

Zootaxa 3626 (1): 055–076 www.mapress.com/zootaxa/

Copyright © 2013 Magnolia Press





http://dx.doi.org/10.11646/zootaxa.3626.1.2

http://zoobank.org/urn:lsid:zoobank.org:pub: AD0253C8-9CAF-4082-93CE-9CD0CE7A6210

# Hypotrichous ciliates (Protozoa: Ciliophora) from a temporary pond in Argentina, with redescription of *Apoamphisiella hymenophora* (Stokes, 1886) Berger, 1999

GABRIELA C. KÜPPERS<sup>1</sup> & MARÍA C. CLAPS

Instituto de Limnología Dr. Raúl A. Ringuelet CONICET-CCT La Plata, Boulevard 120 y 62, (1900) La Plata, Buenos Aires, Argentina

<sup>1</sup>Corresponding author. E-mail: gkuppers@fcnym.unlp.edu.ar

# Abstract

Hypotrichous ciliates collected in the plankton and soil samples from a temporary pond in Buenos Aires province, Argentina, were characterized after live observations and protargol impregnation. *Apoamphisiella hymenophora* (Stokes) Berger is redescribed and the neotype material deposited. *Apoamphisiella hymenophora* differs from its congeners in having 2 macronuclear nodules, 1 contractile vacuole with anterior and posterior collecting canals, the absence of cortical granules, 2 cirri behind the rightmost frontal cirrus, 1 postoral cirrus, 6 dorsal rows of dikinetids along with scattered dikinetids on the right body margin, and 3–9 caudal cirri arranged in groups at the ends of dorsal rows 1, 2, and 4. *Rigidohymena candens, R. quadrinucleata, Histriculus histrio, Gastrostyla steinii*, and *Pseudouroleptus caudatus* are new for the Argentine microfauna. Since especially the soil ciliates have been almost unexplored in South America, the results from the present investigation describe and contribute to the knowledge of the diversity of these microorganisms within this geographical region.

Key words: Hypotricha, morphology, neotype, soil, freshwater, Buenos Aires

# Introduction

Ciliates are among the most diverse groups of microorganisms in the Protozoa kingdom, numbering at about 8,000 known species (Lynn 2008). Nevertheless, ciliate diversity still remains highly underestimated because of a combination of circumstances—namely, undersampling, misidentifications, the lack of trained taxonomists interested in ciliates mostly outside Europe and China, and the lack of conservation programs that focus on microorganisms, among other shortcomings (Foissner 2006, 2008). The number of free living ciliates has been estimated to be as many as 30,000 different species (Foissner 2006). Freshwater and soil ciliates from the Neotropical Region have been only little investigated through modern methods, such as silver impregnation, electron microscopy, and/or molecular-genetic techniques. In the last decade, Paiva and Silva-Neto (2004a, b, c, 2005, 2006, 2007, 2009), Paiva *et al.* (2009, 2012), Küppers *et al.* (2006, 2007a, b, 2009, 2011), and Küppers and Claps (2010, 2012) described the morphology and phylogeny of either new or poorly known ciliates from Brazil and Argentina. Indeed, the soil ciliates in particular are almost uninvestigated in South America.

Hypotrichous ciliates are generally dorsoventrally flattened, substrate-oriented organisms and characterized by the presence of compound cilia called cirri on the ventral surface along with rows of dikinetids on the dorsal surface (Lynn 2008).

The present work provides morphological and biometric data on six hypotrichous ciliates based on observations on live and protargol-impregnated organisms. *Apoamphisiella hymenophora* (Stokes, 1886) Berger, 1999 is redescribed and neotype material deposited. The other ciliates represent new records for the Argentine microfauna.

### Material and methods

Plankton as well as soil samples were taken from a temporary pond located 40 km south of the city of La Plata, near the locality of Poblet in the Buenos Aires province, Argentina (for details see Küppers *et al.* 2007a). Plankton samples were obtained during the hydroperiod, whereas the soil samples from the pond bed were taken during drought phases between the years 2004 and 2008.

During the hydroperiod, specific physicochemical conditions of the water were measured with a multiparameter probe (Horiba, Japan)-e. g., water temperature, dissolved-oxygen concentration, electrical conductivity, and pH. Apoamphisiella hymenophora was also recorded in a temporary pond covered by floating macrophytes, located near the city of Dolores, Buenos Aires province (see Küppers & Claps 2010). In the laboratory, raw cultures were established in Petri dishes with the addition of crushed wheat kernels. Soil ciliates were studied after rewetting soil samples following the non-flooded Petri dish method (Foissner 1992); sometimes crushed wheat kernels were also added to stimulate bacterial growth to serve as food source for the ciliates. Ciliates were observed in vivo under stereoscopic and bright-field microscopes, at magnifications of  $40\times$ ,  $100\times$ ,  $400\times$ , as well as with a high-power 1,000× oil-immersion objective. After fixation with Bouin solution, ciliates were silverimpregnated according to Wilbert (1975) and the impregnated specimens observed, measured, and photographed under the bright-field microscope. Drawings were made with the aid of a camera lucida. Voucher slides have been deposited in the Museo de La Plata with the following accession numbers: Rigidohymena candens MLP-71, R. quadrinucleata MLP-72, Histriculus histrio MLP-73, Gastrostyla steinii MLP-75, and Pseudouroleptus caudatus MLP-74. The neotype of Apoamphisiella hymenophora is MLP-77 and the voucher slide of a population found in Dolores is MLP-76. Terminology follows Lynn (2008) and Berger (1999, 2008), and the taxonomy is after Berger (1999, 2006, 2008) and Foissner and Stoeck (2008). The descriptions of species below follow the order of appearance found in Berger (1999).

### **Results and discussion**

**Phylum Ciliophora Doflein** 

**Order Hypotricha Stein** 

# **Oxytrichidea Ehrenberg**

# Rigidohymena candens (Kahl, 1932) Berger, 2011

(Table 1; Figs. 1, 5 A)

**Morphology.** Body size *in vivo* 126–154  $\times$  35–42 µm, elongate elliptical in outline and rigid. Nuclear apparatus composed of 2 macronuclear nodules and 2–4 micronuclei. Contractile vacuole in mid-body, on the left margin (Fig. 1 A). Without cortical granules. Cytoplasm transparent. Ventral somatic ciliature composed of the typical oxytrichid 18 frontal-ventral-transverse groups of cirri. Transverse cirri slightly or not protruding beyond posterior cell end. Marginal rows of cirri discontinuous posteriorly (Figs. 1 B, 5 A). Dorsally with 6 rows of bristles, with 3 (rarely 4) caudal cirri at the end of kineties 1, 2, and 4 (Fig. 1 C). Oral ciliature composed of 26–39 adoral membranelles and paroral and endoral membranes arranged in the *Cyrtohymena* pattern. Buccal cavity large and deep. Adoral zone of membranelles occupying about 40% of total body length (on average of protargol-impregnated specimens).

**Comments.** The infraciliature of *R. candens* is in agreement with those observed by other authors in different geographic locations; however, the Argentine specimens are smaller in size ( $126-154 \mu m vs$ . greater than  $150 \mu m$  in length) and transverse cirri are located more anteriorly than in other populations (Berger 1999). *Rigidohymena candens* from Argentina resembles *R. inquieta* (Stokes, 1887) Berger, 2011 considering the body size, but the Argentine strain presents 3 postoral and 2 pretransverse ventral cirri against a total of 4 cirri (1 postoral or pretransverse cirrus is absent) in *R. inquieta* (Grolière 1975; Berger 1999). The species described by Pätsch (1974) as *Oxytricha candens* Kahl, 1932 is similar to the Argentine strain in body size and the presence of 3 postoral and 2 pretransverse ventral cirri, although the cited author stated that it is flexible. Regarding the relative position of

transverse cirri, the Argentine isolate resembles the species described by Stein (1859) as *Oxytricha platystoma* Ehrenberg, 1831 (now *Steinia platystoma* (Ehrenberg) Diesing) and preliminary classified as *R. inquieta* by Berger (1999). However, we consider the data available on the specimen described by Stein (1859) rather insufficient in order to treat it as a different species that could be conspecific with the Argentine isolate. For this reason, we prefer to classify the Argentine species as a slightly different population of *R. candens*.

**Occurrence and ecology.** *Rigidohymena candens* had been previously recorded in freshwater and moss samples from Germany, Austria, France, Ivory Coast, Tasmania, and Australia (Berger 1999). In South America, this species had also been found in Peru (Berger 1999) and Brazil (Hardoim & Heckman 1996), although no illustrations or morphometric details were provided. The species represents a new finding for Argentina and was recorded during August 2003, July and August 2004, and June 2005 in plankton samples under the following physicochemical conditions: electrical conductivity 1,233–2,760  $\mu$ S cm<sup>-1</sup>, dissolved oxygen concentration 5.6–9.6 mg L<sup>-1</sup>, temperature 2.4–9.4 °C, and pH 5–8.6. Food vacuoles contained pennate diatoms, desmids, and the ciliates *Halteria grandinella* (Müller) Dujardin and *Cyclydium* sp.

TABLE 1. Morphometric data on Rigidohymena candens (Rc), R. quadrinucleata (Rq), Histriculus histrio (Hh), and
Gastrostyla steinii (Gs). Measurements are in µm. AZM, adoral zone of membranelles; CV, coefficient of variation; M, median;
Max, maximum value; Min, minimum value; n, number of observations; SD, standard deviation; Spp, species; ?, arithmetic
mean; * anterior nodule; ** "amphisiellid cirral row".

Character	Spp	$\overline{x}$	М	Min	Max	SD	CV(%)	n
Body, length in vivo	Rc	135.8	136.5	126.0	154.0	8.8	6.5	10
	Rq	134.0	130.0	130.0	150.0	8.9	6.7	5
	Hh	166.0	168.0	140.0	182.0	18.8	11.3	7
	Gs	151.0	154.0	119.0	168.0	18.7	12.4	8
Body, width in vivo	Rc	39.2	42.0	35.0	42.0	3.6	9.2	10
	Rq	58.0	55.0	50.0	70.0	7.6	13.1	5
	Hh	85.0	84.0	63.0	98.0	14.2	16.7	7
	Gs	70.9	77.0	63.0	77.0	5.8	8.2	8
Body, length	Rc	137.2	140.0	112.0	168.0	16.3	11.9	25
	Rq	168.3	175.0	130.0	210.0	22.8	13.5	15
	Hh	152.2	150.5	140.0	168.0	10.0	6.6	10
	Gs	138.1	133.0	96.6	175.0	18.9	13.7	25
Body, width	Rc	43.1	42.0	35.0	56.0	5.6	13.0	25
	Rq	78.7	80.0	45.0	110.0	17.2	21.9	15
	Hh	72.8	77.0	63.0	77.0	5.9	8.1	10
	Gs	69.3	70.0	49.0	84.0	9.8	14.1	25
AZM, length	Rc	54.6	56.0	49.0	63.0	5.3	9.8	25
	Rq	66.6	70.0	55.0	75.0	7.4	11.2	15
	Hh	76.6	77.0	63.0	84.0	7.7	10.1	10
	Gs	61.9	63.0	49.0	77.0	7.8	12.6	25
Basis of largest membranelle, ength	Rc	8.5	8.0	7.0	10.0	1.0	11.8	10
	Rq	11.4	11.5	9.5	13.0	1.1	9.7	10

# TABLE 1. (Continued)

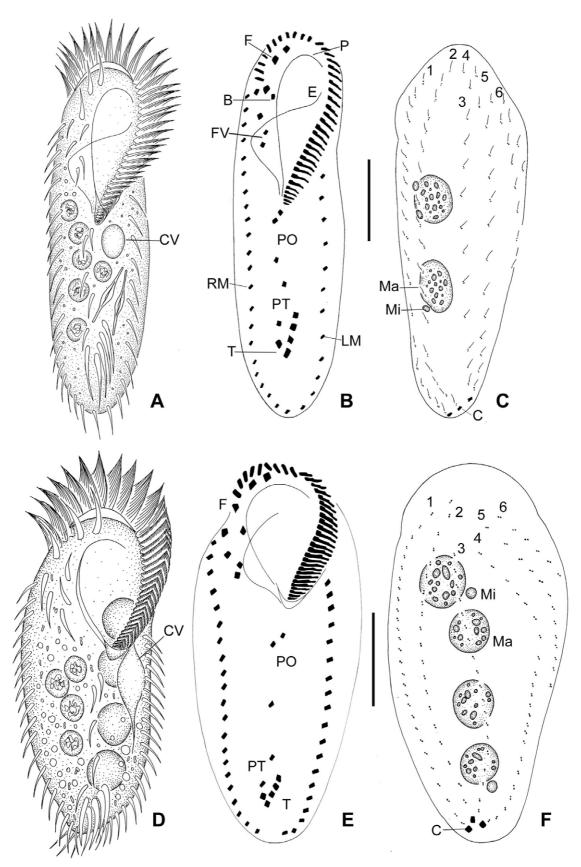
Character	Spp	$\overline{x}$	М	Min	Max	SD	CV(%)	n
	Hh	10.4	10.0	9.0	11.5	1.0	10.4	5
	Gs	13.3	13.5	11.0	17.0	2.0	14.9	10
Anterior end to paroral, distance	Rc	8.8	8.0	6.0	13.0	2.2	26.1	10
	Rq	11.3	11.5	9.0	14.0	1.4	12.5	10
	Hh	23.0	22.0	21.0	26.0	2.0	8.6	5
	Gs	11.7	11.5	10.0	14.0	1.1	9.9	10
Anterior end to buccal cirrus, listance	Rc	17.9	19.5	10.0	22.0	4.1	22.8	10
	Rq	27.1	27.0	20.0	34.0	4.9	18.1	10
	Hh	24.4	24.0	22.0	28.0	2.3	9.4	5
	Gs	23.3	24.0	19.0	26.0	2.2	9.7	10
Anterior end to posteriormost frontoventral cirrus, distance	Rc	38.8	39.0	33.0	43.0	2.7	7.0	10
	Rq	47.8	48.5	35.0	60.0	8.1	17.0	10
	Hh	42.0	41.0	40.0	46.0	2.5	6.0	5
Anterior end to posteriormost postoral ventral cirrus, distance	Rc	76.7	75.5	66.0	85.0	5.7	7.4	10
	Rq	107.0	100.0	85.0	130.0	17.6	16.4	10
Anterior end to postoral ventral cirrus, distance	Gs	68.2	67.0	58.0	84.0	7.1	10.5	10
Postoral ventral cirri V/3–V/4, listance in between	Rc	16.9	15.5	10.0	28.0	5.2	30.8	10
	Rq	23.4	27.0	15.0	30.0	7.7	33.1	10
Posteriromost transverse cirrus to posterior cell end, distance	Rc	19.6	19.5	16.0	24.0	2.8	14.4	10
	Rq	12.3	11.5	9.0	18.0	3.0	24.5	10
	Hh	23.0	24.0	20.0	27.0	2.7	11.9	5
	Gs	12.8	13.0	9.0	18.0	2.5	20.1	10
Macronuclear nodules, number	Rc	2	2	2	2	0	0	25
	Rq	4	4	4	4	0	0	15
	Hh	2	2	2	2	0	0	10
	Gs	4.5	4.0	4	8	1.1	25.4	25
Macronuclear nodules*, length	Rc	22.4	21.2	17.0	29.4	3.0	13.3	25
, 8	Rq	26.2	26.0	20.0	33.0	4.2	16.0	10

# TABLE 1. (Continued)

Character	Spp	$\overline{x}$	М	Min	Max	SD	CV(%)	n
	Hh	33.7	33.9	29.4	39.2	3.0	8.9	10
	Gs	17.8	16.8	11.2	25.9	3.7	20.9	20
Macronuclear nodules*, width	Rc	13.3	12.6	10.0	23.3	3.1	23.3	25
	Rq	20.8	21.5	15.0	25.0	3.1	15.1	10
	Hh	14.3	14.0	11.9	18.2	1.8	12.9	10
	Gs	10.4	10.1	7.7	14.0	1.5	14.7	20
Micronuclei, number	Rc	2.5	2.0	2	4	0.7	27.5	20
	Rq	2.3	2.0	2	3	0.6	24.7	3
	Hh	2	2	2	2	0	0	10
	Gs	2.3	2.0	1	4	1.5	65.4	3
Micronucleus, length	Rq	4.6	4.5	4.0	6.0	0.6	12.6	8
	Hh	6.3	6.3	5.6	7.0	0.6	9.4	10
	Gs	3.2	2.1	2.0	5.6	2.0	63.4	3
Micronucleus, width	Rc	2.9	2.8	2.1	3.7	0.4	14.6	25
	Rq	4.2	4.0	3.0	6.0	0.8	20.1	8
	Hh	4.8	4.9	4.2	5.6	0.4	8.5	10
	Gs	3.2	3.1	2.1	4.5	0.9	27.8	8
Membranelles, number	Rc	35.1	36.0	26	39	3.0	8.7	25
	Rq	34.8	35.0	33	39	1.7	4.8	10
	Hh	52.8	54.0	47	56	2.8	5.3	9
	Gs	37.4	36.0	27	51	6.5	17.3	25
Frontal cirri, number	Rc	3	3	3	3	0	0	25
	Rq	3	3	3	3	0	0	15
	Hh	3	3	3	3	0	0	10
	Gs	3	3	3	3	0	0	25
Buccal cirri, number	Rc	1	1	1	1	0	0	25
	Rq	1	1	1	1	0	0	15
	Hh	1	1	1	1	0	0	10
	Gs	1	1	1	1	0	0	25
	_						_	
Frontoventral cirri, number	Rc	4	4	4	4	0	0	25
	Rq	4	4	4	4	0	0	15
	Hh	4	4	4	4	0	0	10

# TABLE 1. (Continued)

Character	Spp	$\overline{x}$	М	Min	Max	SD	CV(%)	n
Frontoventral row**, number of cirri	Gs	13.1	13.0	12	16	1.0	8.1	25
Postoral ventral cirri, number	Rc	3	3	3	3	0	0	25
	Rq	3	3	3	3	0	0	10
	Hh	3	3	3	3	0	0	10
	Gs	1.0	1.0	1	2	0.2	19.2	25
Pretransverse ventral cirri, number	Rc	2	2	2	2	0	0	25
	Rq	2	2	2	2	0	0	6
	Hh	2	2	2	2	0	0	10
	Gs	2.3	2.0	2	3	0.5	20.7	25
Transverse cirri, number	Rc	5.0	5.0	5	6	0.2	3.9	25
	Rq	5	5	5	5	0	0	15
	Hh	5	5	5	5	0	0	10
	Gs	4.1	4.0	4	5	0.4	9.0	25
Left marginal row, number of cirri	Rc	16.1	16.0	13	22	2.1	12.8	25
	Rq	19.2	19.0	16	21	1.7	9.1	8
	Hh	31.5	29.0	29	43	5.6	17.9	6
	Gs	29.6	30.0	24	35	3.5	11.8	25
Right marginal row, number of cirri	Rc	20.8	21.0	16	24	1.9	9.2	25
	Rq	20.2	20.0	18	22	1.4	6.8	8
	Hh	48.1	46.5	44	57	5.0	10.4	6
	Gs	32.0	33.0	23	38	4.4	13.8	25
Dorsal kineties, number	Rc	6	6	6	6	0	0	25
	Rq	6.1	6.0	6	7	0.3	5.2	10
	Hh	6	6	6	6	0	0	10
	Gs	6	6	6	6	0	0	25
Caudal cirri, number	Rc	3.1	3.0	3	4	0.3	8.9	25
	Rq	3	3	3	3	0	0	6
	Hh	0	0	0	0	0	0	10
	Gs	3.0	3.0	3	3	0	0	25



**FIGURE 1.** Morphology of *Rigidohymena candens* (A–C) and *R. quadrinucleata* (D–F) from life (A, D) and after protargol impregnation (B, C, E, F). **A, B, D, E.** Ventral view. **C, F.** Dorsal view. B, buccal cirrus; C, caudal cirri; CV, contractile vacuole; E, endoral; F, frontal cirri; FV, frontoventral cirri; LM, left marginal cirri; Ma, macronucleus; Mi, micronucleus; P, paroral; PO, postoral cirri; PT, pretransverse cirri; RM, right marginal cirri; T, transverse cirri; 1–6, dorsal rows of bristles 1 to 6. Scale bars= 30 µm (B, C), 50 µm (E, F).

# Rigidohymena quadrinucleata (Dragesco & Njine, 1971) Berger, 2011

(Table 1; Figs. 1 D–E, 5 B, C)

**Morphology.** Body size *in vivo* 130–150 × 50–70  $\mu$ m; elliptical in shape and rigid. Nuclear apparatus composed of 4 macronuclear nodules and 2–3 micronuclei (very often faintly impregnated). Contractile vacuole equatorial on the left body margin, with anterior and posterior collecting canals (Fig. 1 D). Cortical granules absent. Cytoplasm transparent, with 3.7  $\mu$ m-lipid droplets and 2.5  $\mu$ m long refractive crystals. Ventral ciliature in the typical oxytrichid 18-cirri pattern (Figs. 1 E, 5 B). Marginal rows of cirri discontinuous posteriorly. Dorsally with 6 (rarely 7) rows of bristles and 3 caudal cirri at the ends of dorsal kineties 1, 2, and 4 (Fig. 1 F). Oral ciliature composed on average of 35 membranelles and paroral and endoral membranes arranged in the *Cyrtohymena* pattern. Buccal cavity large and deep. Adoral zone of membranelles occupying about 40% of total body length (on average of protargol-impregnated specimens).

We found some individuals in middle and late morphogenetic stages of binary fission. In middle dividers, the cirri originate from 6 independent primordia each for the proter and opisthe. In late dividers, the dorsal kinety 3 fragments posteriorly and two dorsomarginal rows originate in the vicinity of right marginal rows of the proter and opsithe (Fig. 5 C).

**Comments.** The morphometric characteristics of the Argentine population of *R. quadrinucleata* generally coincide with the descriptions of other authors (Dragesco & Njine 1971; Dragesco & Dragesco-Kernéis 1986; Dragesco 2003; Foissner 1984). Some minor differences, however, were observed between the Argentine isolate and other populations from different geographical locations—namely, the presence of collecting canals in the contractile vacuole *vs.* their absence, and the number of dorsal rows of bristles (6, rarely 7 in the Argentine population *vs.* consistently 6). The population from Rwanda, described by Dragesco (2003), also presents a higher number of frontal (frontal plus frontoventral and buccal) and ventral (postoral and pretransverse) cirri than the Argentine population.

Unfortunately, we were unable to obtain molecular-genetic data or witness a complete morphogenesis. According to Berger (2006), the presence of dorsomarginal kineties and the fragmentation of dorsal kinety 3 would place this hypotrich in the oxytrichid dorsomarginalians. Moreover, the rigid body, the absence of cortical granules, and the fact that cirrus V/3 is distinctly displaced posteriorly and possibly not involved in anlage formation led Berger (2011) to transfer this and the formerly described species to the Stylonychinae, within the new genus *Rigidohymena* Berger, 1999. Unfortunately, since we were unable to observe the participation of cirrus V/3 in anlage formation, this trait still must be checked in *R. quadrinucleata*.

**Occurrence.** *Rigidohymena quadrinucleata* had been previously recorded in soil and freshwater samples by other authors in Cameroon, Austria, Namibia, Antarctica, and Brazil (Berger 1999). Dragesco (2003) had also found this species in Rwanda. Foissner *et al.* (2002) had mentioned reliable records of its presence in all biogeographic regions in both soil and freshwater samples. In Argentina, *R. quadrinucleata* represents a new finding, having been recorded in soil samples collected in May and rewetted in August 2008.

# Histriculus histrio (Müller, 1773) Corliss, 1960

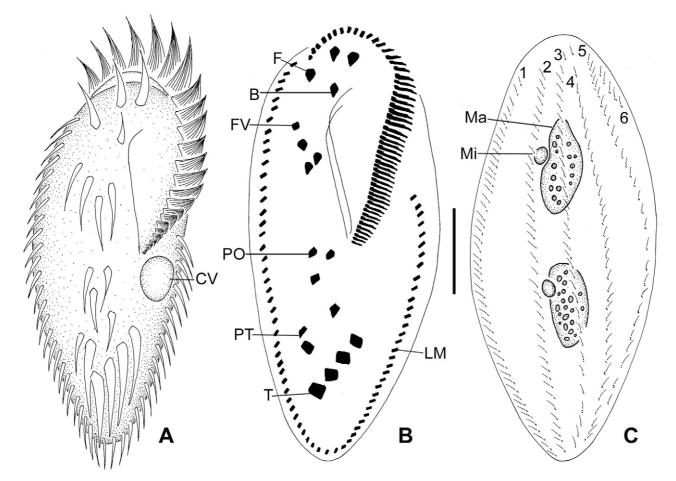
(Table 1; Fig. 2)

**Morphology.** Body size *in vivo* 140–182 × 63–98  $\mu$ m; elliptical in shape and rigid. Nuclear apparatus composed of 2 macronuclear nodules and 2 micronuclei. Contractile vacuole equatorial, on the left body margin (Fig. 2 A). Cortical granules absent. Cytoplasm transparent. Ventral ciliature in the typical oxytrichid 18-cirri pattern (Fig. 2 B). Marginal rows of cirri confluent posteriorly. Dorsally with 6 rows of bristles; caudal cirri absent (Fig. 2 C). Oral ciliature composed of 47–56 membranelles and paroral and endoral membranes arranged in the *Oxytricha* pattern. Adoral zone of membranelles occupying about 50% of total body length (on average of protargol-impregnated specimens).

**Comments.** The morphometric characteristics of this *H. histrio* isolate generally match those observed by other authors in different geographical locations (Foissner & Gschwind 1998; Berger 1999). The undulating membranes are curved and intersect each other as in the neotype (Foissner & Gschwind 1998), instead of being moderately curved or almost parallel (Berger 1999). Unlike the observations of Gupta *et al.* (2006) on the

population from Tübingen, Germany, however, the transverse cirri do not protrude beyond posterior end of the cell in the Argentine isolate.

**Occurrence and ecology.** *Histriculus histrio* has a widespread geographic distribution, occurring in Europe, Asia, Australia, and America (Berger 1999). In Argentina, the species had been previously mentioned by Seckt (1924), although without providing any morphological description. In the present study, the species was found in December 2004 in plankton samples, under the following environmental conditions: electrical conductivity 1,830  $\mu$ S cm<sup>-1</sup>, dissolved oxygen concentration 4.5 mg L<sup>-1</sup>, temperature 19.2 °C, and pH 6.2.



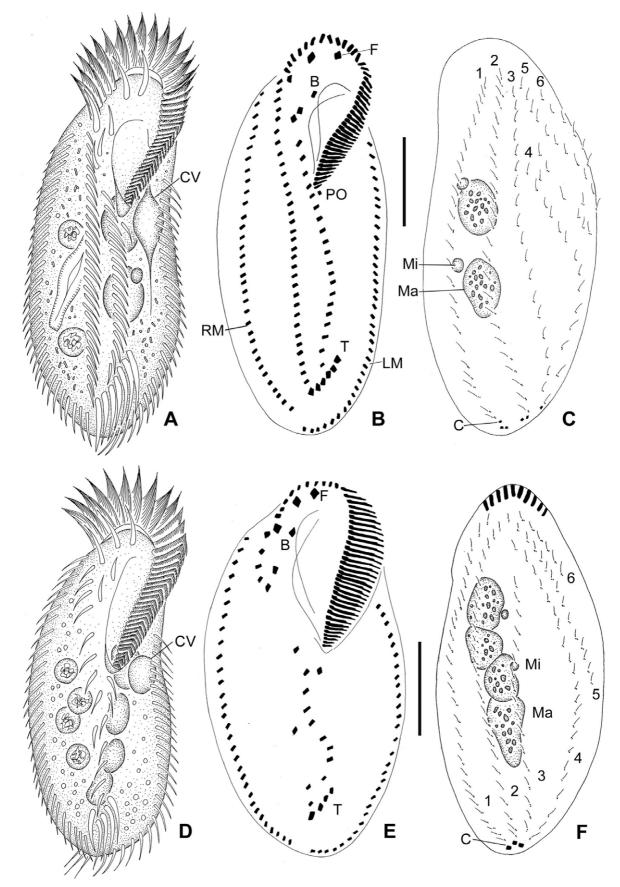
**FIGURE 2.** Morphology of *Histriculus histrio* from life (A) and after protargol impregnation (B, C). **A**, **B**. Ventral view. **C**. Dorsal view. Labels see Fig. 2. Scale bar=  $30 \mu m$ .

# Apoamphisiella hymenophora (Stokes, 1886) Berger, 1999

(Tables 2, 3; Figs. 3 A–C, 6)

**Improved diagnosis.** Body size *in vivo* 160 (112–210) × 63 (56–77)  $\mu$ m. Two macronuclear nodules and one contractile vacuole with anterior and posterior collecting canals. Cortical granules absent. Ventrally with 2 cirri behind the rightmost frontal cirrus and 1 postoral cirrus. Dorsally with 6 rows of dikinetids, with rows1–3 almost as long as body, row 4 conspicuously shorter anteriorly, dorsomarginal row 5 extending from the anterior end up to the equatorial region, dorsomarginal row 6 located in anterior third of body, and scattered dikinetids between row 3 and dorsomarginal row 5; 3–9 caudal cirri, arranged in groups at the ends of dorsal rows 1, 2, and 4.

**Neotype material.** To our knowledge, no type material of *A. hymenophora* has been deposited in a scientific repository. Therefore, the protargol-impregnated slide deposited in the Museo de La Plata, Buenos Aires province, Argentina (accession number MLP-77) is designated as the neotype. According to Article 75.3 of the ICZN (1999), the neotypification of *A. hymenophora* is justified because: i. The taxonomic status of the species is clarified and data and description provided are sufficient to ensure its recognition ii. Differentiation of *A. hymenophora* from



**FIGURE 3.** Morphology of *Apoamphisiella hymenophora* (A–C) and *Gastrostyla steinii* (D–F) from life (A, D) and after protargol impregnation (B, C, E, F). A, B, D, E. Ventral view. C, F. Dorsal view. Labels see Fig. 2. Scale bars= 30 µm (B, C), 50 µm (E, F).

related taxa (mainly *A. tihanyiensis*, see comments below) is accomplished. iii. Type material was inexistent from the original description by Stokes (1886). Grimes and L'Hernault (1978) described the species after electron microscopy and protargol impregnations but type material was not designated. vi. The protargol slide containing the neotype is deposited in the Museo de La Plata, Argentina.

**Type locality.** The locality of isolation is a temporary pond about 40 km south of the city of La Plata, near the locality of Poblet in the Buenos Aires province, Argentina ( $35^{\circ}$  05' S,  $57^{\circ}$  48' W). The species was found in plankton samples.

**Redescription.** Body size  $112-210 \times 56-77 \ \mu m$  in vivo; elliptical in shape with both ends rounded and the anterior end slightly curved to the right. Length to width ratio about 2.5:1, both under live observation and after protargol impregnation. Dorsoventrally flattened and flexible. Nuclear apparatus composed of 2 macronuclear nodules and 3-4 micronuclei. Contractile vacuole on the left margin at midbody with anterior and posterior collecting canals. Cortical granules absent. Cytoplasm colorless with refractive 1-µm crystals irregularly distributed (Fig. 3 A). Somatic ciliature composed of 3 frontal cirri, 2 cirri behind the rightmost frontal cirrus, 1 buccal cirrus, 2 ventral rows of cirri, 1 postoral cirrus, 6-8 transverse cirri, and 2 marginal rows of cirri. Right ventral row of cirri begins behind the distal membranelles and left ventral row at about the level of the posterior third of the oral apparatus (Figs. 3 B, 6 A, C). Rightmost transverse cirri slightly protrude beyond the posterior end of the cell. Marginal rows of cirri not confluent posteriorly. Right marginal row begins at about the same level as the rightmost ventral row, terminating slightly posterior to the right transverse cirri. Left marginal row terminates posteriorly, almost at the end of the cell. The dorsal surface usually presents 6 rows of dikinetids. Dorsal rows 1-3almost as long as body, row 4 being conspicuously shorter anteriorly and extending from the equatorial region up to the cell's posterior end; dorsomarginal row 5 extends from the anterior end up to the equatorial region; dorsomarginal row 6 located in the anterior third of the body; with scattered dikinetids between row 3 and dorsomarginal row 5 anteriorly (Figs. 3 C, 6 B). One individual out of 10 presented a short extra dorsomarginal row. Dorsal bristles 4.2 (3.5–5.0)  $\mu$ m (n=10) in length after protargol impregnation. On average with 6 caudal cirri at the ends of rows 1, 2, and 4; usually separated in groups of 2-3 at the end of row 1, 2-3 at the end of row 2, and 1-3 at the end of row 4. Oral apparatus composed of 40-61 adoral membranelles and paroral and endoral intercepting each other optically and arranged in the *Cyrtohymena* pattern. Buccal cavity rather deep and wide; lateral cilia emerging from the adoral membranelles and buccal lip covering proximal membranelles. The adoral zone of membranelles represents 40% of the total body length (as calculated on the basis of averaged values of protargol-impregnated specimens).

**Occurrence and ecology**. Near the locality of Poblet, the species was recorded in December 2003, November and December 2004 in plankton samples, under the following physicochemical conditions: electrical conductivity 1,600–2,190  $\mu$ S cm<sup>-1</sup>, dissolved oxygen concentration 4.5–7.1 mg L<sup>-1</sup>, temperature 17.8–20.8 °C, and pH 6.2–6.3. The food vacuoles contained pennate diatoms, green algae, euglenids, and ciliates. In February 2004, *A. hymenophora* was also found in Dolores in a temporary pond covered by floating macrophytes (*Lemna* sp., *Limnobium* sp.), located on the margins of the provincial Route 63, km 10 (36° 18' 57" S, 57° 34' 55" W). The following water conditions were recorded in the pond from Dolores: electrical conductivity 3,940  $\mu$ S cm<sup>-1</sup>, temperature 14.3 °C, and pH 7.5. The food vacuoles contained testate amoeba, green algae, and pennate diatoms.

**Comments.** Apoamphisiella hymenophora was originally described by Stokes (1886) as Holosticha hymenophora Stokes, 1886, isolated from freshwater shallow pools, probably in New Jersey, USA (Berger 1999). Later, Lundin and West (1963) found this species in freshwater in Michigan, USA. Borror (1972) transferred *H. hymenophora* to the genus *Paraurostyla* Borror, 1972. Later, Berger (1999) finally reclassified *P. hymenophora* in the genus *Apoamphisiella* Foissner, 1997, but stated that synonymy with *A. tihanyiensis* could not be excluded. Stokes (1886) and Lundin and West (1963) based their descriptions on live observations and coincide in that *A. hymenophora* lacks cortical granules, has two contractile vacuoles, presents straight undulating membranes, the right ventral row of cirri begins near the distal membranelles while the left ventral row commences almost at the buccal vertex, and in the presence of only one cirrus behind the rightmost frontal cirrus. Although Berger (1999) interpreted as possible cortical granules the structures found near the cirri of *H. hymenophora* from figures 8–10 in Grimes & L'Hernault (1978), our findings on live observations coincide with Stokes (1886) and Lundin and West (1963)—namely, *A. hymenophora* from Argentina has only one contractile vacuole with anterior and posterior collecting canals, the undulating membranes are curved

and intersect each other optically, and two cirri are present behind the rightmost frontal cirrus. The presence of two contractile vacuoles in the species described by Stokes (1886) and Lundin and West (1963) was doubtful according to Kahl (1932); moreover, Berger (1999) mentioned that the second contractile vacuole possibly corresponds to the posterior collecting canal. The presence of straight undulating membranes in the species described by the first authors above is obviously a misobservation, and the second cirrus behind the rightmost frontal cirrus cited by them could have been confused with the first cirrus from the left ventral row of cirri.

TABLE 2. Morphometric data on Apoamphisiella hymenophora from the type locality (first row) and Dolores (second row).
Measurements are in µm. Abbreviations as in Table 1.

Character	$\overline{x}$	М	Min	Max	SD	CV(%)	n
Body, length	162.6	160.0	136.5	210.0	18.4	11.3	25
	263.0	260.0	200.0	330.0	38.5	14.6	15
Body, width	66.7	70.0	45.0	84.0	8.7	13.1	25
	95.3	90.0	70.0	120.0	12.6	13.2	15
AZM, length	65.3	63.0	40.0	98.0	13.7	21.1	25
	92.6	100.0	80.0	110.0	10.3	11.1	15
Basis of largest membranelle, length	9.0	9.0	8.0	11.0	0.8	9.4	10
	10.3	10.2	9.0	11.0	0.6	6.1	10
Anterior end to paroral, distance	19.0	19.5	13.0	22.0	2.5	13.5	10
	28.4	28.5	24.0	31.0	2.2	7.8	10
Anterior end to buccal cirrus, distance	26.2	25.5	22.0	35.0	4.1	15.6	10
	40.0	40.0	32.0	48.0	4.5	11.8	10
Anterior end to cirrus behind rigthmost frontal cirrus, distance	32.3	34.5	25.0	37.0	5.2	16.1	10
	52.8	54.0	44.0	60.0	5.1	9.8	10
Anterior end to postoral cirrus, distance	58.8	59.5	50.0	67.0	5.7	9.8	10
	94.2	95.0	86.0	103.0	5.0	5.3	10
Anterior end to right marginal row, distance	25.4	26.5	17.0	32.0	4.7	18.5	10
	38.2	38.5	24.0	45.0	6.1	16.2	10
Anterior end to right ventral row, distance	19.4	19.5	17.0	22.0	2.1	11.1	10
	31.5	32.0	23.0	39.0	5.5	17.5	10
Anterior end to left ventral row, distance	39.8	41.0	30.0	45.0	4.5	11.3	10
,	63.2	64.2	54.0	73.0	5.5	8.8	10

# TABLE 2. (Continued)

Character	$\overline{x}$	М	Min	Max	SD	CV(%)	n
Posteriormost transverse cirrus to posterior end, distance	14.5	13.5	10.0	25.0	4.0	28.2	10
	19.6	20.0	16.0	25.0	2.5	13.1	10
Macronuclear nodules, number	2.0	2.0	2	2	0	0	25
	2.0	2.0	2	2	0	0	15
Macronuclear nodules*, length	26.5	25.0	18.2	40.0	5.0	18.9	25
	41.3	39.5	33.0	60.5	7.5	18.2	12
Macronuclear nodules*, width	17.1	15.0	14.0	25.0	3.1	18.3	25
	16.8	17.0	14.0	18.0	1.1	6.6	12
Micronuclei, number	3.6	4.0	3	4	0.5	13.9	25
	7.7	8.0	6	12	1.6	21.0	15
Micronucleus, length	5.4	5.6	4.2	7.0	0.9	17.1	25
	6.6	6.5	6.0	7.0	0.4	6.3	12
Micronucleus, width	4.8	4.9	3.8	7.0	0.9	17.9	25
	6.3	6.2	6.0	7.0	0.4	6.1	12
Membranelles, number	49.0	49.0	40	61	5.9	12.1	25
	58.8	61.0	54	63	2.8	4.8	15
Frontal cirri, number	3.0	3.0	3	3	0	0	25
	3.0	3.0	3	3	0	0	15
Buccal cirri, number	1.0	1.0	1	1	0	0	25
	1.0	1.0	1	1	0	0	15
Cirri behind right frontal cirrus, number	2.0	2.0	2	2	0	0	25
	2.0	2.0	2	2	0	0	15
Postoral ventral cirri, number	1.0	1.0	1	1	0	0	19
	1.0	1.0	1	1	0	0	15
Right ventral row, number of cirri	32.1	32.0	26	38	3.2	10.0	18
	45.5	44.0	42	54	3.9	8.6	8
Left ventral row, number of cirri	22.0	22	16	28	3.2	14.5	18
	31.7	31	29	37	2.6	8.4	8

Character	$\overline{x}$	М	Min	Max	SD	CV(%)	n
				2			
Transverse cirri, number	7.1	7.0	6	8	0.7	10.7	25
	9.0	9.0	8	10	0.5	5.5	9
Right marginal row, number of cirri	38.6	38.0	32	49	5.0	12.9	24
	59.0	57.5	55	66	4.1	7.0	8
Left marginal row, number of cirri	41.5	41.5	34	51	4.4	10.6	24
Let marginar row, number of entr	51.7	52.0	46	58	3.9	7.5	8
Dorsal kineties, number	6.1	6.0	6	7	0.3	5.2	10
	6.0	6.0	6	6	0	0	3
Caudal cirri, number	5.7	5.0	3	9	2.0	35.9	14
	8.2	7.5	7	11	1.6	19.1	8

TABLE 2. (Continued)

Grimes and L'Hernault (1978) made protargol impregnations, transmission and scanning electron microscopy of interphase and dividing specimens of A. hymenophora isolated in a small stream from New York, USA. Those authors were also able to keep specimens of the species in culture. Unfortunately, their descriptions and the morphometric characterizations are incomplete, thus justifying a redescription upon live observation and after protargol impregnation. The most remarkable features that led us to consider the Argentine strain as conspecific with A. hymenophora are the absence of cortical granules—in accord with the observations of Stokes (1886), Lundin and West (1963), and Grimes and L'Hernault (1978)-along with the presence of 6 dorsal kineties in a rather constant pattern-essentially in agreement with Grimes and L'Hernault (1978), although those authors did not describe this feature in detail. The relative length of right and left ventral rows of the species described by Grimes and L'Hernault (1978) could have also been misinterpreted since they stated and illustrated (see Fig. 30) that both rows begin almost posteriorly to the distal membranelles. An inspection of the scanning electron micrographs, however, reveals that the left row actually begins almost at the level of the posterior third of the buccal apparatus, as occurs in the Argentine strain. These observations on the Argentine population reject the possible synonymy of A. hymenophora with A. tihanyiensis mentioned by Berger (1999) since the former species differs in a fundamental way from the latter because of the absence vs. presence of cortical granules and the dorsal pattern of the ciliature (4 dorsal rows, 2 dorsomarginal rows, and scattered dikinetids between rows 3 and 5 vs. 4 dorsal rows and an irregular field of dikinetids on the right body margin, respectively; Foissner 1997; Paiva & Silva-Neto 2004a). These species also differ in the number of postoral and caudal cirri (see Table 3).

The dorsal pattern of *A. hymenophora* resembles that observed in two specimens (out of 14) of *A. foissneri* Paiva & Silva-Neto, 2004 from Brazil; although, most of the specimens studied by Paiva and Silva-Neto (2004a) possessed 4 dorsal rows and an irregular field of dikinetids on the right margin. In addition, *A. hymenophora* differs from *A. foissneri* in the number of contractile vacuoles (1 *vs.* 2), the number of postoral ventral cirri (1 *vs.* 2), and in the slightly posteriorly protruding rightmost transverse cirri *vs.* the conspicuously protruding ones in the posterior end of body, respectively (Paiva & Silva-Neto 2004a). The differences of *A. hymenophora* from other species are mainly with respect to the presence, pattern, and color of cortical granules; the dorsal pattern of the rows of bristles; the number of contractile vacuoles; and the number of macronuclear nodules (Table 3).

The other population of *A. hymenophora* found in Dolores, Buenos Aires province, has a greater size than the population found near Poblet and other previously cited ones along with a higher number of micronuclei and transverse cirri (Table 2, Fig. 6 C). We consider this species as conspecific with the population found near Poblet mainly because both lack cortical granules and share the same arrangement pattern of the dorsal and ventral ciliature. The higher number of marginal and ventral cirri could be related to the greater size of the Dolores population.

Character	A. hymenophora	A. tihanyiensis	A. jurubatiba	A. foissneri
Cortical granules	Absent	Citrine to pale yellow	Brown	Absent
Contractile vacuoles, number	1, 2* (probably misinterpretation)	1	1	2
Macronuclear nodules, number	2	2	2–4	2
Cirri behind rigthmost frontal cirrus, number	2	2	3	2
Postoral cirri, number	1, up to 4**	1, 2	1	2
Transverse cirri, number	5-8	5–8	5–7	6–11
Dorsal rows of dikinetids, number	6, rarely 7; scattered dikinetids on the right side	Usually 4; scattered dikinetids on the right side	Usually 4; scattered dikinetids on the right side; rarely 3 dorsomarginal kineties	Usually 4; scattered dikinetids on the right side; rarely 2 dorsomarginal kineties
Caudal cirri, number	3–9	3–14	6–11	9–14
	*Stokes (1886); **Grimes & L'Hernault (1978); Berger (1999); this study	Foissner (1997); Berger (1999); Paiva & Silva-Neto (2004a)	Paiva & Silva-Neto (2004a)	Paiva & Silva-Neto (2004a)

TABLE 3. Morphological comparison among Apoamphisiella species. Only variable features were chosen.

# Gastrostyla steinii Engelmann, 1862

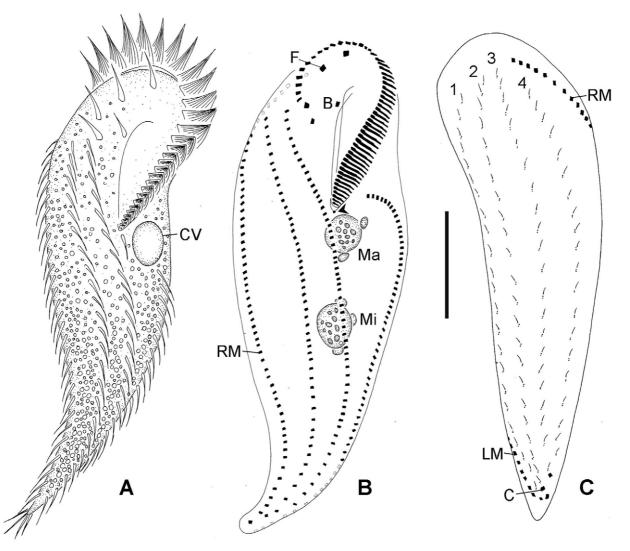
(Table 1; Figs. 3 D-F, 7 A-E)

**Morphology.** Body size *in vivo* 119–168 × 63–77  $\mu$ m; with anterior and posterior ends rounded, left margin concave and right margin convex. Length to width ratio 2:1, both under live observation and after protargol impregnation. Dorsoventrally flattened. Body rigid. Nuclear apparatus with a variable number of macronuclear nodules, usually 4 (64%) but individuals with 5 (19.4%), 6 (9%), 7 (3%), and 8 (4.5%) nodules were also observed (n= 67). Macronuclear nodules ellipsoidal in shape, sometimes elongate ellipsoidal, bilobate, or with truncated ends; 1–4 globular micronuclei. Contractile vacuole equatorial or supraequatorial, on the left body margin (Fig. 3 D). Cortical granules absent. Cytoplasm dark at low magnification (less than 100×), filled with refractive fat globules, mainly at the posterior end and the margins of the body. Somatic ventral ciliature composed of 3 anterior frontal cirri, 1 buccal cirrus, an irregular frontoventral row of 12–16 cirri, usually 1 postoral ventral cirrus, 2–3 pretransverse ventral cirri, 4 (rarely 5) transverse cirri, and marginal rows of cirri that slightly overlap posteriorly (Figs. 3 E, 7 A). Transverse cirri not surpassing posterior end of body; marginal cirri as long as caudal cirri. Dorsally with 6 rows of bristles and 3 caudal cirri at the ends of rows 1, 2, and 4 (Figs. 3 F, 7 B). Oral apparatus composed of 27–51 adoral membranelles and endoral and paroral arranged in the *Oxytricha* pattern. Adoral zone of membranelles occupying on average about 45% of total body length (on average of impregnated cells).

**Comments.** The morphology of *G. steinii* from Argentina is in agreement with the findings of other authors (Grim 1970; Foissner 1982; Foissner *et al.* 1991, 2002; Berger 1999; Dragesco 2003). The Argentine isolate

presents a variable number of macronuclear nodules, although usually 4 nodules were observed. The biometry among individuals with 4 and those with more than 4 macronuclear nodules is very similar. Foissner (1982) found some individuals of *G. steinii* with 6 macronuclear nodules, but at low frequency (?= 4.2, n= 10). Concerning the number of macronuclear nodules, the Argentine isolate should be also compared with *Gastrostyla muscorum* Kahl, 1932. This species was discovered by Kahl (1932) in moss samples near Hamburg, Germany and is different from *G. steinii* mainly because of the presence of 8 macronuclear nodules *vs.* usually 4, respectively. Unfortunately, *G. muscorum* has not been found since its original description, and our observations on *G. steinii* suggest that both species are very likely synonyms. This possibility was already mentioned by Berger (1999). With respect to the ciliature, cirrus IV/3 is slightly displaced to the left of the "frontoventral median row", as in some African individuals (about 30%) recorded by Foissner *et al.* (2002). Certain morphogenetic stages of binary fission found in the protargol slides (Figs. 7 C–E) suggest that the middle portion of the frontoventral row is formed by only one cirrus, and the anlagen of the proter and opisthe develop independently. These characteristics are consistent with the diagnosis of the subgenus *Gastrostyla (Gastrostyla)* proposed by Foissner *et al.* (2002).

**Occurrence and ecology.** Other authors had found this widely distributed species in soil and freshwater (Berger 1999; Foissner *et al.* 2002; Dragesco 2003). In the present study, *G steinii* was recorded in soil samples during January 2004 and rewetted in September–October 2006 and January–February 2007. The food vacuoles contained wheat starch from the culture media, testate amoeba (*Trinema* sp.), and small ciliates such as *Pleuronema* sp., *Cyclidium* sp., *Halteria grandinella*, and *Chilodonella* sp.



**FIGURE 4.** Morphology of *Pseudouroleptus caudatus* from life (A) and after protargol impregnation (B, C). **A**, **B**. Ventral view. **C**. Dorsal view. Labels see Fig. 2. Scale bar= 50 µm.

Character	$\overline{x}$	М	Min	Max	SD	CV(%)	n
Body, length in vivo	217.0	224.0	161.0	252.0	28.7	13.2	10
Body, width in vivo	73.5	70.0	56.0	105.0	13.3	18.1	10
Body, length	257.4	252.0	196.0	308.0	32.9	12.8	30
Body, width	77.0	70.0	49.0	112.0	16.3	21.2	30
AZM, length	73.9	72.4	63.0	91.0	7.8	10.6	24
Basis of largest membranelle, length	11.2	11.5	9.0	12.0	0.9	8.2	10
Anterior end to paroral, distance	18.9	18.0	16.0	24.0	2.6	13.9	10
Anterior end to buccal cirrus, distance	28.2	28.5	23.0	35.0	4.2	14.9	10
Anterior end to posteriormost cirrus behind rigthmost frontal cirrus, distance	35.2	36.5	28.0	43.0	5.3	15.1	10
Anterior end to postoral cirrus, distance	83.8	82.0	71.0	98.0	9.2	11.0	10
Anterior end to right marginal row, distance	19.7	18.0	13.0	31.0	5.8	29.7	10
Anterior end to right Frontoventral row, distance	35.2	33.5	23.0	48.0	8.6	24.6	10
Anterior end to left frontoventral row, distance	27.1	28.0	22.0	32.0	3.6	13.2	10
Macronuclear nodules, number	2	2	2	2	0	0	30
Macronuclear nodules*, length	31.0	30.0	26.6	42.5	3.9	12.7	20
Macronuclear nodules*, width	12.3	11.2	8.4	16.8	2.2	17.7	20
Micronuclei, number	3.1	3.0	2	5	0.8	27.4	30
Micronucleus, length	8.2	8.4	4.9	10.5	1.7	21.1	20
Micronucleus, width	5.5	5.6	4.2	6.3	0.7	13.6	20
Membranelles, number	52.5	52.0	43	63	5.0	9.5	30
Frontal cirri, number	3	3	3	3	0	0	30
Buccal cirri, number	1	1	1	1	0	0	30
Cirri behind right frontal cirrus, number	1	1	1	1	0	0	30
Postoral cirri, number	1.1	1.0	1	2	0.3	28.7	10
Right frontoventral row, number	49.6	50.5	42	56	4.2	8.5	12
Left frontoventral row, number of cirri	58.0	60.0	42	63	6.4	11.1	10
Right marginal row, number of cirri	59.8	60.0	50	75	6.4	10.8	21
Left marginal row, number of cirri	55.3	55.5	40	70	7.4	13.4	20
Dorsal kineties, number	4	4	4	4	0	0	10
Caudal cirri, number	4.1	4.0	4	5	0.3	8.6	8



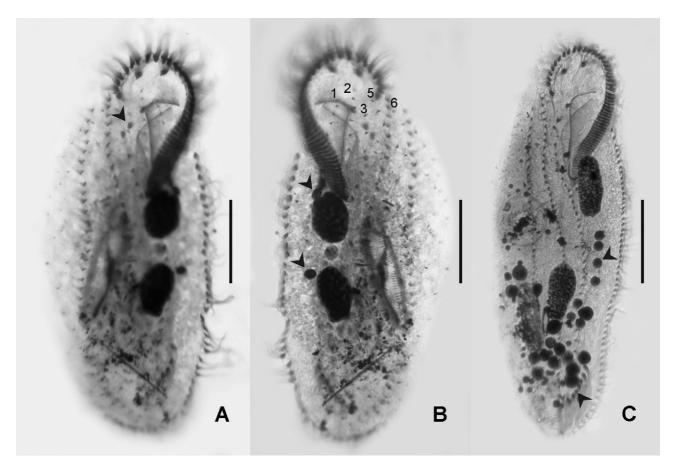
**FIGURE 5.** Micrographs of *Rigidohymena candens* (A), and *R. quadrinucleata* (B–C) after protargol impregnation in ventral view. Late divider of *R. quadrinucleata* (C), showing the presence of two dorsomarginal kineties (arrowhead). Scale bars=  $30 \mu m$  (A),  $50 \mu m$  (B, C).

# Pseudouroleptus caudatus Hemberger, 1985

(Table 4; Figs. 4, 7 F–I)

**Morphology.** Body size *in vivo*  $161-252 \times 56-105 \,\mu\text{m}$ ; sigmoid in shape and flexible. Nuclear apparatus formed by 2 macronuclear nodules and 2–5 micronuclei. Contractile vacuole at the level of the buccal vertex on the left body margin, with two inconspicuous collecting canals. Colorless cortical granules arranged in longitudinal stripes among the dorsal rows of dikinetids. Cytoplasm dark at low magnification (less than  $10\times$ ), due to the presence of refractive globules, sometimes densely packed at posterior end of body (Figs. 4 A, 7 I). Somatic ciliature formed by 3 frontal cirri, 1 cirrus behind the rightmost frontal cirrus, 1 buccal cirrus, usually 1 postoral cirrus (Fig. 7 H), 2 frontoventral rows of cirri, 2 marginal rows of cirri, 4 dorsal rows of bristles, and 4–5 caudal cirri (Figs. 4 B, 7 F). Rows of cirri slightly spiraled. Oral ciliature composed of 43–63 adoral membranelles and paroral and endoral intersect optically (Fig. 7 G). Adoral zone of membranelles occupying about 30% of total body length (on average of impregnated cells).

**Comments.** The Argentine isolate of *P. caudatus* corresponds to the subspecies described by Foissner *et al.* (2002) as *P. caudatus caudatus* from Africa, because of the relative length of the right frontoventral cirral row. The dense colorless cortical granulation, which feature had been overlooked by Hemberger (1985), is confirmed in the Argentine population. Foissner *et al.* (2002) had mentioned finding a Brazilian strain of *P. caudatus caudatus* that also presented colorless cortical granules. Two inconspicuous collecting canals not mentioned by other authors (Berger 1999; Foissner *et al.* 2002) were observed in the contractile vacuole of the Argentine population (Fig. 7 I). The morphometric data generally coincide with previous descriptions (Hemberger 1985; Olmo-Rísquez 1998; Foissner *et al.* 2002). We found a higher number of caudal cirri (4–5 *vs.* 3–4) than had Hemberger (1985) and Olmo-Rísquez (1998).

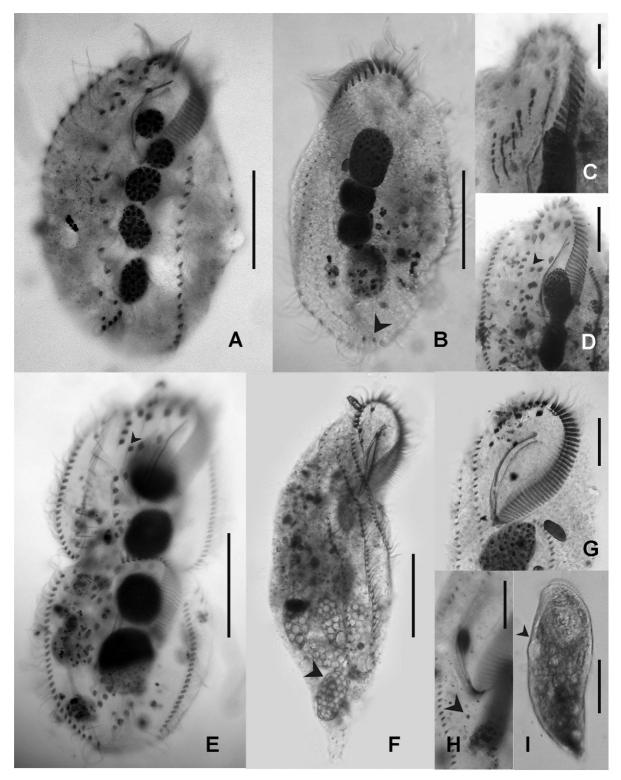


**FIGURE 6.** Micrographs of *Apoamphisiella hymenophora* from the type locality (A, B) and Dolores (C) after protargol impregnation. **A, C.** Ventral view. **B.** Dorsal view. **A.** Arrowhead points to the two cirri behind the frontal cirri. **B.** Micronuclei (arrowheads). **C.** Increased micronuclei number (anterior arrowhead) and transverse cirri (posterior arrowhead). 1–5, 6, dorsal rows of bristles. Scale bars=  $30 \mu m (A, B)$ ,  $50 \mu m (C)$ .

**Occurrence and ecology.** *Pseudouroleptus caudatus* was described from a forest soil and freshwater samples from Tambopata river in Peru (Hemberger 1985) and was subsequently found in sediment samples from the Guadarrama river in Spain (Olmo-Rísquez 1998) as well as in a rice-field soil from Zanzibar, Africa (Foissner et al. 2002). The latter authors mentioned that this species had also been observed in Brazil. Paiva et al. (2009) collected *P. caudatus* in Brazil. In the present study, the species was found in soil samples obtained during January 2004 and rewetted at that time as well as in March-November 2006. It also developed from soil samples obtained during October 2004 and January 2005. These recordings constitute the first ones for the species in Argentina. The food vacuoles contained pennate diatoms, green algae, euglenids, testate amoeba (*Arcella* sp., *Euglypha* sp., *Trinema* sp.), and ciliates (*Cinetochilum margaritaceum* Perty).

# Acknowledgements

The authors wish to thank Drs. Lía Lunaschi and Fabiana Drago for the use of the drawing tube in their laboratory at the Museo de La Plata. We are also grateful to two anonymous reviewers for their valuable suggestions. Dr. Donald Haggerty, a career investigator and native English speaker, edited the final version of the manuscript. Financial support was from the CONICET (PIP 035) and ANPCyT (PICT 2010-1041).



**FIGURE 7.** Micrographs of *Gastrostyla steinii* (A–E) and *Pseudouroleptus caudatus* (F–I) after protargol impregnation (A–H) and from life (I). **A, C–H.** Ventral view. **B, I.** Dorsal view. **A.** Individual with five macronuclear nodules. **B.** Arrowhead points to the caudal cirri. **C–E.** Middle (C) and late (E) dividers of *G steinii* showing independent cirral anlagen in the proter (C) and the single cirrus from the middle part of the frontoventral row (D, E, arrowheads). **F.** Individual with testate amoeba in food vacuoles (arrowhead). **G.** Magnification of the oral zone. **H.** Postoral cirrus (arrowhead). **I.** Contractile vacuole (arrowhead). Scale bars=  $50 \mu m$  (A, B, E, F, G, I),  $20 \mu m$  (C, D),  $30 \mu m$  (G, H).

### Literature cited

- Berger, H. (1999) Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). Monographia Biologicae, 78, 1-1080.
- Berger, H. (2006) Monograph of the Urostyloidea (Ciliophora, Hypotricha). Monographia Biologicae, 85, 1-1304.
- Berger, H. (2008) Monograph of the Amphisiellidae and Trachelostylidae (Ciliophora, Hypotricha). *Monographia Biologicae*, 88, 1–737.
- Berger H. (2011) Monograph of the Gonostomatidae and Kahliellidae (Ciliophora, Hypotricha). *Monographia Biologicae*, 90, 1–741.
- Borror, A.C. (1972) Revision of the order Hypotrichida (Ciliophora, Protozoa). *Journal of Protozoology*, 19, 1–23. http://dx.doi.org/10.1111/j.1550-7408.1972.tb03407.x
- Corliss, J.O. (1960) The problem of homonyms among generic names of ciliated protozoa, with proposal of several new names. *Journal of Protozoology*, 7, 269–278.

http://dx.doi.org/10.1111/j.1550-7408.1960.tb00741.x

- Dragesco, J. (2003) Infaciliature et morphometrie de vingt espèces de ciliés hypotriches recoltès au Rwanda et Burundi, comprenant *Kahliella quadrinucleata* n. sp. *Pleurotricha multinucleata* n. sp. et *Laurentiella bergeri* n. sp. *Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa*", 45, 7–59.
- Dragesco, J. & Dragesco-Kernéis, A. (1986) Ciliés libres de l'Afrique intertropicale. Introduction á la connaissance et á l'étude des ciliés. *Faune Tropicale*, 26, 1–159.
- Dragesco, J. & Njine, T. (1971) Complments la connaissance des ciliés libres du Cameroun. Annales de la Faculté des Sciences, Université Fédérale du Cameroun, 7–8, 97–140.
- Ehrenberg, C.G. (1831) Über die Entlwicklung und Lebensdauer der Infusionsthiere; nebst ferneren Beiträgen zu einer Vergleichung ihrer organischen Systeme. Abhandlungen der Preussischen Akademie der Wissenschaften, Physikalischmathematische Klasse, 1831, 1–154.
- Engelmann, T.W. (1862) Zur Naturgeschichte der Infusionsthiere. Zeitschrift für wissenschaftliche Zoologie, 11, 347-393.
- Foissner, W. (1982) Ökologie and Taxonomie der Hypotrichida (Protozoa: Ciliophora) einiger österreichischer Böden. Archiv für Protistenkunde, 126, 9–17.
- Foissner, W. (1984) Infraciliatur, Silberliniensystem und Biometrie einiger neuer und wenig bekannter terrestrischer, limnischer und mariner Ciliaten (Protozoa: Ciliophora) aus den Klassen Kinetofragminophora, Colpodea und Polyhymenophora. *Stapfia*, 12, 1–165.
- Foissner, W. (1992) Estimating the species richness of soil protozoa using the "non-flooded petri dish method". *In*: Lee, J.J. & Soldo, A.T (Eds.), *Protocols in Protozoology*. Society of Protozoology, Allen Press, USA, B-10.1.
- Foissner, W. (1997) Soil ciliates (Protozoa: Ciliophora) from evergreen rain forests of Australia, South America and Costa Rica: diversity and description of new species. *Biology and Fertility of Soils*, 25, 317–339. http://dx.doi.org/10.1007/s003740050322
- Foissner, W. (2006) Biogeography and dispersal of micro-organisms: a review emphasizing protists. *Acta Protozoologica*, 45, 111–136.
- Foissner W. (2008) Protist diversity and distribution: some basic considerations. *Biodiversity and Conservation*, 17, 235–242. http://dx.doi.org/10.1007/s10531-007-9248-5
- Foissner, W., Agatha, S. & Berger, H. (2002) Soil ciliates (Protozoa, Ciliophora) from Namibia (Southwest Africa), with emphasis on two contrasting environments, the Etosha region and the Namib desert. *Denisia*, 5, 1–1063.
- Foissner, W., Blatterer, H., Berger, H. & Kohmann, F. (1991) Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems - Band I: Cyrtophorida, Oligotrichida, Hypotrichia, Colpodea. Informationsberichte des Bayer, Landesamtes für Wasserwirtschaft 1/91, 478 pp.
- Foissner, W. & Gschwind, K. (1998) Taxonomy of some freshwater ciliates (Protozoa: Ciliophora) from Germany. Berichte der naturwissenschaftlich-medizinischen Vereinigung in Salzburg, 12, 25–76.
- Foissner, W. & Stoeck, T. (2008) Morphology, ontogenesis and molecular phylogeny of *Neokeronopsis (Afrokeronopsis) aurea* nov. subgen., nov. spec. (Ciliophora: Hypotricha), a new African flagship ciliate confirms the CEUU hypothesis. *Acta Protozoologica*, 47, 1–33.
- Grim, J.N. (1970) Gastrostyla steinii: Infraciliature. Transactions of the American Microscopical Society, 89, 486–497. http://dx.doi.org/10.2307/3224558
- Grimes, G.W. & L'Hernault, S.W. (1978) The structure and morphogenesis of the ventral ciliature in *Paraurostyla hymenophora*. *Journal of Protozoology*, 25, 65–74.

http://dx.doi.org/10.1111/j.1550-7408.1978.tb03869.x

- Grolière, C.A. (1975) Descriptions de quelques ciliés hypotriches des tourbières a sphaignes et des étendues d'eau acides. *Protistologica*, 11, 481–498.
- Gupta, R., Kamra, K. & Sapra, G.R. (2006) Morphology and cell division of the oxytrichids *Architricha indica* nov. gen., nov. sp., and *Histriculus histrio* (Müller, 1773), Corliss, 1960 (Ciliophora, Hypotrichida). *European Journal of Protistology*, 42, 29–48. http://dx.doi.org/10.1016/j.ejop.2005.09.004
- Hardoim, E.L. & Heckman, C.W. (1996) The seasonal succession of biotic communities in wetlands of the tropical wet-and-dry climatic zone: IV. The free-living sarcodines and ciliates of the Pantanal of Mato Grosso, Brazil. *Internationale Revue gesanten Hydrobiologie*, 81, 367–384.

http://dx.doi.org/10.1002/iroh.19960810307

Hemberger, H. (1985) Neue Gattungen und Arten hypotricher Ciliaten. Archiv für Protistenkunde, 130, 397–417. http://dx.doi.org/10.1016/S0003-9365(85)80051-8

International Commission on Zoological Nomenclature. (1999) *International Code of Zoological Nomenclature*, Fourth edition. International Trust for Zoological Nomenclature, London. xxix + 306 pp.

- Kahl, A. (1932) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. *In*: Dahl, F. (Ed.), *Die Tierwelt Deutschlands 25*. Gustav Fischer, Jena, pp. 399–650.
- Küppers, G.C. & Claps, M.C. (2010) Morphology and notes on morphogenesis during cell division of *Deviata polycirrata* n. sp. and of *Deviata bacilliformis* (Gelei, 1954) Eigner, 1995 (Ciliophora: Kahliellidae) from Argentina. *Journal of Eukaryotic Microbiology*, 57, 273–284.
  - http://dx.doi.org/10.1111/j.1550-7408.2010.00474.x
- Küppers, G.C. & Claps, M.C. (2012) Freshwater ciliates (Protozoa, Ciliophora) from Argentina: an annotated and updated compilation. *In*: Thangadurai, D., Busso, C.A., Abarca Arenas, L.G. & Jayabalan, S. (Eds.), *Frontiers in Biodiversity Studies*. IK International Publishing, New Delhi, India, pp. 61–100.
- Küppers, G.C., Lopretto, E.C. & Claps, M.C. (2006) *Pelagostrobilidium wilberti* n. sp. (Oligotrichea, Choreotrichida): Morphology and morphogenesis. *Journal of Eukaryotic Microbiology*, 53, 477–484. http://dx.doi.org/10.1111/j.1550-7408.2006.00129.x
- Küppers, G.C., Claps, M.C. & Lopretto, E.C. (2007a) Description of *Notohymena pampasica* n. sp. (Ciliophora, Stichotrichia). *Acta Protozoologica*, 46, 221–227.
- Küppers, G.C., Lopretto, E.C. & Claps, M.C. (2007b) Description of *Deviata rositae* n. sp., a new ciliate species (Ciliophora, Stichotrichia) from Argentina. *Journal of Eukaryotic Microbiology*, 54, 443–447. http://dx.doi.org/10.1111/j.1550-7408.2007.00284.x
- Küppers, G.C., Claps, M.C. & Lopretto, E.C. (2009) Ciliates (Protozoa) from dried sediments of a temporary pond from Argentina. *Revista Mexicana de Biodiversidad*, 80, 581–592.
- Küppers, G.C., da Silva Paiva, T., do Nascimento Borges, B., Harada, M.L., González Garraza, G. & Mataloni, G. (2011) An Antarctic ciliate, *Parasterkiella thompsoni* (Foissner) nov. gen., nov comb., recorded in Argentinean peat-bogs: morphology, morphogenesis, and molecular phylogeny. *European Journal of Protistology*, 47, 103–123. http://dx.doi.org/10.1016/j.ejop.2011.01.002
- Lundin, F.C. & West, L.S. (1963) The free-living protozoa of the Upper Peninsula of Michigan. Northern Michigan College Press, Marquette, Michigan, 175 pp.
- Lynn, D.H. (2008) The Ciliated Protozoa. Characterization, Classification, and Guide to the Literature, 3rd ed. Springer, Dordrecht, The Netherlands, 628 pp.
- Müller, O.F. (1773) Vermium terrestrium et fluviatilium, seu animalium infusoriorum, helminthicorum et testaceorum, non marinorum, succincta historia. Heineck & Faber, Havniae & Lipsiae, 135 pp.
- Olmo-Rísquez, J.L. (1998) *Diversidad local y global de los protozoos ciliados de hábitats de agua dulce*. Tesis Doctoral, Universidad Complutense de Madrid, España, 108 pp.
- Pätsch, B. (1974) Die Aufwuchsciliaten des Naturlehrparks Haus Wildenrath. Monographische Bearbeitung der Morphologie und Ökologie. Arbeiten aus dem Institut für landwirtschaftliche Zoologie und Bienenkunde, 1, 1–82.
- Paiva, T.S. &, Silva Neto, I.D. da (2004a) Comparative morphometric study of three species of *Apoamphisiella* Foissner, 1997 (Ciliophora: Hypotrichea) from Brazilian locations, including a description of *Apoamphisiella foissneri* sp. n. *Zootaxa*, 505, 1–26.
- Paiva, T.S. & Silva Neto, I.D. da (2004b) Description of *Parentocirrus brasiliensis* sp. n. (Ciliophora: Spirotrichea), a new ciliate protist present in activated sludge. *Zootaxa*, 504, 1–10.
- Paiva, T.S. & Silva Neto, I.D. da (2004c) Ciliate protists from Cabiúnas Lagoon (Restinga de Jurubatiba, Macaé Rio de Janeiro) with emphasis on water quality indicator species and description of Oxytricha marcili sp. n. Brazilian Journal of Biology, 64, 467–478.

http://dx.doi.org/10.1590/S1519-69842004000300010

- Paiva, T.S. & Silva Neto, I.D. da (2005) *Deviata estevesi* sp. n. (Ciliophora: Spirotrichea), a new ciliate protist from a restinga lagoon in Rio de Janeiro, Brazil. *Acta Protozoologica*, 44, 351–362.
- Paiva, T.S. & Silva Neto, I.D. da (2006) *Pseudourostyla pelotensis* sp. nov. (Ciliophora, Stichotrichia, Urostylida): a new psammophilic ciliate from the southern Brazil. *Zootaxa*, 1247, 43–58.
- Paiva, T.S. & Silva Neto, I.D. da (2007) Morphology and morphogenesis of *Strongylidium pseudocrassum* Wang and Nie, 1935, with redefinition of *Strongylidium* Sterki, 1878 (Protista: Ciliophora: Stichotrichia). *Zootaxa*, 1559, 31–57.
- Paiva, T.S. & Silva Neto, I.D. da (2009) Morphology and divisional morphogenesis of *Nudiamphisiella interrupta* Foissner, Agatha & Berger, 2002 (Ciliophora: Stichotrichia) based on a Brazilian strain. *European Journal of Protistology*, 45, 271–280. http://dx.doi.org/10.1016/j.ejop.2009.04.001
- Paiva, T.S., Borges, B.N., Harada, M.L. & Silva Neto, I.D. da (2009) Comparative phylogenetic study of Stichotrichia (Alveolata: Ciliophora: Spirotrichea) based on 18S-rDNA sequences. *Genetics and Molecular Research*, 8, 223–246. http://dx.doi.org/10.4238/vol8-1gmr529
- Paiva, T.S., Borges, B.N., Silva Neto, I.D. da & Harada, M.L. (2012) Morphology and 18S rDNA phylogeny of *Hemicycliostyla sphagni* (Ciliophora, Hypotricha) from Brazil with redefinition of the genus *Hemicycliostyla*. *International Journal of Systematic and Evolutionary Microbiology*, 62, 229–241. http://dx.doi.org/10.1099/ijs.0.x00001-0
- Seckt, H. (1924) Estudios hidrobiológicos en la Argentina. Revista de la Universidad Nacional de Córdoba, 11, 55-110.
- Stein, F. (1859) Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischer Reihenfolge bearbeitet. I. Abtheilung. Allgemeiner Theil und Naturgeschichte der hypotrichen Infusionsthiere. W. Engelmann, Leipzig, I–XII, 206 pp., Tafeln I–XIV.

Stokes, A.C. (1886) Some new hypotrichous infusoria. Proceeding of the American Philosophical Society, 23, 21-30.

- Stokes, A.C. (1887) Some new hypotrichous infusoria from American fresh waters. *Annals and Magazine of Natural History*, Serie 5, 20, 104–114.
  - http://dx.doi.org/10.1080/00222938709460018

Wilbert, N. (1975) Eine verbesserte Technik der Protargolimprägnation für Ciliaten. Mikrokosmos, 64, 171–179.