

Further Studies on Morphology, Infraciliature and Morphogenesis of *Pseudouroleptus caudatus* Hemberger, 1985 (Ciliophora, Hypotrichida).

*Estudios adicionales sobre la Morfología, Infraciliatura y Morfogénesis de
Pseudouroleptus caudatus Hemberger, 1985 (Ciliophora, Hypotrichida).*

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ABSTRACT

The oxytrichide hypotrich, *Pseudouroleptus caudatus* Hemberger, 1985 was found in sediment samples from the Guadarrama river (Central Spain). Its morphology, infraciliature, morphogenesis and reorganization has been studied, using observations of living cells and protargol impregnation. The morphology and morphogenesis of the European population are very similar to those of the type population from Peru. 2% methylcellulose induces *P. caudatus* to shed fragments or a capsule shaped like itself. Its morphogenesis is very similar to that of *Hemiamphisiella terricola*, especially in generating a postoral cirrus. A detailed description on the morphology, infraciliature, morphogenesis, reorganization and ecology are provided.

Key Words: Capsule shedding; Ciliophora; Hypotrichida; Morphogenesis; Morphology; *Pseudouroleptus caudatus*.

RESUMEN

El hipotrico oxitriquido, *Pseudouroleptus caudatus* Hemberger, 1985, fue encontrado en el sedimento del río Guadarrama (España). Su morfología, infraciliación, morfogénesis y reorganización han sido estudiada in vivo y utilizando el método del protargol. La morfología y morfogénesis de la población europea es muy similar a la de la población tipo del Perú. Un 2% de metilcelulosa induce en *P. caudatus* la formación de una cápsula envainante. Su morfogénesis es muy similar a la de *Hemiamphisiella terricola*, especialmente en la formación del cirro postoral. Una detallada descripción de su morfología, infraciliación, morfogénesis, reorganización y ecología son realizadas.

Palabras claves: Cápsula envainante; Ciliophora; Hypotrichida; Morfogénesis; Morfología; *Pseudouroleptus caudatus*.

Introduction

The genus *Pseudouroleptus* was erected by Hemberger in 1985. It shows, according to Hemberger (1982, 1985), Eigner and Foissner (1994) and Petz and Foissner (1996) the following characteristics: The oral primordium originates in close contact with the ACR

(Amphisiellid Cirral Row). The ACR commences anlagen formation within-row and originates from the three rightmost anlagen. Usually, one postperistomial cirrus is developed from the third anlage from right. One cirrus left of the ACR is present. All dorsal kineties develop intrakinetally. At transverse cirral row, nearly as long as the body, parallels the ACR and originates from a single anlage. Caudal cirri present.

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The type species is *Pseudouroleptus caudatus* Hemberger, 1985. Hemberger (1982) also described

its morphogenesis in his dissertation. The objective of our study is a more detailed description of its morphology, infraciliature and morphogenesis and to provide new data about its reorganization and ecology.

Material and methods

Pseudouroleptus caudatus was isolated from the sediment of the Guadarrama river 4 km far from Cercedilla (4°3' W, 40°45' N). This site is 1188 m above sea-level.

The species was maintained in a commercial mineral water (Fontbella) enriched with sterile rice grains.

The *in vivo* studies were made with a phase contrast microscope impeding movement using 2% methylcellulose. Its morphology and infraciliature were visualized by protargol impregnation (Wilbert, 1975; Foissner 1991) and scanning electron microscopy (Valbonesi and Luporini 1990).

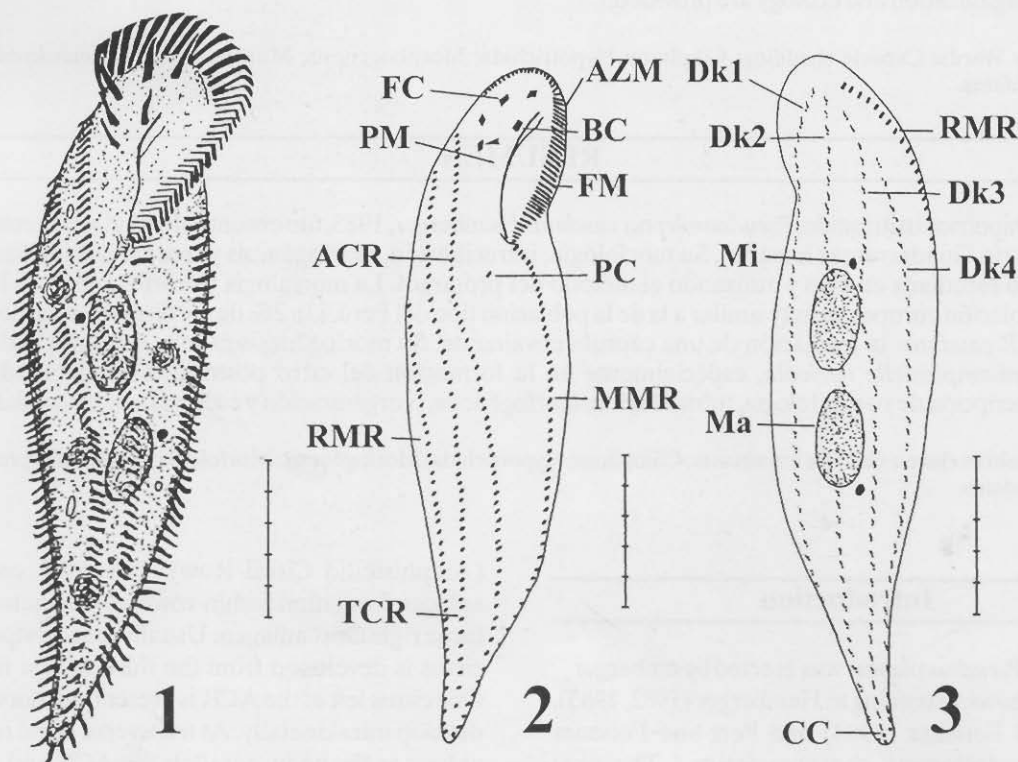
Attempts to establish pure cultures with usual methods failed. The biometrical characterization was based on 30 protargol impregnated specimens (Wilbert, 1975). Standard deviation and coefficient of variation were calculated according to statistics textbooks.

Terminology is according to Borror (1972), Corliss (1979), Foissner (1982), Hemberger (1982), Eigner & Foissner (1994) and Petz & Foissner (1996).

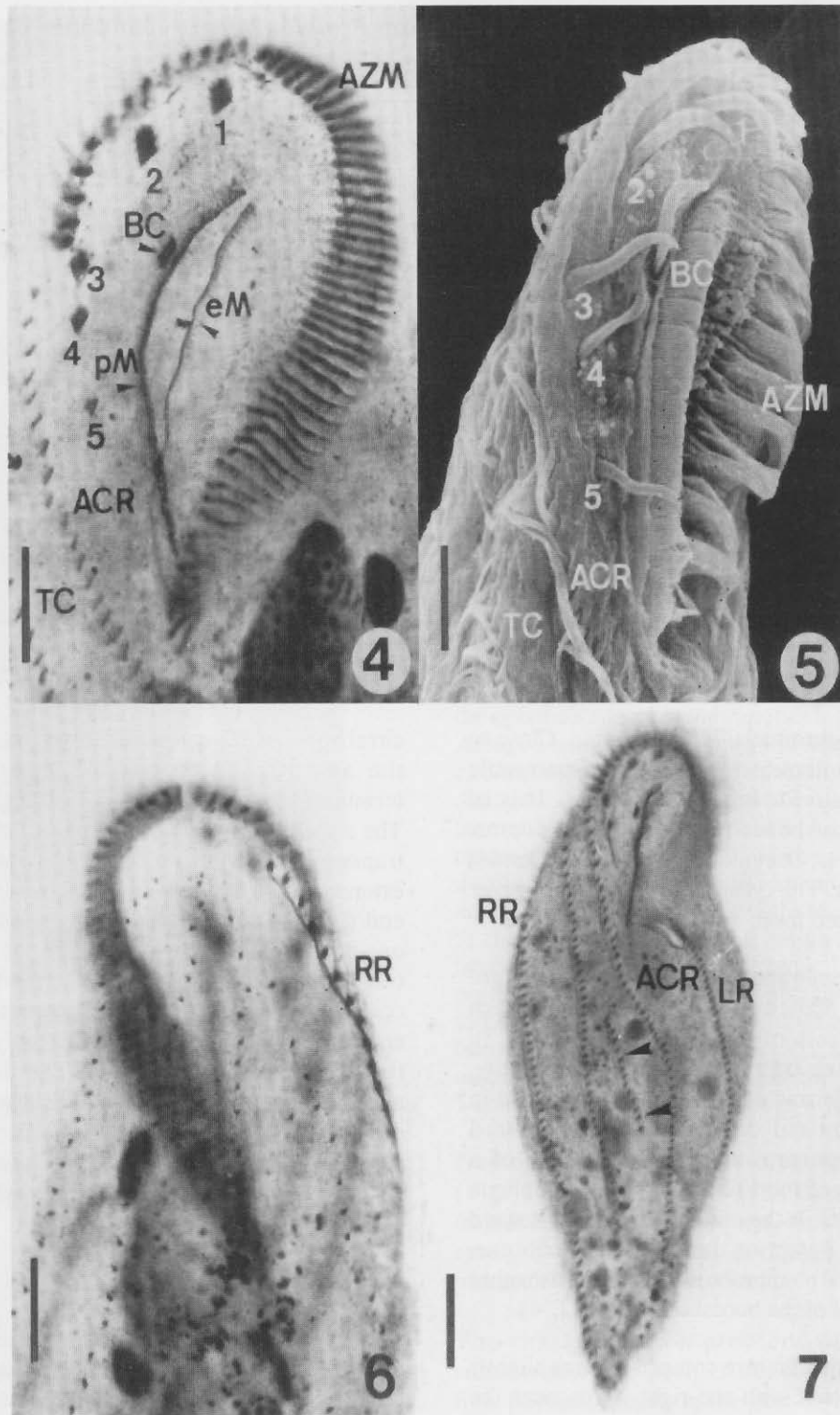
Results

Redescription of the morphology of *Pseudouroleptus caudatus* (Fig. 1 to 7; Table 1)

Size *in vivo* about 150-270 x 40-70 μm . The main biometrical characteristics of *P. caudatus* are shown in Table 1. Body elongated, with rounded anterior and tapered posterior end, flexible, flattened dorso-ventrally and slightly twisted along main body axis (Fig.1,2,7).



Figs. 1 to 3. *Pseudouroleptus caudatus* alive (1) and after protargol impregnation (2,3). 1. Ventral view of a typical specimen. 2,3. Infraciliature, ventral and dorsal view of the specimens. ACR - amphisiellid cirral row; AZM - adoral zone of membranelles; BC - buccal cirrus, CC - caudal cirri; DK 1-4 - dorsal kineties; EM - endoral membrane; FC - frontal cirri; LMR - left marginal row; Ma - macronucleus; Mi - micronucleus; PC - postperistomial cirrus, PM - paroral membrane; RMR - right marginal row; TCR - transverse cirral row. Arrow head marks cirrus left of ACR. Scale bar division 10 μm .



Figs. 4 to 7. *Pseudouroleptus caudatus*, somatic and oral infraciliature of morphostatic cells after protargol impregnation (4,6,7) and in scanning electron microscope (5). 4, 5, 7. Ventral cirral pattern of interphase specimens: 1,2,3 are frontal cirri; 4,5 cirri left of ACR, which produce anlage 3. 6. Anterior dorsal body portion. 7. Arrowhead marks out an additional midventral row. ACR - amphiellid cirral row; AZM - adoral zone of membranelles; BC - buccal cirrus, CC - caudal cirri; eM; endoral membrane; FC; frontal cirri; LR - left marginal row; Ma - macronucli; Mi - micronucli; PC - postperistomial cirrus, pM - paroral membrane; RR - right marginal row; TC - transverse cirral row. Scale bar division 10 μ m.

Table 1. Morphometric data from *Pseudouroleptus caudatus*.

Character	X	M	SM	SE	CV	Min	Max	n
Body, length	217.1	220	27.9	5.1	12.8	160	280	30
Body, width	66	65	11.4	2.0	17.3	45	90	30
Adoral zone of membranelles, length	67.9	65.5	9.0	1.6	13.3	55	85	30
Macronuclear segment, length	43	45	4.6	0.8	10.8	35	50	30
Macronuclear segment, width	21.8	22	2.3	0.4	10.7	18	25	30
Micronucleus, length	10.2	10	1.1	0.2	10.7	8	12	30
Micronucleus, width'	7.6	8	1.2	0.2	16.8	6	10	30
Macronuclear segments, number	2	2	0	0	0	2	2	30
Micronuclei, number	2.6	2	0.9	0.2	35.8	2	5	30
Adoral membranelles, number	49.3	49.5	5.1	0.9	10.3	40	60	30
Right marginal row, number of cirri	59.9	60	4.9	0.9	8.2	48	68	30
Left marginal row, number of cirri	52.0	52.5	5.5	1.0	10.6	39	62	30
Frontal cirri, number	3	3	0	0	0	3	3	30
Buccal cirri, number	1	1	0	0	0	1	1	30
Cirri left of the ACR, number	1.2	1	-	-	-	1	2	30
Transverse cirral row, number of cirri	58.3	58	4.8	0.8	8.2	47	68	30
Amphisiellid cirral row, number of cirri	51.8	54	5.9	1.0	11.4	40	63	30
Postperistomial cirri, number	1.2	1	-	-	-	0	2	30
Caudal cirri, number	3.4	3	-	-	-	3	4	20
Dorsal kineties, number	4.1	4	-	-	-	4	5	30

Data are based on protargol-impregnated specimens. Measurements in μm . X - arithmetic mean; M - median; SD - standard deviation; SE - standard error of arithmetic mean; CV - coefficient of variation; Min- minimum; Max - Maximum; n - sample size.

Two ellipsoidal macronuclei left of median. Close to them 2-5 ovoid micronuclei (Fig.1,3). Contractile vacuole near posterior left side of AZM. It is of channel type and can be seen *in vivo*. The whole cortex is full of colourless, irregularly distributed granules 1-1,3 μm diameter. The cytoplasm is usually brown or grey due to ingested food.

Adoral zone of membranelles extends about one third of body length, composed of 40-60 membranelles, each consisting of 4 rows of kinetosomes (Fig. 4,5,7). Endoral membrane on right side of buccal cavity, consists of a single row of kinetosomes anchored in dorsal wall of buccal cavity, continues toward cytostome. The paroral membrane consists of a longitudinal series of short (2-6 kinetosomes), oblique rows that are longer in the middle and shorter toward the anterior and posterior ends of the membrane. Endoral and paroral membrane usually cross each other in the anterior half of the buccal cavity (Fig. 2, 4).

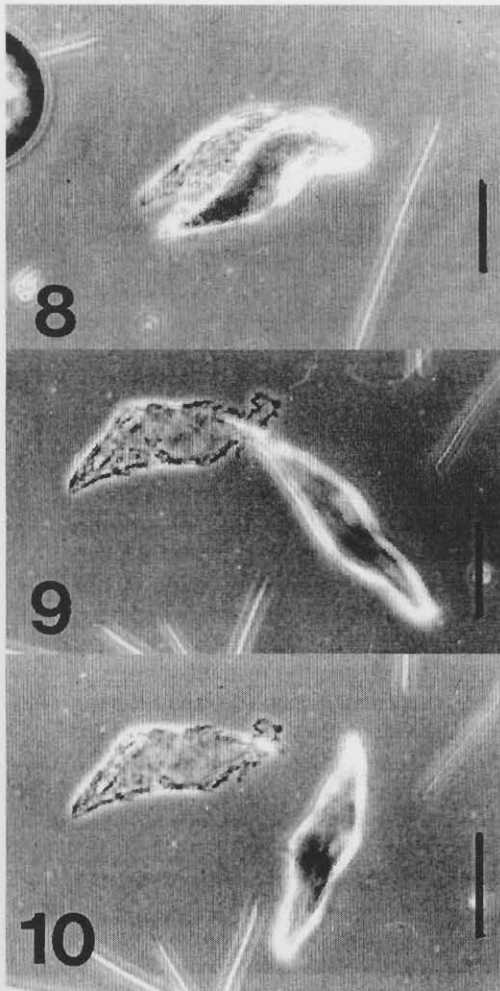
The somatic ventral ciliature comprises three slightly enlarged frontal cirri with the right cirrus near the distal end of the adoral zone of membranelles. (Fig. 2,4,7). One or two cirri occur right of the ACR in the frontal field. Buccal cirrus near anterior end of undulating membranes. Close beneath the peristomial vertex apperars usually one, rarely two, post-peristomial cirri; rarely it is lacking. The amphisiellid

cirral row (ACR) commences near the distal end of the adoral zone of membranelles and usually terminates toward the left side of the posterior end. The rightmost ventral row of cirri is in fact a row of transverse cirri which is longitudinally arranged and extends from the anterior area to the tapered posterior end (Fig. 2, 7). Some impregnated specimens show one additional short row between the ACR and the transverse cirral row (Fig. 7). The right marginal row commences on the dorsal surface near the anterior end of the body (Fig.6); both marginal rows extend to the posterior end of the cell. The dorsal cilia are arranged in four rows, each consisting of 30-55 dikinetids. 3-4 caudal cirri on posterior end of dorsal side (Fig. 3). This species can perform capsule shedding when immersed in 2% methylcellulose (Fig. 8,10) (See Discussion).

Occurrence and ecology

Pseudouroleptus caudatus is a benthonic and omnivorous species which feeds on bacteria, ciliates (e.g. *Chilodonella uncinata*) and diatoms.

It moves slowly through the detritus and can form spherical resting cysts, 50-60 μm in diameter. It is probably a cosmopolitan species as it was originally described in soil samples from Peru. This species has been found in association with ciliate species indicating



Figs. 8 to 10. *Pseudouroleptus caudatus* leaving the capsule shedding.

β -mesosaprobic water conditions such as, *Paramecium aurelia*, *Halteria grandinella* and *Chilodonella uncinata*.

Divisional morphogenesis (Fig. 11-23).

The nuclear apparatus and the marginal rows divide in the usual way and thus demand no further comment.

Stage 1: (Fig.11, 18). Stomatogenesis commences near the middle portion of the ACR. The ACR and the postperistomial cirrus appear unchanged.

Stage 2: (Fig.12). A field of basal bodies develops along the left edge of the ACR and extends to the parental peristomial vertex. The postperistomial cirrus has apparently dissolved and incorporated in this field. So, two portions can be observed in this field: an anterior portion which includes the postperistomial cirrus and a long posterior area.

Stage 3: (Fig.13, 19). The oral primordium splits: the large posterior field differentiates adoral membranelles at its anterior end, the small anterior portion begins to form the opisthe's anlagen 1-3. The buccal cirrus disorganizes to a streak of basal bodies.

Stage 4: (Fig.14, 20). The formation of the opisthe's adoral zone of membranelles proceeds posteriorly. 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 streak (proter's anlage 3). The proter's anlage 4 very likely develops de novo. The cirri in the central portion of the ACR disorganize and form a streak of basal bodies (proter's and opisthe's anlage 5). The rightmost streak (proter's and opisthe's anlage 6) is either generated also by disorganized cirri of the ACR or develops de novo. All cirral streaks align and lengthen. Six anlagen each in the proter and opisthe are recognizable and organize in the same manner.

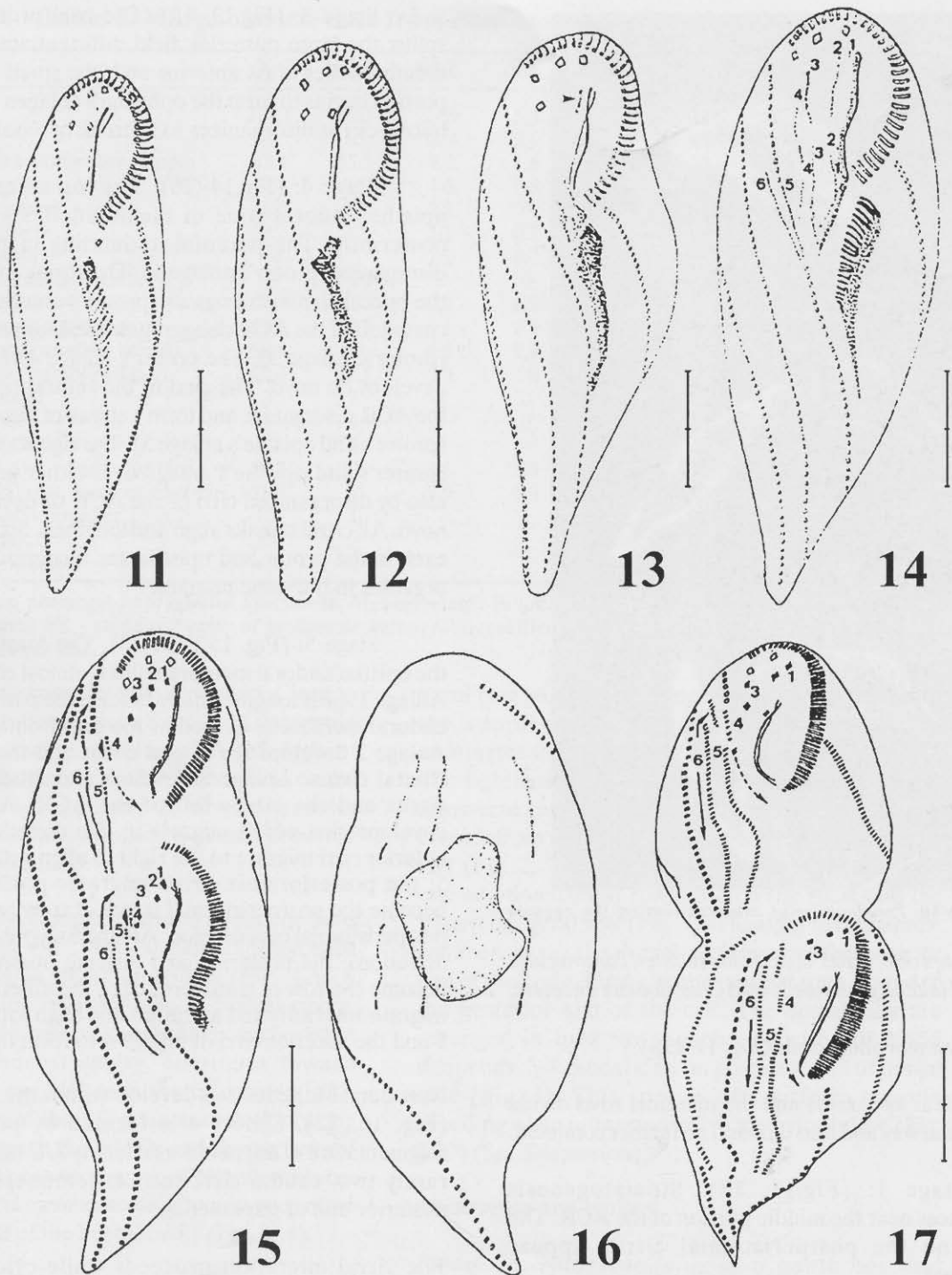
Stage 5: (Fig. 15,17,21,23). The formation of the opisthe's adoral membranelles is almost complete. Anlage 1 splits longitudinally to form the paroral and endoral membrane as well as the first frontal cirrus. Anlage 2 develops the buccal cirrus and the second frontal cirrus. Anlage 3 develops the third 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 to align with anlage 5; the posteriormost cirrus migrates posteriorly to become the postperistomial cirrus. Rarely two or no postperistomial cirri develop. Anlage 6 migrates in two directions: the posterior cirri migrate posteriorly to become the row of transverse cirri; the anterior cirri migrate anteriorly and to the left and align with anlage 5 and the anterior cirri of anlage 4 to form the ACR.

New dorsal kineties 1-3 develop within the old one (Fig. 16, 22). Kinety 4 is formed by posterior fragmentation of the new dorsal kinety 3. Usually one, rarely two, caudal cirri each differentiate at the posterior end of the kineties.

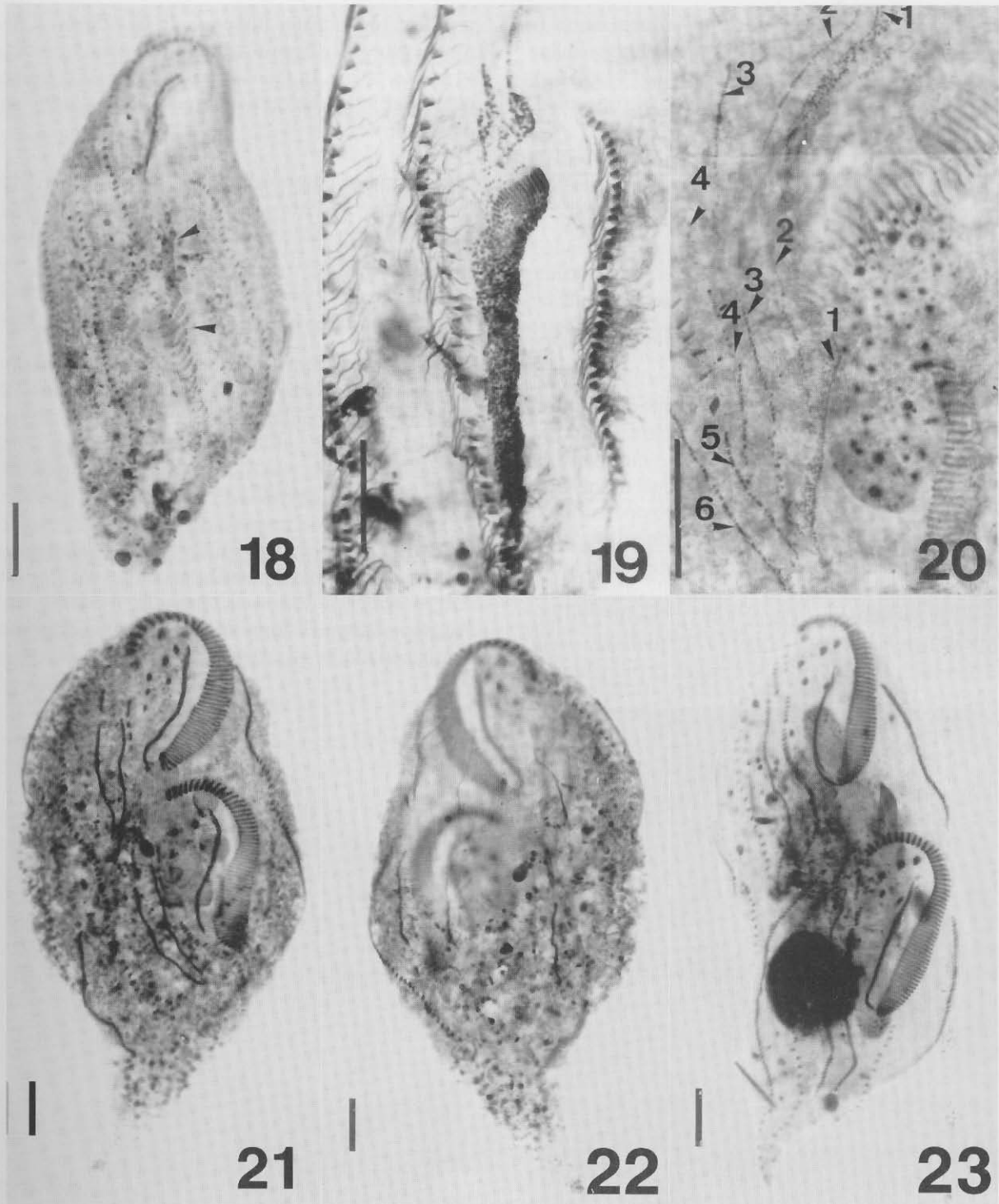
The cirral migration proceeds while cytokinesis commences. The postperistomial cirrus reaches its final position usually in the post-divider.

Reorganisation (Fig. 24, 25).

Physiological regeneration largely resembles the development of the proter. Reorganisers are distinguishable from dividers by the fact that the



Figs. 11 to 17. Morphogenesis of *Pseudouroleptus caudatus*, protargol impregnations (ventral views, except Fig. 16). Fig. 11. Morphogenesis commences with a proliferation of basal bodies near the middle portion of the amphisiellid cirri row. Fig. 12. The oral primordium elongates and extends over the postperistomial cirrus. Fig. 13. Early dividers showing formation of cirral anlagen in the opisthe. Differentiation of adoral membranelles commences at the anterior end of the oral primordium. Arrow marks disintegrating buccal cirrus. Fig. 14. Middle divider showing six streaks, the former 1-4 anlagen in the same way both proter and opisthe. Anlagen 5 and 6 develop from the amphisiellid cirral row in both filial cells; anlage 6 possibly develops de novo. Fig. 15. 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 (short arrow) to align with most cirri of anlage 4 (triangles). The posterior portion of anlage 6 becomes the transverse cirral row (long arrow). The posterior cirri of anlage 4 become the postperistomial cirrus (dotted arrows). Fig. 16. New dorsal kineties develop within old ones. Fig. 17. Cirral migration as described in figure 15 proceeds during cytokinesis. Numbers 1-6 indicate cirral anlagen. Scale bar division 25 μ m.



Figuras.18-23. *Pseudouroleptus caudatus* divisional morphology after protargol impregnation. Fig.18. Early divider showing oral primordium close to ACR (arrowheads). Fig. 19. The oral primordium splits in two fields. Fig.20. The numbers indicate the six streaks in proter and opisthe. Fig.21. Late divider with cirral segregation. Fig.22. Dorsal view of late divider with new dorsal kineties and caudal cirri developing at the posterior end of the new dorsal kineties. Fig.23. Late divider showing migration of cirri and comencing cytokinesis.

proximal portion of the parental adoral zone is reorganised. Six anlagen develop and organise similar as in the proter of cells in division (Fig. 24, 25). A complete sequence, however, has not been observed.

Discussion

Pseudouroleptus is one of the genera that were included by Eigner & Foissner (1994) and Petz & Foissner (1996) in the Family Amphisiellidae and recently by Eigner (1997) in the Family Oxytrichidae ("Neokinetal 3" anlagen and long primary primordia present). The morphology of European populations of *Pseudouroleptus caudatus* matches the description given by Hemberger (1985) based on Peruvian specimens. The only differences we can mention are: (i) Presence of a second cirrus on the left of the CR. (ii) Hemberger described one or no postperistomial cirrus, we observed also two such cirri. (iii) *Pseudouroleptus caudatus* can shed fragments or even show a capsule shedding as it occurs in other ciliate species as *Blepharisma* (Giese, 1973), *Stentor* (Tartar, 1961) or *Tetrahymena* (Tiedtke, 1976). In *Blepharisma* and *Stentor* it is not yet known which cell organelles

produce the capsule material. In *Tetrahymena*, the mucocysts were found to produce this substance (Tiedtke, 1976). In *P. caudatus* the capsule is formed or produced by the cortical granules, which are very likely mucocysts.

The morphogenetic processes fits the description given by Hemberger (1982). Curiously, Martín *et al.* (1981) already described the morphology and morphogenesis of a specimen identified by them as *Uroleptus* sp. that also match the features given for *Pseudouroleptus caudatus*.

The literature mentions four more *Pseudouroleptus* species, viz. *P. terrestris* (Hemberger, 1985), *P. humicola* (Gellert, 1956, Hemberger, 1982), *P. procerus* (Berger & Foissner, 1987) and *P. buitkampii* (Foissner, 1982, Berger & Foissner, 1987). However, morphogenetical data on these species are lacking and thus their systematic position remains doubtful. If we compare the two genera *Hemiamphisiella* and *Pseudouroleptus*, both included in the family Oxytrichidae, with the two single species, *Hemiamphisiella terricola* and *Pseudouroleptus caudatus*, the fundamental difference consists in the length of the transversal cirral row: in

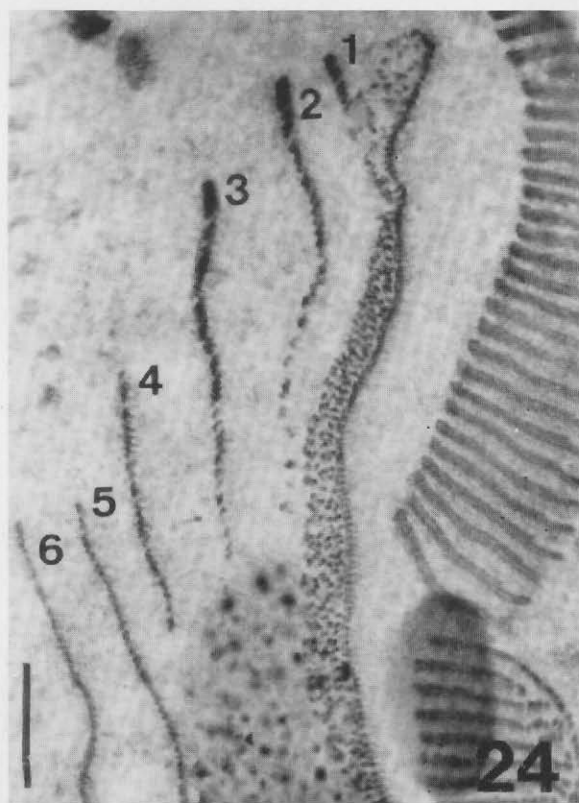


Fig.24. Regeneration. Six anlagen can be observed.

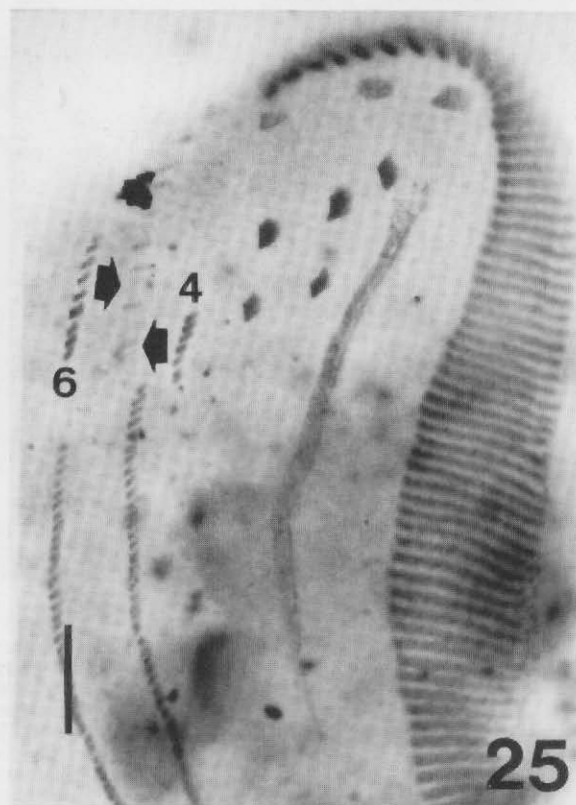


Fig.25. Late state of regeneration showing the migration of anlagen 4 and 6 (arrowheads).

P. caudatus the two nearly body long ventral rows, while in *H. terricola* the middle part of the right most ventral row is missing. The morphogenetic origin of this TCR is, however, the same for both species (from anlage 6) as well as for the other anlagen.

An important difference concerns the origin of the two left dorsal kineties adjacent to the right marginal row: in *P. caudatus* they are formed by splitting of one DK, and thus this species has four DK in interphase, whereas in *H. terricola* no such process occur and only three DK can be observed in morphostatic specimens.

Acknowledgements

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