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Divisional Morphogenesis in *Steinia sphagnicola* (Ciliophora, Hypotrichida): a Comparative Light and Scanning Electron Microscopic Study

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SUMMARY

The morphogenesis of the hypotrichous ciliate *Steinia sphagnicola* Foissner, 1989 was investigated using protargol impregnation and scanning electron microscopy. Morphogenesis commences with the proliferation of basal bodies for the oral primordium close to the anteriormost transverse cirrus, which appears unchanged, indicating that the oral primordium originates apokinetally. The opisthe's adoral zone of membranelles sinks into the cell during its differentiation, which might be an important difference between euhypotrichs and pseudohypotrichs. The proter's oral structures show a cryptic reorganization during divisional morphogenesis, indicated by the decreased length of the cilia, the disappearance of the fragmentation of the endoral membrane and the flattening of the buccal cavity and buccal pit. During their reorganization the undulating membranes undergo a series of transformations resembling patterns found in morphostatic cells of *Notohymena, Stylonychia, Cyrtohymena* and *Oxytricha*. The genus-specific pattern of the undulating membranes and the fragmentation of the endoral membrane are achieved only shortly after cell division, indicating that these characters are young evolutionary acquisitions. The six fronto-ventral-transverse-anlagen of *S. sphagnicola* develop in the usual oxytrichid mode, except for the third postoral cirrus, which is not involved in the formation of cirral primordia, and for the second postoral cirrus, which develops, like in *Stylonychia lemnae*, the anlagen 5 and 6 for both the proter and opisthe. During streak formation new basal bodies are possibly produced at the rear end of the streaks and these push forward the older ones. Our data prove a close relationship between *Steinia* and *Stylonychia* and suggest the following evolutionary sequence within oxytrichids s. str.: *Oxytricha, Cyrtohymena, Notohymena, Stylonychia, Steinia.*

Introduction

The shape and arrangement of the paroral and endoral membrane are useful characters for discriminating oxytrichid hypotrichs [2, 4, 6, 19]. Foissner [6] restricted the genus *Steinia* Diesing, 1866 to species having a sinusoidal and fragmented endoral membrane. For species whose distal part of the paroral membrane is hemispherically curved, the genera *Cyrtohymena* and *Notohymena* were established. *Notohymena* differs from *Cyrtohymena* by the broadened, hooked distal end of the paroral membrane [2]. In *Stylonychia* the undulating membranes are straight and parallel, in *Oxytricha* they are curved and intersect [2, 6, 9, 23, 24, 29, 30].

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Grolière [15] described the morphogenesis of *Steinia platystoma*, but provided only four illustrations with very sparse details on the development of the somatic and oral infraciliature. Therefore, we investigated the morphogenesis of *Steinia sphagnicola*, to complete the detailed ontogenetic characterization of oxytrichid hypotrichs with very similar ventral ciliatures but rather different oral structures [2, 6, 23, 24].

Material and Methods

Steinia sphagnicola Foissner, 1989 was collected in spring 1991 in a small tributary (Windach) to the River Amper near Munich, about 100 m above the purification plant of the village of Eching.

Cultures were set up in petri dishes containing water from the sampling site and a few crushed wheat grains to support growth of indigenous bacteria and small ciliates which served as food organisms. *Steinia sphagnicola* divided readily in these cultures for some weeks.

The infraciliature was revealed with protargol (protocol 2) and by scanning electron microscopy (SEM). See Foissner [7] for detailed descriptions of these methods. Drawings were made with the help of a camera lucida. To make plain the changes during morphogenesis, old (parental) cirri are depicted by contour, whereas new cirri are shaded black. Terminology and morphometric characterization are according to [3, 6, 9, 22, 26].

Results

Interphase Morphology of Steinia sphagnicola, German Population (Figs. 1–3, 6–9; Table 1)

The German population matches the Austrian type population well (Table 1). The figures and a short description should thus suffice to orientate the reader. It is worth noting the deviating number of ventral and transverse cirri in a few specimens probably caused by the culture conditions.

Size in vivo about 160 × 80 µm. Elliptical, right body margin convex, left slightly concave, posterior end narrowed and bluntly pointed, anterior end broadly rounded. Body rigid (as in *Stylonychia*), flattened about 2 : 1 dorso-ventrally. Two macronuclear segments, usually two micronuclei. Contractile vacuole in midbody at left margin, with two collecting canals. No conspicuous cortical granules.

Adoral zone of membranelles about 50% of body length. Buccal field large and deep, i.e. extending almost to dorsal surface, distal portion semicircularly curved to left and spirally rolled up to form peculiar, circular pit also extending to dorsal surface (Figs. 1, 8). Paroral membrane less distinctly curved than buccal area, commences left of first frontal cirrus and extends left of narrow ridge (buccal lip) to proximal fifth of adoral zone of membranelles, made of zigzag lined basal bodies having 25 µm (!) long cilia forming conspicuous membrane covering buccal area (Figs. 2, 6–8). Buccal lip hyaline, broadens anteriorly, covers most of buccal pit; pit bounded in front and at left by cortex, at right by endoral membrane. Endoral membrane sinusoidal, consists of about 10 fragments, commences at same level as paroral membrane but terminates more posteriorly, viz. at proximal end of adoral zone of membranelles.

All cirri remarkably long (about 30–35 µm), arranged in usual oxytrichid pattern; posteriormost frontal cirri and anteriormost postoral cirri, however, remarkably paired. Transverse cirri in two groups, right group includes posteriormost ventral cirrus (Figs. 1, 2, 6, 7). Dorsal cilia in vivo 4 µm long, arranged in 4 rows as long as cell and in 2 rows half as long as cell. Caudal cirri at end of rows 1, 2 and 3 (Fig. 3).

Divisional Morphogenesis (Figs. 4, 5, 10–49; Table 2)

The nuclear apparatus divides in the usual way and hence requires no further comment (Figs. 4, 20, 34–47).

Stage 1 (Figs. 4, 10). A few basal bodies develop at the left edge of the anteriormost transverse cirrus, which appears intact both in the light and scanning electron microscope. Ciliary stubs are recognizable on all basal bodies.

Stage 2 (Figs. 5, 11). The basal bodies increase in number and form an anarchic field (oral primordium) which extends to the peristomial vertex. The oral primordium is club-shaped, curved, and broadly rounded anteriorly, where the ciliary stubs are slightly longer than in the posterior portion of the primordium.

Stage 3 (Figs. 12–17, 22, 23). A short streak of basal bodies, later becoming the opisthe's fronto-ventral-transverse anlagen (FVT-anlagen) 1–3, separates from the right anterior portion of the oral primordium, where adoral membranelles commence to form from anterior to posterior. Simultaneously, the second postoral cirrus disaggregates and organizes proter's and

Figs. 1–5. Morphology and morphogenesis of *Steinia sphagnicola* from life (Fig. 1; from [6]) and after protargol impregnation ► (Figs. 2–5). – Figs. 1, 2. Ventral views. – Fig. 3. Dorsal view. – Figs. 4, 5. Very early dividers. Arrow in Fig. 4 marks oral primordium close to anteriormost transverse cirrus. AFC = anterior frontal cirri, AZM = adoral zone of membranelles, BC = buccal cirrus, CC = caudal cirri, EM = endoral membrane, LMR = left marginal row, MA = macronuclear segment, MI = micronucleus, OP = oral primordium, P = buccal pit, PFC = posterior frontal cirri, PM = paroral membrane, POC = postoral cirri, RB = replication band, RMR = right marginal row, TC = transverse cirri, VC = ventral cirri, numbers 1 to 6 = dorsal kineties. Scale bar division = 10 µm.

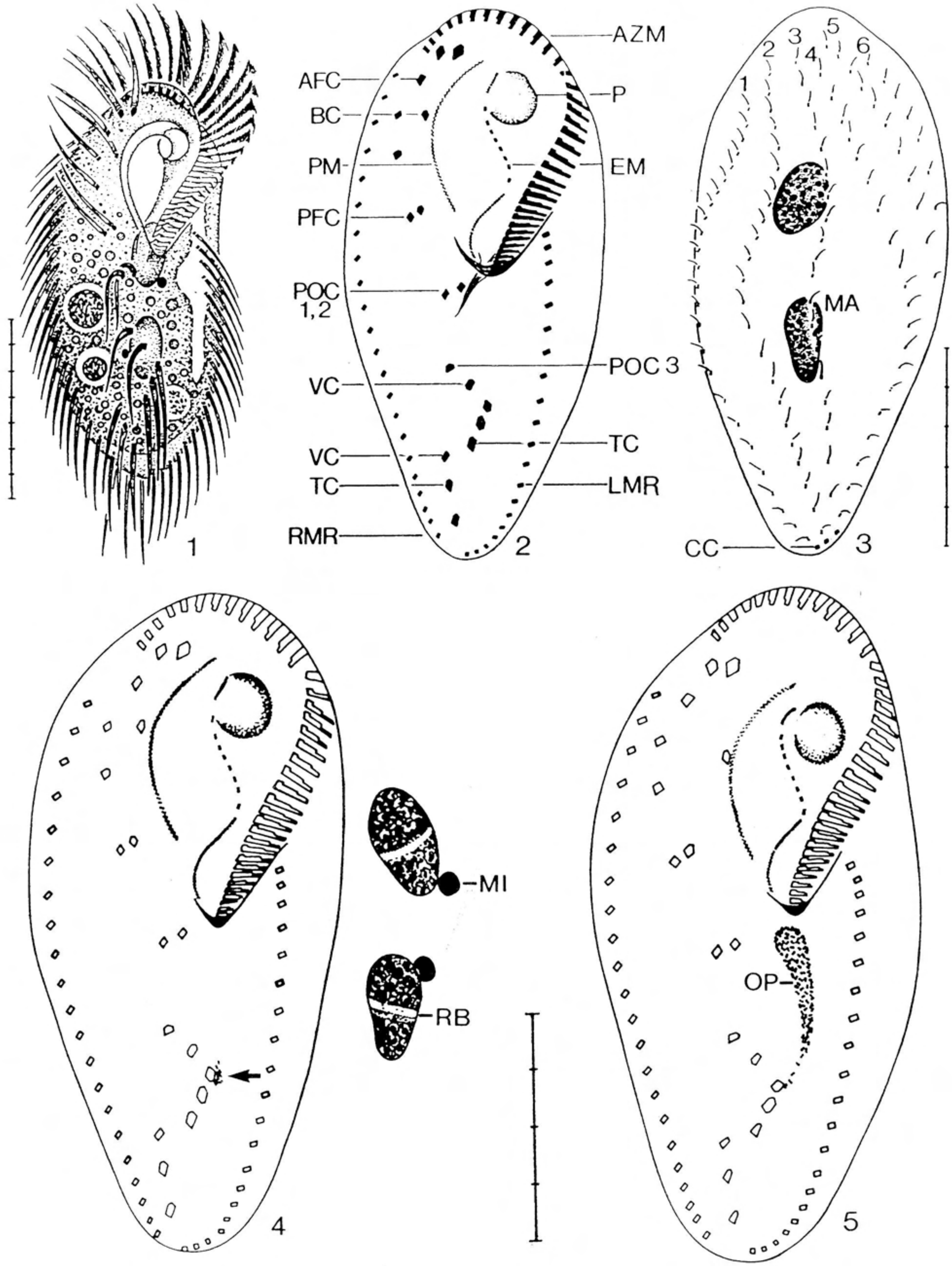
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Table 1. Morphometric characteristics from *Steinia sphagnicola*. Upper line: Austrian type population (from [6]); lower line: German population

Character[1]	x̄	M	SD	SE	CV	Min	Max	n
Body, length	140.7	141.0	11.3	3.0	8.0	122.0	157.0	14
	143.0	138.5	17.5	3.5	12.2	112.2	176.0	25
Body, width	60.4	59.5	7.6	2.0	12.6	46.0	72.0	14
	68.5	66.0	10.3	2.1	15.0	55.0	99.0	25
Macronuclear segments, number	2.0	2.0	0.0	0.0	0.0	2.0	2.0	14
	2.0	2.0	0.0	0.0	0.0	2.0	2.0	25
Macronuclear segment, length	21.2	20.0	2.8	0.8	13.5	17.0	28.0	14
	25.9	26.4	2.9	0.6	11.2	22.0	28.0	25
Macronuclear segment, width	11.2	10.5	2.2	0.6	19.6	8.0	15.0	14
	10.4	11.0	1.9	0.4	18.3	8.8	17.6	25
Distance between macronuclear segments	17.7	17.0	4.8	1.3	27.4	11.0	28.0	14
	9.9	8.8	3.9	0.8	39.4	4.4	19.8	25
Micronuclei, number	1.7	2.0	–	–	–	1.0	2.0	14
	2.4	2.0	0.6	0.1	25.0	2.0	4.0	25
Micronucleus, diameter	3.9	3.9	0.4	0.1	10.0	3.0	5.0	14
	3.8	3.3	0.9	0.2	23.7	2.2	6.6	25
Adoral membranelles, number	36.4	37.5	2.3	0.6	6.3	31.0	39.0	14
	36.4	36.0	2.3	0.5	6.3	32.0	40.0	25
Adoral zone of membranelles, length	66.9	69.0	4.7	1.3	7.1	56.0	72.0	14
	70.0	66.0	10.2	2.0	14.6	52.8	94.2	25
Endoral membrane, number of fragments	10.4	10.5	1.6	0.4	15.4	7.0	13.0	14
	10.4	10.0	0.9	0.2	8.7	9.0	12.0	25
Right marginal row, number of cirri	21.9	22.0	1.3	0.4	6.1	20.0	24.0	14
	23.2	23.0	1.1	0.2	4.7	21.0	25.0	25
Left marginal row, number of cirri	19.6	20.0	2.3	0.6	11.8	14.0	23.0	14
	20.0	20.0	1.3	0.3	6.5	18.0	24.0	25
Anterior frontal cirri, number	3.0	3.0	0.0	0.0	0.0	3.0	3.0	14
	3.0	3.0	0.0	0.0	0.0	3.0	3.0	25
Postorior frontal cirri, number	4.0	4.0	0.0	0.0	0.0	4.0	4.0	14
	4.0	4.0	0.0	0.0	0.0	4.0	4.0	25
Buccal cirrus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	14
	1.0	1.0	0.0	0.0	0.0	1.0	1.0	25
Postoral cirri, number	3.0	3.0	0.0	0.0	0.0	3.0	3.0	14
	3.0	3.0	0.0	0.0	0.0	3.0	3.0	25
Ventral cirri above transverse cirri, number	2.0	2.0	0.0	0.0	0.0	2.0	2.0	14
	2.0	2.0	–	–	–	1.0	3.0	25
Transverse cirri, number	5.0	5.0	0.0	0.0	0.0	5.0	5.0	14
	4.9	5.0	–	–	–	4.0	5.0	25
Caudal cirri, number	3.0	3.0	0.0	0.0	0.0	3.0	3.0	14
	3.0	3.0	0.0	0.0	0.0	3.0	3.0	25
Dorsal kineties, number	6.0	6.0	0.0	0.0	0.0	6.0	6.0	14
	6.0	6.0	0.0	0.0	0.0	6.0	6.0	25

[1] Data based on protargol impregnated specimens from exponentially growing cultures, Measurements in µm. x̄ = arithmetic mean; M = median; SD = standard deviation; SE = standard error of arithmetic mean; CV = coefficient of variation in %; Min = minimum value; Max = maximum value; n = number of individuals investigated.

Table 2. Origin of anlagen and number of cirri produced in the anlagen of *Steinia sphagnicola*

Anlage	Proter	Opisthe	Number of cirri
1	undulating membranes	oral primordium	1
2	buccal cirrus	oral primordium	3
3	posterior frontal cirri	oral primordium	3
4	posterior frontal cirri	1st postoral cirrus	3
5	2nd postoral cirrus	2nd postoral cirrus	4
6	2nd postoral cirrus	2nd postoral cirrus	4

opisthe's FVT-anlagen 5 and 6 (Figs. 12, 13, 22, arrowheads). A small field of basal bodies splits off from the dissolving cirrus and migrates anteriorly, where it enlarges and forms a V-shaped primordium. The rest of the cirrus migrates slightly posteriorly and organizes a V-shaped primordium too, viz. the opisthe's anlagen 5 and 6 (Figs. 14–17, 22, 23). In all anlagen, the anterior basal bodies have longer ciliary stubs than the posterior ones (Fig. 23).

Stage 4 (Figs. 18–20, 24). The anterior portion of the oral primordium proceeds to differentiate adoral membranelles and the whole primordium sinks into a shallow depression of the cell surface (Fig. 24). Ciliary growth proceeds from anterior to posterior. The two posteriormost frontal cirri disorganize to proter's FVT-anlagen 3 and 4 (Figs. 18, 19), and three short streaks evolve at the right anterior end of the oral primordium (Figs. 20, 24).

Stage 5 (Figs. 21, 25, 26, 28, 34, 44; Table 2). The formation of adoral membranelles in the oral primordium as well as ciliary outgrowth and invagination of the new adoral zone of membranelles are still in progress. The frontal scutum of the adoral zone of membranelles develops, i.e. a tiny crest grows out from the pellicle at the anterior end of the opisthe's adoral zone (Fig. 26). The first postoral cirrus disaggregates and becomes FVT-anlage 4 of the opisthe (Fig. 21).

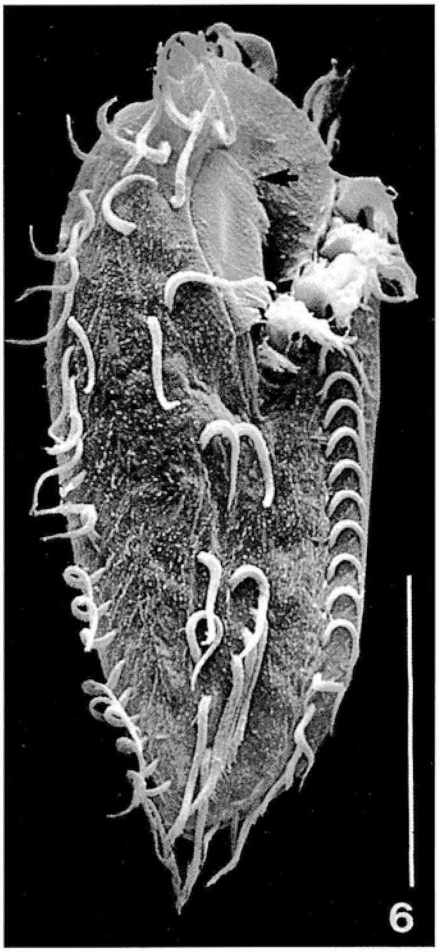
The buccal cirrus and the anterior portion of the proter's paroral membrane disaggregate to anlagen (Fig. 28). The V-shaped primordia mentioned in stage 3 split into two separate streaks. Thus, six anlagen are recognizable in each the proter and opisthe (Figs. 25, 28, 34; Table 2). All anlagen lengthen by continued production of basal bodies and ciliary growth proceeds posteriad in each anlage (Fig. 25).

Scattered pairs of basal bodies occur left of the opisthe's anlagen 1–3 and develop to a long, narrow streak running close to the right side of the new adoral zone of membranelles. These basal bodies, which produce the undulating membranes, have much shorter cilia than those of the cirral anlagen; those in the left half of the anlage have longer cilia than those in the right (Fig. 26).

The third right marginal cirrus and somewhat later a few cirri within the middle portion of the right marginal row develop to marginal primordia (Figs. 28, 34). Within dorsal kineties 1, 2 and 3 basal bodies are proliferated at two sites above and below the prospective division furrow (Fig. 44).

Figs. 6–11. Scanning electron microscope (Figs. 6, 8, 10, 11) and bright field (Figs. 7, 9) light micrographs of morphostatic (Figs. 6–9) and dividing (Figs. 10, 11) *Steinia sphagnicola*. – Fig. 6. Ventral view. The right half of the buccal field is covered by the cilia of the paroral membrane (arrow). – Fig. 7. Ventral view after protargol impregnation. – Fig. 8. The curious buccal pit (P) is recognizable only in specimens with paroral cilia moved upwards. – Fig. 9. Oral area after protargol impregnation showing uninterrupted paroral and fragmented endoral membrane (arrows). – Figs. 10, 11. Very early dividers showing origin (Fig. 10) and development (Fig. 11) of the oral primordium near the anteriormost transverse cirrus which appears unchanged. AFC = anterior frontal cirri, AZM = adoral zone of membranelles, BC = buccal cirrus, EM = endoral membrane, LMR = left marginal row, MA = macronuclear segment, MI = micronucleus, OP = oral primordium, P = buccal pit, PFC = posterior frontal cirri, PM = paroral membrane, POC = postoral cirri, RMR = right marginal row, TC = transverse cirri, VC = ventral cirrus. Figs. 8, 9, 10, 11, bars = 20 µm; Figs. 6, 7, bars = 50 µm. ▶

Figs. 12–21. Morphogenesis of *Steinia sphagnicola* after protargol impregnation. – Fig. 12. Early divider showing anlage originating from oral primordium (arrow). Arrowhead marks disaggregating second postoral cirrus. – Figs. 13–17. FVT-anlagen 5 and 6 originate from postoral cirrus 2 in both proter and opisthe. A small field of basal bodies splits off from the dissolving postoral cirrus 2, migrates anteriorly (Figs. 15, 16, arrows), and organizes to proter's anlagen 5 and 6 (Fig. 17, 18). – Figs. 18–21. Anlagen 3 and 4 of the proter originate from the posteriormost frontal cirri (Figs. 18, 20; arrow). The opisthe's anlagen 3 and 4 originate, respectively, from the oral primordium (Fig. 20, arrowhead) and from postoral cirrus 1 (Fig. 21), which dissolves later than postoral cirrus 2. The whole process is accompanied by cortical growth as indicated by increasing distances between postoral cirri 1 and 2 and between frontal cirri und postoral cirri. Postoral cirrus 3 is not involved in anlagen formation and is resorbed during cytokinesis (Figs. 30, 41). FC = anterior frontal cirri, POC = postoral cirri, numbers 3 to 6 = FVT-anlagen in proter and opisthe. Figs. 12, 20, scale bar division = 10 µm; Figs. 13–19, 21 drawn to scale (bar = 10 µm in Fig. 21). ▶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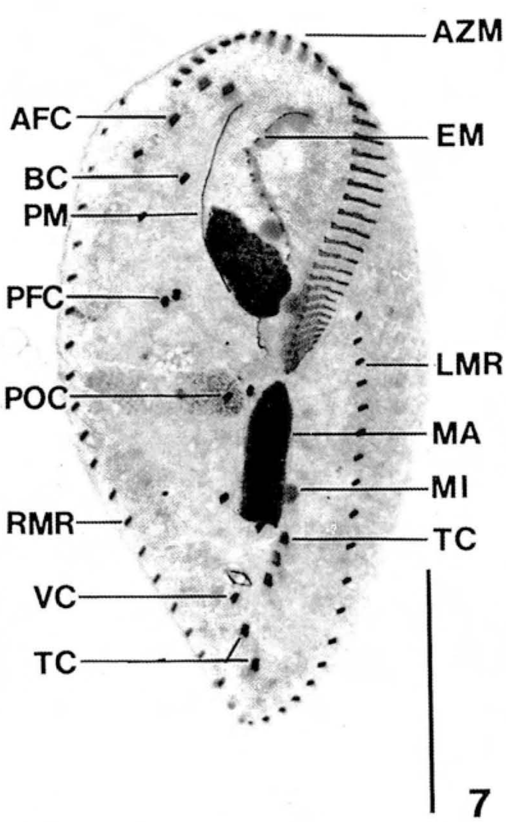
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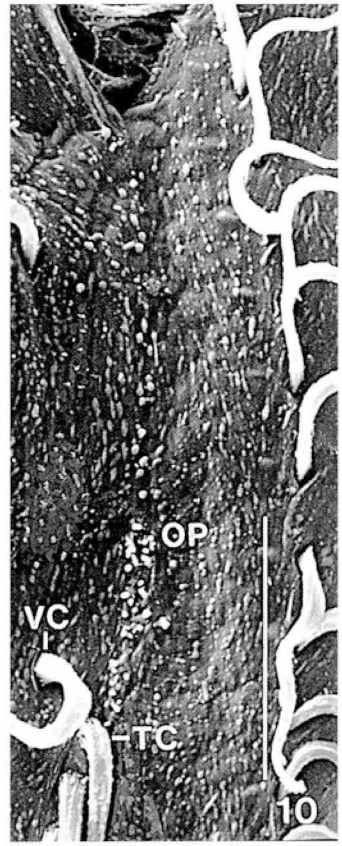
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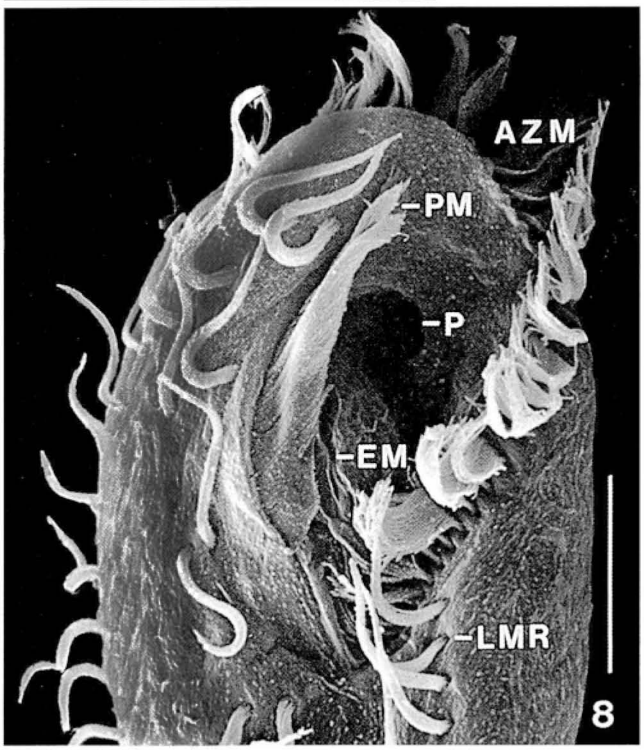
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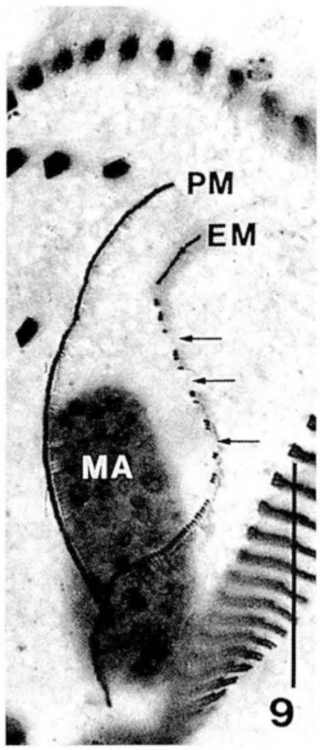
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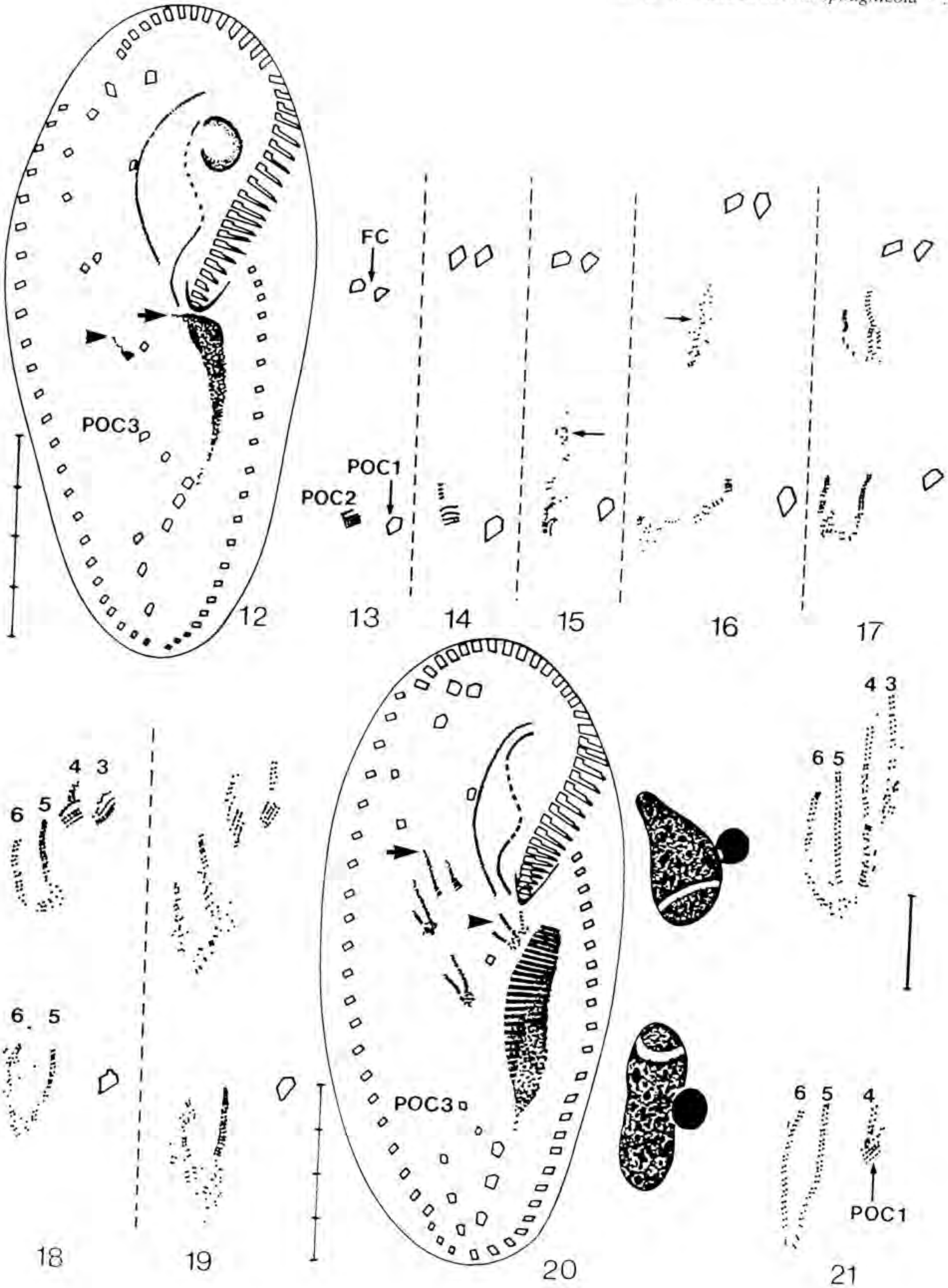
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Stage 6 (Figs. 29, 35). The anterior end of the new adoral zone of membranelles curves slightly to the right. The formation of adoral membranelles is complete except of the last two to four membranelles. The disaggregated anterior portion of the proter's paroral membrane forms a slightly bifurcated patch of basal bodies. Later, the right fork generates the first frontal cirrus, whereas the left fork forms a right-curved hook. All fronto-ventral-transverse anlagen organize to cirri. The anteriormost left marginal cirrus and some cirri near the prospective division furrow of the left marginal row disaggregate to anlagen. Above the anteriormost portion of the proter's and opisthe's right marginal primordia a short streak of paired basal bodies develops, viz. the anlage for the shortened dorsal kinety 5 (Fig. 29).

Stage 7 (Figs. 30, 32, 36, 37, 45). The anterior third of the new adoral zone of membranelles curves distinctly to the right. The proter's paroral membrane straightens almost completely and its upper half is

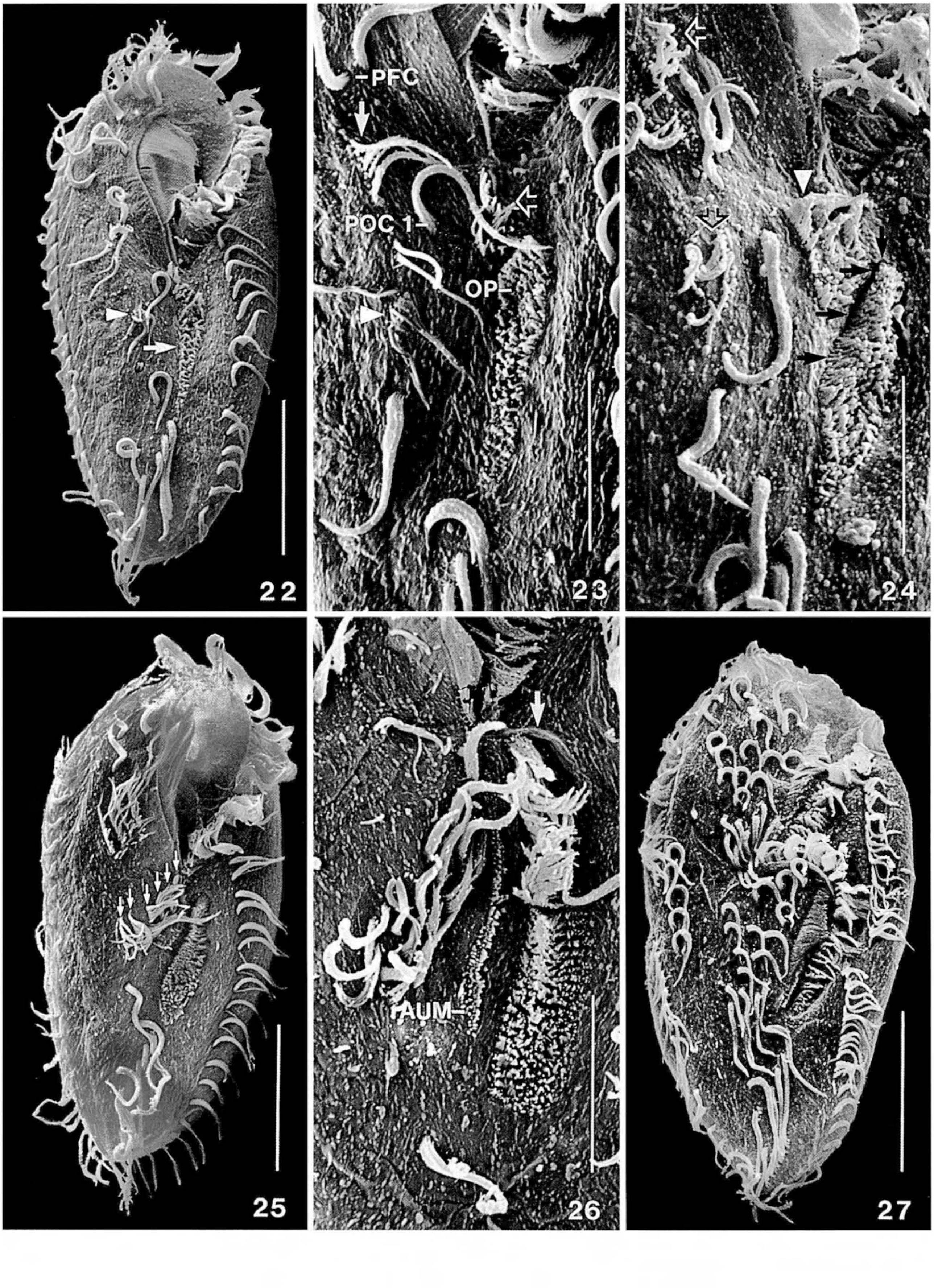
Figs. 22–27. Scanning electron micrographs of dividing *Steinia sphagnicola*. – Fig. 22. Early divider showing developing oral primordium (arrow) and disaggregation of second postoral cirrus (arrowhead). – Fig. 23. Early divider showing organizing FVT-anlagen 5 and 6, respectively, in proter (arrow) and opisthe (arrowhead). These anlagen develop from proter's postoral cirrus 2 (compare Figs. 12–18, 22). Open arrow indicates two anlagen emerging from oral primordium (OP). – Fig. 24. Early divider showing invagination of developing adoral zone of membranelles (arrows). Arrowhead marks three anlagen emerging from oral primordium. Open arrows indicate FVT-anlagen 5 and 6 in proter and opisthe. – Fig. 25. Middle divider showing almost complete new adoral zone of membranelles and FVT-anlagen 1–6 in the opisthe (arrows). – Fig. 26. Middle divider. The opisthe's anlagen have organized to cirri. The anlage for the undulating membranes (AUM) is a long, narrow streak with short cilia clearly separate from the new cirri and the developing adoral zone of membranelles. Arrow marks developing frontal scutum of the adoral zone. – Fig. 27. Middle divider. Cirri in each anlage segregate and move to their final positions. The opisthe's adoral zone of membranelles is distinctly invaginated; the proter's oral area is reorganized, as indicated by the reduced length of the cilia of the paroral membrane and the narrowed buccal area (compare Figs. 6, 33). Figs. 22, 25, 27, bars = 40 µm; Figs. 23; 24, 26, bars = 20 µm. ▶

Figs. 28–33. Morphogenesis of *Steinia sphagnicola* (Figs. 28–30, protargol impregnation; Figs. 31–33, SEM-micrographs). – Fig. 28. Middle divider (compare Fig. 34) with seven anlagen in the opisthe because the first frontal cirrus has separated from the anlage for the undulating membranes. The parental paroral membrane commences to reorganize (arrow) and the buccal cirrus (open arrow) and some right marginal cirri in the prospective division furrow (arrowhead) disaggregate to anlagen. – Fig. 29. Middle divider showing anlagen (arrows) for dorsal kinety 5 which originate from parental marginal cirri. Arrowheads mark primordia in left marginal row. – Fig. 30. Middle divider showing segregation of new cirri and reorganization of the proter's undulating membranes. Note that fragmentation of endoral membrane gradually disappears. – Fig. 31. Higher magnification of opisthe's oral structures of cell shown in Fig. 27. The new cirri segregate and the new adoral zone of membranelles sinks into the cell. The frontal scutum (arrow) has developed and carries the anterior portion of the adoral zone of membranelles. – Fig. 32. Late divider showing developing new marginal cirri (arrowheads) and one of the two anlagen for the shortened dorsal kineties 5 and 6 which move from ventral to lateral (arrow). – Fig. 33. Late divider showing proter's proximal adoral membranelles (arrows) which have shortened cilia, indicating that the posterior portion of the parental adoral zone of membranelles is reorganized. $AD_{5,6}$ = anlagen for shortened dorsal kineties 5 and 6, EM = endoral membrane, PM = paroral membrane, POC = postoral cirrus. Figs. 28–30, scale bar division = 10 µm; Figs. 31, 32, bars = 20 µm; Fig. 33, bar = 10 µm. ▶

Figs. 34–39. Protargol impregnated middle and late dividers of *Steinia sphagnicola*. – Fig. 34. Middle divider showing developing primordia in right marginal row (arrows) and six cirral anlagen each in proter and opisthe (compare Fig. 28). – Fig. 35. Middle divider showing segregation of new cirri. Arrow marks reorganizing anterior portion of proter's paroral membrane. – Fig. 36. Middle divider showing independently developing anlagen for dorsal kineties 5 and 6 (arrow). – Figs. 37–39. Middle and late dividers showing reorganization of proter's undulating membranes, migration of cirri, and commencing cytokinesis. Arrows in Figs. 37 and 39 mark new dorsal kineties 5 and 6 and intersecting undulating membranes, respectively. Figs. 34, 35, 37–39, bars = 40 µm; Fig. 36, bar = 20 µm. ▶

Figs. 40–43. Morphogenesis of *Steinia sphagnicola* after protargol impregnation. – Figs. 40, 41. Middle dividers showing developing dorsal kineties 5 and 6 (arrows), migration of cirri, and reorganizing undulating membranes in the proter. The fragmentation and sinusoidal bending of the endoral membrane have disappeared. – Fig. 42. Late divider showing intersecting undulating membranes. The parental cirri which were not involved in the formation of anlagen are gradually resorbed. – Fig. 43. Very late divider. The anterior end of the paroral membrane becomes curved and the endoral membrane fragments. Most parental cirri have been resorbed and the dorsal infraciliature is fully developed. Scale bar division = 10 µm. ▶

Figs. 44–49. Morphogenesis of the dorsal infraciliature (Figs. 44–47) and post-dividers (Figs. 48, 49) of *Steinia sphagnicola* after protargol impregnation. – Figs. 44, 45. Early and middle dividers showing primordia within dorsal kineties 1, 2 and 3. – Fig. 46. Middle divider showing separation and development of dorsal kinety 4 (AD_4) from kinety 3. – Fig. 47. Late divider showing cytokinesis and differentiation of caudal cirri at posterior end of kineties 1, 2 and 3. – Fig. 48. Early post-divider. The curved paroral membrane crosses (intersects) the endoral membrane which becomes fragmented and sinusoidal. – Fig. 49. Late post-divider showing how the paroral membrane moves rightward producing the genus-specific arrangement of the undulating membranes. The buccal pit (P) develops. Scale bar division = 10 µm. ▶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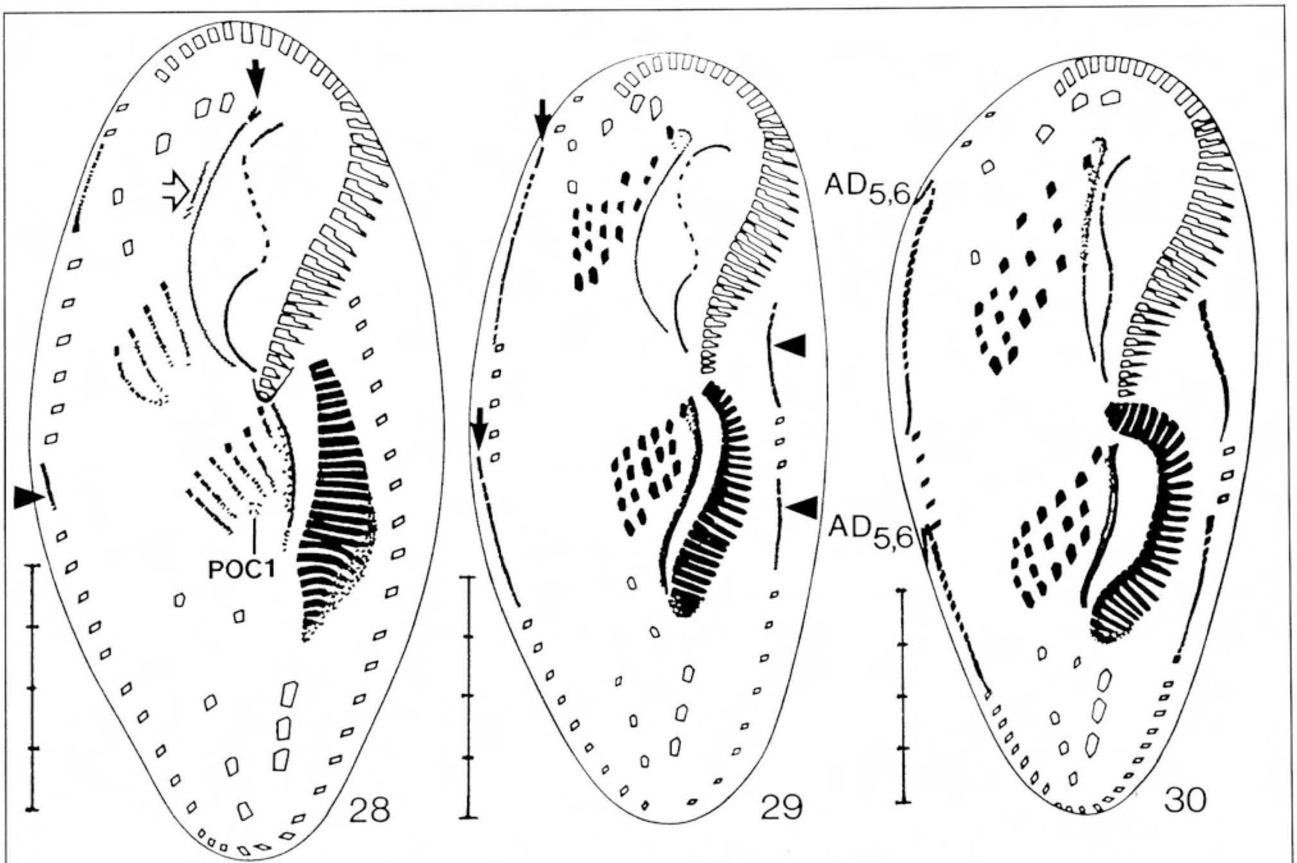
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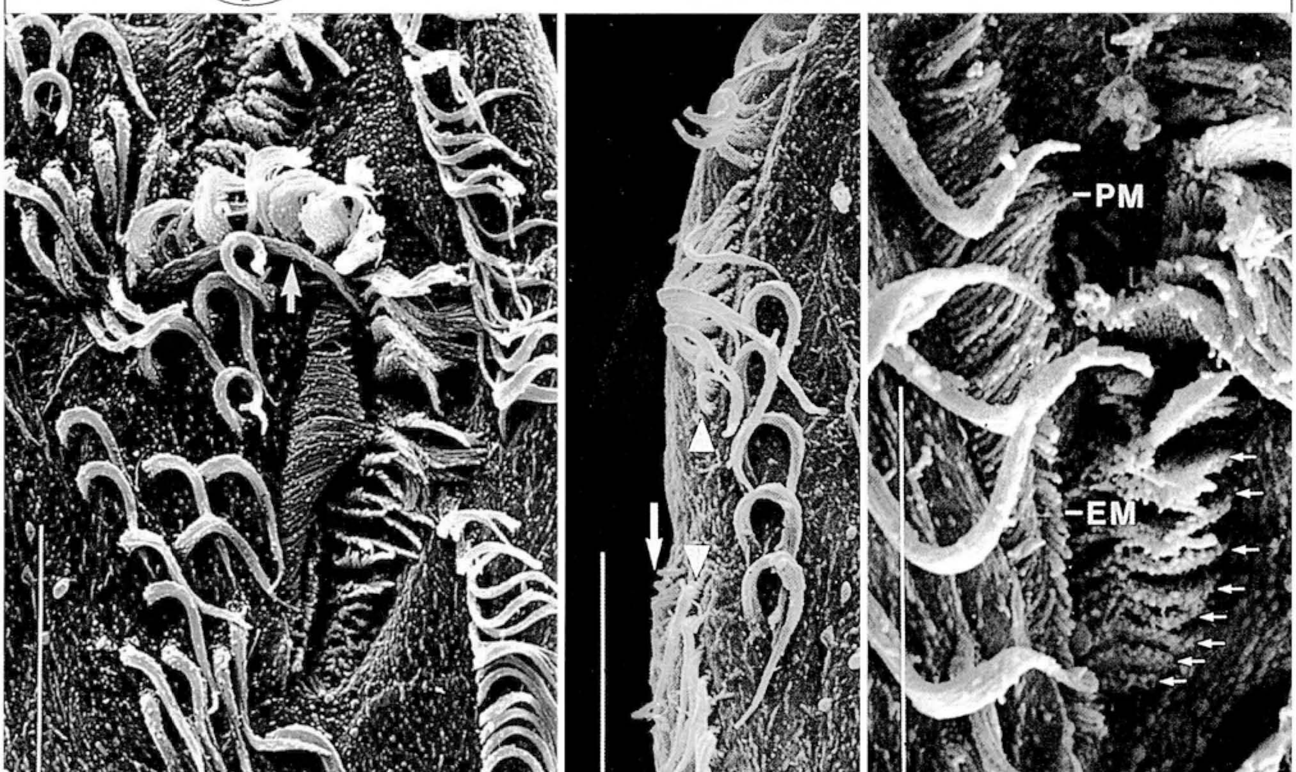
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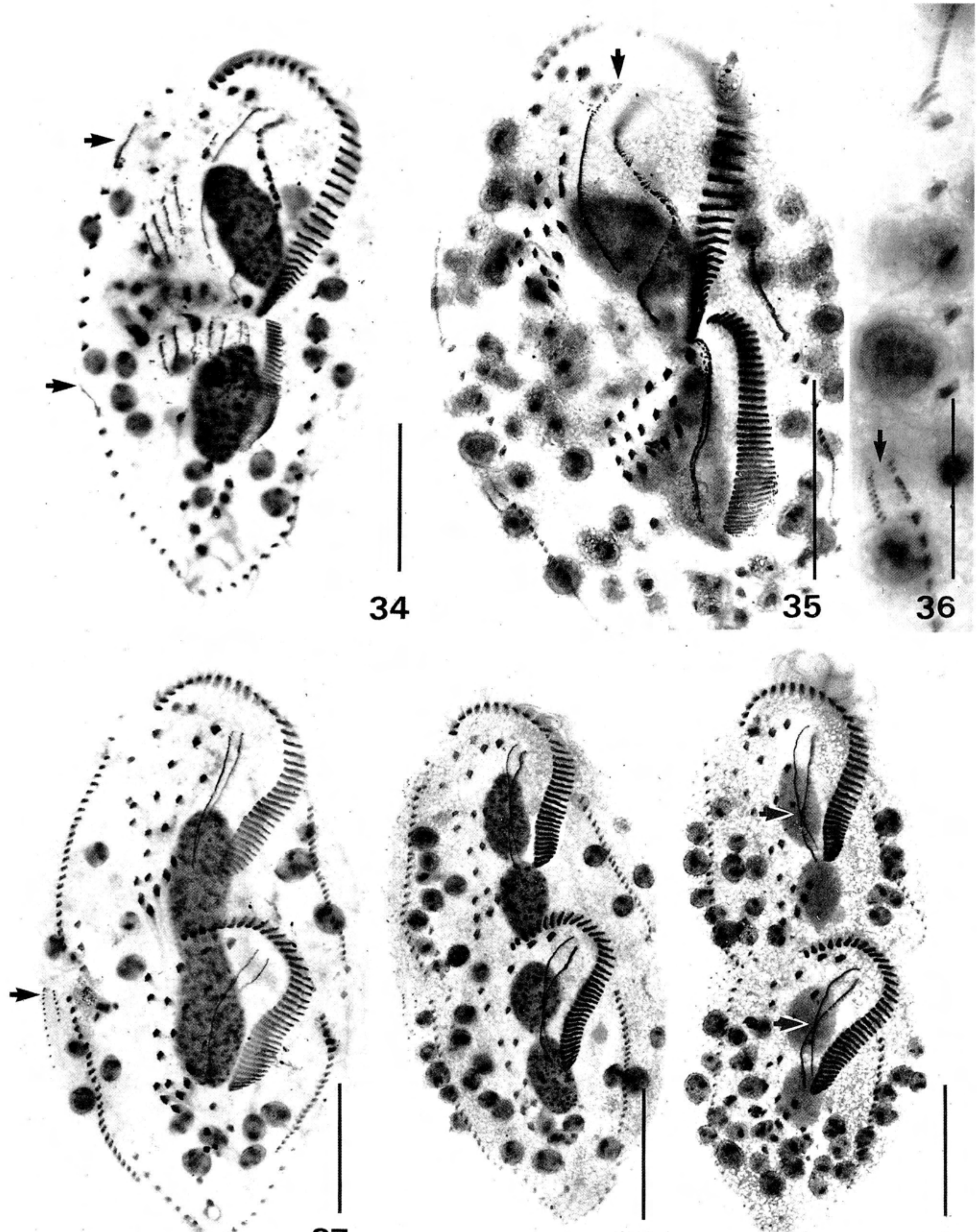
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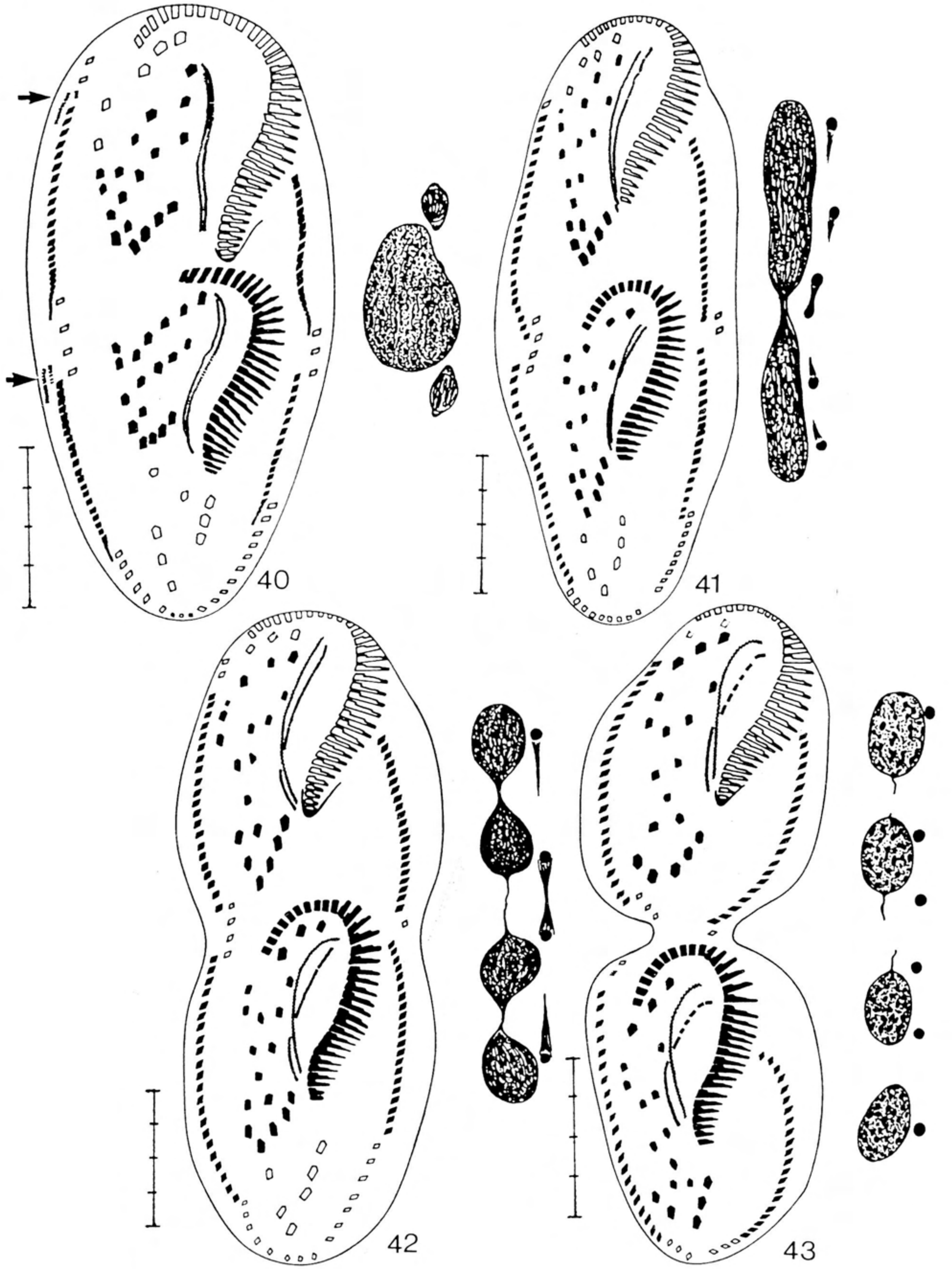
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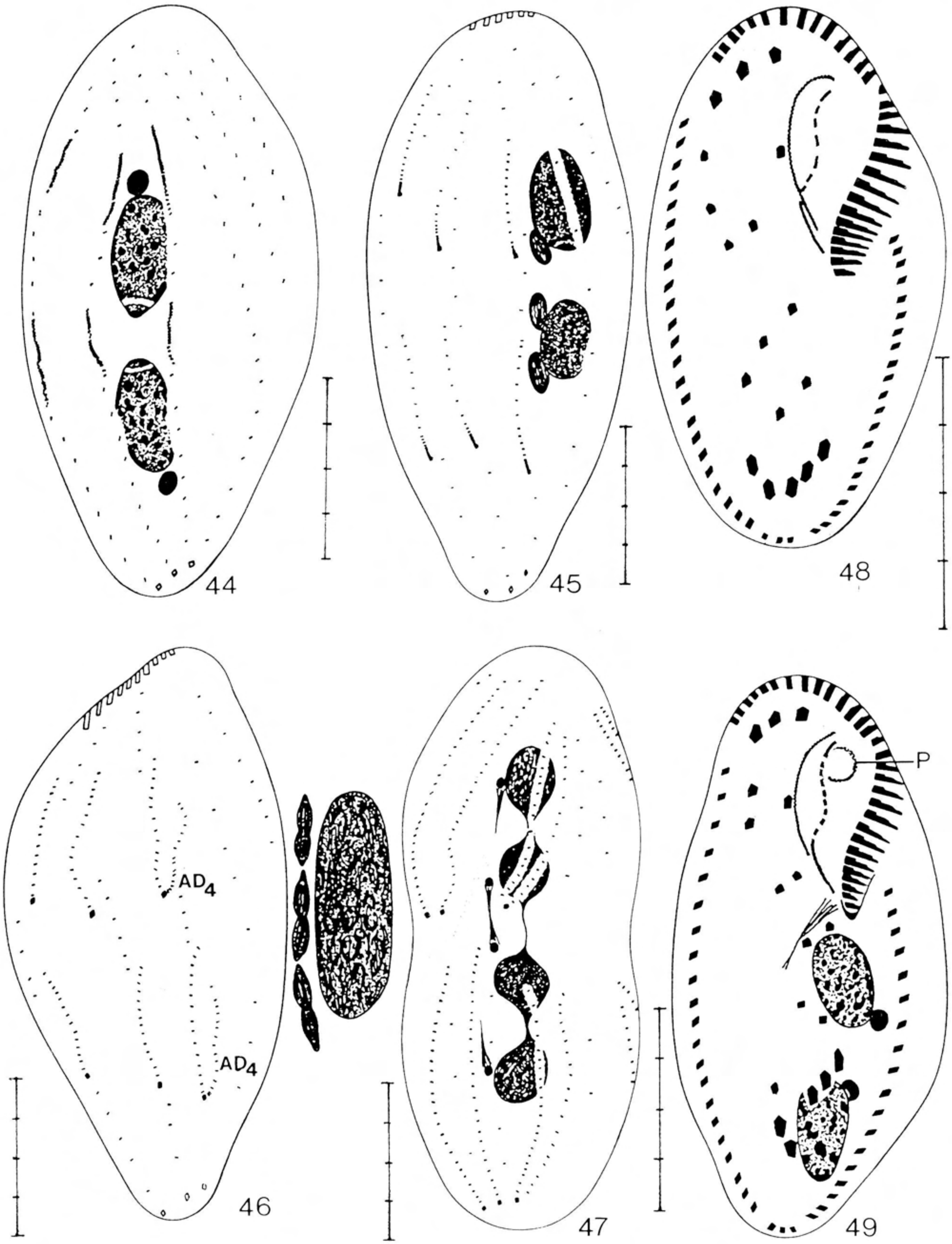
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reorganized. Later the hook at its anterior end disappears. The endoral membrane also reorganizes, i.e. its fragmentation and sinusoidal bending disappears, producing an uninterrupted line of paired basal bodies along the reorganizing paroral membrane (Figs. 30, 37). The opisthe's anlage for the undulating membranes divides into two rows of paired basal bodies (Fig. 30). The basal bodies of the future paroral membrane are arranged in a zigzag, those of the future endoral membrane form two parallel lines. The anlage for dorsal kinety 5 lengthens and moves to the right. A short streak of basal bodies develops between the anlage for dorsal kinety 5 and the anteriormost cirrus of the new marginal row (Figs. 30, 32, 36, 37). This streak is the anlage for the shortened dorsal kinety 6 and has distinct contact neither with the new anteriormost marginal cirri nor with kinety 5, which developed earlier. The posterior ends of the new dorsal kineties 1, 2 and 3 commence with the differentiation of caudal cirri (Fig. 45).

Stage 8 (Figs. 27, 31, 33, 46, 47). The opisthe's adoral zone of membranelles is complete and its posterior half sinks into the cell to produce the peristomial vertex (Figs. 27, 31); the anterior portion is lined by the frontal scutum, which is now a distinct ridge orientated perpendicularly to the cell surface (Fig. 31). The proter's oral apparatus shows distinct signs of reorganization: the length of the paroral cilia and of the proximal eight to ten adoral membranelles is reduced, the buccal area becomes flatter and smaller, and the peculiar buccal pit disappears (Figs. 27, 30, 33). The cirri in each anlage segregate. The new dorsal kineties 5 and 6 move from ventral to lateral and develop short cilia. The posterior portion of dorsal kinety 3 segregates a streak each in the proter and opisthe that moves to the right and becomes dorsal kinety 4 (Figs. 46, 47). The formation of new caudal cirri at the posterior ends of kineties 1–3 is complete.

Stage 9 (Figs. 38, 40, 41). In each daughter cell the new cirri move to their final positions. The anterior portions of the proter's and opisthe's undulating membranes move to the left and diverge, whereas their posterior portions intersect (Fig. 41).

Stage 10 (Figs. 39, 42, 43, 48, 49). Cytokinesis commences and cirri and oral apparatus arrange to the species-specific pattern. Dorsal kineties 5 and 6 move from lateral to dorsal. The parental cirri which did not participate in the formation of primordia are gradually resorbed. The endoral membrane splits into about ten fragments each composed of three to four pairs of basal bodies with the exception of the first and last fragment, which consist of about nine and, respectively, twenty-five pairs of basal bodies (Figs. 48, 49). The undulating membranes still intersect in early post-dividers (Fig. 48); later, however, the paroral membrane moves to the right, producing the genus-specific pattern, viz. paroral and endoral membrane extend side by side (Fig. 49). Likewise, the shaping of the buccal pit and the production of new pharyngeal fibres occur only in post-dividers.

Discussion

The Oral Primordium

Stomatogenesis in *Steinia sphagnicola* commences close to the first left transverse cirrus, which is in accordance with the observations in *S. platystoma* [15]. This mode of oral primordium formation has been observed in various hypotrichs, e.g. in *Amphisiella* [25] and above all in many oxytrichids [1, 10, 24, 29, 30]. However, other oxytrichids develop the oral primordium de novo between the postoral and transverse cirri [8, 9, 11, 23, 29]. Both types even occur in species of the same genus, indicating either misclassification of species or rather a random distribution of this character within the oxytrichids.

Both in the light and scanning electron microscope the anteriormost transverse cirrus, where the oral primordium develops, shows no signs of disintegration. The occurrence of nascent basal bodies adjacent to intact cirral structures during morphogenesis has been reported by Grimes [12] in *Oxytricha fallax* using transmission electron microscopy. On that account, it is reasonable to suppose that the oral primordium of *Steinia sphagnicola* also develops apokinetally.

The invagination of the oral primordium very likely also occurs in other oxytrichids. It is apparently common in urostylids [14, 27], and might in fact be widespread within euhypotrichs. In the pseudohypotrichs, e.g. *Euplotes*, just the reverse occurs: the oral primordium develops in a subsurface pouch and evaginates [16, 21]. This seems to be a rather fundamental difference between euhypotrichs and pseudohypotrichs and is found also in the two main groups of oligotrichids [20].

The Proter's Oral Structures

The fragmentation of *Steinia's* endoral membrane is unique within the oxytrichids. Therefore, it is surprising that its reorganization during divisional morphogenesis occurs as in other members of the family [1, 5, 9, 11, 15, 23, 24, 29, 30]. However, it is worth mentioning that the anterior portion of the reorganizing paroral membrane forms a right-curved hook as in *Cyrtohymena* [23] and, even more remarkable, in morphostatic specimens of *Notohymena* [24]. This indicates a rather close relationship of these genera. However, the undulating membranes also argue for a close relationship with *Stylonychia*, as does the rigid body, because the membranes extend side by side in late dividers, whereas they intersect in the other genera mentioned. Intersecting undulating membranes occur also in a certain stage of the stomatogenesis of *S. sphagnicola* (Figs. 39, 41, 42), indicating that it is a plesiomorphic feature. The genus-specific pattern of the undulating membranes is obtained in *Steinia, Cyrtohymena* and *Notohymena* only shortly after cell division, suggesting that this character is a young evolutionary acquisition.

It is widely assumed that the parental adoral zone of membranelles is transferred unchanged to the next generation in oxytrichids, although some data show that at least the pharyngeal fibres are resorbed and rebuilt [9]. Much more extensive changes occur in *Steinia sphagnicola* (Figs. 27, 30); however, the reorganization is cryptic, i.e. occurs without formation of anlagen.

The Opisthe's Undulating Membranes

Middle dividers of *S. sphagnicola* show groups of scattered pairs of basal bodies near the posterior ends of the opisthe's cirral anlagen 1–3. These basal bodies, which have shorter cilia than those of the cirral streaks, form the anlage for the undulating membranes, and could have been generated by the cirral primordia. However, taking the lengths of the cilia as a chronological parameter another explanation is possible, viz. that the anlage for the undulating membranes develops de novo. Jerka-Dziadosz [18] also could not clear the origin of the anlagen for the undulating membranes of *Paraurostyla weissei,* but supposes that they split off from the anlagen for the adoral membranelles.

Within the anlage for the undulating membranes the leftmost basal bodies have longer cilia than the rightmost, indicating that the endoral membrane develops slightly earlier than the paroral membrane. This is in accordance with the observations by Jerka-Dziadosz [18].

Development of Cirral Primordia

Grolière's [15] data on the development of the cirral primordia in *Steinia platystoma* can be summarized as follows: The first postoral cirrus disorganizes to a primordium which splits longitudinally producing two sets consisting of two streaks each for the proter and opisthe. Afterwards, each set generates five anlagen by further proliferation of basal bodies and splitting of the streaks. All cirral anlagen develop independently of the oral primordium. This is a very uncommon ontogenesis for oxytrichids and not confirmed by our observations on *S. sphagnicola*, which show that the origin and development of the six cirral anlagen occur as described in many other oxytrichids [1, 9, 29, 30].

The origin of the anlagen 4–6 in the opisthe of *S. sphagnicola* resembles that described in *Stylonychia pustulata, S. vorax* [29],and *Histriculus muscorum* [1], a species closely related to *Stylonychia* [30]. In these taxa only two (numbers 1, 2) of the three postoral cirri develop anlagen, whereas in many other oxytrichids all postoral cirri are involved, e.g. in *Cyrtohymena, Notohymena*, and *Oxytricha* [1, 5, 8, 9, 23, 24]. Furthermore it is remarkable that the second postoral cirrus of *Steinia sphagnicola* develops the anlagen 5 and 6 not only for the opisthe but also for the proter. The same has been described for the species of the *Stylonychia mytilus* complex [30]. Other oxytrichids develop the anlagen 5 and 6 of the proter either de novo or from the right posteriormost frontal cirrus, whereas the opisthe's anlagen 5 and 6 are produced either by the second postoral cirrus or by the second and the third postoral cirrus [1, 8, 23-25, 29]. These peculiarities of anlagen formation indicate a close relationship of the genera *Steinia* and *Stylonychia* and separates them from *Cyrtohymena, Notohymena* and *Oxytricha*.

Protargol preparations from many hypotrichs suggest that cirral primordia grow from posterior to anterior, i.e. that new basal bodies are generated at the anterior end of the streaks. However, scanning electron microscopic images of dividing *Paraurostyla* [14], *Thigmokeronopsis* [27] and *S. sphagnicola* suggest just the reverse, because the length of the cilia decreases from anterior to posterior.

Development of Somatic Primordia

Again our results disagree with those of Grolière [15], who describes that the proter's marginal primordia of *S. platystoma* develop from the third cirrus of the left and right marginal row, whereas the opisthe's anlagen develop in the posterior third of each marginal row. Our data show that the proter's marginal primordia develop from the third cirrus of the right and, respectively, from the first cirrus of the left marginal row. The anlagen for the opisthe originate from some marginal cirri near the prospective division furrow.

The anlage for the short dorsal kinety 6 is situated near the anlage for kinety 5, but shows distinct contact neither with it nor with the marginal primordia (Fig. 37). Therefore, we agree with Wirnsberger [28] that this kinety is a reduced and transformed marginal row which originates de novo, as supposed in *Oxytricha fallax* [12].

References

1 Berger H., Foissner W., and Adam H. (1985): Morphological variation and comparative analysis of morphogenesis in *Parakahliella macrostoma* (Foissner, 1982) nov. gen. and *Histriculus muscorum* (Kahl, 1932), (Ciliophora, Hypotrichida). Protistologica, *21,* 295–311.

2 Blatterer H. und Foissner W. (1988): Beitrag zur terricolen Ciliatenfauna (Protozoa: Ciliophora) Australiens. Stapfia, Linz, *17,* 1–84.

3 Borror A. C. (1972): Revision of the order Hypotrichida (Ciliophora, Protozoa). J. Protozool., *19,* 1–23.

4 Diesing K. M. (1866): Revision der Prothelminthen. Abtheilung: Amastigen. I. Amastigen ohne Peristom. Sber. Akad. Wiss. Wien, *52* (year 1865), 505–579.

5 Foissner W. (1983): Die Morphogenese von *Urosoma macrostyla* (Wrześniowski, 1870) (Ciliophora: Oxytrichidae). Arch. Protistenk., *127,* 413–428.

6 Foissner W. (1989): Morphologie und Infraciliatur einiger neuer und wenig bekannter terrestrischer und limnischer Ciliaten (Protozoa, Ciliophora). Sber. Akad. Wiss. Wien, *196,* 173–247.

7 Foissner W. (1991): Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. Europ. J. Protistol., *27,* 313–330.

8 Foissner W. und Adam H. (1983a): Die Morphogenese von *Urosomoida agiliformis* Foissner, 1982 (Ciliophora, Oxytrichidae). Zool. Anz., *211,* 161–176.

9 Foissner W. und Adam H. (1983b): Morphologie und Morphogenese des Bodenciliaten *Oxytricha granulifera* sp. n. (Ciliophora, Oxytrichidae). Zool. Scr., *12,* 1–11.
10 Foissner W., Schlegel M., and Prescott D. M. (1987): Morphology and morphogenesis of *Onychodromus quadricornutus* sp. n. (Ciliophora, Hypotrichida), an extraordinarily large ciliate with dorsal horns. J. Protozool., *34,* 150–159.
11 Ganner B., Foissner W., and Adam H. (1986): Morphogenetic and biometric comparison of four populations of *Urosomoida agiliformis* (Ciliophora, Hypotrichida). Annls. Sci. nat. (Zool.), *8,* 199–207.
12 Grimes G. W. (1973): Origin and development of kinetosomes in *Oxytricha fallax*. J. Cell. Sci., *13,* 43–53.
13 Grimes G. W. and Adler J. A. (1976): The structure and development of the dorsal bristle complex of *Oxytricha fallax* and *Stylonychia pustulata*. J. Protozool., *23,* 135–143.
14 Grimes G. W. and L'Hernault S. W. (1978): The structure and morphogenesis of the ventral ciliature in *Paraurostyla hymenophora*. J. Protozool., *25,* 65–74.
15 Grolière C. A. (1969): Etude comparée de morphogenèse au cour de la bipartition, chez plusieurs espèces de ciliés hypotriches. Annls. Stn. limnol. Besse, *4,* 335–365.
16 Hill B. F. (1981): The cortical morphogenetic cycle associated with cell division in *Diophrys* Dujardin, 1841 (Ciliophora, Hypotrichida). J. Protozool., *28,* 215–221.
17 Jerka-Dziadosz M. (1980): Ultrastructural study on development of the hypotrich ciliate *Paraurostyla weissei*. I. Formation and morphogenetic movements of ventral ciliary primordia. Protistologica, *16,* 571–589.
18 Jerka-Dziadosz M. (1981): Ultrastructural study on development of the hypotrich ciliate *Paraurostyla weissei*. III. Formation of preoral membranelles and an essay on comparative morphogenesis. Protistologica, *17,* 83–97.
19 Kahl A. (1932): Urtiere oder Protozoa. I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. Tierwelt Dtl., *25,* 399–650.
20 Petz W. and Foissner W. (1992): Morphology and morphogenesis of *Strobilidium caudatum* (Fromentel), *Meseres corlissi* n. sp., *Halteria grandinella* (Müller), and *Strombidium rehwaldi* n. sp., and a proposed phylogenetic system for oligotrich ciliates (Protozoa, Ciliophora). J. Protozool., *39,* 159–176.
21 Ruffolo J. J. (1976): Cortical morphogenesis during the cell division in *Euplotes:* an integrated study using light optical, scanning electron and transmission electron microscopy. J. Morph., *148,* 489–528.
22 Sokal R. P. and Rohlf F. J. (1981): Biometry. The principles and practise of statistics in biological research. 2nd ed. W. H. Truman and Company; San Francisco.
23 Voss H. J. (1991a): Die Morphogenese von *Cyrtohymena muscorum* (Kahl, 1932) Foissner, 1989 (Ciliophora, Oxytrichidae). Arch. Protistenk., *140,* 67–81.
24 Voss H. J. (1991b): Die Morphogenese von *Notohymena rubescens* Blatterer und Foissner, 1988 (Ciliophora, Oxytrichidae). Axch. Protistenk., *140,* 219–236.
25 Voss H. J. (1992): Morphogenesis in *Amphisiella australis* Blatterer and Foissner, 1988 (Ciliophora, Hypotrichida). Europ. J. Protistol., *28,* 405–414.
26 Wallengren H. (1900): Zur Kenntnis der vergleichenden Morphologie der hypotrichen Infusorien. Bih. K. svenska Veterisk Akad. Handl., *26,* 1–31.
27 Wicklow B. J. (1981): Evolution within the order Hypotrichida (Ciliophora, Protozoa): ultrastructure and morphogenesis of *Thigmokeronopsis jahodai* (n. gen., n. spec.); phylogeny in the Urostylina (Jankowski, 1979). Protistologica, *17,* 331–351.
28 Wirnsberger E. (1987): Division and reorganization in the genus *Pseudokeronopsis* and relationships between urostylids and oxytrichids (Ciliophora, Hypotrichida). Arch. Protistenk., *134,* 149–160.
29 Wirnsberger E., Foissner W., and Adam H. (1985): Morphological, biometric, and morphogenetic comparison of two closely related species, *Stylonychia vorax* and *S. pustulata* (Ciliophora: Oxytrichidae). J. Protozool., *32,* 261–268.
30 Wirnsberger E., Foissner W., and Adam H. (1986): Biometric and morphogenetic comparison of the sibling species *Stylonychia mytilus* and *S. lemnae,* including a phylogenetic system for the oxytrichids (Ciliophora, Hypotrichida). Arch. Protistenk., *132,* 167–185.

Key words: Ciliophora – Oxytrichidae – *Steinia sphagnicola* – Morphology – Morphogenesis – SEM

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