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Identification and Ontogenesis of the *nomen nudum* Hypotrichs (Protozoa: Ciliophora) *Oxytricha nova* (= *Sterkiella nova* sp. n.) and *O. trifallax* (= *S. histriomuscorum*)

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Summary. *Oxytricha nova* and *O. trifallax* were named and established as viable genetic systems (*via* frozen resting cysts) by molecular biologists, but never determined or described in a scientific way. Thus, their identity is unknown and both are *nomen nudum* species according to the International Code of Zoological Nomenclature. In the present paper, this bewildering situation is rectified by investigating offspring of the original populations. It is shown, by a detailed literature review and morphological and ontogenetical analysis, using live observation, silver impregnation and scanning electron microscopy, that both populations belong to a single morphotype, viz. *Sterkiella histriomuscorum* (Foissner, Blatterer, Berger and Kohmann, 1991), a cosmopolitan species very frequent in limnetic and terrestrial habitats. However, on the molecular level, *O. nova* and *O. trifallax* are very distinct, suggesting that they are different species. Thus, *S. histriomuscorum* is a complex of sibling species. For the sake of nomenclatural continuity and priority, we suggest identifying *O. trifallax* as *S. histriomuscorum* and establishing *O. nova* as a new species, *Sterkiella nova* sp. n. Both species are diagnosed by a combination of morphological, ontogenetical and gene sequence characters. Field populations of S. *histriomuscorum* should be designated as ~~gene sequence~~ "*Sterkiella histriomuscorum* complex" if no molecular data are available to decide whether they belong to *S. nova*, *S. histriomuscorum*, or to another not yet described species of the complex.

Key words: infraciliature, nomenclature, Oxytrichidae, *Oxytricha nova*, *Oxytricha trifallax*, sibling species, *Sterkiella histriomuscorum*, *Sterkiella nova* sp. n.

INTRODUCTION

Since self-splicing introns (ribozymes) were discovered in *Tetrahymena thermophila*, ciliates have become important models for molecular biologists and genome researchers (for review, see Cech 1990). Over the years, model systems have been established with several ciliate species to investigate important phenomena, such as gene scrambling and unscrambling, chromosome fragmentation, gene excision, and telomere function (Prescott 1994). Unfortunately, some of the models were based on organisms which had never been described in a scientific way, namely *Oxytricha nova* and *O. trifallax*. Although both organisms, which were obviously provisionally named,

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were used in many studies since 1980 (see list of synonyms in species descriptions), their identity is not known. Hence, they are *nomen nudum* species, according to articles 13 and 15 of the International Code of Zoological Nomenclature (1985). This situation is untenable not only because *nomen nudum* species do not exist in the official zoological literature but also because such species can hardly be re-sampled if the original strain should be lost.

In the present paper, the morphological identity of *Oxytricha nova* and *O. trifallax* is unscrambled, and both will be firmly established in accordance with the rules of the International Code of Zoological Nomenclature (1985). We shall show that they are sibling species of a *Sterkiella histriomuscorum* complex, which contains taxa that are very similar morphologically and ontogenetically but sufficiently different in several gene sequences to warrant recognizing at least two species, namely *Sterkiella nova* sp. n. and *S. histriomuscorum* (Foissner *et al.*, 1991) Foissner *et al.*, 1991.

MATERIALS AND METHODS, NOMENCLATURE AND TYPE SLIDES

Origin of strains

Oxytricha nova (= *Sterkiella nova* sp. n.; see Nomenclature and Discussion): This species was recovered in 1995 from resting cysts frozen at -70°C by D. M. Prescott on 12.3.1986. It is not known whether the original culture was set up with one or several individuals, i.e. whether the cysts were from a clone or a population. For the morphological investigations, well-growing cultures were obtained by excysting several hundred cysts in Eau de Volvic (French table water) enriched with washed *Chlorogonium* cells and some crushed wheat grains to support growth of bacteria.

The original source of *S. nova* has been described by Klobutcher *et al.* (1981): "The organism used in work reported from this laboratory (University of Colorado at Boulder, Department of Molecular, Cellular, and Developmental Biology) before 1978 was isolated from a Boulder pond and was referred to as *Oxytricha* sp. This organism died out and was replaced in work reported in 1978 and since then by an organism isolated from water obtained from North Carolina and designated *Oxytricha nova*. The original *Oxytricha* sp. and the new *O. nova* are similar in most respects but sufficiently different to suggest that they are different species".

Oxytricha trifallax (= *Sterkiella histriomuscorum*; see Nomenclature and Discussion): a culture was obtained by S. M. Adl (University of British Columbia, Vancouver), who got the isolate from G. Herrick (Salt Lake City), one of the founders of *O. trifallax* (see below and Adl and Berger 1997). In our laboratory, the population was cultivated as described for *S. nova*.

Oxytricha trifallax was established as a viable (*via* frozen resting cysts) genetic system in the laboratory of G. A. Herrick by R. Hammersmith, who isolated it from the Jordan River in Indiana (USA) in the winter of 1985 (G. A. Herrick, Salt Lake City, Utah; pers. comm.). In the literature, *O. trifallax* was mentioned for the first time by Greslin *et al.* (1989) and Hunter *et al.* (1989). Later, Seegmiller *et al.* (1996) mentioned other sources and strains of *O. trifallax*: „Wild *O. trifallax* cells were collected from diverse limnetic sites in Indiana, cloned in the lab and placed into a single fertilely interbreeding group... A PCR screen for new IES-R alleles in 12 additional *O. trifallax* isolates...“. These strains are, according to the molecular data, very similar to the Jordan River isolates of R. Hammersmith (see Fig. 3 in Seegmiller *et al.* 1996).

Morphological methods and terminology

Cells were studied *in vivo* using a high-power oil immersion objective (N.A. 1.32), differential interference contrast, and video microscopy. The infraciliature and other cytological details were revealed by protargol impregnation, methyl green-pyronin staining, and scanning electron microscopy. See Foissner (1991) for a detailed description of all methods mentioned.

Counts and measurements on silvered specimens were performed at a magnification of x 1000. *In vivo* measurements were conducted at a magnification of x 250 - 1000. Although these provide only rough estimates, it is convenient to give such data as specimens may shrink or become inflated in preparations (Table 1). Standard deviation and coefficient of variation were calculated according to statistics textbooks. Drawings of live specimens were based on free-hand sketches and videotape records, those of impregnated cells were made with a camera lucida.

Terminology is according to Berger and Foissner (1997). See this paper especially for numbering and designating of cirri and for diagnosis of genera presently assigned to the Oxytrichidae, to which *Sterkiella nova* (*Oxytricha nova*) and *S. histriomuscorum* (*O. trifallax*) belong.

Nomenclature

Nomenclature of the species and strains treated in this paper is extremely confused and difficult to follow for someone not familiar with the subject and the International Rules of Zoological Nomenclature. Thus, we provide an alphabetically sorted, two-sided index, which shows, in ***boldface***, the bonafide names and allows, for the sake of clarity, to dispense with quotation marks or complicated wordings in the following text. See Berger and Foissner (1997) for literature on original genus and species descriptions.

Histriculus Corliss, 1960: a valid oxytrichid genus characterized by a stiff body, confluent marginal cirral rows, and the lack of caudal cirri (see chapter "Distinguishing the genera *Oxytricha*, *Sterkiella*, *Stylonychia*, and *Histriculus* "). Type species (by original designation): *Paramaecium histrio* Müller, 1773.

Histriculus muscorum (Kahl, 1932) Corliss, 1960 is an outdated combination, that is, the species was assigned to the wrong genus; now it is ***Sterkiella histriomuscorum*** (see chapter "The *Sterkiella histriomuscorum* story").

Histrio Sterki, 1878: invalid because of homonymy (Corliss 1960). Type species (by original designation): *Histrio steinii* Sterki, 1878.

Histrio muscorum Kahl, 1932 is an invalid binomen because of homonymy; now it is ***Sterkiella histriomuscorum*** (see chapter "The *Sterkiella histriomuscorum* story").

Oxytricha Bory de Saint-Vincent, 1824: a valid genus characterized as described in Berger and Foissner (1997). See also chapter "Distinguishing the genera *Oxytricha*, *Sterkiella*, *Stylonychia*, and *Histriculus*"). Type species (by subsequent designation): *Oxytricha granulifera* Foissner and Adam, 1983.

Oxytricha nova, a *nomen nudum* species, is ***Sterkiella nova*** sp. n. in the present paper.

Oxytricha trifallax, a *nomen nudum* species, is ***Sterkiella histriomuscorum*** (Foissner *et al.*, 1991) Foissner *et al.*, 1991 in the present paper.

Sterkiella Foissner, Blatterer, Berger and Kohmann, 1991: Genus erected to contain some oxytrichid stylonychids erroneously assigned to *Histrio* and *Histriculus* (see chapters "Distinguishing the genera *Oxytricha*, *Sterkiella*, *Stylonychia*, and *Histriculus*" and "The *Sterkiella histriomuscorum* story"). Type species (by original designation): *Oxytricha cavicola* Kahl, 1935.

Sterkiella histriomuscorum (Foissner *et al.*, 1991) Foissner *et al.*, 1991 is the valid name for (i) *Histrio muscorum* Kahl, 1932, (ii) *Histriculus muscorum* (Kahl, 1932) Corliss, 1960, and (iii) the *nomen nudum* species *Oxytricha trifallax*.

Sterkiella histriomuscorum* complex** is presently composed of ***Sterkiella histriomuscorum and ***S. nova***.

Sterkiella nova sp. n. is the *nomen nudum* species *Oxytricha nova* in the previous literature.

Type slides

This chapter gives detailed information about the type material of the species and populations under discussion. All slides contain protargol-impregnated specimens and have been deposited in the Oberösterreichische Landesmuseum in Linz (LI), Austria (Natural History Museum of Upper Austria, Linz). Relevant specimens are marked by a black ink circle on the cover glass. The slides can be loan from the curator of the collections: Dr. Erna Aescht, Biologiezentrum des Oberösterreichischen Landesmuseums, Johann-Wilhelm-Klein-Strasse 73, A-4040 Linz, Austria.

Sterkiella nova Foissner and Berger, 1999 (this paper)

This is the *nomen nudum* species *Oxytricha nova* of the previous literature. The population deposited was obtained by D. M. Prescott as described in the Materials and Methods section of the present paper. Accession numbers: 1999/111 (Holotype; prepared with protocol B as described in Foissner 1991) and 1999/112 - 118 (Paratypes; prepared with protocols A and B as described in Foissner 1991). Remarks: The eight slides contain many excellently prepared morphostatic and dividing specimens from a pure culture.

Sterkiella histriomuscorum (Foissner *et al.*, 1991) Foissner *et al.*, 1991

(1) *Histriculus muscorum*, voucher slide from a population of a soil in the Austrian Central Alps. Accession number: 1981/10. Remarks: Detailed description of morphology in Foissner (1982). The slide contains several well-impregnated specimens of *H. muscorum* and many other ciliates because it has been made from material as obtained with the non-flooded Petri dish method.

(2) *Sterkiella histriomuscorum*, two voucher slides of the population from activated sludge in Austria. Accession numbers: 1993/75, 76. Remarks: Detailed description of morphology in Augustin and Foissner (1992). The slides contain many well-impregnated specimens (Foissner's method) of *S. histriomuscorum* and several other ciliates because they were made from a mixed sewage culture.

(3) *Histriculus muscorum*, four voucher slides of the populations investigated by Berger *et al.* (1985) from the Gastein area in Salzburg, Austria. Accession numbers: 1997/131 - 134. Remarks: The slides contain well-impregnated (Foissner's method) morphostatic and dividing specimens of *H. muscorum* and several other ciliates because they were made from non-flooded Petri dish cultures; only slide 1997/132 is from a more pure culture (population 4 in Berger *et al.* 1985), but contains only few dividers.

(4) *Sterkiella histriomuscorum*, one voucher slide from the Antarctic population studied by Petz and Foissner (1997). Accession number: 1997/130. Remarks: The slide contains many excellently prepared (Wilbert's method) morphostatic and dividing specimens from a wheat grain culture.

(5) *Sterkiella histriomuscorum* ("Oxytricha trifallax "), two **neotype** slides from the "Oxytricha trifallax" population described in the Materials and Methods section of the present paper. Accession numbers: 1999/109, 110. Remarks: The slides contain many excellently prepared (protocol A in Foissner 1991) morphostatic and dividing specimens from a pure culture.

Histriculus histrio (Müller, 1773) Corliss, 1960

This is the type species of the genus. Four **neotype** slides with protargol-impregnated (protocol A in Foissner 1991) specimens have been deposited. Accession numbers: 1999/61 - 64. Remarks: Detailed description in Berger and Foissner (1997) and Foissner and Gschwind (1998). The slides contain several well-impregnated morphostatic and

Table 1. Morphometric data from *Sterkiella nova*

Character[1]	Method[2]	$\bar{x}$	M	SD	CV	Min	Max	n
Body, length	IV	122.0	119	9.7	7.9	106	137	29
Body, length	PF	98.1	97	7.2	7.4	87	113	29
Body, length	PW	128.5	132	17.2	13.4	85	150	29
Body, width	IV	57.2	58	4.4	7.7	50	62	29
Body, width	PF	46.5	48	7.0	15.0	25	60	29
Body, width	PW	59.8	60	8.9	14.9	36	75	29
Anterior somatic end to proximal end of adoral zone, distance	PW	52.7	55	7.0	13.2	40	62	29
Anterior macronuclear nodule, length	PW	28.6	29	5.1	17.8	17	39	29
Anterior macronuclear nodule, width	PW	14.0	14	2.6	18.3	9	18	29
Micronuclei, length	PW	3.9	4	0.6	14.5	3	5	29
Micronuclei, width	PW	3.8	4	0.6	14.8	3	5	29
Macronuclear nodules, number[3]	PW	2.0	2	0.0	0.0	2	2	29
Micronuclei, number	PW	1.4	1	0.7	47.4	1	4	29
Adoral membranelles, number	PW	34.2	34	2.0	5.7	30	39	29
Right marginal cirri, number	PW	21.5	21	1.7	7.7	18	25	29
Left marginal cirri, number	PW	19.9	20	1.4	6.9	17	23	29
Frontal cirri, number	PW	3.0	3	0.0	0.0	3	3	29
Frontoventral cirri, number	PW	4.0	4	0.0	0.0	4	4	29
Buccal cirri, number	PW	1.0	1	0.0	0.0	1	1	29
Postoral ventral cirri, number	PW	3.0	3	0.0	0.0	3	3	29
Pretransverse ventral cirri, number	PW	2.0	2	0.0	0.0	2	2	29
Transverse cirri, number[4]	PW	5.0	5	-	-	5	6	29
Caudal cirri, number	PW	3.0	3	0.0	0.0	3	3	29
Dorsal kineties, number	PW	6.0	6	0.0	0.0	6	6	29
Dikinetids in dorsal kinety 2, number	PW	19.7	20	1.4	7.0	16	22	29
Dikinetids in dorsal kinety 6, number	PW	5.8	6	1.2	20.3	4	8	29
4-day-old resting cysts, length	PW	45.6	47	4.5	9.8	38	57	34
4-day-old resting cysts, width	PW	45.4	47	4.6	10.2	38	57	34
4-weeks-old resting cysts, length	PW	44.6	44	4.3	9.6	36	53	42
4-weeks-old resting cysts, width	PW	43.5	44	4.4	10.0	36	53	42

[1] Data based on randomly selected, protargol-impregnated morphostatic specimens. Measurements in µm. CV, coefficient of variation in %; M, median; Max, maximum; Min, minimum; n, number of specimens investigated; SD, standard deviation; $\bar{x}$, arithmetic mean.
[2] IV, *in vivo* (from video tape records); PF, Foissner's (1991) protargol protocol; PW, Wilbert's (1975) protargol protocol.
[3] Of 286 specimens investigated, 265 had two macronuclear nodules, 13 had three, and 8 had only one.
[4] Of 30 specimens investigated, a single one had 6 transverse cirri.

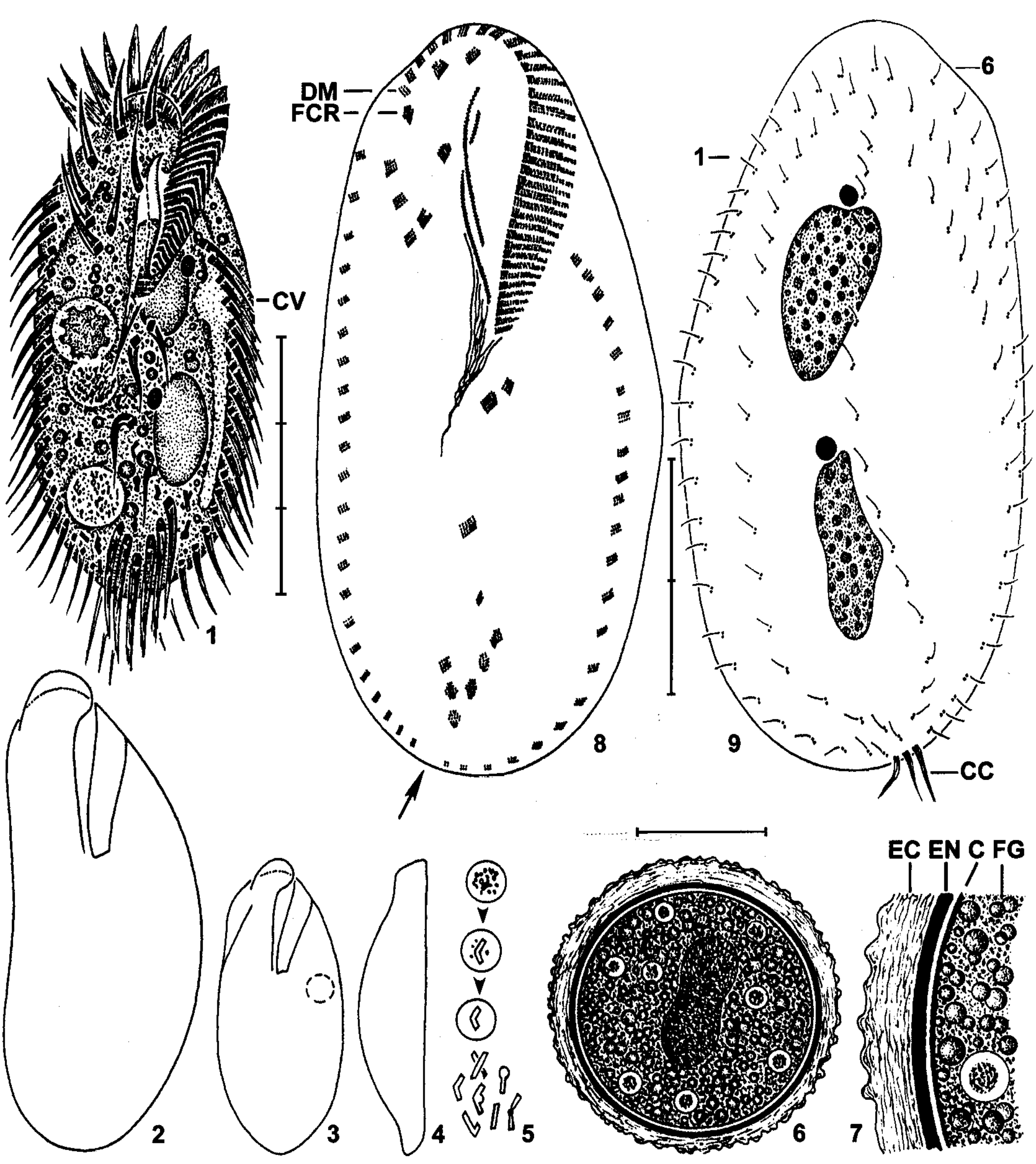
dividing specimens; they were obtained from field material and thus contain many other ciliate species.

RESULTS

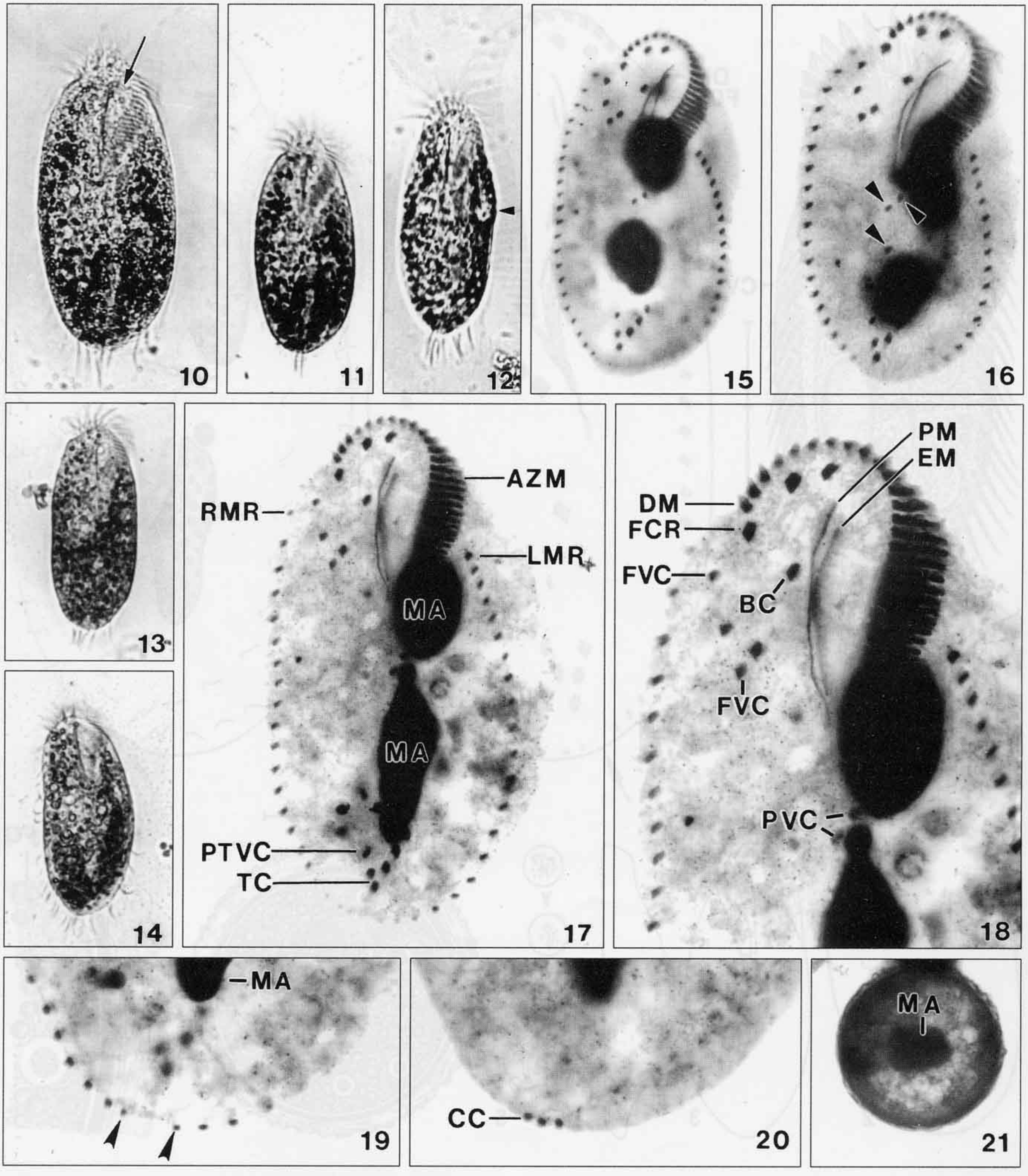
Sterkiella nova sp. n. (Figs. 1-30; Table 1)

Synonymy. *Oxytricha nova*, a *nomen nudum*, first mentioned in Klobutcher *et al.* (1981) and since then in many, mostly gene sequence and phylogenetic studies (see papers marked by asterisk in reference section). Very likely, Fig. 1b in Steinbrück (1986) does not show *O. nova* but a *Stylonychia* sp., as indicated by the large buccal field and the comparatively short, straight paroral membrane.

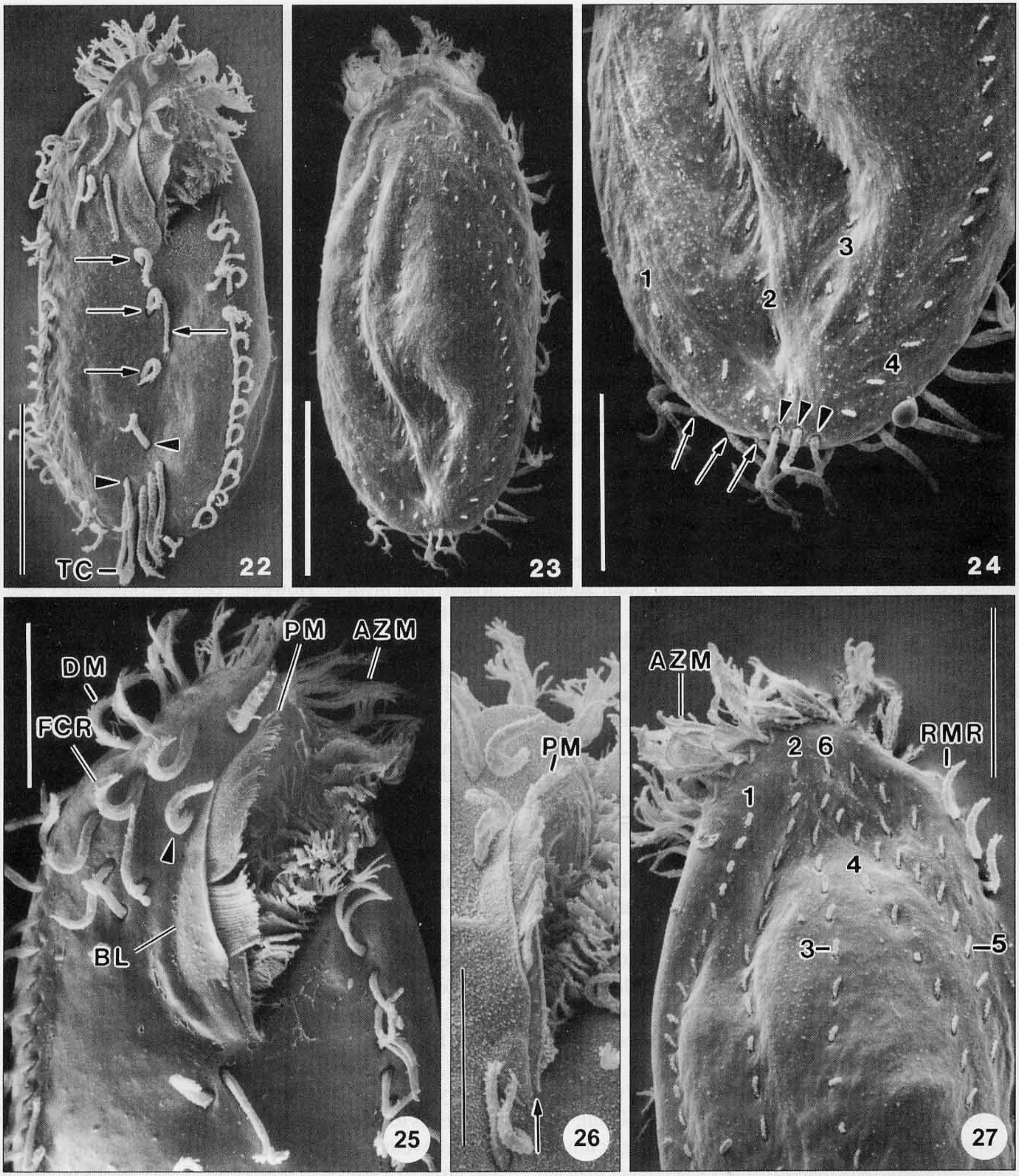
Diagnosis. Size *in vivo* about 120 x 60 µm, ellipsoidal. Two macronuclear nodules. On average 34 adoral membranelles, 21 right and 20 left marginal cirri, and 5 transverse cirri. 6 dorsal kineties with 1 caudal cirrus each associated with kineties 1, 2, 4. Undulating membranes intersecting (*Oxytricha* pattern). Proter and opisthe cirral anlagen separate, proter anlagen 4, 5, 6 originate from cirrus IV/3, opisthe anlagen originate from oral primordium (anlagen 1 - 3), cirrus IV/2 (anlage 4) and cirrus V/4 (anlagen 5, 6). Dorsal kineties generated in *Oxytricha* pattern. Complete nucleotide sequence of macronuclear DNA pol α gene described in Mansour *et al.* (1994) and



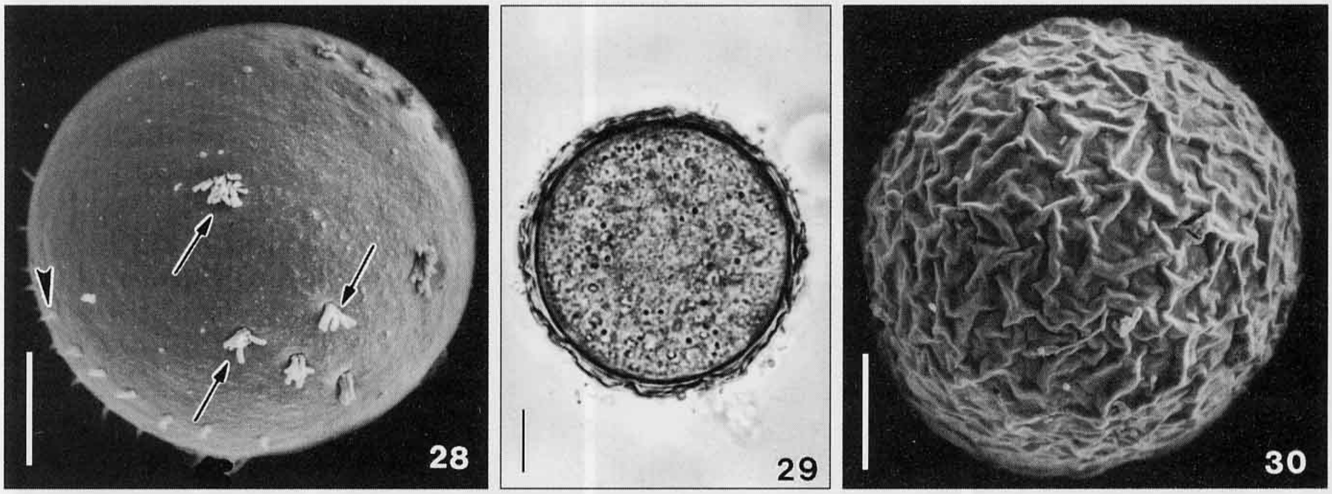
Figs. 1-9. *Sterkiella nova*, morphology of interphase cells and resting cysts from life (1-7) and after protargol impregnation (8, 9). 1 - ventral view of a representative specimen fed with a mixture of bacteria, *Chlorogonium* and wheat starch; 2, 3 - shape and size (135 µm, 110 µm) variants; 4 - narrow side view; *S. nova* is flattened dorsoventrally up to 2:1; 5 - the cytoplasmic crystals develop in small vacuoles; 6, 7 - one month old resting cyst with wrinkled surface and dumb-bell shaped macronucleus; 8, 9 - infraciliature of ventral and dorsal side. The posterior ends of the marginal rows are separated by a small gap (arrow), which is filled, on the dorsal side, by three inconspicuous caudal cirri. For detailed labeling of structures, see Figs. 17, 18, 25. C - cortex of cell, CC - caudal cirri, CV - contractile vacuole with two long collecting canals, DM - distalmost adoral membranelle, EC - ectocyst, EN - endocyst, FCR - right frontal cirrus III/3, FG - fat globules, 1, 6 - dorsal kineties. Scale bar division 20 µm



Figs. 10-21. *Sterkiella nova*, morphology of interphase cells and resting cysts from life (10-14), after protargol impregnation (15-20) and methyl green-pyronin staining (21). 10-14 - ventral views of freely motile specimens showing variability of shape and size (length 110-130 µm). Note narrow buccal field (arrow) and contractile vacuole (arrowhead); 15-18 - infraciliature of ventral side. Arrowheads denote postoral ventral cirri. Squashed, unmounted preparations, length of cells 110-140 µm; 19, 20 - posterior ventral and dorsal side to show location of caudal cirri (CC) on dorsal side between the ends of the ventral marginal rows (arrowheads); 21 - resting cyst showing fused macronuclear nodules. AZM - adoral zone of membranelles, BC - buccal cirrus, CC - caudal cirri, DM - distalmost adoral membranelle, EM - endoral membrane, FCR - right frontal cirrus III/3, FVC - frontoventral cirri, LMR - left row of marginal cirri, MA - macronucleus, PM - paroral membrane, PTVC - pretransverse ventral cirrus, PVC - postoral ventral cirri, RMR - right row of marginal cirri, TC - transverse cirri



Figs. 22-27. *Sterkiella nova*, morphology of interphase cells in the scanning electron microscope. 22, 23 - general ventral and dorsal view. Arrowheads mark pretransverse ventral cirri. The specimen shown in Fig. 22 has four postoral ventral cirri (arrows) instead of the usual three; 24 - posterior dorsal portion showing last cirri (arrows) of left marginal row, which are close to the caudal cirri (arrowheads) at the posterior dorsal margin of the cell (cp. Figs. 19, 20); 25, 26 - anterior ventral portion showing paroral membrane in cleft of buccal lip (arrow). Arrowhead marks buccal cirrus. 27 - anterior dorsal portion showing arrangement of dorsal kineties. AZM - adoral zone of membranelles, BL - buccal lip, DM - distalmost adoral membranelle, FCR - right frontal cirrus III/3, PM - paroral membrane, RMR - right row of marginal cirri, TC - transverse cirri. Numbers 1-6 denote dorsal kineties. Scale bars 40 μm (Figs. 22, 23) and 20 μm (Figs. 24 - 27)



Figs. 28-30. *Sterkiella nova*, resting cysts in the light (29) and scanning electron microscope (28, 30). 28 - encysting specimen with cirri (arrows) and dorsal bristles (arrowhead) still projecting from the forming, smooth cyst wall; 29, 30 - four weeks old resting cysts with wrinkled ectocyst. For labeling of structures, see Fig. 7. Scale bar division 10 µm

deposited in the Gene Bank sequence data base, accession number U 02001. Complete sequence of small subunit rRNA in Elwood *et al.* (1985).

Type location. Freshwater in North Carolina, USA.

Type specimens. One holotype slide and seven paratype slides (all protargol-impregnated) with morphostatic and dividing specimens of *S. nova* have been deposited in the Oberösterreichische Landesmuseum in Linz (LI), Austria, accession numbers 1999/111 - 118. Relevant specimens are marked by a black ink circle on the cover glass.

Etymology. "*nova*" (new) refers to a new isolate of an *Oxytricha* sp. (see Material section).

Interphase morphology (Figs. 1-30, Table 1). Morphometric data shown in Table 1 are repeated in this section only if needed for clarity. All observations are from cultivated material. Description will be very detailed, even containing generic characters, because it should serve not only ciliate taxonomists but also molecular biologists and biochemists usually not familiar with details of ciliate morphology and terminology.

Size in flourishing cultures *in vivo* about 100-140 x 45-65 µm, usually around 120 x 60 µm, very small specimens (< 100 µm) occur in declining cultures. Body ellipsoidal, right margin usually less convex than left, sometimes even concave (Figs. 2, 14), both ends broadly rounded, rarely bluntly pointed posteriorly (Figs. 3, 12, 14); dorsoventrally flattened up to 2:1, depending on nutritional state, ventral side flat, dorsal convex (Fig. 4). Body rather rigid, specimens with sharp-cornered injuries have been observed, very much like those known from *Stylonychia mytilus*. However, cells become rather flexible when overfed and, especially, when slightly squeezed by the cover glass. Hence, body rigidity must be observed in freely motile, untouched specimens and compared with that of common, flexible species, such as *Oxytricha* and *Urostyla* (for details on this character, see Berger and Foissner 1997). Macronuclear nodules in central portion of cell slightly left of midline, ellipsoidal (about 2:1), number slightly variable (Table 1), contain many 1-2.5 µm sized nucleoli. Micronuclei globular, near or attached to macronuclear nodules in variable positions, number highly variable (Table 1). Contractile vacuole slightly above midbody at left margin of cell, with one lacunar collecting canal each extending anteriorly and posteriorly (Fig. 1). No specific cortical granules. Cells colourless, however, well-fed specimens often appear dark in posterior half at low magnification (≤ 100 x) due to food inclusions and many fat globules 1-4 µm (usually 2-3 µm) across (Figs. 10, 11, 14); similarly, small cells from declining cultures usually contain black patches composed of hundreds of colourless to slightly yellowish, variably shaped crystals, which develop in small vacuoles from granular precursors and grow to a size of 2-5 µm (Figs. 5, 12). Feeds on green algae (*Chlorogonium*), bacteria, and wheat starch (Fig. 1). Movement moderately rapid, usually gliding to and fro on slide surface and bottom of culture dish, never rests.

Ventral and dorsal ciliary pattern (infraciliature) very constant, that is, 18 fronto-ventral-transverse cirri on ventral side and 6 kineties (ciliary rows) on dorsal (Figs. 8, 9, 15-18, 22-24, 27; Table 1). Shape and size of cirral bases, as well as number of basal bodies (cilia) in individual cirri, in contrast, highly variable; specimens entirely identical in this respect were not observed, common structure shown in Figure 8.

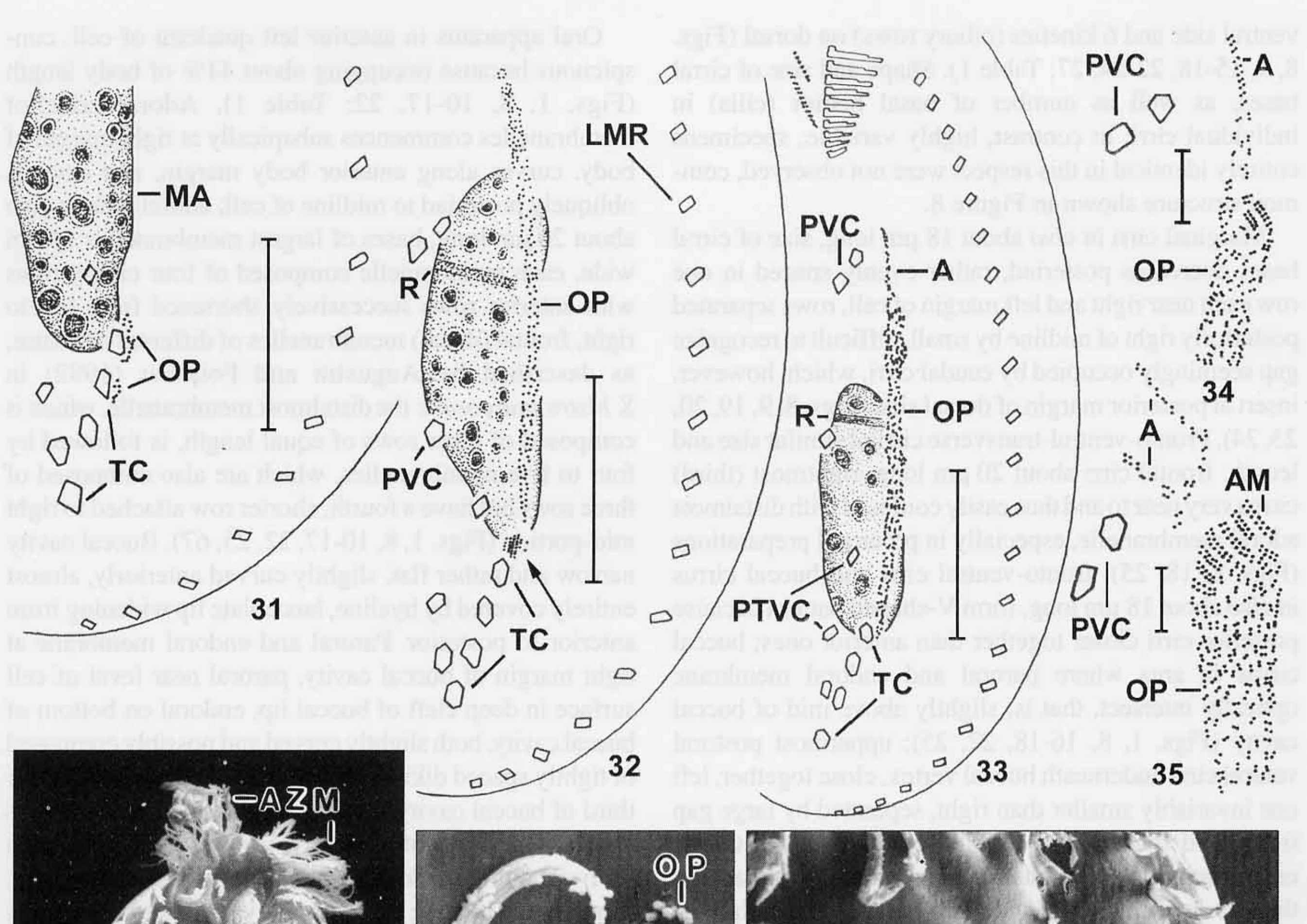
Marginal cirri *in vivo* about 18 µm long, size of cirral bases decreases posteriad, rather evenly spaced in one row each near right and left margin of cell, rows separated posteriorly right of midline by small, difficult to recognize gap seemingly occupied by caudal cirri, which, however, insert at posterior margin of dorsal side (Figs. 8, 9, 19, 20, 23, 24). Fronto-ventral-transverse cirri of similar size and length: frontal cirri about 20 µm long, rightmost (third) cirrus very near to and thus easily confused with distalmost adoral membranelle, especially in protargol preparations (Figs. 8, 18, 25); fronto-ventral cirri and buccal cirrus *in vivo* about 18 µm long, form V-shaped pattern because posterior cirri closer together than anterior ones; buccal cirrus in area where paroral and endoral membrane optically intersect, that is, slightly above mid of buccal cavity (Figs. 1, 8, 16-18, 22, 25); uppermost postoral ventral cirri underneath buccal vertex, close together, left one invariably smaller than right, separated by large gap from third (posterior) postoral ventral cirrus distinctly underneath mid-body; anterior pretransverse cirrus smaller than posterior one, which is very near to the rightmost transverse cirrus (Figs. 1, 8, 15-18, 22, 25); transverse cirri near posterior body end, *in vivo* 25-30 µm long and thus distinctly projecting beyond posterior body margin, distally frayed, form hook-like pattern (Figs. 1, 8, 10-17, 22).

Dorsal cilia *in vivo* 3-4 µm long, originate from anterior basal body of dikinetids comprising dorsal bristle rows (Figs. 9, 23, 24, 27). Rows 1-3 in left half of dorsal side, almost as long as body, follow curvature of body margin, except row 3, which curves right in posterior half producing rather large, barren area between kineties 2 and 3; row 4 commences subapically near midline of cell, curves to right margin in mid-body, and continues posteriad to right caudal cirrus; row 5 slightly shortened anteriorly, ends somewhat above or below mid-body; row 6 very short, on average comprising 6 dikinetids only (Table 1), terminates in anterior third of cell. Caudal cirri at posterior body margin right of midline, narrowly spaced, associated with dorsal kineties 1, 2 and 4 (see ontogenesis), inconspicuous because slender and only slightly longer (22 µm) than marginal cirri (Figs. 9, 19, 20, 23, 24, 60, 64).

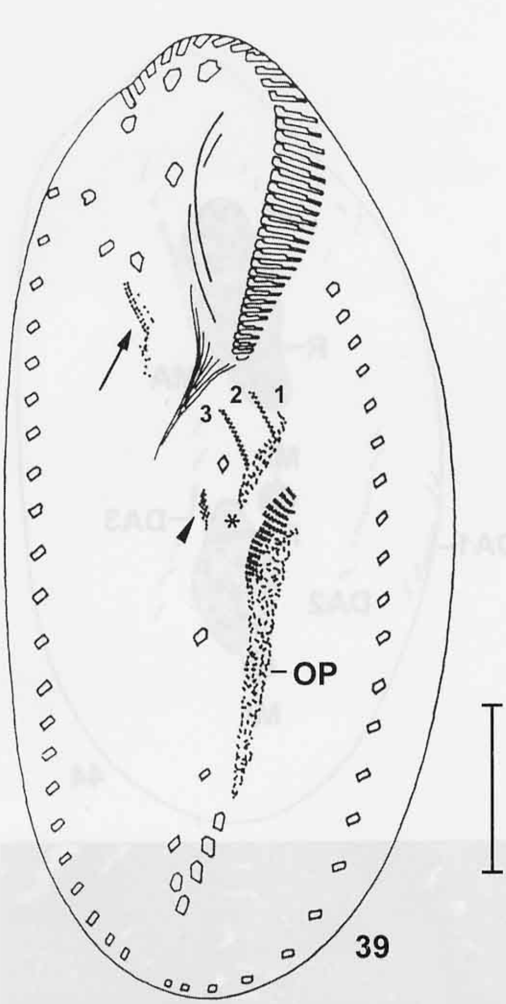
Oral apparatus in anterior left quadrant of cell, conspicuous because occupying about 41% of body length (Figs. 1, 8, 10-17, 22; Table 1). Adoral zone of membranelles commences subapically at right margin of body, curves along anterior body margin, and extends obliquely posteriad to midline of cell; adoral cilia *in vivo* about 20 µm long, bases of largest membranelles 11 µm wide, each membranelle composed of four ciliary rows with anterior rows successively shortened from left to right, frontal (distal) membranelles of different structure, as described by Augustin and Foissner (1992) in *S. histriomuscorum*: the distalmost membranelle, which is composed of three rows of equal length, is followed by four to five membranelles, which are also composed of three rows but have a fourth, shorter row attached to right mid-portion (Figs. 1, 8, 10-17, 22, 25, 67). Buccal cavity narrow and rather flat, slightly curved anteriorly, almost entirely covered by hyaline, lanceolate lip widening from anterior to posterior. Paroral and endoral membrane at right margin of buccal cavity, paroral near level of cell surface in deep cleft of buccal lip, endoral on bottom of buccal cavity, both slightly curved and possibly composed of tightly spaced dikinetids, intersect optically in anterior third of buccal cavity (Figs. 1, 10-14, 18, 22, 25, 26), as also evident from ontogenesis (Figs. 67, 69). Paroral cilia *in vivo* about 10 µm long, endoral cilia at least 15 µm long, form bundle beating into cytopharynx. Pharyngeal fibres inconspicuous, originate from posterior portion of endoral membrane and adoral zone of membranelles (Figs. 1, 8).

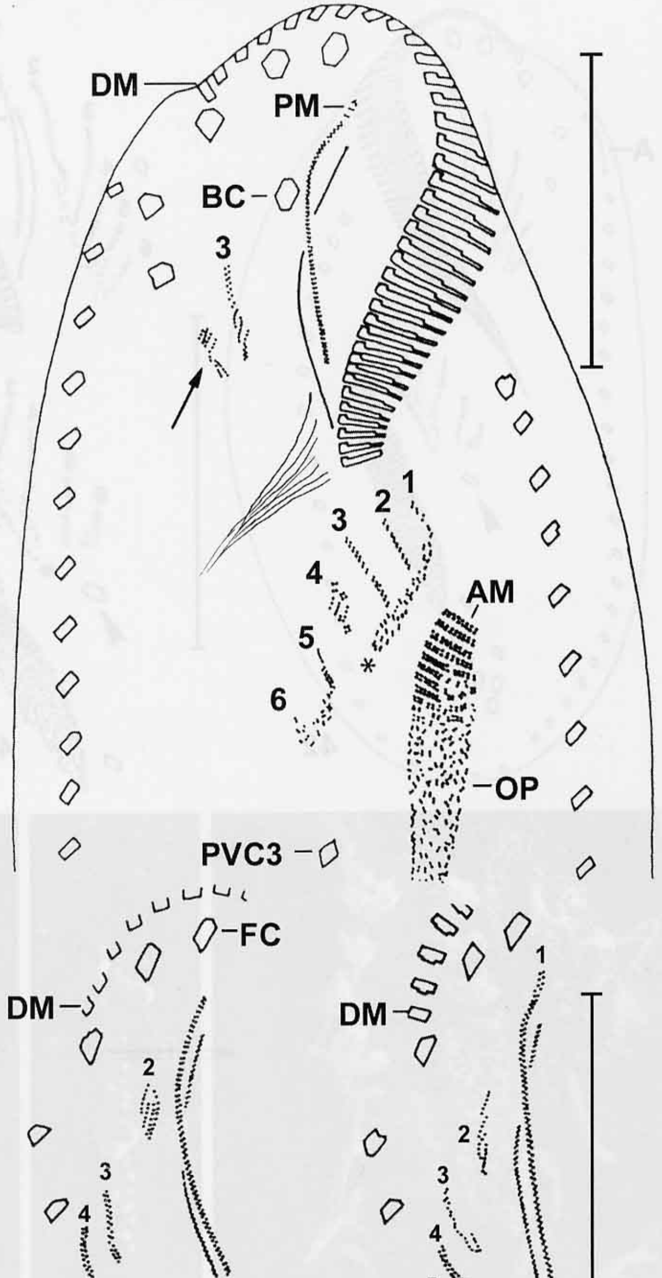
Resting cysts (Figs. 6, 7, 21, 28-30; Table 1). Permanent resting cysts spherical to slightly ellipsoidal, colourless, old cysts slightly smaller than young ones (Table 1). Ectocyst 1.5-3.5 µm, usually 2-3 µm thick, appears to be composed of many tightly spaced membranes, colourless and hyaline, surface smooth in very young cysts (Fig. 28), distinctly wrinkled when finished (Figs. 29, 30), stains lilac with methyl green-pyronin. Endocyst about 1 µm thick and with brownish shimmer, compact, separated from cortex of cell by narrow, hyaline zone. Cyst content comprises countless fat globules 1-2 µm across and some 3-4 µm sized vacuoles with granular, yellowish content, possibly food remnants. Macronuclear nodules fused to reniform or dumb-bell shaped mass (Figs. 6, 21).

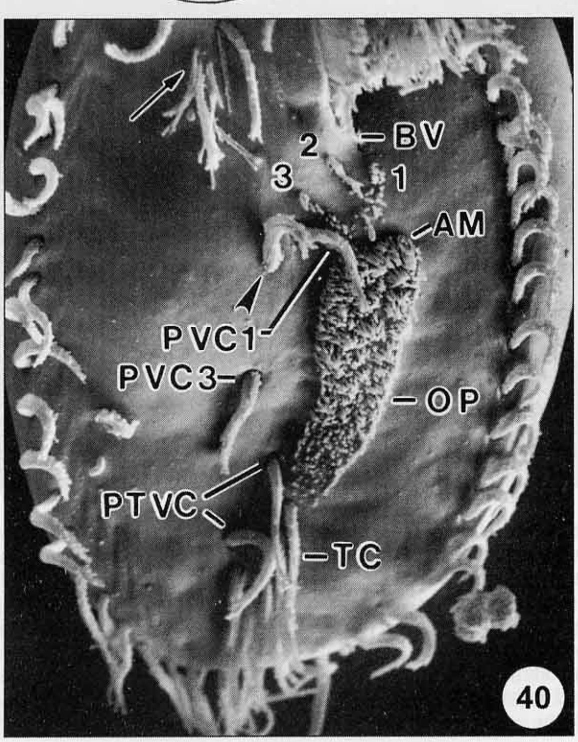
Divisional morphogenesis (Figs. 31-70). To make plain the changes during morphogenesis, old (parental) structures are depicted by contour, whereas newly formed structures are shaded black. For details, see also figure explanations.



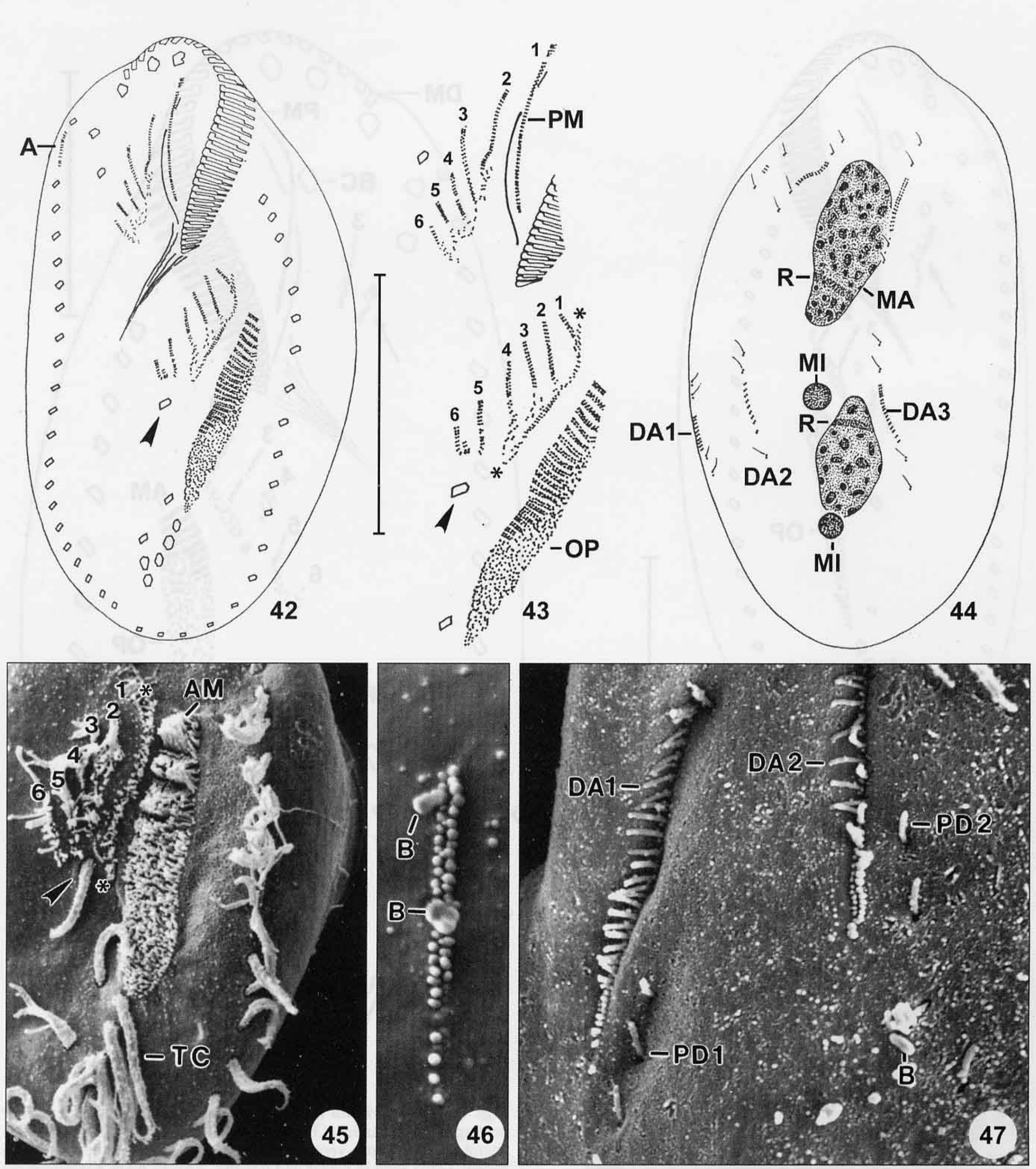
Figs. 31-38. *Sterkiella nova*, very early dividers after protargol impregnation (31-35) and in the SEM (36-38). 31, 36, 37 - basal bodies develop near the uppermost transverse cirri; 32 - an anarchic field of basal bodies develops between buccal vertex and transverse cirri. A supernumerary transverse cirrus (arrow) is incorporated into the oral primordium; 33 - 35, 38 - two cirral anlagen originate from the oral primordium. A - cirral anlagen, AM - adoral membranelles, AZM - adoral zone of membranelles, LMR - left marginal row, MA - posterior macronuclear bead, OP - oral primordium, PTVC - pretransverse ventral cirri, PVC - postoral ventral cirri, R - reorganization band, TC - transverse cirri. Scale bars 20 µm (Figs. 31-33) and 5 µm (Figs. 34, 35)



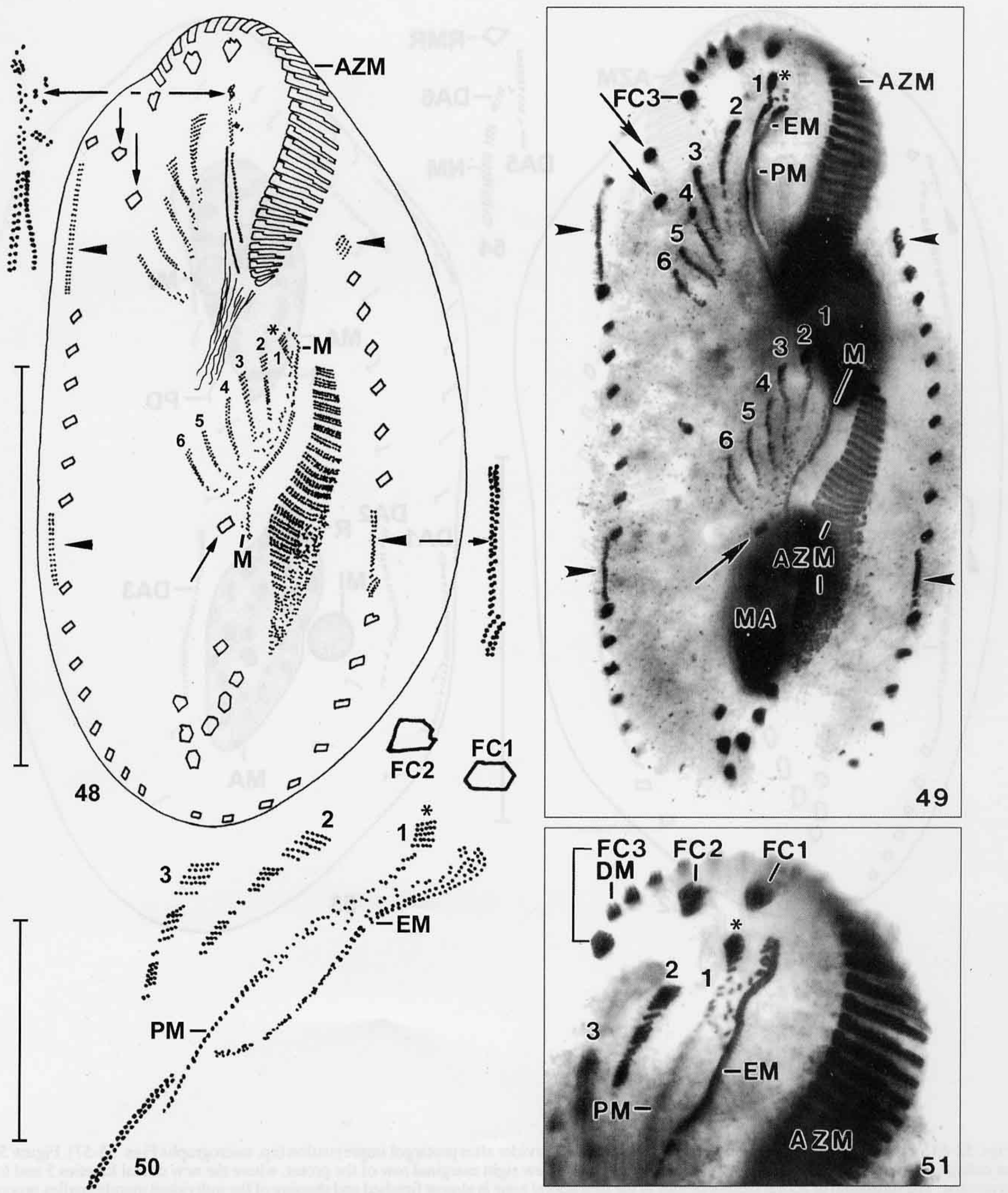




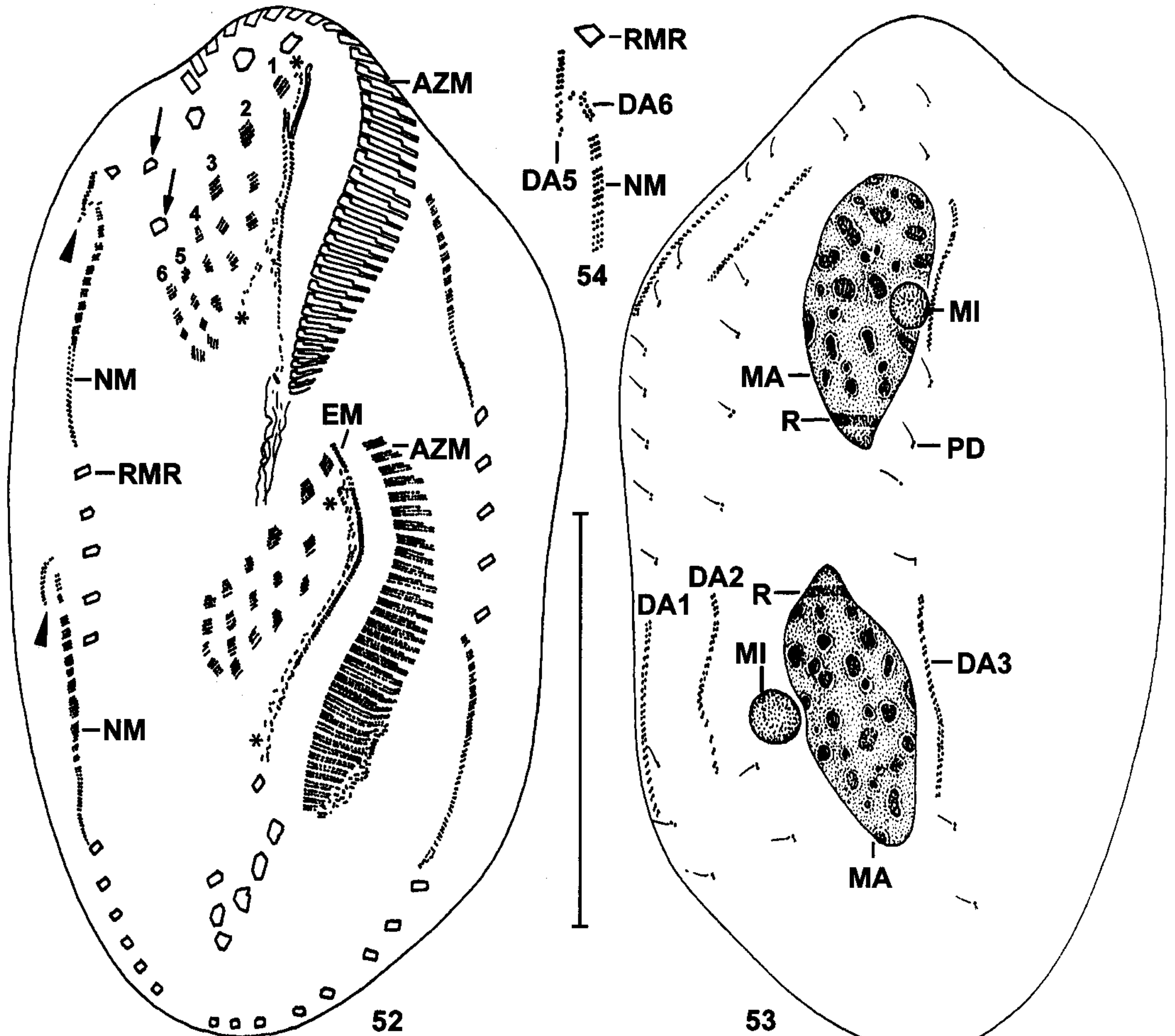
Figs. 39-41b. *Sterkiella nova*, ventral views of early dividers after protargol impregnation (39, 41, 41a, b) and in the SEM (40). Six (numbers 1-6) fronto-ventral-transverse cirral anlagen develop from parental cirri each in proter and opisthe. Arrows mark posteriormost frontoventral cirrus IV/3, which gives rise to proter cirral streaks 4, 5, 6 (Figs. 41a, b). Arrowheads denote postoral ventral cirrus V/4, from which opisthe's cirral streaks 5 and 6 originate. The parental paroral commences to reorganize at the anterior end (Figs. 41, 41b). In the opisthe, the new paroral and endoral are forming at the rear end of the cirral streaks (asterisks). AM - adoral membranelles, BC - buccal cirrus, BV - buccal vertex, DM - distalmost adoral membranelle, FC - frontal cirri, OP - oral primordium, PM - paroral membrane, PTVC - pretransverse ventral cirri, PVC 1, 3 - postoral ventral cirri, TC - transverse cirri. Scale bars 25 μm



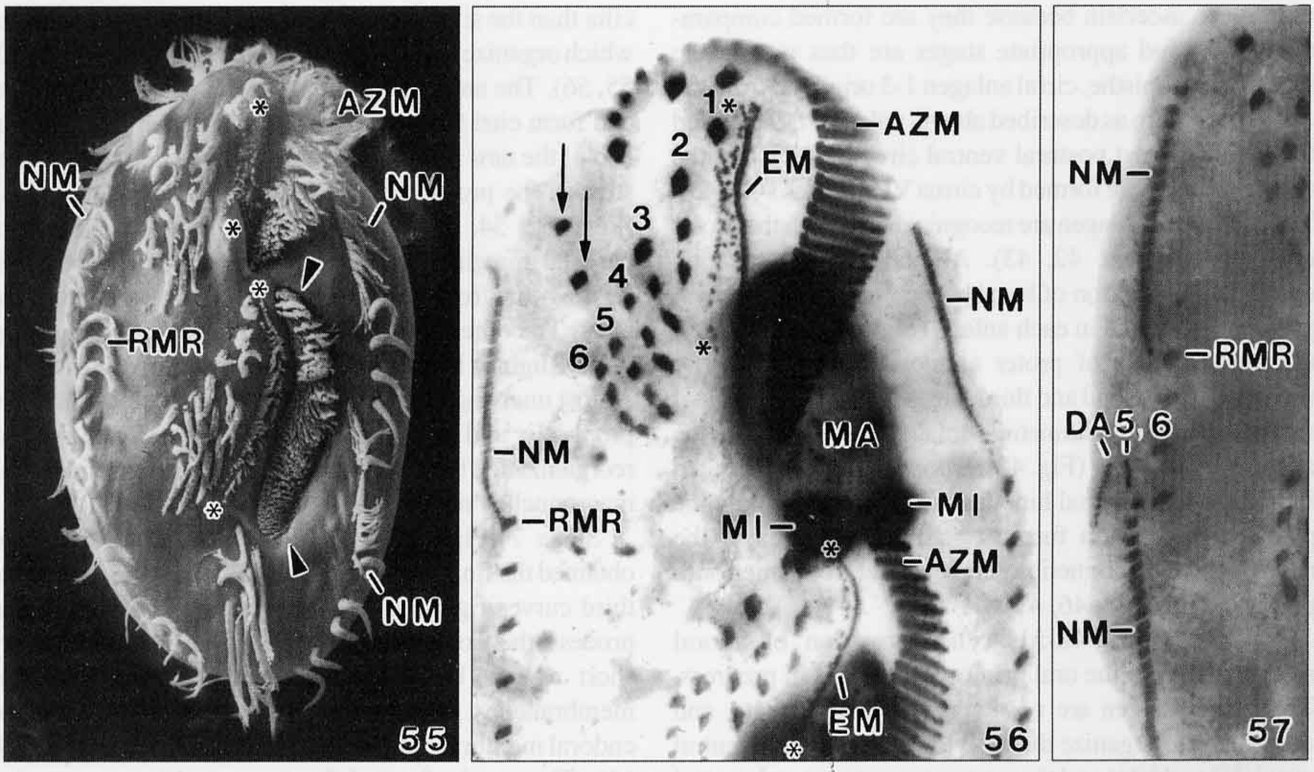
Figs. 42-47. *Sterkiella nova*, ventral (42, 43, 45) and dorsal (44, 46, 47) views of early-middle dividers after protargol impregnation (42 - 44) and in the scanning electron microscope (45-47). 42, 43, 45 - six fronto-ventral-transverse cirral anlagen each (numbers 1 - 6) are recognizable in the proter and opisthe, the second and third cirrus of the right marginal row form an anlage for a new marginal row, and a third row of basal bodies is added to the opisthe's anteriormost adoral membranelles, which commence to invaginate. Asterisks mark anlage for the opisthe's undulating membranes. Arrowheads mark third postoral ventral cirrus (V/3), which does not participate in anlagen formation; 44, 46, 47 - new dorsal kineties originate by anlagen formation within three parental rows. The parental bristles, which do not participate in anlagen formation (Fig. 47, not shown in Fig. 44), will be resorbed later. A - anlage, AM - adoral membranelles, B - bacterial rods from culture medium, DA 1 - 3 - anlagen for dorsal kineties, MA - macronuclear nodule, MI - micronuclei, OP - oral primordium, PD 1, 2 - parental dorsal bristle rows, PM - paroral membrane, R - reorganization band, TC - parental transverse cirri. Scale bar 50 µm (for Figs. 42, 44, which show the same specimen; Fig. 43 is an enlarged detail from Fig. 42)



Figs. 48-51. *Sterkiella nova*, ventral views of middle dividers after protargol impregnation. Figures 50 and 51 detail the reorganization of the proter's paroral and endoral membrane in a slightly later stage than shown in Figures 48 and 49. Six distinct fronto-ventral-transverse cirral anlagen each are now recognizable in the proter and opisthe. Arrows mark two frontoventral cirri (VI/3, VI/4) and a postoral ventral cirrus (V/3), which are morphogenetically inactive. The opisthe's undulating membranes are forming, while those of the proter are reorganizing. During these processes, the anterior end of the paroral splits Y-like; the right fork produces the new frontal cirrus I/1 (asterisks). Arrowheads denote anlagen within the marginal cirral rows; each of the anlagen will produce a new marginal cirral row. AZM - adoral zone of membranelles, DM - distalmost adoral membranelle, EM - proter endoral membrane, FC 1-3 - frontal cirri, M - opisthe undulating membranes, MA - macronuclear nodule, PM - proter paroral membrane, numbers 1-6 - anlagen for the fronto-ventral-transverse cirri. Scale bars 50 µm (Fig. 48) and 15 µm (Fig. 50)



Figs. 52-54. *Sterkiella nova*, ventral and dorsal view of a middle divider after protargol impregnation (cp. micrographs Figs. 55-57). Figure 54 is an enlarged part of Figure 52 and shows the anterior end of the new right marginal row of the proter, where the new dorsal kineties 5 and 6 are generated. The formation of adoral membranelles in the new adoral zone is almost finished and shaping of the individual membranelles proceeds from anterior to posterior. The endoral membrane forms slightly earlier than the paroral (asterisks), which shows a characteristic, oblique tail in the reorganizing proter. Cirri are forming in the fronto-ventral-transverse anlagen. Arrowheads denote anlagen for dorsal kineties 5 and 6 (for details, see Figs. 54, 57). Arrows mark morphogenetically inactive frontoventral cirri; note that cirrus V/3 has been resorbed (cp. Figs. 48, 49). The nuclear apparatus is still almost unchanged, except of the micronuclei, which are prophasic, and the reorganization band, which moved to the proximal end of the macronuclear nodules. AZM - adoral zone of membranelles, DA 1-6 - anlagen for dorsal kineties, EM - endoral membrane, MA - macronuclear nodules, MI - micronuclei, NM - anlagen for the new marginal rows, PD - parental dorsal kineties (only partially shown), R - reorganization band, RMR - parental right marginal row, numbers 1-6 - fronto-ventral-transverse cirral anlagen. Scale bar 50 µm



Figs. 55-57. *Sterkiella nova*, ventral views of middle dividers in the scanning electron microscope (55) and after protargol impregnation (56, 57). These micrographs supplement the camera lucida drawings shown in Figures 52-54. The new adoral zone of membranelles (arrowheads) is slightly but distinctly invaginated, while the reorganizing parental oral area has flattened. The forming (opisthe), respectively, reorganizing (proter) undulating membranes have shorter cilia than the six (numbers 1-6) fronto-ventral-transverse cirral anlagen, where cirri are forming. The endoral membrane forms slightly earlier than the paroral (asterisks), which has a characteristic, oblique tail in the reorganizing proter; the tail is unciliated and thus not recognizable in the scanning electron microscope (Fig. 55). Arrows mark morphogenetically inactive frontoventral cirri VI/3 and VI/4. Note large, prophasic micronuclei (Fig. 56) and anlagen for dorsal kineties 5 and 6, which are generated at the anterior end of the new right marginal rows (Fig. 57). AZM - adoral zone of membranelles, DA 5, 6 - anlagen for dorsal kineties 5 and 6, EM - endoral membrane, MA - macronuclear nodule, MI - micronuclei, NM - anlagen for the new marginal cirral rows, RMR - parental right marginal row, numbers 1-6 - fronto-ventral-transverse cirral anlagen

Stage 1 (Figs. 31, 36, 37). A few basal bodies develop left of the anteriormost transverse cirri, which appear intact both in the light and scanning electron microscope. Ciliary stubs are recognizable on these basal bodies, which belong to the oral primordium.

Stage 2 (Fig. 32). The basal bodies increase in number and form a long, narrow anarchic field (oral primordium), which extends between the buccal vertex and the transverse cirri. The macronuclear nodules show a reorganization band.

Stage 3 (Figs. 33-35, 38-40). A streak of basal bodies with ciliary stubs grows out from the right anterior end of the oral primordium, where dikinetidal adoral membranelles are formed from anterior to posterior (Figs. 33, 34, 38, 40). The streak then separates from the oral primordium, increases the number of basal bodies, and organizes to three oblique, dikinetidal cirral anlagen, which are connected posteriorly by scattered basal bodies, the prospective undulating membranes of the opisthe (Figs. 35, 39). While these cirral streaks are forming, the posteriormost frontal cirrus (IV/3) and postoral ventral cirrus V/4 disaggregate to cirral anlagen (Figs. 39, 40).

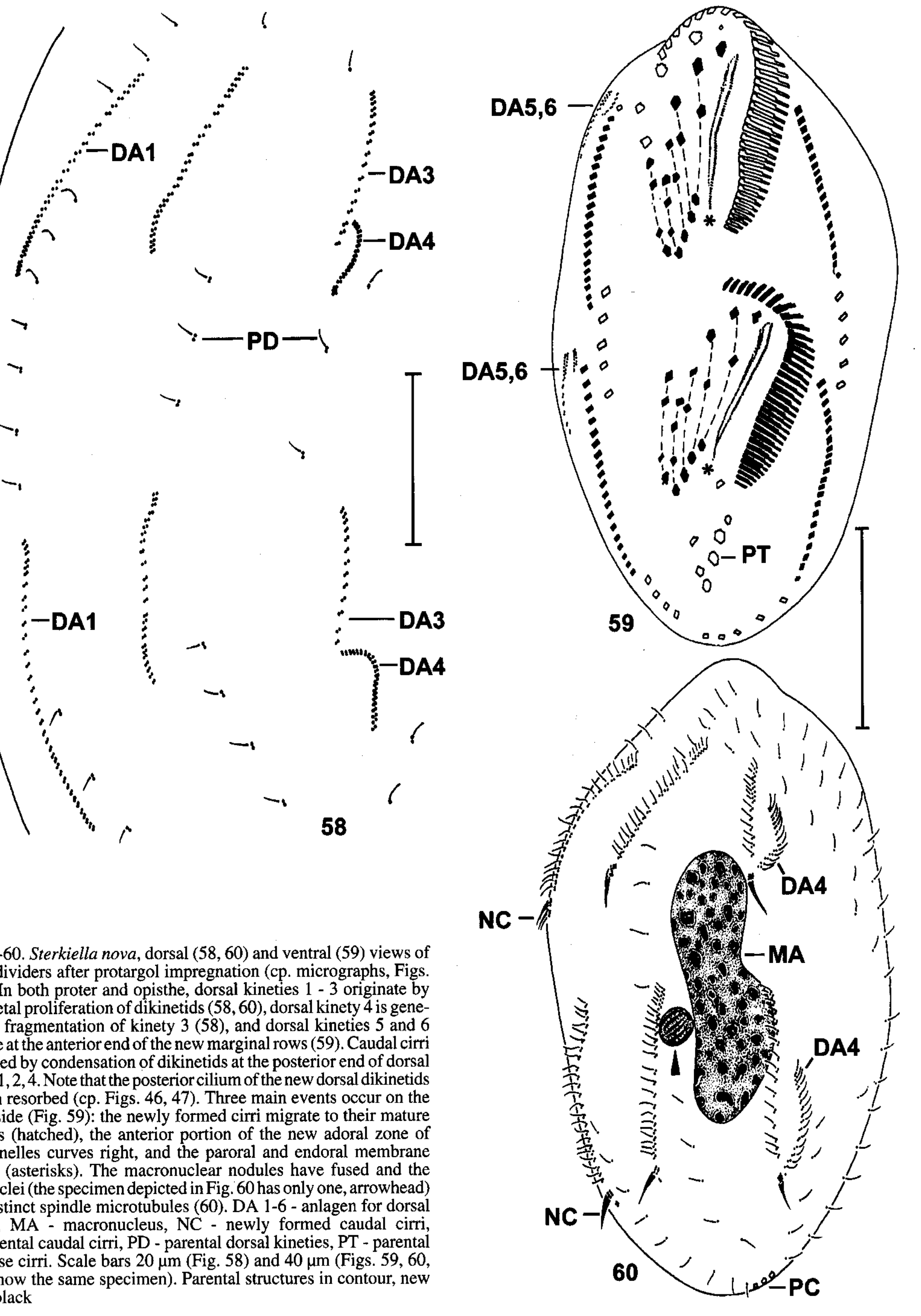
Stage 4 (Figs. 41-47). The oral primordium proceeds to differentiate adoral membranelles and a third row of basal bodies is added to the anteriormost membranelles, which slightly invaginate (Figs. 42, 43, 45). In the proter, the anterior portion of the paroral membrane and the buccal cirrus (II/2) generate cirral anlagen 1 and 2; cirral anlage 3 is formed by the penultimate frontoventral cirrus (III/2), and the anlagen 4, 5 and 6 are generated by the posteriormost frontal cirrus (IV/3), which disintegrates to a rather long, oblique streak (Figs. 39, 41a) assuming the shape of an extended letter W, when the anlagen grow out (Figs. 41b, 42, 43). The origin of proter anlagen 4-6 is difficult to ascertain because they are formed comparatively fast, and appropriate stages are thus rare in the slides. In the opisthe, cirral anlagen 1-3 originate from the oral primordium as described above, anlage 4 is generated by the uppermost postoral ventral cirrus (IV/2), and the anlagen 5 and 6 are formed by cirrus V/4 (Figs. 41-43, 45). Thus, six cirral anlagen are recognizable in each the proter and opisthe (Figs. 42, 43). All anlagen lengthen by continued production of basal bodies, and ciliary growth proceeds posteriad in each anlage (Fig. 45). In all stages, the cirral streaks of proter and opisthe are distinctly separate. The second and third cirrus of the right marginal row reorganize to a dikinetidal anlage, which will become a new marginal row (Fig. 42). Dikinetids are proliferated intrakinetally in dorsal kineties 1-3 above and below the prospective division furrow; both basal bodies of the newly produced dikinetids generate cilia from anterior to posterior (Figs. 44, 46, 47).

Stage 5 (Figs. 48-51). The formation of adoral membranelles in the oral primordium is still in progress. The cirral anlagen are now very distinct, cuneate, and commence to organize the individual cirri. Frontoventral cirri VI/3 and VI/4 and the posteriormost postoral ventral cirrus (V/3) are ontogenetically inactive and will be resorbed (Figs. 48, 49). The scattered dikinetids at the posterior end of the opisthe cirral anlagen arrange to a long streak, the prospective undulating membranes, right of the forming adoral zone. The parental undulating membranes reorganize completely from anterior to posterior, and the anterior portion of the paroral primordium generates the left frontal cirrus (I/1) in both proter and opisthe (Figs. 48, 50, 51). Four anlagen are now recognizable in the marginal rows (Figs. 48, 49): the proter anlagen develop at the anterior end of the rows, while the opisthe anlagen originate slightly underneath the prospective division furrow. These anlagen, which develop from parental marginal cirri and from anterior to posterior, will generate the new marginal rows.

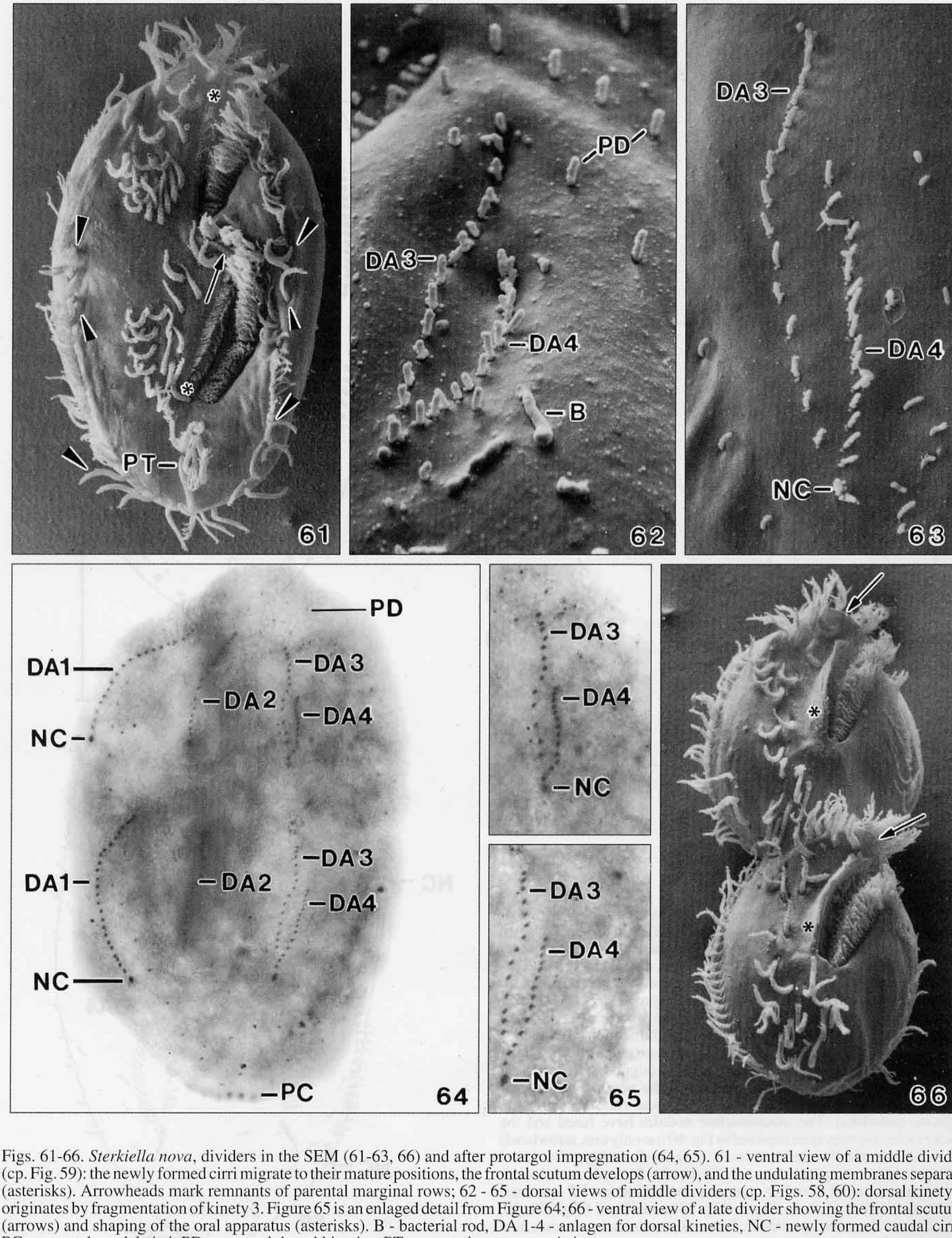
Stage 6 (Figs. 52-57). The new adoral zone of membranelles commences to invaginate (Fig. 55). The formation of adoral membranelles is complete, except for the posterior 5-10 membranelles. Shaping of the individual membranelles proceeds from anterior to posterior, that is, a third, slightly shorter row of cilia is added to each membranelle. The endoral membrane forms slightly earlier than the paroral, which shows a characteristic, oblique tail in the reorganizing proter, whose oral area has flattened (Figs. 52, 55, 56). The forming opisthe, respectively, reorganizing proter undulating membranes have shorter cilia than the six fronto-ventral-transverse cirral anlagen, which organize to cirri from anterior to posterior (Figs. 52, 55, 56). The anlagen for the marginal rows are complete and form cirri from anterior to posterior. At the anterior end of the new right marginal rows two short, dikinetidal streaks, the prospective dorsal kineties 5 and 6 develop (Figs. 52, 54, 57). These streaks do not evolve from parental marginal cirri, which are far away, but either *de novo*, or from the anterior end of the new marginal rows. The other dorsal anlagen are as described in stage 4, but slightly lengthened. The nuclear apparatus is still almost unchanged, except for the micronuclei, which are prophasic and thus rather large (Figs. 53, 56). The reorganization band has moved to the proximal ends of the macronuclear nodules.

Stage 7 (Figs. 58 - 65). The new adoral zone has obtained the final number of membranelles and its anterior third curves right and behind a minute, upright cortical process, the frontal scutum (Figs. 59, 61); a fourth, very short row of basal bodies is added to the mid-zone membranelles. In both proter and opisthe, the paroral and endoral membrane separate and lie side by side (Figs. 59, 61). The newly formed fronto-ventral-transverse cirri have been completed, that is, possess cilia as long as in interphase specimens, and are migrating to their mature positions (Figs. 59, 61). Likewise, the dorsal ciliature is completed by fragmentation of kinety 3, that is, the posterior third proliferates additional dikinetids, separates, and migrates to the left and then anteriad, forming dorsal kinety 4. Thus, the dorsal kineties of *S. nova* originate as follows (Figs. 58-60, 62-65): kineties 1-3 are generated by intrakinetal proliferation of dikinetids, kinety 4 originates by fragmentation of kinety 3, and kineties 5 and 6 are generated at the anterior end of the new marginal row. Caudal cirri are formed by condensation of dikinetids at the posterior end of kineties 1, 2, 4 (Figs. 60, 63-65). The cilium of the posterior basal body of the newly formed dorsal dikinetids has been reduced. The macronuclear nodules have fused and the micronuclei show distinct spindle microtubules (Fig. 60).

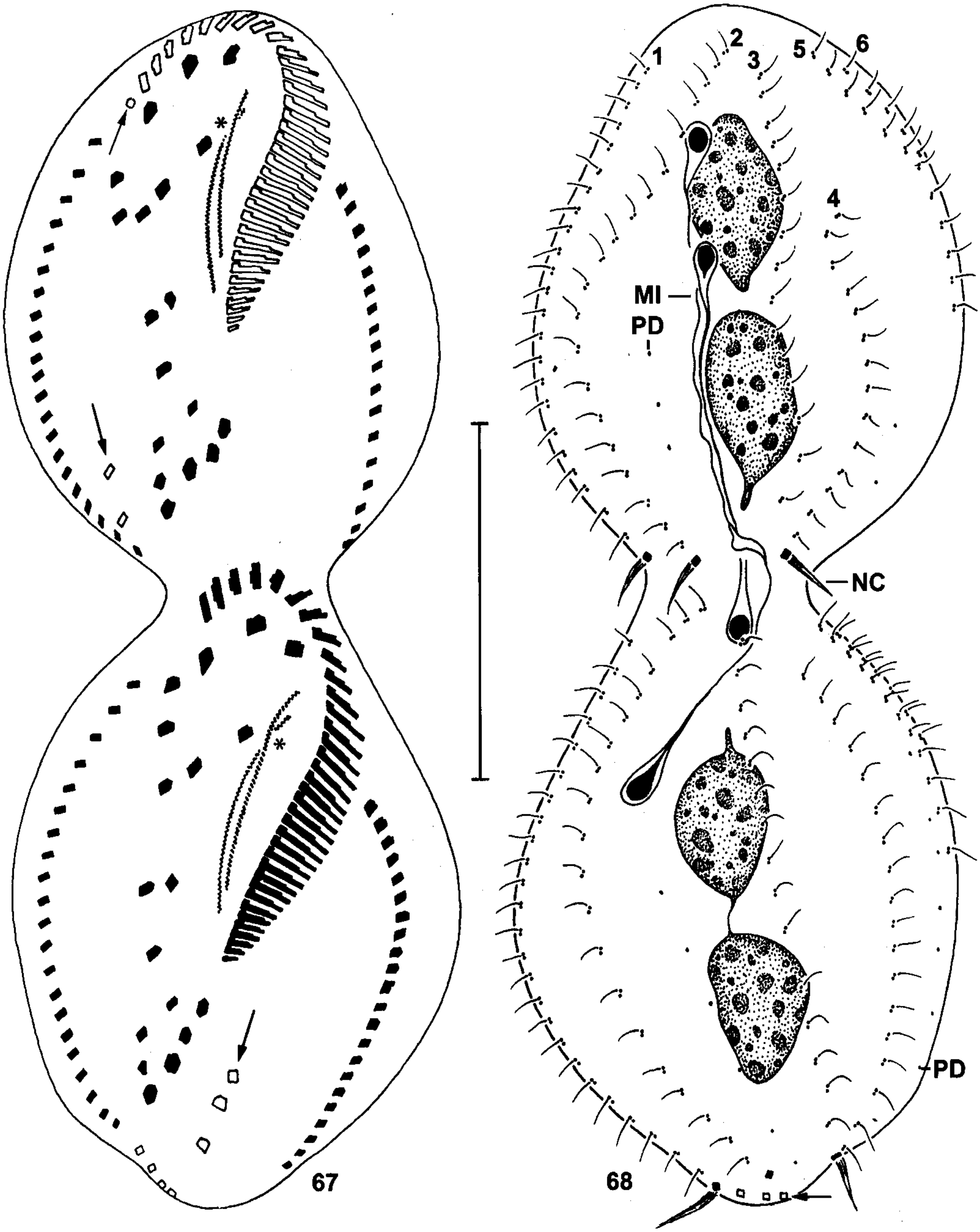
Stage 8 (Figs. 66-70). When cytokinesis commences, shaping of the new adoral membranelles and of the buccal cavity is completed in both proter and opisthe. The shaping of the buccal cavity causes the paroral and endoral membrane to become superimposed and to slightly but distinctly intersect optically in the anterior third when the cell is viewed ventrally (Figs. 66, 67, 69). The fronto-ventral-transverse cirri migrate apart (Fig. 67), obtaining the species-specific pattern and shape only after separa-



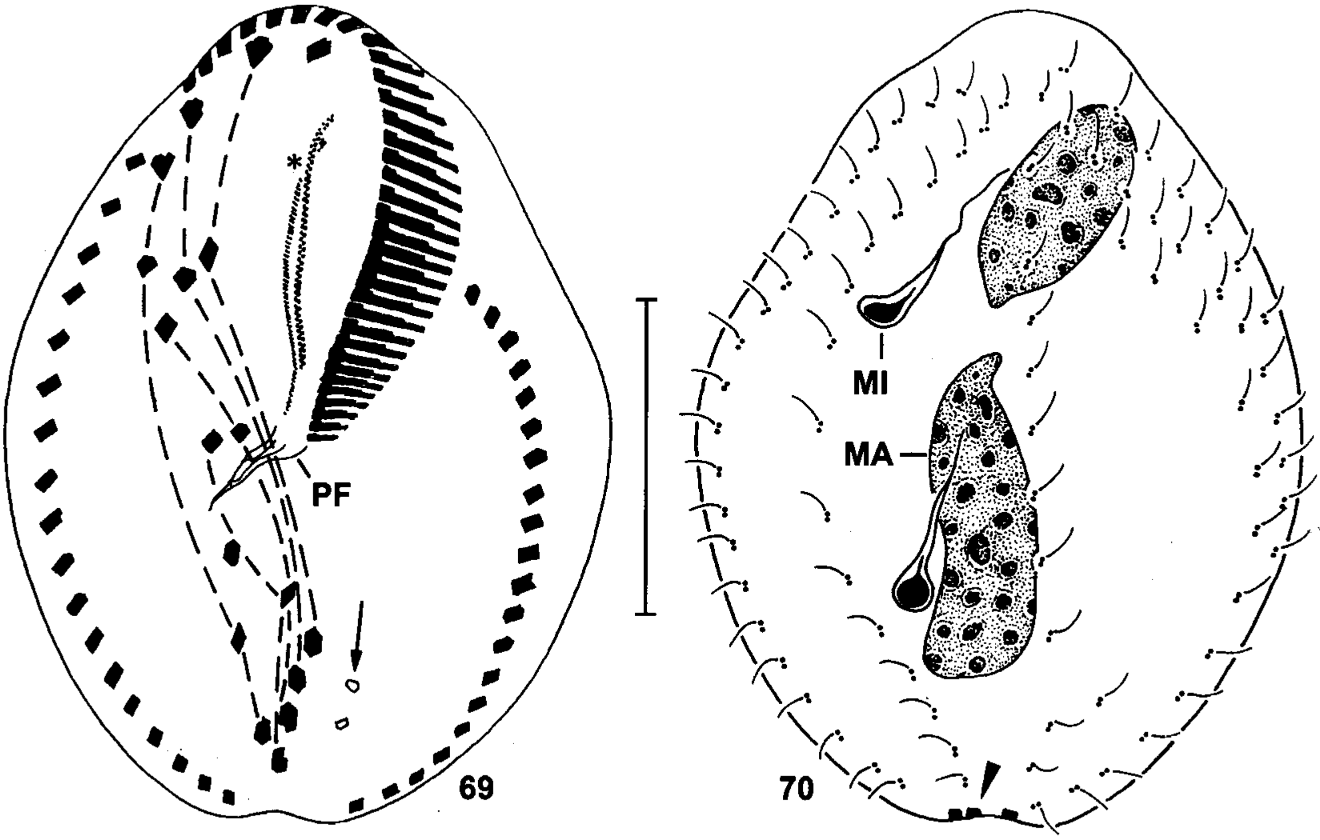
Figs. 58-60. *Sterkiella nova*, dorsal (58, 60) and ventral (59) views of middle dividers after protargol impregnation (cp. micrographs, Figs. 61-65). In both proter and opisthe, dorsal kineties 1 - 3 originate by intrakinetal proliferation of dikinetids (58, 60), dorsal kinety 4 is generated by fragmentation of kinety 3 (58), and dorsal kineties 5 and 6 originate at the anterior end of the new marginal rows (59). Caudal cirri are formed by condensation of dikinetids at the posterior end of dorsal kineties 1, 2, 4. Note that the posterior cilium of the new dorsal dikinetids has been resorbed (cp. Figs. 46, 47). Three main events occur on the ventral side (Fig. 59): the newly formed cirri migrate to their mature positions (hatched), the anterior portion of the new adoral zone of membranelles curves right, and the paroral and endoral membrane separate (asterisks). The macronuclear nodules have fused and the micronuclei (the specimen depicted in Fig. 60 has only one, arrowhead) show distinct spindle microtubules (60). DA 1-6 - anlagen for dorsal kineties, MA - macronucleus, NC - newly formed caudal cirri, PC - parental caudal cirri, PD - parental dorsal kineties, PT - parental transverse cirri. Scale bars 20 µm (Fig. 58) and 40 µm (Figs. 59, 60, which show the same specimen). Parental structures in contour, new shaded black



Figs. 61-66. *Sterkiella nova*, dividers in the SEM (61-63, 66) and after protargol impregnation (64, 65). 61 - ventral view of a middle divider (cp. Fig. 59): the newly formed cirri migrate to their mature positions, the frontal scutum develops (arrow), and the undulating membranes separate (asterisks). Arrowheads mark remnants of parental marginal rows; 62 - 65 - dorsal views of middle dividers (cp. Figs. 58, 60): dorsal kinety 4 originates by fragmentation of kinety 3. Figure 65 is an enlaged detail from Figure 64; 66 - ventral view of a late divider showing the frontal scutum (arrows) and shaping of the oral apparatus (asterisks). B - bacterial rod, DA 1-4 - anlagen for dorsal kineties, NC - newly formed caudal cirri, PC - parental caudal cirri, PD - parental dorsal kineties, PT - parental transverse cirri



Figs. 67, 68. *Sterkiella nova*, ventral and dorsal view of a late divider after protargol impregnation (cp. Figure 66). The shaping of the buccal cavity (Fig. 66) causes the paroral and endoral membrane to intersect optically (asterisks). Some parental cirri (arrows) which did not participate in anlagen formation are still present. The fused macronuclear nodules (Fig. 60) and the micronuclei divided. MI - micronuclei, NC - newly formed caudal cirri, PD - parental dorsal bristles, 1-6 - newly formed dorsal kineties. Scale bar 50 μm. Parental structures in contour, new shaded black



Figs. 69, 70. *Sterkiella nova*, ventral and dorsal view of an early opisthe postdivider after protargol impregnation. Early postdividers are distinctly smaller and broader than mature interphase specimens. Fronto-ventral-transverse cirri, which originated from the same anlage, are connected by hatched lines (cp. Fig. 59); most of them have not yet obtained the final location and shape as shown by the irregular outline. Arrow marks some not yet resorbed parental transverse cirri; arrowhead denotes newly formed caudal cirri. Note that the anterior portions of the paroral and endoral membrane intersect optically (asterisk), which is an important difference to *Stylonychia*. MA - macronuclear nodule, MI - micronucleus with adhering spindle microtubules, PF - growing pharyngeal fibres. Scale bar 30 µm

tion of the daughters (Fig. 69); the dorsal infraciliature, however, is complete already in late dividers (Fig. 68). The six fronto-ventral-transverse anlagen in the proter and opisthe each produce 18 cirri, the typical number for oxytrichids s. str. (Berger 1999): 1(1), 2(3), 3(3), 4(3), 5(4), 6(4). The parental cirri and dorsal bristles, which did not participate in anlagen formation, are resorbed in very late dividers and early postdividers (Figs. 67, 69). The fused macronuclear mass (Fig. 60) divides twice so that each offspring obtains two nodules. The divided micronuclei are still connected by a long, fine strand, possibly spindle microtubules (Figs. 68, 70).

Molecular data. The complete nucleotide sequence of the macronuclear DNA pol α gene, genes encoding actin I, histone H-4, β telomere protein and the telomere binding protein, number of internal eliminated segments in the micronuclear gene encoding β telomerase protein and actin I, as well as intron length of the α and β telomerase protein have been described and/or reviewed by Greslin *et al.* (1989), Hoffman and Prescott (1997a, b), Mansour *et al.* (1994), Prescott (1994), and Prescott and DuBois (1996). For details, see Discussion.

Sterkiella histriomuscorum (Foissner et al., 1991) Foissner et al., 1991 (Figs. 71-78, Table 2)

Synonymy (according to Berger 1999; as an aid for non-taxonomists, some explanations are included). 1932 *Histrio muscorum* Kahl, Tierwelt Dtl., 25: 617 (original description, Fig. 71); 1938 *Stylonychia curvata* - Giese and Alden, J. exp. Zool., 78: 117 (misidentification); 1953 *Histrio similis* (Quennerstedt 1867) - Wenzel, Arch. Protistenk., 99: 113 (misidentification); 1954 *Oxytricha minor* - Mote, Proc. Iowa Acad. Sci., 61: 578, 588 (misidentification); 1956 *Opistotricha terrestris* Horváth, Arch. Protistenk., 101: 275 (synonym); 1957 *Oxytricha histrioides* Gellért, Annls Inst. biol. Tihany, 24: 20 (synonym); 1958 *Histrio macrostoma* Gellért and Tamás, Annls Inst. biol. Tihany, 25: 229, 240 (synonym); 1960 *Histriculus muscorum* (Kahl, 1932) - Corliss, J. Protozool., 7: 275 (replacement name for genus because *Histrio* was preoccupied); 1970 *Opistotricha terrestris* Horváth - Delhez and Chardez, Annls Spéléol., 25: 135 (redescription); 1970 *Histriculus muscorum* Kahl, 1932 - Dragesco, Annls Fac. Sci. Univ. féd. Cameroun (numéro hors-série): 116 (redescription); 1972 *Oxytricha bimembranata* Shibuya, 1929 - Matis and Danišková, Acta Fac. Rerum nat. Univ. comen. Bratisl., 17: 49 (misidentification); 1982 *Histriculus muscorum* Kahl, 1932 - Foissner, Arch. Protistenk., 126: 80 (Figs. 72, 73; authoritative redescription; 1985 *Histriculus muscorum* (Kahl, 1932) - Berger *et al.*, Protistologica, 21: 303 (morphometric comparison of four populations and description of morphogenesis); 1986 *Histriculus muscorum* Kahl, 1932 - Dragesco and Dragesco-Kernéis, Faune tropicale, 26: 483 (brief review); 1986 *Oxytricha terrestris* (Horváth, 1958) - Dragesco and Dragesco-Kernéis, Faune tropicale, 26: 471; 1989 *Oxytricha trifallax*, a *nomen nudum* species, first mentioned in the paper by Hunter *et al.* (1989), and since then used in the following studies: DuBois and Prescott (1995), Greslin *et al.* (1989), Klobutcher and Herrick (1997), Lingner *et al.* (1994), Prescott and DuBois (1996), Seegmiller *et al.* (1996, 1997), and Williams *et al.* (1993); 1991 *Oxytricha histriomuscorum* nom. nov. - Foissner *et al.*, Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft, 1/91: 311 (replacement name for species because of objective homonymy); 1991 *Sterkiella histriomuscorum* (Foissner, Blatterer, Berger and Kohmann, 1991) nov. comb. - Foissner *et al.*, Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft, 1/91: 311 (brief review and transfer to genus *Sterkiella* established in the same paper with *Oxytricha cavicola* Kahl, 1935 as type); 1992 *Histriculus muscorum* Kahl - Zou and Zhang, Acta zool. sin., 38: 345 (morphology and morphogenesis); 1992 *Sterkiella histriomuscorum* (Foissner, Blatterer, Berger and Kohmann, 1991) Foissner, Blatterer, Berger and Kohmann, 1991 - Augustin and Foissner, Arch. Protistenk., 141: 279 (redescription of a population from activated sludge); 1994 *Histriculus muscorum* Kahl, 1932 - Shin and Kim, Korean J. Zool., 37: 115 (redescription); 1997 *Sterkiella histriomuscorum* (Foissner *et al.*, 1991) - Adl and Berger, Europ. J. Protistol., 33: 99 (life cycle); 1997 *Sterkiella histriomuscorum* (Foissner *et al.*, 1991) Foissner *et al.*, 1991 - Petz and Foissner, Polar Record, 33: 323 (morphology and morphogenesis of an Antarctic population).

Improved diagnosis. Morphology and morphogenesis very similar to *Sterkiella nova* (= sibling species). Differences in nucleotide sequences of the complete pol α gene and the small subunit rDNA significant, however (for details, see Discussion). Complete nucleotide sequence of macronuclear DNA pol α gene described in Hoffman and Prescott (1997b) and deposited in the Gene Bank sequence data base, accession number U59426.

Type material. No type material is available from Kahl's population of *Histrio muscorum* (now *Sterkiella histriomuscorum*, see Discussion). Clearly, Kahl's species needs an unambiguous identity to put an end to the existing confusion. Thus, we suggest fixing the *nomen nudum* species *Oxytricha trifallax* as neotype of *Histrio muscorum* Kahl, 1932. Two neotype slides with protargol-impregnated morphostatic and dividing specimens of *Oxytricha trifallax* (now *Sterkiella histriomuscorum*, see Discussion) have been deposited in the Oberösterreichische Landesmuseum in Linz (LI), Austria, accession numbers: 1999/109, 110.

Morphological and molecular biological characterization. The morphology and ontogenesis of *S. histriomuscorum*, as described by Berger *et al.* (1985) and Petz and Foissner (1997), and of its *nomen nudum* synonym, *Oxytricha trifallax*, are very similar to those of *S. nova* described above. Hence, there is no need for a detailed (re)description; main morphometric and morphologic characters of nine populations are compiled in Table 2 and Figures 72 - 78. The life cycle and standardized growth conditions are described in Adl and Berger (1997). Note that the *Histriculus muscorum* of Matsusaka's group is another species, namely *Sterkiella cavicola* (Foissner *et al.* 1991, Nakamura and Matsusaka 1991).

As concerns *Oxytricha trifallax* (now *Sterkiella histriomuscorum*, see nomenclature), the following small differences to *Sterkiella nova* should be mentioned: (1) the caudal cirri of *O. trifallax* are slightly larger than those of *S. nova*; (2) the buccal cirrus is often nearer to the anterior end of the paroral in *O. trifallax* than in *S. nova*; (3) the paroral and endoral intersect near mid of buccal cavity in *O. trifallax* and in the anterior third in *S. nova*;

(4) *Oxytricha trifallax* is, on average, slightly smaller than *S. nova* in most morphometric characters (Table 2), including the resting cysts (diameter 35-45 μm, $\bar{x}$ 38.9, SD 2.8, CV 7.1%, n 28). However, data must not be over-interpreted because they were obtained with different methods. For instance, specimens prepared with Foissner's protargol protocol are distinctly smaller (length 98 μm) than those prepared with Wilbert's protocol (length 129 μm, Table 1). The first value (98 μm) matches most other data well (Table 2).

Ontogenesis is also very similar in *Oxytricha trifallax* and *S. nova*, although there are small differences in the temporal relationships of the events. A representative example is shown in Figures 41 and 76: both populations (species) agree in the opisthe development but differ in the development of the proter anlagen 4, 5, 6, which are more advanced in *O. trifallax* than in *S. nova*.

The molecular composition of *O. trifallax* has been described and/or reviewed by Doak *et al.* (1997), Hoffman and Prescott (1997a, b), Klobutcher and Herrick (1997), Prescott (1994), Prescott and DuBois (1996), Seegmiller *et al.* (1996), and Witherspoon *et al.* (1997). For details, see Discussion.

Sterkiella histriomuscorum is very frequent in limnetic and, especially, terrestrial habitats. It has been recorded from all main biogeographical regions (Foissner 1998). Very likely, it has a broad ecological range; however, some of the range might be caused by different, morphologically inseparable species.

Conjugation

In both, *Sterkiella nova* (*Oxytricha nova*) and *S. histriomuscorum* (*O. trifallax*), conjugation was never observed under the culture conditions used. Likewise, no sexual processes occurred when cultures of *S. nova* and *S. histriomuscorum* were mixed, indicating that they cannot mate. Prescott (1994) could not find mating types, but observed selfing from time to time in laboratory cultures. Usually, all of the cells that result from selfing die without resuming vegetative growth.

DISCUSSION

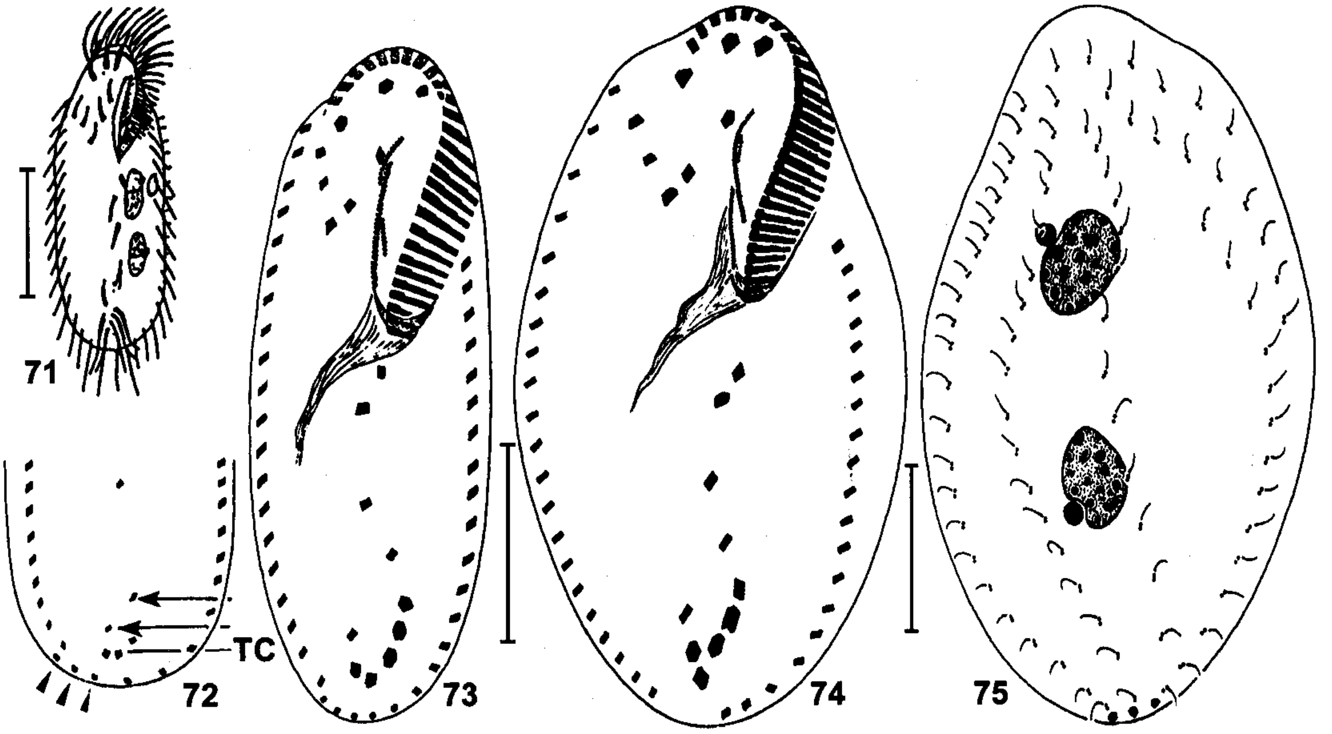
Distinguishing the genera *Oxytricha*, *Sterkiella*, *Stylonychia*, and *Histriculus*

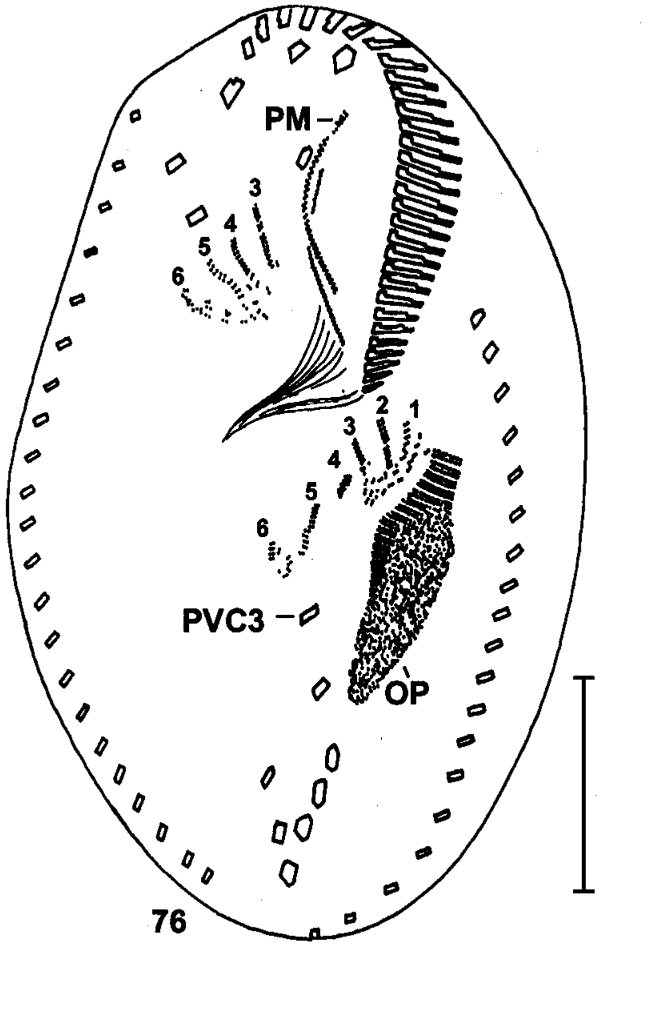
Species of these genera frequently look alike to untrained workers. Thus, they have often been confused (for review, see Berger 1999). Recently, however, Berger and Foissner (1997) solved the puzzle by using morphological and ontogenetic traits, which clearly distinguish these and other oxytrichid genera from each other. Briefly, *Oxytricha* is distinctly different from the other genera by its morphogenetically active postoral cirrus V/3 (Berger and Foissner 1997) and the small subunit ribosomal RNA gene sequences (Schlegel *et al.* 1991). Thus, it belongs to the subfamily Oxytrichinae, whereas the other genera, in which cirrus V/3 does not participate in anlagen formation, belong to the Stylonychinae. Within this group, only *Histriculus* lacks caudal cirri and has confluent marginal cirral rows, clearly separating it from *Sterkiella* and *Stylonychia*. The latter genera differ morphologically, at the present state of knowledge, mainly in the arrangement of the undulating membranes (intersecting in *Sterkiella*, parallel in *Stylonychia* Figs. 85, 86) and the buccal field (narrow in *Sterkiella*, rather broad-triangular in *Stylonychia*; Figs. 79, 80, 83, 84). The ontogenetic difference noted by Berger and Foissner (1997), namely, that cirral streaks V and VI of the opisthe originate de novo, holds only for *Sterkiella cavicola*, type of the genus. In *S. histriomuscorum* (*Oxytricha trifallax*) and *S. nova*, these anlagen are generated by cirrus V/4, as in *Stylonychia* spp. (Wirnsberger *et al.* 1985, 1986). However, *Sterkiella nova* and *Stylonychia mytilus* (type of the genus) differ distinctly in certain gene sequences (Hoffman and Prescott 1997b) and the allozyme pattern (Schlegel 1985, Schlegel and Steinbrück 1986), that is, are distinct genera, in spite of the rather inconspicuous morphological and ontogenetic differences.

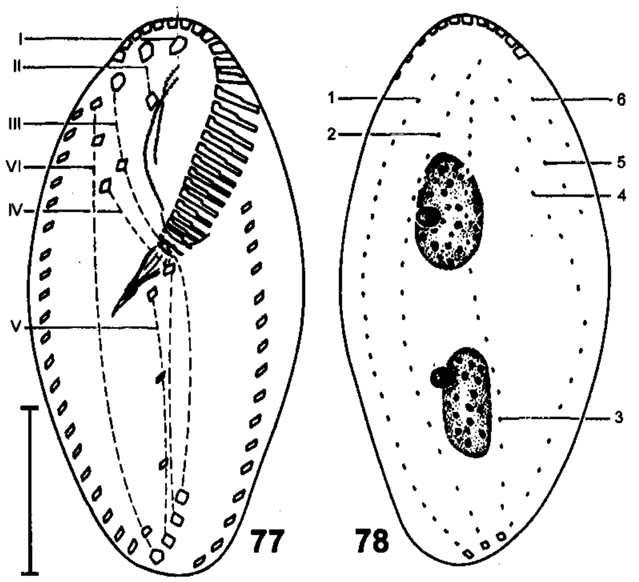
Identification of *Sterkiella histriomuscorum*

The original description of *S. histriomuscorum* (*Histrio muscorum* in Kahl 1932, p. 617) is brief and rather general (translated from German; includes characters mentioned in the subgenus description and the key to species): "Length *in vivo* 100-150 μm, length:width ratio slightly variable. Body rather rigid and distinctly flattened, parallel-sided with posterior end broadly rounded. Rightmost transverse cirri 1/3-1/2 projecting beyond posterior body margin. Last three cirri of left marginal row form rather distinct bristles, which, however, are soft and only slightly elongated. Frequently found in mosses from the German Alps and California ".

Fortunately, Kahl (1932) provided an excellent figure (Fig. 71), which not only perfectly matches later redescriptions (Foissner 1982, Shin and Kim 1994) but also *Oxytricha nova* (Figs. 1, 13) and *Oxytricha trifallax* (Fig. 74). Kahl's description of the caudal cirri, which he







Figs. 71-78. *Sterkiella histriomuscorum* (71-73, 77, 78) and its *nomen nudum* synonym *Oxytricha trifallax* (74-76) from life (71) and after protargol impregnation (72-78). 71- ventral view of type population (from Kahl 1932); 72, 73 - ventral views of soil populations from the Austrian Alps (from Foissner 1982). Note that there are three to five transverse cirri. Arrows mark pretransverse ventral cirri, arrowheads denote caudal cirri; 74, 75 - infraciliature of ventral and dorsal side of *O. trifallax*; 76 - early divider of *O. trifallax* showing that cirral anlagen (numbers 1-6) originate as in *S. nova*; 77, 78 - infraciliature of ventral and dorsal side of the soil population investigated by Berger *et al.* (1985); most specimens have only four transverse cirri. Roman numerals denote cirri originating from same anlage. Arabic numerals denote dorsal kineties. OP - oral primordium, PM - paroral membrane, PVC 3 - postoral ventral cirrus V/3, which does not participate in anlagen formation, TC - transverse cirri. Scale bars 40 μm (Fig. 71) and 20 μm (other figures)

misinterpreted as „the last three cirri of the left marginal row", exactly matches our observations; they are indeed inconspicuous and easily misidentified as marginal cirri (Figs. 1, 8, 9, 19, 20, 23, 24).

Sterkiella comprises, according to Berger's (1999) recent revision of oxytrichid hypotrichs, seven reliable species. Most have more than two macronuclear nodules and are thus easily distinguished from the *S. histriomuscorum* complex, which has two. The only other species with two macronuclear nodules is *S. tricirrata* (Buitkamp), which differs from *S. histriomuscorum* by a slightly reduced number of transverse cirri (3 vs. 3 - 5) and dorsal kineties (5 vs. 6). Thus, this species might well be another member of the *S. histriomuscorum* complex. As concerns separation from species of related genera, see next chapter and chapter "Distinguishing the genera *Oxytricha*, *Sterkiella*, *Stylonychia*, and *Histriculus*".

Proposed synonymy of *Sterkiella histriomuscorum* with *Stylonychia pustulata/vorax* rejected

Very recently, Eigner (1999) vaguely speculated about synonymy of *S. histriomuscorum* with *Stylonychia pustulata* and/or *S. vorax*, for which he erected the new genus *Tetmemena*. Indeed, such speculations and misidentifications are common in this kind of hypotrichs (Borror 1972 and synonymy list by *S. histriomuscorum*), whose separation needs a rather sophisticated set of characters difficult to experience by workers not fully familiar with the group. It is thus necessary to discuss the subject in some detail, using Eigner's paper as a representative example.

(1) Eigner (1999) correctly states that Berger and Foissner (1997) put much emphasis on the arrangement of the undulating membranes (parallel in *Stylonychia*, optically intersecting in *Sterkiella* and *Oxytricha*). We still hold this view, although the character is sometimes inconspicuous, because it is supported by a lot of ontogenetic data (for a review, see Berger and Foissner 1997). Eigner (1999) argues that, depending on the preparation conditions and the orientation of the specimens, the undulating membranes may appear parallel or intersecting in *Bakuella pampinaria*. We agree and thus do not use this character in large and soft hypotrichs, but only in the medium-sized oxytrichids *s. str.* Furthermore, it is quite common that the same character has different weight in different groups and exceptions exist within the group. Thus, Eigner (1999) mixes two different subjects.

(2) Eigner (1999) overlooked that *Stylonychia vorax* in Wirnsberger *et al.* (1985) is a misidentified *S. bifaria* (Foissner *et al.* 1991, p. 334).

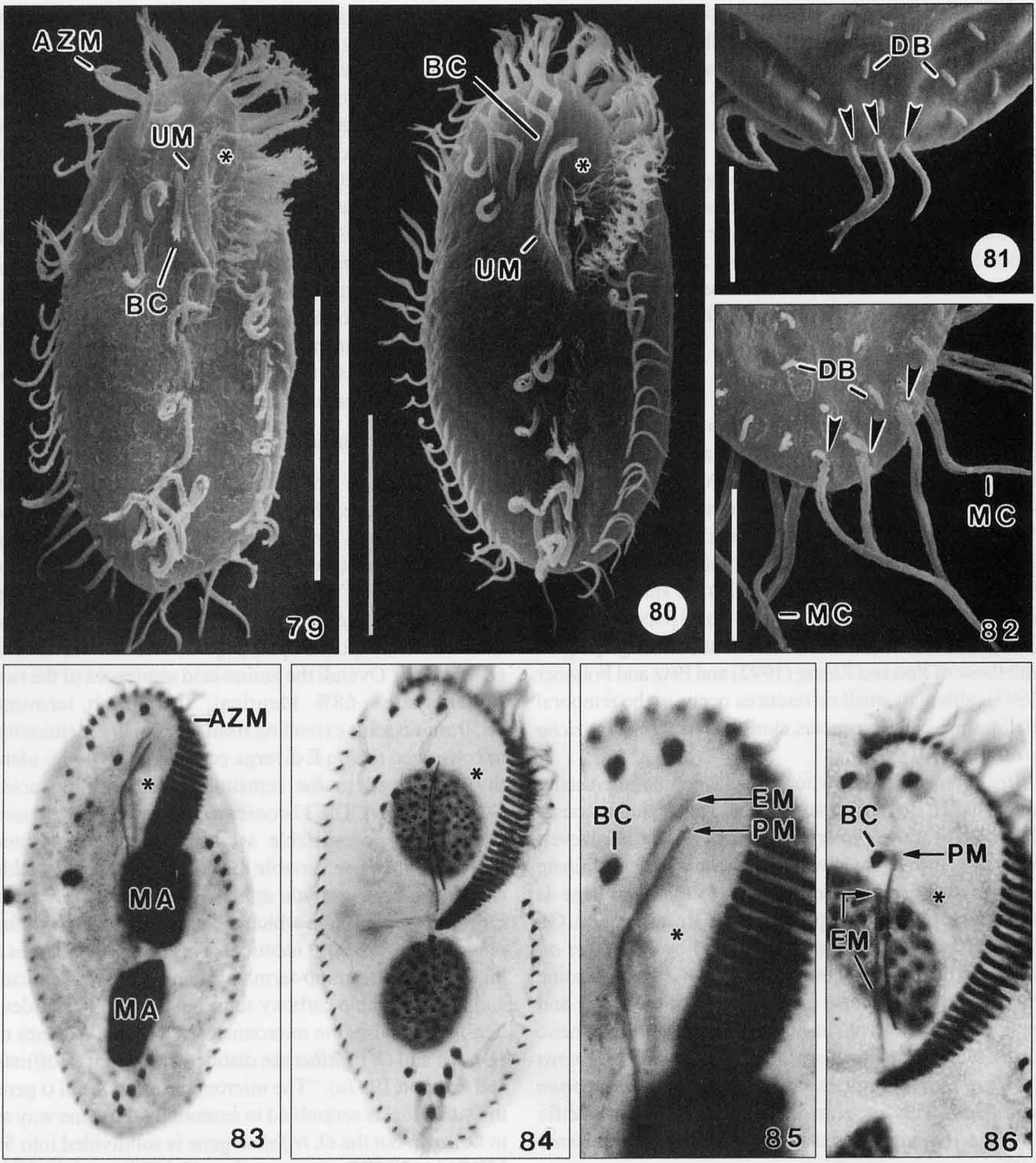
(3) Eigner (1999) considers almost entirely ontogenetic characters in separating the species under discussion. However, species are usually not separated by specific ontogenetic features, which are of significance mainly at genus, family, and ordinal level (Foissner 1996). As a consequence, Eigner (1999) lost most species characters (see following paragraph).

(4) *Stylonychia pustulata* differs from members of the *Sterkiella histriomuscorum* complex by the following features (Figs. 79 - 86 and Foissner *et al.* 1991): (i) the arrangement of the undulating membranes (parallel vs. intersecting), (ii) the shape of the buccal field (moderately broad-triangular vs. narrow elliptical), (iii) the location of the buccal cirrus (at anterior end of paroral vs. slightly above mid-buccal cavity), (iv) dorsal kinety 4 (unshortened anteriorly vs. shortened), (v) the arrangement and distinctiveness of the caudal cirri (widely spaced and distinct vs. narrowly spaced and indistinct), (vi) the resting cyst (with conspicuous tubercles or spines vs. irregularly wrinkled or almost smooth; Figs. 29, 30), (vii) the origin of the oral primordium (near upper postoral ventral cirri vs. near transverse cirri; Figs. 31, 32, 37), (viii) the allozyme pattern (Schlegel 1985, Schlegel and Steinbrück 1986), and (ix) the 16S-like rRNAs, which show an identity of 99,3%, indicating relatedness but not identity (Lynn and Sogin 1988, Schlegel *et al.* 1991).

(5) Data are, unfortunately, much less detailed for *Stylonychia vorax* (for a review, see Foissner *et al.* 1991). However, the studies available show clearly that it is much more similar to *Stylonychia pustulata* than to members of the *Sterkiella histriomuscorum* complex.

(6) *Stylonychia bifaria* (misidentified as *S. vorax* by Wirnsberger *et al.* 1985, see above) differs from members of the *Sterkiella histriomuscorum* complex mainly by the arrangement of the transverse cirri (two distinct groups vs. single group) and the structure of the resting cyst (with conspicuous tubercles vs. wrinkled; Kay 1945 and Figs. 29, 30).

(7) Although Petz and Foissner (1997) showed by clear figures that the Antarctic *Sterkiella histriomuscorum* population has a smooth or slightly wrinkled cyst wall (with conspicuous tubercles or spines in *Stylonychia pustulata*; Foissner *et al.* 1991) and develops the oral primordium near the transverse cirri (near the upper postoral cirri in *S. pustulata*; Wirnsberger *et al.* 1985), Eigner (1999, p. 45) states: "At least the species described by Petz and Foissner (1997) as *Sterkiella histriomuscorum* is probably *Stylonychia vorax* or *S. pustulata*". Although one can be of different opinion about the generic significance of these characters (Berger and Foissner 1997,



Figs. 79 - 86. Comparison of the *Sterkiella histriomuscorum* complex with *Stylonychia pustulata* in the scanning electron microscope (79-82) and after protargol impregnation (83-86). These species, which look similar at first glance (79, 80, 83, 84), are frequently confused or even synonymized, although they differ in many features, some of which are shown in the micrographs: (i) arrangement of undulating membranes (UM; endoral [EM] and paroral [PM] optically intersecting in mid-buccal cavity vs. parallel), (ii) buccal field (asterisk; narrow-elliptical vs. moderately broad-triangular), (iii) location of buccal cirrus (BC; slightly above mid-buccal cavity vs. anterior end of paroral), and (iv) arrangement and distinctiveness of caudal cirri (arrowheads; narrowly spaced and short vs. widely spaced and long). AZM - adoral zone of membranelles, BC - buccal cirrus, DB - dorsal bristles, EM - endoral membrane, MA - macronuclear nodules, MC - marginal cirri, PM - paroral membrane, UM - undulating membranes (paroral + endoral). Scale bars 40 µm (79, 80) and 10 µm (81, 82)

Eigner 1999), they unequivocally distinguish species. Thus, Eigner's speculation is groundless.

All data mentioned above were available to Eigner (1999). He did not discuss, why he discarded most of them. Furthermore, he neglected a basic "rule" in species taxonomy, namely, to reinvestigate the available type material. Slides with protargol-impregnated specimens of most populations under discussion are deposited in the Museum of Natural History in Linz (LI), Austria, and available to any worker. In sum, Eigner's proposed synonymies must be rejected because they are based on highly selected characters and insufficient literature and slide (species) knowledge.

Ontogenetic comparison

Ontogenetic data are available from four populations of *S. histriomuscorum*, which occurred in soils from Austria (Berger *et al.* 1985) and continental Antarctica (Petz and Foissner 1997) and in freshwaters from Spain (Nieto *et al.* 1984) and China (Zou and Zhang 1992). However, detailed illustrations were provided only by Berger *et al.* (1985) and Petz and Foissner (1997). In spite of this, it is obvious that ontogenesis is similar in all populations and to that described here for *S. nova*. Our data largely agree with those of Zou and Zhang (1992) and Petz and Foissner (1997), although small differences occur in the temporal relationships of the processes, similar as between *Oxytricha nova* and *O. trifallax*.

Berger *et al.* (1985), who studied a population usually having only four transverse cirri, could not unambiguously clarify the origin of proter's anlagen 4, 5 and 6. However, the figures provided show that they very likely originate as in *S. nova*, that is, from cirrus IV/3. This stage is difficult to observe, as explained in the Result section. On the other hand, the population studied by Berger *et al.* (1985) is clearly different from *S. nova* in that some fronto-ventral-transverse cirral anlagen of the proter and opisthe are confluent during the early morphogenetic stages, while all anlagen are distinctly separate in *S. nova* and *O. trifallax*. This might indicate that *S. histriomuscorum* populations with four transverse cirri are not conspecific with those having five transverse cirri (Petz and Foissner 1997).

The micrographs and description by Nieto *et al.* (1984) are more difficult to interpret: „The next step is the formation of the fronto-ventral-transverse primordium and paroral primordium of the future opisthe. As two ciliary streaks extend from the right anterior margin of the oral primordium, two ventral cirri disaggregate and the subsequent stringing out of their kinetosomes forms two primordial streaks each. From these six streaks, the closest cirral streak to the oral primordium will form the paroral primordium and the remainder one will give rise to the fronto-ventral-transverse system". However, micrographs 7 and 8 in Nieto *et al.* (1984) indicate that the oral primordium develops three anlagen and cirrus IV/2 only one, as in *S. nova* and *S. histriomuscorum* (Petz and Foissner 1997). Furthermore, Nieto *et al.* (1982, 1984) very likely misidentified the species. The figures show an organism which highly resembles *Stylonychia pustulata* (for review, see Foissner *et al.* 1991) in the location of the buccal cirrus (at anterior end of paroral), in the shape and size of the buccal field (broadly triangular), and the arrangement of the undulating membranes (parallel).

Molecular comparison

This has been performed by Hoffman and Prescott (1997b) who state: "Molecular data from both actin I and DNA pol α show that *Oxytricha* (now *Sterkiella*) *nova* and *O. trifallax* (now *Sterkiella histriomuscorum*) are different species". Specifically, Hoffman and Prescott (1997b) emphasize the following differences in the macronuclear DNA pol α polypeptides of *O. nova* and *O. trifallax*: "Overall the amino acid sequences of the two proteins are ~68% identical; the amino terminal ~350 amino acids extending from the initiator methionine to conserved region E diverge considerably (~46% identity) compared to the remaining ~1150 amino acids (~72% identity). The 12 conserved domains are separated by "spacers" of variable sequence and length; some "spacers" are more variable than others are. It is possible to define a core catalytic domain extending from region E through region V, in which the *O. nova* and *O. trifallax* polypeptides are 80% identical. This domain separates a highly variable amino-terminal domain (46% identical) and a less variable carboxy-terminal domain (63% identical)". Likewise, the micronuclear DNA pol α genes of *O. nova* and *O. trifallax* are distinctly different (Hoffman and Prescott 1997a): "The micronuclear DNA pol α gene in *O. trifallax* is scrambled in essentially the same way as in *O. nova*, but the *O. trifallax* gene is subdivided into 51 MDSs by 50 IESs, compared to 45 MDSs and 44 IESs in *O. nova*. PCR experiments failed to detect any non-scrambled or alternatively scrambled copies of the gene in the micronuclear genome. The first 1234 bp in the *O. trifallax* gene are subdivided into four non-scrambled MDSs, and the first 1233 bp in *O. nova* are subdivided into three non-scrambled MDSs. In *O. nova* the 3'-end of the gene is divided into MDSs 44 and 45 by a single long IES of 223 bp, but in *O. trifallax* this region is divided into MDSs 49-51 by two short IESs of 69 and 10 bp. The other four additional MDSs in *O. trifallax* compared to *O. nova* are scrambled. The eight MDSs that are missing from the main body of the micronuclear gene of *O. trifallax* correspond closely in position in the ORF to the eight MDSs that are missing from the cloned micronuclear gene of *O. nova*. One scrambled MDS (MDS 8) is not present in the cloned micronuclear PCR product from *O. trifallax*. There is no corresponding MDS in *O. nova*. The micronuclear DNA pol α gene in *O. trifallax* contains an inversion in the same position as in *O. nova*. This strongly suggests that the DNA pol α gene became scrambled before *O. trifallax* and *O. nova* diverged from their common ancestor". There are also distinct differences in the internal eliminated sequences of these species (Prescott and DuBois 1996).

Neighbour-joining trees from the amino acid and DNA pol α sequences place *O. nova* and *O. trifallax* in the same clade, which is distinctly distant from the *Stylonychia mytilus/lemnae* clade; in contrast, actin I sequences separate *O. nova* from *O. trifallax*, which forms a clade with *S. mytilus/lemnae* (Hoffman and Prescott 1997b). However, 16S-like ribosomal RNA trees unambiguously show *O. nova* as sister group of *Stylonychia pustulata* (Lynn and Sogin 1988, Schlegel *et al.* 1991), which is in accordance with morphological and ontogenetical findings (Wirnsberger *et al.* 1985, 1986).

The *Sterkiella histriomuscorum* story

Before establishing *Sterkiella histriomuscorum* and *S. nova* as sibling species of a *Sterkiella histriomuscorum* complex, it seems appropriate to present in detail the complex history of the genus *Sterkiella*. The chapter is based on recent reviews (Berger 1999, Berger and Foissner 1997).

In 1878 Sterki established the genus *Histrio* to separate stylonychid hypotrichs with (*Stylonychia*) or without (*Histrio*) caudal cirri and with posteriorly open (*Stylonychia*) or confluent (*Histrio*) marginal cirral rows. Unfortunately, the generic name was preoccupied by *Histrio* Fischer, 1813, a fish. Thus, Corliss (1960) replaced the homonym by naming the ciliate genus *Histriculus* and combining, among others, *Oxytricha (Histrio) muscorum* Kahl, 1932 with the new name to *Histriculus muscorum* (Kahl, 1932) Corliss, 1960. Kahl (1932) and Borror (1972) added to *Histrio* and *Histriculus* species with inconspicuous caudal cirri and indistinctly separated marginal rows, obviously assuming that Sterki (1878) misinterpreted these characters in the type species *Histrio steinii* Sterki, 1878, a junior (objective?) synonym of *Histriculus histrio* (Müller, 1773). However, ontogenetic studies proved that species, as added by Kahl (1932) and Borror (1972) to *Histriculus*, indeed have caudal cirri and open marginal cirral rows, especially *Histriculus muscorum* (now *Sterkiella histriomuscorum*, see below), one of the most widespread oxytrichids (Berger *et al.* 1985). Thus, separation of *Histriculus* from *Stylonychia* became indistinct, suggesting synonymy (Wirnsberger *et al.* 1986). It was only recently that Foissner *et al.* (1991) and Berger and Foissner (1997) showed the existence of stylonychid hypotrichs without caudal cirri, as defined by Sterki (1878), requiring that species assigned to *Histrio* and *Histriculus* mainly by Kahl (1932) and Borror (1972) be referred to a new genus, *Sterkiella* Foissner *et al.*, 1991. Thus, a single species has accumulated three generic combinations over time: *Oxytricha (Histrio) muscorum* Kahl, 1932; *Histriculus muscorum* (Kahl, 1932) Corliss, 1960; and *Sterkiella histriomuscorum* (Foissner *et al.*, 1991) Foissner *et al.*, 1991. In the latter binomen, the species name has also changed because Kahl (1932) named three other species *Oxytricha muscorum*, namely *Oxytricha (Opisthotricha) muscorum*, *O. (Steinia) muscorum*, and *O. (Stylonychia) muscorum*. All these are primary homonyms because a subgeneric name does not affect homonymy [article 57(d) of the ICZN (1985)]. Thus, three species had to be renamed, among them also *Histrio muscorum* Kahl, 1932, now called *Sterkiella histriomuscorum* (Foissner *et al.*, 1991) Foissner *et al.*, 1991. The doubling of "Foissner *et al.*, 1991" behind the species name is caused by a formal requirement, viz. that we had to combine the species with *Oxytricha* (to *Oxytricha histriomuscorum* nom. nov.) before transferring it to *Sterkiella*, which is indicated by parentheses [article 51(c) of the ICZN (1985)]. In other words, *Histrio muscorum* Kahl, 1932 (p. 617) was renamed because of homonymy of the genus name and of primary homonymy of the species name [thus, Kahl loses authorship of the species; article 60 of the ICZN (1985)], and then transferred to the genus *Sterkiella* because of new taxonomic findings.

When we discovered and rectified homonymy in 1991, we did not consider *Opisthotricha terrestris* Horváth, 1956, *Oxytricha histrioides* Gellért, 1957 and *Histrio macrostoma* (Gellért and Tamás, 1958) as junior synonyms of *Histrio muscorum* Kahl, 1932. Recently, however, Berger (1999) suggested this synonymy (see Results section). Thus, the oldest name (*O. terrestris*) could be used as a replacement name for *Histrio muscorum*, respectively, *Sterkiella histriomuscorum* [articles 52 (b) and 60 of the ICZN (1985)]. However, changing the name again would not only cause further instability but could require changing back the name in future, if other authors reach a different conclusion, viz. that the above mentioned synonyms are distinct, valid species. Thus, we maintain our replacement name from 1991, which is unique and ensures stability.

Oxytricha nova and *O. trifallax*, sibling species of a *Sterkiella histriomuscorum* complex

Fortunately, detailed morphological and morphometric data are available from many populations of *S. histriomuscorum* collected worldwide in mainly terrestrial habitats. Except the population from activated sludge in Austria, all are very similar to each other and to *O. nova* and *O. trifallax* (Table 2). The mean values for the number of adoral membranelles, one of the best species

Table 2. Morphometric comparison of main characters of 9 populations of *Sterkiella histriomuscorum* (formerly *Histriculus muscorum*) with *Sterkiella nova* sp. n. and *Oxytricha trifallax*

Character[1]	Reference[2]	Species[3]	Method[4]	$\bar{x}$	SD	CV	Min	Max	n
Body, length	1	SH	PF	67.4	3.8	5.7	60	73	10
	1	SH	PF	71.6	6.0	8.3	60	80	8
	2	SH	PF	81.7	4.5	5.5	72	88	15
	2	SH	PF	73.6	8.4	11.5	52	82	15
	2	SH	PF	71.5	6.2	8.7	61	82	15
	2	SH	PF	64.9	5.4	8.3	57	75	15
	3	SH	PF	99.0	13.2	13.3	85	129	20
	4	SH	PW	105.8	4.8	4.6	100	114	10
	5	SH	PW	83.7	8.4	10.1	66	102	31
	6	SN	PW	128.5	17.2	13.4	85	150	29
	6	OT	PF	83.7	5.4	6.5	73	96	29
Adoral zone of membranelles, length	1	SH	PF	26.3	1.2	4.5	24	28	10
	1	SH	PF	30.5	1.9	6.3	27	33	8
	2	SH	PF	31.1	2.6	8.2	27	37	15
	2	SH	PF	29.4	2.8	9.4	22	33	15
	2	SH	PF	28.1	2.0	7.1	24	31	15
	2	SH	PF	27.5	1.9	6.9	25	32	15
	3	SH	PF	45.2	3.8	8.5	39	53	20
	4	SH	PW	43.4	2.0	4.6	41	47	10
	5	SH	PW	37.4	3.5	9.4	31	45	31
	6	SN	PW	52.7	7.0	13.2	40	62	29
	6	OT	PF	35.7	2.1	5.9	32	40	29
Adoral membranelles, number	1	SH	PF	31.2	2.1	6.7	28	35	10
	1	SH	PF	30.1	1.2	3.9	28	32	8
	2	SH	PF	31.0	2.3	7.4	26	34	9
	2	SH	PF	31.2	1.4	4.5	29	33	9
	2	SH	PF	30.1	3.2	10.6	24	34	9
	2	SH	PF	29.2	1.7	5.9	27	32	9
	3	SH	PF	39.3	2.9	7.3	34	44	20
	4	SH	PW	28.4	1.3	4.5	27	31	10
	5	SH	PW	27.9	1.1	4.0	26	31	31
	6	SN	PW	34.2	2.0	5.7	30	39	29
	6	OT	PF	29.7	1.3	4.5	26	32	29
Right marginal cirri, number	1	SH	PF	19.9	1.4	7.3	17	22	10
	1	SH	PF	22.6	1.3	5.8	20	25	8
	2	SH	PF	20.7	2.1	10.2	17	24	12
	2	SH	PF	18.8	1.1	5.6	17	21	12
	2	SH	PF	18.4	1.5	8.2	16	22	12
	2	SH	PF	19.0	1.0	5.5	17	21	12
	3	SH	PF	27.2	2.0	7.4	24	32	20
	4	SH	PW	21.6	1.2	5.4	20	23	10
	5	SH	PW	20.5	1.1	5.3	18	22	31
	6	SN	PW	21.5	1.7	7.7	18	25	29
	6	OT	PF	21.8	1.1	5.2	20	24	29

Table 2 (contd)

Character[1]	Reference[2]	Species[3]	Method[4]	$\bar{x}$	SD	CV	Min	Max	n
Transverse cirri, number	1	SH	PF	3.8	-	-	3	4	10
	1	SH	PF	5.0	0.0	0.0	5	5	8
	2	SH	PF	4.0	0.4	10.2	3	5	13
	2	SH	PF	3.9	-	-	3	4	13
	2	SH	PF	3.9	-	-	3	4	13
	2	SH	PF	4.2	-	-	4	5	13
	3	SH	PF	4.7	0.6	12.6	4	6	20
	4	SH	PW	4.0	0.0	0.0	4	4	10
	5	SH	PW	5.0	0.0	0.0	5	5	31
	6	SN	PW	5.0	-	-	5	6	29
	6	OT	PF	5.0	0.0	0.0	5	5	29
Dorsal kineties, number	1	SH	PF	6.0	0.0	0.0	6	6	10
	1	SH	PF	6.0	0.0	0.0	6	6	8
	2	SH	PF	6.0	0.0	0.0	6	6	12
	2	SH	PF	6.0	0.0	0.0	6	6	12
	2	SH	PF	6.0	0.0	0.0	6	6	12
	2	SH	PF	6.0	0.0	0.0	6	6	12
	3	SH	PF	6.2	-	-	6	7	20
	4	SH	PW	6.0	0.0	0.0	6	6	8
	5	SH	PW	5.8	-	-	5	6	31
	6	SN	PW	6.0	0.0	0.0	6	6	29
	6	OT	PF	6.0	0.0	0.0	6	6	29

[1] Data based on randomly selected, protargol-impregnated morphostatic specimens. Measurements in µm. CV - coefficient of variation in %, Max - maximum, Min - minimum, n - number of specimens investigated, SD - standard deviation, $\bar{x}$ - arithmetic mean.
[2] 1- Foissner (1982): two populations from soils of the Austrian Central Alps, field material; 2 - Berger *et al.* (1985): four populations from soils of the Austrian Central Alps, field material (populations 1 - 3) and a cultivated strain (population 4) were investigated; 3 - Augustin and Foissner (1992): from activated sludge in Austria, cultivated on sludge; 4 - Shin and Kim (1994): from soil of cultivated field in Korea, cultivated on bacteria; 5 - Petz and Foissner (1997): from Antarctic soil, cultivated on bacteria; 6 - this paper, see "Materials and Methods" and "Type location".
[3] SH - *Sterkiella histriomuscorum*; SN - *Sterkiella nova*; OT - *Oxytricha trifallax*.
[4] PF -Foissner's (1991) protargol protocol; PW - Wilbert's (1975) protargol protocol.

characters in hypotrichs, vary between 28 and 34 only, if the population from activated sludge (39.3) is excluded. Similar low variation is obtained for the number of marginal and transverse cirri. Furthermore, all populations have two macronuclear nodules, six dorsal kineties, and three inconspicuous caudal cirri. Metric parameters, like length of body and adoral zone of membranelles, vary to a greater extent. However, this is at least partially caused by the different preparation and culture methods used (Table 1), and should thus not be over-interpreted (see Results section).

Certainly, the populations listed in Table 2 cannot be reliably separated by morphological and morphometric criteria because the extreme values highly overlap in most cases. Furthermore, the ontogenesis pattern is very similar in several *S. histriomuscorum* populations (see above) and in *O. nova* and *O. trifallax*. On the other hand, *O. nova* and *O. trifallax* are clearly different in their genetic material (see Discussion above), suggesting that *S. histriomuscorum* is a complex of sibling species, like *Stylonychia mytilus* (Ammermann and Schlegel 1983, Steinbrück and Schlegel 1983), *Tetrahymena pyriformis* (Nanney and McCoy 1976), and *Paramecium aurelia* (Sonneborn 1975). Looking at the data compiled in Table 2 in more detail, it seems reasonable to assume that the complex contains more than two species, that is, *Sterkiella nova* (formerly *Oxytricha nova*) and *Sterkiella histriomuscorum* (formerly *Oxytricha trifallax*). For instance, the population from activated sludge, which has a distinctly higher number of adoral membranelles, and some alpine populations, which usually have only four transverse cirri, might be sufficiently different at the molecular level to give them species status. *Sterkiella tricirrata*, mentioned above, might belong to the complex, too. And Seegmiller *et al.* (1996) and Witherspoon *et al.* (1997) mention "two sibling *Oxytricha* species, *O. fallax* and *O. trifallax*". However, the molecular differences between these populations are less distinct than those between *O. nova* and *O. trifallax*.[1]

Taxonomic - nomenclatural consequences

There is no possibility of knowing whether the populations of *S. histriomuscorum* studied by Kahl (1932) and others were *O. nova*, *O. trifallax*, or other (sibling) species. Thus, one could argue to classify both, *O. nova* and *O. trifallax*, as species nova, considering their molecular distinctiveness. However, this certainly would break the spirit of the International Code of Zoological Nomenclature (1985) to maintain nomenclatural continuity and priority. There can be no doubt that *O. nova* and *O. trifallax* are, from a morphological point of view, populations of Kahl's *Histrio muscorum*. Thus, we arbitrarily identify *O. trifallax* as *Histrio muscorum* Kahl, 1932 (now *Sterkiella histriomuscorum*) and establish *O. nova* as a new species, *Sterkiella nova*, simply because *O. nova* was used in more studies than *O. trifallax*. Furthermore, we maintain the specific epithet "*nova*" (although it is rather trivial) to ensure continuity with the previous literature. Synonymization of *O. trifallax* with *S. histriomuscorum* is not necessary, simply because the former is a *nomen nudum* and thus non-existent in the official zoological literature.

Our proposal follows that used by Nanney and McCoy (1976) for the *Tetrahymena pyriformis* complex. Furthermore, we suggest to follow Corliss and Daggett (1983) in designating field populations of *S. histriomuscorum* as "*Sterkiella histriomuscorum* complex", if molecular data are lacking. Alternatively, one can follow the concept of Génermont and Lamotte (1980) and designate it as "*Sterkiella* supraspecies *histriomuscorum* (Foissner *et al.*, 1991) Foissner *et al.*, 1991".

As mentioned above, the complex very likely contains more than the two species diagnosed here. If such species should be discovered, probably the synonyms of *S. histriomuscorum* (see synonymy list in Results section) should be used for naming. This would be an important contribution to reduce the vast number of species names. However, the name "*trifallax*" should be abandoned forever to avoid further confusion.

CONCLUDING REMARKS

The long-practiced cavalier assignment of a taxon name to random ciliate isolates by molecular biologists has generated tremendous confusion which will contaminate the literature for all time. Morphologists also contributed to the confusion by unjustified synonymies and nomenclatural mistakes. To order this mass and find moderate solutions for the problems, was a difficult job. Thus, we hope that our suggestions will be followed by both molecular biologists and classical taxonomists, otherwise the confusion will increase to an unresolvable mass. Furthermore, a more close and fair co-operation between morphologists and molecular biologists is needed.

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[1]It is beyond the scope of the present paper to discuss the *Oxytricha fallax* problem in detail. However, some background information is necessary to fully understand the rationale of the suggestions in the following chapter. Unfortunately, the morphological identity of the *O. fallax* population now used by molecular biologists is also not known because it is a re-isolate, that is, not that studied by Grimes (1972) and then used by Hammersmith during the 1970s (Hammersmith, pers. comm.). The population studied by Grimes died out, although cysts are still maintained in Hammersmith's laboratory (pers. comm.). The organism studied by Grimes and determined by A. C. Borror as "unnamed subspecies of *Oxytricha fallax* Stein" (Grimes 1972, p. 428) is about 80 µm long and possibly not very flexible, whereas Stein's (1859) *O. fallax* is 150 - 180 µm long and highly flexible and contractile (Stein 1859: "*Oxytricha fallax* is a real *Oxytricha* because it is highly flexible and contractile and thus cannot be assigned to *Stylonychia*. If specimens get between two obstacles, they contract or extend trying to force themselves through the obstacles by winding left and right. Such movements cannot be performed by any *Stylonychia*".). Thus, the organism studied by Grimes (1972) and Grimes and Adler (1976) cannot be identical with Stein's species. The data provided suggest that it was a member of the *Sterkiella histriomuscorum* complex.

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