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Morphogenesis in Some Freshwater Tintinnids (Ciliophora, Oligotrichida)

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Summary

The morphogenesis of four freshwater tintinnids was investigated using protargol silver impregnation and scanning electron microscopy. Division is rather similar in *Tintinnopsis cylindrata*, *Tintinnidium pusillum* and *T. semiciliatum*, e.g. the oral primordium develops apokinetally posterior of somatic kinety 10 and the somatic ciliature originates by two rounds of intrakinetal basal body proliferations before cytokinesis. The peculiar ventral organelles form without apparent contact with parental ciliary structures as do the adoral membranelles and the paroral membrane; thus, the ventral organelles might be part of the oral apparatus. The morphogenesis of *Codonella cratera* differs from that of the other species by the reorganization of some parental ciliary rows and by a second round of somatic basal body proliferation after cytokinesis. Based on morphologic and morphogenetic similarities, *Tps. cylindrata* is transferred from the Codonellidae to the Tintinnidiidae, and *Tps. baltica* and *Tps. subacuta* are newly combined with *Codonella*: *C. baltica* nov. comb., *C. subacuta* nov. comb. We could not discern a unique character defining oligotrichs as a monophyletic group because the enantiotropic cell division is possibly less pronounced in some tintinnids and occurs also in peritrichs and a few prostomatids. We suggest, however, that the enantiotropic cell division evolved convergently in these taxa, and thus adhere to our view that this special mode of cell division is the most reliable apomorphy for the oligotrichs. In addition, the character *combination*, polar oral apparatus *and* apokinetal origin of the oral primordium, occurs only in oligotrichs. Morphogenetic similarities suggest a sister group relationship between tintinnids and strobilidiids.

Introduction

Observations on tintinnid division date back to the turn of the century [6, 7, 12–15, 28, 36, 46]. More recently, fission was studied by Biernacka [1], Brownlee [5], Coats & Heinbokel [9], Gold [24] and Laval-Peuto [37]; sexual reproduction was investigated by Gold & Pollingher [26] and Laval-Peuto [38]. All these studies, however, lack a detailed description of the origin and development of the oral and somatic ciliature. We therefore investigated the morphogenesis of 4 freshwater tintinnids from 3 different genera using protargol silver impregnation and scanning electron microscopy.

Material and Methods

Tintinnopsis cylindrata and *C. cratera* were collected in an eutrophic pond, the Salzachsee, City of Salzburg, Austria. See [23] for detailed site description.

Tintinnidium pusillum occurred in the plankton of a small eutrophic pond, the Poppelsdorfer Weiher, Bonn, Germany. See [23] for detailed site description.

Tintinnidium semiciliatum was found in the Aufwuchs of the River Amper, southern Germany. See [3] for detailed site description.

Field material was used for the investigations. Protargol silver impregnation (Wilbert's protocol with centrifuge [19]) and scanning electron microscopy as described in [19] were applied to reveal the infraciliature. Counts and measurements on stained specimens were performed at a magnification of X1000. Cells were drawn using a camera lucida. Diagrams of kineties are from [23].

Results

Terminology and Orientation of Cell

Detailed accounts of the interphasic morphology and infraciliature of the species investigated have been published earlier [3, 20, 23]. Thus, we provide only very short

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descriptions, some figures and kinety diagrams to make plain the changes occurring during cell division.

According to the Chatton-Lwoff convention, the somatic kineties are numbered clockwise when viewed from the apical pole of the cell, with kinety 1 being the rightmost postoral row [8, 10]. However, in oligotrichs counterclockwise numbering has been widely used, e.g. in strobilidiids by Deroux [11] and Lynn & Montagnes [41] and in tintinnids by Foissner & Wilbert [23]. This view was strongly opposed by a reviewer. We thus changed the clockwise numbering, although with reservations because oligotrichs lack a stomatogenic kinety which is usually kinety 1. The other terminology of the kineties follows Foissner & Wilbert [23], who provided the first kinety diagrams for tintinnids. More recent suggestions [8, 48, 49] are in our opinion not superior to the original proposal.

A "ventral organelle" is a short kinety situated at the anterior ventral body surface near the oral cavity [23]. Usually, there are 2 such organelles which have no obvious relationship to the somatic ciliature. More likely, they belong to the oral apparatus (see morphogenesis). According to our [3, 23] and other data [39], these organelles are composed of ciliated dikinetids. The "ventral kinety" (VK) and the right kinety 2 of *Nolaclusilis bicornis* [49] are possibly homologous to the ventral organelles found in *Tintinnidium* and *Tintinnopsis;* they consist, however, of single basal bodies.

The orientation of the cell is shown in Fig. 6. The acentric oral cavity clearly defines the ventral side. Accordingly, dorsal and lateral surfaces can be distinguished.

Tintinnopsis cylindrata Kofoid & Campbell, 1929 [20, 23, 33]

Interphasic infraciliature (Figs. 3, 7). 9–11, usually 10, meridional somatic kineties of slightly different length arranged in 2 groups: 5–7 kineties left and 4–5 right of ventral organelles; therefore, kinety-free area on ventral side. Somatic kineties consist of ciliated monokinetids. 11–13, usually 11, adoral membranelles, 2–3 of them increasingly elongated and extending into oral cavity. 1 single-rowed paroral membrane.

Transverse ventral organelle (organelle 1) composed of 9–13 ciliated dikinetids, oblique ventral organelle (organelle 2) composed of 5–6 dikinetids with only 1 ciliated basal body each.

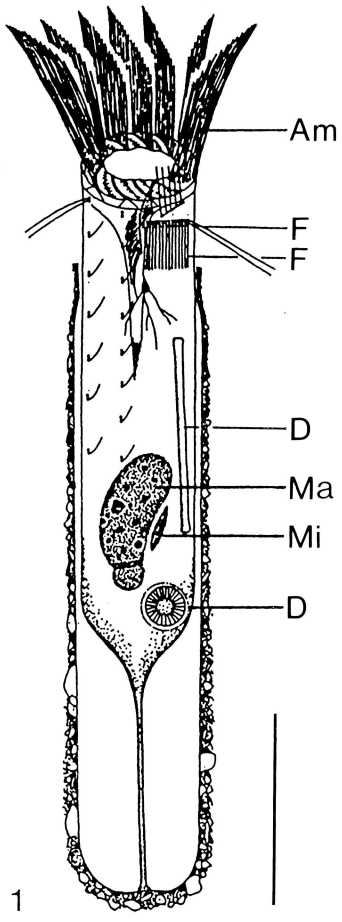
Morphogenesis (Figs. 8–14, 25, 29, 30). To avoid confusion, numbering of kineties is based on an average of 10 somatic kineties as shown in Fig. 7.

Division starts with the appearance of a replication band in the posterior portion of the macronucleus. Then, the oral primordium originates apokinetally on the cell surface in a ventro-lateral position, i.e. distinctly posterior and slightly to the right of somatic kinety 10 (Figs. 8, 25). The anarchic field soon enlarges and moves below the cell surface, where the adoral membranelles differentiate (Fig. 9; cp. Figs. 22, 23 from *T. semiciliatum*). To the right of these, the paroral membrane forms without apparent contact with developing membranelles or parental ciliary structures, indicating a de novo origin (Fig. 9). Some new basal bodies are formed within the parental rows (first round of proliferation), which thus appear slightly elongated (e.g. in somatic kineties 8–10 the basal body number increases by about 18%, n = 23). Kinety 10 possibly lengthens and splits slightly later than kineties 1–9.

Next, the cilia of the adoral membranelles become distinct but remain below the cell surface (Fig. 10). The paroral membrane, composed of a single row of tightly spaced basal bodies, has elongated distinctly. The somatic ciliary rows split at the level of the newly forming oral apparatus, i.e. slightly below mid-body. The opisthe's kineties 1–5 are already ciliated, whereas rows 6–10 are still barren, suggesting that development starts earlier in kineties 1–5 (Fig. 10). The ventral organelles originate de

Figs. 1–6. Interphasic morphology in some tintinnids (from [3, 20, 23]). – Fig. 1. Ventral view of *Tintinnidium pusillum.* – Fig. 2. Ventro-lateral view of *Tintinnidium semiciliatum* showing lorica attached to substrate. – Fig. 3. Ventro-lateral view of *Tintinnopsis cylindrata.* – Figs. 4, 5. Dorsal and ventral view of *Codonella cratera.* – Fig. 6. Schematized top view of *T. pusillum.* The acentric oral cavity defines the ventral side. Bars = 40 μm. Am = adoral membranelles; C = cytostome; Cv = contractile vacuole; D = diatom; E = elongated adoral membranelles; F = fibers of ventral organelles and paroral membrane, respectively; Fv = food vacuole; L = lateral kinety field; Lo = lorica; M = mineral grain; Ma = macronucleus; Mi = micronucleus; My = myoneme; O = oral cavity; P = peristomial bottom; Pm = paroral membrane; S = sharp-edged particle; Ss = silverline system after dry silver nitrate impregnation; V = ventral kinety; VL = ventro-lateral kinety; V1 = transverse, V2 = oblique ventral organelle.

Figs. 7–14. Morphogenesis in *Tintinnopsis cylindrata* (protargol impregnation). Paroral membrane of proter not shown; ciliature depicted only schematically. – Fig. 7. Diagram of interphasic infraciliature. – Fig. 8. Left lateral view of very early divider having anarchic field of basal bodies (Op) on cell surface. All somatic basal bodies are ciliated. – Fig. 9. Left lateral view of early divider showing oral primordium composed of anlagen for adoral membranelles and paroral membrane (arrowhead) already situated beneath cell surface. Sometimes, 1 basal body non-ciliated at posterior ends of somatic kineties (arrow). – Fig. 10. Left lateral view of early divider with oral primordium in side view. Somatic kineties have separated, parental kineties and opisthe's kineties 1–5 are ciliated, whereas opisthe's rows 6–11 are non-ciliated. – Figs. 11, 12. Ventral and dorsal view of middle divider. The oral membranelles evaginate and the new ventral organelles differentiate. 1–4 barren basal bodies occur at posterior ends of proter's somatic kineties (arrow). Opisthe's kineties completely ciliated, except rows 1–4 which have some barren basal bodies (arrowheads). – Fig. 13. Left lateral view of late divider showing proter's posterior end connected with opisthe's dorsal region. All basal bodies have cilia. – Fig. 14. Very late divider. All basal bodies have cilia. Scale bar divisions = 10 μm. Ma = macronucleus; Mi = micronucleus; Op = oral primordium; V1, V2 = ventral organelles; numbers denote somatic kineties.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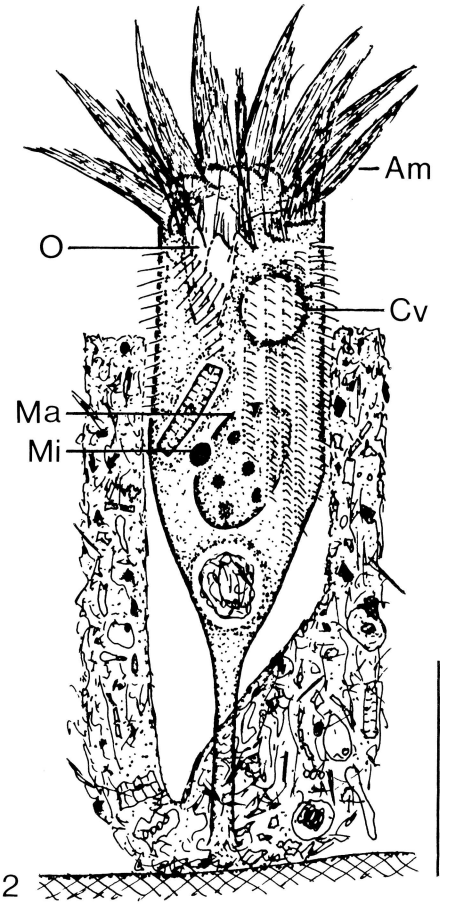
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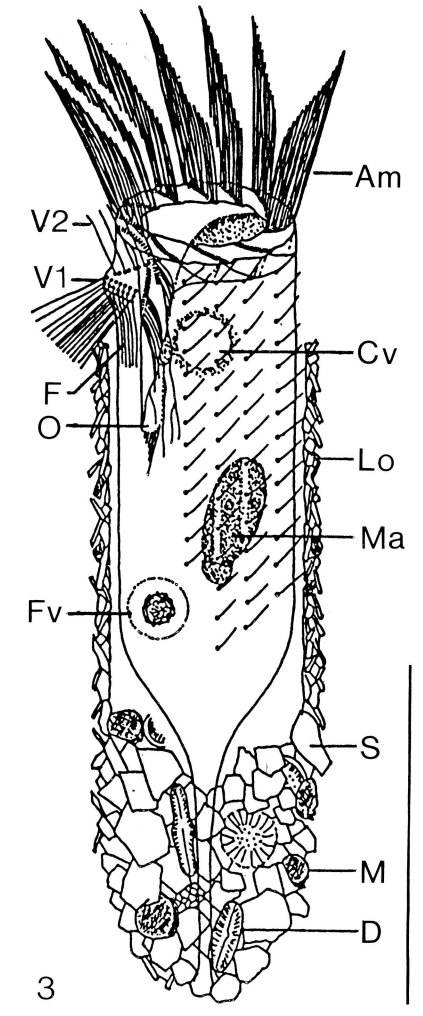
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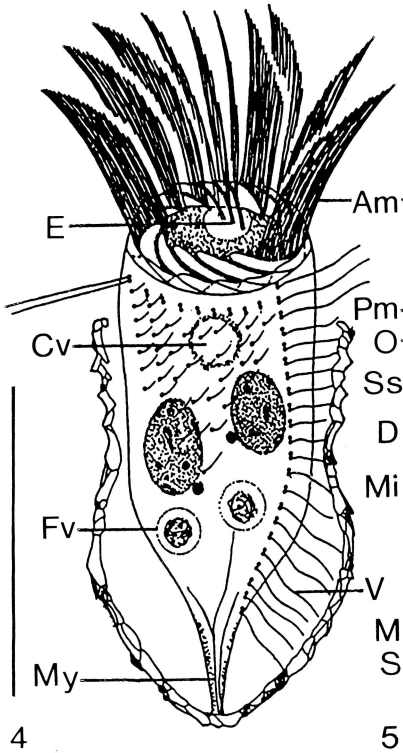
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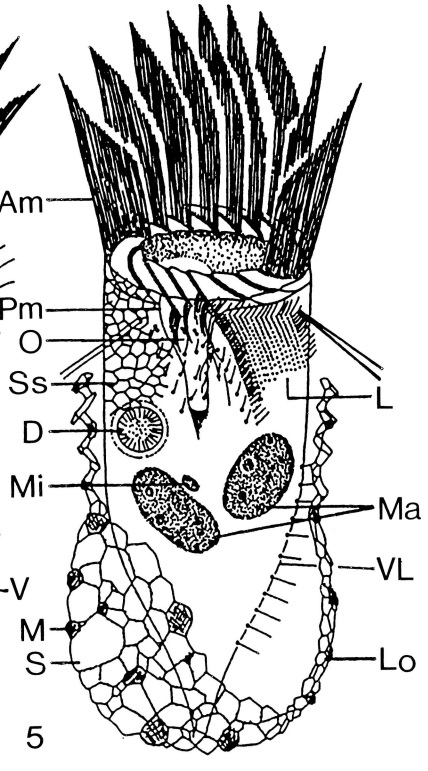
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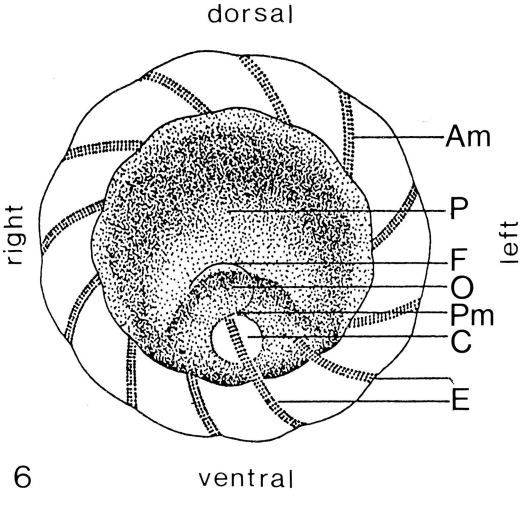
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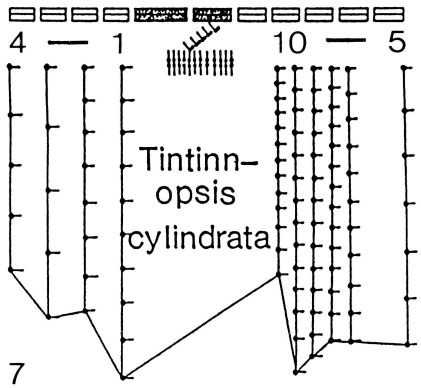

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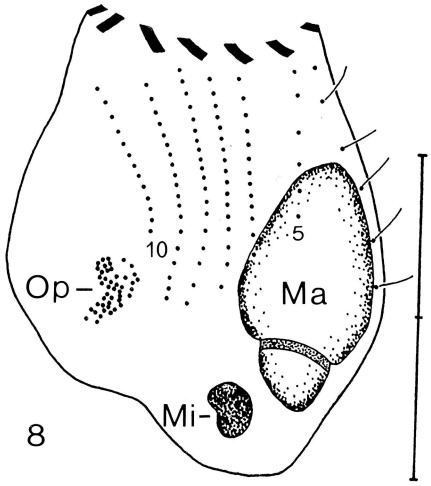

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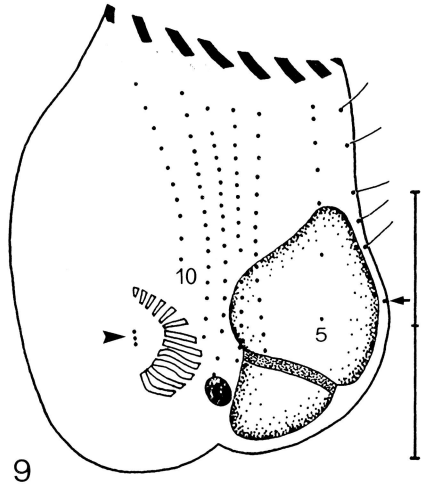

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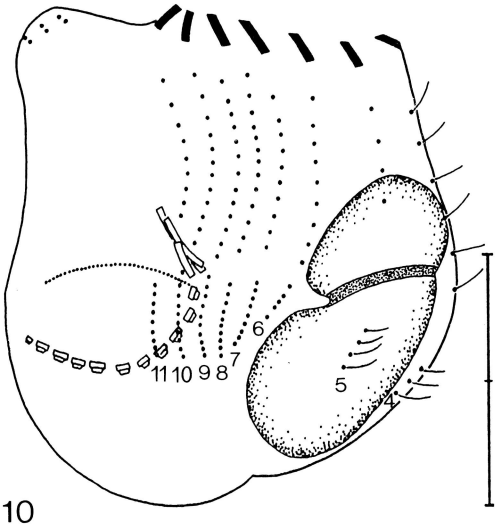

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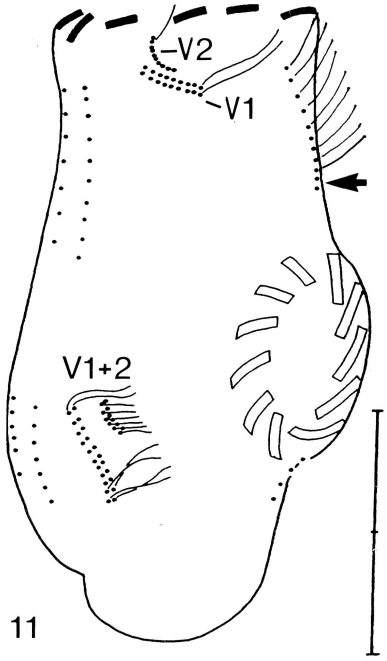

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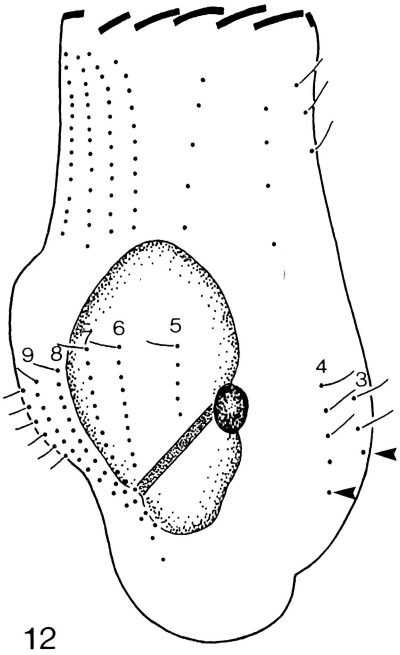

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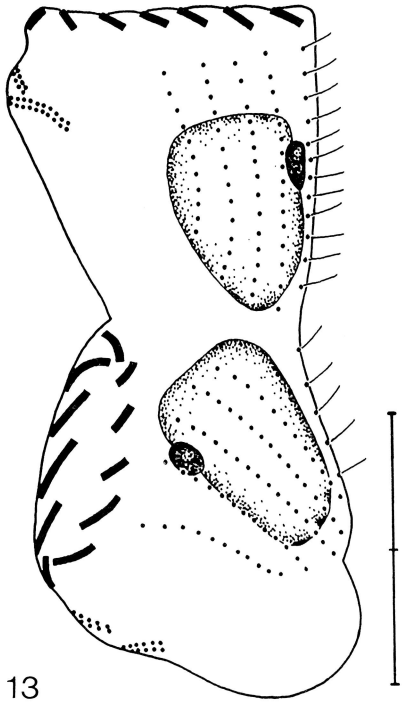

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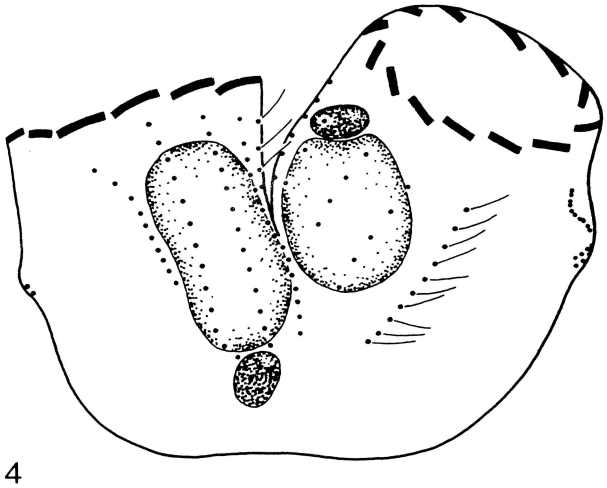

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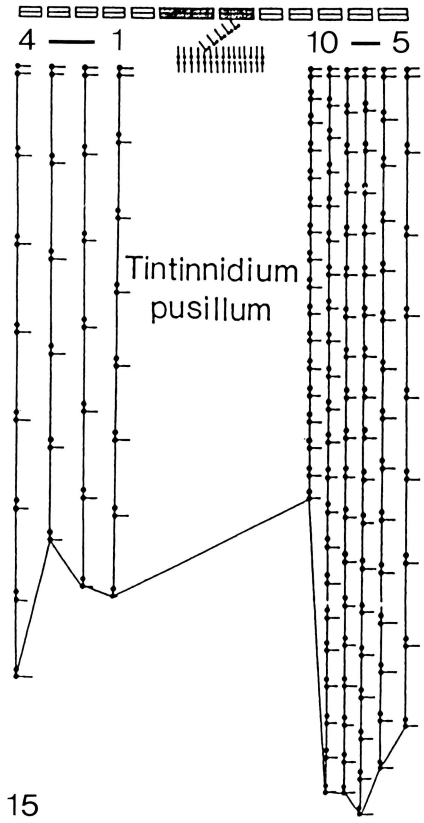

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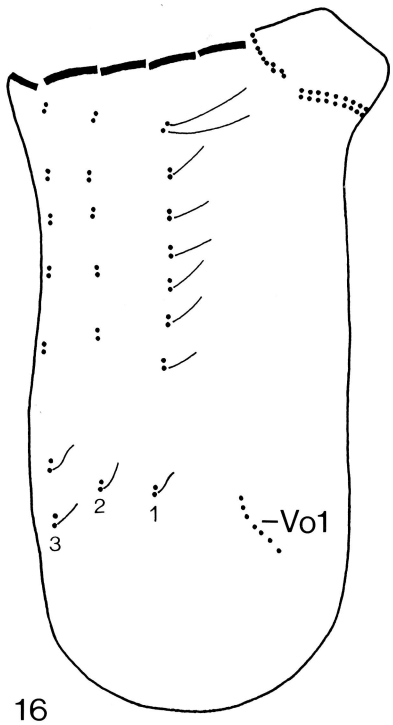

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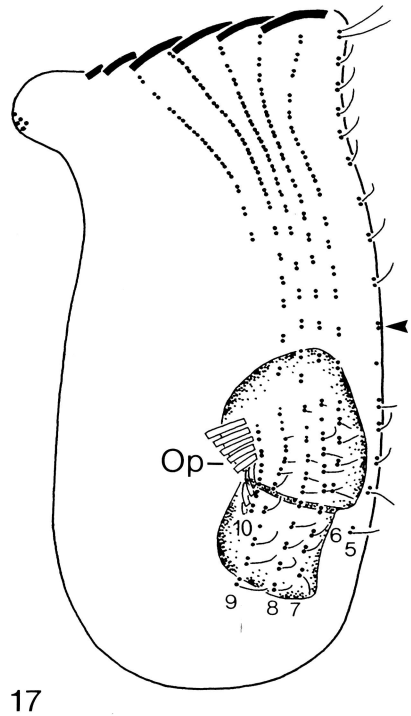

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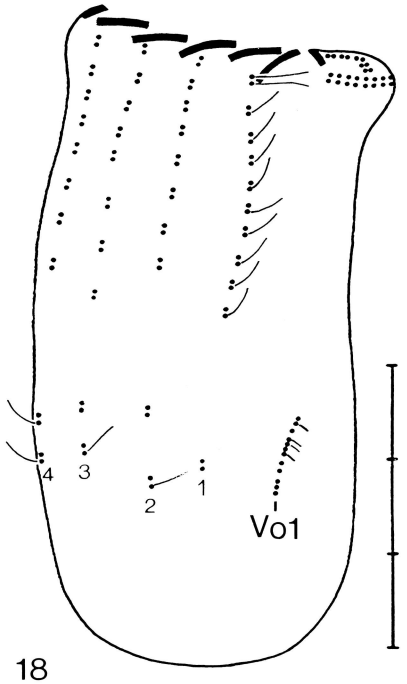

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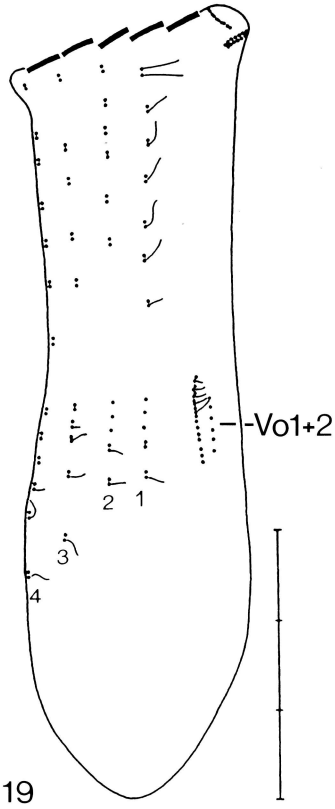

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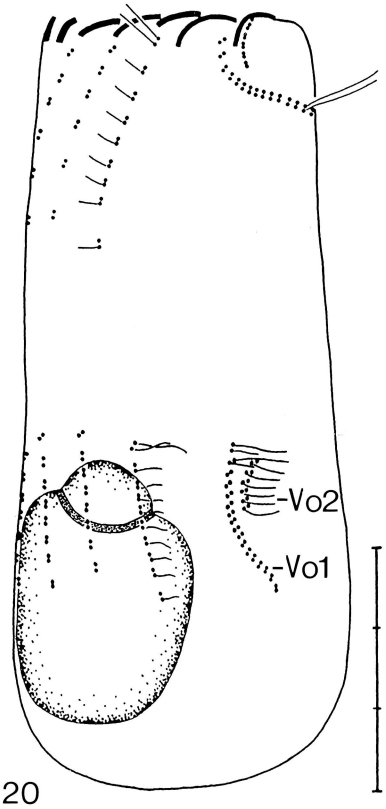

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novo on the posterior third of the ventral side, i.e. left of the opisthe's kinety 1 (Fig. 11). Each of these organelles is initially composed of a single row of basal bodies which later become dikinetids and develop their specific ciliation (cp. Figs. 16, 18, 19, 26 from *T. pusillum*).

In the next stage, the oral membranelles evaginate after forming a complete ring or a very flat spiral which increases in diameter by spreading of the membranelles (Fig. 11). 1–4 basal bodies are recognizable at the posterior ends of the proter's somatic kineties. They are still non-ciliated, indicating that a second round of basal body proliferation is commencing which provides the kineties with their species-specific number of cilia (Figs. 11, 12). The ventral organelles of the opisthe have been completed but are still arranged almost meridionally. Subsequently the micronucleus and then the macronucleus divide and move, respectively, into the proter and opisthe (Figs. 13, 30). The opisthe's oral apparatus is obviously fully differentiated now. An oblique fission furrow develops between the proter's posterior region and the anterior *dorsal* half of the opisthe (Figs. 13, 30).

In the final stages, the daughter cells lie side by side, i.e. only their posterior portions are connected (Figs. 14, 29). Compared with earlier stages, the opisthe is now rotated 180° relative to the proter, i.e. their dorsal sides face each other. Ventral organelle 1 rotates clockwise by about 90° to its specific transverse position.

No signs of reorganization were observed in the parental oral and somatic ciliature.

Tintinnidium pusillum Entz, 1909a [12, 20, 23]

Interphasic infraciliature (Figs. 1, 15). 9–11, usually 10, meridional somatic kineties of different length arranged in 2 groups: 5–6 kineties to the left and 4–5 to the right of ventral organelles; therefore, kinety-free area on ventral side. Somatic kineties composed of dikinetids, only posterior basal bodies ciliated. First dikinetid of each kinety with 2 long cilia. 11–13 adoral membranelles, 2–3 of them increasingly elongated and extending into oral cavity. 1 single-rowed paroral membrane.

Transverse ventral organelle (organelle 1) composed of 13–17 ciliated dikinetids, oblique ventral organelle (organelle 2) composed of 5–6 dikinetids with only 1 ciliated basal body each.

Morphogenesis (Figs. 16–20, 26). To avoid confusion, numbering of kineties is based on an average of 10 somatic rows as shown in Fig. 15.

Division starts with the appearance of a replication band in the posterior portion of the macronucleus. The oral primordium then originates apokinetally as an anarchic field of basal bodies on the cell surface distinctly posterior of somatic kinety 10, i.e. in a lateral to dorso-lateral position (like in *Tps. cylindrata*, thus not figured). Further development and differentiation of the adoral membranelles is also as described in *Tps. cylindrata*; however, the paroral membrane originates slightly later.

During differentiation of the adoral membranelles, the somatic kineties elongate slightly by intrakinetal proliferation of some basal bodies (first round; e.g. in somatic kineties 7–10 the basal body number increases by about 19%, n = 20). The proliferation usually commences in rows 6–8 (but this may be variable); sometimes kinety 10 elongates last. The rows then separate in their posterior third (Fig. 16). In rows 1–4 sometimes only 1 pair of basal bodies splits from the parental kineties (Figs. 16, 18). Starting in rows 6–10 of the opisthe, additional basal bodies proliferate at the anterior ends (second round; Figs. 17, 19). These new basal bodies originate as single, non-ciliated and closely spaced granules which move gradually posteriad whereby pairs of basal bodies and cilia are formed (Figs. 17, 19). Later, the anteriormost dikinetid of the opisthe's somatic rows develops 2 elongated cilia (Fig. 20).

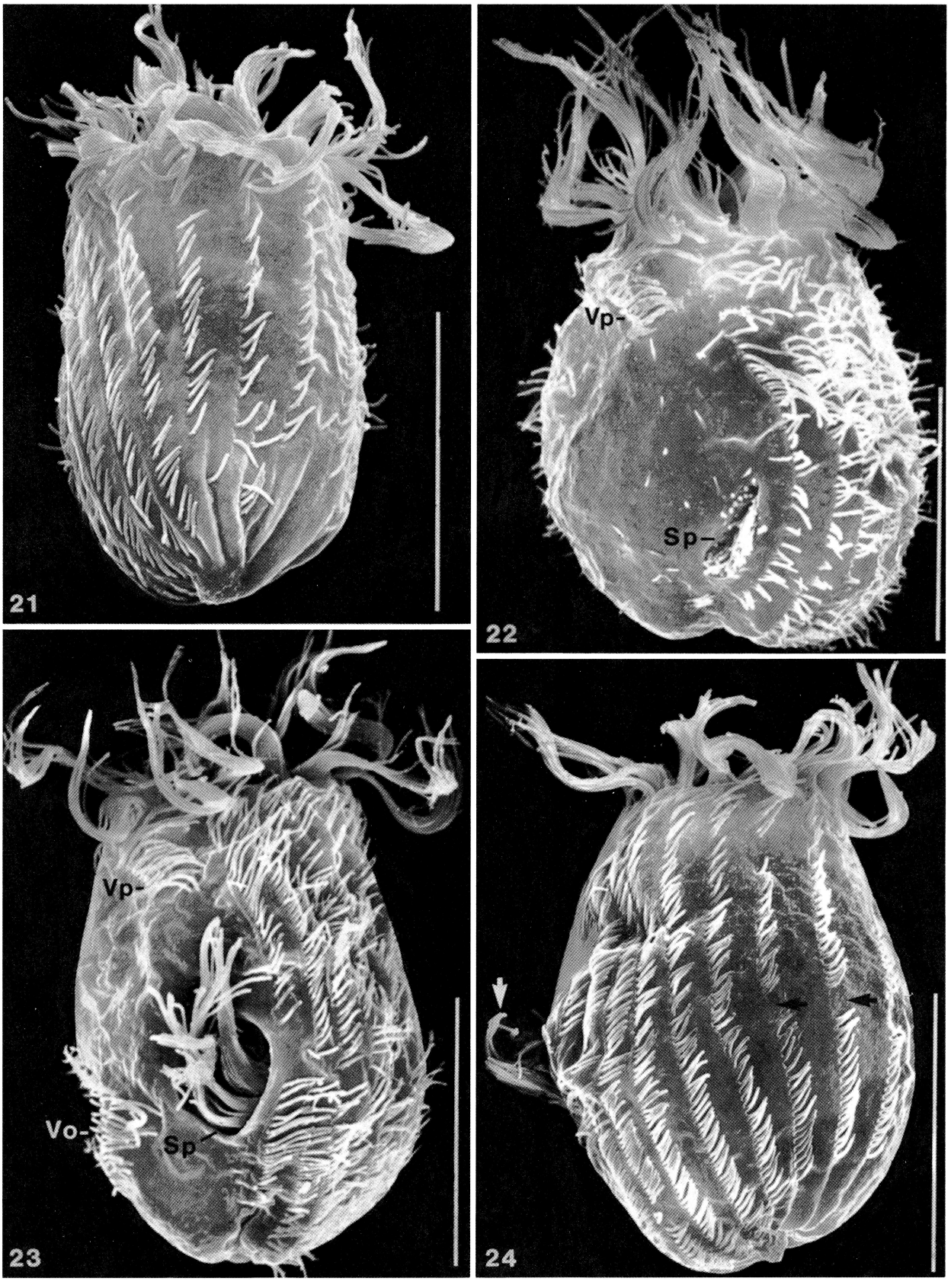
The ventral organelles and the paroral membrane form de novo at about the same time. The ventral organelles originate in the posterior third of the ventral side, i.e. left of the new kinety 1. Organelle 1 is first differentiated as a single row of few basal bodies (Fig. 16). The row elongates and some basal bodies soon become ciliated (Figs. 18, 26). Subsequently, the second organelle originates as a row of 5–6 basal bodies left of the first organelle (Fig. 19). Starting at the anterior ends of both organelles, pairs of basal bodies are formed. At this stage only the posterior basal bodies are ciliated. Later, all basal bodies of the first ventral organelle develop cilia (Fig. 20). Exactly the same mode of origin of the ventral organelles has been observed in *Tps. cylindrata* and *T. semiciliatum*.

Like in *Tps. cylindrata*, no signs of reorganization were observed in the parental oral and somatic ciliature. We did not, however, observe very late dividers.

Tintinnidium semiciliatum (Sterki, 1879) Kent, 1881 [3, 20, 32, 51]

Interphasic infraciliature (Figs. 2, 21). 12–15, usually 14, meridional somatic kineties of different length

◀ Figs. 15–20. Morphogenesis in *Tintinnidium pusillum* (protargol impregnation). Paroral membrane of proter not shown; ciliature depicted schematically. – Fig. 15. Diagram of interphasic infraciliature. – Figs. 16–18. Left and right lateral views (Figs. 17, 18 same specimen) of early dividers showing anlage of ventral organelle 1 and second round of basal body proliferation at anterior ends of opisthe's kineties 6–10. Last dikinetid of proter's somatic kineties sometimes non-ciliated (arrowhead); opisthe's ciliature completely depicted. – Figs. 19, 20. Right lateral views of middle dividers showing development and completion of ventral organelles and somatic kineties. All basal bodies are ciliated, except those formed at the anterior ends of the opisthe's kineties and in the posterior half of the ventral organelles (Fig. 19). Scale bar divisions = 10 μm. Op = oral primordium; Vo1, Vo2 = opisthe's ventral organelles; numbers denote somatic kineties.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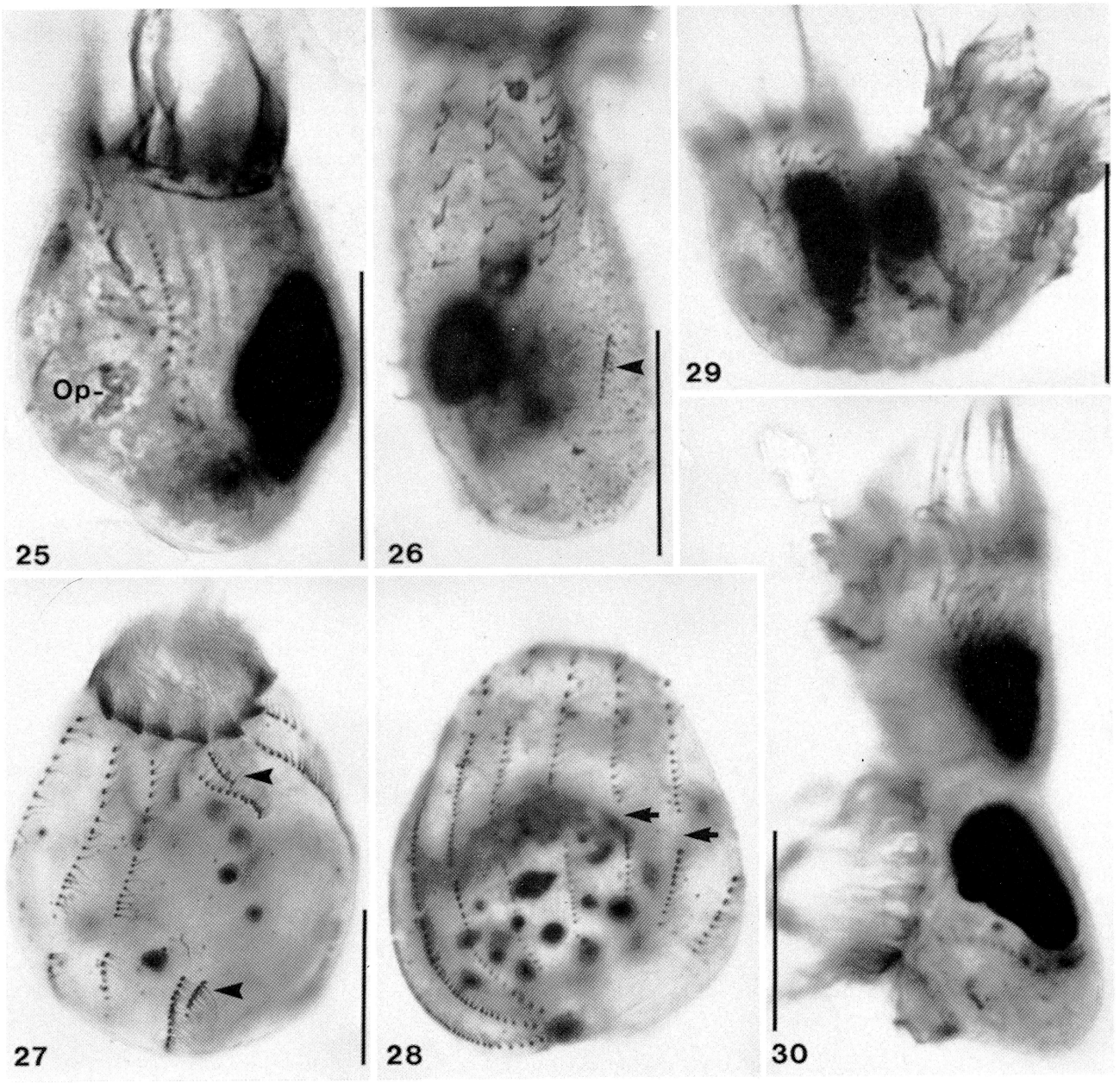


Figs. 25–30. Light micrographs of successive stages of the tintinnid morphogenesis from different species (protargol impregnation). – Fig. 25. Left lateral view of very early divider of *Tintinnopsis cylindrata* showing oral primordium composed of an anarchic field of basal bodies. – Fig. 26. Right lateral view of early divider of *Tintinnidium pusillum* showing anlage of ventral organelle 1 (arrowhead). – Figs. 27, 28. Ventral and dorsal view of middle divider of *T. semiciliatum* with fully differentiated ventral organelles (arrowheads) and separated somatic kineties (arrows). – Figs. 29, 30. Late (Fig. 30) and very late (Fig. 29) dividers of *Tps. cylindrata* showing daughters nearly at right angles (Fig. 30) and connected at their posterior portions (Fig. 29). Bars = 20 μm.

◀ Figs. 21–24. Morphology (Fig. 21) and morphogenesis (Figs. 22–24) in *Tintinnidium semiciliatum* (scanning electron micrographs). – Fig. 21. Dorsal view of interphasic specimen. – Fig. 22. Ventro-lateral view of early divider showing opening of subsurface pouch (Sp). – Fig. 23. Ventro-lateral view of middle divider with long, newly formed adoral cilia emerging from subsurface pouch. – Fig. 24. Dorsal view of middle divider showing increased number of somatic cilia due to intrakinetal proliferation of basal bodies. Black arrows mark separated somatic kineties, white arrow points to opisthe's adoral cilia. Bars = 20 μm. Vo, Vp = ventral organelle 1 of the opisthe and proter, respectively; Sp = subsurface pouch.

arranged in 2 groups: about 7 kineties to the left and about 7 to the right of ventral organelles; therefore, kinety-free area on ventral side. Somatic kineties consist of 4–7 dikinetids with long cilia (not always both basal bodies ciliated) and many monokinetids with short cilia. 14–15 adoral membranelles, 3 of them increasingly elongated and extending into oral cavity. 1 single-rowed paroral membrane. Transverse ventral organelle (organelle 1) composed of 8–19 ciliated dikinetids, oblique ventral organelle (organelle 2) composed of 6–9 dikinetids with only 1 ciliated basal body each (ventral organelles numbered incorrectly in [3]).

Morphogenesis (Figs. 22–24, 27, 28). This is very similar to that in *T. pusillum* and *Tps. cylindrata*; thus only selected stages are illustrated. *Tintinnidium semiciliatum* was studied also with the scanning electron microscope to demonstrate the subsurface development of the oral primordium and the intrakinetal proliferation of basal bodies.

Even early divisional stages show a narrow opening in the cortex containing the oral anlage, i.e. basal bodies with very short cilia (Fig. 22). The opening later widens and very long adoral cilia project (Fig. 23). The somatic kineties elongate slightly by intrakinetal proliferation of basal bodies (first round) and subsequently split in their posterior third (Figs. 23, 24, 27, 28). The ventral organelles originate de novo on the posterior third of the ventral side, i.e. left of the new kinety 1 (Fig. 27). We did not find very late dividers.

Codonella cratera (Leidy, 1877) Imhof, 1885 [20, 23, 30, 40]

Interphasic infraciliature (Figs. 4, 5, 31). 29–32 slightly spirally coursing somatic kineties of very different length, most composed of monokinetids. Sometimes, posteriormost basal body of kineties non-ciliated (cilium not impregnated?). Ventral kinety to the right of oral cavity composed of dikinetids, only posterior basal bodies with single, long cilium each. Conspicuous lateral field of kineties to the left of oral cavity, composed of about 16 closely spaced rows. One, very rarely 2, ventro-lateral kineties on posterior half of left-lateral side. Other kineties, i.e. numbers 2–10 and 27–32 each with ciliated dikinetid at anterior end. 14–17, usually 15, adoral membranelles, 3–4 of them slightly elongated and extending into oral cavity. 1 single-rowed paroral membrane; no ventral organelles.

Morphogenesis (Figs. 32–35). To avoid confusion, numbering of kineties is based on 32 somatic rows as shown in Fig. 31. As some processes are rather similar to those described in the other species, only selected stages are illustrated.

Division commences with the appearance of a replication band in the macronuclei. The oral primordium then originates apokinetally on the cell surface close behind the lateral ciliary field and to the left of kinety 26. Kinety 26 elongates and curves posteriad, lining the oral anlage at the right. This row, as well as rarely kinety 28, also elongates distinctly anteriad (Figs. 32, 33). Occasionally an anterior elongation of kinety 26 was observed in seemingly non-dividing specimens; possibly, these represent very early morphogenetic stages or post-dividers (proter).

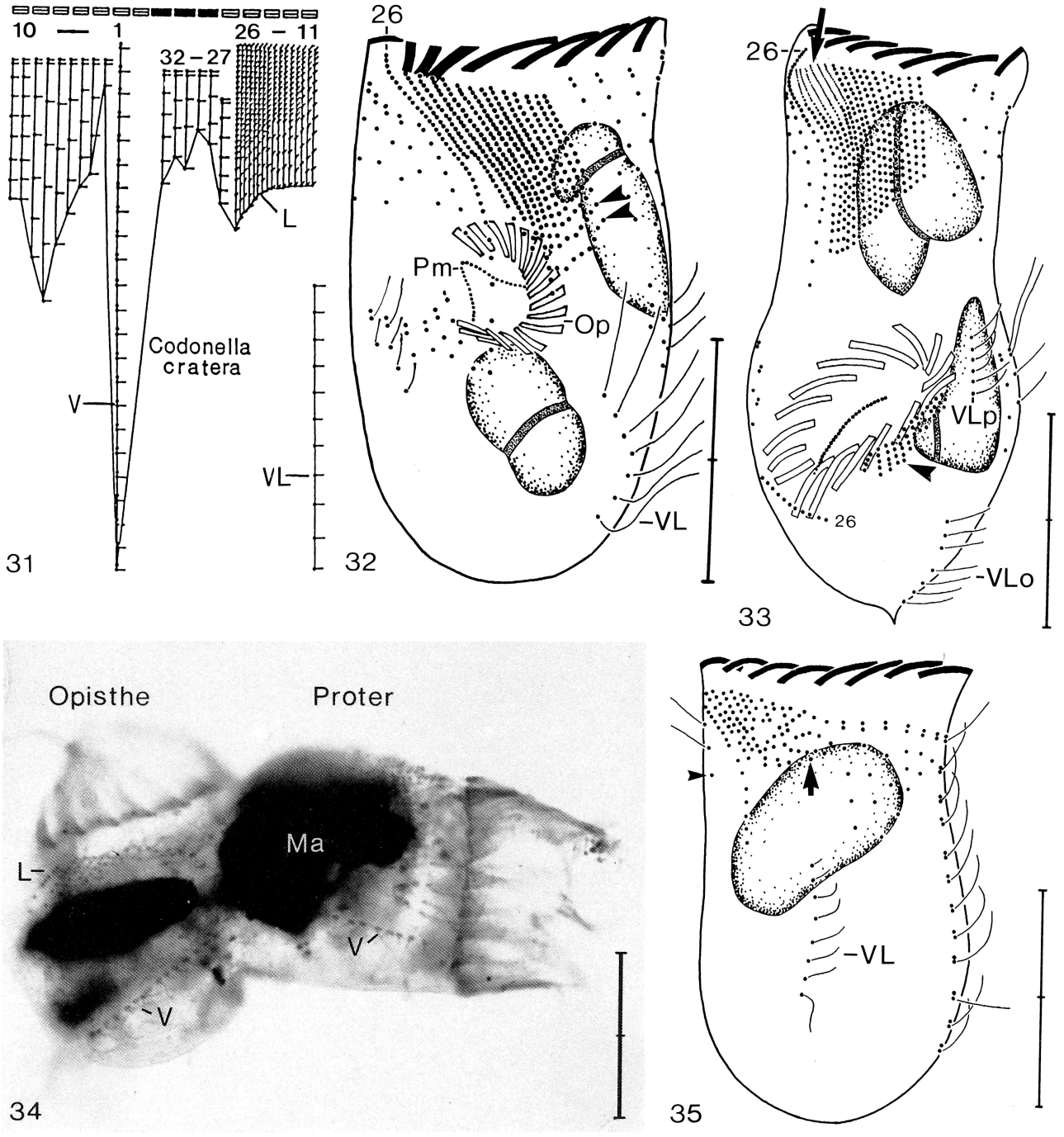
After the small anarchic field has moved into the cell and the adoral membranelles have differentiated short cilia, the paroral membrane forms de novo in the area enclosed by the membranelles. The somatic kineties elongate by intrakinetal proliferation of basal bodies (first round) which is indicated by non-ciliated granules appearing within the rows. Kineties 27–32 split slightly before rows 2–10 (Fig. 32). The posterior portions of these kineties move posteriad to become somatic anlagen of the opisthe. The faster development of the opisthe's rows 27–32, as compared to rows 2–10, is indicated by the early formation of dikinetids at their anterior ends (Fig. 32). Kinety 1 (ventral kinety), which is composed of dikinetids and extends from the adoral membranelles to the posterior end of the cell, separates in mid-body after the dikinetid number has increased slightly (Fig. 34).

Subsequently, the posterior ends of rows 11–25 loosen and a few basal bodies of each kinety migrate posteriad to form the anlage of the opisthe's lateral kinety field (Figs. 32, 33). The anteriormost membranelles of the oral primordium bend towards the elongated (buccal) adoral membranelles to form a small ring or very flat spiral.

During this process, kinety 26 splits and the posterior portion moves around the oral anlage to become the opisthe's kinety 26 (Figs. 32, 33). Subsequently, the diameter of the oral anlage increases distinctly by spreading of the membranelles which now have long cilia. Their bases, however, are still situated below the cell surface (Fig. 33). The very short kineties of the new lateral kinety field (rows 11–25), which already bear short cilia, are close to the left lower half of the oral membranellar ring (Fig. 33). The ventro-lateral kinety splits and the posterior portion moves back to the end of the cell; the anterior portion remains near mid-body (Fig. 33). Probably, basal bodies are now reorganized in the parental lateral kinety field because very densely spaced granules appear in several regions of these kineties, e.g. in the anterior portions of rows 20–25 (Fig. 33). The other parental somatic kineties and the oral structures are apparently not renewed.

When cytokinesis commences, macronuclear segments fuse and divide only once before cell division is accomplished. Thus, each daughter has only 1 macronucleus which divides into 2 segments after the cells have separated. The fission of the micronucleus could not be observed because it did not impregnate. During the single macronuclear stage an oblique fission furrow develops between the proter's posterior region and the opisthe's dorsal to right-lateral side (Fig. 34). This is rather similar to *Tps. cylindrata*. Likewise, immediately before separation the daughters are only connected at their posterior portions.

After cell division, the opisthe's very short kineties 11–25 (lateral kinety field) and the ventro-lateral row evidently achieve their final number of cilia by a second round of basal body proliferation. The new basal bodies of the ventro-lateral kinety originate at the anterior end, as



Figs. 31–35. Morphogenesis in *Codonella cratera* (protargol impregnation). Paroral membrane of proter not shown; ciliature depicted schematically. – Fig. 31. Diagram of interphasic infraciliature. – Fig. 32. Left lateral view of early divider. Basal bodies from parental lateral kinety field migrate posteriad to form anlagen for the opisthe's kinety field (arrowheads). All basal bodies of the proter are ciliated except, sometimes, a few at the posterior end of kinety 26; cilia of opisthe are growing. – Fig. 33. Left lateral view of middle divider showing anlagen for opisthe's kinety 26 and lateral kinety field (arrowhead); both have short cilia. Basal bodies are reorganized in parental lateral kinety field (arrow) and ventro-lateral kinety has divided. – Fig. 34. Light micrograph of very late divider showing longitudinal axes of proter and opisthe nearly at right angles. – Fig. 35. Left lateral view of newly formed daughter cell. A second round of basal body proliferation occurs in the lateral kinety field (arrow) and at the anterior end of the ventro-lateral kinety which show outgrowing cilia. Last basal body of somatic kineties sometimes non-ciliated (arrowhead). Scale bar divisions = 10 μm. L = lateral kinety field; Ma = macronucleus; Op = oral primordium; Pm = paroral membrane; V = ventral kinety; VL = ventro-lateral kinety; VLo, VLp = ventro-lateral kinety of opisthe and proter, respectively; numbers denote somatic kineties.

indicated by the gradual ciliation of basal bodies (Fig. 35).

Discussion

Nuclear Division and Cortical Morphogenesis

Our observations on nuclear division in binucleate species, viz. *C. cratera*, agree with those of Entz [12], Hofker [28] and Schweyer [46]. The macronuclei fuse during middle morphogenetic stages and divide once just before proter and opisthe separate. The binucleate condition is achieved by a second round of macronuclear division after the daughters have separated. Biernacka [1] and Laackmann [36], in contrast, observed 3 macronuclear segments before cell separation, viz. 2 segments in the proter and 1 in the opisthe, in *Codonella subacuta*, *Tps. lohmanni*, *Tps. meuneri*, *Tps. campanula* and *Cyttarocylis helix*. This could be caused by a premature second division of the proter's single macronucleus before the cells have disconnected as suggested by Biernacka's [1] Fig. 39 from *Tps. lohmanni*. In three other tintinnids (*Tps. acuminata*, *Tps. levigata*, *Stylicauda platensis*), Coats & Heinbokel [9] observed the second macronuclear division concurrently with cytokinesis. These results suggest that macronuclear division is different in various species. However, data are rather weak. Campbell [7], for instance, obviously missed the fusion of the macronuclear segments, as is apparent from the figures on his plate 22. As concerns the mononucleate species, our observations correspond with the few published data available [12, 14].

Earlier investigations on cortical morphogenesis of tintinnids were performed without using silver impregnation and merely show the development of the adoral membranelles and gross figures of dividers [1, 6, 7, 9, 12, 14, 24, 28, 36, 37, 45, 46]. Some of these observations do not agree with our data. According to Entz [12] the oral primordium of *Favella ehrenbergii* (alias *Cyttarocylis ehrenbergii*) and *Tps. campanula* develops on the dorsal side. In our species and in those studied by others [6, 9, 28, 37] it originates on the ventral or lateral surface.

The oral apparatus of the tintinnids and other oligotrichs (for literature see [43]) develops apokinetally (Figs. 8, 17, 25, 32), as also very briefly mentioned by Brownlee [5]. The enigmatic ventral organelles were first found in freshwater tintinnids [3, 23] and later in some marine species, e.g. *Tintinnidium mucicola* [39] and, possibly, *Nolaclusilis bicornis* (see terminology). These organelles develop de novo just as the adoral membranelles and the paroral membrane. Thus, they might be part of the oral structures since the somatic ciliature develops without special primordia, i.e. by intrakinetal proliferation.

The intrakinetal origin of the somatic infraciliature in tintinnids and strobilidiids supports the sister group relationship suggested by Petz & Foissner [43]. In contrast, the somatic infraciliature develops de novo, i.e. between the parental somatic kineties or in a subsurface tube in halteriids and very likely also in strombidiids. The proposed sister group relationship is sustained by the very early invagination of the tintinnid and strobilidiid oral primordium (Fig. 22; Figs. 6, 16 in [43]), whereas the halteriid oral apparatus develops entirely on the cell surface or in a tubular pouch in freshwater strombidiids [43].

It is noteworthy that basal bodies are added intrakinetally at the anterior (opisthe; e.g. *T. pusillum*, *C. cratera*) or posterior (proter; e.g. *Tps. cylindrata*) ends of the somatic kineties. The significance of this finding is not yet clear. This second round of basal body proliferation occurs in *Tintinnidium* and *Tintinnopsis* during cytokinesis. In *C. cratera*, however, it takes place after cell division. This was also observed by Brownlee [5] in *Favella* spp. and *Tintinnopsis* sp.

We could not observe any reorganization of the somatic and oral infraciliature in *Tintinnidium* and *Tintinnopsis*. In *C. cratera*, parental basal bodies are apparently renewed in the lateral kinety field. The other parts of the infraciliature are obviously not reorganized.

Is the Tintinnid Division Enantiotropic?

Fauré-Fremiet [17] introduced the term "enantiotropic" to characterize the inverted polarity of the oligotrich daughter cells, specifically the middle stages of the halteriid morphogenesis, where the daughters' adoral zones of membranelles are opposed (Fig. 36). He provided no strict definition but mentioned that the axes of proter and opisthe may be at right angles in other stages; Fauré-Fremiet also compared the enantiotropic division with the more common homeotropic (homothetogenic) fission, where proter and opisthe have the same orientation (Fig. 36).

According to Corliss [10], the enantiotropic division "involves a condition of inverse homothety and shifting body axes via pronounced morphogenetic movements during stomatogenesis". Following this more concise definition the division of the tintinnids is enantiotropic: there is a pronounced shifting of the body axes causing proter and opisthe to be connected with their posterior dorsal portions, i.e. showing "a condition of inverse homothety" (Figs. 13, 14, 29, 30, 34). In our species, however, there is no stage where the daughters are as distinctly opposed as in the middle stages of the halteriid morphogenesis. Thus, the enantiotropy would seem to be less pronounced in tintinnids although there are figures in the literature indicating that the daughters' axes in other tintinnid species are as strongly shifted as in *Halteria* [24, 25, 45]. Some conflicting findings exist. Biernacka [1], Coats & Heinbokel [9] and Schweyer [46] in tintinnids, as well as Kormos & Kormos [35] in *Strobilidium*, reported very late dividers with almost corresponding axes, i.e. the proter is connected with a short stalk to the anterior dorso-lateral surface of the opisthe (Fig. 36) slightly resembling our Fig. 30. We cannot reject these observations entirely, as we found only dividers outside their lorica, which is usually deserted during the samples' transport to the laboratory. Cell division is probably modified by this. However, Campbell [6] observed posteriorly connected very late dividers of *Tps. nucula* within the lorica.

An Improved Characterization of Oligotrich Ciliates

Several definitions of the oligotrichs are available [10, 44, 47]. However, all are rather incomplete or vague, e.g. that of Small & Lynn [47], when they erected the superfluous subclass Choreotrichia for the oligotrich ciliates: "Body generally conical or bell-shaped; body cilia poorly developed; oral cilia make almost complete circle of polykinetids around broad end, used to locomote and feed (some are raptors); most are planktonic".

We could not discern a unique character which defines the oligotrichs as a monophyletic group. Even the enantiotropic cell division suggested by Petz & Foissner [43] is not unique among the ciliates because it occurs also in peritrichs and in the prostomatid *Pseudobalanion*. Very late dividers of these are, like oligotrichs, connected with

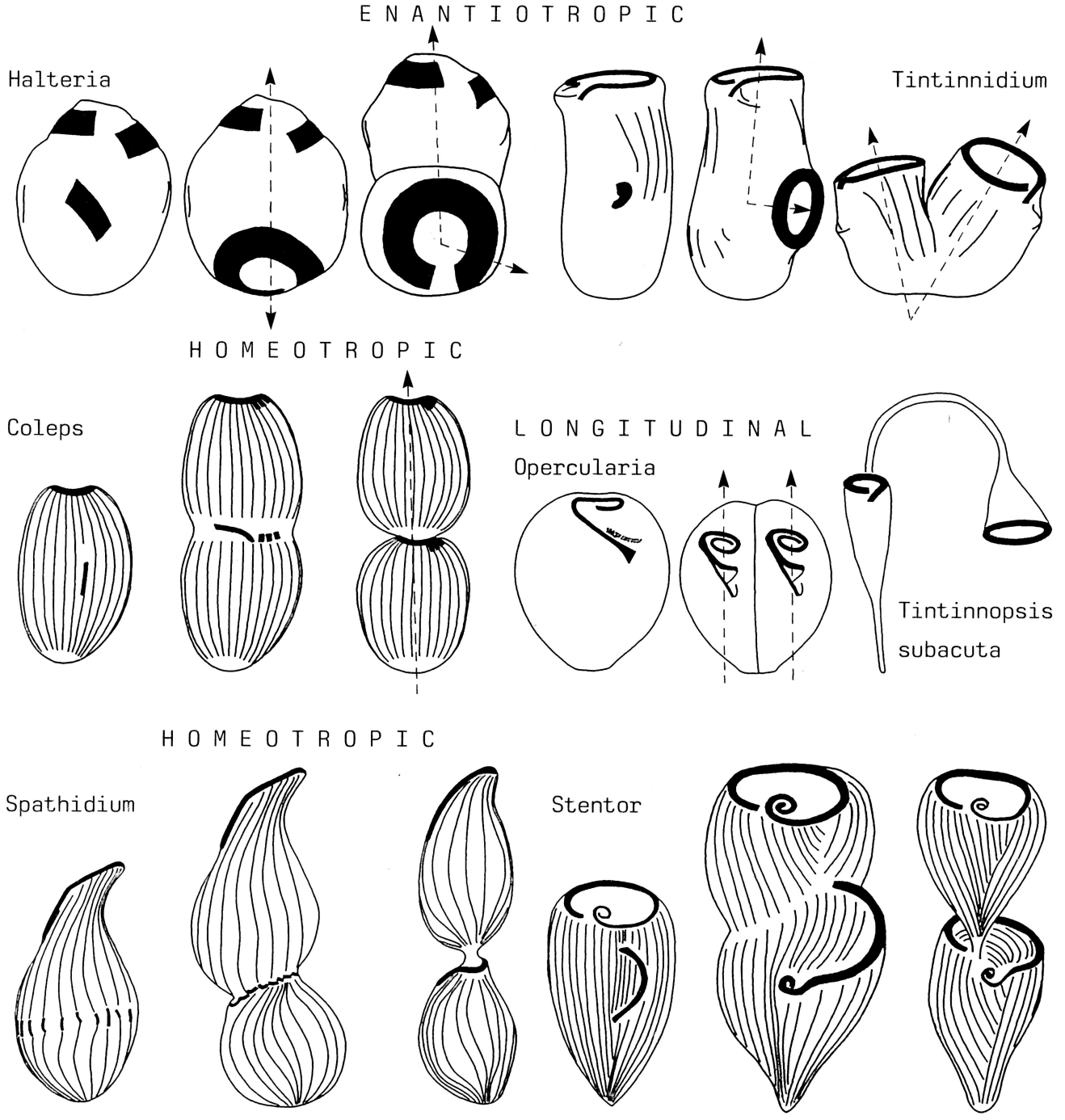


Fig. 36. Division modes and orientation of daughters (broken arrows) in ciliates with a polar oral apparatus (strongly schematized after [1, 2, 17, 18, 29, 52]).

their posterior portions [18, 21]. However, both lack a macronuclear replication band, and peritrich ciliates divide longitudinally, causing parallel daughter axes (Fig. 36). *Pseudobalanion* has a telokinetal stomatogenesis and lacks adoral membranelles [21]. This suggests that the enantiotropic cell division evolved convergently in oligotrichs, peritrichs and *Pseudobalanion*. Thus, this special kind of cell division is still the best apomorphy for the oligotrichs, although weakened by some incompatible data for tintinnids (see above). Furthermore, oligotrichs have a unique character *combination*, viz. the polar oral apparatus *and* the apokinetal origin of the oral primordium. Such a combination is not known for any other ciliate. There are apparently only two ciliate groups which develop their oral primordium apokinetally, viz. all oligotrichs and some hypotrichs, supporting other evidence that these groups are closely related [e.g. 39, 42].

A further highly characteristic feature of the oligotrichs is the early completion of the opisthe's oral structures during stomatogenesis and their restriction to a small region in or below mid-body, which later becomes the apical pole of the cell. This restriction of the daughter's completed oral primordium to a limited area of the cell is very likely the actual reason for the enantiotropic cell division and also causes a slightly oblique division furrow. Such a conclusion seems justified considering other ciliates with a polar oral apparatus, where the newly formed oral structures encircle the perimeter of the cell in mid-body and the division plane is thus transverse (Fig. 36).

Based on our present and earlier investigations [43], a considerably improved characterization of the oligotrichs is possible: Conical or bell-shaped ciliates with polar oral apparatus also used for locomotion. Stomatogenesis apokinetal; newly formed oral apparatus restricted to small area in or below mid-body, which later becomes anterior cell pole. Division enantiotropic. This characterization contains no ultrastructural data simply because none have been published [44, 47].

Classification

Only few tintinnids have been studied using silver impregnation. Their classification is thus still based exclusively on lorica morphology and many families have been suggested [10, 47]. This is, however, rather arbitrary; *Favella ehrenbergii* produces at least three differently shaped and structured loricae during its life cycle; these were previously considered to be separate species from different families [37].

According to the sparse infraciliary data available, two groups of tintinnids can be distinguished: one with ventral organelles (e.g., *Tps. cylindrata* [23], *Tintinnidium* spp. [3, 23, 39]) and another without (e.g., *C. cratera* [23], *Stenosemella lacustris* [22], *Tps. baltica* [39], *Tps. subacuta* [47], *Tps. bütschlii*, *Tps. campanula* and *Tps. ventricosa* [16]). This is in rough accordance with current systematic schemes [10, 47] separating codonellids (*Codonella*, *Tintinnopsis*) and tintinnidiids (*Tintinnidium*) at familial level. Further groups (families?) might be constituted by genera like *Favella*, *Eutintinnus*, *Amphorellopsis* and *Salpingacantha* [39, 47].

The lorica of the type species of *Codonella*, *C. galea* [27, 34], is very similar in shape and structure to that of *C. cratera* [23, 40]. It is thus reasonable to assume congenerity. As only *C. cratera* is well known, its infraciliature might be taken as representative for the genus. We therefore reject the suggestion of Laval-Peuto & Brownlee [39] to transfer *C. cratera* to *Tintinnopsis*. This is sustained by the differently shaped, i.e. tubular, lorica of *Tps. beroidea*, type species of *Tintinnopsis* [34, 50]. Based on this argumentation *Tps. baltica* and *Tps. subacuta*, both having the typical codonellid lateral kinety field and lorica shape [39, 47], should obviously be transferred to *Codonella: C. baltica* (Brandt, 1896) [4] n. comb. and *C. subacuta* (Jörgensen, 1899) [31] n. comb. Very likely *Tps. campanula, Tps. bütschlii* and *Tps. ventricosa* belong to *Codonella,* too [16]; however, silver impregnated specimens should be investigated before a final decision is made.

We agree with earlier suggestions [23, 39] that *Tps. cylindrata* belongs to the Tintinnidiidae. Whether *Tps. cylindrata* should be included in the genus *Tintinnidium* on account of its very similar infraciliature and morphogenesis must await a reinvestigation of the type species, *Tps. beroidea*.

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