppt; temperature = 22 °C. Water characteristics at the bottom: conductivity = 3.958 µS/cm; dissolved [O2] = 7.9 mg/L; salinity = 2.3 ppt; temperature = 21.9 °C.

**Deposition of type-specimens.** Protargol-impregnation slides containing the holotype and several paratypes were deposited in the collection of Laboratório de Protistologia, Dept. de Zoologia, Inst. de Biologia, Universidade Federal do Rio de Janeiro – UFRJ. Slides access number: IBZ0008-11 – holotype (marked with ink on the slide) and IBZ-0008-12 – paratypes.

**Description.**

**Interphase:** Specimens roughly ovoid in outline, only slightly dorsoventrally flattened, with conspicuous...
apical protrusion at right anterior end; colorless under stereoscopic microscope. Invariably with an adhesive tail near centre of posterior end of body, flexible at its base, non-retractile, and lacking basal bodies (Figures 1a–e, 2a–c, f, i, j), which allows temporary attachment to substrata (Figures 1b, 2a, b). Cytoplasm with abundant globular bodies. Rod-shaped extrusomes attached slight above girdle kinety and along sides of ventral kinety, seldom observed in vivo, sometimes impregnate with protargol (Figures 1a, 2g, 3d, f–i). Hemitheca present, partially around posterior region of body, delimited by girdle kinety (Figure 2k); presence of subpellicular platelets could not be checked due to body fragility. Contractile vacuole not observed (absent?).

Adoral zone of membranelles (AZM) extends from infundibular opening to ventral side of apical protrusion, rising in a spring-like open spiral; comprises 11–14 ventral, two thigmotactic, and 33–45 anterior membranelles (Figures 1a, d, e, 2a–h, j). Proximal ventral membranelles with bases usually shorter (~2–3 µm) than those of anterior membranelles (~4–6 µm). Bases of thigmotactic membranelles ~8 µm long. Each thigmotactic membranelle likely formed by one ventral or one anterior membranelle plus an additional membranelle, since structures have an inconspicuous horizontal gap (Figures 1d, 5g). Two sets of fibers associated with AZM; one fiber links proximal end of AZM to inner side of thigmotactic membranelle bases, then it links posterior parts of bases of anterior membranelles, extending to distal end of AZM. Small knots associated to this fiber, with perpendicular fibers extending through spaces between membranelles; knots and fibers conspicuous near proximal membranelles, but become less obvious towards distal end of AZM. Another fiber runs along anterior part of ventral membranelle bases, from near oral opening to two or three anterior membranelles after thigmotactic ones (Figures 1d; 3a–c). Endoral membrane composed of single row of basal bodies, extending from base of infundibular opening to distal end of buccal lip (Figures 1a, d; 4c).

Somatic ciliature composed of girdle and ventral kinety. Girdle kinety has 39–54 kinetids, commences on ventral surface, on right side, at 29–35 µm from anterior end of body and below distal ventral membranelles, runs parallel to proximal part of AZM, then twists to dorsal surface and extends to left-lateral region of body, bending posteriorly and terminating near posterior end of ventral kinety. Ventral kinety has 11–23 kinetids, begins immediately below right end of girdle kinety, extends longitudinally towards posterior end of body and sometimes gently curves leftwards (Figures 1d, e, 2f, g, i). Remarkably, two argentophylic lines run parallel to each side of ventral kinety, where extrusomes attach to (Figure 3d). Kinetids of both rows monociliated, likely dikinetids.

Macronucleus roughly globular, sometimes irregular-shaped, 11–17 µm x 11–15 µm, with numerous chromatin nodules; located near midbody (Figures. 1e, 2h, 3h). Micronucleus not observed.

Divisional morphogenesis: Stomatogenesis occurs hypoapokinetally. Oral primordium appears near middle of ventral side, below the level of thigmotactic membranelles, left of ventral kinety and above left portion of girdle kinety (Figure 4a, b). During early morphogenesis, adoral zone of opisthe curves counterclockwise at anterior region and clockwise at posterior region (Figures 4a–e). Endoral of opisthe very likely originates de novo, right of proximal posterior membranelles. The
Table 1. Morphometric characterization of *Novistrombidium rufinoi* sp. nov. AP – apical protrusion; CV – coefficient of variation; M – median; Max – maximum value observed; Mean – arithmetic mean; Min – minimum value observed; N – sample size; GK – girdle kinety; SD – standard deviation; SE – standard error; VK – ventral kinety.

<table>
<thead>
<tr>
<th>Character</th>
<th>Mean</th>
<th>M</th>
<th>SD</th>
<th>SE</th>
<th>CV(%)</th>
<th>Min</th>
<th>Max</th>
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<tr>
<td>Body length (without tail)</td>
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<td>2.6</td>
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<td>6.7</td>
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<td>31.0</td>
<td>2.7</td>
<td>0.6</td>
<td>8.7</td>
<td>26.0</td>
<td>37.0</td>
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<td>Distance from AP to buccal vertex</td>
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<td>19.0</td>
<td>2.4</td>
<td>0.7</td>
<td>12.4</td>
<td>15.0</td>
<td>23.0</td>
<td>13</td>
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<td>29.0</td>
<td>35.0</td>
<td>13</td>
</tr>
<tr>
<td>Distance from AP to macronucleus</td>
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<td>21.0</td>
<td>2.1</td>
<td>0.6</td>
<td>9.8</td>
<td>19.0</td>
<td>25.0</td>
<td>15</td>
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<td>33</td>
<td>45</td>
<td>19</td>
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<td>15.6</td>
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Figure 1. Schematic diagrams of *Novistrombidium rufinoi* sp. nov. a: Habitus of live specimen; b: Specimen temporarily adhered to substratum via mucus thread (arrowhead); c: Course of girdle and ventral kineties; d: Ventral side after protargol-impregnation. Arrowheads indicate fibers associated to adoral zone; e: Dorsal side after protargol-impregnation. AM – anterior membranelles; AP – apical protrusion; E – endoral; Ma – macronucleus; GK – girdle kinety; T – tail; TM – thigmotactic membranelles; VK – ventral kinety; VM – ventral membranelles. Scale bars = 10 µm.
Figure 2. *Novistrombidium rufinoi* sp. nov. a–e: From life; f–k: After protargol-impregnation. a: Free-swimming specimen; b, c: Specimen adhered to substratum by tail; d, e: Specimen seen from above. Arrowheads mark peristome aperture; f: Ventral side of holotype; g: Ventral side, white arrowhead marking an extrusome associated to girdle kinety; black arrowhead mark extrusomes attachment sites adjacent to ventral kinety; h: Detail of macronucleus.; i: Specimen in which tail became impregnated with protargol; j: Dorsal side; k: Detail of hemitheca (arrowheads). AM – anterior membranelles; AP – apical protrusion, AZM – adoral zone (of membranelles); E – endoral; GK – girdle kinety, Ma – macronucleus, T – tail; TM – thigmotactic membranelles; VK – ventral kinety, VM – ventral membranelles. Scale bars f–h, j = 10 µm; k = 50 µm.
Figure 3. *Novistrombidium rufinoi* sp. nov., after protargol-impregnation. a: Fibers associated to ventral region of proximal anterior membranelles. Black arrowhead mark a knot; white arrowhead indicate fibers; b: Proximal region of the adoral zone with its associated fibers marked by arrows. c: Detail of apical protrusion showing endoral and fiber associated with distal anterior membranelles (arrow); d: Detail of ventral kinety to show argentophylic lines (white arrowheads) and extrusomes (black arrowheads); e: Ingested pennate diatom (asterisk); f: Left lateral view of ventral kinety showing extrusome attachment sites (arrowheads); g: Ventrolateral view of specimen showing extusomes associated to girdle kinety (arrowheads); h, i: Dorsal view of specimen in different focal planes showing extrusomes (white arrowheads) and attachment sites (black arrowheads). AM – anterior membranelles; E – endoral; Ma – macronucleus; TM – thigmotactic membranelles; membrane, VM – ventral membranelles. Scale bars: a–c, e = 5 µm; d = 4 µm; h, i = 10 µm.
Figure 4. Divisional morphogenesis of *Novistrombidium rufinoi* sp. nov., after protargol-impregnation. 

- **a, b**: Early dividers. Arrowheads show early developing oral primordium of opisthe.
- **c**: Left lateral side of specimen showing oral primordium (arrowheads).
- **d**: Oral primordium (white arrowheads) with early developing endoral (black arrowhead).
- **e**: Oral primordium (white arrowheads) in a subsequent stage. Black arrowheads indicate endoral; double arrowhead marks proximal end of oral primordium.
- **f**: Beginning of spiralling of proximal end (double arrowhead) of oral primordium. Black arrowhead indicates endoral.

GK – girdle kinety; Ma – macronucleus; TM – thigmotactic membranelles; VK – ventral kinety. Scale bars = 20 µm.
Figure 5. Divisional morphogenesis of *Novistrombidium rufinoi* sp. nov., after protargol-impregnation. **a**: Same specimen as in 4f, different focal plane to show macronuclear DNA replication band (arrows) and posterior end of oral primordium, which has begun spiralling (double arrowhead); **b–f**: Multiple focal planes of specimen to show the coiling of posterior end of oral primordium (double arrowheads); **g–i**: Middle-to-late divider. White arrowhead shows emerging opisthe’s adoral zone; black arrows show endoral; double arrowhead indicate small gap in thigmotactic membranelles. GK – girdle kinety; Ma – macronucleus; TM – thigmotactic membranelles; VK – ventral kinety. Scale bars = 20 µm.
developing adoral zone then spirals helicoidally at posterior end, appearing as a coiled-like funnel (Figures 4f, 5a–f), which then opens as the structure rotates ~90° clockwise in middle-to-late dividers (Figures 4f–i). Oral ciliature of proter not renewed, parental structures being retained. Thigmotactic membranelles are formed once the oral primordium is almost complete and probably developed associated to already formed last two ventral or first two anterior membranelles (Fig 4f).

Parental girdle kinety shared by both dividers at least until late morphogenesis (Figures 4e, f), exhibits homogeneously increased amount of closely spaced kinetids in middle to late dividers, indicating intrakinetal proliferation of basal bodies. Replication of ventral kinety could not be observed.

Macronucleus shows typical replication band during early to middle morphogenesis (Figure 4a); in late dividers, elongates and becomes dumbbell shaped before dividing (Figure 4f).

**Additional remarks.** Specimens of *N. rufinoi* swim very fast and were relatively very abundant in freshly collected samples, but could not be kept for more than a week in cultures. They occurred simultaneously with minute scuticociliates, *Uronychia* sp., and *Pseudokeronopsis erythrina* Chen et al., 2011. *Novistrombidium rufinoi* feeds mostly on bacteria, but eventually can capture small diatoms (Figure 2e). Thigmotactic membranelles were not seen to be used for adhesion. Instead, *N. rufinoi* uses its tail to adhere temporarily to substrata, where it spins around its longitudinal axis and slowly swims forward, held by a tiny mucous filament connecting the tail to substratum (Figure 1b, 2a, c), a behavior resembling that of *Strobilidium caudatum* (Fromentel, 1876) Foissner, 1987 (Foissner et al., 1999). After about 10–15 seconds, the organism abruptly releases from filament and swims very fast. When it finds a new substratum, it swims in circles around it, descends and adheres by its tail, repeating above-mentioned process again.

**DISCUSSION**

The presented species was classified in *Novistrombidium* because its morphology and morphogenesis agree with both original description (SONG & BRADBURY, 1998) and the improved diagnosis: “Strombiidiidae with left portion of dextrally spiralled girdle kinety posterior to oral primordium” (AGATHA, 2003). Moreover, classification in the similar genera *Parallelostrombidium* Agatha, 2003 or *Spirostrombidium* Jankowski, 1978 are precluded because posterior end of girdle kinety does not run parallel to ventral kinety, as in the former, neither is inversely oriented to it, as in the latter (AGATHA, 2004a, b).

When this subdivision is adopted, *N. rufinoi* is to be placed in subgenus *Propecingulum* Agatha & Srüder-Kypke, 2014, because extrusomes are attached along girdle kinety. In *N. rufinoi*, extrusomes are also associated to the ventral kinety. In this respect, Modeo et al. (2003) mentioned that 3–4 groups of extrusomes may insert near the ventral kinety in *N. testaceum*.

The species herein presented is regarded as new based on three conspicuous and unique features among congeners: (i) presence of a tail; (ii) the peculiar spring-like conformation of the AZM; (iii) and the relatively high number of ventral membranelles (33–45). Aside from such features, when further compared with congeners, *S. rufinoi* is readily distinguished from *N. testaceum*, *N. apsheronicum* and *N. ioanum* by the shape of macronucleus. While it consists of a single, roughly globular nodule in *S. rufinoi*, it is mostly C-shaped (often divided or constricted into two nodules) in *N. testaceum*; mostly shaped as a question mark in *N. apsheronicum*; and irregularly shaped, subdivided in two to four nodules in *N. ioanum* (Anigstein, 1913; Lynn & Gilron, 1993; Alekperov & Asadullayeva, 1997; Song & Bradbury, 1998; Agatha, 2003; Modeo et al., 2003).

*Novistrombidium rufinoi* also differs from *N. orientale* in size (34–45 x 26–37 µm vs. 18–32 x 12–22 µm), position of right end of girdle kinety (below distal ventral membranelles, with ventral kinety placed immediately below right end of girdle kinety vs. below proximal ventral membranelles, with ventral kinety right and below right end of girdle kinety), number of ventral membranelles (11–14 vs. 5–8), and number of kinetids in girdle kinety (39–54 vs. 24–36) (Liu et al., 2009). *Novistrombidium rufinoi* can be further distinguished from *N. platum* by the number of ventral membranelles (11–14 vs. 8–11), presence of thigmotactic membranelles (vs. absent in *N. platum*), position of ventral kinety (immediately below anterior end of girdle kinety vs. right and below anterior end of girdle kinety), and size of macronucleus (11–17 x 11–15 µm vs. 12–49 x 9–21 µm) (Song & Packroff, 1997). Lastly, *S. rufinoi* differs from *N. sinicum* in number of thigmotactic membranelles (two vs. three), position of right end of girdle kinety (below distal ventral membranelles, with ventral kinety placed immediately below right end of girdle kinety vs. below thigmotactic membranelles, with ventral kinety right and below right end of girdle kinety), and length of macronucleus (11–17 vs. 18–29 µm) (Liu et al., 2009).

Within the Oligotrichia, the presence of a retractile tail, located in the latero-posterior region, is an autapomorphy of the family Tontoniidae Agatha, 2004 (Agatha, 2004b). In contrast to these, the tail observed in *N. rufinoi* is neither retractile nor extensible. It is worthy of note that similar tails are present in some other representatives of Strombidiidae, such as *Strombidium foissneri* Xu et al., 2008; *S. longipes* Meunier, 1910; *S. minor* (Kahl, 1932) Maeda & Carey, 1985; *S. parastylifer* Song et al., 2009; *S. rapulum* (Yagiu, 1933) Kahl, 1934; *S. rassoulzadegani* McManus et al., 2010; and *S. stylifer* Levander, 1894, albeit their systematic implications remain to be investigated.

Among *Novistrombidium* species, divisional morphogenesis was investigated with now days standard techniques only in *N. apsheronicum*; however, few middle and late dividers were observed (Agatha, 2003). Previously, Anigstein (1913) described and illustrated some divisional stages of *N. testaceum* after live observations. Like in the aforementioned species, in *N. rufinoi* the oral primordium develops above the left portion
of girdle kinety, and somatic kineties enlarge by intrakinetal proliferation of basal bodies.

ACKNOWLEDGEMENTS

We are thankful to the anonymous reviewers for their comments and suggestions. Financial support: CAPES, CNPq (Universal 014/2013, 485974/2013-4), FAPESP (2015/17856-0) and FAPERJ (BIOTA E-26/110.022/2011).

REFERENCES


ANIGSTEIN L. 1913. Über *Strombidium testaceum* nov. spec. eine marine oligotrichiche Ciliate.

ARCHIV FÜR PROTISTENKUNDE 32: 79-110.


