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Morphology and Morphogenesis of *Onychodromus quadricornutus* n. sp. (Ciliophora, Hypotrichida), an Extraordinarily Large Ciliate with Dorsal Horns[1]

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ABSTRACT. The morphology and the morphogenesis of the freshwater hypotrich ciliate *Onychodromus quadricornutus* n. sp. have been investigated using living organisms, protargol impregnation, and scanning electron microscopy. Some preliminary and supplementary results about the morphogenesis of *O. grandis* and *Laurentiella acuminata* are included. The new species is unique among all described hypotrichs in having four dorsal horns, whose function is unknown. In addition, *O. quadricornutus* is probably the most voluminous hypotrich ciliate known (2×10^6–5×10^6 μm^3). Its morphogenetic pattern resembles the oxytrichids *O. grandis* and *L. acuminata*. The strongest apomorphic character, which unites these three species, is probably the multiple fragmentation of the dorsal primordia during morphogenesis. This fragmentation causes the characteristic high number and more or less irregular distribution of the dorsal kineties in the non-dividing individuals.

HYPOTRICHOUS ciliates have been well known for a long time and were carefully studied by many authors; however, their classification remains controversial to date (5). The new species described is unique among all known hypotrichous ciliates in having horns and presents new data that may contribute to a deeper understanding of systematics and evolution of the family Oxytrichidae in the order Hypotrichida.

MATERIALS AND METHODS

Onychodromus quadricornutus was isolated by D. M. Prescott from a freshwater aquarium in Boulder (Colorado, USA) where tropical aquarium fish are sold, so this hypotrich is probably from some tropical climate. Unfortunately, we were unable to find out the exact source.

Cells were either cultured in bottled spring water (Eau de Volvic, France) enriched with wheat grains, small ciliates (*Chilodonella uncinata, Cyclidium glaucoma, Tetrahymena thermophila*) and zooflagellates as food organisms or in Pringsheim's solution (25) with *Chlorogonium elongatum* as food organism. Cultures (petri dishes, 15-cm diam.) were kept at room temperature, and the medium was changed every two weeks.

Onychodromus quadricornutus was examined in vivo and in silver-impregnated slides. A combination of the protargol methods of Wilbert (27) and Foissner (5) was used to reveal the infraciliature. Preparation for scanning electron microscopy followed the method described in ref. 1. All counts and measurements were performed at a magnification of 1000× on individuals from well fed, spring-water cultures in the exponential growth phase. With the oil immersion objective, one scale mark of the ocular micrometer corresponded to 1.4 μm. Statistical procedures follow methods as described in ref. 22.

Estimates of the wet weight of the ciliate were made by calculating the volume of the cell (reducing the complicated shape to a simple geometric figure and using the measurements shown in Table I) and assuming a specific-gravity of 1.0. This method is widely used by hydrobiologists (20).

The drawings of the impregnated specimens were made with the help of a camera lucida. To make plain the changes during the morphogenetic processes, old cirri are depicted only by contour, whereas the new ones are filled in. The descriptive terminology is according to refs. 3, 16, and 26.

RESULTS

Onychodromus quadricornutus n. sp.

Diagnoses. In vivo about 370 × 200 × 70 μm (n = 6). Body extraordinarily large, stiff; outline elliptical; four prominent horns on the dorsal side; 130 adoral membranelles; 20 macronuclear segments; 11 ventral cirral rows, 12 transverse cirri on the average. Numerous (>20) dorsal kineties.

Type location. Unknown; the population investigated was isolated from a freshwater aquarium in Boulder, Colorado.[2]

Type specimens. One slide of holotype specimens and one slide of paratype specimens have been deposited in the collection of microscope slides of the Upper Austrian Museum in Linz.

Description (Figs. 1, 2, 5–11, Table I). Cells cultured in

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[2] Dr. J. O. Corliss informed us after our paper had been submitted for review that he and Y.-B. Pang found probably the same species in China.

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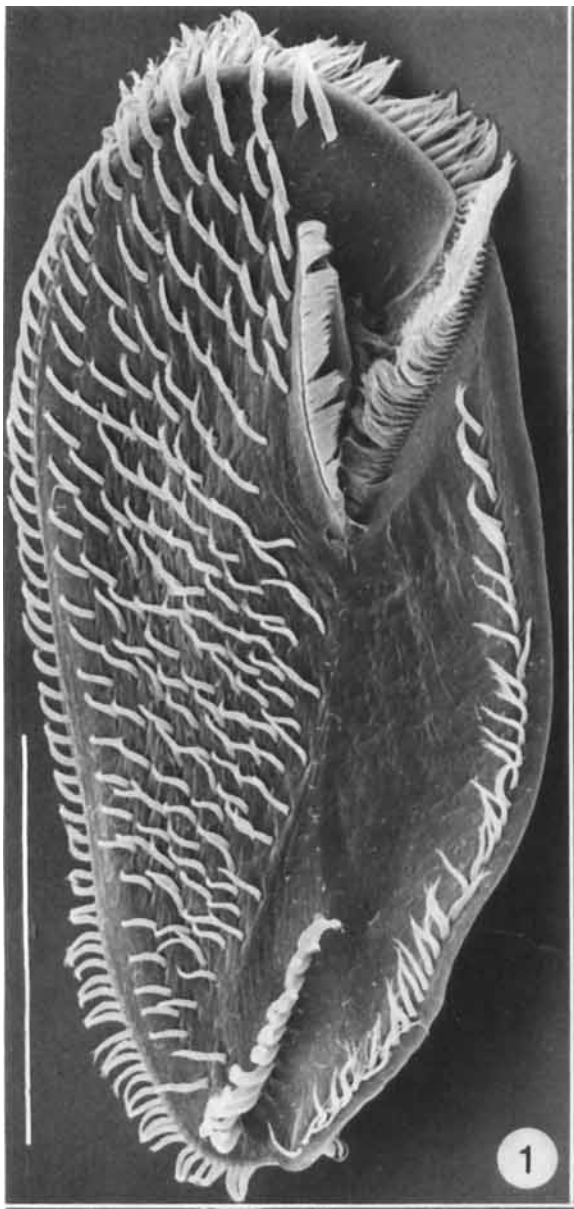
Figs. **1–24, 26, 27.** *Onychodromus quadricornutus.* Figures 1, 2, 5, 18, and 19 are scanning electron micrographs; Figs. 3, 4, 6, 8, 9, 12–17, 20–24, 26, and 27 are drawings and light micrographs from protargol-impregnated specimens; Figs. 7, 10, and 11 are from life. Figures 1, 2, and 5–11 show non-dividers; Figs. 3, 4, 12–24, 26, and 27 are from dividing specimens.

Figs. **1–6.** General organization and early morphogenetic stages. **1, 2, 5.** Ventral and dorsal views showing the cirral pattern and the four prominent horns on the dorsal side. The caudal horn (arrow) and the dorsal cilia (Dc), which are arranged in many long and short rows, are shown at higher magnification in Fig. 5. One caudal cirrus (CC) is inserted near the tip of the caudal horn. Bars = 100 μm, 100 μm, 20 μm. **3, 4.** Early stages of the development of the oral primordium (OP), which arises at the left and close to the uppermost transverse cirri. Note the round clusters of basal bodies at the posterior end of the primordium (triangle). Bars = 20 μm, 30 μm. **6.** Infraciliature of the ventral side. Note the macronucleus arranged as a question mark. The arrows denote the three posterior horns. Bar = 100 μm.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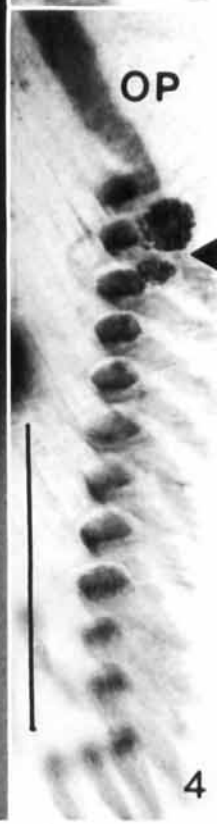
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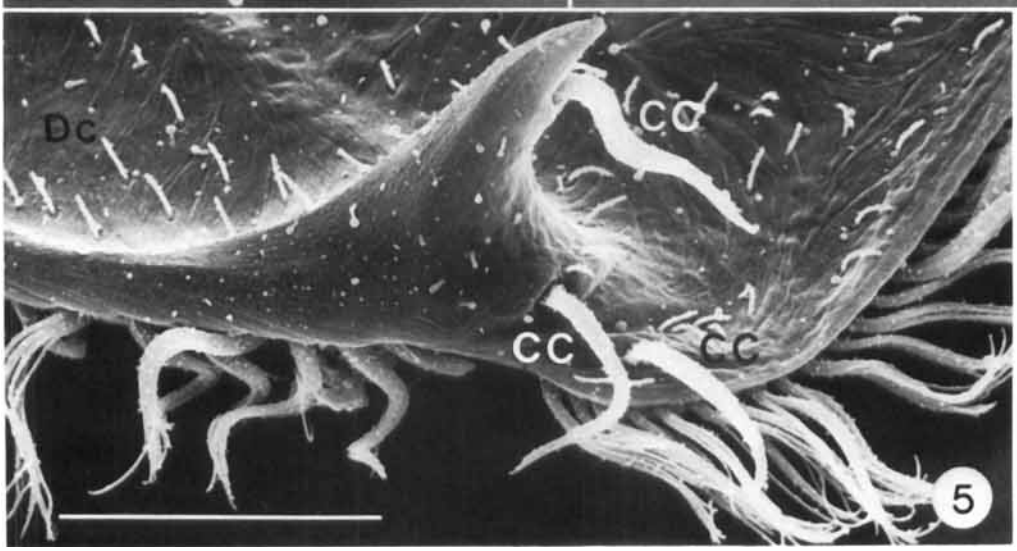
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TABLE I. *Biometric characterization of* Onychodromus quadricornutus *n. sp.*[a]

Character	$\bar{x}$	SD	CV	Min	Max	n
Body, length (spring-water culture)	272.5	48.5	17.8	170	361	50
Body, length (Pringsheim's solution)	423.3	112.8	26.7	222	694	50
Body, width (spring-water culture)	157.4	33.7	21.4	83	230	50
Body, width (Pringsheim's solution)	235.6	71.5	30.4	124	375	50
Adoral zone of membranelles, length	134.3	20.2	15.0	92	165	25
Nuclear figure, length	192.4	34.6	18.0	143	270	25
Macronuclear segment, length	18.3	4.8	26.2	11	28	25
Macronuclear segment, width	9.2	2.0	22.2	6	14	25
Micronucleus, length	5.2	0.7	13.3	4	6	25
Micronucleus, width	4.9	0.6	13.0	4	6	25
No. adoral membranelles	130.5	21.7	16.6	89	180	25
No. macronuclear segments	19.6	4.8	24.7	11	28	25
No. micronuclei	9.6	4.9	51.2	4	26	25
No. dorsal horns	4.0	0.0	0.0	4	4	50
No. ventral cirral rows	10.6	1.3	12.4	8	13	25
No. cirri in right marginal row	57.7	7.1	12.2	45	74	25
No. cirri in left marginal row	43.5	5.3	12.3	34	54	25
No. cirri in buccal row	5.6	1.2	20.5	3	8	25
No. transverse cirri	12.2	1.5	12.7	9	16	25
No. ventral cirri near transverse cirri	2.0	0.0	0.0	2	2	25
No. caudal cirri	3.0	0.0	0.0	3	3	25
No. dorsal anlagen during division	3.0	0.0	0.0	3	3	25
No. cirral primordia (proter)	12.6	1.5	11.9	10	16	25
No. cirral primordia (opisthe)	13.0	1.4	11.0	10	16	25

[a] All data are based, if not stated otherwise, on the investigation of randomly selected protargol-impregnated specimens originating from spring-water cultures. All measurements in micrometers. Legend: CV, coefficient of variation in %; Max, maximum; Min, minimum; n, number of investigated individuals; No., number; SD, standard deviation; $\bar{x}$, arithmetic mean.

Pringsheim's medium with *Chlorogonium elongatum* as food organism are considerably larger than those from the spring-water cultures (Table I). The reason is unknown, but suboptimal culture conditions may probably be excluded because ciliates divided readily and reached high abundance in the spring-water cultures too. A more detailed investigation of the size differences under various culture conditions was beyond the scope of this study. The differences, however, should be considered in the interpretation of the biometrical data, which surely do not reflect the whole potential variability of this species. A comparison with a natural (non-cultured) population would be of great interest. If not otherwise stated, all data in this study refer to individuals cultured in spring water.

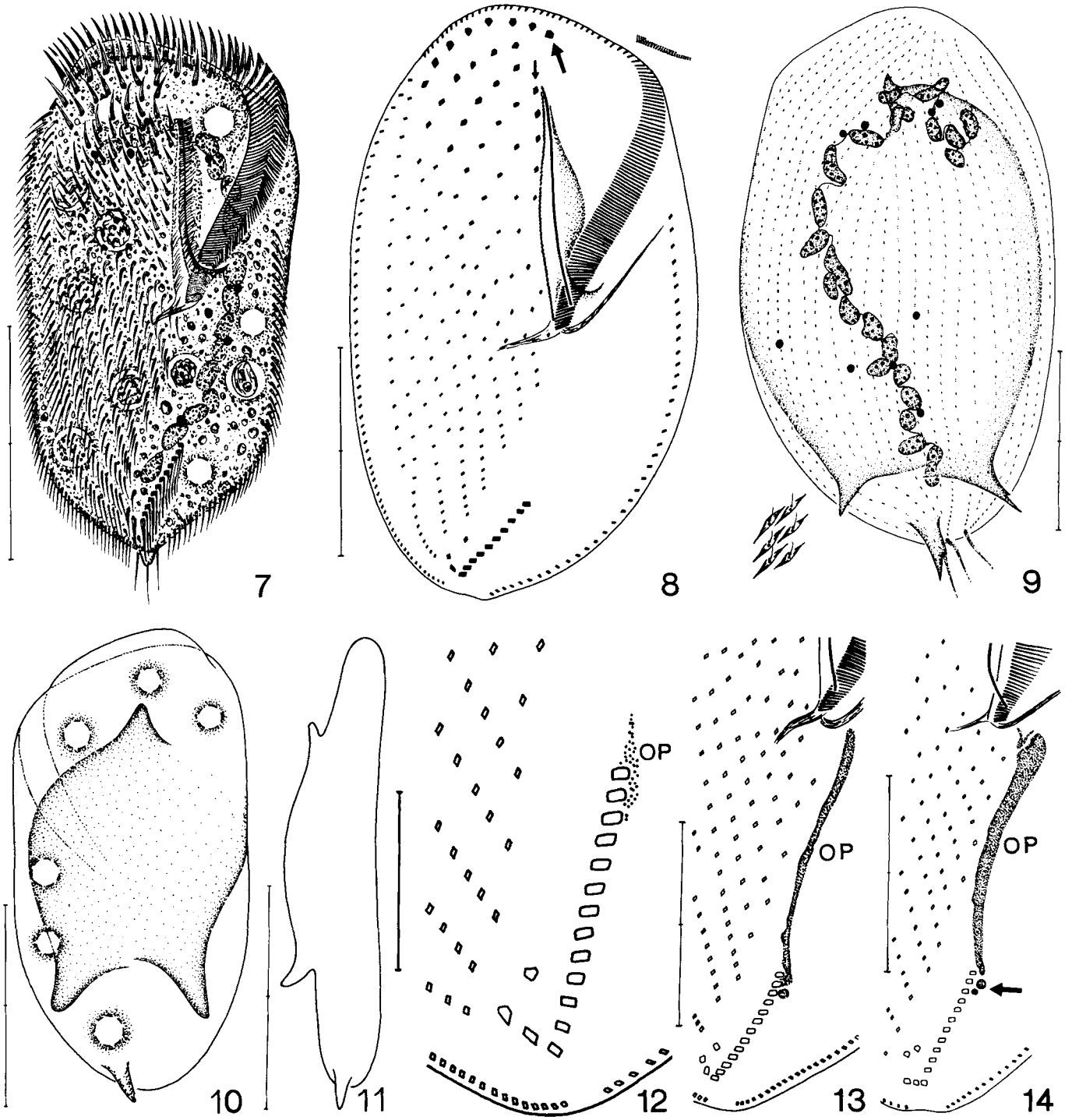
Body shape in vivo and in growing cultures rather variable, outline elliptical to lanceolate, sometimes distinctly truncated posteriorly. Many undersized individuals of very variable shape develop in old cultures. Body stiff, a little flexible under the cover glass, conspicuously (2–3:1) flattened dorso-ventrally, especially the rims. Constantly four prominent horns on the dorsal side, one horn in the upper half of the body near its center, two horns subterminal right and left of the median, and one smaller caudal horn. Horns cone-shaped, immobile, tips sometimes bifurcated, function unknown (Figs. 2, 5). The number and shape of the horns did not change with culture conditions and culture age. Macronuclear segments elliptical, connected by a fine strand, arranged like a question mark left of the median (Fig. 6). Micronuclei spherical, most of them lying near the macronucleus, some scattered in the cytoplasm. About four to six contractile vacuoles along the left and anterior body margin. Pellicle without subpellicular granules, very brittle; sharp-edged fragments can be broken out by touching the cell with a hair or fine needle. Cytoplasm colorless, filled with numerous fatty shining globules, 1–5 μm in diameter. No cytoplasmic crystals. Movement strikingly slow and jerky. Feeds on algae (*Chlorogonium elongatum*), zooflagellates, ciliates (*Chilodonella uncinata, Cyclidium glaucoma, Tetrahymena thermophila*), and wheat starch. Cannibalistic when starved.

Adoral zone of membranelles nearly ½ of body length, its posterior part covered by the pellicle. Bases of the largest membranelles in vivo about 25 μm wide, their fine details as in other hypotrichs. Buccal area large, considerably deepened, a small bulge at its right side. Two straight undulating membranes, cilia about 12 μm long. Cirral pattern constant. One left and right row of marginal cirri, about 20 μm long; those of the right row are sometimes rather irregularly distributed. Transverse cirri conspicuously enlarged and during movement of the ciliate, strikingly motionless, about 25 μm long; only the lowermost ones project slightly above the posterior body margin. Two slightly enlarged ventral cirri near the transverse cirri. Caudal cirri fine, the left cirrus being constantly inserted near the tip of the caudal horn (Fig. 5). One row of buccal cirri, no clustered (oxytrichid) postoral cirri. Ventral cirri arranged in 8–13 straight rows at the right of the undulating membranes. The rows elongate from right to left, with exception of the rightmost row, which is nearly as long as the body. Thus, a suture ("systeme secant") is produced at the right part of the ventral cirral field (Figs. 6, 8). The first 2–4 cirri of the leftmost rows are conspicuously enlarged, about 25 μm long, and form 2–3 arched "rows" along the anterior body margin.

Dorsal cilia in vivo 4 μm long, arranged in many (>20) rows, most of them being more or less shortened. This shortening and the small distances between the rows were the reason why it was impossible to do exact counting. All dorsal kineties appear composed of densely packed pairs of basal bodies, only the anterior being ciliated. Each pair is surrounded by fibrils forming a rhomboid figure (Figs. 9, 22).

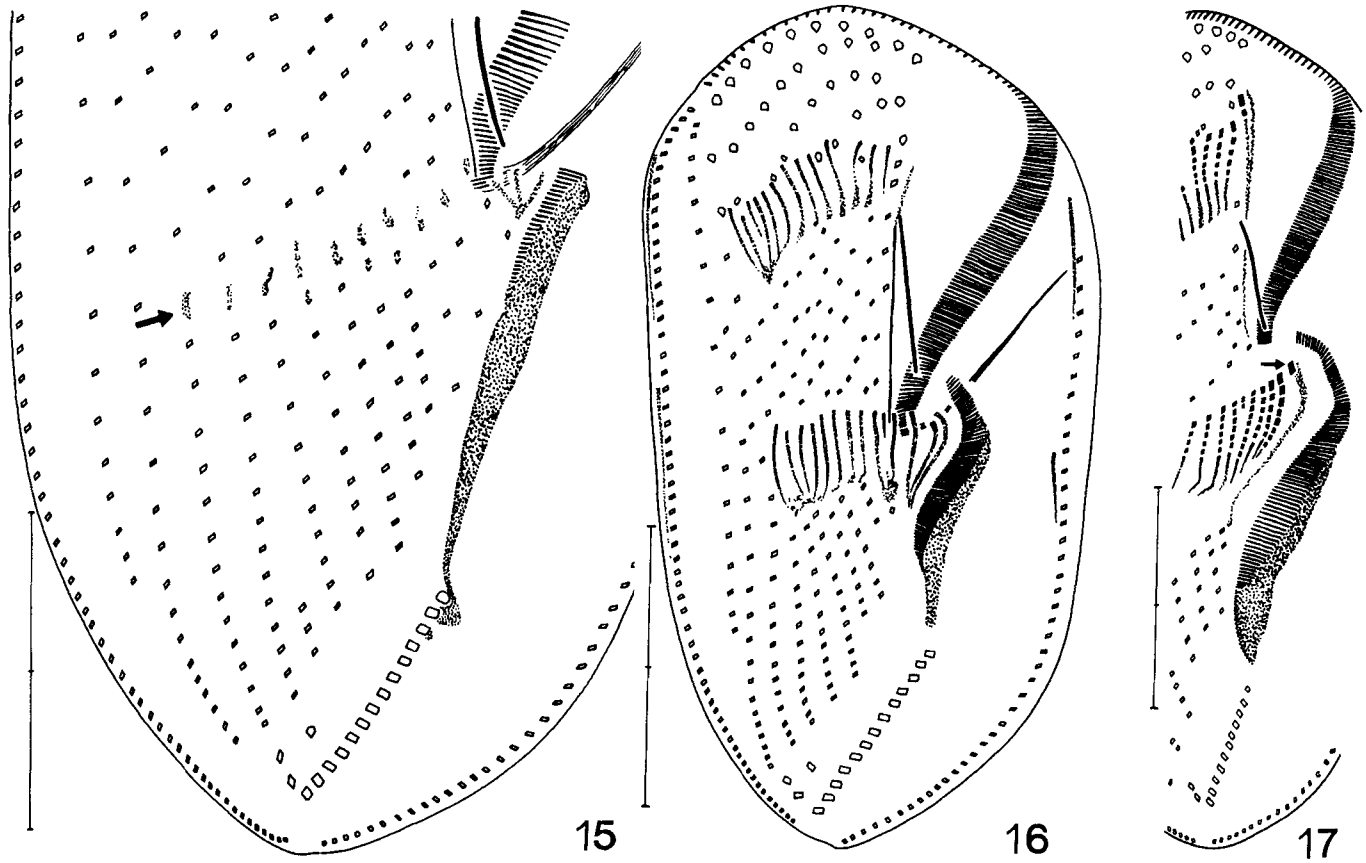
Cortical development during cell division (Figs. 3, 4, 12–24, 26, 27). The morphogenesis of *O. quadricornutus* is most similar to that of *Laurentiella acuminata.* To make a comparison easy, the style of description follows closely the paper of Martin et al. (18).

A few basal bodies develop at the left border of the first one to three transverse cirri, which appear intact (Figs. 3, 12). Basal bodies increase in number forming a narrow patch that extends anteriorly and reaches the proximity of the peristomial vertex. The resulting oral primordium (OP) is club-shaped, rounded anteriorly, and pointed posteriorly. About 50% of the dividing cells show one or two round clusters of basal bodies at the posterior end of the anarchic field (Figs. 4, 13, 14). From the anterior end of the OP, three streaks separate; the left one becomes the paroral primordium, the middle one the buccal primordium, and the right one develops to a ventral row (Figs. 15–17). The buccal row of the proter is formed by an anlage that develops near the parental row, whose cirri are resorbed at the end of the division process. Some parental cirri of the ventral



Figs. 7–14. General organization and early morphogenetic stages. 7, **10**, **11**. Ventral, dorsal, and lateral aspect of typical individuals from life. Note the contractile vacuoles forming a C-shaped figure. Bars = 200 μm, 170 μm, 190 μm. **8**, **9**. Infraciliature of the ventral and dorsal side. An enlargement of an adoral membranelle and some dorsal cilia is shown in the upper right and the lower left corner, respectively. The large arrow in Fig. 8 denotes the first frontal cirrus; the small arrow marks the buccal row. Bars = 100 μm. **12–14**. Development of the oral primordium (OP). Note the round clusters of basal bodies near the upper transverse cirri (arrow). Bars = 30 μm, 100 μm, 100 μm.

rows disintegrate and rearrange their basal bodies into the ciliary streaks appearing next, which average 13 for each tomite (Figs. 15–20, Table I). Those of the proter evolve a little bit later than those of the opisthe. On the average there are two frontal-ventral-transverse (FVT) anlagen more in the filial products than ventral rows in the interphase individuals, suggesting that some reduction occurs in later division phases. This reduction probably begins after the new transverse cirri have been separated, because the number of transverse cirri of the non-dividers is on the average the same as the number of FVT anlagen (Table I). No FVT anlagen develop within the two to three rightmost ventral rows, which remain unchanged during the whole divi-



Figs. **15–17**. Ventral view of middle morphogenetic stages. **15**, **16**. Three streaks split off from the upper end of the oral primordium. Cirral primordia evolve within the parental ventral rows (with exception of the two rightmost rows and the leftmost buccal row) by disintegration of some cirri (arrow). Bars = 90 μm. **17**. New cirri differentiate within the cirral streaks. The first frontal cirrus (comp. Fig. 8) splits off from the primordium of the undulating membranes (arrow). Bar = 100 μm.

sion process. These rows are produced by splitting of some of the rightmost FVT anlagen, in both the proter and the opisthe (Figs. 16, 18, 23).

Development of cirral primordia is accomplished by growth, in both length and width, of thin streaks and by posterior fragmentation in cirri, which progresses as an anteroposterior wave, transverse cirri being the last and largest, except for the frontal cirri that derive from the approximately eight leftmost cirral streaks (Figs. 16–18, 20, 23, 24). While this happens, the rest of the OP differentiates into membranelles. Alignment of membranelles starts at the anterior right edge of the OP. The paroral primordium of the opisthe develops as a long anarchic field of basal bodies parallel to the right border of the OP and remains connected with streaks II and III until the start of organization of the endoral membrane and the first (leftmost) frontal cirrus (Figs. 16, 17, 23, 24). Thus, the FVT system of the opisthe derives from usually 13 cirral primordia: three of these come from the OP and 10 originate by proliferation and rearrangement of basal bodies from old ventral cirri.

The paroral and endoral membrane, the buccal area, and some fibrillar structures of the proter undergo a partial disorganization. The basal bodies lose temporarily their linear arrangement and later rearrange and form again the paroral and endoral membrane and a new first frontal cirrus. The buccal area appears smaller, less deep, and the fibrils in the peristomial vertex disappear (Figs. 18, 23, 24). The parental adoral zone of membranelles is inherited without any appreciable changes. Thus, the FVT system of the proter evolves from one paroral primordium and, on the average, 12 primordia which originate by proliferation and rearrangement of basal bodies from parental ventral cirri (Figs. 16–18, 23, 24).

All cirral streaks of the proter develop *without* participation of the primordia of the opisthe. The evidence is the high number of unchanged parental cirri and the large gap between the two

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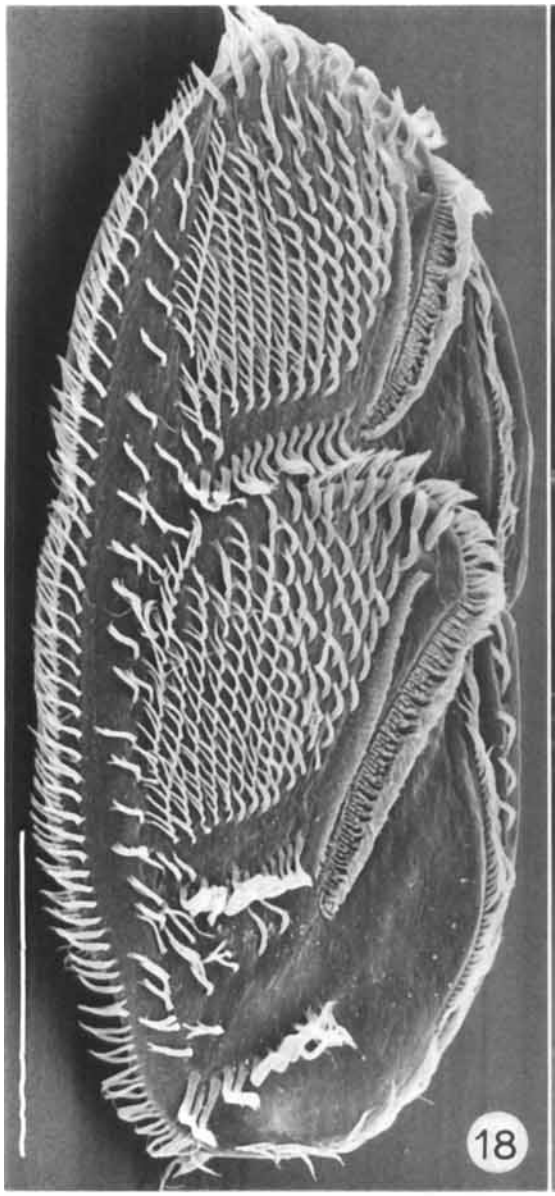
Figs. **18–22**. Middle and late morphogenetic stages. **18**, **19**. Ventral and dorsal view of late stages. The right parental marginal row and the rightmost parental ventral row are still unchanged. The oral apparatus of the proter shows clear symptoms of some reorganization (comp. Fig. 1), e.g. lack of the cilia of the undulating membranes. The triangles mark the developing new dorsal kineties, which evolve by multiple fragmentation of single main streaks (comp. Fig. 21). Bars = 100 μm. **20**. Ventral view of a middle morphogenetic stage. The small arrow denotes a splitting cirral streak; the two larger arrows mark the developing marginal rows. Bar = 100 μm. **21**, **22**. Early and late stage of the development of a dorsal ciliary streak. A new caudal cirrus evolves at the posterior end of the main kinety (arrow), at right of which many short kineties develop. The parental dorsal cilia are still visible (small arrows). Bars = 50 μm.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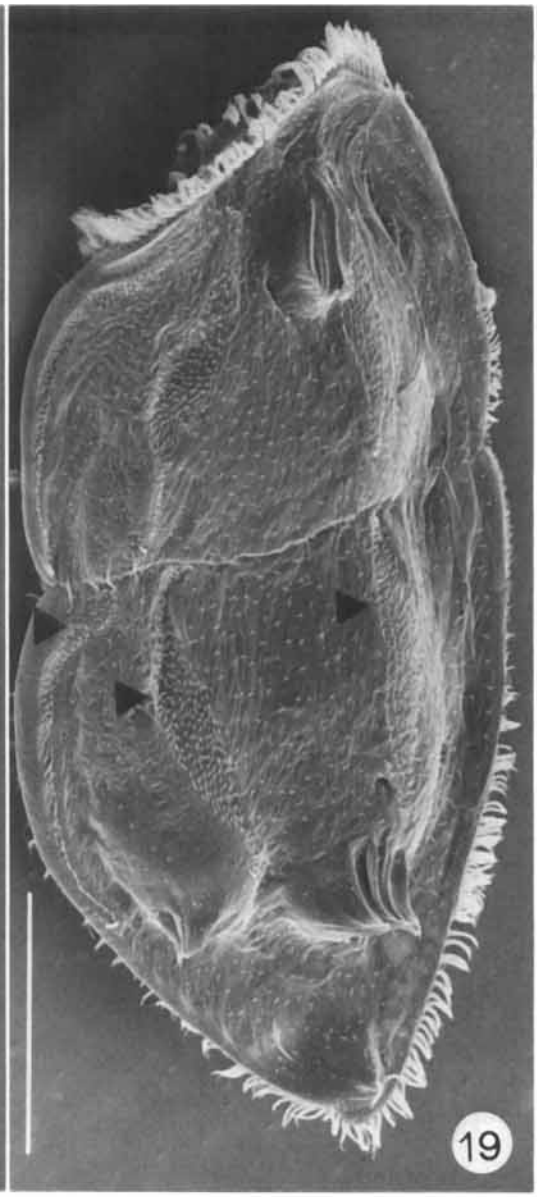
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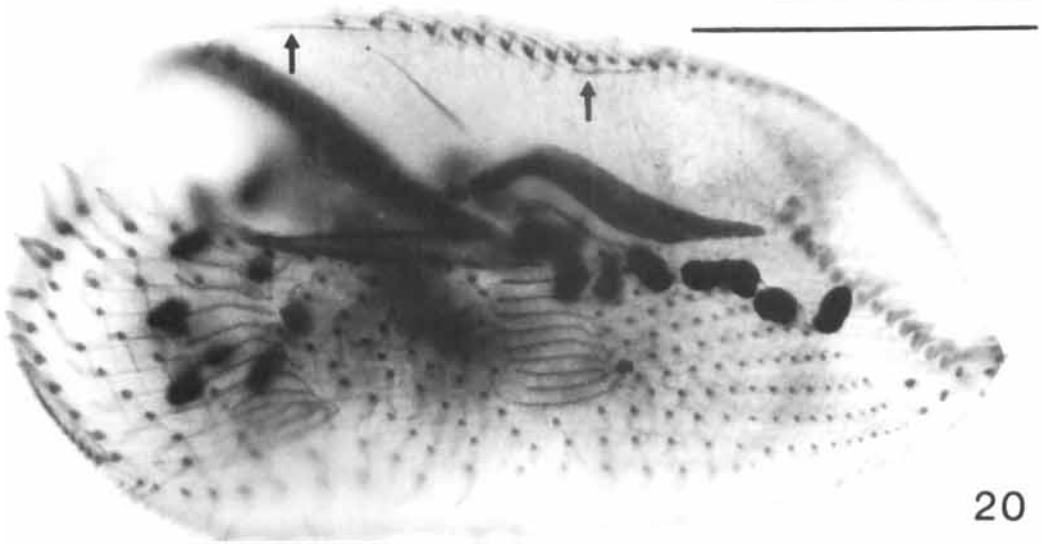
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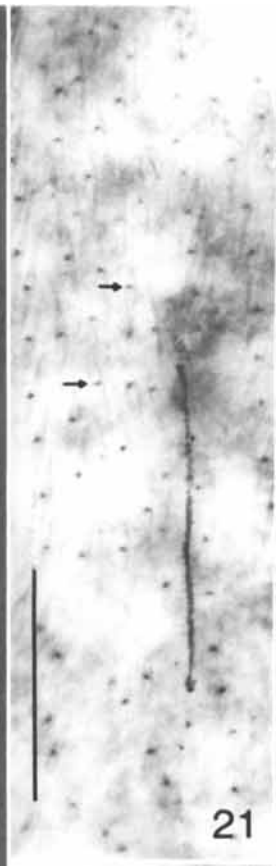
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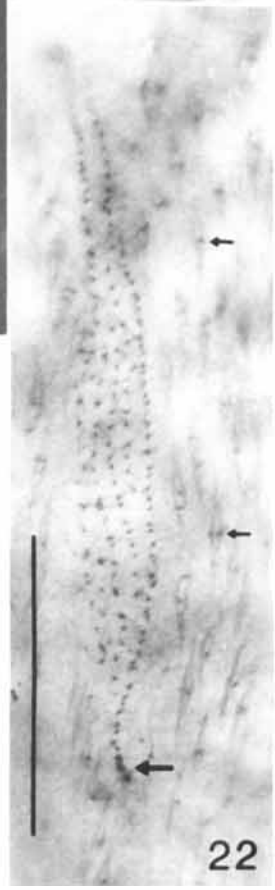
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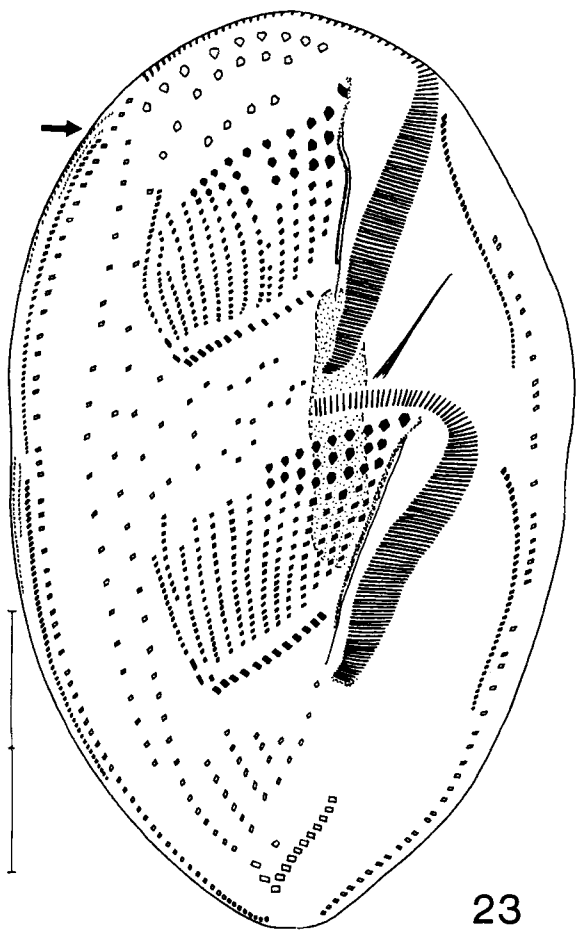

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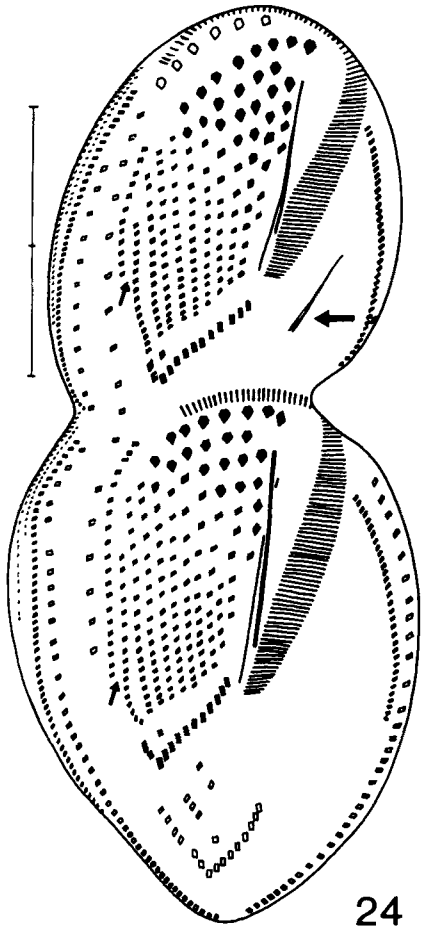

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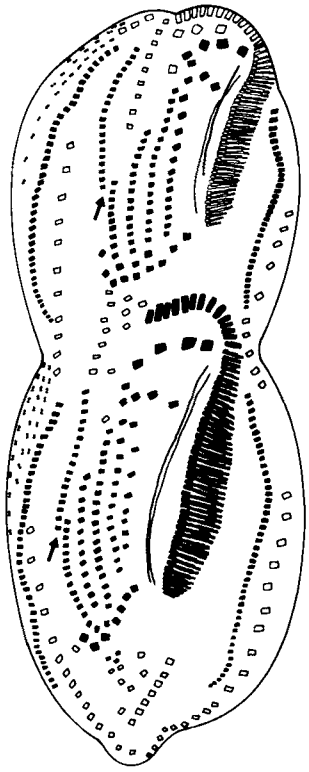

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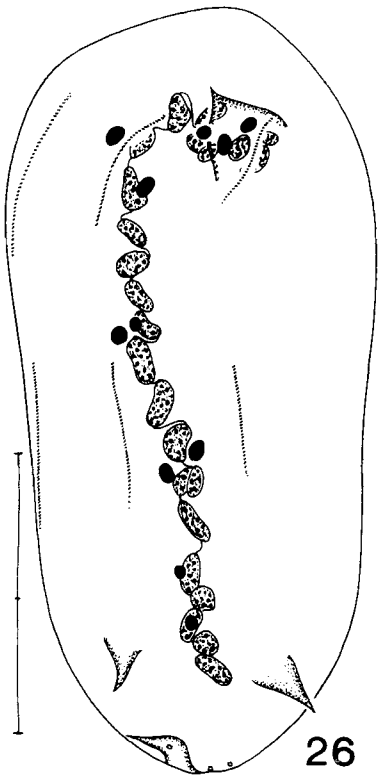

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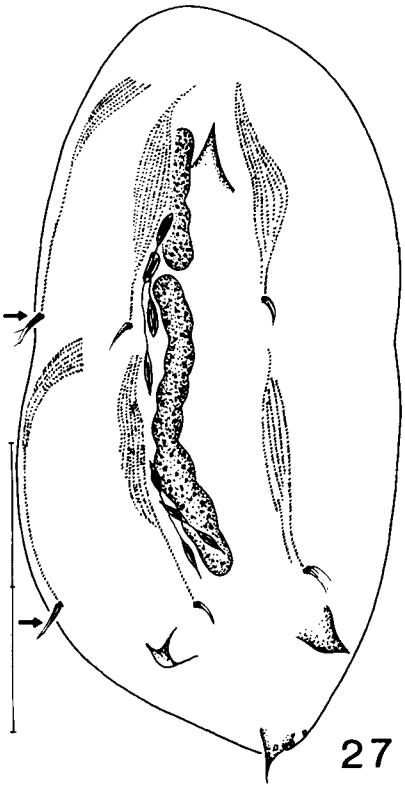

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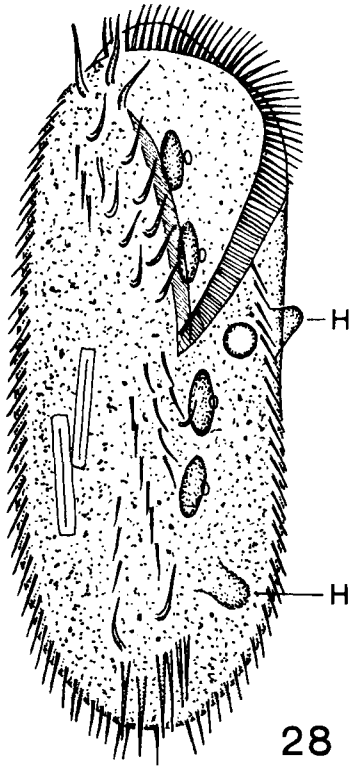

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anlagen fields in the early and middle stages of the division process (Figs. 15–17, 20, 23).

New marginal cirri appear a little bit later than the FVT anlagen and are built from streaks always formed closely at the right of the old rows at two sites on each side, one anterior and the other posterior to the future fission plane. No parental marginal cirri are involved in this process, as indicated by the unchanged parental right marginal row in the early and late division stages (Figs. 16, 18, 20, 23). Anterior and posterior elongation of each primordium and subsequent fragmentation form the new marginal cirral rows.

The dorsal ciliature derives from many kineties in three sets. One set of three kineties develops by proliferation of some basal bodies of three parental dorsal ciliary rows (Figs. 21, 26). At the posterior end of these, kineties organize the new caudal cirri (Figs. 22, 27). A second set of two kineties develops near the anterior limit of each newly developing right marginal row and then extends to the anterior half of the cell (Figs. 23, 24). A third set of many short kineties develops by multiple fragmentation of the anterior ends of the three dorsal ciliary rows which produce the caudal cirri (Figs. 19, 22, 27).

All ciliary organelles—ventral, marginal, and dorsal—that do not participate in morphogenesis disappear after cytokinesis. The dorsal horns develop at the end of the fission process. The macronuclear segments fuse to a rod-like mass during the middle stages of the division. Later, this mass segregates into the typical nodulated macronucleus of the interphase individuals (Figs. 23, 27).

DISCUSSION

Nomenclatural rectification. Lin & Prescott (17) introduced the name "*Styx* sp." for our species but gave no description that would satisfy the rules of the International Code of Zoological Nomenclature. Thus, this name is a *nomen nudum* and needs not be considered further. In addition, the "*Styx* sp." of these authors is a junior homonym of *Styx* Staudinger, 1875, a butterfly, and *Styx* Oppenheim, 1895, a mollusc (19).

Comparison with other species. Onychodromus quadricornutus is unique among all described hypotrichs by having four dorsal horns. It is probably the most voluminous hypotrich ciliate known (ca. 2×10^6 μm^3 for individuals from springwater cultures and ca. 5×10^6 μm^3 for those cultured in Pringsheim's medium, reaching peak values up to 20×10^6 μm^3 for the largest cells). To our knowledge, there is only one other species with horns: *O. grandis* Stein, 1859 (23, 24). This species has two bulges near the left margin, clearly depicted by Stein (24, Taf. V, Figs. 3, 4; redrawn in Fig. 28 of this paper). Unfortunately, this important character is lacking in later descriptions of this species (e.g. 15, 21) and even in Kahl (16), indicating misidentification of species or superficial observation. The caudal projections of some other hypotrichs, e.g. *Psilotricha* (7), *Aspidisca* (16), and various terrestrial species (9), are very probably not homologous to the dorsal horns of *O. quadricornutus* because they are simple elongations at the end of the cell, whereas the horns of *O. quadricornutus* and *O. grandis* are a special differentiation of the dorsal side.

Concerning the general appearance, the nodulated macronucleus, and the infraciliary pattern, *O. quadricornutus* resembles species of the genus *Laurentiella* and *Coniculostomum*. The bewildering nomenclatural and taxonomic problems within this and related genera have been discussed by others (12 and H. Hemberger, Dissertation, University of Bonn, 1982) and are beyond the scope of this paper. With respect to the size, the shape, the moniliform macronucleus, and the extensive ventral and dorsal ciliature, *Coniculostomum monilata* is probably the most similar species (see ref. 4). This species lacks, however, dorsal horns, and the left half of its ventral ciliature is much more irregularly arranged than in *O. quadricornutus*.

Morphogenetic comparison and systematic position of O. quadricornutus *(Table II). Onychodromus quadricornutus* and *Laurentiella acuminata* share some important characters (cirral pattern, number of dorsal kineties and FVT anlagen, fragmentation of dorsal primordia) with the oxytrichids sensu lato (e.g. *Paraurostyla*) in the phylogenetic system of the oxytrichids (29). *Parakahliella* and *Pleurotricha* clearly belong to other lineages as indicated by their increased number of self-replicating marginal rows and—in *Pleurotricha*—by the clustered frontal cirri and postoral ventral cirri. There is, however, no clear *generic* separation possible with non-morphogenetic characters between species like *Onychodromus quadricornutus, O. grandis, Laurentiella acuminata, Parakahliella macrostoma,* and *Paraurostyla weissei*. The differences are rather vague (characters 1, 2 in Table II) or simply quantitative (characters 6, 8).

A main difference between *O. quadricornutus* and *L. acuminata* concerns the development of the cirral primordia of the proter and the opisthe. They evolve independently in *O. quadricornutus* and in some continuity in *L. acuminata*. This character has been used to separate some oxytrichid genera (6) and is perhaps an argument for the validity of the genus *Laurentiella*, which has been synonymized with *Onychodromus* (H. Hemberger, Dissertation, University of Bonn, 1982). Unfortunately, nothing has been published about the morphogenesis of the type species, *O. grandis*. Thus, it is impossible to make any defensible conclusion about the generic status of *Laurentiella acuminata* and *Onychodromus quadricornutus*; however, the dorsal horns occurring in both, *O. grandis* and *O. quadricornutus*, are a strong apomorphic character suggesting congenerity of these species.

[3] We are obliged to Dr. J. Martin for his kindness in sending us protargol-impregnated slides of this species. A reinvestigation showed clearly multiple fragmentation in the dorsal primordia; it is, however, a little different from that occurring in *O. quadricornutus*. The left kinety (observed from the ventral surface) fragments as in *O. quadricornutus*, the middle one does not fragment, and the right kinety has a simple fragmentation like kinety 3 in *Oxytricha granulifera* (10) and *Histriculus muscorum* (2). In addition, the posterior fragment shows some multiple fragmentation at its anterior end.

←

Figs. 23–28. Middle and late morphogenetic stages and comparison with other species. **23, 24.** Ventral view of late morphogenetic stages. Positioning of new structures is in progress when macronuclear segments fuse. Two dorsomarginal kineties evolve close to the new right marginal cirral rows (arrow in Fig. 23). The small arrows in Fig. 24 point to the shifting rightmost ventral rows; the large arrow denotes the rest of the fibrils of the parental oral apparatus (comp. Fig. 8). Bars = 100 μm. **25.** *Paraurostyla weissei*. Ventral view of a late morphogenetic stage. Note shifting of the rightmost ventral row (from ref. 28). **26, 27.** Early and middle stages in the development of the dorsal infraciliature. To make details clearer, parental dorsal cilia have been omitted. Three anlagen evolve near parental cilia in the proter and opisthe (comp. Fig. 21). Later, many dorsal kineties of different lengths develop by multiple fragmentation of these three primordia (comp. Figs. 19, 22). New caudal cirri develop at the posterior end of the main kineties (arrows in Fig. 27). Bars = 130 μm. **28.** *Onychodromus grandis* has two lateral horns (H) (redrawn from ref. 24).

TABLE II. *Morphologic and morphogenetic comparison of seven morphologically similar hypotrich ciliate species.*

Character	Species[a]						
	O. q.	*O. g.*	*L. a.*	*P. w.*	*P. m.*	*P. l.*	*K. p.*
1 Frontal cirri clearly separated from ventral rows?	no	yes	yes	yes	yes	yes	no
2 Ventral cirri in highly ordered rows?	yes	no	yes	yes	yes	yes	yes
3 Clustered posterior frontal cirri and postoral ventral cirri?	no	no	no	no	no	yes	no
4 Number of right marginal rows	1	1	1	1	>1	1	1
5 Number of left marginal rows	1	1	1	1	>1	1 to >1	1
6 Number of caudal cirri	3	3	3	~13	~5	3	3
7 Transverse cirri present?	yes	yes	yes	yes	no	yes	yes
8 Number of FVT anlagen	~13	6	~6–8	~6–9	5	6	6–7
9 Continuity between the cirral primordia of proter and opisthe?	no	no	yes	no	no	?	no
10 Parental buccal cirrus (i) involved in development of cirral primordia?	no	no	no	yes	yes	?	yes
11 Multiple fragmentation of dorsal kineties during morphogenesis?	yes	yes	yes[b]	no	no	?	yes
12 Number of dorsomarginal kineties	2	6	6	2–3	1	?	2
13 Parts of parental somatic infraciliature preserved in post-dividers?	no	no	no	no	yes	?	no

[a] Species and data source: *Onychodromus quadricornutus* (present paper), *O. grandis* (ref. 24 and unpubl. results of Foissner), *Laurentiella acuminata* (18), *Paraurostyla weissei* (28), *Parakahliella macrostoma* (2), *Pleurotricha lanceolata* and *P.* sp. (Hemberger, Dissertation, University of Bonn, 1982, 14), *Kerona pediculus* (11; for nomenclature see ref. 8).

[b] Although not mentioned in ref. 18, multiple fragmentation occurs (see footnote 3).

Likewise, the occurrence of multiple fragmentation of the dorsal primordia in *L. acuminata*[3] support the suggested inclusion of this species into the genus *Onychodromus* (13). This is strengthened by a study on the morphogenesis of *O. grandis* which is in progress (Foissner, unpubl.). The main results are 1) the oral primordium originates apokinetally and has no contact with the transverse cirri; 2) six cirral streaks are formed as in the oxytrichids s. str. (see ref. 29); 3) multiple fragmentation of the dorsal primordia occurs and it is very similar to that described in *L. acuminata* ([39], footnote 3 of this paper) especially in developing numerous small dorsomarginal kineties close to the new right marginal cirri.

Unfortunately, the phylogenetic significance of the dorsal infraciliature is not well understood, but some authors propose that it is important (10, 18). Different modes of origin, however, occur in species with a nearly identical ventral morphogenetic pattern and which are thus at present in the same genus (10). Multiple fragmentation is not very common but occurs also in *Kerona pediculus,* which belongs to a different family from *Onychodromus* and *Laurentiella* with regard to the development of the frontal cirri (11). Nevertheless, *O. quadricornutus* and *K. pediculus* have many morphological and morphogenetic characters in common (Table II), indicating a close relationship of these families or highly convergent evolution.

Another striking similarity exists between *O. quadricornutus* and *Paraurostyla weissei.* The rightmost ventral row of *P. weissei* evolves from two cirral streaks and appears very similar to the rightmost ventral rows of *O. quadricornutus* during the late divisional stages (comp. Figs. 24, 25). Another species, *Urostyla hologama* (very probably a *Paraurostyla* species; see ref. 28) even shows the same pattern as *O. quadricornutus* during the interphase. It is, however, very likely an analogy because these rows are self-replicating in *P. weissei,* whereas they evolve by division of the cirral streaks of the neighboring ventral rows in *O. quadricornutus.*

Considering this discussion and the data in Table II, hypotrich classification does not look very satisfactory. Our studies produce more and more evidence that many convergent characters exist. Furthermore, the situation is complicated, at least for ecologists and classical morphologists, by the occurrence of sibling species (e.g. 1). Much more work at all taxonomic and methodological levels is necessary to elucidate the true phylogenetic relationships within the hypotrichs. Nevertheless, *O. quadricornutus* can be reliably classified as a member of the superfamily Oxytrichoidea Jankowski, 1979 (12) and is probably in the same evolutionary line as *Paraurostyla* and *Laurentiella.*

Two of the three reviewers of the paper considered our decision to ally this conspicuous species with *Onychodromus* as being too conservative. They suggested a new genus. As already mentioned and shown in Table II, however, the ventral and dorsal cirral pattern and the morphogenetic events are very similar in *O. quadricornutus, O. grandis,* and *L. acuminata.* The rather different appearance of the interphase individuals is simply caused by the very different number of cortical units (cirri etc.), but not by a basic difference of the pattern. Thus, we prefer to be conservative at this stage of knowledge. There are already too many monotypic and/or poorly defined hypotrich ciliate genera.

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A Multinucleated Marine Amoeba Which Digests Seaweeds[1]

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ABSTRACT. Marine amoebae were isolated during a search for organisms which degrade cell walls of seaweed. One of the isolates, a multinucleated amoeba (referred to here as Amoeba-I-7 or Am-I-7) was isolated from live tissues of the brown seaweed *Sargassum muticum*. It digested a variety of brown and red seaweeds including their walls and cuticles. Axenic clone cultures were isolated from cells that migrated on agar. Cultures were grown on agar or in liquid media. Seaweeds, seaweed wall extracts, and unicellular algae were tested as food sources.

AMOEBAE are a diverse group of protozoa with widely varied characteristics and habitats. Most amoebae in the aquatic environment are believed to be associated with detritus and to feed on bacteria, decaying plant and animal matter, or microscopic algae (3). Some amoebae were isolated from drifting specimens of the brown seaweed *Sargassum*. Since the same species were also isolated from water samples, it was assumed that the amoebae isolated from the seaweed fed on bacteria associated with the surfaces rather than on the plant tissue itself (5).

During our search for microorganisms capable of decomposing seaweed cell walls, several species of amoebae were isolated from intact seaweed tissues. These amoebae were clone-cultured axenically, and flourished on seaweed tissues free of debris or bacteria. Amoebae-I-7 (Am-I-7) was one of the isolates which was able to digest rapidly and completely a variety of brown and red seaweeds. This paper describes Am-I-7, its isolation and culture techniques, and the effectivity of various seaweeds as food sources.

MATERIALS AND METHODS

Isolation of Am-I-7. Young plants of *Sargassum muticum*, carrying amoebae, were collected in January 1984 from rocky shores at Alegria beach, Hollister Ranch, Santa Barbara County, California. Reproductive plants of *Sargassum* which were also infected were collected at the same site in April 1985. Moist

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