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Divisional Morphogenesis in *Amphisiellides illuvialis* N. Sp., *Paramphisiella caudata* (Hemberger) and *Hemiamphisiella terricola* Foissner, and Redefinition of the Amphisiellidae (Ciliophora, Hypotrichida)

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ABSTRACT. Classification of hypotrich ciliates is bewildering, possibly due to many unrecognized convergencies and the lack of detailed ontogenetic data in most species. A puzzling case are hypotrichs which have an obliquely extending "median cirral row" on the ventral surface between the right and left marginal cirral row. Such species are often assigned to the poorly defined family Amphisiellidae. However, we show by a comparative analysis of the divisional morphogenesis of three amphisiellid morphotypes and by a reevaluation of literature data that a median cirral row can be formed by at least four non-homologous processes. These data are used to define the "amphisiellid median cirral row" (a row containing all or most cirri from at least two rightmost anlagen, arranged one behind the other during cytokinesis), to redefine the Amphisiellidae (Euhypotrichina with an amphisiellid median cirral row of which the anterior segment is formed by cirri of the rightmost ventral anlage and the posterior segment by cirri of the second ventral anlage from right; a middle segment may be formed by neighboring anlagen), and to improve the diagnoses of the seven genera assigned by us to this family. Attempts to reconstruct the evolution within the Amphisiellidae failed, in spite of the detailed morphological and ontogenetic data available. We thus conclude that such data, although highly valuable, are insufficient to light up the supergeneric phylogeny of hypotrich ciliates. For this, molecular markers and investigations of the morphogenetic processes at electron microscopic level appear indispensable.

Supplementary key words. *Amphisiella, Gastrostyla, Lamtostyla, Paragastrostyla,* phylogeny, *Pseudouroleptus, Tachysoma,* taxonomy.

MANY hypotrichs have an obliquely extending cirral row on the ventral surface between the right and left marginal row. Using ontogenetic data, Borror & Wicklow recognized that this "median cirral row" is of different origin in several species, despite the superficial interphase similarity of such hypotrichs (Borror, A. C. & Wicklow, B. J. 1982. Non-homology of median rows in hypotrichs with only three longitudinal rows of cirri. *J. Protozool.*, **29**:285A). However, data were too scarce and bewildering for a conclusive interpretation, i.e. for establishing higher taxa. In spite of this, Small & Lynn [23], having hardly more data, installed the new family Amphisiellidae. Their definition "at least 1 or more frontoventral cirral files extends well past mid-ventrum" applies to almost any hypotrich taxon. Furthermore, their new family is a junior synonym of Jankowski's [21] Amphisiellidae and includes genera, e.g. *Kahliella* and *Onychodromus,* which clearly belong to other families.

A wealth of data on hypotrichs with a median cirral row has become available since Borror and Wicklow's (1982 abstract) classic formulation of the problem [3, 9, 27, 32]. These findings and those presented in our paper provide a firm basis for a comprehensive redefinition of the Amphisiellidae.

MATERIALS AND METHODS

Amphisiellides illuvialis n. sp. was discovered 7 December 1991 in a litter sample from a disused pigpen in village of Schrötten, Deutsch Goritz, Austria. A second population (Fig. 21–24) was found near Eching (Bavaria) in a tiny track puddle, which was heavily polluted by sewage from an activated sludge plant. This population was very similar to the type material and was not studied in detail.

Paramphisiella caudata (Hemberger, 1985) was found on the Shimba Hills near Mombasa (Kenya, Africa) in July 1985. It was isolated from a sandy soil collected near a small river in the vicinity of the Sheldrick waterfalls.

Hemiamphisiella terricola Foissner, 1988 was found in February 1987 in mosses covering the soil of an autochthonous pine forest (*Callitris* sp.) near Adelaide (Tailem Bend; Australia).

All species were isolated from raw cultures set up with the non-flooded Petri dish method [13]. Pure cultures were started with several individuals in lettuce-medium enriched with some squeezed wheat grains. Body shapes of living specimens were drawn from slides without coverslip. Details were studied on slightly to heavily squeezed individuals using an oil immersion objective and interference contrast optics. The infraciliature was revealed by Foissner's [16] protargol protocol. Drawings were made with the help of a camera lucida.

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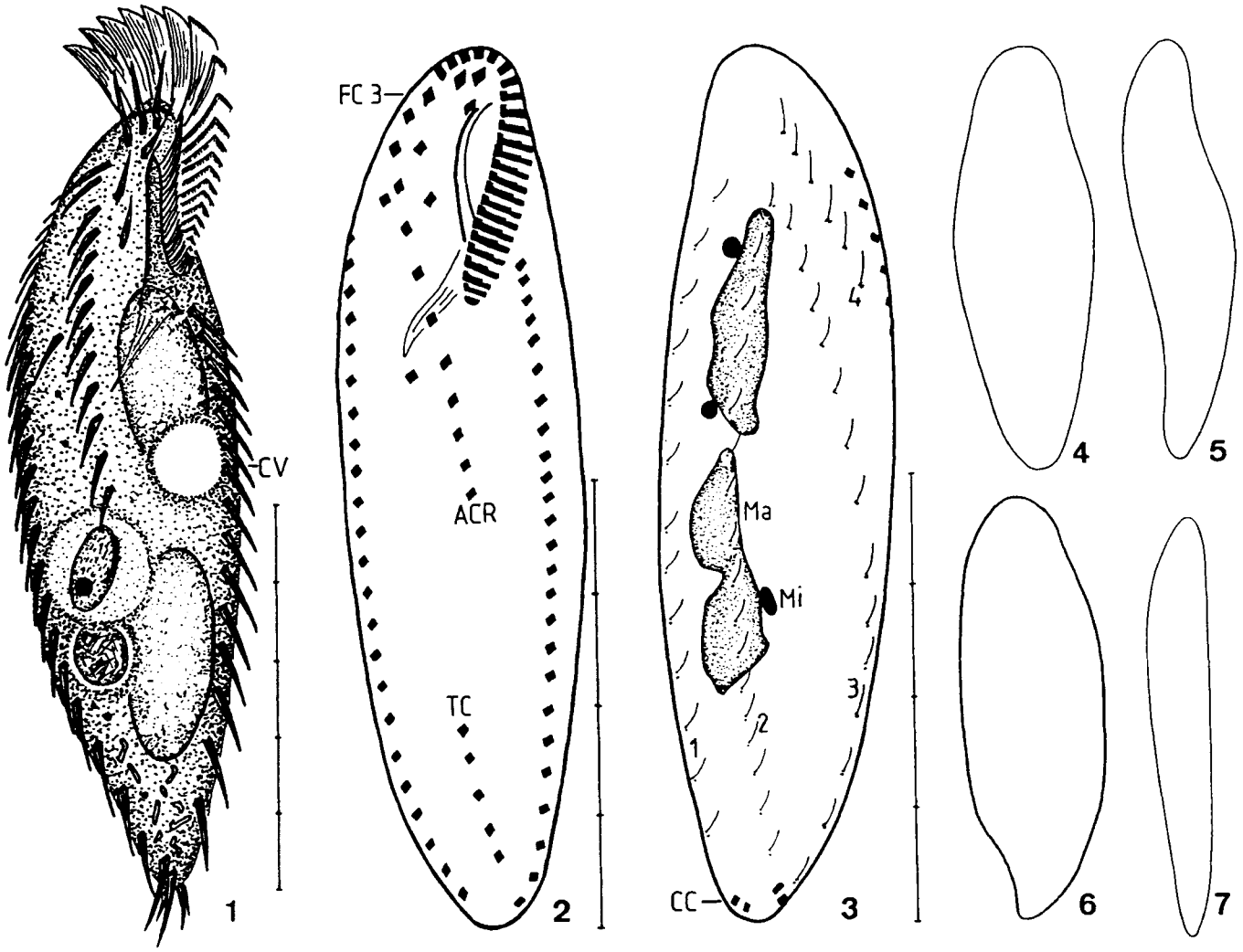

Fig. 1–7. Interphase morphology of *Amphisiellides illuvialis* n. sp. from life (**1**, **4–7**) and after protargol impregnation (**2**, **3**). **1**. Ventral view of typical specimen. **2**, **3**. Infraciliature of ventral (cp. Fig. 21) and dorsal side. Numbers in Fig. 3 denote dorsal kineties. **4–6**. Ventral views of more rarely occurring body shapes. **7**. Lateral view. Scale bar division = 10 μm. ACR, amphisiellid cirral row; CC, caudal cirri; CV, contractile vacuole; FC 3, frontal cirrus 3; Ma, macronuclear segment; Mi, micronucleus; TC, transverse cirri.

To illustrate the changes during morphogenetical processes, old (parental) cirri are depicted by contour, whereas new cirri are shaded black. Statistical procedures are according to [24].

Terminology is according to [7]. We have, however, introduced the new terms "amphisiellid median cirral row" (ACR) and "discocephalid median cirral row" (DCR) to distinguish both from the median cirral row of other hypotrichs. The amphisiellid cirral row is defined as containing all or most cirri, i.e. complete cirral rows and/or cirral row fragments, from at least two rightmost anlagen, arranged one behind the other during and shortly after cytokinesis. It usually extends obliquely from the right anterior ventral surface to the posterior half of the cell (Fig. 45, 46, 52, 53, 61–67). In contrast to the ACR, the discocephalid cirral row is composed of single cirri, each originating from a different ventral anlage (Fig. 60). Another new term is "postperistomial cirrus." It designates a curious isolated cirrus close beneath the peristomial vertex. The postperistomial cirrus is produced from the middle segment of the ACR, i.e. from the third anlage from right, and migrates from the frontal area to the peristomial vertex (Fig. 45, 46, dotted arrows; 49, 50, 53, arrows; 65–67, white arrows). This cirrus is rather variable; it is absent in a few individuals while others have two or three.

RESULTS

Amphisiellides illuvialis n. sp.

(Fig. 1–24; Table 1)

Diagnosis. Lanceolate, in vivo 50–140 × 20–50 μm. 2 macronuclear segments, 25 adoral membranelles, 1 buccal cirrus at anterior end of paroral membrane, 2 cirri right and 3 cirri left of amphisiellid cirral row, 2 transverse cirri, and 3 caudal cirri on average.

Derivatio nominis. "illuvies" (Lat.), dirty water.

Type location. Village of Schrötten, Deutsch Goritz, Austria, 46° 47′ N, 15° 49′ E, 320 m alt.

Type specimens. A holotype and a paratype of *A. illuvialis* mounted as two slides of protargol-impregnated cells have been deposited in the collection of microscope slides of the Oberösterreichische Landesmuseum in Linz. Accession numbers: 10, 11/1994.

Description (Fig. 1–11, 21). Size and shape highly variable,

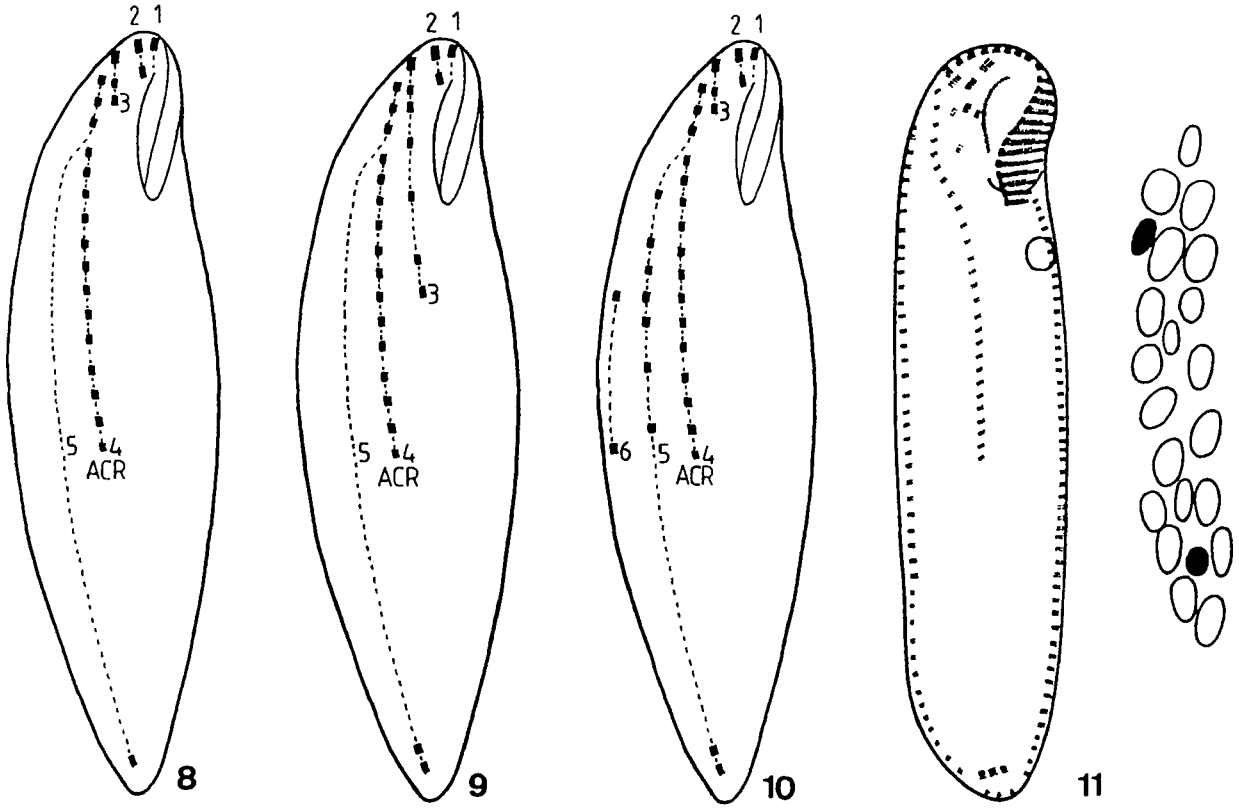

Fig. 8–10. Variability of cirral pattern in *Amphisiellides illuvialis* n. sp. after protargol impregnation (cp. Fig. 21). Numbers denote anlagen from which cirral rows originate. **8.** No cirral row right or left of amphisiellid cirral row (ACR). **9.** Anlage 3 produces a cirral row left of the ACR. **10.** Anlage 5 produces a cirral row right of the ACR. Some cirri are produced by an occasionally occurring 6th anlage. **11.** Ventral infraciliature and nuclear apparatus (right) of *Amphisiellides atypicus* (Hemberger, 1985) (from [19]).

typical cells in vivo about 120 × 30 μm and lanceolate, i.e. distinctly tapering anteriorly and posteriorly (Fig. 1); shapes shown in Fig. 4–6 occur more rarely. Dorso-ventrally flattened about 2:1 (Fig. 7); highly flexible. Two ellipsoid macronuclear segments in left body half, shape and size highly variable (Fig. 3, 21); nucleoli in vivo 1.5–2.5 μm in diameter. Several micronuclei (2–3.5 μm) adjacent to macronuclear segments, only faintly stained by protargol. Contractile vacuole slightly above midbody at left body margin. Cytoplasm colorless, yellowish crystals about 4 μm long mainly in posterior portion. Food vacuoles 10–20 μm in diameter, contain small ciliates and bacteria. No conspicuous cortical granules.

Adoral zone of membranelles about 30% of body length. Bases of membranelles in vivo 8 μm, cilia of membranelles 13 μm, all cirri about 10 μm long. Buccal cavity inconspicuous, i.e. flat and narrow; oral lip slightly curved, parallels undulating membranes (Fig. 1, 2, 21). Three slightly enlarged frontal cirri, right frontal cirrus at distal end of adoral zone of membranelles. Amphisiellid cirral row (ACR) commences at right frontal cirrus and terminates near center of ventral surface. In about 45% of cells 1–2 cirri occur right of the ACR (Fig. 1, 2, 21); and they also lack in about 45% (Fig. 8); many cirri right of the ACR forming a row occur in about 10% (Fig. 10, anlage 5); occasionally, a sixth anlage develops 2–4 cirri (Fig. 10, anlage 6). Usually 2–3 cirri left of ACR (Fig. 8, 10, 21); numerous cirri left of the ACR forming a row occur in about 3% of cells (Fig. 9, anlage 3). One buccal cirrus (rarely two) at anterior end of paroral membrane. Transverse cirri arranged longitudinally in single row containing usually two (Fig. 1, 21), rarely up to five cirri (Fig. 2); rarely they arrange in two rows (Fig. 13). Right marginal row commences on dorsal surface distinctly below anterior end of body; both marginal rows extend to posterior end of cell, leaving small gap.

Dorsal cilia 3–4 μm long, arranged in three rows almost as long as cell; one short kinety on right anterior surface (Fig. 3), occasionally accompanied by faintly stained second kinety from previous generation. Caudal cirri usually at posterior end of kineties 1 and 2.

Comparison with related species. *Amphisiellides illuvialis* differs from the sole congener, *Amphisiellides atypicus* (Hemberger, 1985) Foissner, 1988, mainly in the number of macronuclear segments (2 versus 23; Fig. 11). Further differences relate to the arrangement of the transverse cirri (transverse in type species, longitudinal in *A. illuvialis*), the smaller body size (100 μm versus 200 μm) and the fewer cirri in the ventral and marginal rows of *A. illuvialis* as well as in the morphogenesis (see Discussion). Live specimens of *A. illuvialis* are difficult to distinguish from other binucleate amphisiellid hypotrichs, viz. *Amphisiella binucleata* (Hemberger, 1985) Foissner, 1988 (buccal cirrus in posterior half of paroral membrane, no caudal cirri), *Amphisiella australis* Blatterer & Foissner, 1988 (no caudal cirri), *Amphisiella terricola* Gellért, 1955 (redescription [12]; transverse cirri obliquely arranged, no caudal cirri), *Amphisiella magnigranulosa* Foissner, 1988 (conspicuous cortical granules, buccal cirrus in posterior half of paroral membrane, transverse cirri obliquely arranged, no caudal cirri) and *Amphisiella polycirrata* Berger & Foissner, 1989 (conspicuous row of buccal cirri). Likewise, distinction from binucleate species of the genus *Gastrostyla* [22] may be difficult. They differ from *A. illuvialis* mainly by their obliquely arranged transverse cirri (longitudinally in *A.*

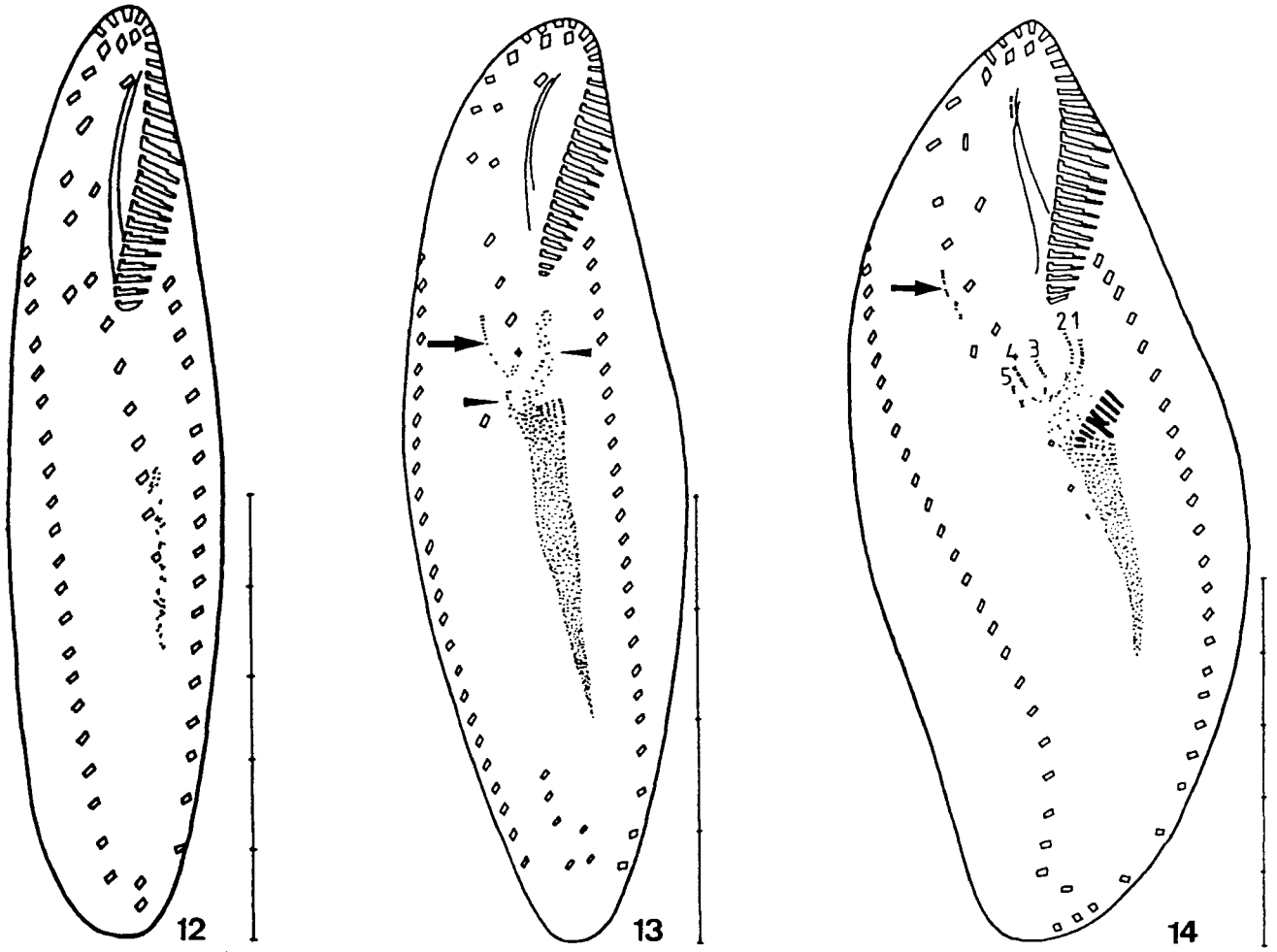

Fig. 12–14. Ventral views of early dividers of *Amphisiellides illuvialis* n. sp. after protargol impregnation. **12.** Morphogenesis commences with a proliferation of basal bodies near the posterior cirri of the amphisiellid cirral row (cp. Fig. 22). **13.** Two streaks extend from the enlarged oral primordium (arrowheads). One streak (arrow) develops from cirri of the ACR or, less likely, de novo. **14.** Five anlagen (numbers 1–5) for the cirri of the opisthe are recognizable. The cirral primordium right of the amphisiellid cirral row appears nearly unchanged (arrow). The parental buccal cirrus disorganizes. Scale bar division = 10 μm.

illuvialis) and the postperistomial cirrus. Protargol impregnation should thus be used for determining binucleate hypotrichs with a more or less distinct median cirral row.

Divisional morphogenesis (Fig. 12–20, 22–24). The nuclear apparatus and the marginal rows divide in the usual way and thus demand no further comment (Fig. 16–18).

Stage 1: (Fig. 12, 22) Stomatogenesis commences with the proliferation of basal bodies near the posterior cirri of the ACR which, however, appears unchanged.

Stage 2: (Fig. 13) A large, wedge-shaped field of basal bodies develops in the middle portion of the ventral surface left of the ACR; most probably cirri from the posterior segment of the ACR disaggregate and contribute to this field. Membranelles differentiate at the left anterior end of the oral primordium. One long and one very short streak originate from the right anterior end of the oral primordium and extend left and right of the ACR, respectively (Fig. 13, arrowheads). A third streak develops right of the ACR from a disaggregated cirrus of the ACR or, possibly, de novo (Fig. 13, arrow).

Stage 3: (Fig. 14) The formation of membranelles in the oral primordium proceeds posteriorly. Five cirral anlagen for the opisthe are recognizable. The cirral primordium right of the ACR appears nearly unchanged (Fig. 14, arrow). The parental buccal cirrus disorganizes to a short streak of basal bodies.

Stage 4: (Fig. 15–17) Five anlagen each are recognizable in the proter and opisthe. The anlagen of the proter develop slightly later than those of the opisthe. All anlagen of the opisthe derive from the oral primordium, whereas those of the proter evolve from parental frontoventral cirri, with the possible exception of anlage 5 (see stage 2). Anlage 1 of the proter is generated by the parental undulating membranes; anlage 2 by the buccal cirrus; anlage 3 by cirri left of the ACR; anlagen 4 and 5 by the posterior (left) segment of the ACR, or (anlage 5), possibly, de novo (Fig. 15, 16). Some cirri may develop between the posterior portions of anlagen 4 and 5 (Fig. 16, inset), possibly forming the second row of transverse cirri found in about 3% of cells (Fig. 13).

Dorsal kineties 1 to 3 and the caudal cirri develop within the parental kineties. Dorsal kinety 4 (Fig. 17, triangle) is produced by an anlage which develops within the right marginal row (Fig. 16, arrowheads).

Stage 5: (Fig. 18–19, 23) The new adoral zone of membranelles is almost complete. The anlagen of the proter and opisthe organize in the same manner (Fig. 16, 18, 20). Some posterior cirri of anlagen 2–5 are usually resorbed during cytokinesis; thus,

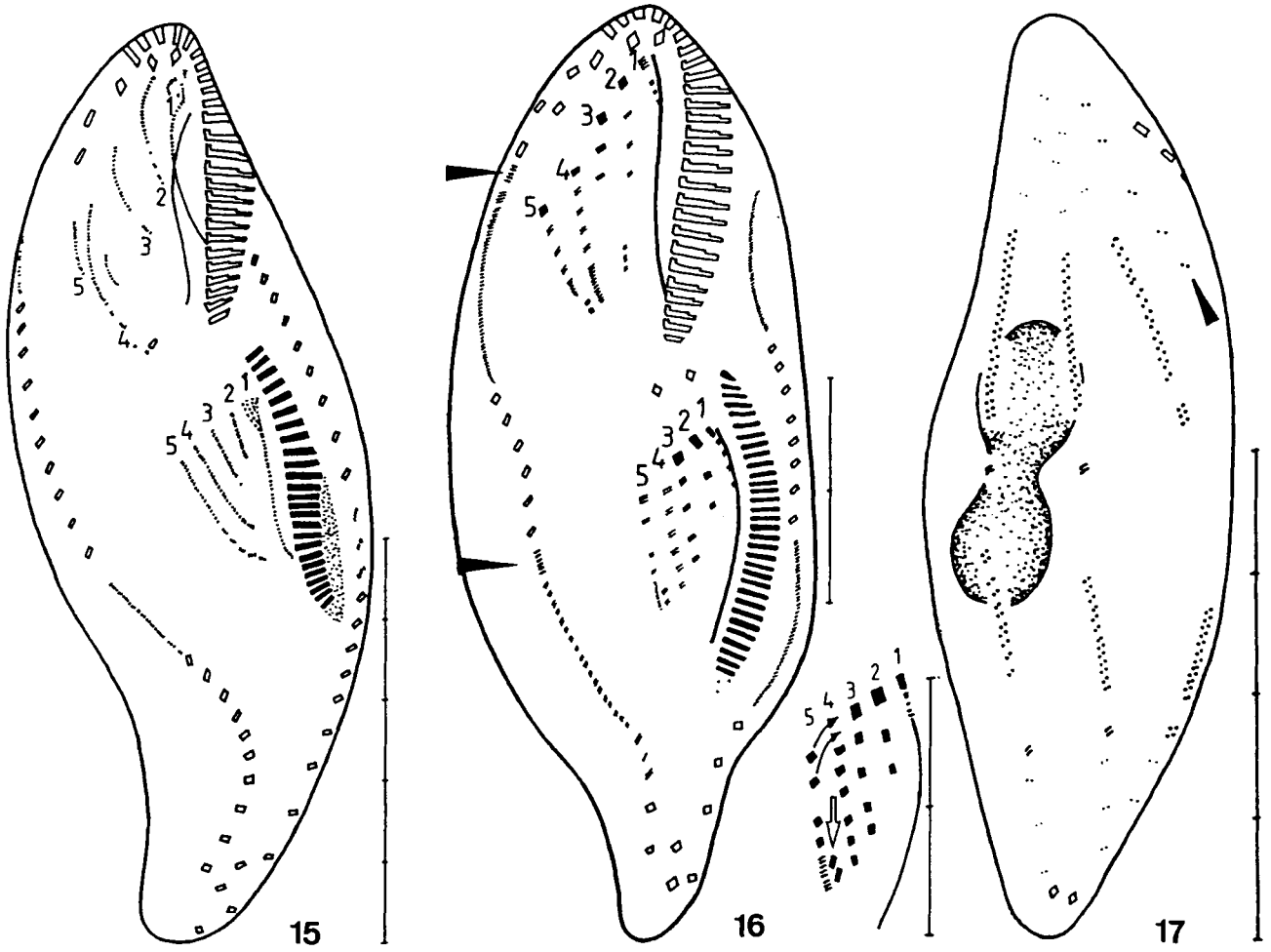

Fig. 15–17. Middle dividers of *Amphisiellides illuvialis* n. sp. after protargol impregnation. Numbers in figures denote cirral anlagen. **15.** Five ventral anlagen each are recognizable in proter and opisthe. Anlagen develop also within marginal rows. **16.** Anlage 1 splits longitudinally to form the first frontal cirrus and the undulating membranes. Arrowheads mark anlagen for dorsal kinety 4. The inset shows the migration of the anterior cirri of anlage 5 (paired arrows). It also shows two cirri (white arrow) between anlagen 4 and 5, which possibly form a second row of transverse cirri (cp. Fig. 13). **17.** Anlagen development in dorsal kineties. The arrowhead marks parental kinety 4 which originates from an anlage produced within the right marginal row (cp. Fig. 16). Scale bar division = 10 μm.

their number is smaller in interphase than in dividing specimens. Anlage 1 splits longitudinally to form the paroral and endoral membrane as well as the first frontal cirrus. Anlage 2 develops one or two buccal cirri and the second frontal cirrus. Anlage 3 develops the third frontal cirrus and two or more cirri in the frontal field which may form a row (Fig. 9). Anlage 4 does not migrate and forms the posterior (left) segment of the ACR. Anlage 5 develops three kinds of cirri: (Fig. 18, 23) the anterior portion migrates anteriorly and to the left forming the anterior segment of the ACR (Fig. 18, paired arrows); the posterior portion migrates posteriorly to form longitudinally arranged transverse cirri (Fig. 18, single arrows); the middle portion forms the cirri right of the ACR.

The caudal cirri develop at the posterior end of kineties 1 and 2 (Fig. 19), rarely also in kinety 3. The short dorsal kineties which originate near the right marginal row migrate on the dorsal surface (Fig. 18, 19, arrowheads; Fig. 23, thick arrow).

Stage 6: (Fig. 20, 24) The new ACR is nearly complete, i.e. the anterior portion of anlage 5 aligns with anlage 4. The posterior portion of anlage 5 migrates posteriorly forming the transverse cirri.

Redescription of *Paramphisiella caudata* (Hemberger, 1985) Foissner, 1988

(Fig. 25–39, Table 2)

A redescription of *P. caudata* is necessary because Hemberger [19] studied only protargol-impregnated cells. Thus, he did not recognize the conspicuous cortical granules, which usually do not impregnate with protargol. However, we cannot exclude that a very similar (in terms of the infraciliature) species without cortical granules exists in Peruvian soils, where Hemberger [19] discovered his species. If so, our population must be given species status. Likewise, a redescription of the morphogenesis is necessary because Hemberger's description differs in a key stage, either because he misinterpreted his data or our form is another species (Hemberger, H. 1982. Revision der Ordnung Hypotrichida Stein (Ciliophora, Protozoa) an Hand von Protargolpräparaten und Morphogenesedarstellungen. Dissertation, University of Bonn).

We thus deposit two protargol-impregnated slides of our *P. caudata* population in the collection of microscope slides of the Oberösterreichische Landesmuseum in Linz. Accession numbers: 12, 13/1994.

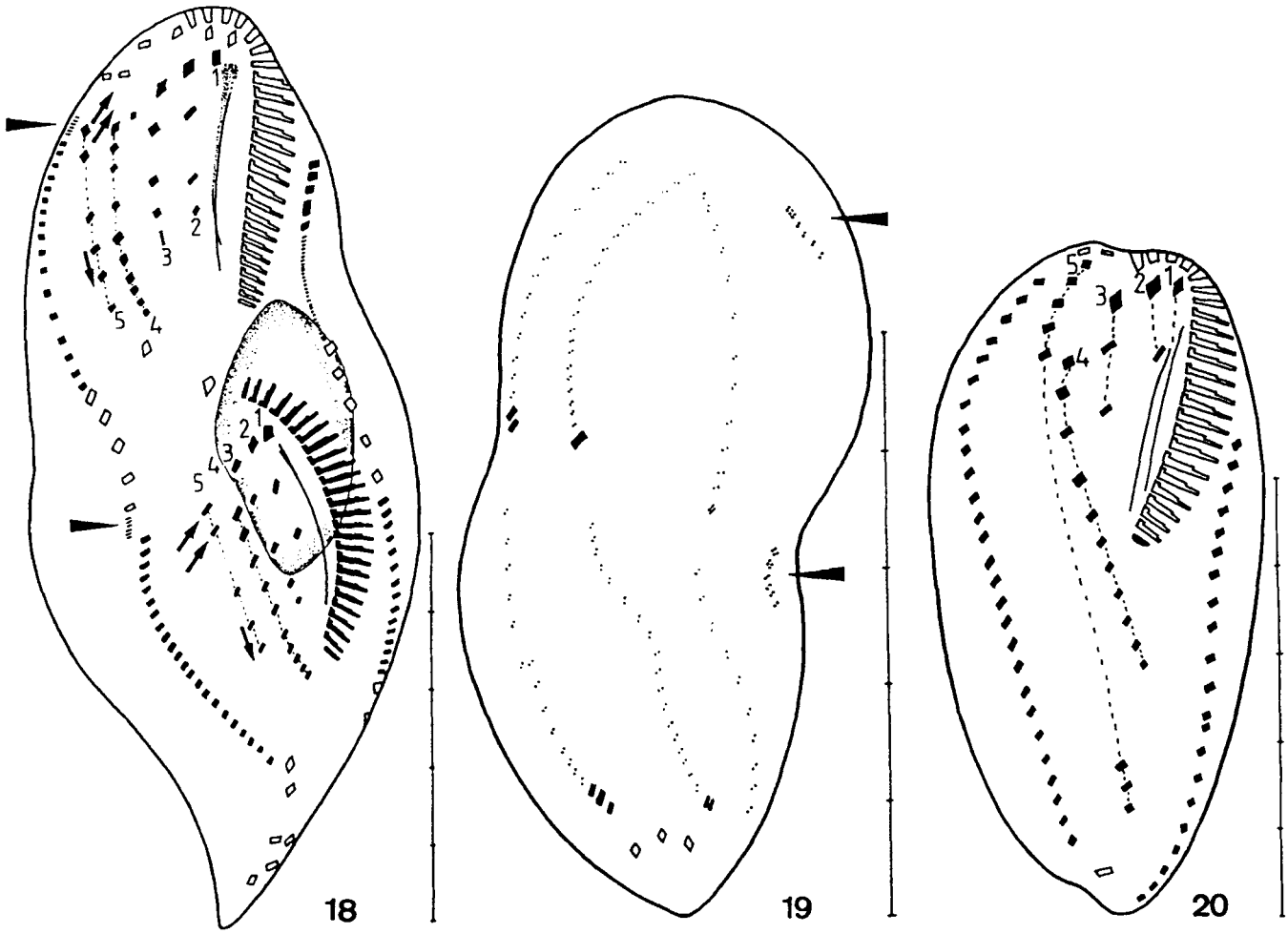

Fig. **18–20**. Late dividers of *Amphisiellides illuvialis* n. sp. after protargol impregnation (cp. Fig. 23, 24). Numbers in figures denote cirral anlagen. **18**. Ventral view showing migration of cirri in the rightmost anlage: the anterior cirri migrate anteriorly and to the left to align with anlage 4 (paired arrows); the posterior cirri migrate posteriorly to become transverse cirri (single arrows). Arrowheads mark anlagen for dorsal kinety 4 within the right marginal row. **19**. Dorsal view showing formation of caudal cirri at posterior end of new dorsal kineties. Arrowheads mark dorsal kinety 4 produced within the right marginal row (cp. Fig. 18, 23). **20**. Ventral view of post-divider. The migration of the cirri and the alignment of the amphisiellid cirral row (anlagen 4 and 5) are almost complete. Scale bar division = 10 μm.

Table **1**. Morphometric characterization of *Amphisiellides illuvialis* n. sp. Data are based on randomly selected protargol-impregnated and mounted interphase specimens from two exponentially growing stock cultures. Measurements in μm. CV, coefficient of variation; M, median; Max, maximum value; Min, minimum value; n, sample size; nc, not calculated because not distributed normally; SD, standard deviation; SE, standard error or arithmetic mean; $\bar{x}$, arithmetic mean.

Character	$\bar{x}$	M	SD	CV	Min	Max	n
Body, length	98.0	103.0	14.3	14.6	56.0	132.0	28
Body, width	26.7	26.5	6.8	25.6	16.0	46.0	28
Adoral zone of membranelles, length	28.7	29.0	2.7	9.5	24.0	35.0	28
Amphisiellid cirral row, length	53.6	51.0	12.3	23.0	19.0	80.0	28
Anterior macronuclear segment, length	24.3	27.0	7.0	28.8	9.0	32.0	27
Anterior macronuclear segment, width	7.2	7.0	2.0	27.6	4.0	12.0	27
Posterior macronuclear segment, length	21.2	19.5	10.4	49.3	9.0	40.0	12
Posterior macronuclear segment, width	7.0	6.0	2.3	33.5	4.0	12.0	12
Macronuclear segments, number	2.0	2.0	nc	nc	1.0	4.0	28
Adoral membranelles, number	24.6	25.0	2.7	10.8	21.0	35.0	28
Right marginal row, number of cirri	25.6	26.0	4.3	16.7	15.0	36.0	28
Left marginal row, number of cirri	19.4	20.5	4.3	22.0	8.0	27.0	28
Buccal cirri, number	1.0	1.0	nc	nc	1.0	2.0	28
Amphisiellid cirral row, number of cirri	13.0	12.0	3.6	27.6	5.0	27.0	28
Cirri right of amphisiellid cirral row	2.1	0.0	3.8	181.0	0.0	13.0	28
Cirri left of amphisiellid cirral row	3.0	3.0	1.5	50.0	1.0	6.0	14
Transverse cirri, number	2.3	2.0	0.8	34.8	1.0	5.0	25
Caudal cirri, number	3.5	3.0	1.1	31.4	2.0	7.0	28

Interphase morphology (Fig. 25–29). Lanceolate, in vivo about 150 × 40 μm, posterior end usually more distinctly tapered than anterior (Fig. 25, 29). Highly flexible and about 30% contractile. Macronuclear segments in vivo about 10 × 6 μm, usually in left body half. Contractile vacuole above mid-body at left margin, with indistinct collecting canals. Cortical granules in conspicuous longitudinal rows, 1–1.2 μm in diameter, colorless (Fig. 28). Cytoplasm contains lipid inclusions 1–5 μm in diameter, yellowish crystals mainly in posterior portion of body, and food vacuoles with ciliates (*Colpoda inflata*), heterotrophic flagellates (*Polytoma* sp.) and bacteria (Fig. 25). Movement rather slow and clumsy.

Adoral zone of membranelles about 25% of body length. Bases of membranelles in vivo 7 μm, frontal cirri 15 μm, marginal and ventral cirri 10 μm long. Buccal cavity flat and narrow. Oral lip parallels slightly curved undulating membranes, left edge slightly thickened and wavy. Three enlarged frontal cirri, right cirrus at distal end of adoral zone of membranelles. ACR commences near distal end of adoral zone of membranelles and usually terminates in posterior half of cell near left marginal row. One cirrus left of ACR in frontal field. Buccal cirrus near anterior end of undulating membranes. Transverse cirri arranged longitudinally, difficult to distinguish from marginal and caudal cirri (Fig. 26). Right marginal row commences on dorsal surface near anterior end of body; both marginal rows extend to posterior end of cell.

Dorsal cilia 3 μm long, arranged in 3 rows; kinety 3 in about 80% of specimens posteriorly shortened (Fig. 27). Caudal cirri tiny, in vivo 15–20 μm long.

Divisional morphogenesis (Fig. 30–39). The nuclear apparatus and the marginal rows divide in the usual way and hence require no further comment (Fig. 34–38).

Stage 1: (Fig. 30–32). Stomatogenesis commences with the proliferation of basal bodies near the middle portion of the ACR (Fig. 30). A large field of basal bodies develops and splits in a larger posterior and a smaller anterior portion; the anterior portion extends to the proximal end of the parental undulating membranes (Fig. 31). The ACR appears unchanged.

Small streaks of basal bodies develop within the dorsal kineties (Fig. 32).

Stage 2: (Fig. 33) The large posterior field of basal bodies differentiates adoral membranelles at its anterior end. The small anterior portion generates one short (anlage 1) and two long (anlagen 2 and 3) streaks. The middle streak (anlage 2) extends to the parental buccal cirrus which has disorganized to a short streak of basal bodies. The cirri in the central portion of the ACR disorganize and form a short streak of basal bodies (anlage 4). The rightmost streak (anlage 5) is either generated also by disorganized cirri of the ACR or develops de novo. The organizing center in the ACR is obviously the site where the anterior and posterior portion of the anlagen 4 and 5 aligned in the previous generation.

Hemberger (1982 dissertation) depicts a similar stage with all cirral anlagen originating from the central portion of the ACR, whereas our specimens definitely show that only cirri for the new ACR originate from this portion.

Stage 3: (Fig. 34) The opisthe's adoral membranelles differentiate posteriorly. Five long cirral streaks ("primary primordia" [11]) are recognizable. Streak 1 contains basal bodies from the disorganizing parental paroral membrane and, possibly, from the opisthe's oral primordium. Streak 2 develops from the oral primordium and joins the parental buccal cirral streak. Streak 3 originates from the oral primordium. Streak 4 develops from disaggregating cirri of the anterior segment of the ACR. Streak 5 possibly develops de novo or from the ACR too; streaks 4 and 5 form a V-shaped pattern.

Stage 4: (Fig. 35) The primary primordia split in the middle producing 5 anlagen each in proter and opisthe.

Stage 5: (Fig. 36, 37) The formation of the opisthe's adoral

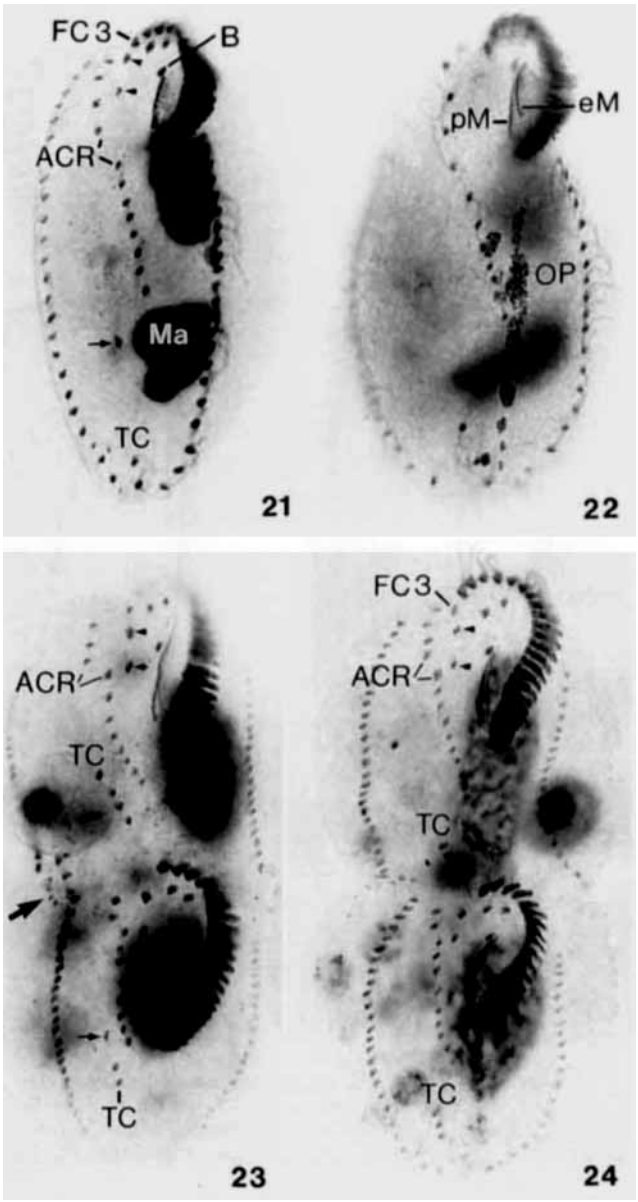

Fig. 21–24. *Amphisiellides illuvialis* n. sp., interphase (**21**) and divisional (**22–24**) morphology after protargol impregnation. These figures are from population II, which differs slightly from the type material in the location of frontal cirrus 3 (cp. Fig. 2, 21). **21**. Infraciliature of ventral side (cp. Fig. 2, 8–10). Note buccal cirrus (B) at anterior end of paroral membrane, and frontal cirrus 3 (FC 3) at distal end of adoral zone of membranelles. Arrow marks single cirrus right of ACR. Arrowheads mark two cirri left of ACR. **22**. Early divider showing oral primordium developing at posterior end of ACR (cp. Fig. 12, 13). **23**, **24**. Late dividers (cp. Fig. 18, 20). The ACR forms from the anterior portion of anlage 5 and the posterior portion of anlage 4. The posterior portion of anlage 5 migrates rearwards to become the transverse cirral row; one of these cirri does not completely migrate posteriad and remains as a cirrus right of the ACR (thin arrow; cp. Fig. 21). Thick arrow marks dorsal kinety 4, which originates from anlagen produced near or within the right marginal row (cp. Fig. 16, 18, 19, arrowheads). Arrowheads mark two cirri left of the ACR originating from anlage 3. ACR, amphisiellid cirral row; B, buccal cirrus; eM, endoral membrane; FC 3, frontal cirrus 3; Ma, macronuclear segment; OP, oral primordium; pM, paroral membrane; TC, transverse cirral row. Figures purposely without scale bars because the applied staining technique (squashed, unmounted specimens) leads to inavoidable distortions of cells which would give meaningless measurements.

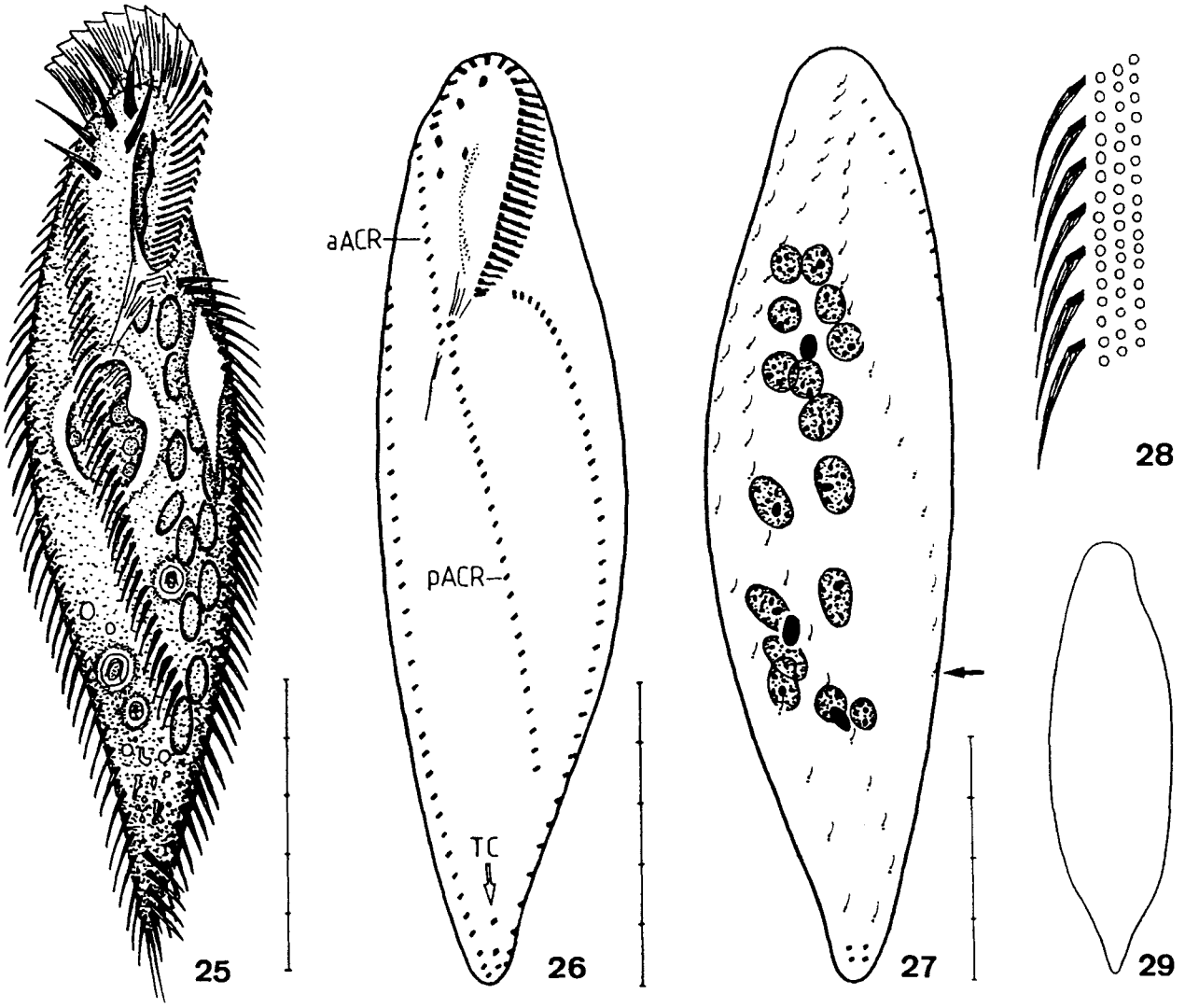

Fig. 25–29. Interphase morphology of *Paramphisiella caudata* (Hemberger) from life (**25**, **28**, **29**) and after protargol impregnation (**26**, **27**). **25**. Ventral view of typical specimen. **26**, **27**. Infraciliature of ventral and dorsal side. White arrow points to transverse cirri; black arrow marks end of dorsal kinety 3. **28**. Surface view showing conspicuous rows of colorless, cortical granules. **29**. More rarely occurring body shape. Scale bar division = 10 μm. aACR, anterior segment of amphisiellid cirral row; pACR, posterior segment of amphisiellid cirral row; TC, transverse cirri.

zone of membranelles is almost complete. The anlage for the undulating membranes split to form the paroral and endoral membrane. The anlagen in the proter and opisthe organize in the same manner (Fig. 36, 39). Anlage 1 splits longitudinally to form the paroral and endoral membrane as well as the first frontal cirrus. Anlage 2 develops the buccal cirrus and the second frontal cirrus. Anlage 3 develops the third frontal cirrus and the cirrus between the ACR and the buccal cirrus. Anlage 4 does not migrate and forms the posterior (left) segment of the ACR. Anlage 5 develops the anterior (right) segment of the ACR and the transverse cirri.

Occasionally, an additional anlage occurs between the anlagen 3 and 4 (resulting in a total of six anlagen), in one or both filial products to form one or two additional frontal cirri.

New dorsal kineties develop within the old ones. The basal bodies at the posterior end of dorsal kineties 2 and 3 aggregate to form inconspicuous caudal cirri (Fig. 37, 38).

Stage 6: (Fig. 38, 39) Most cirri of anlage 5 migrate anteriorly in each proter and opisthe. These cirri align more or less perfectly with those of anlage 4 to form the ACR. The posterior portion of anlage 5 migrates posteriorly to form longitudinally arranged transverse cirri.

Divisional morphogenesis in *Hemiamphisiella terricola* Foissner, 1988
(Fig. 40–55)

The population studied is described in [5] and shown here by micrographs for the first time (Fig. 49, 50, 52, 53). *Uroleptoides qingdaoensis* Song Weibo & Wilbert, 1989 is possibly a junior synonym of *Hemiamphisiella terricola*. The frontoterminal cirri of this species belong very likely to the right marginal row.

The nuclear apparatus and the marginal rows divide in the usual way and hence require no further comment (Fig. 44, 47, 48).

Stage 1: (Fig. 41) Stomatogenesis commences near the middle portion of the ACR. The ACR and the postperistomial cirrus appear unchanged.

Stage 2: (Fig. 42, 54) A narrow field of basal bodies develops along the left edge of the ACR and extends to the parental undulating membranes. The postperistomial cirrus has apparently dissolved and been incorporated in this field. The buccal cirrus disorganizes to a streak of basal bodies. The cirrus left of the anterior portion of the ACR commences to disaggregate.

Stage 3: (Fig. 43) The oral primordium splits: the large pos-

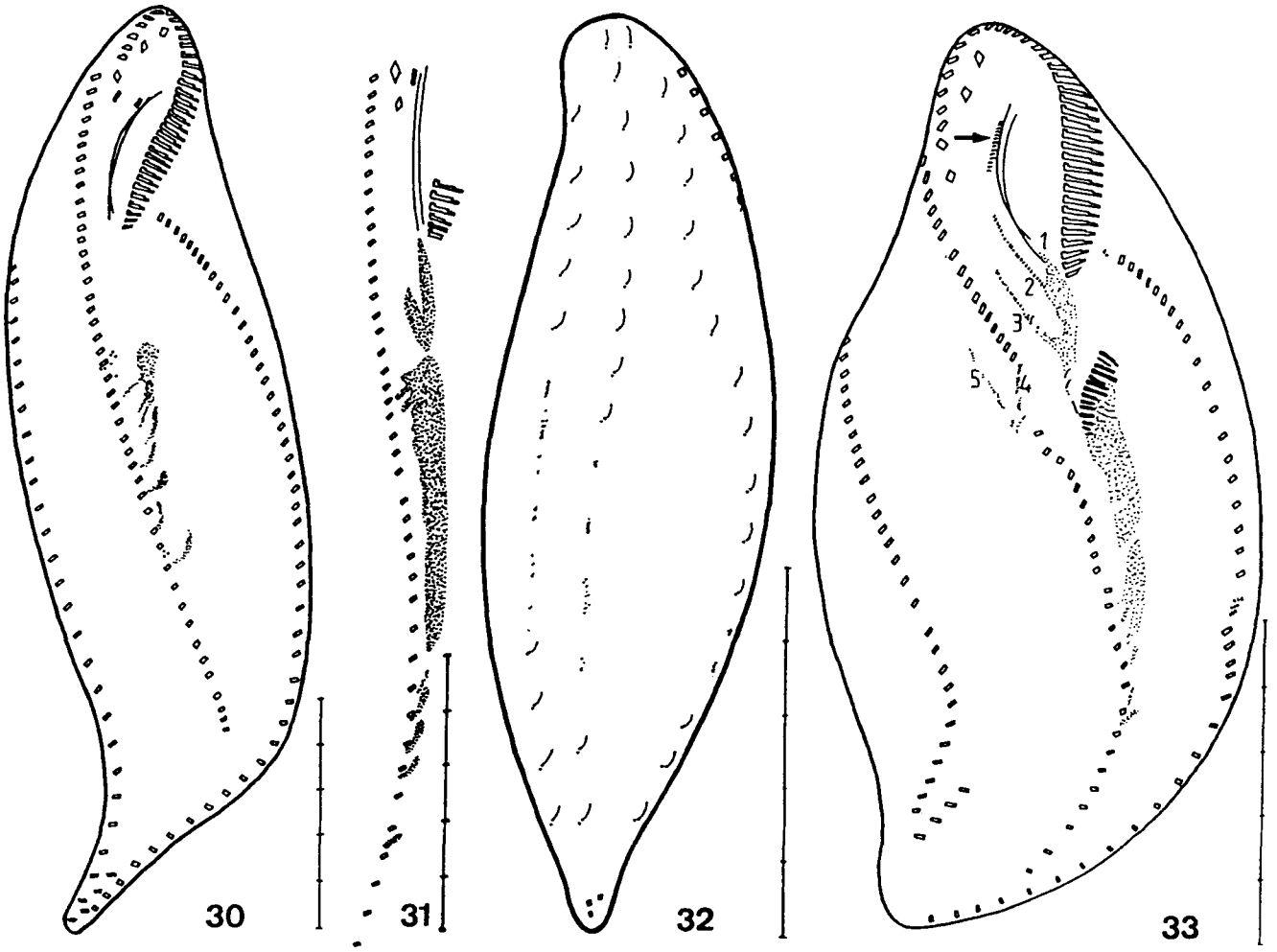

Fig. 30–33. Early dividers of *Paramphisiella caudata* after protargol impregnation. **30**. Morphogenesis commences with a proliferation of basal bodies near the middle portion of the amphisiellid cirral row. **31**. The oral primordium splits into a smaller anterior and a larger posterior portion. **32**. Small streaks of basal bodies develop within three dorsal kineties. **33**. Anlagen (numbered) 1–3 are generated by the oral primordium, anlage 4 by the ACR, and anlage 5 possibly develops de novo. The parental buccal cirrus disorganizes (arrow). Scale bar division = 10 μm.

terior portion organizes adoral membranelles, the small anterior portion forms the opisthe's anlagen 1–4. The parental undulating membranes disorganize (proter's anlage 1). The streak formed by the buccal cirrus elongates (proter's anlage 2). The cirrus left of the ACR disaggregates and forms a twisted streak (proter's anlage 3) above the proter's anlage 4, which possibly develops de novo or from migrating basal bodies of anlage 2. The cirri of the middle segment of the ACR (cp. Fig. 46, anlage 4) disorganize and form V-shaped streaks (proter's and opisthe's anlagen 5, 6); however, we cannot exclude that anlage 6 develops de novo.

Stage 4: (Fig. 44, 45, 47, 48) The formation of the opisthe's adoral zone of membranelles proceeds posteriorly. All cirral streaks align and lengthen. Six anlagen each in the proter and opisthe are recognizable and organize in the same manner (Fig. 44–46). Anlage 1 splits longitudinally to form the paroral and endoral membrane as well as the first frontal cirrus. Anlage 2 develops the buccal cirrus and the second frontal cirrus. Anlage 3 develops the third frontal cirrus and the cirrus left of the ACR. Anlage 4 develops cirri which migrate in two directions: the anterior cirri migrate to the right (Fig. 45, triangles) to align with anlage 5; the posteriormost cirrus migrates posteriad to become the postperistomial cirrus (Fig. 45, 46, dotted arrows). Rarely do two, three or no postperistomial cirri develop. Likewise, anlage 6 develops cirri which migrate in two directions: the posterior cirri migrate posteriorly to become the transverse cirri (Fig. 45, short arrows); the anterior cirri migrate anteriorly and to the left (Fig. 45, large arrows) and align with anlage 5 and the anterior cirri of anlage 4 to form the ACR. This process of forming the ACR from three segments is also well recognizable in late dividers (Fig. 46).

New dorsal kineties develop within the old ones. Basal bodies aggregate at the posterior ends to form inconspicuous caudal cirri (Fig. 47, 48).

Stage 5: (Fig. 46, 55) The cirral migration as described in stage five proceeds and cytokinesis commences. The postperistomial cirrus reaches its final position usually in the post-divider.

DISCUSSION

The amphisiellid median cirral row (ACR). Migration of cirri takes an essential part in forming the final cirral pattern in many hypotrichs. The migration is often most distinct in the rightmost anlage and results in so-called frontoterminal cirri (urostylids and oxytrichids) or, as in amphisiellids, in a median cirral row composed of two or more anlagen segments. However, a median cirral row can be formed by at least four basic, non-homologous processes: It may develop in the proter and opisthe from a single

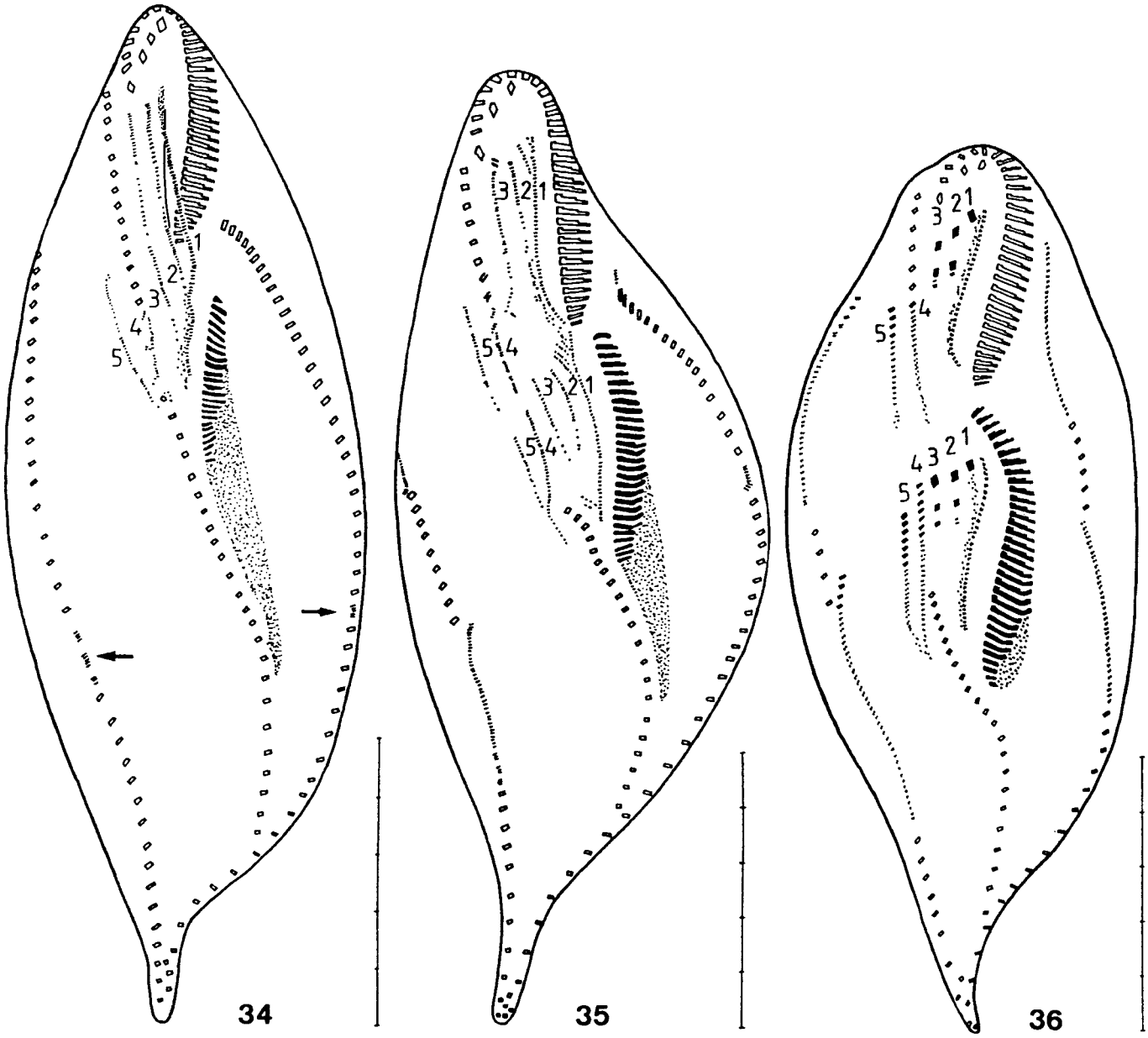

Fig. 34–36. Ventral views of middle dividers of *Paramphisiella caudata* after protargol impregnation. Numbers in figures denote anlagen. **34.** The cirral anlagen form five long primary primordia. Anlagen develop within the marginal rows (arrows). **35.** The primary primordia split transversely to form five anlagen each in proter and opisthe. **36.** Anlage 1 splits longitudinally to form the first frontal cirrus and the undulating membranes. Cirri organize within anlagen 2–5. Scale bar division = 10 μm.

anlage within the parental median row (Fig. 56). This mode occurs in *Orthoamphisiella* spp. [9]. Hemberger (1982 dissertation) depicts similar development in *Uroleptoides atypicus, A. terricola* and *Trachelochaeta gonostomoida*; these taxa possibly constitute a new family. Furthermore, the median cirral row may develop from a single anlage each in proter and opisthe (Fig. 57). This mode occurs in *Cladotricha koltzowii* [6] and in *Engelmanniella mobilis* [32]. Alternatively, the median cirral row of amphisiellids develops from two or more rightmost anlagen in both proter and opisthe (Fig. 58, 59). This mode occurs, e.g. in *Hemiamphisiella terricola* (Fig. 66), *Amphisiellides illuvialis* (Fig. 62) and *Paramphisiella caudata* (Fig. 63). All species and genera mentioned above form their median row by complete anlagen or at least large portions of them. In contrast, the median cirral row of the Discocephalidae, e.g. *Psammocephalus faurei* [29], is formed by individual cirri each originating from a different anlage (Fig. 60).

Paraurostyla weissei also possesses a cirral row that develops from two rightmost anlagen [30]. However, several ventral rows, which evolve from independent primordia, occur left of this composite row. This produces a cirral pattern which is rather different from that of the amphisiellids. Nevertheless, a certain relationship between *Paraurostyla, Amphisiella* and *Gastrostyla* is apparent and supported by a phylogenetic approach using other apomorphies [31].

In *Onychodromus quadricornutus* the rightmost anlage migrates only slightly anteriorly; a composite cirral row is not formed [17]. Moreover, as in *P. weissei,* several ventral rows

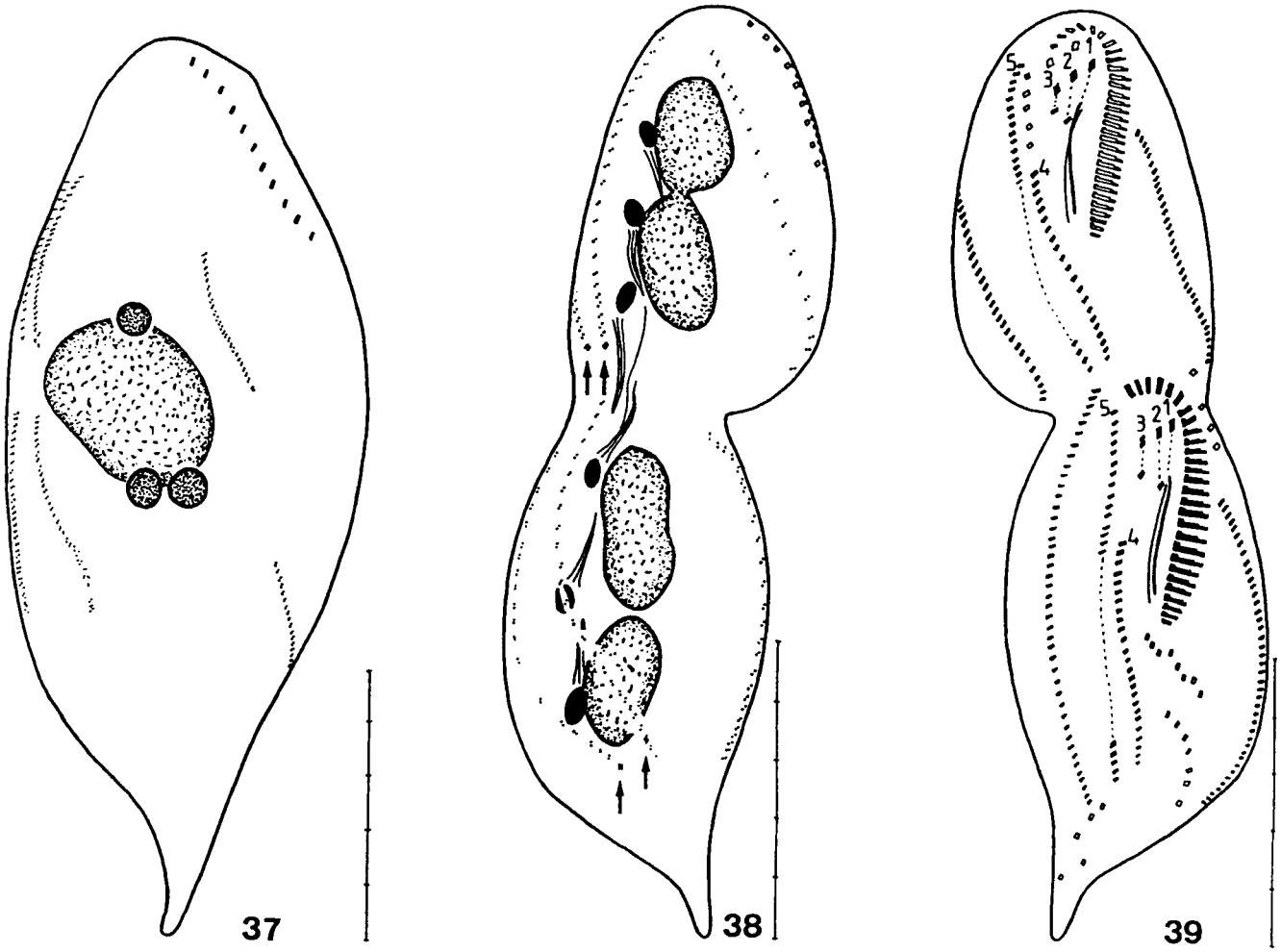

Fig. 37–39. Late dividers of *Paramphisiella caudata* after protargol impregnation. **37**, **38**. New dorsal kineties develop within old ones and inconspicuous caudal cirri develop at posterior end of kineties 1 and 2 (arrows). **39**. The anterior portion of anlage 5 migrates anteriorly to align with anlage 4, forming the amphisiellid cirral row. The posterior portion of anlage 5 becomes the transverse cirral row. Numbers denote anlagen. Dotted lines indicate migration. Scale bar division = 10 μm.

occur in this species, which also separates it from the amphisiellids.

Seven types (genera) of forming the ACR, the postperistomial cirrus, and the transverse cirri can be distinguished in amphisiellid hypotrichs (Fig. 61–67). The first type, found in *Amphisiella* spp., builds the ACR from cirri of the two rightmost anlagen (Fig. 61). The anterior segment is formed by anteriorly migrated cirri of the rightmost anlage 6; the posterior segment is formed by cirri from anlage 5. Transverse cirri are formed either by posteriorly migrated cirri from these two rightmost anlagen (*A. australis* [27]) or from more than two anlagen (*A. marioni* [29]), as in *Gastrostyla* (Fig. 65).

The second type, found in *Amphisiellides illuvialis*, builds the ACR as *Amphisiella*, but several cirri of the rightmost anlage remain right of the ACR (Fig. 62). Several posterior cirri of the rightmost anlage 5 migrate posteriorly to form a short, longitudinally arranged transverse cirral row. The second anlage from right (4) does not contribute to the transverse cirri.

The third type, found in *Paramphisiella caudata*, builds the ACR and the transverse cirri as *Amphisiellides* (Fig. 63). However, no cirri remain right of the ACR.

The fourth type, found in *Paragastrostyla lanceolata*, builds the ACR from three or four rightmost anlagen (Fig. 64). The anterior segment of the ACR is formed by all anteriorly migrated cirri of the rightmost anlage 7; thus, no transverse cirri are formed in this genus. A middle segment is formed by posteriorly migrated cirri of anlage 5; possibly anlage 4 is also involved in forming the ACR. The posterior segment is formed by all cirri of the second anlage from right (6).

The fifth type, found in *Gastrostyla steinii*, builds the ACR from the three rightmost anlagen as *Paragastrostyla* (Fig. 65). However, only the first (anteriormost) cirrus of the third anlage from right (4) forms the middle segment of the ACR; the second cirrus of this anlage (4) migrates further posteriad to form the postperistomial cirrus. Transverse cirri are formed by posteriorly migrated cirri of the rightmost and several neighboring anlagen. Hemberger (1982 dissertation) describes the ventral row (ACR) of *G. steinii* as being composed of only two anlagen (segments). We assume, however, after a careful examination of his drawings, the ACR to be usually composed of an additional middle segment, viz. the anteriormost cirrus of anlage 4. However, alignment of this cirrus with the ACR may be imperfect in some specimens.

The sixth type, found in *Hemiamphisiella terricola*, forms the ACR and the postperistomial cirrus by processes as described for *Gastrostyla*, whereas the transverse cirri are formed as in *Amphisiellides* and *Paramphisiella* (Fig. 66).

The seventh type, found in *Pseudouroleptus caudatus*, forms

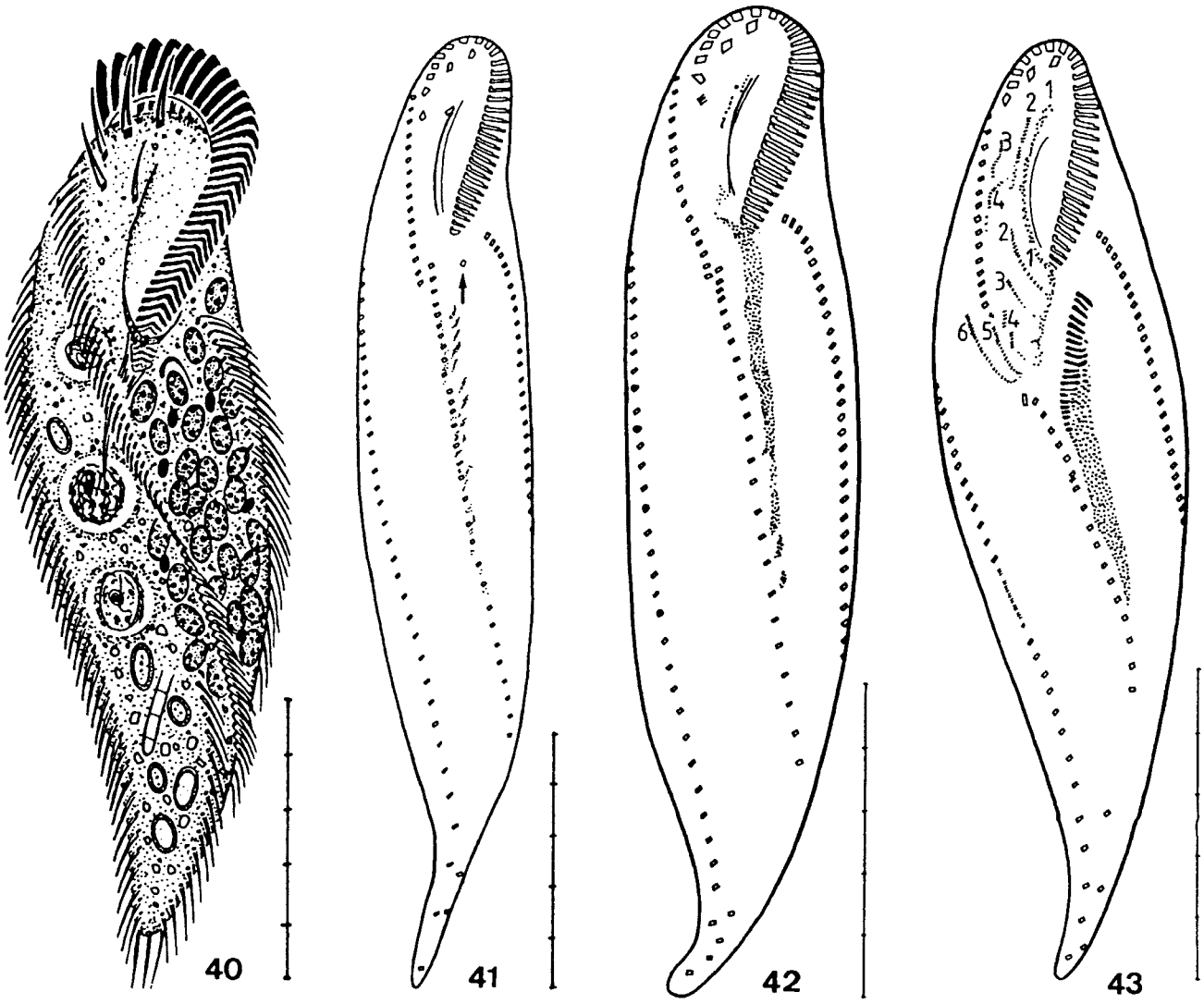

Fig. **40–43**. Interphase morphology (**40**, from [12]) and early dividers (**41–43**) of *Hemiamphisiella terricola* Foissner, 1988. **40**. Ventral view from life. **41**. Morphogenesis commences with a proliferation of basal bodies near the middle portion of the amphisiellid cirral row. Arrow marks postperistomial cirrus. **42**. The oral primordium elongates and extends over the postperistomial cirrus (cp. Fig. 54). The buccal cirrus and the cirrus behind the third frontal cirrus disaggregate. **43**. Anlagen (numbered) 1 to 4 of the proter develop from parental frontoventral cirri (anlage 4 possibly originates de novo). Anlagen 1 to 4 of the opisthe develop from the oral primordium. Anlagen 5 and 6 develop from the amphisiellid cirral row in both filial products; anlage 6 possibly develops de novo. Anlagen originate within the marginal rows (cp. Fig. 44). Scale bar division = 10 μm.

the ACR and the postperistomial cirrus as the former two types (Fig. 67). However, the rightmost anlage 6 produces a great number of cirri, of which most remain right of the ACR. These cirri form a distinct second ventral row which is obviously homologous to the transverse cirri and the cirri right of the ACR found in *Amphisiellides*.

These data show that the median cirral row of amphisiellid hypotrichs is built by at least two rightmost anlagen which arrange one upon the other during cytokinesis and in post-dividers. We consider this unique median cirral row formation as an apomorphy defining the family Amphisiellidae. The alignment of the anlagen is frequently imperfect, i.e. the ACR usually appears more or less distinctly interrupted in interphase specimens. This irregularity is highly characteristic for amphisiellid hypotrichs.

Genus separation in amphisiellid ciliates and improved diagnoses. In spite of the homologous development of the ACR, morphogenetic differences in amphisiellid ciliates are distinct enough to maintain the genera proposed in the literature. We did not find any significant variation in the main characters traditionally used for genus distinction in hypotrich ciliates, e.g. presence/absence of transverse and/or caudal cirri. However, when analyzing a great number of specimens of a certain species or genus, there are usually some individuals which deviate, e.g. with a missing postperistomial cirrus or lacking transverse cirri.

Based on the ontogenetic data discussed above the Amphisiellidae Jankowski, 1979 and the genera contained therein can be defined more properly. We recognize seven genera, two of which were originally assigned to the Oxytrichidae.

Family Amphisiellidae Jankowski, 1979. Euhypotrichina with an amphisiellid median cirral row (ACR) of which the anterior segment is formed by cirri of the rightmost ventral anlage and the posterior segment by cirri of the second ventral anlage from right. A middle segment may be formed by neighboring anlagen.

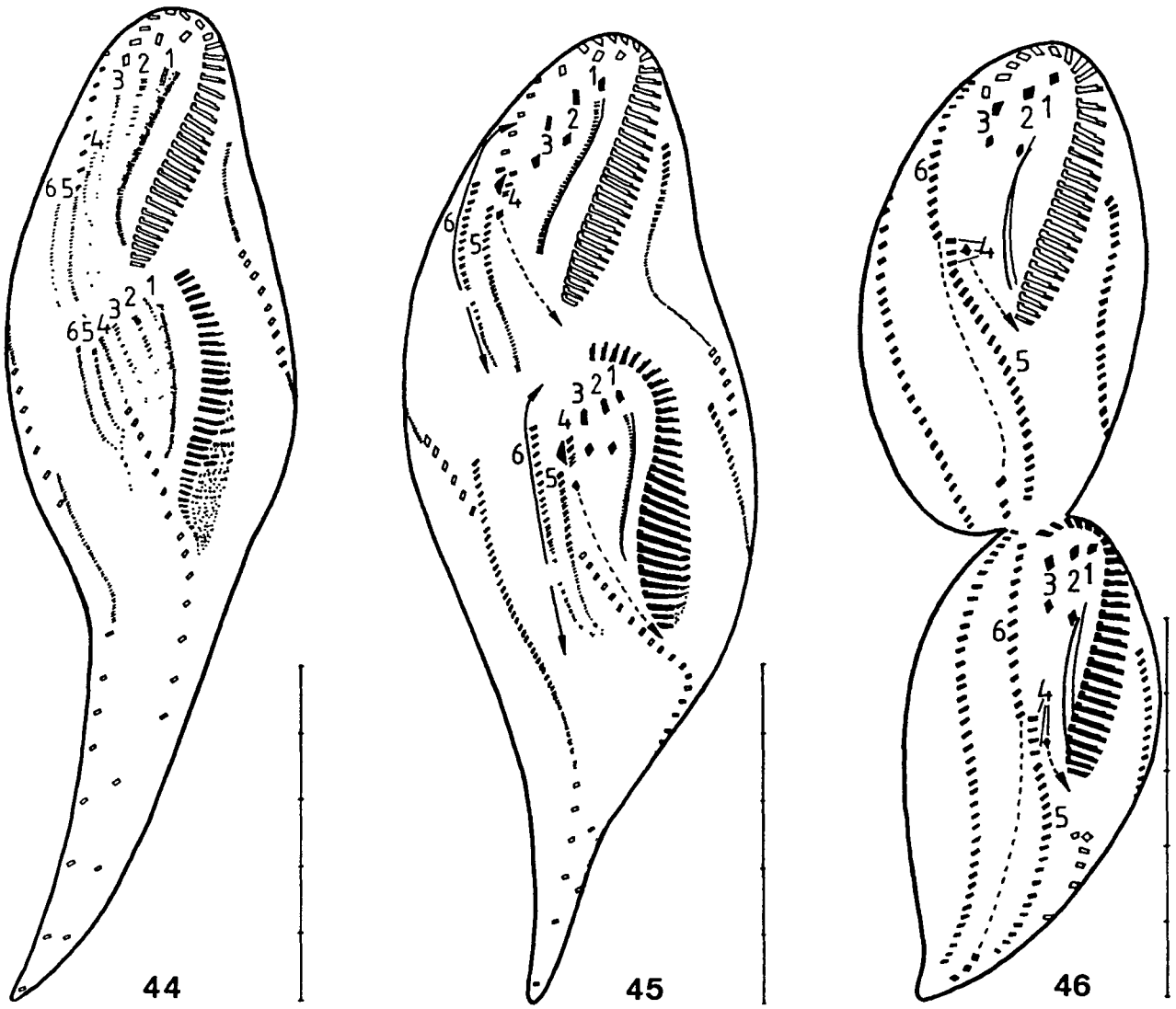

Fig. 44–46. Ventral views of middle (44) and late (45, 46) dividers of *Hemiamphisiella terricola* after protargol impregnation. Numbers in figures denote anlagen. **44.** Six cirral anlagen are recognizable in both proter and opisthe. Anlage 1 splits longitudinally to form the first frontal cirrus and the undulating membranes. **45.** The amphisiellid cirral row is built by all cirri of anlage 5 and by the cirri of the anterior portion of anlage 6, which migrates anteriorly (large arrows) to align with most cirri of anlage 4 (triangles). The posterior portion of anlage 6 becomes the transverse cirral row (short arrows). The posterior cirrus of anlage 4 becomes the postperistomial cirrus (dotted arrows). **46.** Cirral migration as described in figure 45 proceeds (cp. Fig. 55). Dotted lines denote migration of transverse cirri. Dotted arrows indicate migration of (postperistomial) posteriormost cirrus of anlage 4. Scale bar division = 10 μm.

This diagnosis does not clearly exclude the Amphisiellidae from the Discocephalina as diagnosed by Wicklow [29]. His diagnosis of these obligatorily psammolittoral forms is rather wide and was sufficient for the data available at that time. To separate these families unequivocally, a refined (narrower) diagnosis of the Discocephalina is necessary: Euhypotrichina with a discocephalid median cirral row of which each cirrus originates from a different ventral anlage.

Our diagnoses elucidate the different modes of forming the median cirral row in the Amphisiellidae and the Discocephalidae, respectively. To strengthen this difference, the use of different terms, viz. "amphisiellid median cirral row" and "discocephalid median cirral row," seems appropriate. Wicklow [29] suggested the term "midfrontal cirral row" for the Discocephalidae. Although this term is well chosen, there may be problems with finding appropriate terms for the other modes of forming a median cirral row, e.g. for *Orthoamphisiella*. We thus suggest, to put simply the name of the main taxon (family) in front of the "median cirral row".

Genus *Amphisiella* Gourret & Roeser, 1888. The ACR originates from two rightmost anlagen. More than one cirrus left of ACR. Transverse cirri obliquely arranged, originate from more than one anlage. Caudal cirri lacking (Fig. 61). Species assignable: *A. marioni* Gourret & Roeser, 1888 (type species; description of morphogenesis in [29]), *A. australis* Blatterer & Foissner, 1988 (description of morphogenesis in [27]) and *A. perisincirra* (Hemberger, 1985) nov. comb. (basionym: *Tachysoma perisincirra*). *Tachysoma perisincirra* (description of morphogenesis in Hemberger [1982 dissertation] and in [3]) obviously possesses an ACR, though late morphogenetic stages are not known. The data available show the same peculiarities as known from *A. australis* [27], i.e. a second conspicuous primordium is generated by the last cirri of the ACR.

Genus *Amphisiellides* Foissner, 1988. The ACR originates

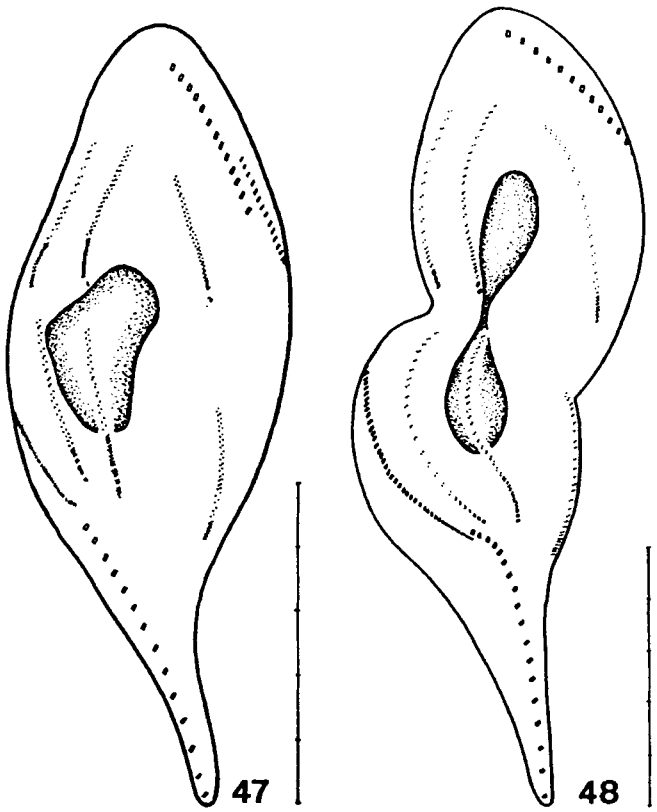

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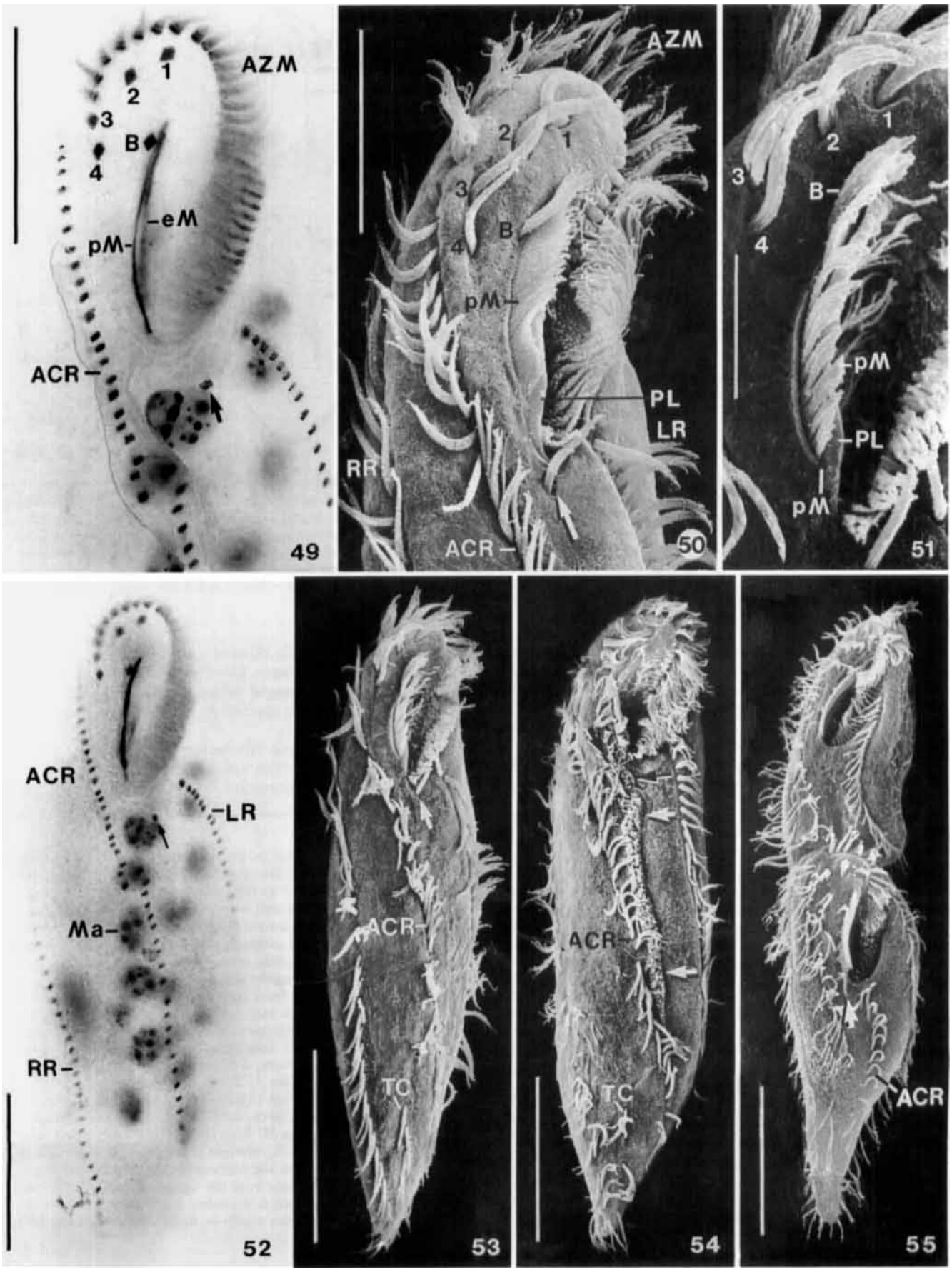
Fig. 49–55. *Hemiamphisiella terricola* after protargol impregnation (**49**, **52**) and in the scanning electron microscope (**50**, **51**, **53–55**). **49**, **52**, **53**. Ventral cirral pattern of interphase specimens. Arrows mark postperistomial cirrus. **50**, **51**. Ventral anterior body portion of interphase specimens. The paroral membrane originates from a narrow cleft at the base of the peristomial lip. Arrow marks postperistomial cirrus. **54**. Early divider (cp. Fig. 42) having a long, ciliated oral primordium (white arrows) from which a short streak of basal bodies projects to the proter's frontal area (black arrows). **55**. Late divider (cp. Fig. 45, 46). The postperistomial cirrus of the opisthe has migrated to its final position (arrow; cp. Fig. 50), that of the proter not yet. The posterior portion of the parental ACR is still recognizable. ACR, amphisiellid cirral row; AZM, adoral zone of membranelles; B, buccal cirrus; eM, endoral membrane; LR, left marginal row; Ma, macronuclear segment; PL, peristomial lip; RR, right marginal row; TC, transverse cirral row; 1, 2, 3, frontal cirri; 4, cirrus left of ACR, produces anlage 3. Fig. 49, 50, bars = 20 μm; 51, bar = 10 μm; 52–55, bars = 40 μm.

←

Fig. 47–48. Dorsal views of late dividers of *Hemiamphisiella terricola* after protargol impregnation. **47**. New dorsal kineties develop within old ones. **48**. Inconspicuous caudal cirri develop at the posterior end of the new dorsal kineties. Scale bar division = 10 μm.

Table 2. Morphometric characterization of *Paramphisiella caudata*. Upper line: population from Kenya. Data are based on randomly selected protargol-impregnated and mounted interphase specimens from an exponentially growing raw culture. Lower line: type population (from [19]). Measurements in μm. Abbreviations: nd, no data available; for others see Table 1.

Character	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length	148.1	146.5	9.6	2.8	6.5	134.0	168.0	12
	200.0	nd	nd	nd	nd	nd	nd	nd
Body, width	41.0	41.0	5.1	1.5	12.5	32.0	51.0	12
	50.0	nd	nd	nd	nd	nd	nd	nd
Adoral zone of membranelles, length	37.8	38.0	1.7	0.5	4.4	35.0	41.0	12
	40.0	nd	nd	nd	nd	nd	nd	nd
Amphisiellid cirral row, length	121.3	123.5	8.6	2.5	7.1	105.0	136.0	12
Macronuclear segment, length	10.4	10.5	1.9	0.5	18.1	8.0	14.0	12
Macronuclear segment, width	6.8	7.0	1.0	0.3	14.3	5.0	8.0	12
Micronuclei, length	4.5	4.4	0.5	0.1	11.4	4.2	5.6	12
Micronuclei, width	3.7	3.8	0.5	0.1	12.3	3.0	4.0	12
Macronuclear segments, number	14.8	15.0	1.1	0.3	7.2	12.0	16.0	12
	14.0	nd	nd	nd	nd	8.0	nd	nd
Micronuclei, number	4.0	4.0	0.6	0.2	15.1	3.0	5.0	12
	5.0	nd	nd	nd	nd	4.0	6.0	nd
Adoral membranelles, number	31.8	32.0	1.3	0.4	4.2	30.0	34.0	12
	35.0	nd	nd	nd	nd	nd	nd	nd
Right marginal row, number of cirri	44.6	44.0	2.7	0.8	6.2	41.0	50.0	12
	38.0	nd	nd	nd	nd	37.0	39.0	nd
Left marginal row, number of cirri	41.0	40.5	3.3	1.0	8.1	37.0	48.0	12
	29.5	nd	nd	nd	nd	24.0	35.0	nd
Buccal cirri, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	12
	1.0	nd	nd	nd	nd	nd	nd	nd
Amphisiellid cirral row, number of cirri	42.3	42.5	1.4	0.4	3.4	41.0	46.0	12
	38.0	nd	nd	nd	nd	nd	nd	nd
Frontal cirri, number	4.2	4.0	0.6	0.2	13.6	4.0	6.0	12
	4.0	nd	nd	nd	nd	nd	nd	nd
Transverse cirri, number	3.5	3.0	0.7	0.2	19.3	3.0	5.0	12
	2.5	nd	nd	nd	nd	nd	nd	nd
Dorsal kineties, number	3.0	3.0	0.0	0.0	0.0	3.0	3.0	12
	3.0	nd	nd	nd	nd	nd	nd	nd
Caudal cirri, number	3.3	3.0	0.7	0.2	19.5	3.0	5.0	12
	3.0	nd	nd	nd	nd	nd	nd	nd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ACR
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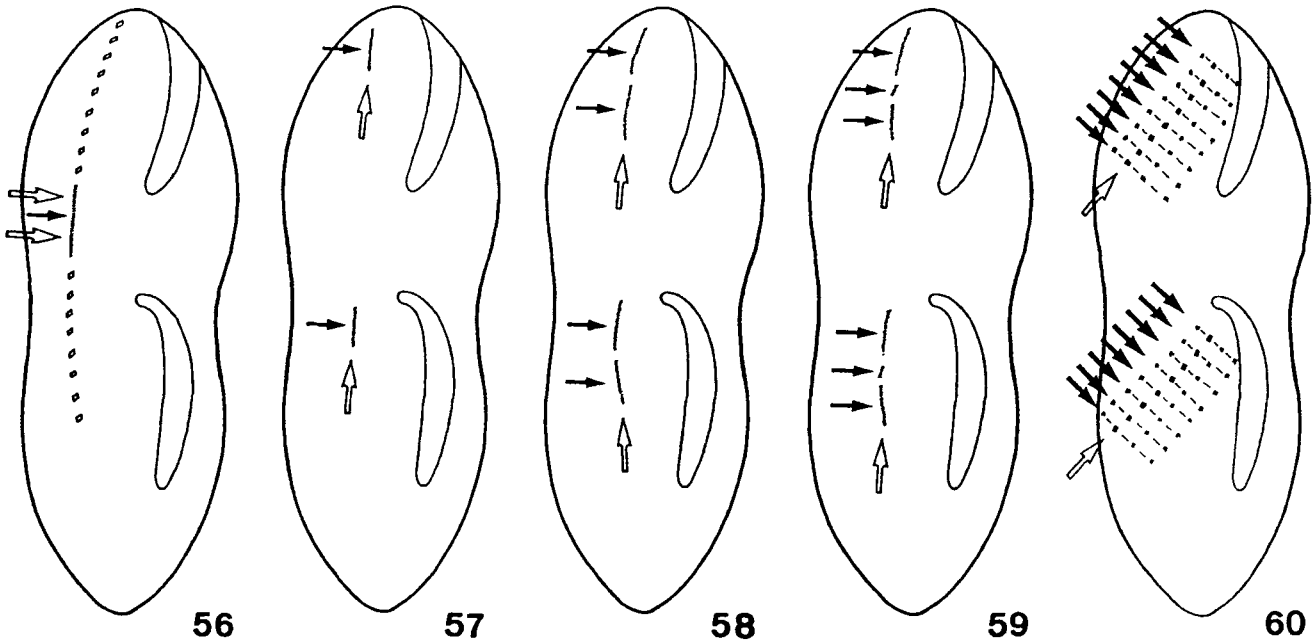


Fig. **56–60**. Origin of the median cirral row in hypotrichs. Black arrows mark anlagen; white arrows mark prospective median cirral rows. **56**. The median cirral row of the proter and opisthe develops from a single anlage within the parental median row. This particular formation occurs in *Orthoamphisiella* spp. [9] and, possibly, in *Amphisiellides atypicus, Amphisiella terricola* and *Trachelochaeta gonostomoida* (Hemberger, 1982 dissertation). **57**. The median cirral row develops from independent anlagen in both proter and opisthe. This type is known to occur in *Cladotricha koltzowii* [6] and *Engelmanniella mobilis* [32]. **58, 59**. The Amphisiellidae develop the median cirral row from two (**58**) or more (**59**) rightmost anlagen in both proter and opisthe. The anlagen develop side by side and get their final position through migration and alignment during cytokinesis and in post-dividers. **60**. *Psammocephalus faurei,* a discocephaline hypotrich, develops the median cirral row from several anlagen of which each contribute only one cirrus [29].

from two rightmost anlagen. One dorsal kinety develops from right marginal row. More than one cirrus left of ACR. Usually two cirri right of ACR. Transverse cirri longitudinally arranged, usually originating from single anlage. Caudal cirri present (Fig. 21, 23, 24, 62). Species assignable: *A. illuvialis* n. sp. and *A. atypicus* (Hemberger, 1985) Foissner, 1988. A complete description of the morphogenesis in *A. atypicus* is not available, thus family assignment is uncertain (see discussion above).

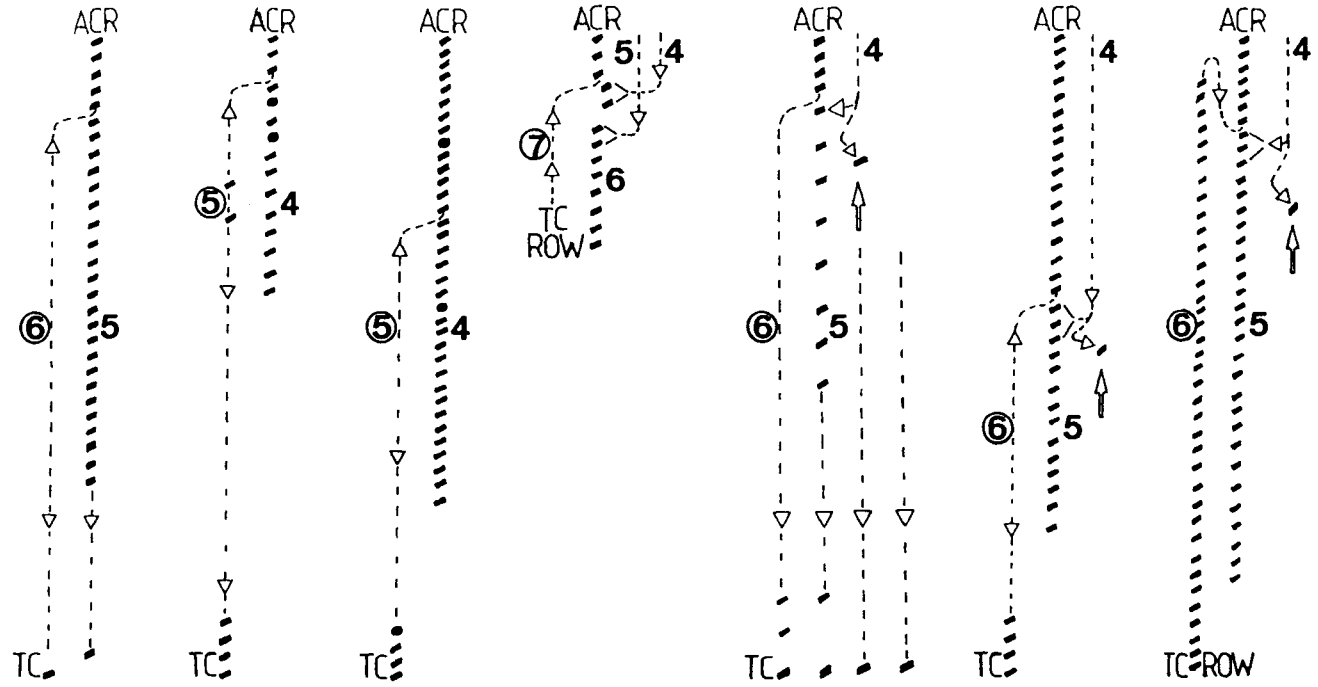
Genus *Paramphisiella* Foissner, 1988. The ACR originates from two rightmost anlagen. One cirrus left of ACR. Transverse cirri longitudinally arranged, originate from single anlage. Caudal cirri present. Single species, *P. caudata* (Hemberger, 1985) Foissner, 1988 (Fig. 63).

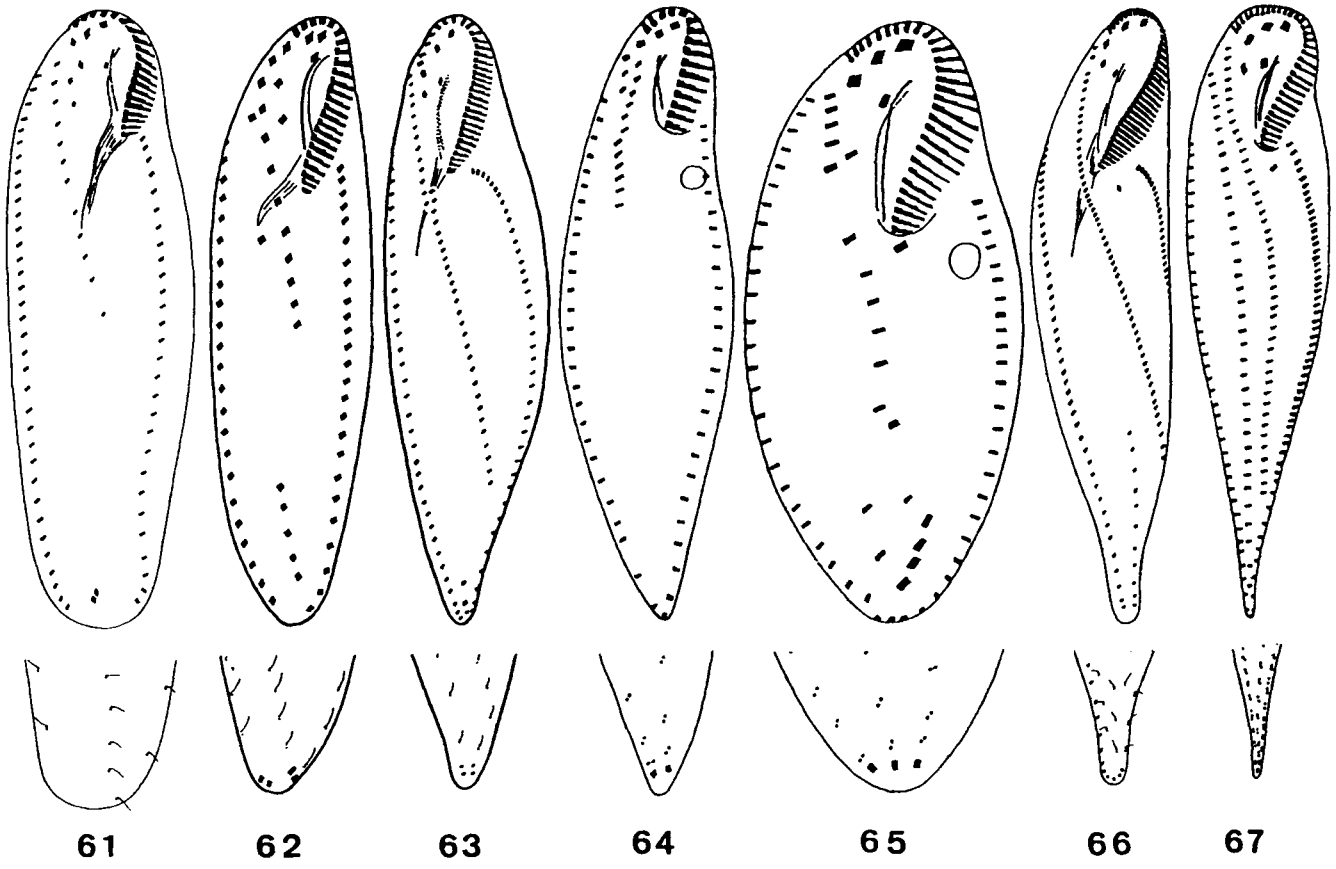
Genus *Paragastrostyla* Hemberger, 1985. The ACR originates from three rightmost anlagen. More than one cirrus left of ACR. Caudal cirri present, transverse cirri absent (Fig. 64).

→

Fig. **61–67**. Summary of data from the seven genera we assign to the family Amphisiellidae, based on the present investigations and on data from literature [3, 12, 14, 27, 29] (Hemberger, 1982 dissertation). The upper series of figures shows the origin of the amphisiellid median cirral row (ACR), the postperistomial cirrus (white arrows), and the transverse cirri (TC). Cirri are drawn in their final position, i.e. after migration. Dotted arrows indicate migration of cirri during and after cytokinesis. No or almost no migration of cirri occurs in the second anlage from right. Ventral anlagen (numbers 4–7) during divisional morphogenesis are designated as usual, those encircled are the rightmost anlagen. Note that cirri of the rightmost anlage invariably form the anterior segment of the ACR, whereas those of the second anlage from right form its posterior segment. This indicates homology in the development of the median cirral row in the genera depicted. The middle series of figures shows the ventral cirral pattern of interphase specimens. The bottom series depicts the posterior dorsal infraciliature, showing the absence of caudal cirri in *Amphisiella*. **61**. The rightmost anlage 6 forms the anterior segment of the ACR, whereas the second anlage (5) from right forms its posterior segment. The posteriormost cirri from both anlagen migrate posteriorly and become obliquely arranged transverse cirri (in *Amphisiella marioni* [29], type of the genus, contribute more than two anlagen to the transverse cirri). **62**. The anterior portion of the rightmost anlage 5 forms the anterior segment of the ACR, whereas the posterior segment of the ACR is formed by all cirri of the second anlage (4) from right. Several cirri of anlage 5 remain right of the ACR, whereas the posterior portion of this anlage forms longitudinally arranged transverse cirri (cp. Fig. 18, 20, 23, 24). **63**. The ACR and the transverse cirri are formed as described in *Amphisiellides,* but no cirri remain right of the ACR (cp. Fig. 36, 39). **64**. All cirri of the rightmost anlage 7 form the anterior segment of the ACR; this genus thus lacks transverse cirri. Anlage 5 builds the middle segment of the ACR; anlage 4 is possibly also involved in this process. All cirri of anlage 6 become the posterior segment of the ACR. **65**. The anterior portion of the rightmost anlage 6 forms the anterior segment of the ACR, the anterior portion of the second anlage (5) from right forms the posterior segment of the ACR. The first cirrus of the third anlage (4) from right forms the middle segment of the ACR, whereas the second cirrus of this anlage becomes the postperistomial cirrus. Posterior cirri of the rightmost and its neighboring anlagen form the transverse cirri. **66**. The ACR and the postperistomial cirrus are constructed as described in *Gastrostyla,* whereas the transverse cirri originate from the rightmost anlage 6 only and are thus longitudinally arranged (cp. 45, 46). **67**. The ACR and the postperistomial cirrus are constructed as described in *Gastrostyla.* However, the posterior segment of the rightmost anlage 6 does not migrate and forms a distinct second ventral row which is, in fact, a row of longitudinally arranged transverse cirri.

Amphisi-ella | Amphisi-ellides | Param-phisiella | Para-gastrostyla | Gastro-styla | Hemi-amphisiella | Pseudo-uroleptus





Single species, *P. lanceolata* Hemberger, 1985 (description of morphogenesis in the Hemberger dissertation, 1982). The alignment of the ACR is least perfect in this species; data indicate that an additional anlage may be involved in forming the ACR.

Genus *Gastrostyla* Engelmann, 1862. The ACR originates from three rightmost anlagen. Two dorsal kineties develop from right marginal row. One or two postperistomial cirri originate from third anlage from right. One cirrus left of ACR. Transverse cirri obliquely arranged, originate from more than two anlagen. Caudal cirri present (Fig. 65). Species assignable: *G. steinii* Engelmann, 1862 (description of morphogenesis in [28] and in the Hemberger dissertation, 1982).

Genus *Hemiamphisiella* Foissner, 1988. The ACR originates from three rightmost anlagen. Usually one postperistomial cirrus develops from the third anlage from right. One cirrus left of ACR. Transverse cirri longitudinally arranged, originate from single anlage. Caudal cirri present (Fig. 49–55, 66). Single species, *H. terricola* Foissner, 1988.

Genus *Pseudouroleptus* Hemberger, 1985. The ACR originates from three rightmost anlagen. Usually one postperistomial cirrus develops from third anlage from right. One cirrus left of ACR. Transverse cirral row nearly as long as body, parallels ACR, originates from single anlage. Caudal cirri present (Fig. 67). Single species, *P. caudatus* Hemberger, 1985 (description of morphogenesis in Hemberger 1982).

Phylogeny of amphisiellid ciliates. The seven genera assigned by us to the Amphisiellidae have a rather similar interphase morphology and construct the median cirral row in a complex and very likely homologous manner (Fig. 61–67). We thus expected that it should be easy to elucidate their phylogeny with Hennig's cladistic method [1, 20]. Unfortunately, all trials to find convincing synapomorphies for most generic groups failed, possibly because of the occurrence of many convergencies which make determination of the character states (apomorphies, plesiomorphies) extremely difficult. To mention only two main problems: The number of anlagen comprising the ACR is very likely correlated with the occurrence of a postperistomial cirrus formed by a complicated cirral migration (Fig. 65–67). Unfortunately, these two characters do not completely match and thus one has to assume that the postperistomial cirrus has been secondarily lost in *Paragastrostyla* (Fig. 64). Likewise, dorsomarginal rows, independent primordia, and development of transverse cirri from more than one anlage varied rather arbitrarily in the schemes examined.

Considering these problems, we even cannot exclude the possibility that our family character, viz. the specific mode of forming the median cirral row, evolved more than one time. Apparently, even very detailed interphase and ontogenetic data are insufficient to puzzle out the bewildering evolutionary paths in amphisiellid (and other!?) hypotrichs. Complementary methods, like molecular markers and transmission electron microscopy of dividing cells must be applied.

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Randomly Amplified Polymorphic DNA (RAPD) and Isoenzyme Analysis of *Trypanosoma rangeli* Strains

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ABSTRACT. Sixteen *Trypanosoma rangeli* strains were compared by isoenzyme and randomly amplified polymorphic DNA (RAPD) analysis. Eight strains were isolated from either *Rhodnius prolixus* or *Homo sapiens* from Honduras, Colombia and Venezuela. Another eight strains were isolated from either *Panstrongylus megistus* or the rodent *Echimys dasythrix* from the State of Santa Catarina, southern Brazil. All six *T. rangeli* strains isolated from *P. megistus* were co-infections with *Trypanosoma cruzi*, demonstrating an overlap of the sylvatic cycles of these parasites and that the accurate identification of species is of utmost importance. Both isoenzyme and RAPD analysis revealed two distinct groups of *T. rangeli* strains, one formed by the strains from Santa Catarina and the other, by the strains from Honduras, Colombia and Venezuela. With the five enzymes used, all the strains from Santa Catarina had identical profiles which overlapped with those of the other regions only in the pattern obtained with malic enzyme. Analysis of 138 RAPD bands by means of an unweighted pair group method analysis (UPGMA) phenogram using the Dice similarity coefficient allowed the separation of the two groups based on their divergence at a lower level of similarity than the phenon line. We show that the identification of *T. cruzi* and *T. rangeli* in naturally mixed infections is readily achieved by either RAPD or isoenzyme analysis.

Supplementary key words. Genetic variation, isoenzymes, randomly amplified polymorphic DNA analysis (RAPD), phenogram, *Trypanosoma cruzi, Trypanosoma rangeli.*

TRYPANOSOMA rangeli, first described by Tejera in 1920 [17], is a trypanosome species that infects man in Central and South America where it can be found in mixed infections together with *T. cruzi* in both invertebrate and vertebrate hosts. While *T. cruzi* causes Chagas' disease, *T. rangeli* is considered nonpathogenic to man [4]. The high cross reactivity between *T. cruzi* and *T. rangeli* in different immunological assays is a major problem in the diagnosis of chronic Chagas' disease in those areas where both species are present [7].

The first unequivocal report of *T. rangeli* in Brazil was in the Amazon Basin [9]. More recently, the organism has also been isolated in the south of the country in the State of Santa Catarina [15]. Isoenzyme analysis of the single isolate reported from Santa Catarina suggested that it was distinct from *T. rangeli* from Honduras but was identified as being of the same species on the basis of its development in the insect vector, transmission to mice by bite, WGA lectin agglutination and complement lysis [15].

In this communication we report a genetic comparison of eight strains of *T. rangeli* from Santa Catarina, Brazil; two from Venezuela; three from Colombia and three from Honduras by means of isoenzymes and randomly amplified polymorphic DNA (RAPD) [16, 20, 21, 22]. RAPD are produced using the polymerase chain reaction (PCR) with arbitrarily chosen primers that are annealed to the template DNA (derived from the parasite) at low temperatures. The amplified DNA fragments represent anonymous regions distributed randomly throughout the genome of the parasite and provide a fingerprint of the isolate or species being studied. Phylogenetic relationships between organisms are estimated by measuring the number of amplified DNA fragments in common.

The results of both techniques confirm that *T. rangeli* from Brazil is genetically distinct from *T. rangeli* from Honduras, Colombia and Venezuela. In addition, the studies emphasize that RAPD represent a powerful means of identifying trypanosome strains and species in both single and mixed infections.

MATERIALS AND METHODS

Parasites. Sixteen *T. rangeli* strains from different geographical regions were studied (Table 1). The six strains (SC-66, SC-67, SC-68, SC-70, SC-71 and SC-72), isolated from *Panstrongylus megistus* were found in mixed *T. cruzi/T. rangeli* infections. The purification of *T. rangeli* was achieved by infecting *Rhodnius domesticus* and transmitting the parasite to mice via the bite of the infected insects. Two *T. cruzi* strains (SC-69 and SC-73) were isolated from *P. megistus* from the same geographical region by xenoculture [2]. The parasites were identified as being *T. cruzi* or *T. rangeli* on the basis of their ability to be transmitted from triatomines to mice via the anterior route [15]. Confirmatory studies on the course of infection in these hosts, isoenzyme patterns, WGA lectin agglutination as well as sensitivity of epimastigote culture forms to complement lysis were also undertaken as previously described [15].

All parasites were grown in LIT medium at 28° C, washed 3 times in PBS pH 7.4, collected by microcentrifugation at 13,000 *g* for 10 min at room temperature and stored at −70° C until use.

Isoenzyme analysis. Pellets of parasite material were submitted to osmotic lysis in an enzyme stabilizer (2 mM dithiothreitol, 2 mM ϵ- aminocaproic acid and 2 mM Na_2-EDTA, pH 7.0) at 4° C, at the ratio 1:1. The lysates were then centrifuged at 15,000 *g* for 1 h at 4° C and the supernatant, (enzymatic extract) cryopreserved in liquid nitrogen. Isoenzymes were separated by refrigerated, horizontal thin-layer starch gel electro-

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