

An Antarctic hypotrichous ciliate, *Parasterkiella thompsoni* (Foissner) nov. gen., nov. comb., recorded in Argentinean peat-bogs: Morphology, morphogenesis, and molecular phylogeny

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Abstract

The ciliate *Parasterkiella thompsoni* (Foissner, 1996) nov. gen., nov. comb. was originally described from Antarctica. In the present study, we report the morphology, morphogenesis during cell division, and molecular phylogeny inferred from the 18S-rDNA sequence of a population isolated from the Rancho Hambre peat bog, Tierra del Fuego Province (Argentina). The study is based on live and protargol-impregnated specimens. Molecular phylogeny was inferred from trees constructed by means of the maximum parsimony, neighbor joining, and Bayesian analyses. The interphase morphology matches the original description of the species. During the cell division, stomatogenesis begins with the de novo proliferation of two fields of basal bodies, each one left of the postoral ventral cirri and of transverse cirri, which later unify. Primordia IV–VI of the proter develop from disaggregation of cirrus IV/3, while primordium IV of the opisthe develops from cirrus IV/2 and primordia V and VI from cirrus V/4. Dorsal morphogenesis occurs in the *Urosomoida* pattern—that is, the fragmentation of kinety 3 is lacking. Three macronuclear nodules are generated before cytokinesis. Phylogenetic analyses consistently placed *P. thompsoni* within the stylonychines. New data on the morphogenesis of the dorsal ciliature justifies the transference of *Sterkiella thompsoni* to a new genus *Parasterkiella*.

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Keywords: *Parasterkiella thompsoni* nov. gen., nov. comb.; Stylonychinae; Ontogeny; 18S-rDNA gene; Peat bogs; Argentina

Introduction

The hypotrichous ciliate *Sterkiella thompsoni* Foissner, 1996 was originally described from samples of the moss

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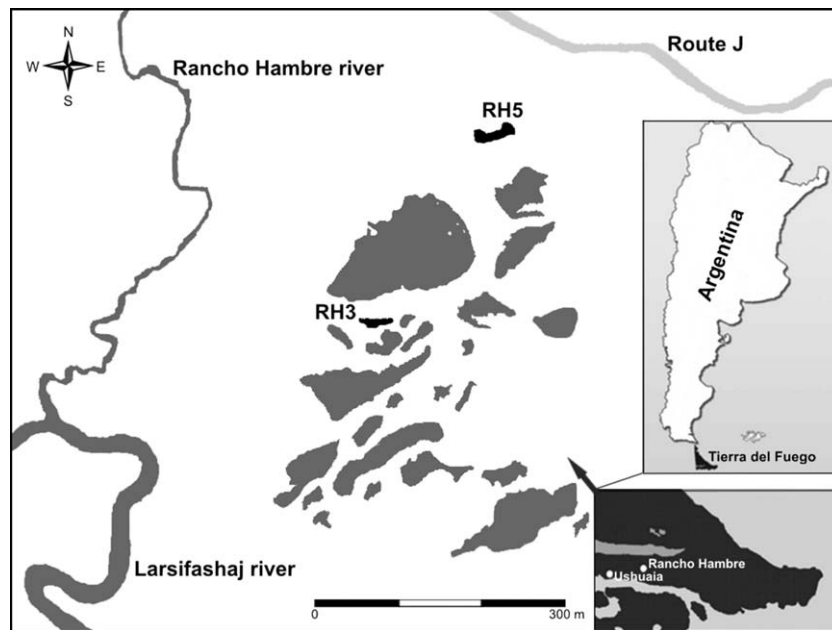


Fig. 1. Sampling sites (ponds RH3 and RH5) in Rancho Hambre peat bog, Tierra del Fuego Province, Argentina.

Sanionia uncinata (Hedwig, 1801) Loeske, 1907 (formerly *Drepanocladus uncinatus*) from Signy Island, South Orkney Islands, in the maritime Antarctic region (Foissner 1996). Since then, this species has only been recorded from moss samples and in a barren soil fellfield near Casey Station, Wilkes Land—a very distant location on Continental Antarctica (Petz and Foissner 1997). This ciliate is easily recognizable because of the typical presence of three macronuclear nodules, a distinct feature that is unusual among oxytrichids (Berger 1999, p. 4). Before the formal description of *S. thompsoni* by Foissner (1996), Sudzuki (1964) found an Antarctic ciliate with two to three macronuclear nodules, recording it under the name *Opisthotricha* sp. Later, Thompson (1972) described an *Oxytricha* sp. with three macronuclear nodules from a rock pool in the Antarctic Peninsula. Foissner (1996) regarded these forms as possibly conspecific with his new species and consequently *S. thompsoni* could be widespread throughout the Antarctic region. Until now, this ciliate has been considered as possibly endemic from Antarctica (Berger 1999; Petz et al. 2007). The province of Tierra del Fuego (Argentina) is located about 1000 km from the Antarctic Peninsula and encompasses extensive areas of peat bogs dominated by *Sphagnum* mosses. Among aquatic ecosystems, peat bogs are rare environments found in particular climatic conditions; such as low temperatures, high humidity, and evenly distributed precipitations (Mataloni 1999). In the course of an integrated limnological research, this oxytrichid ciliate was found in two bodies of water in the locality of Rancho Hambre. This contribution aims at describing the morphology of the Fuegian population along with its morphogenesis during cell division and hypothesizing its molecular phylogeny from analyses of 18S-rDNA genetic sequences. The new data indicate that this

species is a representative of a new genus, *Parasterkiella* nov. gen.

Material and Methods

Study area and samplings

The Rancho Hambre peat bog is located in the Tierra Mayor Valley, about 50 km from Ushuaia city (54°47'S, 68°19'W). This area is dominated by the peat-forming moss *Sphagnum magellanicum* Bridel, 1798 and constitutes an ombrotrophic peat bog, since its only source of nutrients comes from precipitations and snow melt.

A large number of bodies of water of different sizes, depths, and physicochemical and biological conditions characterize the landscape of Rancho Hambre. Two such water bodies, named RH3 (54°44'46.3"S, 67°49'32.9"W) and RH5 (54°44'39.4"S, 67°49'27.1"W), were sampled in April and October 2008 and 2009 during the course of a limnological investigation (Fig. 1). Water samples were preserved in the cold for the establishment of cultures. Temperature, pH, dissolved oxygen, and conductivity were measured in situ with a multiparametric probe (HORIBA, Japan). Photosynthetically active radiation (PAR) was measured with a spherical quantum sensor (Li-COR Biosciences, USA). Water samples for dissolved nutrients were filtered through Millipore APFF membrane. Samples for PO₄-P and NO₃-N concentration measurements were preserved at −20 °C until analysis within four weeks' time at the Universidad de Buenos Aires, while those for determination of NH₄-N concentration were fixed with sulfuric acid at 4 °C. Concentrations of dissolved nutrients (NH₄-N, NO₃-N, PO₄-P) were measured with a

spectrophotometer (Hach Company, USA), with the appropriate reactants for each analysis.

Morphology

In the laboratory, the samples were kept in a culture chamber, under controlled temperature (4–10 °C) and photoperiod (12:12 light/dark cycles). Raw cultures were established in Petri dishes by enriching samples with wheat kernels to stimulate growth of bacteria as a food source for the ciliates. The ciliates were observed *in vivo* under the stereo microscope and bright-field microscope. In order to visualize the infraciliature and the nuclear apparatus, cells were fixed in Bouin solution and silver-impregnated with protargol (Wilbert 1975). Measurements were taken under the bright-field microscope with a calibrated ocular micrometer. Living cells were free-hand sketched, based on live observations and micrographs. Impregnated cells were illustrated with the aid of a drawing tube attached to the microscope. Observations were performed under the stereo microscope at magnifications of 20× and 40×, and under the bright-field microscope at 40×, 100×, 400×, and 1000×.

Resting cysts of *Parasterkiella thompsoni* were obtained from old cultures, by scratching the bottom of the Petri dish with a micropipette. Cysts were observed *in vivo* by bright-field microscopy, stained with methyl-green pyronin (Foissner 1991), and impregnated with protargol.

Voucher slides of *Parasterkiella thompsoni* from Tierra del Fuego (accession numbers MLP66, MLP67, MLP68) were deposited in the Colección de Invertebrados of the Museo de La Plata, Argentina, with relevant cells marked.

The general terminology used is after Berger (1999, 2006, 2008) and Lynn (2008). The terminology concerning body flexibility is according to Foissner and Stoeck (2008) and for the oral apparatus after Foissner and Al-Rasheid (2006). The cirri and developing primordia were numbered according to Wallengren (1900). Resting cysts were described following Walker and Maugel (1980) and Berger (1999).

DNA extraction, amplification, and sequencing

Specimens of *Parasterkiella thompsoni* were picked from cultures from pool RH3 and transferred to embryo dishes, where the isolates were thoroughly washed with mineral and distilled water, then fixed with 70% (v/v) aqueous ethanol. The individuals were then submitted to DNA extraction according to Sambrook et al. (1989). The 18S-rDNA was amplified via PCR, through the use of the Hypotricha-specific primers designed by Paiva et al. (2009). Amplified fragments were purified according to Sambrook et al. (1989) and sequenced by the dideoxymethod (Sanger et al. 1977) by means of an ABI 3130 (Applied Biosystems) automatic sequencer. The resulting sequence was uploaded to the NCBI/GenBank and is available under the accession code HM569264.

Nucleotide matrix assembly and alignment

The nucleotide matrix used in the present study contained 29 representatives of supposedly related taxa (not restricted to species with 18 frontal–ventral–transverse cirri) as ingroup, plus nine urostylid sequences as outgroup. This approach allows a proper accessing of the monophyly of the stylonychines to the inclusion of *P. thompsoni* in unconstrained phylogenetic analyses (Kitching et al. 1998; Nixon and Carpenter 1993).

The nucleotides were aligned through the so-called progressive approach of multiple-sequence alignment (Feng and Doolittle 1987) implemented in the Clustal X 1.81 computer software (Thompson et al. 1997), with the adoption of the gap-opening and -extension parameters determined in Paiva et al. (2009). The resulting matrix was further modified in the software BioEdit v7.0.5 (Hall 1999) for manual refinement and trimming.

Phylogenetic analyses

Phylogenetic hypotheses were constructed under Bayesian, likelihood, and parsimony frameworks, thus permitting the assessment of data sensitivity to different analytic criteria.

A Bayesian phylogenetic inference (BI) was performed in the computer software MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). The BI was based on two Markov chain Monte Carlo (MCMC) simulations that were run with four chains of 1,000,000 generations, of which trees were sampled each 200 generations (temperature of heat chains = 0.2). The first 100,000 generations were then discarded as burn-in, and a majority-rule consensus of the remaining trees was used to calculate the Bayesian posterior probabilities of the recovered kinships (Schneider 2007).

Under the likelihood framework, the software PhyML 3.0 (Guindon and Gascuel 2003) was used to build an initial BioNJ (Gascuel 1997) pairwise-distance tree, which had its likelihood improved via “subtree pruning and regrafting” branch-swapping moves to generate a maximum-likelihood (ML) tree. To evaluate the stability of the nodes obtained, a nonparametric bootstrap analysis was conducted based on 1000 replicates.

Both BI and ML analyses made use of the GTR+I (=0.6671)+G (=0.5653) nucleotide-substitution model (Rodriguez et al. 1990), which was chosen according to the “minimum theoretical information criterion” (Akaike 1974; Bos and Posada 2005), via the software MODELTEST 3.7 (Posada and Crandall 1998).

Finally, a maximum parsimony (MP) analysis was performed in the software PAUP* 4b10 (Swofford 2003), through the use of only parsimony-informative characters and gaps scored as a “fifth base” (Giribet and Wheeler 1999; Schneider 2007) along with the successive-weighting approach (Farris 1969). Initially, the “parsimony ratchet”

(Nixon 1999) strategy implemented in PAUP* via the accessory software PaupRat (Sikes and Lewis 2001) was adopted to speed up the search for fundamental most-parsimonious trees, based on 250 iterations with 15% of the characters perturbed. The characters were then reweighted after their rescaled consistency index (base weight = 10), and a further ordinary heuristic search was conducted with 300 replicates of random sequence addition. This reweighting and heuristic search cycle was successively applied until the length of the optimal trees stabilized (Kitching et al. 1998). Node support in MP was assessed via 1000 jackknife replicates (with JAC emulation enabled in PAUP*). The “tree bisection and reconnection” branch swapping algorithm was employed in all MP-tree searches.

The optimal trees obtained under each framework were rooted a posteriori according to the outgroup position (Nixon

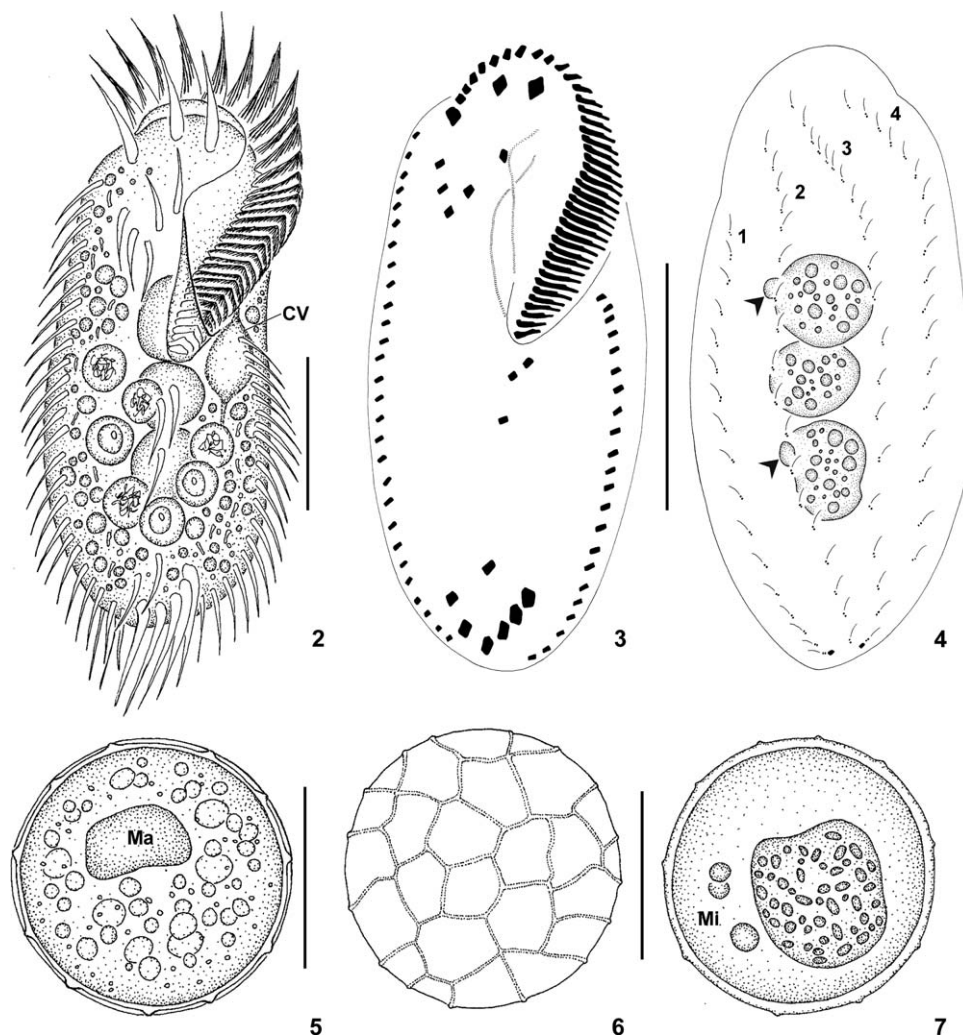
and Carpenter 1993), visualized, and edited for aesthetics and publishing in the software MEGA 4 (Tamura et al. 2007).

Results

Parasterkiella nov. gen.

Diagnosis. Stylonychinae with undulating membranes in *Oxytricha* pattern. With 18 frontal–ventral–transverse cirri, one left and one right marginal rows of cirri separated posteriorly, and four dorsal kineties. Caudal cirri present. Dorsal kineties develop without fragmentation.

Etymology. Composite of *para* (Greek, beside) and the generic name *Sterkiella*. Feminine gender.



Figs 2–7. Morphostatic specimen (2–4) and resting cysts (5–7) of *Parasterkiella thompsoni* from Tierra del Fuego after live observation (2 and 5) and protargol impregnation (3, 4, 6, and 7). (2) Ventral view of a representative specimen. (3 and 4) Infraciliature of ventral and dorsal side and nuclear apparatus of the same specimen. Arrowheads mark micronuclei. (5) Cross section of a mature resting cyst. (6) Polygonal ridges on the surface of the resting cyst. (7) Fused macronucleus and micronuclei of the kinetosome-resorbing resting cyst. CV, contractile vacuole; Ma, macronucleus; Mi, micronuclei; 1–4, dorsal kineties 1–4 (kinety 4 is a dorsomarginal row). Scale bars = 30 μ m (2 and 5–7), 50 μ m (3 and 4).

Type species. *Parasterkiella thompsoni* (Foissner, 1996) nov. comb. (basonym: *Sterkiella thompsoni* Foissner, 1996).

Species assignable. Presently only the type species is assigned to *Parasterkiella*.

***Parasterkiella thompsoni* (Foissner, 1996) nov. comb.**

Improved diagnosis. Size in vivo 90–130 $\mu\text{m} \times 40\text{--}60 \mu\text{m}$ (Foissner 1996) or 95–142 $\mu\text{m} \times 52\text{--}70 \mu\text{m}$. Body semirigid. Three macronuclear nodules. On average 34 (Foissner 1996) or 37 adoral membranelles, 22 (Foissner 1996) or 29 right marginal cirri, 17 (Foissner 1996) or 22 left marginal cirri, five transverse cirri, and four dorsal kineties with one caudal cirrus each associated with kineties 1 and 3. Oral primordium

originates de novo from two anarchic fields of basal bodies; primordia IV and V of the proter develop from cirrus IV/3, and primordia V and VI of the opisthe develop from cirrus V/4.

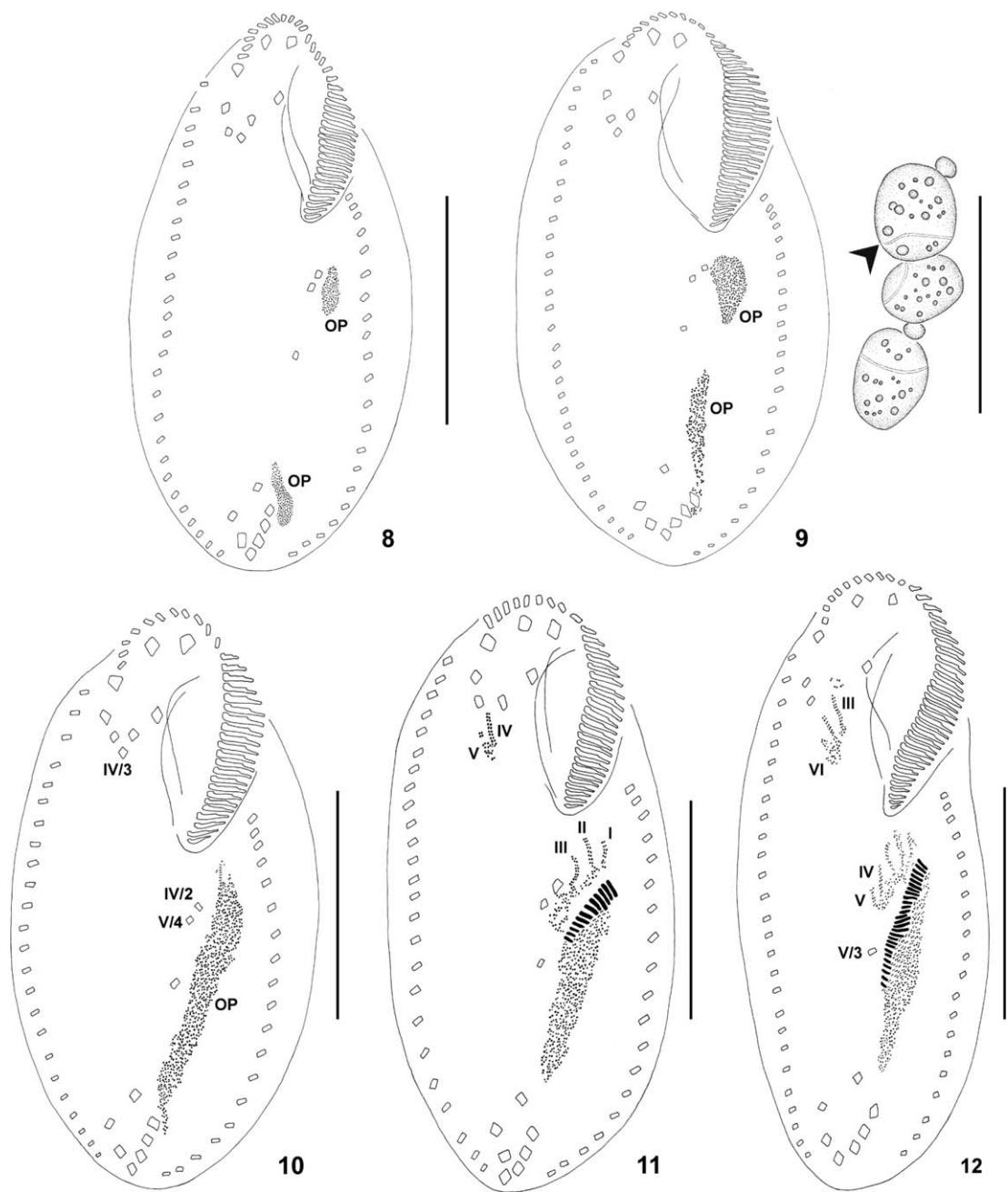
Morphology of *Parasterkiella thompsoni* from Tierra del Fuego Province, Argentina (Figs 2–7, 24–29 and Table 1)

Size in vivo about 95–142 $\mu\text{m} \times 52\text{--}70 \mu\text{m}$, usually 122 $\mu\text{m} \times 58 \mu\text{m}$; length to width ratio 2:1 in vivo and after protargol impregnation; dorsoventrally flattened about 2:1. Body with parallel margins, anteriorly and posteriorly rounded, sometimes broadly rounded posteriorly or slightly pointed (Figs 2–4). Well fed organisms rather bursiform. Semirigid—i.e., the species cannot be classified unambigu-

Table 1. Morphometric characterization of *Parasterkiella thompsoni* from Tierra de Fuego.

Character	$\bar{X}$	<i>M</i>	SD	CV	Min	Max	<i>n</i>
Body, length in vivo	122.1	121.2	15.8	12.9	95.0	142.5	10
Body, width in vivo	58.4	56.5	5.4	9.2	52.2	70.0	10
Body, length	134.4	140.0	22.7	16.8	91.0	168.0	20
Body, width	63.0	63.0	12.6	20.0	42.0	84.0	20
AZM, length	59.8	63.0	7.7	12.8	42.0	70.0	20
Anterior body end to anteriormost transverse cirrus, distance	117.1	121.8	17.7	15.1	82.6	145.6	20
Postoral ventral cirrus V/4 to postoral ventral cirrus V/3, distance in between	14.9	15.4	3.2	21.4	8.4	19.6	20
Posterior body end to posteriormost transverse cirrus, distance	4.3	4.2	0.9	20.9	2.8	5.6	20
Anterior body end to first macronuclear nodule, distance	37.5	38.5	5.6	14.9	28.0	46.2	20
Anterior macronuclear nodule, length	22.8	23.8	3.6	15.7	15.4	28.0	20
Anterior macronuclear nodule, width	17.9	18.2	2.7	15.0	12.6	22.4	20
Middle macronuclear nodule, length	18.9	18.9	4.2	22.2	12.6	29.4	20
Middle macronuclear nodule, width	18.1	18.2	3.5	19.3	12.6	25.2	20
Posterior macronuclear nodule, length	24.2	23.8	4.8	19.8	15.4	33.6	20
Posterior macronuclear nodule, width	17.6	17.1	3.0	17.0	12.6	22.4	20
Macronuclear figure, length	64.3	64.4	12.0	18.6	44.1	85.4	20
Macronuclear nodules, number	3.0	3.0	0.2	6.6	2	4	50
Micronuclei, length	4.6	4.9	0.5	10.8	3.5	5.6	20
Micronuclei, width	3.9	4.2	0.5	12.8	2.8	4.9	20
Micronuclei, number	2.5	2.0	0.9	36.0	2	6	50
Adoral membranelles, number	36.8	37.0	2.0	5.4	32	40	20
Frontal cirri, number	3.0	3.0	0.0	0.0	3	3	20
Buccal cirri, number	1.0	1.0	0.0	0.0	1	1	20
Frontoventral cirri, number	4.0	4.0	0.0	0.0	4	4	20
Postoral ventral cirri, number	3.0	3.0	0.0	0.0	3	3	20
Pretransverse ventral cirri, number	2.0	2.0	0.0	0.0	2	2	20
Transverse cirri, number	5.0	5.0	0.2	4.0	5	6	20
Right marginal cirri, number	28.6	28.0	2.4	8.3	25	33	20
Left marginal cirri, number	21.9	22.0	2.1	9.5	19	27	20
Caudal cirri, number	2.0	2.0	0.0	10.0	2	3	20
Dorsal kineties, number	4.0	4.0	0.0	0.0	4	4	20
Dorsal bristles, length	4.5	4.5	0.3	6.6	4.2	4.9	10
Dikinetids in dorsal kinety 1, number	19.5	20.0	2.1	10.7	15	22	10
Dikinetids in dorsal kinety 2, number	20.8	20.0	2.1	10.0	19	24	7
Dikinetids in dorsal kinety 3, number	21.1	21.0	1.6	7.5	19	23	6
Dikinetids in dorsal kinety 4, number	20.8	21.5	2.4	11.5	17	25	8
Resting cyst, diameter	45.8	45.2	2.7	5.8	41.0	51.0	10

Measurements are in μm and, unless indicated, based on protargol-impregnated specimens. AZM, adoral zone of membranelles; CV, coefficient of variation in %; *M*, median; Max, maximum value; Min, minimum value; *n*, number of observations; SD, standard deviation; $\bar{X}$, arithmetic mean.



Figs 8–12. Morphogenesis of ventral side of *Parasterkiella thompsoni* after protargol impregnation. (8) Oral primordium development from two fields of basal bodies. (9) Enlargement of both basal body fields. Replication bands in macronuclear nodules (arrowhead). (10) Oral primordium as a unique field of basal bodies. (11) Membranelles differentiation and formation of the primordia I–III of the opisthe and the primordia IV, V of the proter. (12) Development of the primordia IV, V of the opisthe and the primordia III, VI of the proter. OP, oral primordium; IV/3, IV/2, V/4, ontogenetically active parental cirri; V/3, ontogenetically inactive parental postoral ventral cirrus; I–VI, frontal–ventral–transverse cirri primordia. Scale bars = 50 µm.

Table 2. Origin of the frontal–ventral–transverse cirri primordia during morphogenesis of *Parasterkiella thompsoni*.

	Primordium					
	I	II	III	IV	V	VI
Proter	UM(I/1)	II/2(II/3, II/2, II/1)	III/2(III/3, III/2, III/1)	IV/3(IV/3, IV/2, IV/1)	IV/3(V/4, V/3, V/2, V/1)	IV/3(VI/4, VI/3, VI/2, VI/1)
Opisthe	OP(I/1)	OP(II/3, II/2, II/1)	OP(III/3, III/2, III/1)	IV/2(IV/3, IV/2, IV/1)	V/4(V/4, V/3, V/2, V/1)	V/4(VI/4, VI/3, VI/2, VI/1)

Cirri formed per primordium are in parenthesis. OP, oral primordium; UM, undulating membrane.

ously in the rigid (e.g., *Stylonychia*) or the flexible (e.g., *Oxytricha*) group (see Foissner and Stoeck 2008). Usually with three macronuclear nodules (one individual out of 50 with two, and one with four nodules) in central body portion. Anterior and posterior macronuclear nodules ellipsoidal; middle macronuclear nodule almost spherical or ellipsoidal. Two globular micronuclei (one individual out of 50 with six micronuclei), sometimes faintly impregnated with protargol (Figs 4, 28). Contractile vacuole in midbody near to the left body margin, with an anterior and a posterior collecting canal (Figs 2, 24). Cortical granules absent. Cytoplasm transparent, with several refractive cytoplasmic inclusions, such as 2.0–3.5 µm long crystals and 5–7 µm-sized lipid globules, mainly along the margins of the cell. Food vacuoles with bacteria, *Chlorella*-like algae, flagellates, small ciliates, and also wheat starch in the individuals from cultures. Moves moderately to very fast, crawling on the bottom of the Petri dish, sometimes remaining still for a while. Somatic ventral ciliature composed of 18 frontal–ventral–transverse cirri, arranged in the typical oxytrichid pattern (Figs 3, 28). Rarely with six transverse cirri. Thick fibres associated with ventral cirri, mainly with the enlarged frontal and transverse cirri. Marginal cirri about 12 µm in length in vivo; transverse cirri about 17 µm in length in vivo and extending beyond posterior end of cell; caudal cirri about 14 µm long in vivo. Dorsal bristles about 4–5 µm long after protargol impregnation, invariably arranged in four kineties. Dorsal kinety 1 slightly shortened anteriorly, kineties 2 and 3 almost as long as body, and kinety 4 posteriorly shortened. Dikinetids surrounded by oblique fibres, distinctly impregnated around dikinetids of rows 2 and 3. Usually two delicate and inconspicuous caudal cirri at the rear ends of dorsal kineties 1 and 3 (Figs 4, 29). One specimen out of 20 with three caudal cirri at the ends of dorsal kineties 1–3. Adoral zone of membranelles representing 44.5% of total body length (calculated on the average values measured on silver-impregnated cells). Buccal cavity rather large and deep, with a conspicuous hyaline lip covering proximal part of adoral zone of membranelles (Figs 24, 25). On average 37 adoral membranelles, with lateral membranelar cilia extending to the right of the buccal cavity; distal membranelles about 18 µm long in vivo. Paroral and endoral intersect each other optically behind the buccal cirrus; both membranes composed of dikinetids. Short pharyngeal fibres extend obliquely backwards.

Resting cysts of kinetosome-resorbing type, on average 45.8 µm in diameter, golden to light brown colored at 40–100× (Figs 5–7, 26, 27 and Table 1). Surface slightly wrinkled, formed by 0.5–1.0 µm high polygonal ridges. Cyst wall 1.5–2.0 µm thick, with a 0.5–1.0 µm thin outer hyaline (mucous?) layer; stains red with methyl-green pyronin (with its ridges intensely stained). Cytoplasm with 3–5 µm lipid droplets and 1.0–1.5 µm refractive globular inclusions. Macronucleus organized in a single reniform or rather dumb bell-shaped mass. Resting cysts were viable even 14 months after encystment.

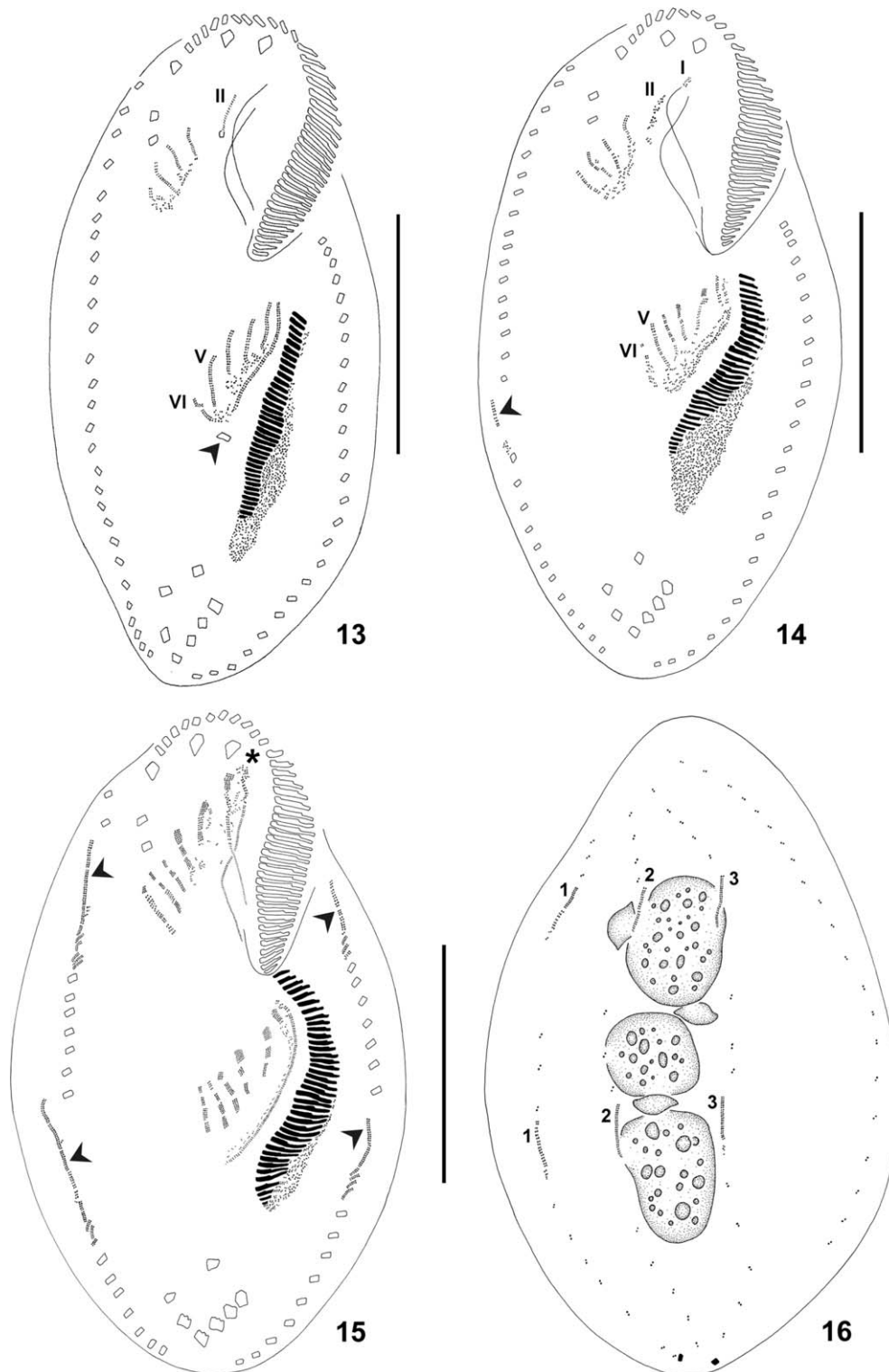
Morphogenesis during cell division (Figs 8–23, 30–48 and Table 2)

Stomatogenesis of the opisthe begins with the proliferation of basal bodies near the anteriormost postoral ventral cirrus IV/2, and near the leftmost transverse cirri II/1 and III/1, resulting in the formation of two anarchic fields of kinetosomes (Figs 8, 9, 30–33). As new basal bodies proliferate, these two fields enlarge posteriad and anteriad, respectively, and become a unified anarchic field of basal bodies (Figs 10, 34). Primordium I of the opisthe develops from the oral primordium, and detaches as a streak of basal bodies which proliferates anteriad. Some presumptive membranelles develop anteriorly, through the alignment of paired kinetosomes in two rows. The macronuclear nodules show replication bands at this stage.

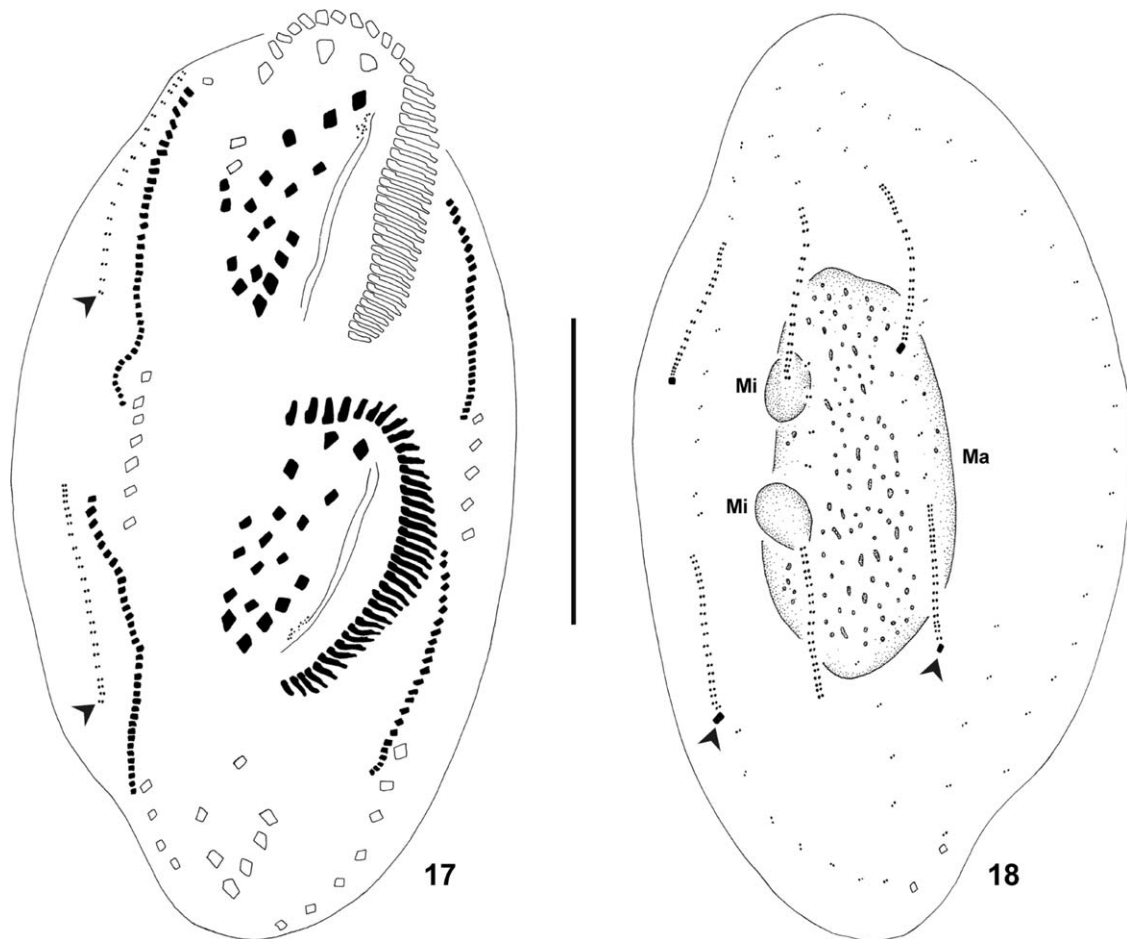
Primordia II and III of the opisthe are also formed from the oral primordium (Figs 11, 35, 37). The posteriormost frontoventral cirrus IV/3 disaggregates to form primordia IV–VI of the proter (Figs 11, 12, 36, 38, 40). The frontoventral cirrus III/2 dedifferentiates and generates primordium III of the proter (Figs 12, 39). The buccal cirrus produces primordium II of the proter, while the paroral dedifferentiates distally to become primordium I of the proter (Figs 13, 14, 41). The anterior part of the oral primordium of the opisthe continues with the differentiation of membranelles in a posteriad direction. A third row of kinetosomes is added in the anterior two-rowed membranelles. Primordia I–III of the opisthe lengthen and presumptive undulating membranes align longitudinally and parallel to the forming adoral zone of membranelles. Primordium IV of the opisthe arises from the disaggregated postoral ventral cirrus IV/2 and primordia V and VI from the disaggregated postoral ventral cirrus V/4 (Figs 12–14, 42, 43). Consequently, a total of six frontal–ventral–transverse cirri primordia are produced in each divider. Cirri II/1, III/1, IV/1, V/1–3, VI/1–4, and the frontal cirri are not involved in primordia formation and will be resorbed in later stages of division or in the postdivider.

Two marginal rows primordia develop within each parental marginal row; the anterior right primordium is generated by the third or fourth cirrus, while the posterior right primordium is generated from the cirrus behind the presumptive divisional furrow. The two left marginal rows primordia are generated somewhat later than the right ones, and the anterior one develops from the first left cirrus (Figs 15, 44).

The dorsal kineties primordia develop intrakinetally within the dorsal rows 1–3 at the level of the marginal row primordia (Fig. 16). In the following stage of division, the endoral of the proter dedifferentiates distally. Whether the endoral dedifferentiates completely could not be observed. New cirri begin to segregate within the streaks of both proter and opisthe. The membranelles of the opisthe are almost completely formed. The undulating membrane primordium of the opisthe is parallel to the adoral zone of membranelles and the leftmost frontal cirrus is segregated (Figs 15, 44). The parental adoral zone of membranelles is fully retained in the proter



Figs 13–16. Morphogenesis of ventral (13–15) and dorsal (16) side of *Parasterkiella thompsoni* after protargol impregnation. (13) Development of primordium VI of the opisthe and primordium II of the proter. Arrowhead marks parental cirrus V/3. (14) Development of the primordia I, II of the proter and right marginal cirri primordium of the opisthe (arrowhead). Parental cirrus V/3 is resorbed. (15) Segregation of new cirri. Arrowheads mark the primordia of the right and left marginal cirri; asterisk marks dedifferentiation of the undulating membranes of the proter. (16) Proliferation of the dorsal row primordia 1–3 in the same specimen as in (15). The macronucleus remains tripartite and micronuclei become spindle-shaped. I, II, V, VI, frontal–ventral–transverse cirri primordia; 1–3, dorsal kinety primordia. Parental cirri, white; new cirri, black. Scale bars = 50 μ m.



Figs 17, 18. Morphogenesis of ventral (17) and dorsal (18) side of *Parasterkiella thompsoni* after protargol impregnation. (17) Late divider showing the opisthe membranelles and frontal–ventral–transverse cirri completely formed. Arrowheads mark dorsomarginal rows. (18) The dorsal row primordia and condensed macronucleus of the same specimen as in (17). Caudal cirri are formed only on kineties 1 and 3 (arrowheads). Ma, macronucleus; Mi, micronucleus. Parental cirri, white; new cirri, black. Scale bar = 50 μ m.

though a cryptic reorganization could not be excluded (see Voss and Foissner 1996). Some extra cirri were observed next to the right anterior and/or posterior marginal row primordia, although these cirri could also be parental (Figs 19–21). These extra cirri seem to be resorbed later since they were not observed in interphase specimens. In late divisional stages, the streaks differentiate into cirri (Table 2). The adoral zone of the opisthe is completely formed and curves to the right anteriorly, typically adopting the shape of a question mark. The undulating membranes have already split longitudinally, but are still parallel to each other (Figs 17, 45). The new cirri begin to migrate to form the characteristic 18-cirri pattern.

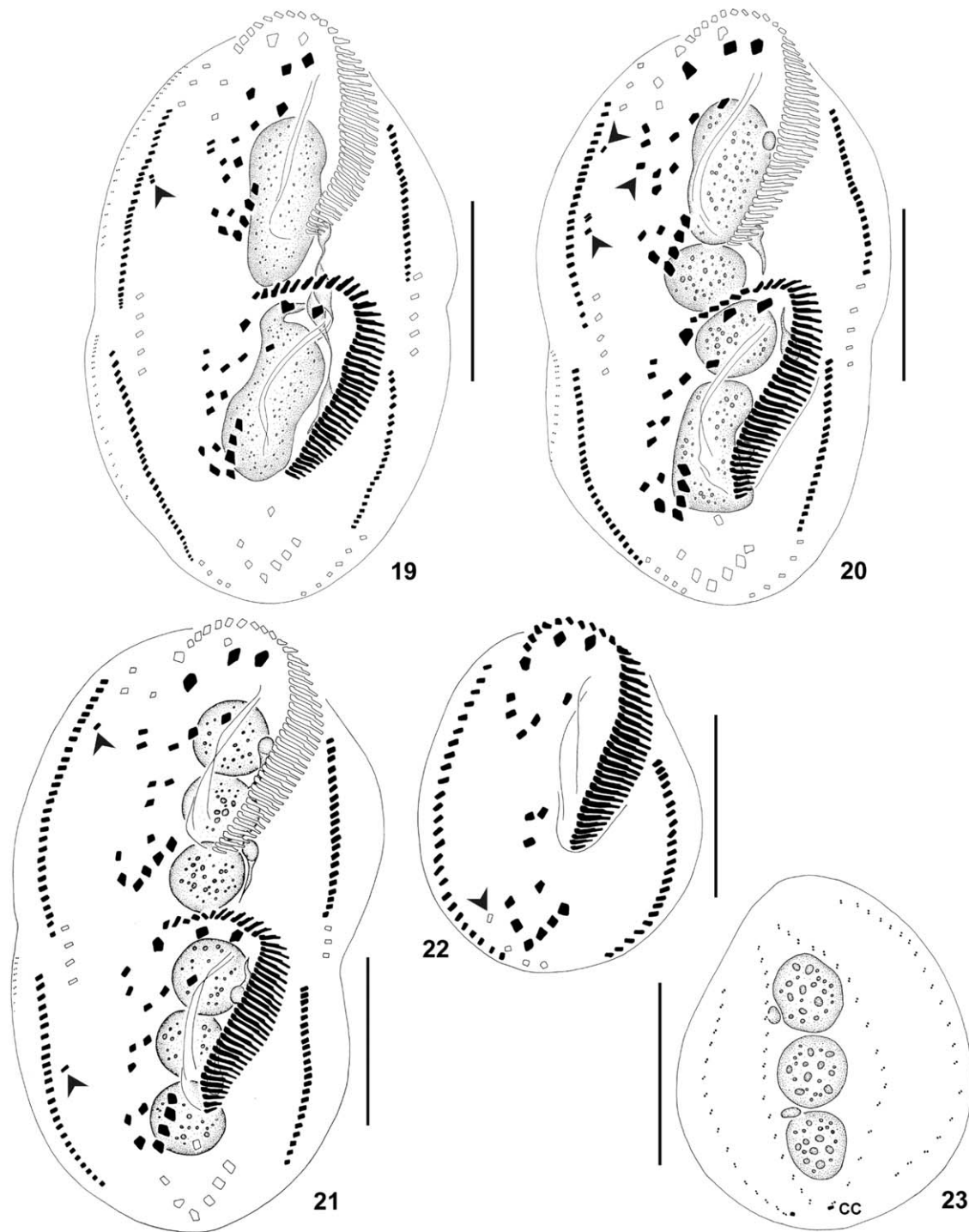
Dorsal kineties primordia are lengthened, and one caudal cirrus is formed at the end of dorsal kineties 1 and 3 (Figs 18, 46). One dorsomarginal row differentiates from the right marginal row primordium (Figs 17, 44). Parental dikinetids remain among the new developing dorsal kineties, but these parental kineties are resorbed later or in the postdivider.

The three macronuclear nodules condense in a single mass and the micronuclei become elliptic. The macronuclear mass

elongates and divides into two pieces, one for the proter and the other for the opisthe. Later, the macronuclear mass of the proter divides again into two nodules, with the anterior being larger than the posterior; while the macronuclear mass of the opisthe divides into an anterior rounded nodule and a posterior enlarged nodule. Both enlarged macronuclear nodules constrict again, resulting in the typical tripartite macronucleus for each divider. Meanwhile, the micronuclei divide mitotically (Figs 18–21, 45–48). The undulating membranes of the proter reorganize and the membranes of both the proter and the opisthe intersect each other optically (Figs 21, 48). In the postdivider, the cirri have already achieved their appropriate positions but some parental marginal (proter) or transverse cirri (opisthe) are still not resorbed. The nuclear apparatus already consists of the typical three macronuclear nodules and the two or three micronuclei (Figs 22, 23).

Occurrence and ecology

Parasterkiella thompsoni was found in two small, shallow water pools (about 500 m², 0.33 m deep) from a Fuegian

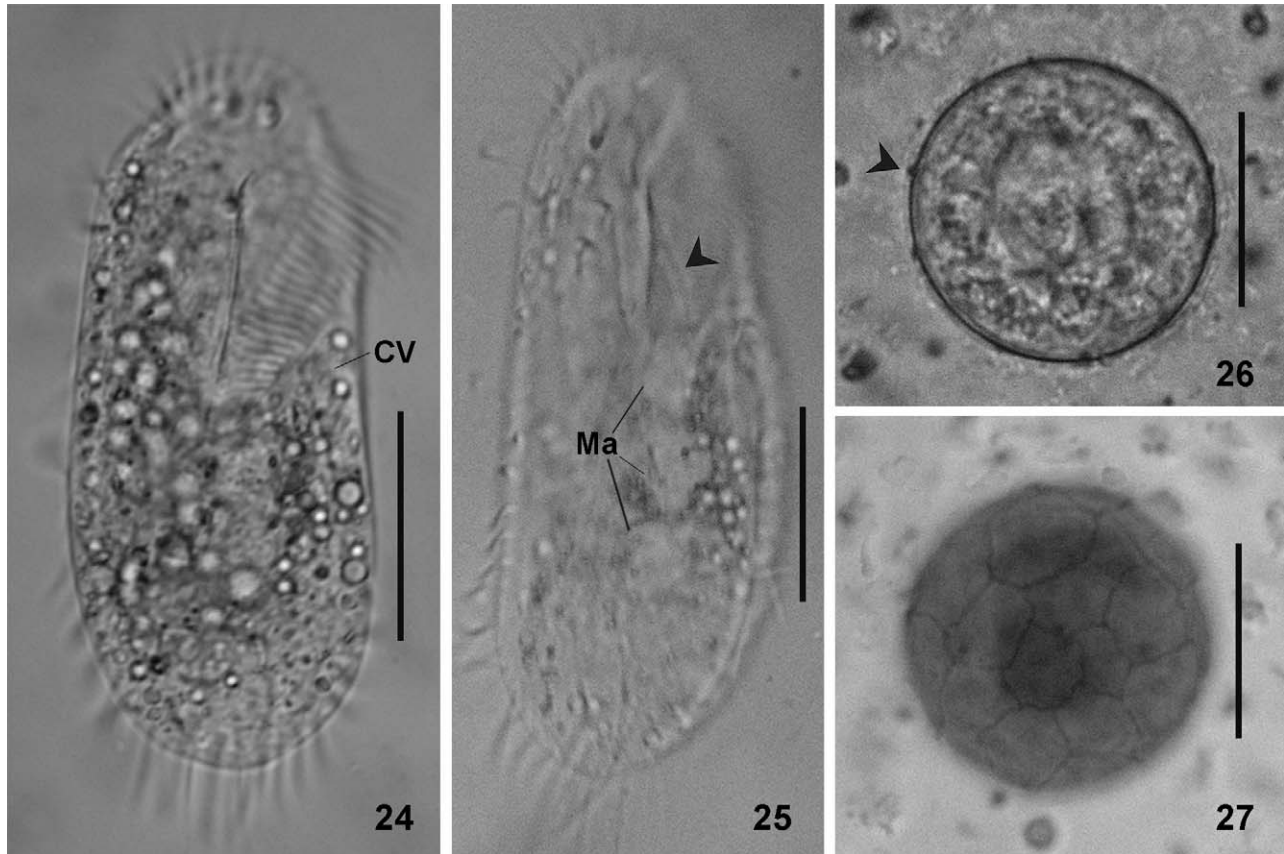


Figs 19–23. Morphogenesis of ventral (19–22) and dorsal (23) side of *Parasterkiella thompsoni* after protargol impregnation. (19) Late divider with macronucleus in two masses and dividing micronuclei. Arrowhead marks extra cirri. (20) Late divider with two macronuclear masses in each divider and crossed undulating membranes of the opisthe. Arrowheads mark extra cirri. (21) Very late divider with three macronuclear nodules and crossed undulating membranes in each divider. Arrowheads mark extra cirri. (22 and 23) Postdivider. Arrowhead marks unresorbed parental marginal cirri. CC, caudal cirri. Parental cirri, white; new cirri, black. Scale bars = 50 μm .

peat bog dominated by *Sphagnum magellanicum*. Low conductivity and pH values (Table 3) are typical of this type of environment (Mataloni 1999), as well as low contents of dissolved nutrients. Waters were well illuminated and oxy-

genated, and both ponds presented a 10 cm-thick ice cover during October 2009.

Remarkably, during April 2009, *Chlorella*-like green algae were densely packed in the cytoplasm of *P. thompsoni*



Figs 24–27. Interphase morphology (24 and 25) and resting cyst (26 and 27) of *Parasterkiella thompsoni* in vivo (24–26) and after protargol impregnation (27). (24) Ventral view of a specimen with food. (25) Ventral view of a slender specimen. Arrowhead marks the conspicuous buccal lip. (26) Optical cross section of a mature resting cyst. Arrowhead marks a ridge on the cyst wall. (27) View of polygonal ridges on the surface of the resting cyst. CV, contractile vacuole; Ma, macronucleus. Scale bars = 30 μm .

Table 3. Physicochemical variables in sampling sites RH3 and RH5, where *Parasterkiella thompsoni* was found.

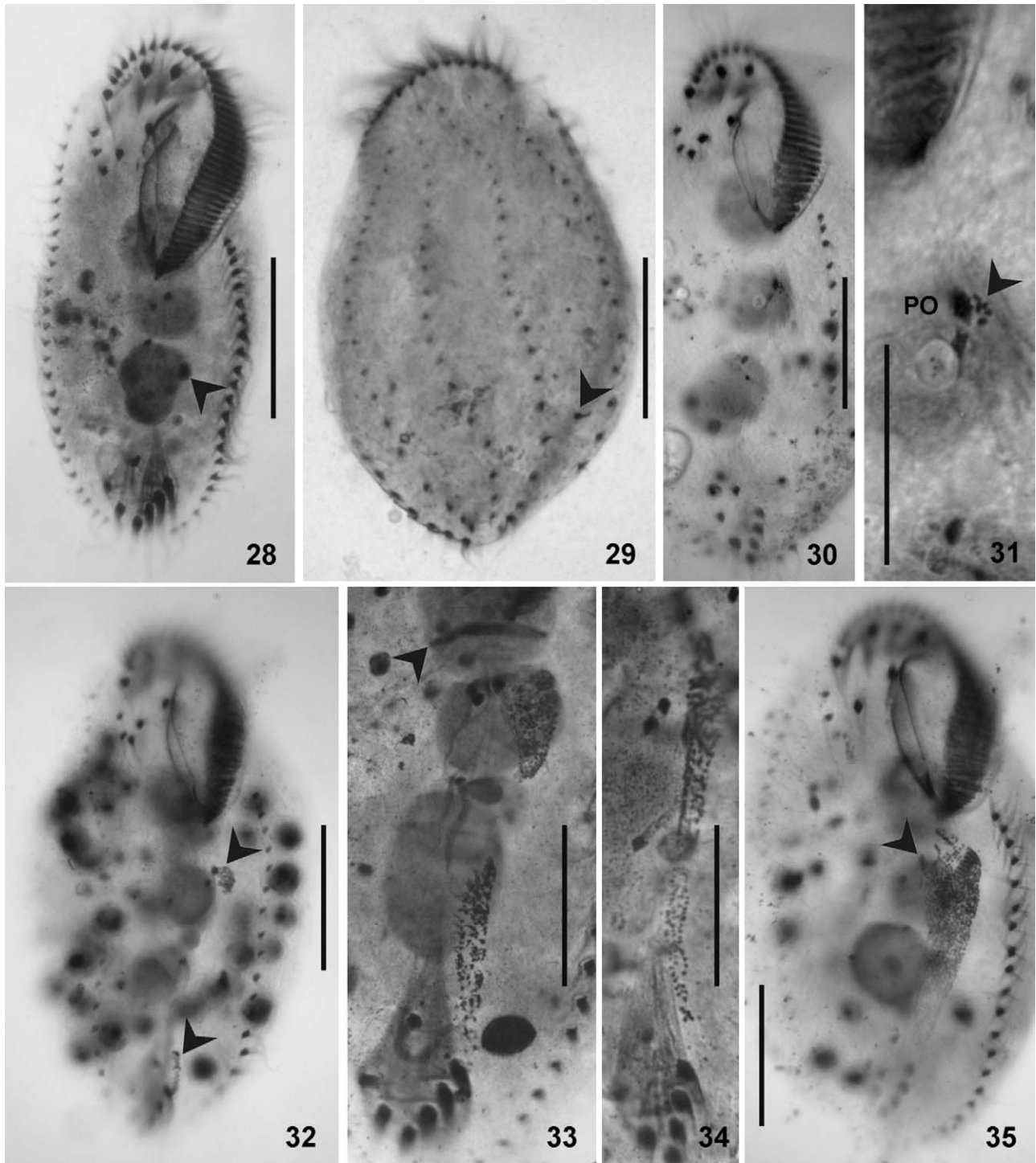
Environmental variable	RH3	RH5
Temperature ($^{\circ}\text{C}$)	7.1 (3.2–11.4)	5.4 (1.7–7.5)
pH	4.5 (4.4–4.7)	4.5 (4.4–4.8)
Conductivity ($\mu\text{S cm}^{-1}$)	14 (10–17)	11 (5.5–18)
Dissolved oxygen (mg L^{-1})	8.8 (8.7–8.9)	8.7 (8.7–8.8)
PAR ($\mu\text{ photons m}^{-2} \text{ s}^{-1}$)	707.4 (410.3–1,184)	635.5 (244.8–1026)
DIN ($\mu\text{g L}^{-1}$)	30.4 (10–51)	42.9 (31.3–54.4)
$\text{PO}_4\text{-P}$ ($\mu\text{g L}^{-1}$)	80 (30–130)	30 (20–40)
Total N ($\mu\text{g L}^{-1}$)	6800 (5500–9400)	6033 (4900–8300)
Total P ($\mu\text{g L}^{-1}$)	143 (90–170)	307 (80–420)

The arithmetic mean is followed by minimum and maximum observations in parentheses ($n = 3$). DIN, dissolved inorganic nitrogen; PAR, photosynthetically active radiation; $\text{PO}_4\text{-P}$, phosphate; Total N, total nitrogen; Total P, total phosphorus.

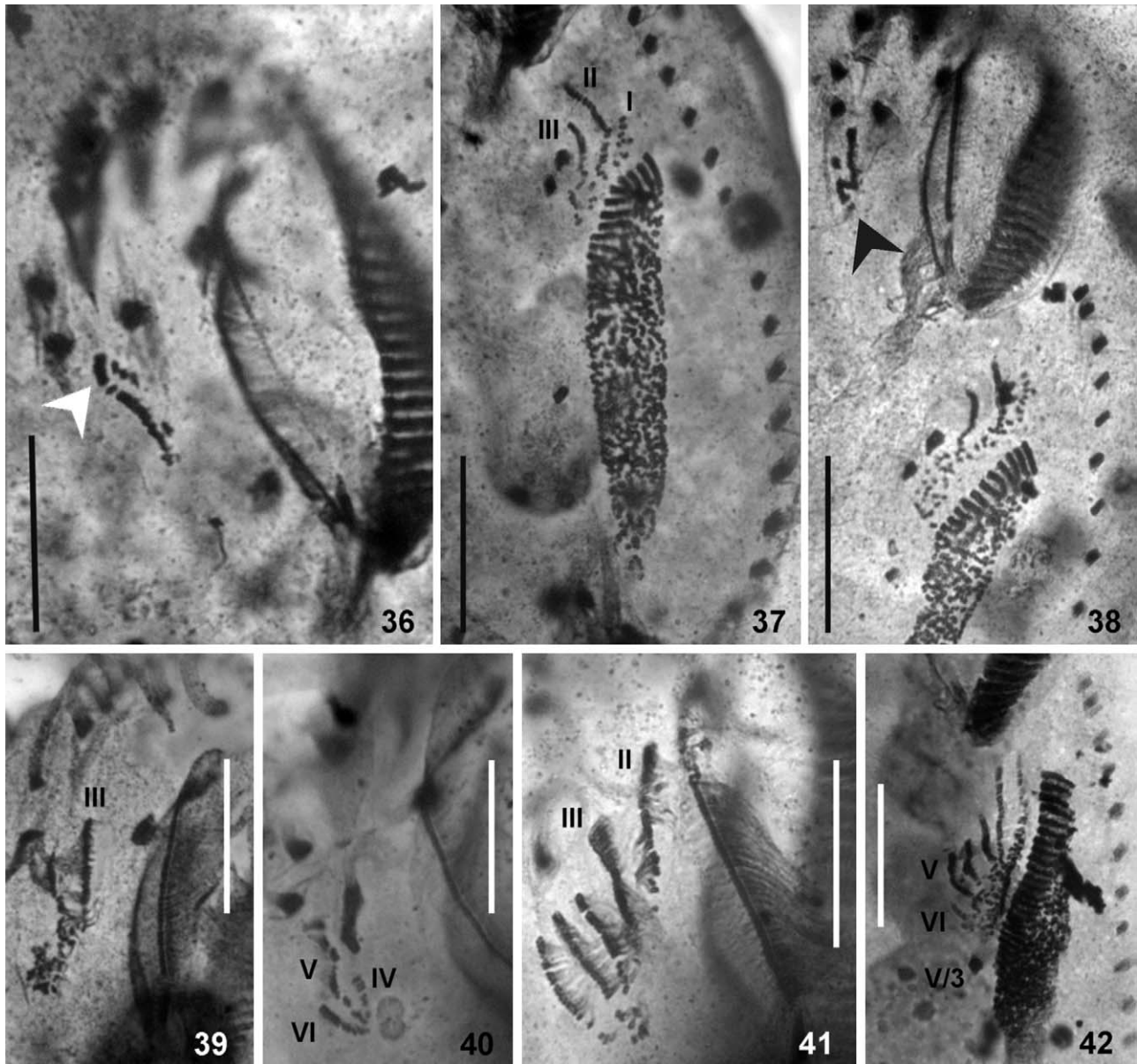
obtained from the pool RH5. Protargol impregnation of these cells showed no difference from the specimens lacking the algae (i.e., cells from RH5 obtained during other sampling occasions and from RH3). Unfortunately, we could not obtain DNA from the specimens with algae, thus we assume both kinds of specimens to be conspecific until additional data become available.

The 18S-rDNA data and phylogenetic analyses (Figs 49–51 and Table 4)

The obtained 18S-rDNA fragment of *Parasterkiella thompsoni* had 1621 nucleotides and a CG content of 44.81%. After aligning and trimming, the nucleotide matrix yielded a total of 1671 characters, of which 1332 were constant and



Figs 28–35. Interphase morphology (28 and 29) and morphogenesis (30–35) of *Parasterkiella thompsoni* after protargol impregnation. (28) Ventral infraciliature. Arrowhead marks micronucleus. (29) Dorsal kineties. Arrowhead marks an extra dikinetid, possibly a still unresorbed parental dikinetid. (30 and 31) Very early divider. Arrowhead marks the beginning of stomatogenesis, with proliferation of basal bodies near the postoral ventral cirri (30); detail of the proliferation of basal bodies near postoral ventral cirri (31, arrowhead). (32) Very early divider showing proliferation of a second field of basal bodies near the transverse cirri. Arrowheads mark both fields of basal bodies. (33) Detail of enlarged fields of basal bodies. Arrowhead marks replication band in macronucleus. (34) Detail of unique field of basal bodies of an early divider. (35) Early to middle divider showing the oral primordium. Arrowhead marks disaggregated cirrus IV/2. PO, postoral ventral cirri. Scale bars = 20 μm (31 and 34), 30 μm (28–30, 32, 33, and 35).

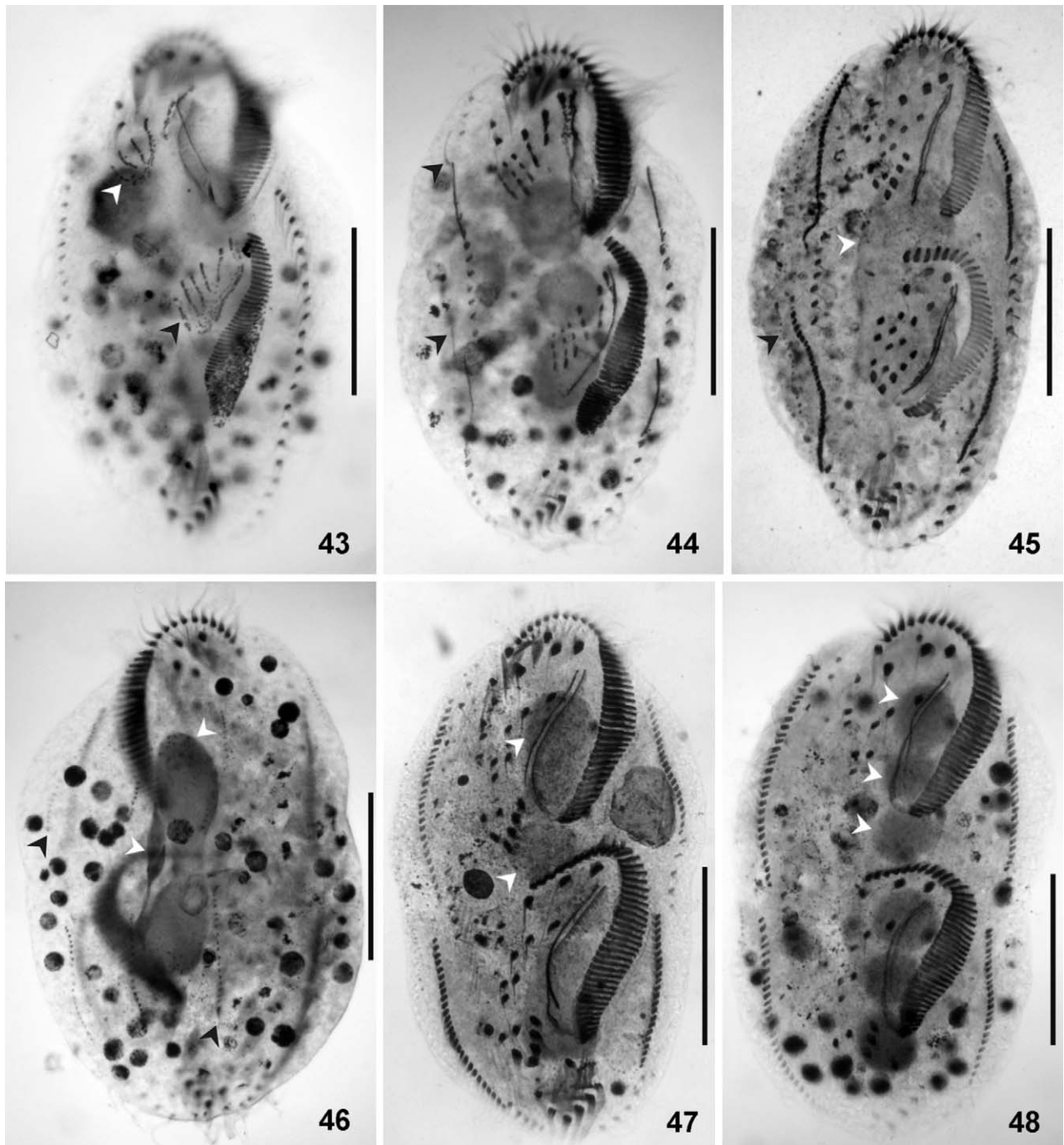


Figs 36–42. Middle dividers of *Parasterkiella thompsoni* after protargol impregnation. (36) Disaggregating cirrus IV/3 and primordium IV of the proter (arrowhead). (37) Oral primordium and primordia I–III of the opisthe. (38) Primordia IV, V of the proter (arrowhead). (39) Primordium III of the proter. (40) Primordia IV–VI of the proter. (41) Primordia II–VI of the proter. (42) Oral primordium and primordia I–VI of the opisthe; postoral ventral cirrus V/3 still unresorbed. I–VI, frontal–ventral–transverse cirri primordia. Scale bars = 20 μm (36 and 39–42), 30 μm (37 and 38).

222 were parsimony-informative. The 18S-rDNA sequence of *P. thompsoni* differs by up to 3.5% from the analogous sequences of *Sterkiella* species (Table 4).

The phylogenetic patterns obtained from BI, MP, and ML consistently hypothesized a monophyly of the Stylonychiinae (Figs 49–51). The internal kinships of the ingroup were slightly variable as a result of different analytic criteria. The BI and ML results yielded almost identical topologies, differing because of a consensus compromise of the relationships within the group formed by *Cyrtohymena citrina* (Berger and Foissner, 1987) Foissner, 1989,

Afrokeronopsis aurea (Foissner and Stoeck, 2008) Foissner et al., 2010, *Onychodromopsis flexilis* Stokes, 1887, *Rubrioxysticha ferruginea* (Stein, 1859) Berger, 1999, and *Paraurostyla weissei* (Stein, 1859) Borror, 1972 in the BI tree. The genera *Sterkiella* Foissner et al., 1991, *Stylonychia* Ehrenberg, 1830, and *Tetmemena* Eigner, 1999 are all polyphyletic. *Parasterkiella thompsoni* was consistently placed among the stylonychiines. In BI and ML trees, *P. thompsoni* branched after the *Laurentiella* + *Stylonychia ammermanni-lemnae-mytilus* group; while in the MP trees both groups switched their positions, so that *P. thompsoni* split at the



Figs 43–48. Morphogenesis of ventral (43–45, 47, and 48) and dorsal (46) side and behavior of nuclear apparatus of *Parasterkiella thompsoni* after protargol impregnation. (43) Middle divider with six frontal–ventral–transverse cirri primordia; arrowheads mark primordium VI of the proter (white) and of the opisthe (black). (44) Cirri segregation in a middle divider; arrowheads mark the formation of dorsomarginal kineties. (45) Late divider with macronucleus fused in a single mass (white arrowhead); black arrowhead marks the dorsomarginal kinety of the opisthe. (46) Late divider with caudal cirri at the ends of dorsal kineties 1 and 3 (black arrowheads); white arrowheads mark elongated macronucleus and dividing micronuclei. (47) Late divider with two macronuclear masses each in the proter (arrowheads) and the opisthe. (48) Late divider with three macronuclear nodules in the proter (arrowheads) and opisthe. Scale bars = 50 μm .

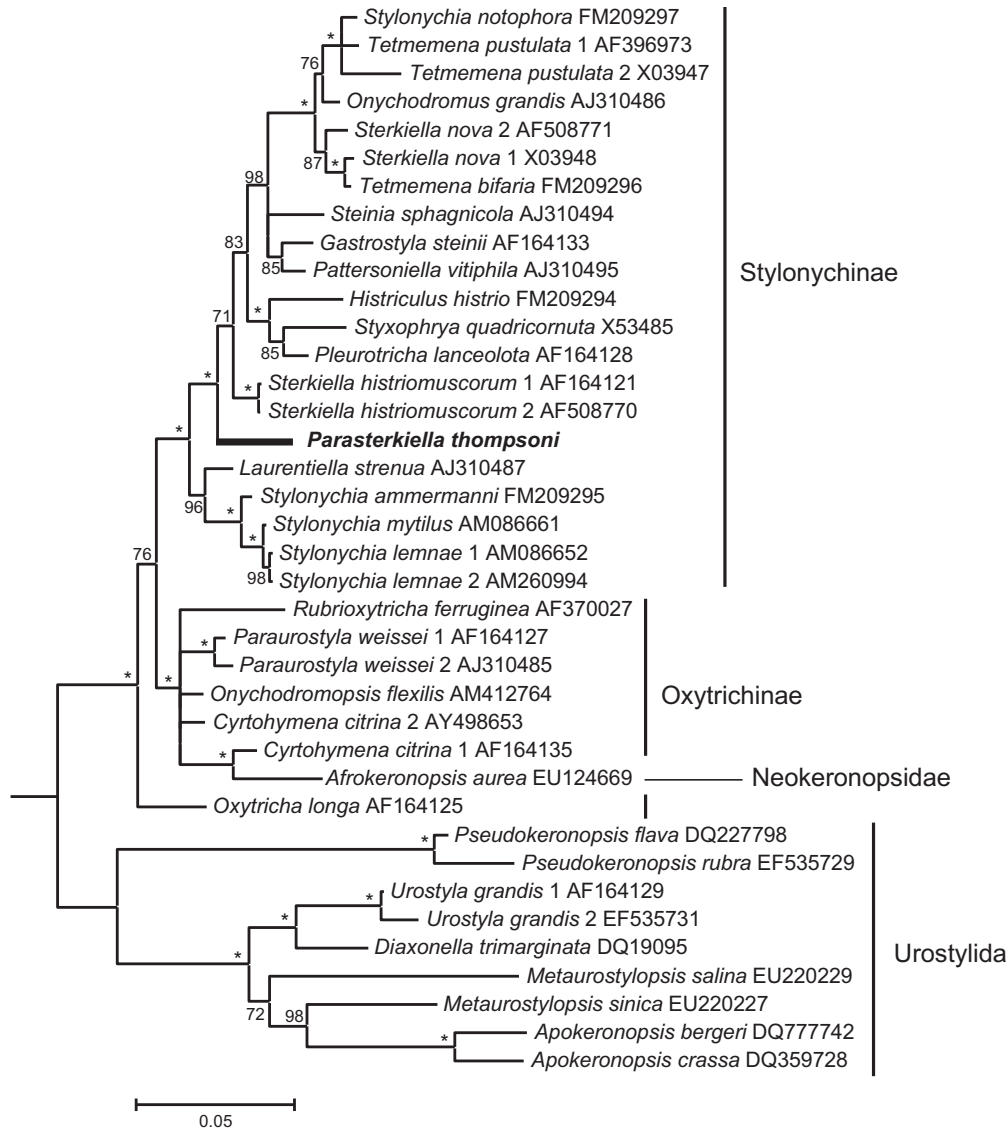


Fig. 49. Majority rule (50%) consensus of the Bayesian trees remaining after burn-in. The numbers associated with nodes indicate the posterior probabilities (shown in %). Asterisks indicate full support, and values smaller than 50% are omitted. NCBI accession codes are provided after the species names. Mean $\ln L = -6734.644$; standard deviation = 0.32; scale bar: five substitutions per 100 nucleotide positions.

base of the Stylonychinae. The divergence of *P. thompsoni* from its sister-taxa had low bootstrap and jackknife support values (Figs 50, 51), but high Bayesian probability (Fig. 49).

Table 4. Model-corrected pairwise genetic distances (in %) between species of *Sterkiella* and *Parasterkiella thompsoni*.

Species	1	2	3	4
1 <i>Sterkiella histriomuscorum</i> 1 AF164121				
2 <i>S. histriomuscorum</i> 2 AF508770	<0.1			
3 <i>S. nova</i> 1 X03948	2.2	2.1		
4 <i>S. nova</i> 2 AF508771	2.2	2.1	0.8	
5 <i>Parasterkiella thompsoni</i> HM569264	1.9	1.9	3.3	3.5

Discussion

Interphase morphology and resting cyst

The morphology of *Parasterkiella thompsoni* from Tierra del Fuego matches the original description of the Antarctic population by Foissner (1996). At first, Foissner (1996) supposed that the Antarctic specimens, with an unusual number of macronuclear nodules, could represent a teratological population of species having normally two or four macronuclear nodules, such as *Sterkiella histriomuscorum* (Foissner et al., 1991) Foissner et al., 1991 or *S. cavicola* (Kahl, 1935) Foissner et al., 1991, respectively. Specimens with two or four macronuclear nodules were also observed in Tierra del Fuego, although with a very low frequency.

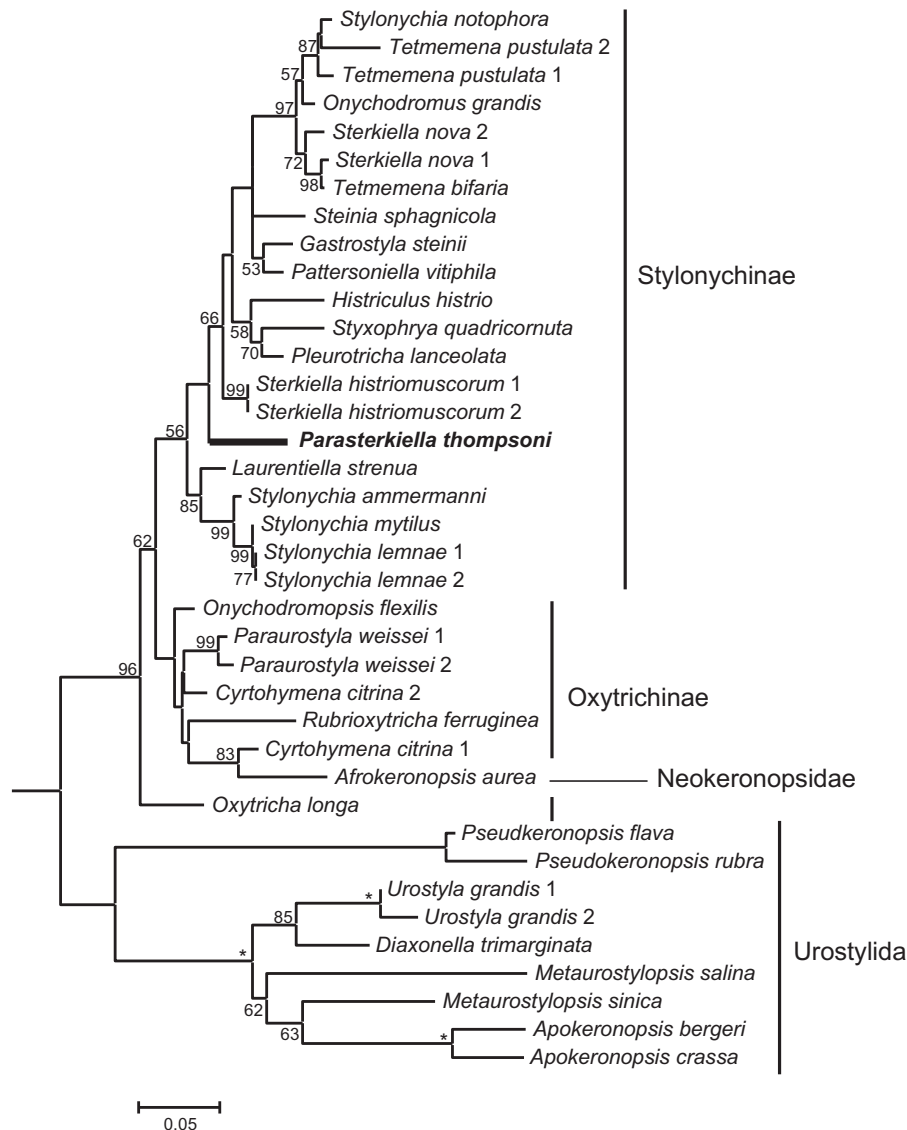


Fig. 50. Maximum likelihood tree. The numbers associated with nodes indicate bootstrap percentages. Asterisks indicate full support, and values smaller than 50% are omitted. $\ln L = -6648.801467$. Scale bar: five substitutions per 100 nucleotide positions.

Other authors described Antarctic oxytrichids with three macronuclear nodules that were considered conspecific with *Parasterkiella thompsoni* (Foissner 1996). Sudzuki (1964) recorded under the name *Opisthotricha* sp. a ciliate from moss samples collected in a petrel rookery near Langhovde, Ongul Islands. That isolate differs from the Fuegian species recorded here, as well as from the other described populations, with respect to the high number of micronuclei (more than 26 [a possible misinterpretation?] vs. usually 2–3). Thompson (1972) described and sketched in more detail after silver impregnation an *Oxytricha* sp. from water samples collected in a rock pool behind Old Palmer station on the Antarctic Peninsula. The specimens from Tierra del Fuego are very similar to those described by Thompson (1972), who also observed four dorsal rows of bristles. Outside Antarctica, Seshachar and Katsuri Bai (1963) described an *Oxytricha* sp. from a laboratory fish tank in Bangalore, India,

with three macronuclear nodules, but much larger in size than the populations from Antarctica and Tierra del Fuego (200–450 μm vs. 80–170 μm , Foissner 1996; Thompson 1972; this study). Beside the greater size, the Indian isolate has a higher number of micronuclei (7–14 vs. usually 2–3 in *P. thompsoni*) and, as was pointed out by Foissner (1996) and Berger (1999), that isolate could represent a different species.

The resting cysts of styloynchines are known to have various patterns of wall ornamentation (Berger 1999). Our results show that the cysts of *P. thompsoni* are similar to those of *S. cavicola* and *Gastrostyla steinii* considering the presence of polygonal ridges on the wall surface (Berger and Foissner 1987; Foissner 1987). Generally, the cyst wall of hypotrichs is composed of four layers (Gutiérrez et al. 2003). Unfortunately, the occurrence of such layers could not be properly checked for *P. thompsoni* in the present study.

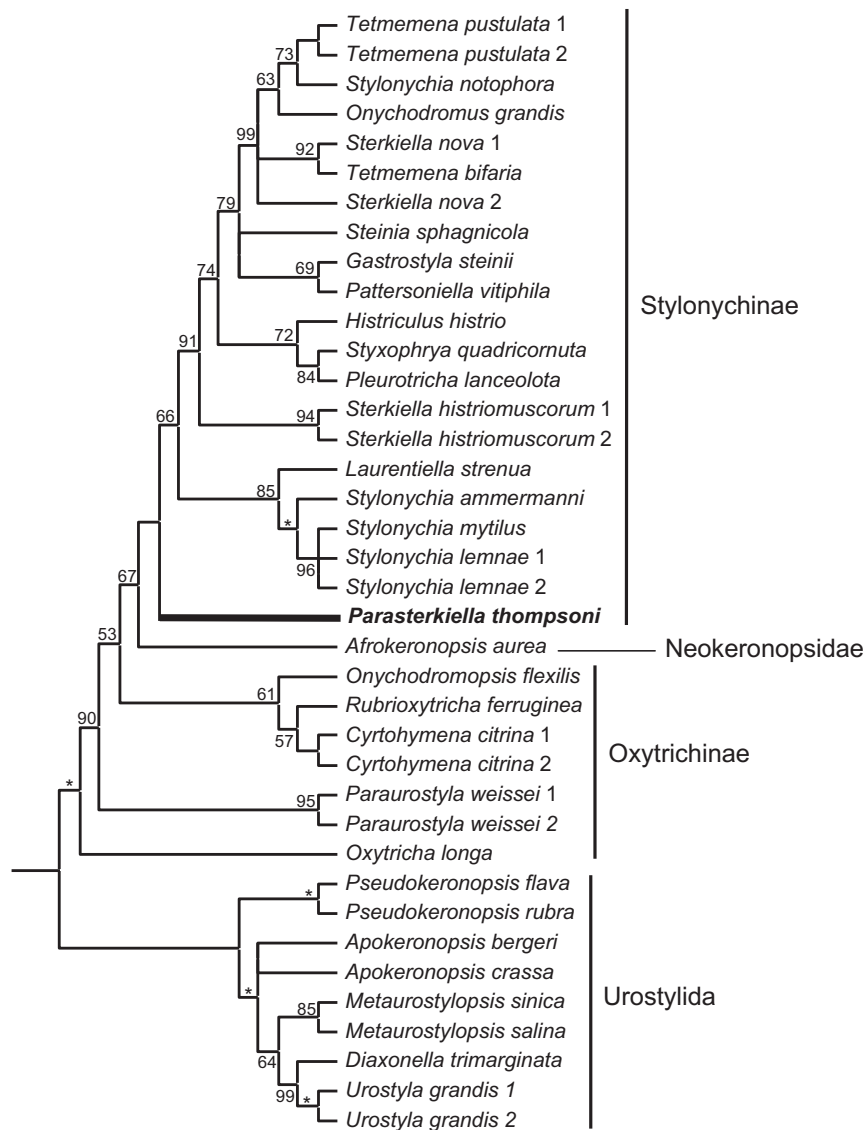


Fig. 51. Strict consensus of four maximum parsimony trees. The numbers associated to branches indicate jackknife percentages. Asterisks indicate full support, and values smaller than 50% are not displayed. Tree length (in steps) = 2514; consistency index = 0.7041; retention index = 0.8631; rescaled consistency index = 0.6077.

Divisional morphogenesis

The morphogenesis of *P. thompsoni* shows some differences compared to the morphogenesis of *Sterkiella* species (Berger et al. 1985; Foissner and Berger 1999; Foissner et al. 2002; Fryd-Versavel et al. 2010; Petz and Foissner 1997). The most noticeable difference lies in the development of the dorsal ciliature, which occurs in the *Urosomoida* pattern (see Berger 1999). In *P. thompsoni* the presence of only four dorsal rows of bristles results from the lack of fragmentation in dorsal kinety 3 and the development of only one dorso-marginal kinety from the right marginal row primordium, as occurs in the Oxytrichinae genera *Urosoma* Kowalewskiego, 1882 and *Urosomoida* Hemberger in Foissner, 1982.

Further differences were observed with respect to the origin of the oral primordium of the opisthe. The development

of this primordium in *P. thompsoni* begins with the proliferation of two separated anarchic fields of basal bodies, one generated near the anteriormost postoral ventral cirrus IV/2 and the other near the leftmost transverse cirri II/1 and III/1. In *S. caviticola*, a small patch of basal bodies, in fact, develops close to cirrus IV/2 and probably contributes to the oral primordium. This contribution, however, would occur after the basal bodies originating near the transverse cirri have proliferated and reached the level of the postoral ventral cirri (Foissner et al. 2002). Such pattern differs from that of *Sterkiella histriomuscorum* and *S. nova* Foissner and Berger, 1999, where the oral primordium develops solely from basal bodies that originate near the leftmost transverse cirri (Berger et al. 1985; Foissner and Berger 1999; Fryd-Versavel et al. 2010; Petz and Foissner 1997). Indeed, the pattern of early formation of the oral primordium observed in *P. thompsoni*

rarely occurs among the Stylonychinae – e.g., *Pattersoniella vitiphila* Foissner, 1987 – and is apparently more common among the flexible-body oxytrichids – e.g., *Oxytricha longigranulosa* Berger and Foissner, 1989, *Rubrioxysticha indica* Naqvi et al., 2006, *Tachysoma terricola* Hemberger, 1985 (Berger 1999; Berger and Foissner 1989; Foissner 1987; Hemberger 1985; Naqvi et al. 2006).

With respect to macronucleus development in *Oxytricha* sp. with three macronuclear nodules (Seshachar and Katsuri Bai 1963), the typical number of nodules is achieved in the postdivider about 24 h after fission, whereas in *P. thompsoni* usually three nodules are generated in each divider before cytokinesis.

Foundation for the creation of the new genus

The genus *Sterkiella* was created to include those species with caudal cirri previously assigned to *Histriculus* Corliss, 1960 (Foissner et al. 1991). At the present time, this genus is defined by a combination of characters – e.g., the rigid or semirigid body, curved and intersecting undulating membranes, separated marginal rows of cirri at the posterior end, and the presence of caudal cirri. All these features, however, occur in other genera and thus are not unique. The supposed sole autapomorphy of the genus was the de novo formation of the primordia V and VI of the opisthe (Berger 1999). Later, Foissner et al. (2002) recognized this origin as a mistake and confirmed that both primordia V and VI develop from the postoral ventral cirrus V/4.

Foissner (1996) and Berger (1999) considered the classification of the Antarctic ciliate in the genus *Sterkiella* as tentative, since this ciliate possesses characteristics common to several oxytrichids. The dorsal ciliature resembles the Oxytrichinae genera *Urosoma* and *Urosomoida* (Berger 1999) and, as confirmed in the present study, the dorsal ciliature actually develops in the *Urosomoida* pattern. Nevertheless, with respect to features like the semirigid body, the lack of cortical granules, the adoral zone of membranelles occupying about 50% of body length, the presence of oblique fibres around dorsal cilia, and the morphogenetic inactivity of cirrus V/3, this species belongs to the Stylonychinae (Berger 1999). This classification is confirmed by our phylogenetic analyses (see below).

In *Sterkiella* the rightmost dorsal primordium fragments and forms two dorsal kineties (Foissner et al. 2002). Hence, the lack of fragmentation in the dorsal primordia of *P. thompsoni* clearly separates this genus not only from *Sterkiella*, but also from all other 18-cirri stylonychine genera (Berger 1999). This difference in the morphogenesis of the dorsal ciliature is herein regarded as justifiable for the creation of a new genus, namely *Parasterkiella*, for *S. thompsoni*. For the present, though, *P. thompsoni* remains monotypic.

Among the non-stylonychine 18-cirri genera in which morphogenetic data also confirmed the occurrence of the *Urosomoida* pattern, *Parasterkiella* is comparable to *Uro-*

soma, *Urosomoida*, and some species currently assigned to *Oxytricha* Bory De Saint-Vincent in Lamouroux, Bory De Saint-Vincent and Deslongchamps (e.g., *O. longa* Gelei and Szabados, 1950 and *O. setigera* Stokes, 1891). These genera all differ in a fundamental way from *Parasterkiella* by having flexible bodies, by exhibiting participation of the postoral ventral cirrus V/3 in primordia formation, and by having an adoral zone of membranelles that occupies less than 50% of the body length (Berger 1999; Berger and Foissner 1988; Foissner 1983; Ganner et al. 1987).

Phylogenetic position

The pairwise genetic distances between populations of *Sterkiella histriomuscorum* and of *S. nova* were smaller than interspecific distances, including *P. thompsoni*. Nevertheless, we found, in accordance with others (Foissner 2007; Foissner et al. 2003; Schmidt et al. 2007), that such comparisons could be misleading as an objective criterion for species or even genus discrimination among stylonychines when performed independently of a morphological study. Although the 18S-rDNA of *P. thompsoni* differs by up to 3.5% from the analogous sequence from *Sterkiella* spp., the *P. thompsoni* sequence does so less when compared with the 18S-rDNAs of certain stylonychines that are morphologically more dissimilar. For instance, the 18S-rDNA sequence of *P. thompsoni* differed by 2.7% from those of *Gastrostyla steinii* and *Laurentiella strenua* (Dingfelder, 1962) Berger and Foissner, 1989 – which have conspicuous ventral row[s] of cirri formed by the ventral primordia – and by 2.6% from the corresponding nucleotide sequences of *Pattersoniella vitiphila*; in which the ventral cirri form a midventral complex and multiple dorsal kinety fragmentation occurs (Berger 1999; Foissner et al. 2002; Martin et al. 1983).

The phylogenetic patterns obtained from BI, MP, and ML did not reject the ingroup monophyly with respect to the urostylid outgroup and consistently hypothesized the monophyly of the Stylonychinae, thus corroborating previous analyses along the same lines (e.g., Bernhard et al. 2001; Foissner et al., 2004; Hewitt et al. 2003; Paiva et al., 2009; Schmidt et al. 2007, 2008; Shao et al. 2007; Yi et al. 2008, 2009). The MP trees were generally in agreement with those obtained by Paiva et al. (2009) concerning the Stylonychinae kinships and the pectinate distribution of its clades. *Paraurostyla weissei* and *Oxytricha longa*, however, occupied slightly different positions, branching before the cyrtohymenids. The morphologically similar genera *Sterkiella*, *Stylonychia*, and *Tetmemena* (Berger 1999) were all polyphyletic within the present analyses, thus corroborating the results from Schmidt et al. (2008). We found little inconsistency regarding the placement of *P. thompsoni* among stylonychines. In the BI and ML trees, *P. thompsoni* consistently branched after the *Laurentiella* + *Stylonychia ammermanni-lemnae-mytilus* group, while in the MP trees *P. thompsoni* split at the base of the Stylonychinae. Although the

divergence of *P. thompsoni* from its sister-taxa had low bootstrap and jackknife support values, the Bayesian probability was high. Worthy of note is that the Bayesian probabilities are often inflated and thus their reliability as a measure of support should be interpreted with extreme caution, especially when their conclusions contradict other supports (Cummings et al. 2003; Randle and Prickett 2010; Schneider 2007; Simmons et al. 2004).

Berger (2006, 2008) established the Dorsomarginalia for the Hypotricha Stein, 1859 with dorsomarginal kineties as supposed morphological synapomorphy. The Oxytrichidae (including the Stylonychinae) belong to the former group, with the fragmentation of dorsal kinety 3 as a robust synapomorphy (Berger 1999, 2006, 2008). Accordingly, *Parasterkiella* would represent a non-oxytrichid Dorsomarginalia, since fragmentation of dorsal kinety 3 is lacking. The phylogeny of the 18S-rDNA marker, however, places *P. thompsoni* as either a basal (MP) or internal (BI, ML) stylonychine branch. In conclusion, if the classification of *P. thompsoni* in the stylonychines is correct, then the *Urosomoida* pattern likely evolved convergently within other hypotrich lineages, as has been previously conjectured by Berger (1999).

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