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Ontogenesis in a Trachelocercid Ciliate (Ciliophora: Karyorelictea), *Sultanophrys arabica*, with an Account of Evolution at the Base of the Ciliate Tree

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Summary. Ontogenesis in *Sultanophrys arabica* Foissner & AL-Rasheid, 1999, a trachelocercid karyorelictid ciliate, was investigated using live observation, silver impregnation, and scanning electron microscopy. Division is homothetogenic and occurs in freely motile (non-encysted) condition. The parental oral apparatus does not reorganise and cell shape is maintained. Stomatogenesis is parakinetal, that is, the anlage for the opisthe oral apparatus is derived directly from the first ordinary somatic ciliary row right of the glabrous stripe and has no connection with parental mouth structures. The oral primordium appears slightly subequatorially and consists of an anarchic field of basal bodies, from which many dikinetidal kinetofragments differentiate. The kinetofragments migrate centrifugally and assemble to a circumoral kinety and three minute brosse kineties. The somatic kineties, the bristle kinety, and the lateral kinety divide without anlagen formation, that is, are disrupted by cytokinesis. Thus, morphogenesis of trachelocercid karyorelictids is distinctly different from that of loxodid karyorelictids, which generate the oral primordium buccokinetally. This shows that different stomatogenic modes developed early in ciliate evolution, which is emphasised by the heterotrichs, whose parakinetal stomatogenesis is rather different from that of the trachelocercids. Nevertheless, our data give some support for the subphyletic division suggested by Lynn (1996), but do not corroborate any of the hypotheses on evolution of ciliate cytoarchitecture. Both, loxodid and trachelocercid karyorelictids have conspicuous ontogenetic similarities (scutica-like structure, "director meridian") with oligohymenophoreans. This is sustained by the trachelocercid brosse kineties, which resemble oligohymenophorean and prostomatid adoral membranelles. Whether these traits evolved convergently or have a deeper meaning needs further investigations.

Key words: interstitial ciliates, morphogenesis, Postciliodesmatophora, stomatogenesis.

INTRODUCTION

SSrRNA gene sequences indicate a common ancestor for karyorelictids, such as *Loxodes* and *Trachelocerca*, which have non-dividing macronuclei, and heterotrichs, such as *Climacostomum* and *Eufolliculina*, which have dividing macronuclei (for reviews, see Lynn and Small 1997, Hirt *et al.* 1998). A close relationship between karyorelictids and heterotrichs is also suggested by the unique somatic cortical fine structure, that is, the postciliary microtubular ribbons overlap to form conspicuous postciliodesmata right of the ciliary rows (Raikov *et al.* 1975, Gerassimova and Seravin 1976). Accordingly, Lynn (1996) united karyorelictids and heterotrichs in the subphylum Postciliodesmatophora Gerassimova & Seravin,

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1976 and established the subphylum Intramacronucleata to contain all other ciliates. Lynn's proposal, which requires that macronuclear division has arisen independently twice during ciliate evolution, is supported to some extent by ontogenetic data, which show that at least heterotrichs have a specific (parakinetal) stomatogenic mode, where the new mouth originates, without any participation of parental oral structures, from an anarchic field of basal bodies produced postorally by a few stomatogenic ciliary rows (for reviews, see Foissner 1996b, Aescht and Foissner 1998). A parakinetal stomatogenesis was also proposed for *Loxodes* (Tuffrau 1961), but recently disproved by Bardele and Klindworth (1996), who showed that *Loxodes* generates the opisthe's oral apparatus buccokinetally, that is, with participation of parental oral structures.

Karyorelictids continuously played an important role in understanding ciliate nuclear dimorphism and phylogeny because their diploid macronuclei are incapable to divide and originate from micronuclei during and after cell fission (Raikov 1958, 1982; Corliss 1974; Orias 1976, 1991; Small 1984; Lynn and Small 1997). Unfortunately, most karyorelictids are, for unknown reasons, fragile and difficult to impregnate with silver compounds. Thus, detailed observations on the ontogenesis of their ciliary pattern were published only recently (Bardele and Klindworth 1996), and ontogenetic data from the largest group of karyorelictids, the trachelocercids, are entirely lacking (Foissner 1996b), although their nuclear division was thoroughly studied already in the sixties (for review, see Raikov 1982). It was only recently that Foissner and Dragesco (1996a, b) invented an appropriate technique, which we used to investigate the ontogenesis of the ciliary pattern in *Sultanophrys arabica*, a trachelocercid karyorelictid discovered recently in a brackish pond at the Saudi Arabian Gulf coast (Foissner and AL-Rasheid 1999). *Sultanophrys* has a parakinetal stomatogenesis, similar to heterotrichs, and thus supports Lynn's subphyletic division.

MATERIAL AND METHODS

Sultanophrys arabica Foissner & AL-Rasheid, 1999 was isolated from a brackish pond at the Saudi Arabian Gulf coast and cultivated as described in AL-Rasheid and Foissner (1999). Specimens divided readily and thus ample material was available.

Dividing specimens were studied *in vivo* and with the scanning electron microscope, as advised in Foissner (1991), using the fixative described by Foissner and Dragesco (1996a). The infraciliature and the nuclear apparatus were revealed with a combination of Wilbert's protargol and Fernandez-Galiano's silver carbonate technique, as described in Foissner and AL-Rasheid (1999). Preparations of extraordinary clarity were obtained with this method, which is, unfortunately, not yet fully reproducible. We were advised by one of the reviewers to emphasise that the micrographs were not touched with ink for the sake of clarity but result from the superb quality of the preparations.

Drawings were made with a camera lucida and are slightly schematised. Note that all cells are strongly contracted and inflated due to the preparation procedures. Terminology is according to Corliss (1979), Foissner (1996b) and Foissner and Dragesco (1996a).

RESULTS

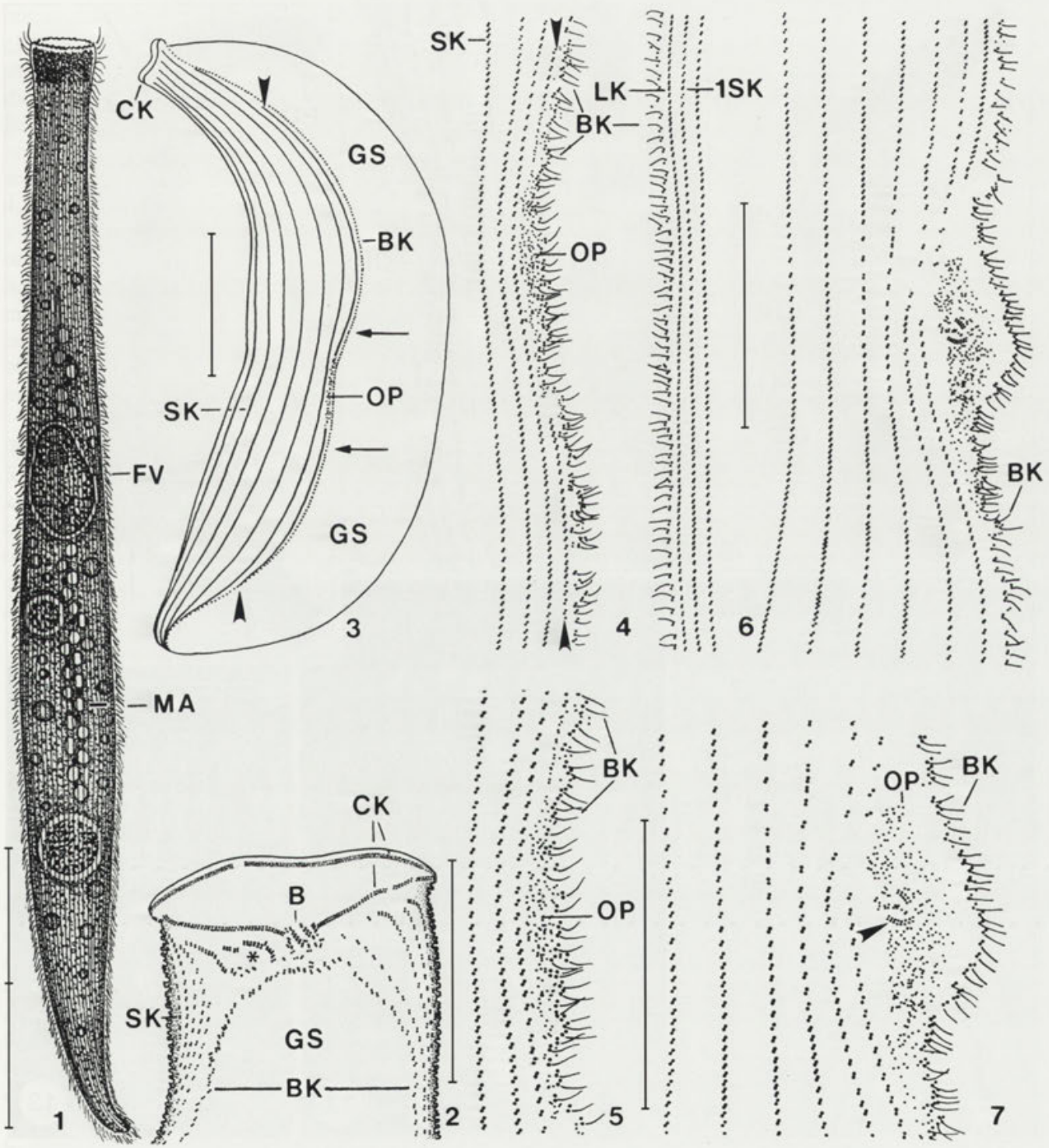
Interphase morphology

See Foissner and AL-Rasheid (1999) for a detailed description of *S. arabica*. Here, we mention only details, which are important for understanding the ontogenetic processes. *In vivo*, the organism is about 800 µm long, 70 µm wide, distinctly flattened, and can contract up to half the body length (Fig. 1). On the right side are about 34 longitudinal ciliary rows, while the left is barren except for the bristle kinety, which borders the barren area, the so-called glabrous stripe (Figs. 2, 3). At the right side of the glabrous stripe are several shortened kineties, which abut to the bristle kinety forming the anterior and posterior secant system (Figs. 3, 10). Furthermore, there is a special (lateral) kinety between the left branch of the bristle kinety and the first ordinary ciliary row (Fig. 4). The lateral kinety shows various specialisations, most notably subcortical fibres forming long bundles in the posterior region of the cell (Fig. 50). The cylindroidal apical end (head) contains distinct oral structures consisting of a circumoral ciliary row and, in midline of left side, a brosse composed of three minute, oblique kineties (Fig. 2).

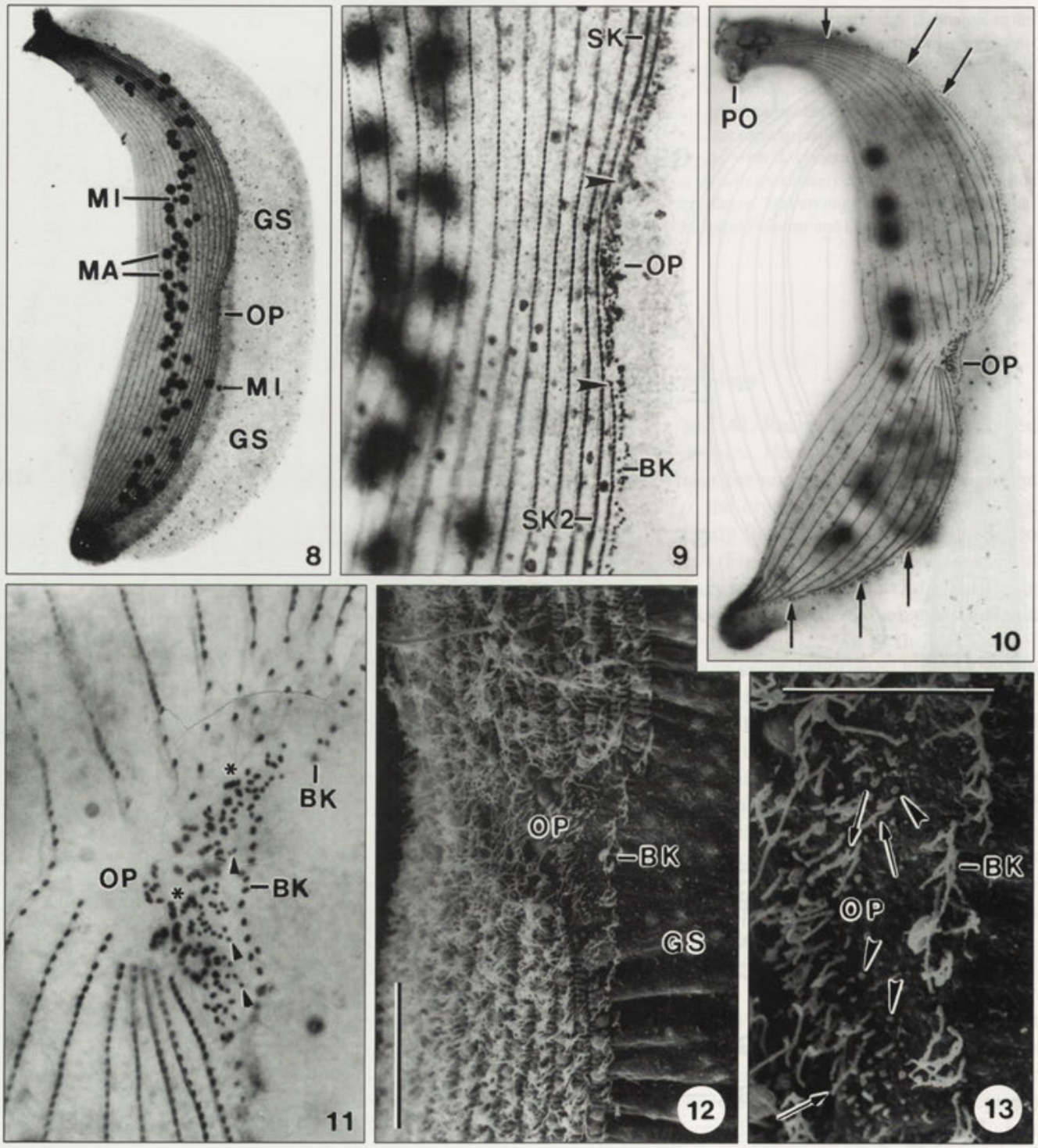
Ontogenesis

All divisional stages shown were observed in at least three specimens. For descriptive purposes, the process was divided into 6 stages according to characteristic events. Division occurs in freely motile (non-encysted) condition and is homothetogenic. The parental daughter (proter) does not reorganise, that is, cell shape, oral apparatus and somatic infraciliature are maintained.

Stage 1 (Figs. 3-5, 8, 9, 14). Division commences with the formation of an oral primordium for the posterior daughter (opisthe) and a flat indentation in the prospective cleavage region. Thus, stomatogenesis and cytokinesis proceed concomitantly. The oral primordium develops near the cell surface slightly subequatorially in the first



Figs. 1-7. *Sultanophrys arabica*, morphostatic (1, 2) and dividing cells (3-7) from life (1) and after silver impregnation (2-7). 1 - right side view of a fully extended specimen; 2 - left side view of anterior end. Asterisk marks kinetofragments, possibly remnants from oral primordium formation; 3-5 - very early divider, overview (3; for clarity only every second kinety is illustrated) and details of oral primordium (4, 5). Figure 4 shows the infraciliature at the right and left margin of the glabrous stripe. Arrows in Figure 3 border region shown at higher magnification in Figures 4 and 5. Arrowheads in Figure 3 mark anterior and posterior end of the stomatogenic kinety. Arrowheads in Figure 4 mark stomatogenic kinety. The oral primordium (OP) consists of an anarchic field of mainly single basal bodies. 6,7 - a slightly advanced stage showing that the bristle kinety is not involved in oral primordium formation. Dikinetidal fragments (arrowhead) commence to form in the oral primordium. B - brosse, BK - bristle kinety, CK - circumoral kinety, FV - food vacuole, GS - glabrous stripe, LK - lateral kinety, MA - macronuclear nodules, OP - oral primordium, SK - ordinary somatic kinety, 1SK - first ordinary left side somatic kinety. Bar division: 100 µm (Figs. 1, 3) and 30 µm (Figs. 2, 4-7)



Figs. 8-13. *Sultanophrys arabica*, early dividers after silver impregnation (8 - 11) and in the scanning electron microscope (12, 13). 8, 9 - very early divider, overview and detail of oral primordium (bordered by arrowheads), which develops near mid-body in the first somatic kinety right of the glabrous stripe; 10, 11 - early divider, overview and detail showing that the bristle kinety is not involved in oral primordium formation (cp. Fig. 13). Arrows mark the anterior and posterior secant system, which is, uniquely, at the right side of the glabrous stripe in *S. arabica*. Arrowheads mark single basal bodies within the oral primordium. Asterisks denote developing dikinetidal fragments. Note that the somatic kineties divide without proliferation of basal bodies in the fission area; 12, 13 - oral primordium of an early divider, similar to that shown in Figures 10 and 11. Arrowheads mark stubs of growing cilia, arrows denote almost fully grown cilia. The bristle kinety is not involved in oral primordium formation (cp. Fig. 11). BK - bristle kinety, GS - glabrous stripe, MA - macronuclear nodules, MI - micronuclei, OP - oral primordium, PO - parental oral apparatus, SK2 - somatic kinety 2. Scale bars: 20 µm (Fig. 12) and 10 µm (Fig. 13)

ordinary somatic ciliary row at the right side of the glabrous stripe. This (stomatogenic) kinety is distinctly shortened anteriorly and posteriorly because it belongs to the secant system (Figs. 3, 10). The oral primordium is generated within an about 30 µm long area, where the ordered arrangement of the dikinetids disappears and an anarchic field of unciliated monokinetids develops (Figs. 4, 5, 9). The newly formed kinetids stain lighter and have a smaller diameter than ordinary somatic and bristle basal bodies. Within the bristle kinety, which is not involved in oral primordium formation (see next stage), rather many minute, unciliated granules appear, very likely forming basal bodies (Figs. 4, 5). The infraciliature at the left side of the glabrous stripe and the nuclear apparatus are still morphostatic (Figs. 4, 8, 14).

Stage 2 (Figs. 6, 7, 10-13, 15-17). This stage shows that the bristle kinety is not involved in oral primordium formation because a barren area develops between the kinety and the primordium. The oral primordium increases in breadth, but not in length, by further proliferation of basal bodies. The elliptical accumulation of disordered kinetids produced is clearly separate from the somatic kineties and the bristle kinety, which commences to bulge leftwards (Figs. 6, 7, 15, 16). Within the oral primordium, ciliary outgrowth commences and the kinetids become dikinetidal and some arrange to minute kinetofragments (Figs. 7, 11-13, 17). The somatic kineties right of the oral primordium become disrupted, as evident from the increased distances between the dikinetids in the prospective cleavage area (Figs. 6, 7, 10, 11). Possibly, some kinetids are also resorbed, as indicated by the rather large size of the barren area (Figs. 11, 19, 20).

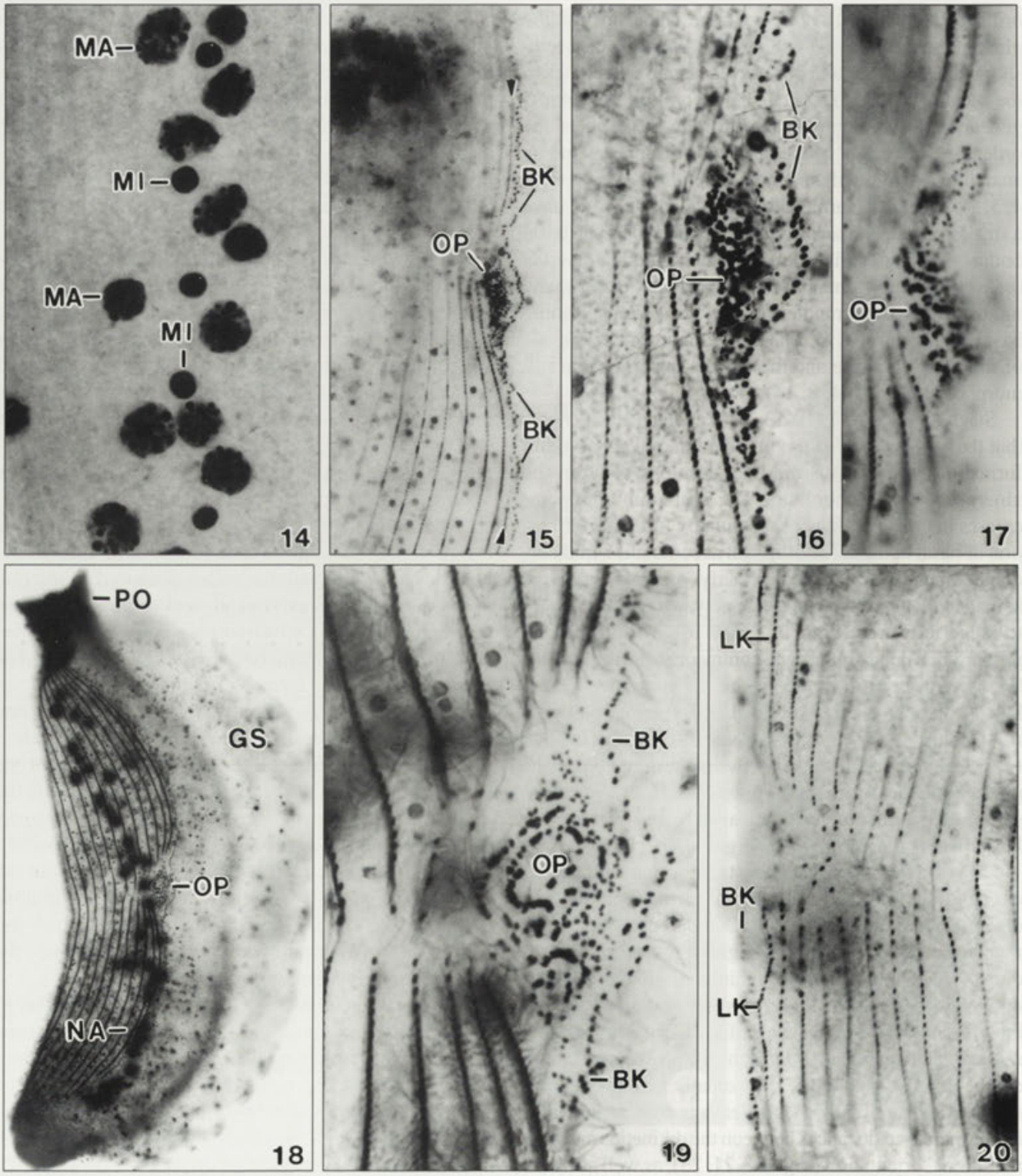
Stage 3 (Figs. 18-24). This stage is characterised by curved kinetofragments in the oral primordium and the onset of micronuclear division. Compared to stage 2, the number of kinetids increases slightly in the oral primordium, which now consists mainly of dikinetids. The dikinetids commence to form short and long, curved kinetofragments which have cilia of usual length and migrate centrifugally to leave blank the prospective buccal cavity (Figs. 19, 22, 23). The somatic kineties become disrupted by stretching of the cleavage area, as indicated by the increased distances between the dikinetids and their meridional orientation (Figs. 18-21, 23), as well as by the subkinetal fibres, which project into the bare area (Fig. 20). Interestingly, kinety separation commences at both sides of the glabrous stripe and proceeds to midline of cell. Furthermore, disruption commences earlier in the kineties at the right side than at the left side of the glabrous stripe and is, at the left side, weakly correlated with stomatogenesis because it may commence in early or middle dividers. Anterior and posterior to the oral primordium, the bristle kinetids are more narrowly spaced than in morphostatic specimens, indicating that new bristles were produced, which is emphasised by rather many unciliated granules, some of which are very likely developing bristle kinetids (Fig. 23). The lateral kinety divides like the bristle kinety, that is, without anlagen formation (Fig. 23).

Usually, the nuclear apparatus commences to divide when the kinetofragments arrange around the prospective oral cavity. However, nuclear division may commence also earlier or later, indicating that it is only roughly correlated with stomatogenesis. The nuclear strand separates more or less distinctly in the cleavage area and the micronuclei get a more distinct membrane, increase in size, and stain lighter (Fig. 21). Sausage-shaped structures, possibly chromosomes, are recognisable within the micronuclei. The macronuclei do not divide.

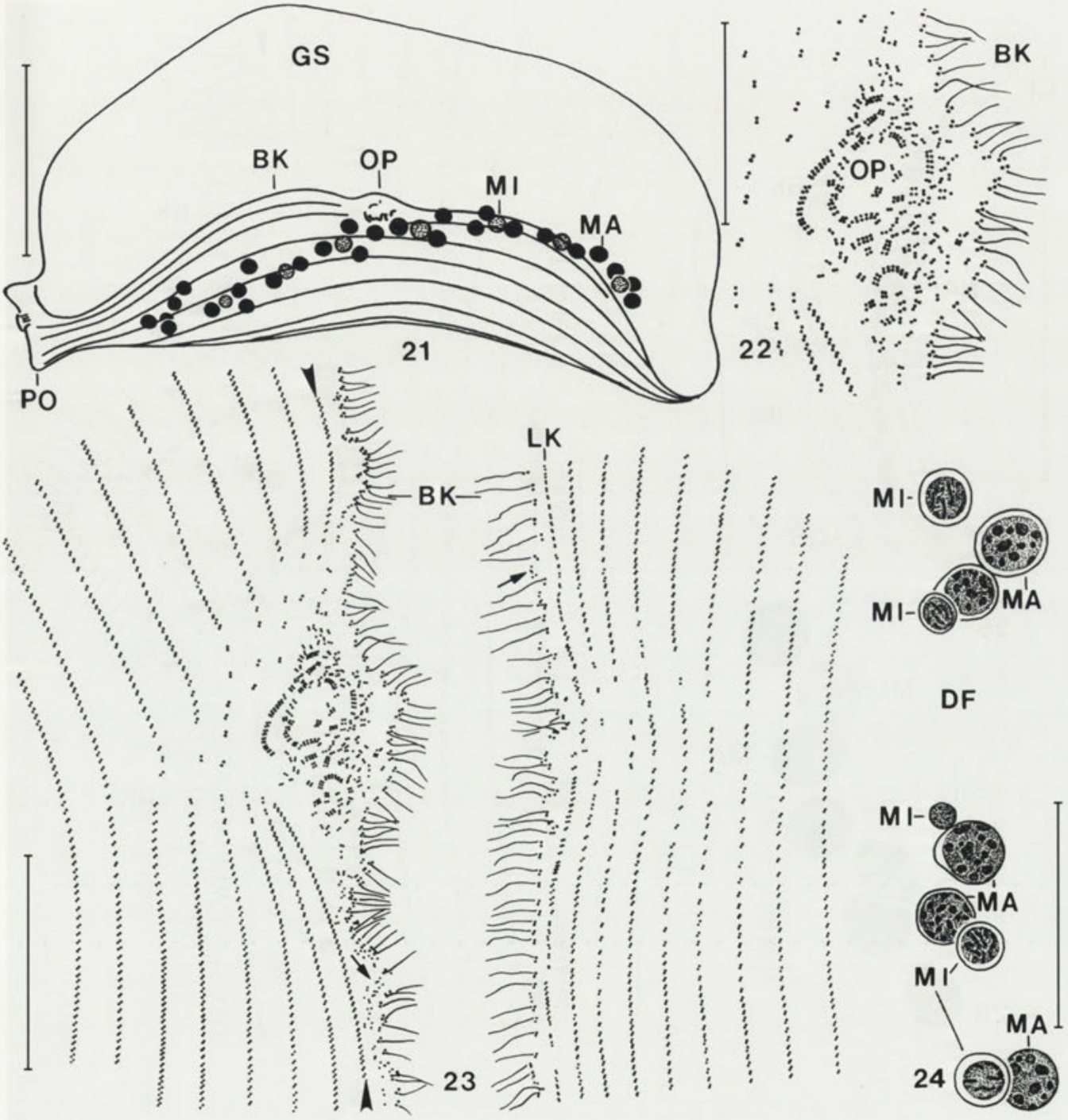
Stage 4 (Figs. 25-33, 40, 44). Middle dividers assemble the opisthe's circumoral kinety and have metaphasic micronuclei. The kinetofragments produced in the oral primordium migrate centrifugally and assemble to a circular circumoral kinety, which surrounds a slightly elevated area, the prospective oral bulge (Figs. 25, 27-32, 40, 44). Between the newly formed circumoral kinety and the bristle kinety, which is distinctly bulged by the forming oral apparatus, remain scattered dikinetids and some short kinetofragments (Figs. 27, 29, 30, 40, 44). Most of the scattered dikinetids remain in the brosse area, while the kinetofragments become brosse kineties or migrate to the right of the brosse, where they remain (Fig. 2). Possibly, these fragments are reserved to replace lost parts of the circumoral kinety. Somatic division progresses to midline of right side. The division furrow is now distinct and slightly underneath mid-body (Figs. 28, 33). Thus, the proter is slightly larger than the opisthe.

The micronuclei increase in size approaching that of macronuclei and show metaphasic chromosomes and distinct spindle microtubules (Figs. 25, 26). Unfortunately, chromosome number could not be estimated because they were too tightly spaced.

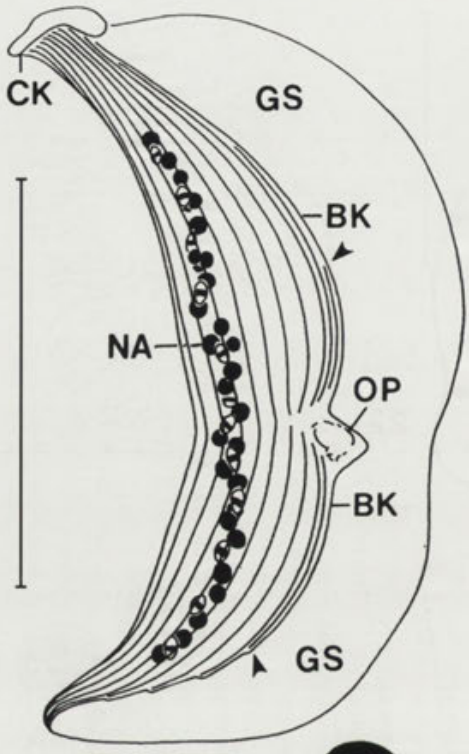
Stage 5 (Figs. 34-39, 41-43, 47-49). Late dividers are characterised by a conspicuous division furrow (Figs. 41, 47), the disruption of the bristle kinety (Figs. 34 - 39), and the formation of the secant system (Figs. 35-39, 48, 49). The nuclear apparatus and the new (opisthe) oral apparatus are similar to those in middle dividers (cp. Figs. 29, 40, 44, 48). However, the opisthe oral apparatus accumulates highly refractive granules, like those found in the parental



Figs. 14-20. *Sultanophrys arabica*, infraciliature and nuclear apparatus (14) of early dividers after silver impregnation. 14-17 - details from early dividers showing that the bristle kinety is not involved in oral primordium formation (15, 16) and kinetofragments are generated in the anarchic field (17). Arrowheads mark the stomatogenic kinety anterior and posterior of the oral primordium; 18-20 - overview and details of an early-middle divider; figures 19 and 20 show corresponding region at right and left margin of glabrous stripe. The kinetofragments commence to migrate around the prospective oral cavity and the somatic kineties disrupt in the fission area. BK - bristle kinety, GS - glabrous stripe, LK - lateral kinety, MA - macronuclei, MI - micronuclei, NA - nuclear apparatus, OP - oral primordium, PO - proter oral apparatus



Figs. 21-24. *Sultanophrys arabica*, infraciliature of early dividers after silver impregnation. 21-23 - overview (21; for clarity only every second kinety is illustrated) and details from same specimen, showing the ciliary pattern of the division area at the right and left margin of the glabrous stripe (23). The oral primordium consists mainly of dikinetids, which arrange to curved kinetofragments around the prospective oral bulge. The somatic kineties become disrupted in the fission area; those right of the oral primordium will form proter's posterior secant system (cp. Figs. 34-39). Arrowheads mark somatic kinety 1, which generated the oral primordium (cp. Fig. 4). The bristle kinety and the lateral kinety are not involved in oral primordium formation and divide without anlagen, that is, are disrupted and new kinetids, some of which are still unciliated (arrows), are generated intrakinetally; 24 - middle region of nuclear apparatus of a similar specimen as shown in Figure 21. The nuclear strand is separating in the fission area and the micronuclei increase in size and become lighter stained (Fig. 21). The macronuclei do not divide. BK - bristle kinety, DF - division furrow, GS - glabrous stripe, MA - macronuclear nodules, MI - micronuclei, OP - oral primordium, PO - parental (proter) oral apparatus. Scale bars: 100 µm (Fig. 21), 30 µm (Figs. 23, 24), and 20 µm (Fig. 22)



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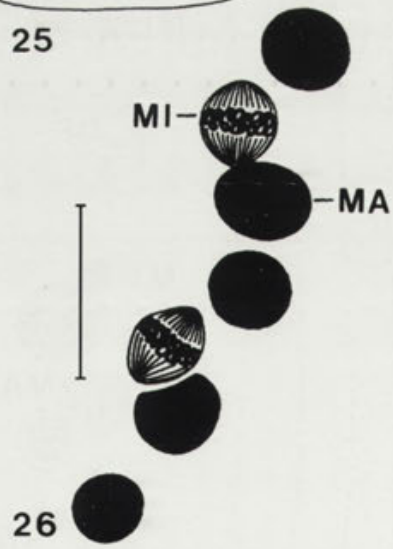

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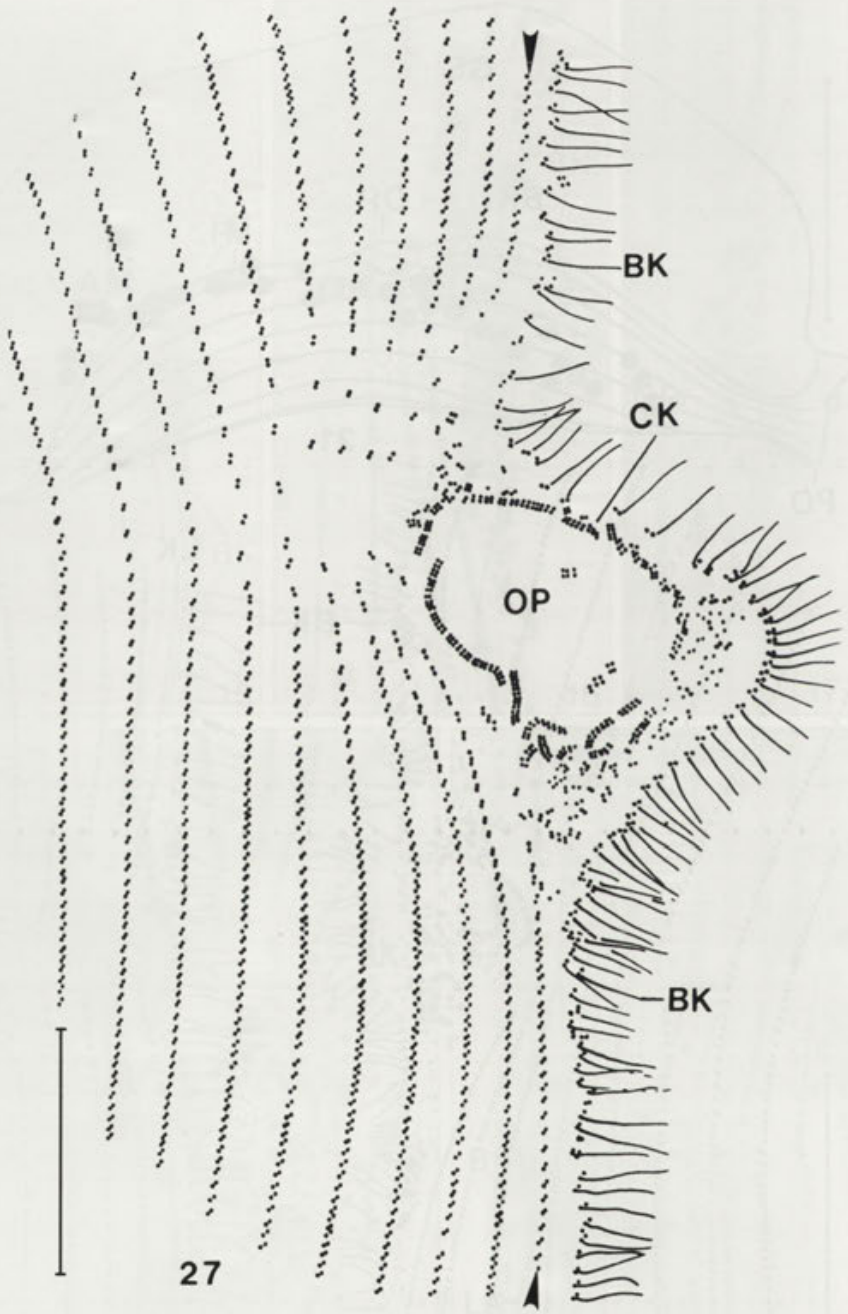

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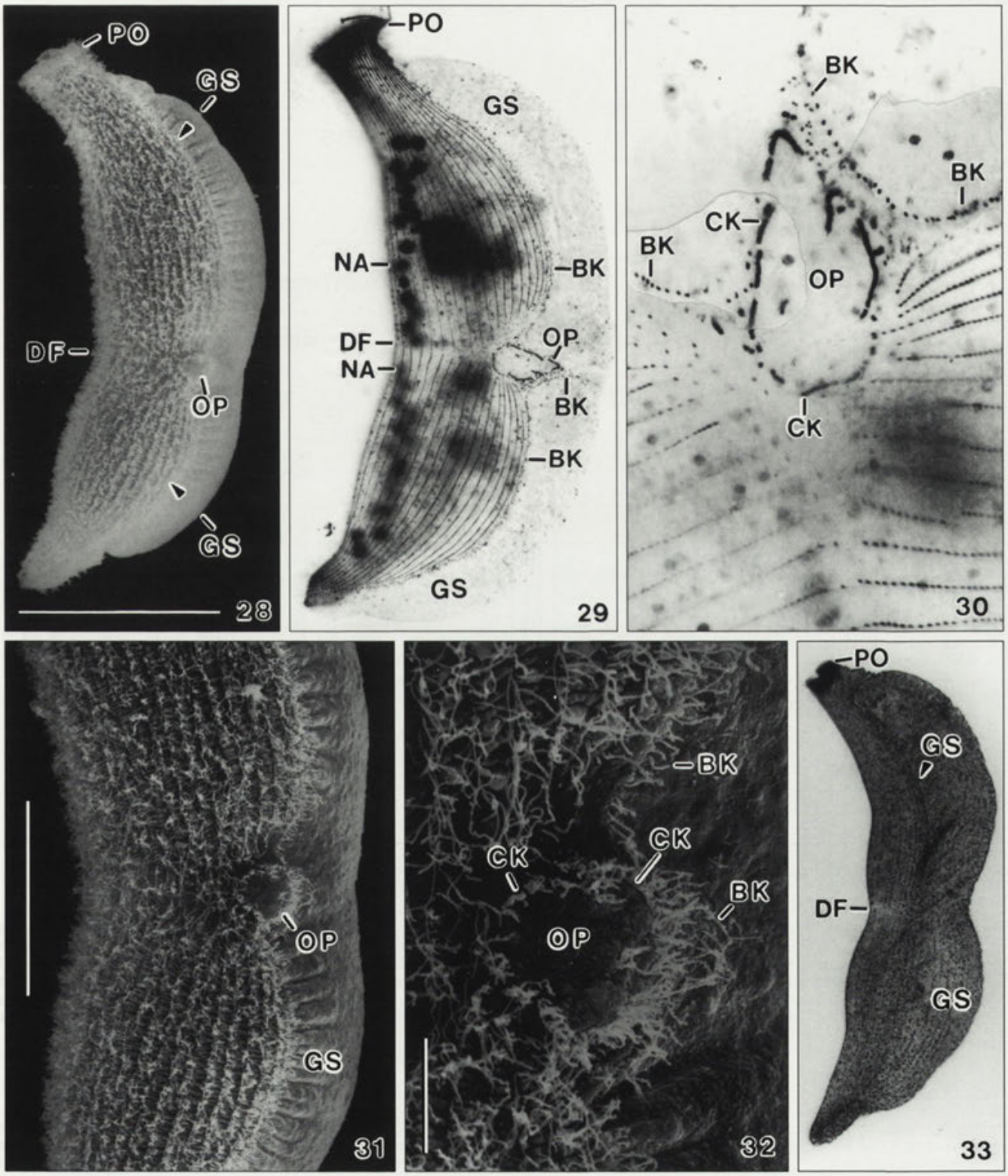
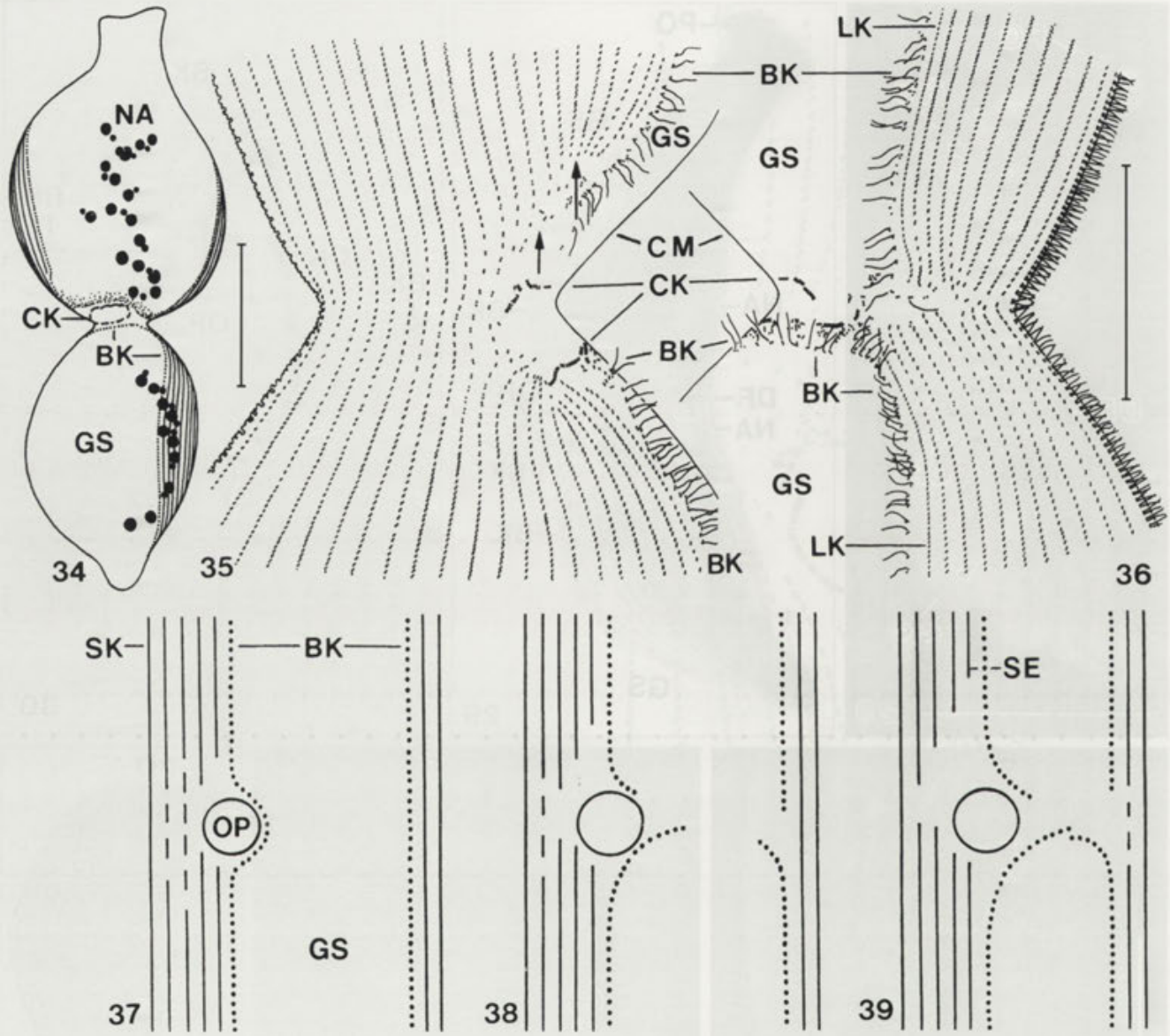
Figs. 25-27. *Sultanophrys arabica*, infraciliature and nuclear apparatus of a middle divider after silver impregnation (cp. Figs. 28, 33, 40, 44). Note that only half of the ciliary rows are illustrated in the overview, Figure 25. 25, 27 - middle dividers are furrowed slightly underneath mid-body and have assembled the newly formed oral kinetofragments to a distinct circumoral kinety, which surrounds the prospective oral bulge and strongly bulges the bristle kinety. Note that kinetids in the bristle kinety are more narrowly spaced than in morphostatic specimens, indicating that new kinetids were produced intrakinetally. In late dividers, the bristle kinety breaks at the summit of the bulge and orientates transversely to the cell's main axis to meet the bristle kinety at the left side of the glabrous stripe (Figs. 37-39). At the posterior margin of the circumoral kinety there are many scattered dikinetids and some small kinetofragments, some of which will become brosse kineties. Somatic division progresses to midline of right side. Arrowheads mark stomatogenic kinety anterior and posterior of the oral primordium; 26 - part of the nuclear apparatus of the specimen shown in Figure 25. The micronuclei multiply by mitosis and show distinct spindle microtubules and metaphasic chromosomes. The macronuclei do not divide. BK - bristle kinety, CK - circumoral kinety, GS - glabrous stripe, MA - macronuclear nodule, MI - micronucleus, NA - nuclear apparatus, OP - oral primordium. Scale bars: 200 µm (Fig. 25) and 20 µm (Figs. 26, 27)

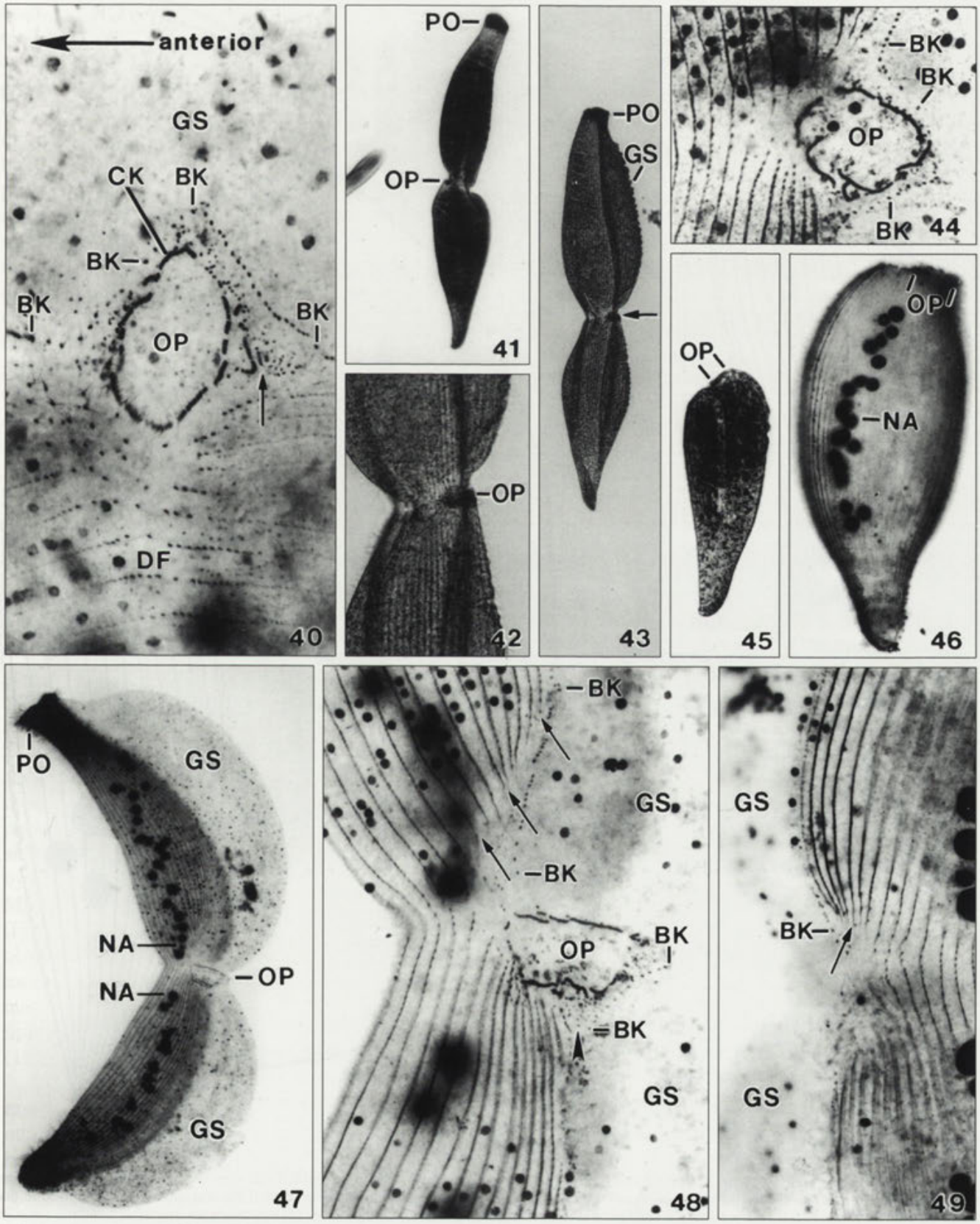


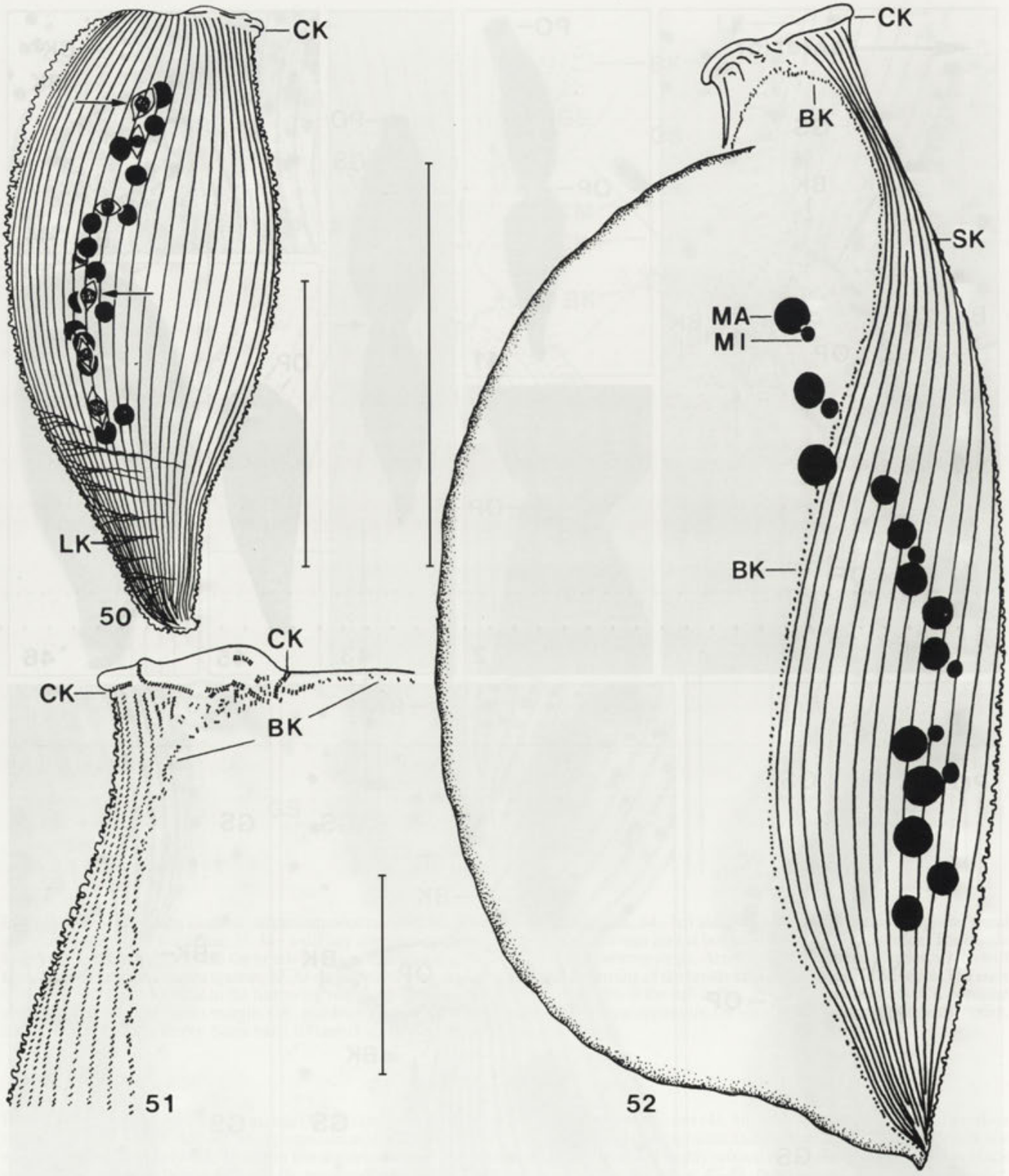
Figs. 28-33. *Sultanophrys arabica*, middle dividers in the scanning electron microscope (28, 31, 32; same specimen, overview and details of opisthe oral apparatus), after silver impregnation (29, 30; same specimen, overview and detail of opisthe oral apparatus), and from life (33). Middle dividers are furrowed slightly underneath mid-body and have assembled the newly formed oral kinetofragments to a distinct circumoral kinety, which surrounds the prospective, slightly elevated oral area. Somatic division proceeds to midline of right side. BK - bristle kinety, CK - circumoral kinety, DF - division furrow, GS - glabrous stripe, NA - nuclear apparatus, OP - oral primordium, PO - parental (proter) oral apparatus. Scale bars: 100 μm (Fig. 28), 50 μm (Fig. 31), and 10 μm (Fig. 32)



Figs. 34-39. *Sultanophrys arabica*, infraciliature of late dividers after silver impregnation. 34 - left side view showing location of opisthe's oral apparatus right of cell's midline; 35, 36 - a slightly distorted specimen, showing the cleavage area at both sides of the glabrous stripe. The bristle kinety disrupted and united with the bristle kinety extending along the left side of the glabrous stripe. Arrows mark shortened kineties, which will form proter's posterior secant system; 37-39 - scheme showing the disruption and patterning of the bristle kinety and the secant kineties. The ends of the bristle kinety unite due to the furrowing of the cell (indicated by decreased breadth of the cell in Fig. 39). BK - bristle kinety, CK - opisthe circumoral kinety, CM - cell's margin, GS - glabrous stripe, LK - lateral kinety, NA - nuclear apparatus, OP - oral primordium, SE - secant kineties, SK - ordinary somatic kinety. Scale bars: 100 μm (Fig. 34) and 50 μm (Figs. 35, 36)

Figs. 40-49. *Sultanophrys arabica*, middle (40, 44) and late (41-43, 47-49) dividers and postdividers (45, 46) from life (41-43, 45) and after silver impregnation (40, 44, 46-49). 40, 44 - opisthe oral area of middle dividers. Arrow marks kinetofragments and scattered dikinetids. For details, see explanations to Figures 27-33; 41-43 - in late dividers the new oral apparatus has accumulated highly refractive granules, which appear as black spot in the micrographs (arrow, OP); 45, 46 - postdividers have a highly characteristic, triangular shape; 47-49 - overview and details of a late divider. Figures 48 and 49 show the cleavage area right and left of the glabrous stripe. The organism is distinctly furrowed and the bristle kinety has been disrupted by cytokinesis: the posterior ends of the proter kinety will unite in postdividers, while the new (opisthe) anterior end curves left to unite with the bristle kinety at the left margin of the glabrous stripe (for a scheme, see Figs. 37-39). Arrowhead marks kinetofragments and scattered dikinetids, some of which will become brosse kineties. The shortened kineties (arrows) will form the posterior secant system, which is inconspicuous at the left margin of the glabrous stripe. BK - bristle kinety, CK - circumoral kinety, DF - division furrow, GS - glabrous stripe, NA - nuclear apparatus, OP - oral primordium (opisthe oral apparatus), PO - parental (proter) oral apparatus





Figs. 50-52. *Sultanophrys arabica*, opisthe postdividers after silver impregnation. 50, 51 - right and left side view of an early postdivider. Arrows mark dividing micronuclei. Young opisthe postdividers have a characteristic triangular shape (cp. Fig. 45, 46), the oral apparatus left of midline, and the oral structures not yet fully assembled; 52 - late postdivider with growing neck and head. The oral structures are assembled and micronuclei division is complete. BK - bristle kinety, CK - circumoral kinety, LK - lateral kinety with conspicuous fibres, MA - macronuclear nodule, MI - micronucleus, SK - ordinary somatic kineties. Scale bars: 100 µm (Figs. 50, 52) and 30 µm (Fig. 51)

head, and thus appears as black spot, which is easily recognisable even at low magnification (x 40; Figs. 41-43). The opisthe, which is slightly smaller than the proter due to the subequatorial division furrow, obtains a very characteristic triangular shape, which is maintained in young postdividers (Figs. 41, 45). The bristle kinety breaks at the summit of the bulge formed by the oral primordium and unites, due to the decreasing diameter of the cell at the cleavage furrow, with the broken and rightwards curved end of the bristle kinety extending along the left margin of the glabrous stripe (Figs. 34-39). Details of this process are difficult to observe because the cleavage area of late dividers is usually rather distorted in the preparations. Indeed, late dividers are easily disrupted mechanically and then some oral kinetofragments of the opisthe remain at the posterior end of the proter.

Somatic division is not yet finished, that is, the kineties in the midline of the cell are still intact. The disrupted kineties along the right side of the oral primordium do not proliferate basal bodies posteriorly. Thus, the kineties, which abut to the opisthe's oral apparatus become shortened and form, when the daughters grow, the anterior and posterior secant system (Figs. 29, 30, 37-39, 47-49). The bare area is greater at the anterior than at the posterior margin of the oral primordium. Accordingly, the posterior secant system is more distinct than the anterior one. No or only an indistinct bare area occurs in the kineties at the left side of the glabrous stripe. Thus, *S. arabica* lacks a left side secant system (Foissner and AL-Rasheid 1999).

Stage 6 (Figs. 45, 46, 50-52). This stage comprises cell shaping of postdividers, which are about half the size of morphostatic specimens. Accordingly, there is distinct postdivisional growth in both cell size and kinetid number, although some are produced during division, as indicated by the decreased distances between the individual kinetids. Micronuclear division usually finishes in late dividers (Fig. 34) or young postdividers (Fig. 50). We never observed dividing micronuclei in morphostatic specimens, indicating that regulation of macronuclear and micronuclear number occurs in late dividers and postdividers by multiple division of micronuclei, as described by Raikov (1982).

Young postdivisional proters are flask-shaped, that is, much stouter than morphostatic specimens (Fig. 41); otherwise, however, they are similar to interphase cells because no changes occur in the proter during division. The posterior ends of the disrupted bristle kinety come close together during tail outgrowing. The fibres in the posterior portion of the lateral kinety increase in length and unite to the conspicuous bundles typical of interphase specimens (Fig. 50; Foissner and AL-Rasheid 1999).

Young postdivisional opisthes have a characteristic triangular shape because the parental tail is maintained and the neck and head are not yet developed (Fig. 45, 46, 50). The newly produced oral apparatus is still at the left margin of the cell and usually rather distorted, that is, the kinetofragments composing the circumoral kinety are disordered and the brosse has not yet fully assembled (Figs. 46, 50, 51). All oral structures obtain their final pattern and location only during postdivisional growth (Fig. 52).

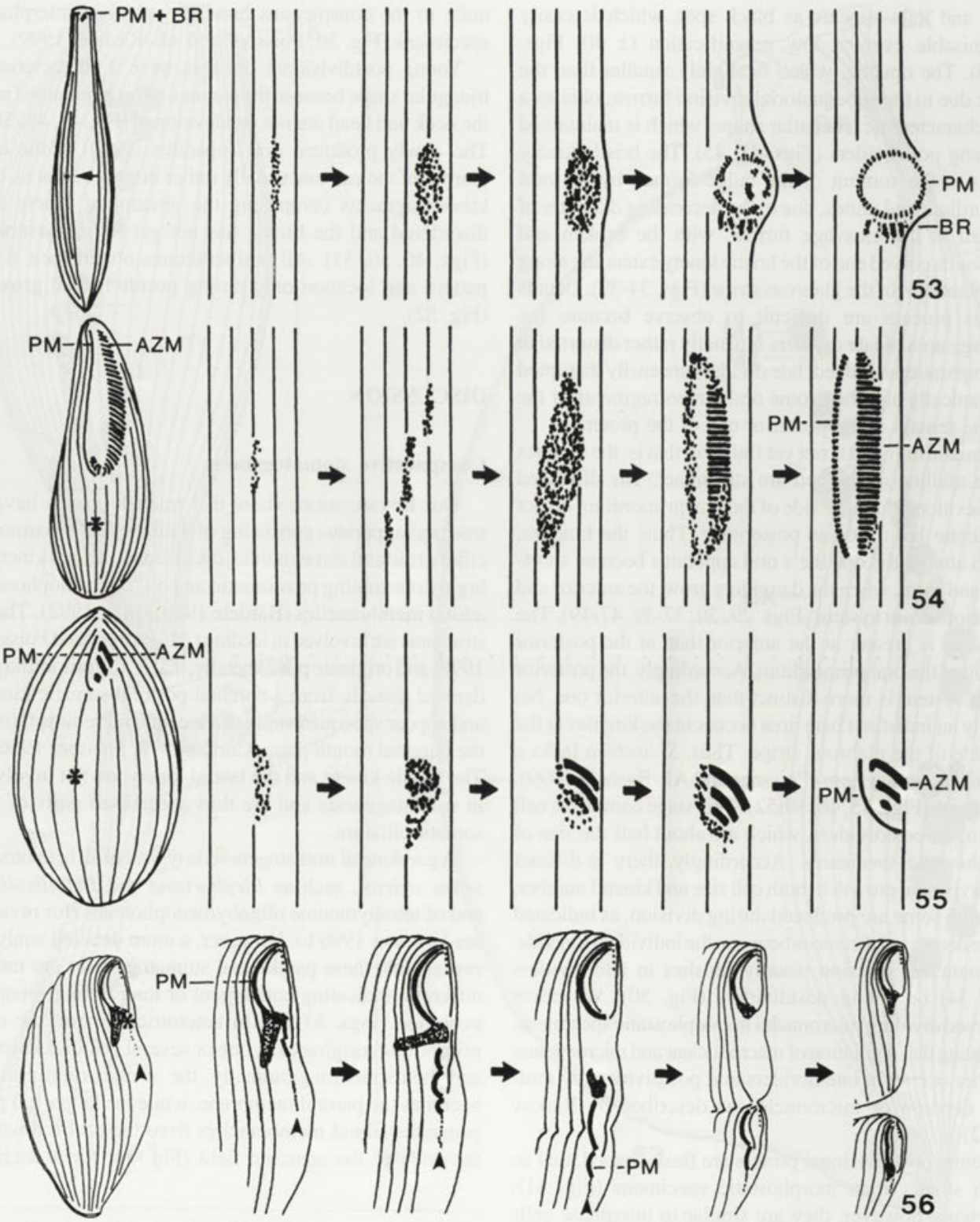
DISCUSSION

Comparative stomatogenesis

Our investigations show that trachelocercids have a true oral apparatus consisting of a dikinetidal circumoral ciliary row and some minute, dikinetidal (brosse) kineties highly resembling prostomatid and oligohymenophorean adoral membranelles (Bardele 1989, Hiller 1992). These structures are involved in feeding (AL-Rasheid and Foissner 1999) and originate parakinetally, that is, the primordia are derived directly from a parental postoral somatic kinety and appear subequatorially at a location far removed from the parental mouth parts (Corliss 1979, Foissner 1996b). The bristle kinety and the lateral kinety are not involved in stomatogenesis and are thus specialised parts of the somatic ciliature.

A parakinetal stomatogenesis is typical of all heterotrichs *sensu stricto*[1], such as *Blepharisma* and *Eufolliculina*, and of tetrahymenine oligohymenophoreans (for review, see Foissner 1996 b). However, a more detailed analysis reveals that these parakinetal stomatogeneses are rather different, indicating convergent or long lasting separate evolution (Figs. 53-55). In heterotrichs *s. str.*, the oral primordium originates in one or several postoral kineties and then splits longitudinally: the smaller right portion becomes the paroral membrane, while the larger left part generates adoral membranelles from the centre towards the ends of the anarchic field (Fig. 54; for a detailed

[1] Note that ontogenesis in *Metopus* and relatives, considered as typical heterotrichs for more than 100 years, is very different from that of *Blepharisma* and relatives (Foissner and Agatha 1999). Thus, metopids do not belong to the heterotrichs, as also indicated by gene sequences (Lynn and Small 1997, Hirt *et al.* 1998).



Figs. 53-56. Diagrams of parakinetal (53-55) and bucocinetal (56) stomatogenesis, 53 - a trachelocercid karyorelictean (*Sultanophrys*), 54 - a heterotrich *s. str.* (*Blepharisma*; after Aescht and Foissner 1998), 55 - a hymenostome oligohymenophorean (*Tetrahymena*, after Foissner 1996b), and 56 - buccokinetal stomatogenesis in the karyorelictean *Loxodes* (from Bardele and Klindworth 1996). Figures in the left column show the somatic and oral ciliary pattern, and the site, where the oral primordium originates, is marked by an asterisk (plus arrow in *Sultanophrys*). The somatic ciliary rows are shown as simple, straight lines. Arrowheads in *Loxodes* mark a scutica-like kinetofragment. AZM - adoral zone of membranelles, BR - brosse (possibly homologous to AZM), PM - paroral membrane (circumoral kinety). See text for detailed explanation

analysis, see Aescht and Foissner 1998). There are no migrating kinetofragments, as in *Sultanophrys*, which, on the other hand, does not split the anarchic field in a right and a left portion. In this respect and by the minute brosse kineties, *Sultanophrys* resembles hymenostome oligohymenophoreans, some of which even have minute, migrating kinetofragments, which assemble to adoral membranelles, for instance *Ichthyophthirius multifiliis* (Foissner 1996b). Furthermore, the oral primordium of *Sultanophrys* develops, like in tetrahymenine oligohymenophoreans (Foissner 1996b), within a "director meridian", that is, a specialised (shortened) postoral kinety. This kinety is generated by the special location of the forming oral apparatus within the right lateral ciliary rows. Here, the oral primordium intersects some somatic ciliary rows, which thus become postoral, at least during ontogenesis (Figs. 11, 19, 29, 37-39), and form the secant system in morphostatic cells (Fig. 10; Foissner and AL-Rasheid 1999). Thus, considered from a descriptive view, stomatogenesis of *Sultanophrys* is more similar to that of hymenostome oligohymenophoreans than to that of heterotrichs. However, the somatic ultrastructures of hymenostomes and karyorelictids are very different (for review, see Lynn 1981), indicating that the stomatogenic similarities evolved convergently.

The migrating kinetofragments of *Sultanophrys* are a conspicuous character, as they are in prostomatid ciliates, where they form, as in *Sultanophrys*, the circumoral kinety and the brosse, which, in prostomatids, is very likely homologous to the hymenostome adoral membranelles (Bardele 1989, Hiller 1992). However, these similarities are likely superficial and convergent because the prostomatid kinetofragments do not originate from an anarchic field, as in *Sultanophrys* (Fig. 19), but at the broken ends of several ventral kineties (Bardele 1989, Hiller 1992, Foissner 1996b). The same applies to the gymnostomes (litostomes), which produce kinetofragments holotelokinetally, that is, at the anterior end of *all* somatic kineties. By a slight rotation, the fragments unite to a circumoral kinety (for review, see Foissner 1996b).

Finally, we have to discuss stomatogenesis of *Loxodes*, the only other karyorelictid investigated so far. *Loxodes* has a buccokinetal stomatogenesis, where dikinetidal kinetofragments are generated at or from the posterior end of the parental paroral (Bardele and Klindworth 1996). Several such fragments are produced, migrate posteriorly, and assemble to the opisthe's paroral in mid-body. The left side oral ciliature is not generated from a special anlage but by proliferation of kinetids within the parental structures (Fig. 56). Furthermore, *Loxodes* has a ventral kinetofragment, which, in terms of location and divisional behaviour, highly resembles the scutica of oligohymenophorean scuticociliates (Bardele and Klindworth 1996, Foissner 1996b). Both a scutica-like structure and a contribution of parental mouth structures to the opisthe oral apparatus are lacking in *Sultanophrys*. Accordingly, ontogenesis looks very different in loxodid and trachelocercid karyorelictids. On the other hand, we must not overlook that the paroral ciliature is produced in both taxa by *migrating* kinetofragments, whose origin is, however, different. Of particular interest is the left side oral ciliature of *Loxodes*, which is, like ordinary somatic kineties, produced by intrakinetal proliferation of kinetids. This could indicate that these kineties are not yet fully oralised somatic ciliature (Bardele and Klindworth 1996, Foissner 1996b) and, in turn, that the oral ciliature evolved from somatic ciliature, as proposed by Small (1984) and Foissner (1995b). See last chapter for a more detailed discussion of this matter.

Evolution of stomatogenic modes

Karyorelictids and heterotrichs are sister groups and at the base of the ciliate tree, according to the somatic cortical ultrastructure and gene sequences (for reviews, see Lynn and Small 1997, Hirt *et al.* 1998). Each of these groups has a distinct stomatogenesis, and even two modes are found in the karyorelictids. Accordingly, three stomatogenic modes occur at the base of the ciliate tree, indicating an explosive radiation of this trait. Furthermore, we are confronted with the fact that the ontogenetic pattern of both loxodids and trachelocercids shows remarkable similarities with that of oligohymenophorean ciliates: the ventral kinetofragment of loxodids resembles the scutica of oligohymenophorean scuticociliates (Bardele and Klindworth 1996, Foissner 1996a), while the parakinetal stomatogenesis of trachelocercids resembles hymenostome oligohymenophoreans (for reviews, see Foissner 1995a, 1996b). Although we tend to interpret these similarities as convergently evolved, we can not exclude a deeper meaning at the present state of knowledge.

The data available do not provide unambiguous evidence, which of the three stomatogenic modes found in the Postciliodesmatophora is ancestral. There are, however, two indicators which favour the parakinetal mode: (1) it occurs in both heterotrichs and the largest group of karyorelictids, and (2) somatic division is more similar in heterotrichs and trachelocercids than in loxodids and trachelocercids (see next chapter). Bardele and Klindworth (1996), on the contrary, suggest that the parakinetal mode

evolved from the buccokinetal mode. They hypothesise that detailed analyses would probably show a scutica-like structure, similar to that found in *Loxodes*, also in typical heterotrichs, such as *Blepharisma* and *Stentor*. However, such structures were not found in a detailed reinvestigation of the stomatogenesis of *Blepharisma*, although the reorganisation anlage for the parental oral apparatus shows some similarities with a scutica (Aescht and Foissner 1998).

Comparative somatic ontogenesis

The somatic ontogenesis of *Sultanophrys* shows four peculiarities: (1) the division furrow is recognisable very early, that is, when stomatogenesis commences (Figs. 8, 10); (2) there is a distinct cleavage gradient proceeding from the margins to the centre of the ciliary field (Figs. 19, 20, 23); (3) there is not, as is usual, proliferation but rarefaction of kinetids in the cleavage area; (4) there is pronounced postdivisional patterning, which is very likely associated with the slender shape and high contractility of the trachelocercids. The rarefaction of kinetids in the cleavage area is possibly the most important feature because it is also found in *Blepharisma* (Aescht and Foissner 1998), while in *Loxodes* "the first sign of cell division is the proliferation of kinetosomes in the somatic kineties in the middle of the cell at a site of the future cleavage furrow" (Bardele and Klindworth 1996).

Loxodid and trachelocercid karyorelictids have a special (bristle) kinety, which frames a more or less broad, non-ciliated (glabrous) stripe (Foissner and Dragesco 1996b). Both the bristle kinety and the glabrous stripe are lacking in the geleiids (Dragesco and Dragesco-Kernéis 1986, own unpublished observation), the third and most enigmatic group of karyorelictids (Foissner 1998). The bristle kinety has a specialised infraciliature and very peculiar ciliation, first described by Foissner and Dragesco (1996a, b): "The bristle kinety commences subapically at the right margin of the glabrous stripe, extends posteriorly, then anteriorly at the left, to end up at the right margin again. The dikinetids of the right posterior portion of the bristle kinety have the *posterior* basal body ciliated, whereas the *anterior* basal bodies are ciliated in its left and right anterior portion". Thus, the ciliation of the dikinetids is opposed by 180° where the ends of the kinety meet. These features suggested that the bristle kinety could play a special role in ontogenesis (Foissner and Dragesco 1996b), which is, however, disproved by the present results: it simply disrupts during cytokinesis. Unfortunately, we could not observe how the peculiar ciliation at the anterior end of the bristle kinety is brought about because all very late dividers were too strongly distorted or the cilia had not impregnated clearly enough. It is, however, clear that the process occurs only in very late dividers (because the morphostatic pattern is still recognisable in late dividers, Fig. 27) and the circumoral ciliature is not involved, as speculated by Foissner and Dragesco (1996b).

In any case, the bristle kinety, although being "silent" ontogenetically, is a highly interesting specialisation, clearly defining loxodids and trachelocercids as sister groups. It is a pity that neither ontogenesis nor comparative morphology gives any information about its origin and history. We do not know of any other ciliates with a similar kinety. Thus, it is possibly a young, special trait, which could provide us with important information about the history of the karyorelictids, when we would be lucky to discover a ciliate with the plesiomorphic state.

Evolution of ciliate cytoarchitecture

This matter is thoroughly discussed in recent reviews by Bardele and Klindworth (1996) and Schlegel and Eisler (1996), to which the reader is referred for detailed information. Here, we shall discuss only some aspects which are related to the karyorelictids and the present results.

Karyorelictids have been postulated as a model for ancestral ciliates because they have non-dividing macronuclei and one of the genera, *Kentrophoros*, is even mouthless (Corliss 1974, Orias 1976, Small 1984). *Kentrophoros* has a symbiotic kitchen garden of sulphur bacteria on the glabrous stripe. The bacteria are ingested through the stripe, which stimulated Small (1984), among others, to suppose that trachelocercids also feed through the glabrous stripe. This has been disproved meanwhile (AL-Rasheid and Foissner 1999). Likewise, detailed investigations showed that *Kentrophoros* has vestiges of an oral apparatus at and near the anterior end, indicating that it is secondarily mouthless (Foissner 1995c), as also proposed by Bardele and Klindworth (1996). Accordingly, the hypotheses of Orias (1976) and Small (1984) lost a basic assumption.

Orias' (1976) and Small's (1984) hypotheses require that the oral ciliature evolved from somatic ciliature, while Eisler (1992; for an update, see Schlegel and Eisler 1996) assumes just the reverse. Based on detailed ultrastructural and ontogenetical investigations of ventrostomial ciliates, Eisler (1992) suggests that a paroral kinety (undulating membrane) gave rise, due to lateral multiplication, to

somatic ciliary rows, which, in turn, produced adoral membranelles. Accordingly, Eisler (1992) assumes that ancestral ciliates had a buccokinetal stomatogenesis. This is supported by recent data on *Loxodes* (Bardele and Klindworth 1996); however, Bardele and Klindworth (1996) conclude "none of the extant karyorelictean makes a good model for the ancestral karyorelictean species". We agree because extant karyorelictids have a highly specialised somatic ciliary pattern and trachelocercids and heterotrichs have a somatic (parakinetal) stomatogenesis, indicating that *Loxodes* is derived. Furthermore, our studies on several loxodids indicate that their oral ciliature evolved from somatic ciliature (Foissner 1995a, b).

Lynn's (1996) subphyletic division of ciliates

The present data and those from *Metopus* (Foissner and Agatha 1999) provide little insights into the subphyletic division suggested by Lynn (1996) and Lynn and Small (1997). However, at least the main stomatogenic mode is the same in heterotrichs *s. str.* and trachelocercid karyorelictids, that is, in two groups classified by Lynn and Small (1997) in the subphylum Postciliodesmatophora. If the parakinetal stomatogenesis of certain oligohymenophoreans is considered as convergently evolved or as at all different from that of heterotrichs and karyorelictids, as suggested by Foissner (1996b), then the parakinetal stomatogenic mode is unique to the Postciliodesmatophora. Loxodids then must be considered as derived, and the Geleiidae await further investigations.

Due to the experience with metopids and trachelocercids, we agree with Lynn (personal communication) that stomatogenic modes can hardly be used to unravel the main paths of ciliate evolution, as long as we do not know the underlying mechanisms to differentiate between analogies and homologies. Our present terms (parakinetal, buccokinetal....) are purely descriptive for *where* the replication and patterning of the oral apparatus occur.

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