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Divisional morphogenesis in *Blepharisma americanum*, *B. undulans*, and *B. hyalinum* (Ciliophora: Heterotrichida)

Erna AESCHT[1] and Wilhelm FOISSNER[2]

[1]Oberösterreichisches Landesmuseum, Biologiezentrum, Linz; [2]Universität Salzburg, Institut für Zoologie, Salzburg, Austria

Summary. Cortical development during cell division of *Blepharisma americanum* Suzuki, 1954, *B. undulans* Stein, 1867, and *B. hyalinum* Perty, 1849 was investigated using protargol impregnation and scanning electron microscopy. Stomatogenesis usually commences in a postoral kinety. Depending on species, 0-3 neighbouring kineties are involved in the anarchic field production. The anterior, non-proliferating portion of kinety 2 is frequently retained as shortened fragment right of the paroral, thus resembling the amphiparakinetal stomatogenic subtype found in stentorids and folliculinids. The oral anlage divides longitudinally to form the right paroral and the left adoral primordium. Differentiation of adoral membranelles proceeds, as is usual, from right to left and, unlike in all other ciliates with true adoral membranelles (hypotrichs, oligotrichs, tetrahymenid hymenostomes), from the centre towards the ends of the primordium. The paroral primordium of the opisthe generates the paroral and an apical, cirrus-like structure, as in *Condylostoma*, which is, however, resorbed when cytokinesis commences. Only the posterior (zigzag) segment of the parental paroral is reorganized. The adoral structures of the proter are maintained in *B. hyalinum*, but reorganized in *B. americanum* and *B. undulans*. The anterior and leftmost part of the adoral zone of membranelles are reorganized *in situ*, while about 5 proximal membranelles arise, possibly only partially, from a unique reorganization anlage developing on the vertex of the buccal cavity. The reorganization anlage was lacking in about one third of the dividers. The asymmetry of the blepharismid somatic infraciliature is caused by a proliferation gradient from anterior right to posterior left. The family status of the Blepharismidae and the subgeneric classification of *Blepharisma* based on nuclear configuration are not supported by the ontogenetic data available; rather blepharismids should remain in the Spirostomidae. Our study strongly suggests that the classical heterotrichs form a natural group distinctly different from other taxa presently assigned to the heterotrichs, such as clevellandellids, armophorids, licnophorids, and odontostomatids. Loxodid karyorelictids and heterotrichs cannot be founded as monophyletic group based on stomatogenic modes, which are markedly different, viz. buccokinetal and parakinetal, respectively. However, both have structures reminiscent to a scutica, viz. a postoral ventral kinetofragment in loxodids and a special reorganization anlage for the parental oral apparatus in heterotrichs.

Key words: ciliates, Heterotrichida, Karyorelictida, ontogenesis, phylogeny.

INTRODUCTION

Recent molecular and ontogenetic evidence indicates that heterotrichs are a melting pot of phylogenetically widely separated organisms. Hirt *et al.* (1995) and Foissner (1996) thus suggested that their complex mouth architectures and cortical ontogenetic processes should be reassessed. While detailed information is available for some heterotrichs *sensu stricto*, viz. *Climacostomum* (Dubochet *et al.* 1979), *Condylostoma* (Bohatier *et al.* 1976), and *Eufolliculina* (Mulisch 1987, Mulisch and Patterson 1987), such data are very incomplete in heterotrichs *sensu lato*,

Address for correspondence: Wilhelm Foissner, Universität Salzburg, Institut für Zoologie, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria; Fax: +43 (0)662 8044-5698

such as armophorids, odontostomatids, clevellandellids, and licnophorids (for references, see Foissner 1996). However, ontogenesis of some other classical heterotrichs, viz. *Blepharisma* and *Stentor*, has also never been investigated in detail, though some species belong to the favourites of microsurgeons (Suzuki 1957; Eberhardt 1961, 1962; Wilfert 1972; further references, see Frankel 1989) and comprehensive data are available on the nuclear apparatus during division, reorganization, and conjugation (Young 1939, Weisz 1949, Suzuki 1954, McLaughlin 1957, Tartar 1961, Giese 1973). Thus, we performed a detailed study on *Blepharisma*, using protargol impregnation and scanning electron microscopy. Three species with a different nuclear configuration were chosen to explore the macronucleus-based subgeneric classification proposed by Hirshfield *et al.* (1965). Furthermore, our study should set a solid basis for a detailed comparison of the ontogenesis in heterotrichs and karyorelictids, which are sister groups according to ultrastructural and molecular data (Gerassimova and Seravin 1976, Baroin-Tourancheau *et al.* 1992, Fleury *et al.* 1992, Hammerschmidt *et al.* 1996).

MATERIALS, METHODS AND TERMINOLOGY

Blepharisma americanum Suzuki, 1954 was found in the coastal rain forest near Punta Pirikkihi, about 54 km south of Limon, Carribean Sea coast of Costa Rica, Central America, W 82°40', N 9°40'. It occurred in a soil sample (pH 6) collected on 23.2.1991 on the bank of a small freshwater pond about 20 m distant from the sea shore.

Blepharisma undulans Stein, 1867 was found in an alluvial soil of the Tullnerfeld, Lower Austria [detailed site description in Foissner *et al.* (1985)].

Blepharisma hyalinum Perty, 1849 was found in a mixed forest on the Pfennigberg near Linz, Upper Austria. It occurred in oak litter (0-3 cm) collected on 28.4.1994. The rewetted litter had pH 5.5 ($CaCl_2$). *Blepharisma hyalinum* became abundant one week after rewetting.

All species were isolated from dried, rewetted material with the non-flooded Petri dish method as described by Foissner (1987). Pure cultures of *B. americanum* and *B. undulans* were set up with a few cells from the raw cultures and maintained as described by Lüftenegger *et al.* (1985). Results from *B. hyalinum* are based on raw cultures, where the specimens readily divided.

Species were identified according to Foissner (1989) and Foissner and O'Donoghue (1990). The infraciliature was revealed with protargol as described by Foissner (1991); procedure A was used for *B. undulans* and *B. hyalinum*, and procedure B for *B. americanum*. Preparation for scanning electron microscopy followed the technique described in Foissner (1991). Counts and measurements on silvered specimens were performed at a magnification of x 1000. Standard deviation and coefficient of variation were calculated according to statistics textbooks.

Terminology is according to Corliss (1979) and Foissner (1996). Kinety 1 (K1) is, as usual (Corliss 1979), that which first shows a distinct sign of kinetosome proliferation. However, a definite assigment of the kineties is frequently impossible because several kineties are involved in anlagen production and the number, arrangement, and length of the postoral kineties vary considerably. For example, of 44 early dividers investigated in *B. americanum*, kinety 1 abuts to the proximal oral vertex in 73 % or accompanies the paroral membrane for a short distance in 27%. In *B. hyalinum* even the first bipolar kinety right of the paroral may be sometimes kinety 1 (Fig. 50). Very likely, not a certain kinety but a narrow postoral field is morphogenetically active. Considering these uncertainties and that we could not find any explanatory power of numbering the kineties in *Blepharisma*, we did it only when needed for clarity.

We distinguish between heterotrichs *sensu stricto* (*s. str.*) and heterotrichs *sensu lato* (*s. l.*). The first mainly comprise those united by Stein (1867) and Kahl (1932) in the families Spirostomidae, Condylostomatidae, Stentoridae, and Folliculinidae. Heterotrichs *s. l.* include those later assigned to the heterotrichs by various authors, e.g. the families Clevelandellidae, Nyctotheridae, Sicuophoridae, Epalxellidae, Discomorphellidae, and Mylestomatidae.

RESULTS

Morphostatic cells

The morphology and particularly the infraciliature of *Blepharisma* species are quite uniform and well known (Foissner 1989, Fig. 1). Thus, we provide only a brief description of the key characters and detailed morphometrics (Table 1).

Blepharisma americanum has a moniliform macronucleus with 4-6, rarely up to 9 (Table 1, Repak *et al.* 1977), nodules, and thus belongs to the *Blepharisma s. str.* group according to Hirshfield *et al.* (1965). The terminal nodules are usually slightly enlarged, as in the Japanese type population (Suzuki 1954) and the Australian specimens studied by Foissner and O'Donoghue (1990). Young cultures of *B. americanum* are conspicuously red because the cells have red to pink stripes of cortical granules and a diffusely red-coloured cytoplasm. In aged cultures or in cultures kept in dark, the pigment granules become bluish, almost colourless. The oral apparatus occupies on average 49% of body length (Table 1). The anterior third of the paroral membrane consists of a line of closely spaced basal bodies, while the posterior two thirds consist of zigzagging dikinetids having only the right basal body ciliated. Single long fibres originate from the left basal bodies in the posterior third of the paroral membrane and extend over the buccal cavity to enter the cytopharynx as oral ribs (Fig. 3).

The population of *Blepharisma undulans* used in the present study has been described by Foissner (1989). Briefly, this species has a binodal macronucleus connected by a thin strand; thus it also belongs to the *Blepharisma*

Table 1. Morphometric characteristics from *Blepharisma americanum* (first line), *B. undulans* (second line; from Foissner 1989), and *B. hyalinum* (third line)[1]

Character	x̄	M	SD	SE	CV	Min	Max	n
Body, length	202.7	203.0	34.3	8.6	16.9	151	253	16
	149.4	145.0	13.0	3.4	8.7	133	175	15
	69.9	71.0	5.6	1.4	8.0	61	79	16
Body, maximum width	55.3	49.0	12.9	3.2	23.4	37	79	16
	49.8	49.0	7.5	1.9	15.1	35	63	15
	18.9	19.0	2.0	0.5	10.8	16	24	16
Anterior somatic end to proximal end of adoral zone of membranelles, distance	99.7	102.5	12.5	3.1	12.5	67	115	16
	61.5	61.0	6.1	1.6	9.9	52	75	15
	38.6	39.0	3.2	0.8	8.4	32	43	16
Anterior somatic end to macronucleus, distance	54.0	55.0	11.3	2.8	21.0	35	80	16
	37.7	40.0	5.5	1.4	14.5	30	48	15
	19.8	20.0	2.4	0.6	12.3	15	24	16
Base of longest adoral membranelle, length	5.6	6.0	0.5	0.1	9.2	5	6	16
	4.1	4.0	0.3	0.1	6.2	4	5	15
	3.6	4.0	0.5	0.1	13.8	3	4	16
Macronucleus or macronuclear nodules, length	16.3	16.0	4.5	1.1	27.7	11	27	16
	22.1	22.0	3.1	0.8	13.9	17	28	15
	14.6	14.5	2.4	0.6	16.2	11	19	16
Macronucleus or macronuclear nodules, width	12.5	13.0	2.6	0.6	20.4	8	16	16
	9.6	10.0	2.1	0.5	21.5	7	14	15
	6.2	6.0	0.7	0.2	12.1	5	8	16
Micronuclei, diameter	1.9	2.0	0.1	0.0	7.1	1.5	2	13
	1.7	1.8	0.2	0.1	12.6	1.4	2	15
	1.7	2.0	0.4	0.1	23.2	1	2	12
Macronuclear nodules, number	5.4	5.0	–	–	–	5	6	16
	2.0	2.0	–	–	–	2	2	15
	1.0	1.0	–	–	–	1	1	16
Micronuclei, number[2]	8.2	8.0	1.9	0.2	22.7	4	12	75
	6.6	6.0	1.2	0.3	17.9	5	9	15
	1.1	1.0	0.3	0.1	27.6	1	2	11
Somatic kineties, number postoral	25.8	26.0	1.2	0.3	4.5	24	29	16
	26.1	26.0	1.0	0.3	4.0	25	28	15
	13.4	14.0	0.8	0.2	6.0	12	14	16
Dikinetids in a right lateral kinety, number	100.1	96.5	17.4	4.4	17.3	78	134	16
	140.3	135.0	20.0	5.2	14.3	105	170	15
	46.9	47.0	6.8	1.7	14.4	34	58	16
Adoral membranelles, number	58.9	58.5	2.8	0.7	4.8	55	66	16
	58.4	59.0	2.9	0.7	5.0	53	63	15
	28.0	28.0	1.4	0.3	4.9	26	30	16

[1] Data based on randomly selected protargol - impregnated specimens from pure cultures in exponential growth phase (*B. americanum* and *B. undulans*) or from raw cultures (*B. hyalinum*). Measurements in µm. CV – coefficient of variation in %, M – median, Max – maximum, Min – minimum, n – number of individuals investigated, SD – standard deviation, SE – standard error of the mean, x̄ – arithmethic mean

[2] Due to the small size of the micronuclei in *B. americanum*, inferring with similarly coloured and sized cell inclusions, they were counted in early dividers

s. str. group according to Hirshfield *et al.* (1965). The cortical granules are pink to brick-red. The oral apparatus occupies on average 41% of body length (Table 1) and is structured as described in *B. americanum*.

Blepharisma hyalinum can be easily identified by its small size (about 70 µm), the colourless cortical granules and the single, ellipsoidal macronucleus characterizing the *Compactus* group (Hirshfield *et al.* 1965). The oral apparatus extends about 55% of body length (Table 1). The anterior segment of the paroral membrane, where the basal bodies are arranged in a simple line, is relatively shorter than in the other two species (Fig. 48).

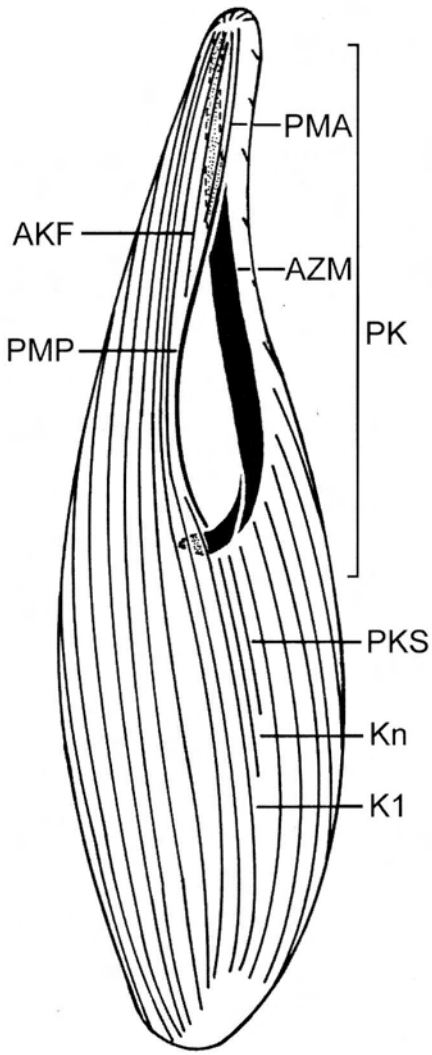


Fig. 1. Descriptive terminology of *Blepharisma*. As concerns K1 see Terminology section. It should be kept in mind that kinety 1 may abut to the proximal oral vertex (73 %) or may accompany the paroral membrane for a short distance as shown in this figure (27%; n - 44 early dividers). AKF - anterior kinety fragment, AZM - adoral zone of membranelles, K1 - stomatogenic (somatic) kinety 1, Kn - somatic kinety n, PK - postoral kineties abutting to posterior and left lateral margin of buccal cavity, PKS - shortened postoral kinety, PMA - anterior portion of paroral membrane composed of a line of single basal bodies, PMP - posterior portion of paroral membrane composed of paired, zigzagging basal bodies

Divisional morphogenesis

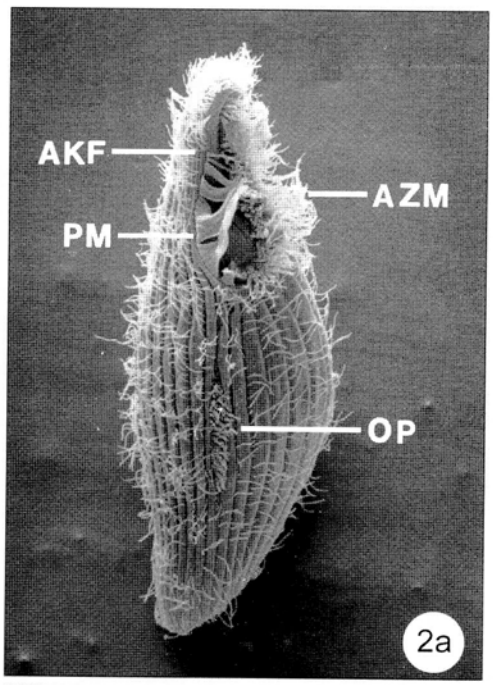
349 dividing specimens of *Blepharisma americanum*, 31 of *B. undulans*, and 46 of *B. hyalinum* were found in the protargol slides. Only few differences could be detected in the ontogenetic processes. Thus, observations and drawings from *B. americanum* (Figs. 2-4, 7-32, 35-47) and *B. hyalinum* (Figs. 33, 34, 48-54) also apply to *B. undulans* (Fig. 6), if not stated otherwise.

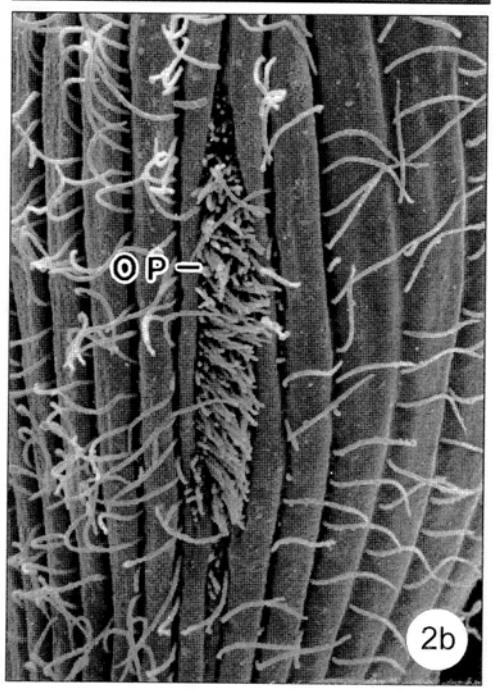
Opisthe stomatogenesis

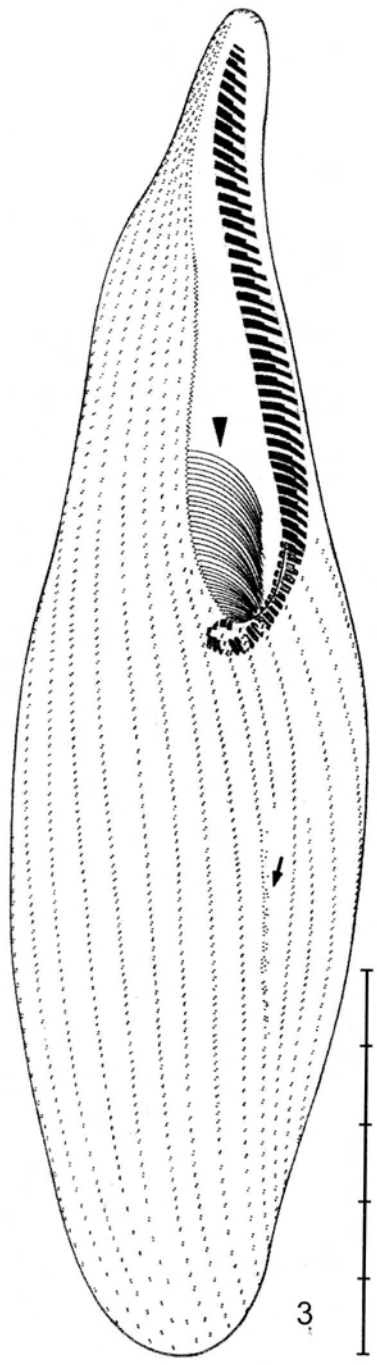
Stomatogenesis commences with the formation of some small groups of basal bodies within and to the left of the middle portion of a postoral kinety (kinety 1 per definition, but see Terminology ; Figs. 2, 3, 6, 15, 33, 48). Next, some neighbouring kineties proliferate basal bodies, at least one kinety but usually two kineties in *Blepharisma americanum* (Figs. 7, 8, 10, 16-18) and *B. undulans*, and rarely none usually one in *B. hyalinum* (Figs. 34, 50). In about 10% of the *B. americanum* specimens (n = 82) kinety n-1 shows some small groups of basal bodies, i.e. four kineties are involved in oral primordium formation. Proliferation proceeds in a sequential fashion, occurring first along "central" kinety 1 and then in kinety 2 and/or n (Figs. 15-17, 33, 34, 48, 50); kinety 2 produces basal bodies earlier than kinety n in 74% of cases (n = 69). Furthermore, proliferation occurs in an oblique fashion, i.e. kineties 2 and n proliferate slightly posteriad and anteriad, respectively, to the elongate field generated by kinety 1 (Figs. 7, 16-18, 34, 50). The elongate anarchic fields, which include the parental basal bodies, soon consist of unoriented kinetosomal pairs (cp. Bohatier 1979, Mulisch and Hausmann 1988), and finally fuse to form a single oral anlage, which progressively becomes larger by continued proliferation of basal bodies (Figs. 18, 51). The anlage develops in a flat groove, which originates by spreading of the furrows in which the stomatogenic kineties extend (Fig. 2b).

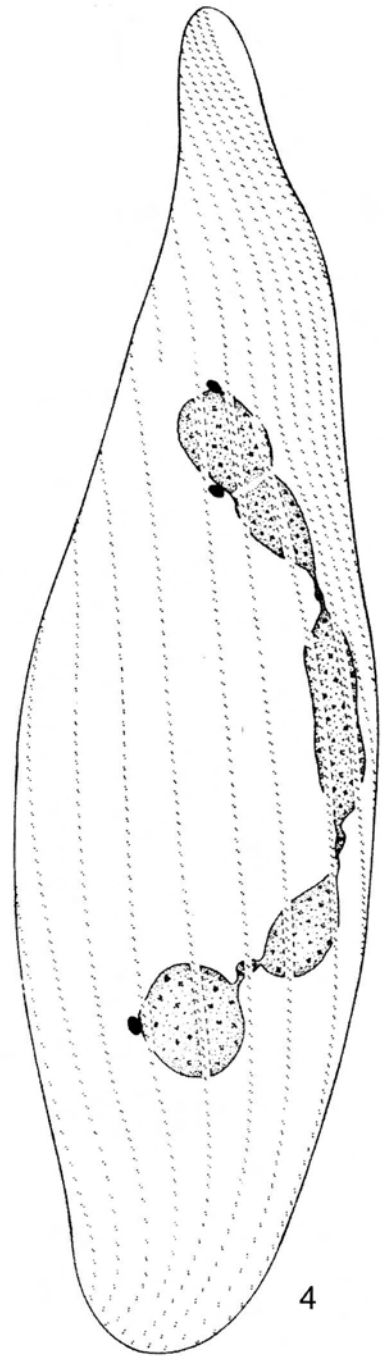
In the next series of events, the adoral membranelles and the paroral membrane are formed. The oral primordium splits longitudinally into two kinetosomal fields of unequal width: the left, which occupies two thirds of the width of the primordium, produces the adoral membranelles, while the narrow right field generates the paroral. Splitting commences centrally and proceeds towards the ends of the anlage (Figs. 8, 10, 18, 19, 51). Likewise, formation of membranelles begins by alignment of dikinetids in the middle part of the primordium and proceeds anteriad and posteriad as well as from right to left (Figs. 8, 10, 18-20, 37, 51, 52). The shortened third row of membranellar kinetosomes is added only when the posterior membranelles, which later form a short spiral, curve to the right (Figs. 11, 13, 21, 53).

The paroral primordium consists of dispersed and probably paired basal bodies (Figs. 8, 18, 19, 51). Subsequently, kinetosomes become more narrowly spaced and form a posterior segment consisting of obliquely (zigzag) arranged dikinetids and an anterior segment made of narrowly spaced kinetids in single file (Figs. 9, 11, 20, 21,

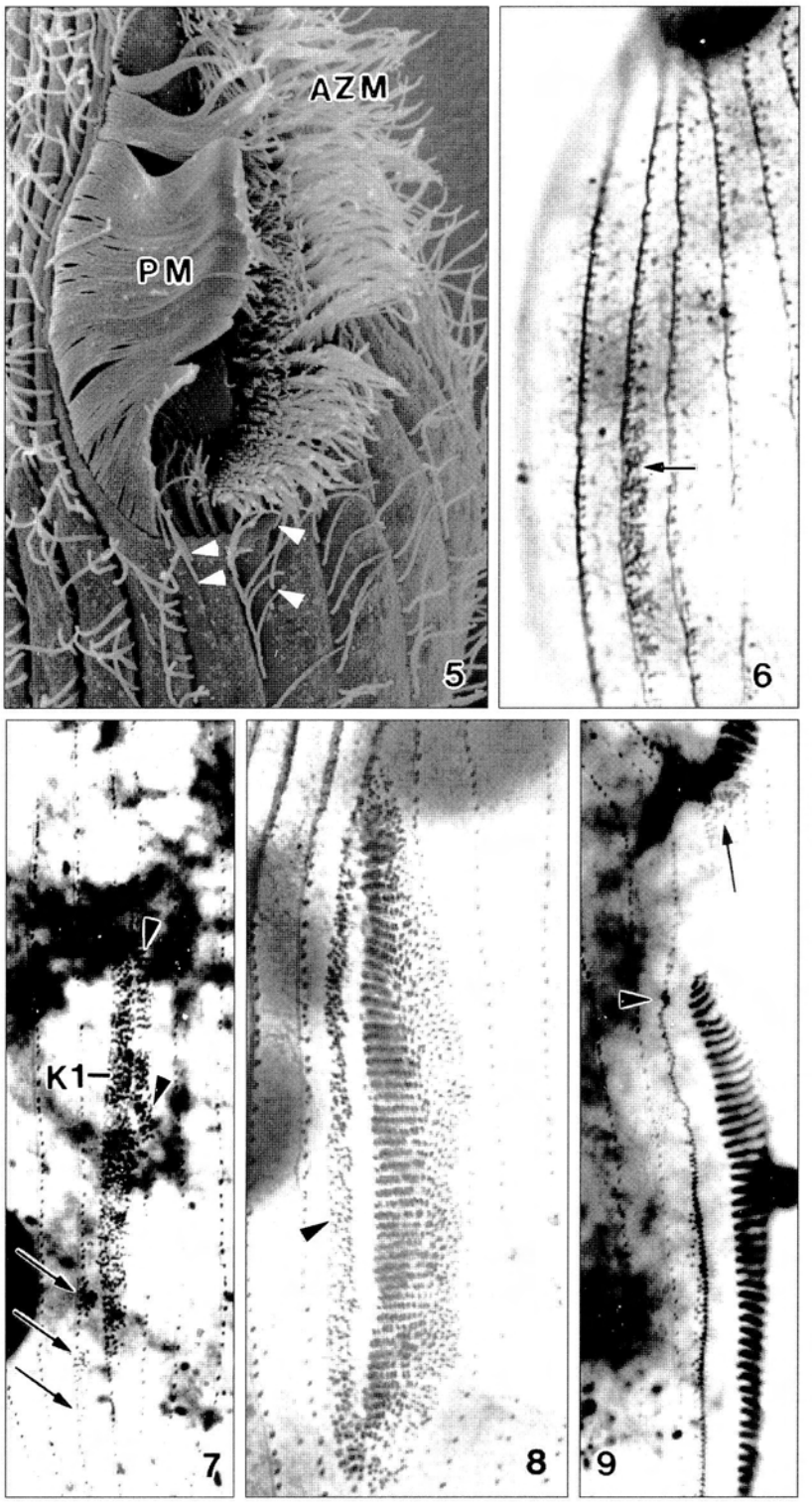




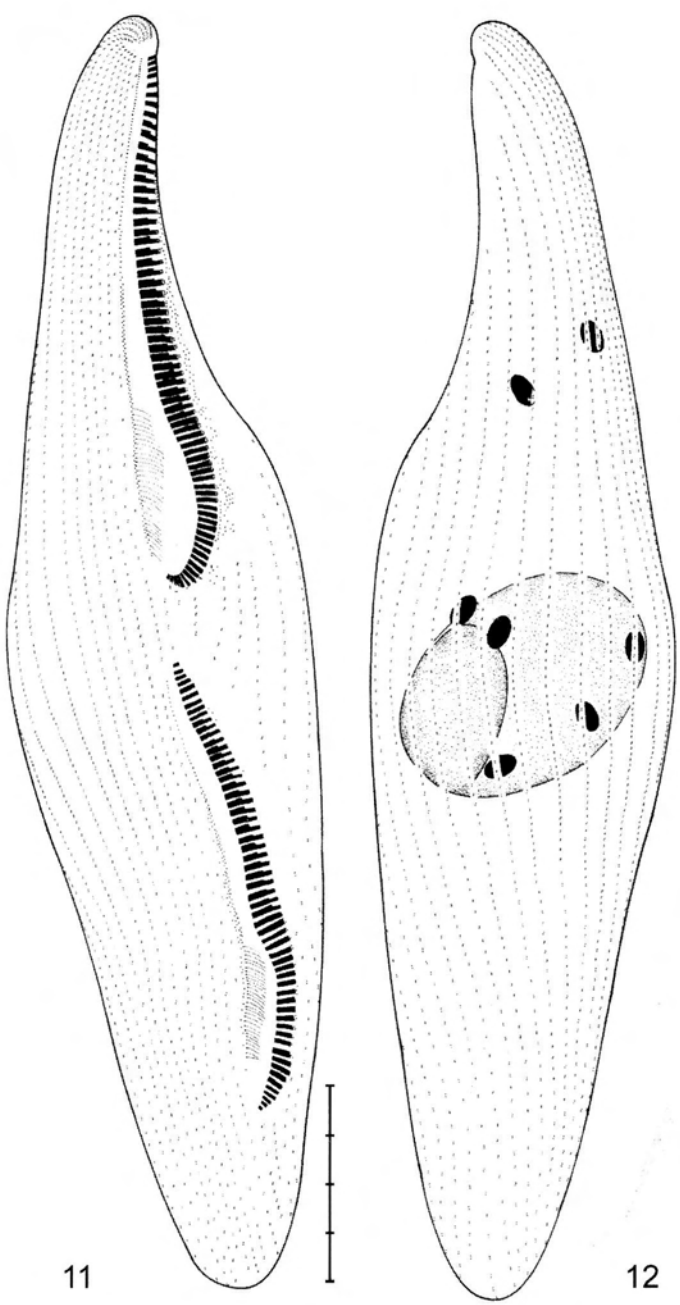


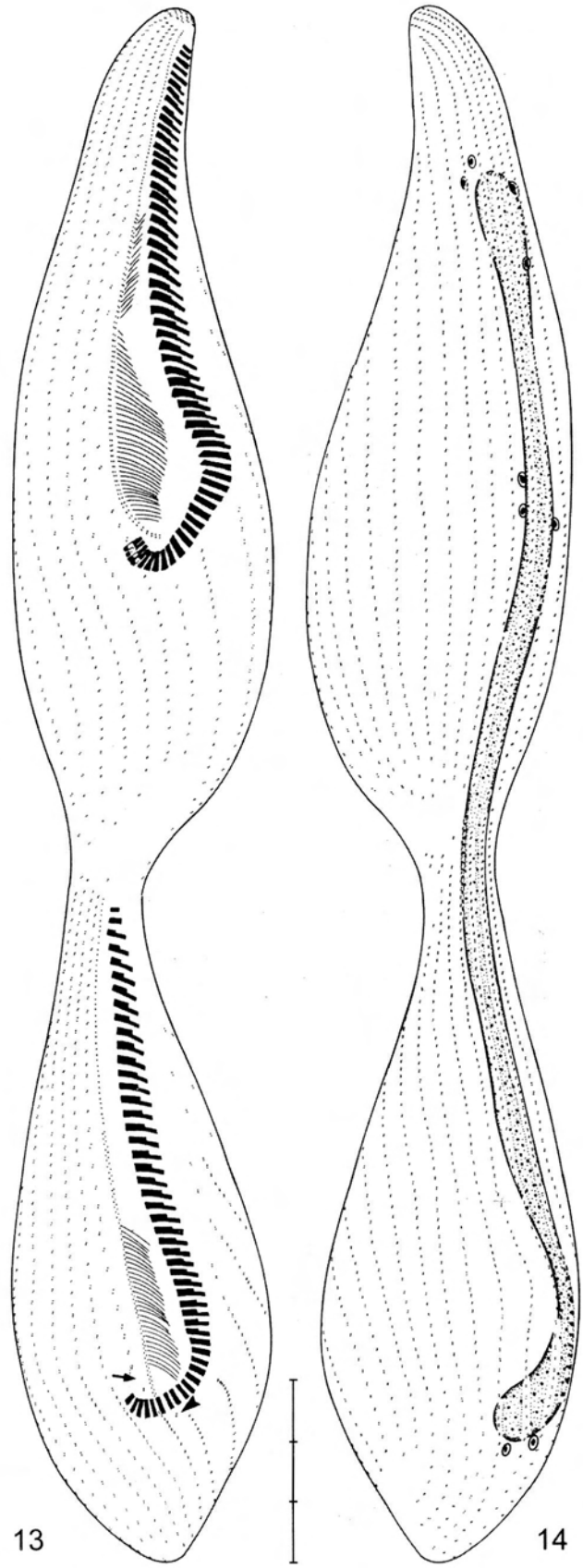


Figs. 2-4. Ventral (Figs. 2a, b, 3) and dorsal (Fig. 4) views of early dividers of *Blepharisma americanum* in the scanning electron microscope (Figs. 2a, b) and after protargol impregnation (Figs. 3, 4). 2a - *Blepharisma americanum* is about 200 µm long, has stripes of red cortical granules, and a moniliform macronucleus with 5 or 6 nodules (Fig. 4). The oral primordium for the opisthe originates subequatorially in a position distinctly apart from the parental oral apparatus; 2b - the oral primordium develops in a flat groove, which originates by spreading of the furrows in which the stomatogenic kineties extend; 3, 4 - ventral and dorsal infraciliature. The anterior third of the paroral membrane is composed of a line of closely spaced basal bodies, while the posterior two thirds consist of zigzagging dikinetids having ciliated only the right basal body. Long fibres (arrowhead) originate from the left basal bodies in the posterior third of the paroral membrane and extend over the buccal cavity to enter the cytopharynx as oral ribs (cp. Fig. 38). Stomatogenesis commences with a proliferation of small groups of basal bodies (arrow) within and to the left of postoral somatic kinety 1; note also the irregular kineties to the left. Some macronuclear nodules are fusing. Scale bar division 10 µm. AKF - anterior kinety fragments, AZM - adoral zone of membranelles, OP - oral primordium, PM - paroral membrane

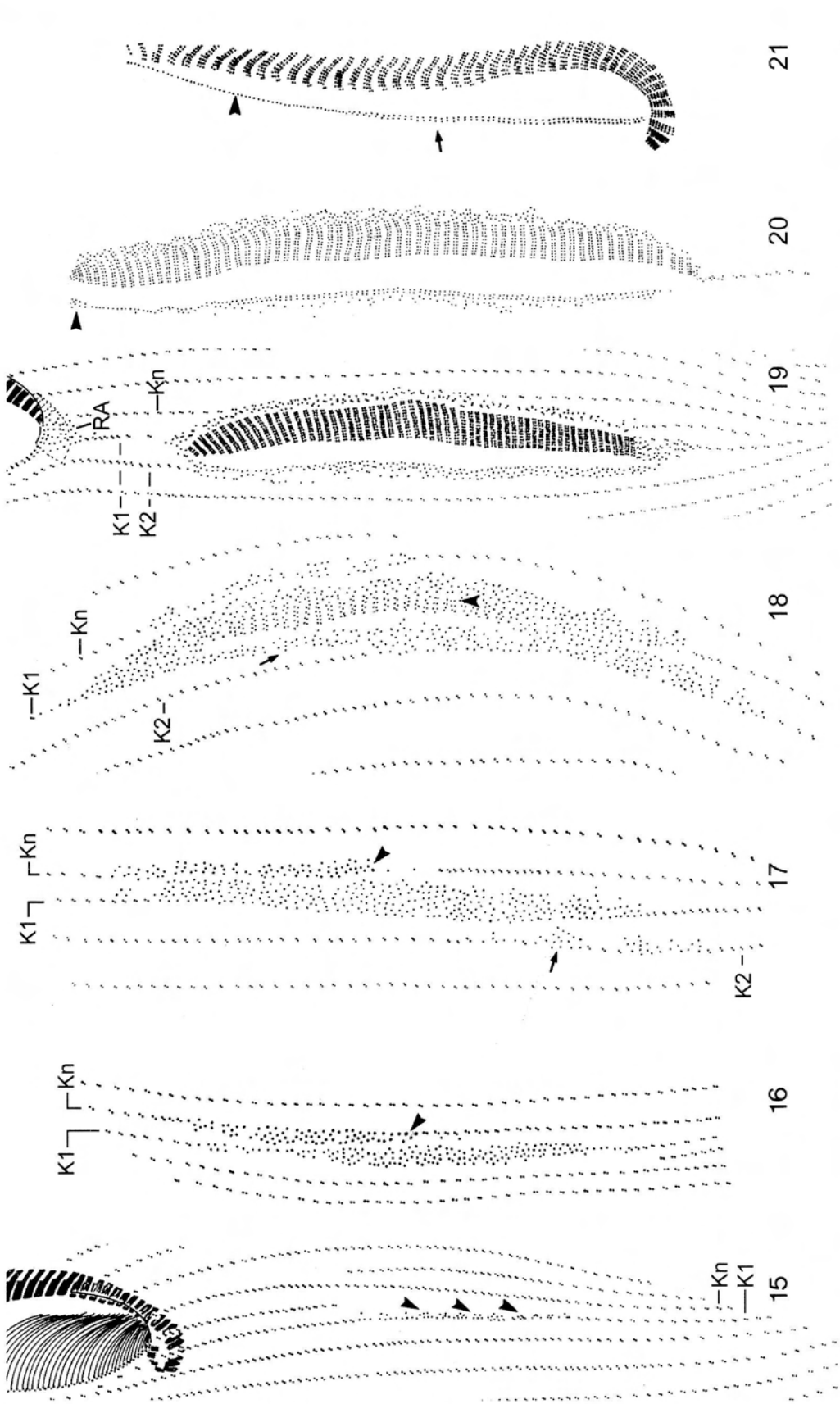


Figs. 5-10. Ventral views of a morphostatic specimen (Fig. 5) and of early (Figs. 6, 7) and middle (Figs. 8-10) dividers of *Blepharisma americanum* (Figs. 5, 7-10) and *B. undulans* (Fig. 6) in the scanning electron microscope (Fig. 5) and after protargol impregnation (Figs. 6-10). 5 - scattered kinetosomal pairs (arrowheads), which have ciliated only one basal body, occur along the buccal vertex; 6 - stomatogenesis commences in postoral kinety 1 (arrow); 7 - kinety 2 proliferates slightly posteriad (arrows) and kinety n slightly anteriad (arrowheads) to the elongated field of basal bodies in kinety 1; 8 - the oral primordium divides longitudinally to form a narrow right paroral (arrowhead) and a broad left adoral anlage; 9 - note cirrus-like patch of basal bodies (arrowhead) at anterior end of newly formed paroral. Arrow marks reorganization anlage of proter; 10 - differentiation of oral structures in the opisthe and reorganization of parental oral apparatus (cp. Figs. 8, 15-21). Arrowheads mark reorganizing paroral membrane; arrow denotes reorganization anlage. In this specimen, kinety 1 abuts to the proximal oral vertex, thus kinety 2 and n have contributed to the oral anlage. Scale bar division 10 µm. AZM - adoral zone of membranelles, K1 - stomatogenic kinety 1, PM - paroral membrane

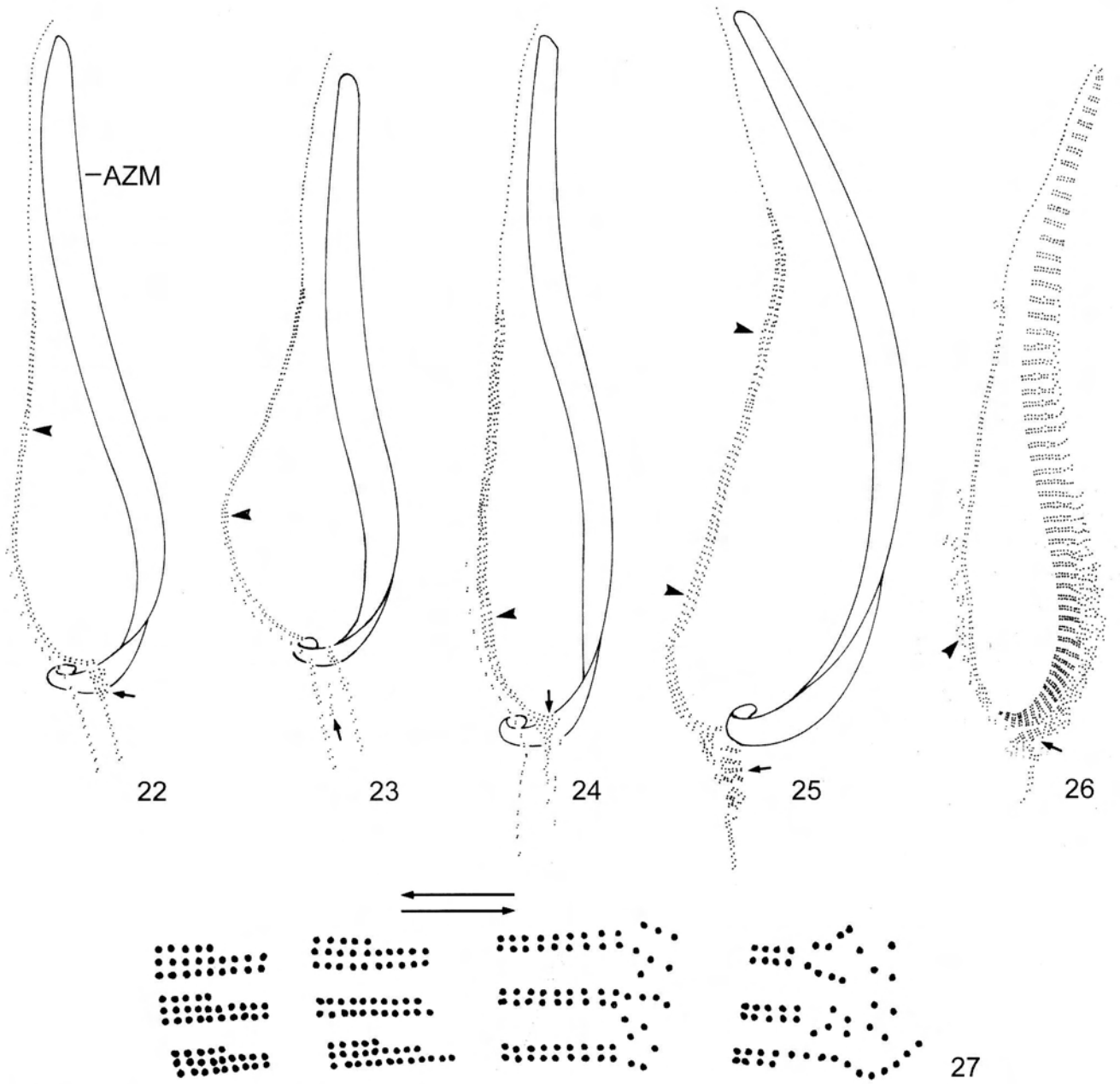




Figs. 11-14. Ventral (Figs. 11, 13) and dorsal (Figs. 12, 14) infraciliature of middle and late dividers of *B. americanum* after protargol impregnation. 11 - the shortened third row of membranellar kinetosomes is added when the posterior membranelles curve to the right (for details, see Figs. 20, 21); note proter's despiralized zone of adoral membranelles; 12 - the macronuclear nodules are fused and the prophasic micronuclei are enlarged; 13 - the proximal portion of the adoral zone of membranelles invaginates and spirals around the forming buccal cavity in each filial product. The arrowhead denotes a scattered kinetosomal pair on the buccal vertex of the opisthe; the arrow marks splitting first kinety to the right of the paroral membrane; 14 - the elongated macronucleus divides. Scale bar division 10 μm



Figs. 15-21. Details of stomatogenesis in the opisthe of *Blepharisma americanum* after protargol impregnation. 15 - basal bodies originate within and to the left of the middle portion of postoral (stomatogenic) kinety 1 (arrowheads); 16, 17 - some neighbouring kineties proliferate basal bodies in a sequential and oblique fashion, i.e. kineties 2 and n proliferate later than kinety 1 and slightly posteriad (Fig. 17, arrow) and anteriad (Figs. 16, 17, arrowheads), respectively; 18, 19 (detail of Fig. 10) - the oral primordium divides longitudinally to form the narrow right paroral (arrow) and the broad left adoral anlage (arrowhead). Splitting commences centrally and proceeds towards the ends of the primordium. Likewise, formation of membranelles begins by alignment of dikinetids in the middle part of the primordium and proceeds anteriad and posteriad as well as from right to left; 20 - kinetosomes of the paroral primordium become aligned and a cirrus-like patch of basal bodies (arrowhead) is recognizable at the anterior end of the newly forming paroral; 21 - the paroral membrane is composed of a dikinetidal posterior segment (arrow) and a monokinetidal anterior segment (arrowhead). A short third row of kinetosomes is added to the adoral membranelles when the posterior membranelles curve to the right. K1 - stomatogenic kinety 1, K2 - kinety 2, Kn - kinety n, RA - reorganization anlage



Figs. 22-27. Details of reorganization in the proter oral apparatus of *Blepharisma americanum* after protargol impregnation. 22, 23 - new basal bodies originate very near to the parental paroral membrane concomitantely at various positions (arrowheads). The reorganization anlage for the posterior adoral membranelles originates by proliferation of basal bodies (arrows) within (Fig. 22) and between (Fig. 23) the anterior ends of the postoral kineties; 24, 25 - by further proliferation of basal bodies quadruplets are formed, which separate into two rows of dikinetids. Splitting commences at several sites (arrowheads). The reorganization anlage comprises a small, triangular anarchic field of basal bodies, which abuts to the proximal end of the reorganizing paroral membrane (Fig. 24, arrow). Four to eight adoral membranelles differentiate from the reorganization anlage and replace the proximal parental adoral membranelles (Figs. 25, 26, arrows); 26, 27 - the right dikinetidal row is almost completely resorbed (arrowhead); the left one constitutes the new paroral membrane. The left, two-rowed part of the adoral membranelles is reorganized *in situ*, while the right portion remains unchanged, except of the shortened third row of basal bodies, which is resorbed (Fig. 27). The arrow marks the reorganization anlage, which replaces the posteriormost parental adoral membranelles. The reconstruction of the adoral membranelles takes place very much like their dedifferentiation, but *vice versa*, i.e. the disorganized kinetids arrange to rows and the third row is rebuilt. AZM - adoral zone of membranelles

52, 53). Furthermore, a cirrus-like patch of basal bodies is recognizable at the anterior end of the forming paroral (Figs. 9, 20, 53). This patch consists of 2-3 dikinetids and is resorbed when cytokinesis commences (Figs. 11,

13, 21). Some kinetosomes to the right of the renewed paroral membrane (Fig. 20) are obviously not used for paroral formation and are resorbed during cytokinesis.

Finally, the proximal end of the adoral zone of membranelles invaginates and spirals around the forming cytostome in about one turn (Figs. 13, 38). Some isolated kinetosomal pairs remain at the posterior end of the newly formed oral apparatus in about 40% of the *B. americanum* (Figs. 13, 41, 45-47) and *B. undulans* specimens; in most specimens they disappear during cytokinesis. These pairs, which were never seen in *B. hyalinum*, are highly reminiscent of a scutica and have at least one basal body ciliated (Fig. 5). The same can be observed in reorganizing proters (Figs. 42-44).

Proter reorganization

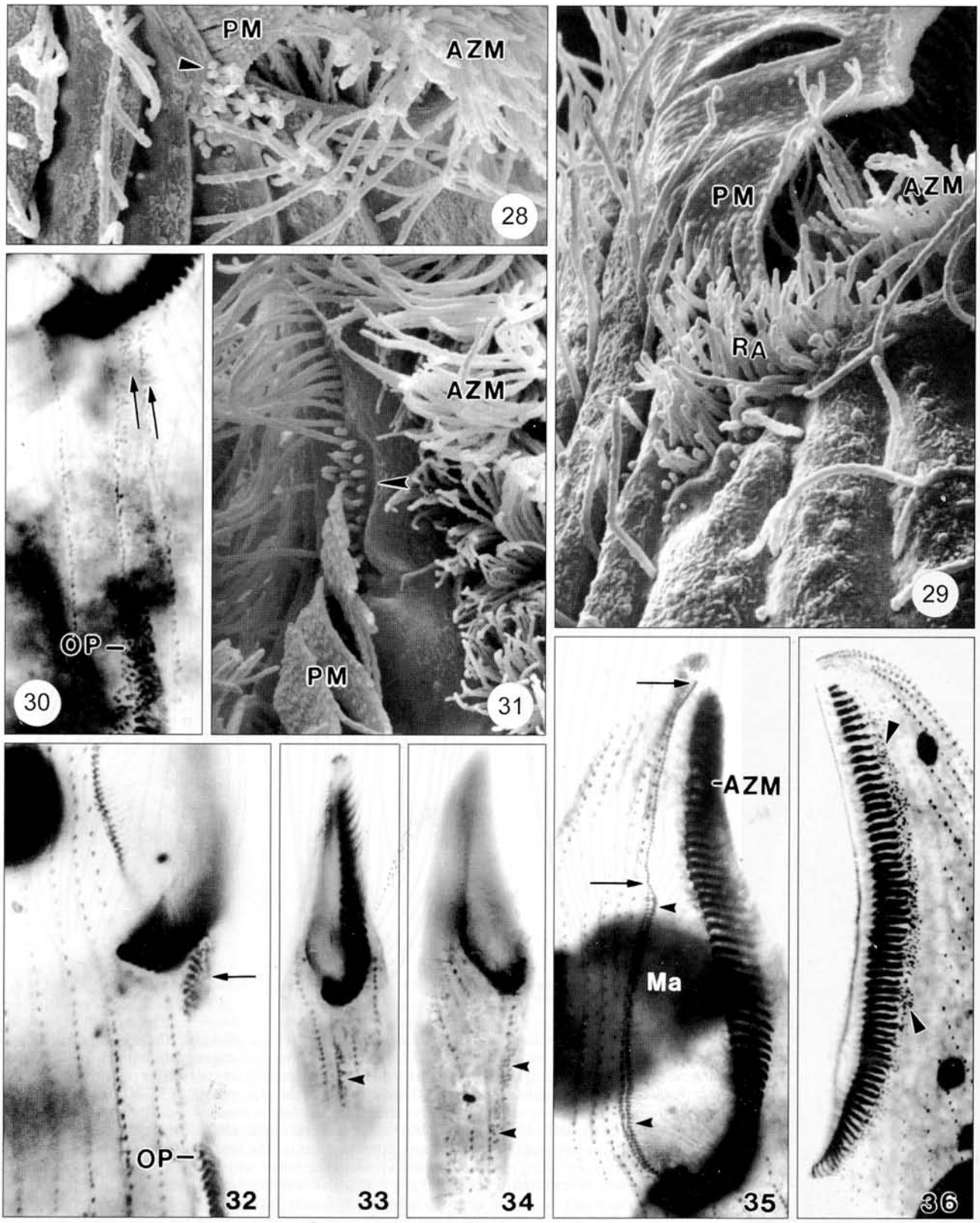
The reorganization of the parental oral apparatus differs in the *Blepharisma* species investigated, especially as concerns the adoral zone of membranelles. While the adoral zone of *B. hyalinum* shows no indication of reorganization, at least in the light microscope, two distinct reorganization processes occur in the adoral zone of *B. americanum* and *B. undulans*, viz. an *in situ* reorganization of the left portion of the individual membranelles and a partial or complete renewal of the proximal membranelles by a special reorganization anlage. However, the reorganization anlage is absent in about one third of the respective stages of *B. americanum*, i.e. when the left margin of the adoral membranelles becomes disordered and the cytopharyngeal spiral unwinds. Very likely, the same is true for *B. undulans*, although this could not be definitely proved because too few appropriate stages were found.

The *in situ* reorganization of the adoral membranelles includes a disorganization and fan-like spreading of the left, two-rowed part of the membranelles, while the right portion remains unchanged, except of the shortened third row of basal bodies, which is resorbed (Figs. 26, 27, 36). The reconstruction of the membranelles, which occurs when the reorganized posterior portion of the adoral zone spiralizes (see below), takes place very much like their dedifferentiation, but *vice versa*, i.e. the disorganized kinetids arrange to rows and the third row is rebuilt (Fig. 27). Whether parental and/or new basal bodies are used for the reconstruction could not be clarified. However, at least the third row of the membranelles is very likely made of newly formed basal bodies because kinetosomes migrating from left to right were never observed.

The proximal membranelles of the parental adoral zone are partially or completely reorganized by a special primordium developing on the vertex of the buccal cavity. This primordium was named "Regenerationsanlage" by Eberhardt (1961) and later, more appropriately, "reorganization anlage" (Suzuki 1973). The renewal, which is only loosely coupled with the opisthe stomatogenesis (see below), includes two concomitantly passing events, viz. the despiralization of the parental adoral zone and the formation of the reorganization anlage. The basal bodies of the reorganization anlage originate by proliferation within, to the left and between ("apokinetally"; see Discussion) the anterior ends of somatic kineties 1 and n (Figs. 9, 10, 22-24, 28, 30). They form a small, triangular anarchic field which abuts to the proximal end of the reorganizing paroral membrane (Figs. 24, 29). Probably, the parental paroral participates in the formation of the anlage (Figs. 24, 25, 28), from which 4-8 adoral membranelles or membranellar fragments differentiate. Whether these fragments, which consist of two rows of kinetosomes, become attached to the left margin of the proximal parental adoral membranelles (Fig. 26) or entirely replace some parental membranelles (Figs. 25, 32) could not be clarified. However, some scanning electron micrographs suggest that the right (inner) half of the parental membranelles is retained. Finally, at the end of cytokinesis, the posterior portion of the adoral zone spiralizes again (Fig. 13).

The posterior (zigzag) segment of the paroral membrane is reorganized in all species, while the anterior segment remains unchanged, except in one specimen of *B. americanum*, where it showed some disorder. The

Figs. 28-36. Reorganization of the parental oral apparatus in *Blepharisma americanum* (Figs. 28-32, 35, 36), and early dividers of *B. hyalinum* (Figs. 33, 34) in the scanning electron microscope (Figs. 28, 29, 31) and after protargol impregnation (Figs. 30, 32-36). 28, 29 - the reorganization anlage is a small, triangular field of ciliated basal bodies on the buccal vertex and usually very near to the parental paroral membrane (arrowhead in Fig. 28); 30 - new basal bodies (arrows) for the reorganization anlage originate by proliferation within and/or between the anterior ends of postoral kineties; 31 - the paroral primordium (arrowhead) originates very near to the parental paroral membrane; 32 - up to eight adoral membranelles (arrow) differentiate from the reorganization anlage; 33, 34 - one or two somatic kineties (arrowheads) proliferate basal bodies for the oral primordium in the opisthe of *B. hyalinum*; 35 - the quadruplets of the paroral primordium separate into two double-rows by longitudinal splitting (arrowheads). Note that the anterior, monokinetidal portion of the paroral membrane is not reorganized (arrows); 36 - the left part of the adoral zone of membranelles is reorganized *in situ* (arrowheads) and the third kinetosomal row of the adoral membranelles is resorbed (Fig. 27). AZM - adoral zone of membranelles, MA - macronucleus, OP - oral primordium, PM - paroral membrane, RA - reorganization anlage


PM
AZM
28
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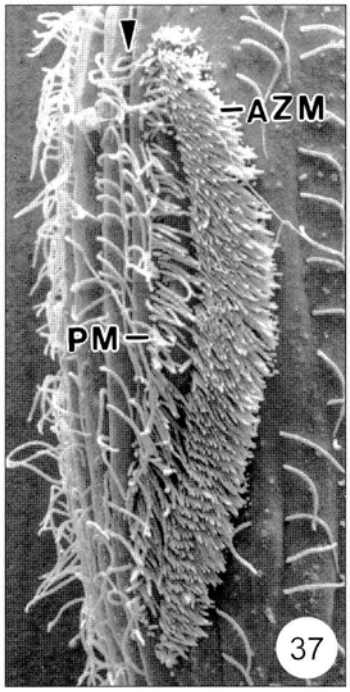
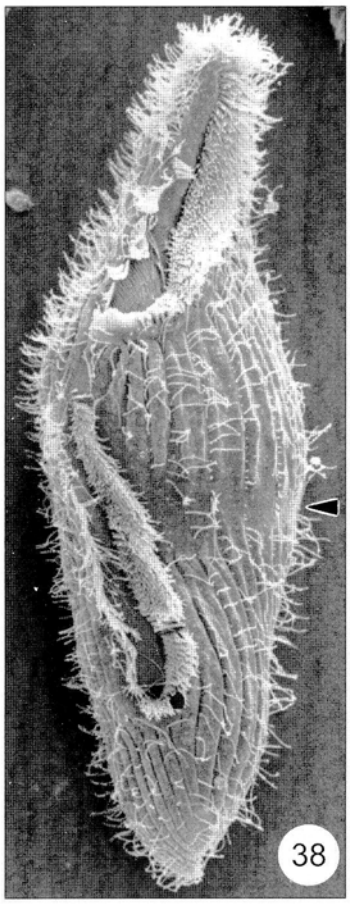
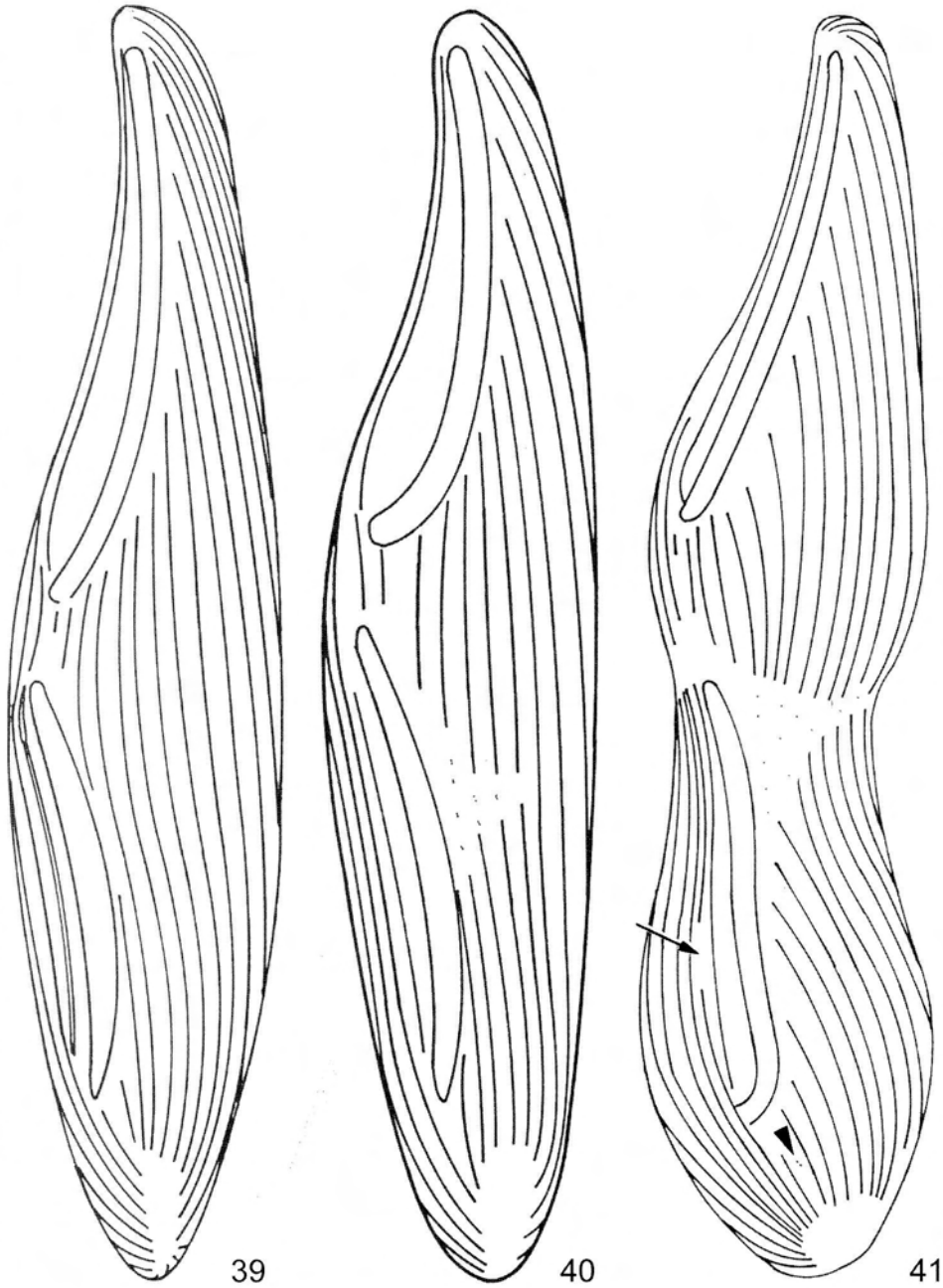
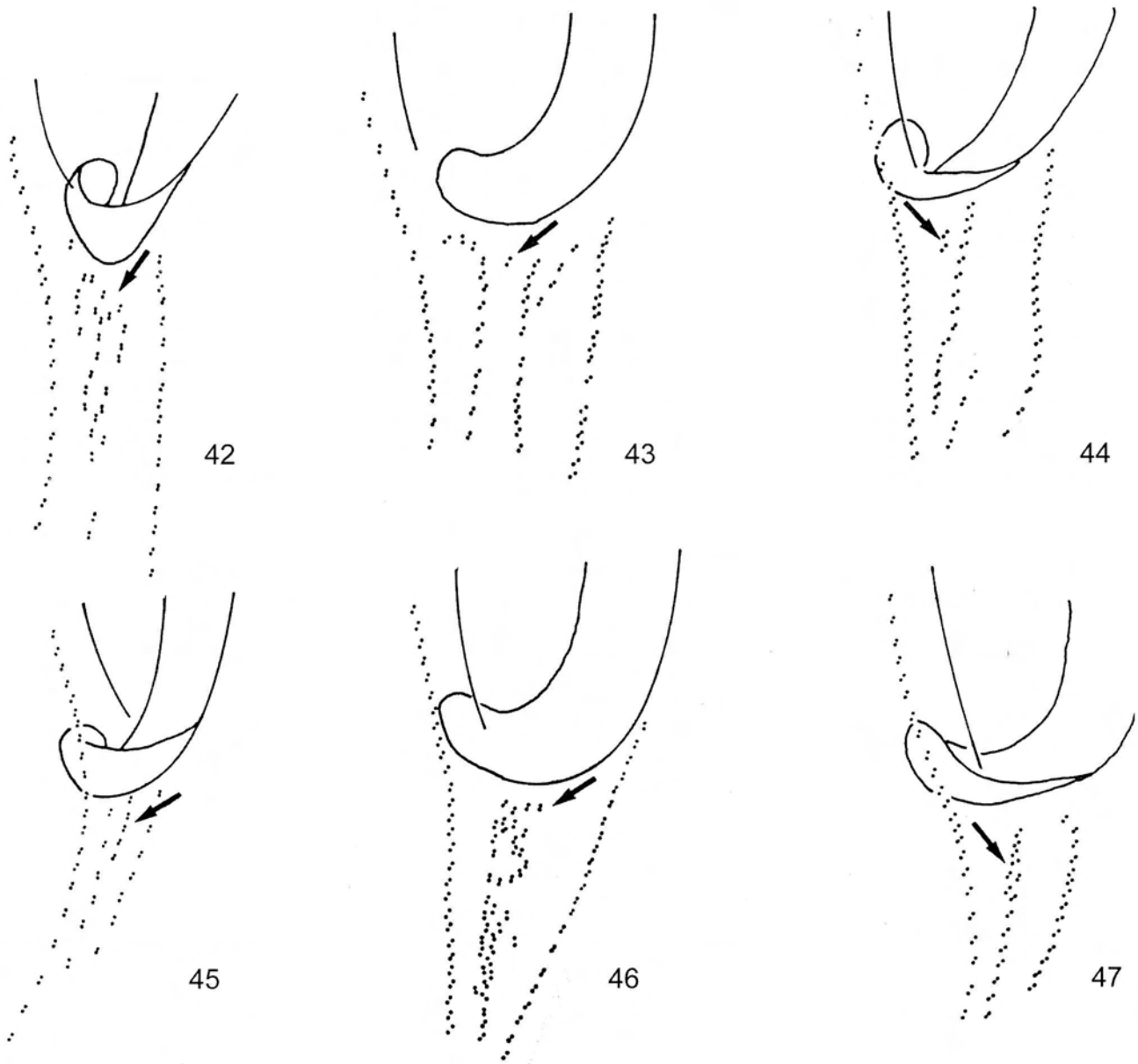




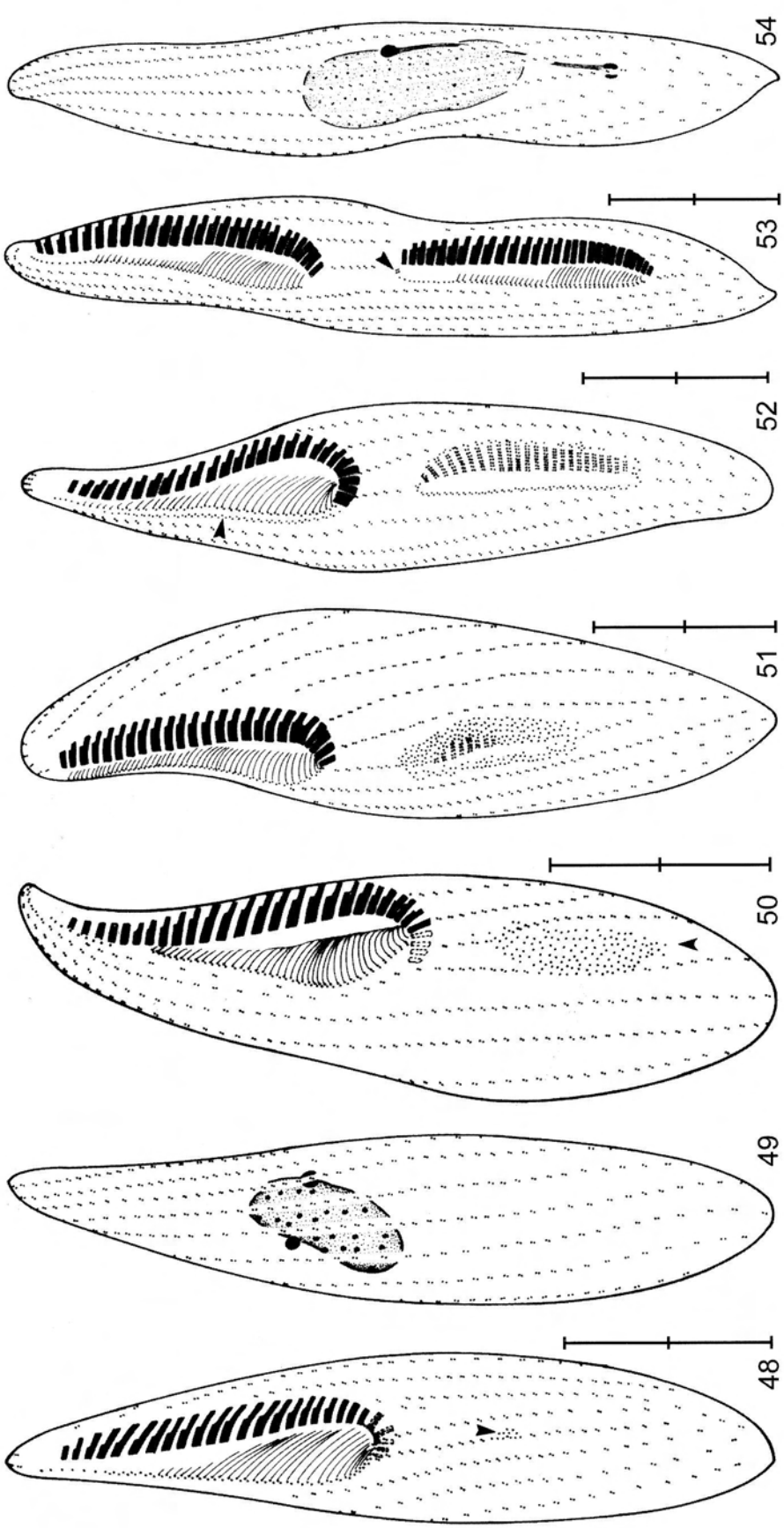


Figs. 37-41. Oral primordium (Fig. 37) and somatic division (Figs. 38-41) of *Blepharisma americanum* in the scanning electron microscope (Figs. 37, 38) and after protargol impregnation (Figs. 39-41). 37 - the anterior portion of stomatogenic kinety 2 (arrowhead), which proliferates only in the posterior region, does not disintegrate and remains as shortened fragment right of the oral apparatus; 38-40 - kineties at the left side of the oral primordium separate slightly subequatorially (arrowhead); 41 - during cytokinesis the new anterior ends of the left lateral kineties abut to the new oral apparatus, while the rear ends are used in posterior pole formation of the proter. Note splitting (arrow) of the first bipolar kinety to the right of the paroral membrane, resulting in an anterior and a posterior segment, which usually becomes somatic kinety 1 in postdividers. A scattered kinetosomal pair is also visible (arrowhead). Note also that the short kinety fragment right of the paroral can be produced either by kinety 2 (Figs. 37, 41, 52, 53) or kinety 3 (Figs. 9, 10, 19), depending on the number and extent of kineties involved in oral primordium formation (see Results)

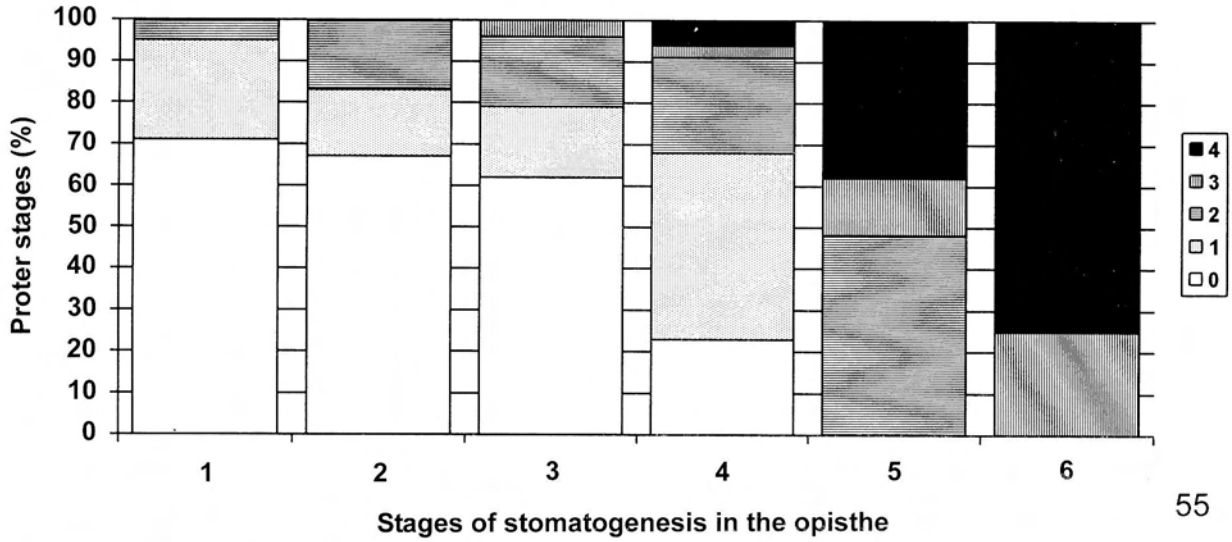


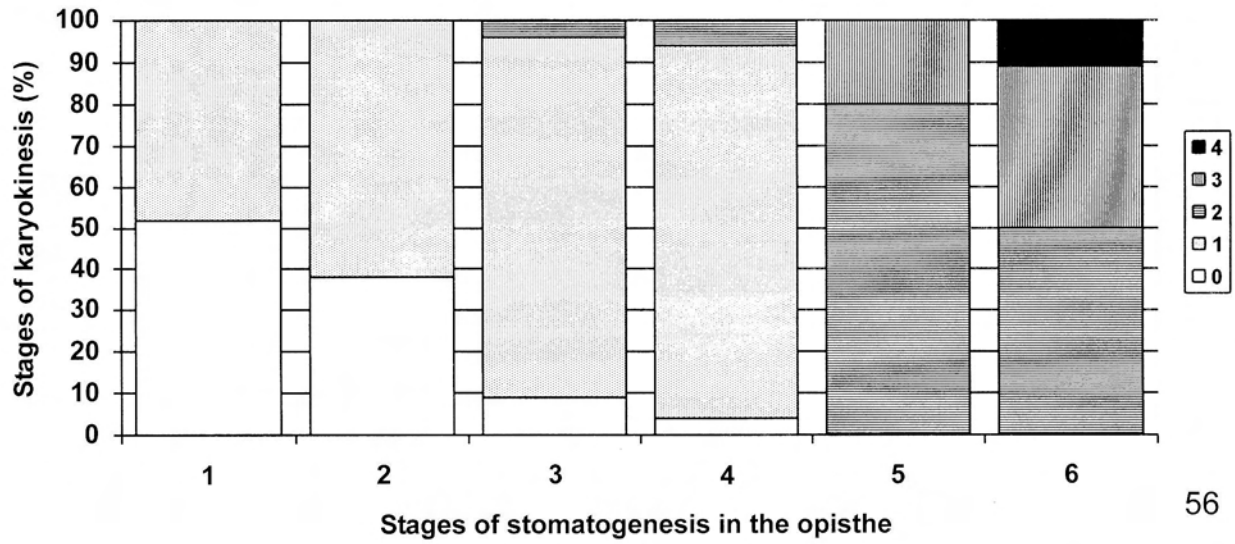
Figs. 42-47. Late proters (Figs. 42-44) and opisthes (Figs. 45-47) of *Blepharisma americanum* after protargol impregnation, showing variability in number and arrangement of kinetosomal pairs (arrows) remaining from the reorganization anlage, respectively, the oral primordium. The kinetosomal pairs, which are resorbed during cytokinesis, are between the postoral kineties. An exact designation of these kineties is impossible for the reasons explained in Terminology

exact sequence of proliferation of individual kinetosomes could not be clarified. However, we could clearly recognize the following details because of the extraordinary quality of the protargol preparations: in early stages, triplets and quadruplets of kinetosomes arise concomitantely at various positions within or possibly to the left of the parental paroral (Figs. 22-24, 31). By further proliferation of basal bodies the entire posterior segment finally consists of quadruplets and longitudinally splits into two double-rows of kinetosomes (Figs. 25, 35, 52). Splitting starts, like proliferation, at several sites (Figs. 10, 24, 35). The right double-row is successively resorbed



Figs. 48-54. Morphogenesis in *Blepharisma hyalinum*, infraciliature and nuclear apparatus after protargol impregnation. 48, 49 - ventral and dorsal view of a very early divider showing oral primordium (arrowhead) close to postoral kinety 1 (cp. Fig. 33); 50 - ventral view of an early divider. The oral primordium (arrowhead) has enlarged by continued proliferation of basal bodies and extends between kinety 1 and n. Usually, two postoral kineties are involved in oral primordium formation (cp. Fig. 34); 51 - ventral view of a middle divider. The oral primordium splits in a narrow right paroral and a broad adoral anlage, which develops membranelles from the centre to the ends as well as from right to left. The development of membranelles from the centre to the ends of the oral primordium is probably a unique feature of heterotrichs *s. str.*; 52 - ventral view of a late divider. Only the posterior (zigzag) segment of the parental paroral is reorganized; the arrowhead denotes resorbing remnants of the paroral primordium. The adoral structures of the proter do not reorganize in *B. hyalinum*; 53, 54 - ventral and dorsal view of a late divider. The paroral primordium of the opisthe shows an apical, cirrus-like structure (arrowhead), which is, however, resorbed when cytokinesis commences. The single macronucleus simply divides (Fig. 54). Scale bar division 10 µm





Figs. 55, 56. Temporal relationships of divisional processes (proter and opisthe stomatogenesis as well as karyokinesis and opisthe stomatogenesis, respectively) in *Blepharisma americanum* (n=290). Stages of stomatogenesis in the opisthe: (1) kinetosome proliferation in kinety 1 (Fig. 15); (2) kinetosome proliferation in kineties 1 and 2 and/or n (Figs. 7, 16, 17); (3) separation of oral primordium in centre of anlage and onset of adoral membranelle formation (Fig. 18); (4) adoral membranelles consist of 2 rows of basal bodies and kinetosomes become more narrowly spaced in the paroral primordium (Figs. 8, 10, 19, 20); (5) third row of kinetosomes is added to the adoral membranelles; new paroral differentiated (Figs. 9, 21, 36); (6) spiralization of the posterior portion of the adoral zone of membranelles and of the paroral membrane. Stages of reorganization in the parental oral apparatus: (0) no reorganization events (Fig. 3); (1) kinetosome proliferation beside kinety 1 and/or the posterior segment of the paroral membrane (Figs. 22, 23, 30); (2) reorganization anlage consists of kinetosomal fields beside and between kineties 1 and n and/or the paroral primordium and the parental paroral are partially separated (Figs. 10, 24, 28); (3) triangular reorganization anlage, parental and newly formed paroral membrane completely separated, reorganization of adoral membranelles (Figs. 11, 25, 29, 35, 36); (4) resorption of parental paroral membrane, spiralization of posterior portion of parental adoral zone of membranelles (Fig. 26). Stages of karyokinesis: (0) no nuclear events (Fig. 49); (1) micronuclei prophasic and/or two or more macronuclear nodules fusing (Fig. 4); (2) micronuclei metaphasic and/or macronuclear nodules fused to an ellipsoidal mass (Fig. 12); (3) micronuclear division and/or one elongated (dividing) macronucleus (Fig. 14); (4) micronuclei and macronucleus divided

(Fig. 26); the left one constitutes the newly built paroral membrane. Oral ribs were always present, however, it could not be clarified whether they persisted or were cryptically reorganized (Figs. 11, 13, 35, 38, 52, 53).

Somatic division

Somatic division commences with an intrakinetal proliferation of basal bodies in some kineties right of the

anterior end of the oral primordium (Fig. 10). This is evident from the close spacing of the dikinetids in this section. The 6-10 kineties adjacent to the left side of the oral primordium do not proliferate, but gradually separate slightly subequatorially at the level of the differentiating opisthe oral apparatus (Figs. 11, 39, 40). The new anterior ends of these kineties converge to the new adoral membranelles during cytokinesis, while their rear ends are used in posterior pole formation of the proter (Figs. 13, 14, 38, 41). In contrast, the right lateral kineties of each filial product remain bipolar during division, except of the first unshortened kinety right of the paroral, which very likely splits during cytokinesis slightly above the cytostome, resulting in a rather long anterior segment and a short posterior segment which becomes K1 in the next generation (Figs. 13, 41, 52, 53). The first bipolar kinety right of the paroral may consist of kinety 2 or 3 depending on the extent of contribution of kinety 2 to stomatogenesis; it may even be different in a dividing cell, i.e. kinety 2 in the proter and kinety 3 in the opisthe (Fig. 53). Extensive proliferation of basal bodies occurs in the postoral kineties of the postdividers. This proliferation, which occurs intrakinetally and/or telokinetally, results in a gradual elongation of the postoral and left lateral kineties. Proliferation may be less intense in one or two postoral kineties resulting in some kind of postoral secant system, formed by short kinety fragments occasionally having a branched appearance in morphostatic specimens and early dividers (Figs. 1, 3, 5, 48).

One shortened kinety is present to the right of the paroral in 33% (n = 105) of the interphase specimens, two such fragments occur in 61% (Figs. 1, 5), and three in 6%. Obviously, at least one (the outermost) of these fragments originates by splitting of the first kinety right of the paroral, as described above. The other fragments, often consisting of 2-3 dikinetids only, are very likely remnants from previous generations, because the anterior portion of stomatogenic kinety 2 often does not disintegrate (Figs. 7, 11, 17, 18, 37, 53). This interpretation, i.e. that one of the fragments originates by splitting of the first unshortened kinety right of the paroral, is supported by the observation that kinety 3 is never (in 82 appropriate stages analyzed) involved in primordium formation; accordingly, only one fragment, that from kinety 2, can remain right of the oral apparatus.

Nuclear division

Nuclear fission matches literature data. The single macronucleus of *B. hyalinum* simply divides (Fig. 54). In the binucleate, respectively monilate species, the nodules fuse to a globular mass in middle dividers (Wilfert 1972; Suzuki 1973; Figs. 4, 12, 35). The macronuclear condensation shows considerable individual variability, as also observed by Suzuki (1973). The mass elongates and divides during cytokinesis (Fig. 14). Extrusion of chromatin material, as observed by Suzuki (1954), occurred also in our strain of *B. americanum*. In 67 out of 88 specimens (76%) of *B. americanum* the micronuclei show divisional processes before the macronucleus appears altered. In 69% of specimens (n = 16) the micronuclei are distributed unequally between the daughter cells (Fig. 14).

Temporal relationships of divisional processes

Fine-tuned temporal relationships between opisthe and proter stomatogenesis as well as between stomatogenesis and nuclear fission are lacking (Figs. 55, 56). The four stages distinguished in the proter are especially poorly related to the events in the opisthe, possibly due to the varying extent of reorganization of the parental oral structures. Specifically, the early stomatogenic events are often more advanced in the proter than in the opisthe. Timing of nuclear division, particularly of the first two stages, also varies to a considerable extent, while the final processes are obviously comparatively fast.

DISCUSSION

Interphase morphology

Few of the numerous *Blepharisma* species have been characterized morphometrically in sufficient detail. Repak *et al.* (1977) measured six limnetic strains of *B. americanum* and found great variability. Our population of *B. americanum* collected from soil, but maintained in pure "limnetic" culture for some years, showed the greatest mean length of all isolates measured so far (Repak *et al.* 1977, Foissner and O'Donoghue 1990, Table 1). This can partially be attributed to the impregnation method used (protargol procedure B, viz. Wilbert's protocol), which often causes some swelling of specimens. Such swelling possibly also accounted for the low number of dikinetids (about 100 *vs.* 173) in the right lateral kineties of our strain compared to that of the smaller Australian pond population studied by Foissner and O'Donoghue (1990). The number of adoral membranelles matched that of most strains studied by Repak *et al.* (1977), while it hardly corresponded to that of the Australian population (55-66 *vs.* 65-100), even showing distinctly different means, i.e. 59 *vs.* 81 (Foissner and O'Donoghue 1990, Table 1).

The soil population of *B. undulans* has, as compared to the limnetic isolates studied by Repak *et al.* (1977) and Dragesco and Dragesco-Kernéis (1991), a larger body size (149x49 *vs.* 111x26 *vs.* 123x33 µm) and more adoral membranelles (58 *vs.* 37 *vs.* 40). The morphometrics of *B. hyalinum* agrees well with that of another soil population investigated by Foissner (1989).

Familial and generic classification

The genera *Blepharisma*, *Anigsteinia*, *Parablepharisma*, and *Pseudoblepharisma* were separated from *Spirostomum* and *Gruberia* and united in a weakly founded [body pyriform or ellipsoid, somewhat anteriorly narrowed, laterally compressed; peristome on left margin; oral dikinetid (= paroral) forward of cytostome] family Blepharismidae by Jankowski in Small and Lynn (1985). Irrespective of the family characters recognized in heterotrichs, the overall similarities in the ontogenesis of *Blepharisma* and *Spirostomum* (Eberhardt 1962; present results), especially the occurrence of a particular reorganization anlage in the proter (very likely present also in *Pseudoblepharisma*, see Grolière 1977), do not support such separation. *Anigsteinia*, though superficially similar to *Blepharisma*, has a quite different nuclear division, viz. the about 150 macronuclear segments do not fuse to a single mass, but to 15-50 nodules dividing amitotically (Larsen 1994); unfortunately, cortical ontogenesis has, as far as we know, not yet been described in this genus. Similar nuclear processes were observed in *Blepharisma* (?) *candidum* (Yagiu and Shigenaka 1956). Therefore, these taxa possibly need a family of their own.

A main character for the intrageneric classification of *Blepharisma* is the shape of the macronucleus, which may be ellipsoidal, vermiform, binodal or multinodal. Such differences, which also occur in other heterotrichs like *Stentor* and *Spirostomum*, were used by Hirshfield *et al.* (1965) to split *Blepharisma* in the subgenera *Compactum*, *Filiformis*, *Halteroides*, and *Blepharisma*. This classification is not supported by our results, which show that, irrespective of the group the species belongs to, divisional processes are almost identical. The number of somatic kineties involved in the formation of the oral primordium is obviously rather variable and related to cell size. Moreover, the extent to which the parental adoral structures are reorganized is different in *B. hyalinum* (none) and *B. bimicronucleatum* (reorganization anlage; unpubl. observations), both belonging to the same (*Compactum*) group.

Comparative morphogenesis in *Blepharisma*

According to Sawyer and Jenkins (1977), the first sign of morphogenetic activity in *Blepharisma japonicum* was the appearance of branched kineties in the area directly subtending the oral apparatus. We also observed branch-like kineties in some dividers of *B. americanum* and *B. undulans*, but not in *B. hyalinum*; they were also absent in the *B. japonicum* population investigated by Dubochet *et al.* (1979), who mention some observations on this species in their paper on *Climacostomum virens*. However, a branch-like kinety pattern occurs also in interphase specimens (see section on somatic division and Foissner and O'Donoghue 1990) and results from previous divisions, where the postoral kineties became divided in more or less long anterior and posterior fragments, some of which did not proliferate basal bodies during and after cytokinesis (Figs. 1, 3, 38, 48, 53). Certainly, branching in the sense of Sawyer and Jenkins (1977) is not related to morphogenesis.

The oral anlage of the opisthe invariably appeared subequatorially in a position distinctly apart from the parental oral apparatus. Furthermore, all basal bodies for the oral primordium originated, depending on species, from one or more parental somatic kineties. Thus, stomatogenesis of *Blepharisma* perfectly matches the parakinetal mode as defined by Corliss (1979) and Foissner (1996). Depending on the number of postoral kineties involved in the formation of the oral anlage, a monoparakinetal (only one, the "director meridian" involved) and a polyparakinetal (two or more involved) subtype have been distinguished (Foissner 1996). The species investigated by us and *B. japonicum* (Sawyer and Jenkins 1977) basically belong to the polyparakinetal subtype, although only one kinety was often involved in the stomatogenesis of *B. hyalinum* and especially *B. bimicronucleatum* (Villeneuve-Brachon 1940 and own unpubl. data). Our observations confirmed Sawyer and Jenkins (1977) in that proliferation proceeded sequentially, occurring first along the centrally located postoral kinety and subsequently in the kineties to the left and right. The oblique proliferation, not mentioned by Sawyer and Jenkins (1977) and Eberhardt (1962), is, however, recognizable in their figures of *B. japonicum* and *B. americanum*, respectively.

Our Figs. 8, 10, 18, and 51 show convincingly that the differentiation of the adoral membranelles commences in the centre of the anarchic field and proceeds in an anterior and posterior direction as well as from right to left. Again,

this contradicts Sawyer and Jenkins (1977), who emphasized that membranelle formation occurred sequentially in an anterior-to-posterior direction in *B. japonicum*. Sawyer and Jenkins (1977) did not provide convincing evidence for their statement and, in our opinion, their Fig. 15 even shows that membranelle formation occurred as in our species. Thus, their assumption of a general gradient of membranelle formation in various groups of ciliates, is groundless. Likewise, Suzuki (1957) did not provide reliable figures for his statement that membranelles develop synchronically in the anarchic field of *B. japonicum*.

The parental oral apparatus of all *Blepharisma* species investigated so far was reorganized during cell division, but to a varying extent. An *in situ* reorganization of the adoral membranelles and a particular reorganization anlage were observed in *B. americanum* (Eberhardt 1962; present results), *B. undulans* (present results), *B. bimicronucleatum* (unpubl. observations), and *B. japonicum* (Sawyer and Jenkins 1977), but not in *B. hyalinum* (present results), at least at the light microscopic level; however, a reorganization anlage was lacking in about one third of the respective stages of *B. americanum*. The facultative appearance of this particular anlage is possibly related to the age of the oral apparatus, i.e. former proters will usually reorganize, while opisthes will not. This would roughly explain the observed ratio. The exact origin of the reorganization anlage was difficult to determine because of the underlying (darkly coloured) adoral membranelle spiral. Eberhardt (1962) and Sawyer and Jenkins (1977) proposed that it is generated by postoral kineties. We observed kinetosomal proliferation beside and between K1 and Kn (Figs. 9, 10, 22, 23, 30), as well as a contact of the anlage with the parental paroral membrane (Figs. 10, 22, 24, 28). We could not clarify whether the contact is due to simple spatial constraints or by a real contribution of the proter paroral to the reorganization anlage.

The posterior zigzag segment of the paroral membrane was completely reorganized in *B. americanum*, *B. undulans*, and *B. hyalinum* (Eberhardt 1962; present study). Sawyer and Jenkins (1977) did not mention such reorganization in *B. japonicum*, but some of their figures (Figs. 18, 19, 21) indicate that it occurred (cp. also Dubochet *et al.* 1979). The anterior portion of the paroral membrane remained intact in all species, as also mentioned by Dubochet *et al.* (1979).

We observed elimination of chromatin during macronuclear division of *B. americanum*, but not in *B. undulans* and *B. hyalinum*. It was reported to occur facultatively in *B. americanum* (Suzuki 1954) and obligatorily in *B. trinodatum* and *B. multinodatum* (Young 1939, Weisz 1949, McLaughling 1957).

Comparative morphogenesis in heterotrichs

Heterotrichs *s. str.* have a parakinetal stomatogenesis, i. e. the oral primordium evolves entirely from parental somatic ciliature. Recently, Foissner (1996) distinguished several parakinetal subtypes in heterotrichs, depending on the number of postoral kineties involved in oral primordium production, the formation of the peristomial ciliature, and the number of oral primordia produced. Most heterotrichs have a polyparakinetal stomatogenesis, i.e. more than one postoral kinety is involved in the formation of the oral anlage (Foissner 1996). The monoparakinetal subtype has been observed in *Blepharisma* spp., *Chattonidium setense* and *Spirostomum teres* (Villeneuve-Brachon 1940; present study). It occurs, at least initially, also in *Nyctotherus ovalis*, *Stentor niger*, and *Pseudoblepharisma crassum*, as well as in regenerating *Condylostoma magnum* (Dragesco 1966, Albaret 1975, Bohatier *et al.* 1976, Grolière 1977). However, it is frequently difficult to ascertain whether one or more postoral kineties are involved in oral primordium formation, even if a fine-scaled analysis is performed, because the postoral kineties are often narrowly spaced and shortened fragments occur which may fuse with the oral primoridum. Our analysis of *Blepharisma hyalinum* and *B. bimicronucleatum* (unpubl. observations) showed that at least part of the specimens formed the oral primordium from a single postoral kinety. Thus, it is reasonable to assume that a true monoparakinetal stomatogenesis occurs in at least some heterotrichs.

Stomatogenesis is amphiparakinetal if the oral primordium intersects many postoral kineties at two sites (Foissner 1996), as, e.g., in *Fabrea*, stentorids and folliculinids. Accordingly, the oral primordium encloses few to many short, non-proliferating parental somatic kinety fragments, which become somatic ciliary rows on the peristomial field of the opisthe (Pelvat and Haller 1979, Foissner 1996). The oral primordium of *Blepharisma* spp. (present results) and *Pseudoblepharisma crassum* (Grolière 1977) resembles the posterior half of the stentorid primordium in that the anterior portion of stomatogenic kinety 2 is frequently inactive and retained (Fig. 18). Consequently, at least one (if two or three are present; remember that one kinety fragment originates by splitting of kinety 3; see Results) of the shortened kineties right of the paroral is usually a kind of intersected kinety and thus very likely homologous to the somatic peristomial ciliature of stentorids and folliculinids.

The discovery of a transient, cirrus-like structure at the anterior end of the developing paroral membrane was another surprising result of the present study. It is highly reminiscent of *Condylostoma*, which develops several such cirri and retains them as conspicuous ciliary tuft (Bohatier *et al.* 1976). Interestingly, the paroral membrane of *Pseudoblepharisma* and *Gruberia*, two *Blepharisma*-like heterotrichs, is entirely or partially composed of such kinetosomal groups (Grolière 1977).

Folliculinids have a biparakinetal stomatogenic mode, i.e. the proter and opisthe each form an oral anlage independently and amphiparakinetally. The proter anlage is very small and remains in a primordial stage, producing only few membranelles, which are resorbed when the swarmer rebuilds the oral apparatus from a large, normal oral primordium (Mulisch 1987, Mulisch and Patterson 1987). Thus, we aggree with Mulisch and Patterson (1987) that the proter reorganization anlage of *Blepharisma* and *Spirostomum* is likely homologous to the proter anlage occurring in folliculinids.

Another feature linking heterotrichs *s. str.* is the unique formation of membranelles, which proceeds from the centre towards the ends of the primordium. Mulisch (1987), working with the highly specialized folliculinids and having only the incorrect data of Suzuki (1957) and Sawyer and Jenkins (1977) for comparison, supposed that the peculiar adoral zone formation could be a derived trait related to the development of large peristomial wings. This is not supported by our results; rather, the special adoral zone formation, although widely unrecognized (e. g. Pelvat and Haller 1979, Dubochet *et al.* 1979), is likely a common feature of heterotrichs *s. str.* providing a superb autapomorphy for the group. Thus, *Phacodinium* and *Plagiotoma*, which form the adoral zone of membranelles from anterior to posterior, as do hypotrichs, oligotrichs, and hymenostomes, cannot belong to the heterotrichs, as frequently assumed (e.g. Small and Lynn 1985), but should be grouped with the hypotrichs, as also suggested by conventional morphological traits (Fernández-Galiano and Calvo 1992).

A further, admittedly rather general character linking the heterotrichs *s. str.* is the partial or complete reorganization of the parental oral apparatus during division, although data on this are still scant (Foissner 1996). Thus, it is not yet possible to find a meaningful route, if such exists, between families and genera.

In conclusion, numerous and close morphological and morphogenetic relationships exist between heterotrichs *s. str.*, i. e. spirostomid, stentorid and folliculinid ciliates, convincingly arguing for a common ancestor. These "classical" and mostly aerobic heterotrichs form a natural group distinctly different from other taxa, such as clevellandellids, armophorids, licnophorids and odontostomatids, presently assigned to the heterotrichs but showing quite different stomatogenic modes (see Table 2 in Foissner 1996, and References). This is also evident from recent molecular biological data (Hirt *et al.* 1995, Hammerschmidt *et al.* 1996).

Reorganization of the parental paroral membrane in *Blepharisma*: an exception from the rule?

Quite different modes of reorganization of the parental paroral membrane may proposed considering the data available and its diverse organization and degree of dedifferentiation during division. However, Eisler (1989) proposed a general mode for ciliates from very different systematic groups, viz. that the old left row is retained while the right row is newly formed, and that the process invariably involves a longitudinal splitting of the organelle. As concerns our data on *Blepharisma*, one of the reviewers made a very important comment, obviously refering to Eisler's general scheme: "If the paroral of *Blepharisma* is, in principle, organized like in most other ciliates, than proliferation of new kinetosomes automatically should take place towards the right and not towards the left as it is stated. That is due to the orientation of the kinetosomes with their postciliary fibres running to the left towards the cytostome and their direction of proliferation to the right. Therefore, if it would be true, that in *Blepharisma* an existing paroral double-row would produce a new double-row, this newly formed structure would be to the right of the old one and not to the left. But in most ciliates like many hymenostomes, scuticociliates, nassulids, and hypotrichs (for review see Foissner 1996) an existing paroral composed of dyads splits longitudinally during reorganization in the following manner: at first the anterior kinetosomes of the dyads separate from their posterior partners and move to the right. Then, at least the posterior kinetosomes of the dyads get new anterior daughter kinetosomes thus forming a new paroral for the proter. The fate of the former anterior kinetosomes of the dyads is rather different in different ciliate species, they may be resorbed like in *Tetrahymena* (Nelsen 1981), they may form a new kinety like in *Furgasonia* (Eisler 1989) or they may serve as oral anlagen for the opisthe like in scuticociliates (Grolière 1974), but in any case the former posterior kinetosome of the parental paroral dyads are also the posterior kinetosomes of the dyads of the new paroral for the proter. For this reason I cannot believe, that in *Blepharisma*, in contrast to all other ciliates investigated

so far, the parental paroral membrane proliferates towards the left, thus producing a complete new paroral and resorbing the whole old one." Certainly, this argumentation cannot be ignored. On the other hand, our preparations leave no doubt that not the inner left but the outer right row of dikinetids is resorbed. Obviously, transmission electron microscopic investigations are required to solve the problem. Generally, data are still very scant and easily over-interpreted. Eisler (1989), for instance, has given a schematic drawing of the development of the paroral membrane of the proter in *Paraurostyla weissei*, although in the original paper Jerka-Dziadosz (1981) has given only one electron micrograph (p. 89) showing an early stage of a "regenerating promer" (p. 88) and, more importantly, has stated that "Ultrastructural details of reorganization of the anterior preoral membranelles [= inner and outer paroral membrane] have not been followed in detail" (p. 85).

Phylogenetic affinities of heterotrich ciliates

Heterotrichs and karyorelictids represent the earliest branch on molecular ciliate trees (Greenwood *et al.* 1991, Baroin-Tourancheau *et al.* 1992, Fleury *et al.* 1992, Leipe *et al.* 1994, Hammerschmidt et al. 1996). Unfortunately, we know almost nothing about stomatogenesis in karyorelictids (Foissner 1996). However, very recently Bardele and Klindworth (1996) provided convincing evidence that *Loxodes striatus* has a buccokinetal stomatogenesis distinctly different from the parakinetal mode found in heterotrichs *s. str.* Thus, although ultrastructural and molecular characters indicate a close relationship between karyorelictids and heterotrichs (Gerassimova and Seravin 1976, Baroin-Tourancheau *et al.* 1992, Fleury *et al.* 1992, Hammerschmidt *et al.* 1996), they cannot be founded as a monophyletic group with the ontogenetic data available.

Our study suggests, however, that some buccokinetal remnants exist in the heterotrichs *s. str.*, viz. scattered postoral basal bodies (Figs. 5, 13, 41-47) resembling the scutica-like vestiges found in *Protocruzia*, *Loxodes*, and tetrahymenid hymenostomes (Grolière 1974, Puytorac *et al.* 1974, Grolière *et al.* 1980, Bardele and Klindworth 1996, Foissner 1996, and references therein). However, only 40% of our specimens had such scattered dikinetids, and at least some of these were ciliated (Fig. 5), indicating that it is not a classical scuticus, which usually lacks cilia and has a hook-like or whiplash configuration never observed in the *Blepharisma* spp. investigated. This also applies to the scutica-like vestige found in *Tetrahymena*. There is, however, an even more important difference between the classical "hymenostome" scutica and the vestiges mentioned above, viz. they are never included in the formation of the opisthe's oral structures; at best, they play some role in the reorganization of the parental oral apparatus, producing a few posteriormost adoral membranelles. On the other hand, the scutica of some typical scuticociliates, viz. *Paralembus* and *Pseudolembus* is rather inactive, i. e. produces only few oral structures (Grolière 1974).

A further similarity between *Blepharisma* (Figs. 10, 24, 25, 35, 52), *Tetrahymena* (Nelsen 1981) and typical scuticociliates (Grolière 1974) concerns the parental paroral membrane, which is reorganized by longitudinal splitting. However, the parental paroral remnants are completely resorbed in *Blepharisma* and *Tetrahymena*, while they form a major portion of the opisthe oral anlage in the scuticociliates, *viz.* the paroral and the scutica (Grolière 1977, Puytorac *et al.* 1974). This corroborates previous suggestions (Foissner 1996, and References) that the stomatogenic function of the paroral kinety was secondarily transferred to the first somatic (postoral) kinety. Accordingly, the so-called "director meridian" (stomatogenic kinety 1) may be considered a strongly modified scutica or *vice versa*. *Loxodes striatus* would then represent a perfect transitional step because its postoral ventral kinetofragment of 8-10 barren dikinetids is positioned and behaves like a scutica, i. e. is involved in stomatogenesis of the opisthe (Bardele and Klindworth 1996); unfortunately its origin is still unknown.

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