Acta Protozool. (2006) 45: 41 - 52

ACTA
PROTOZOOLOGICA

Observation on a Japanese Population of *Pseudoamphisiella alveolata* (Kahl, 1932) Song *et* Warren, 2000 (Ciliophora: Hypotrichida): Morphology and Morphogenesis

Xiaozhong HU[1] and Toshikazu SUZUKI[2]

[1]Laboratory of Protozoology, KLM, Ocean University of China, Qingdao, China; [2]Faculty of Fisheries, Nagasaki University, Bunkyo machi, Nagasaki, Japan

Summary. A population of *Pseudoamphisiella alveolata* was isolated from the coastal waters off Nagasaki, Japan. Its morphology and morphogenesis were studied by observations on organisms from life and following protargol impregnation. Morphometrical data of this form correspond well with those of the Chinese population, thus the validity of this species is confirmed. Its morphogenetic processes show a few that differ from those of *P. lacazei*, the type species of this genus, and main events are documented here: (1) The oral primordium of the opisthe and primary primordium of the fronto-ventral transverse cirri are possibly derived from an anarchic field of kinetosomes originating *de novo* on cell surface, and most ventral cirri do not contribute to the formation of these primordia; (2) The posterior portion of the parental adoral zone of membranelles is renewed *in situ* while the anterior part is retained by the proter; (3) Oblique streaks of FVT-anlagen in both dividers are generated from the division of primary primordium. Two buccal cirri are derived from the anterior two streaks of the FVT-anlagen; (4) The left marginal rows and dorsal kineties equally develop by "within proliferation"; the caudal cirri develop from the posterior end of each dorsal kinety anlage; (5) The anlagen for the right marginal rows originate parallel to FVT-anlagen; (6) So-called caudal cirri near the rightmost transverse cirri are actually migratory right marginal cirri. The present study demonstrates that morphogenetic data can be useful in differentiating species and genera with similar morphologies.

Key words: Hypotrichida, marine ciliate, morphology, morphogenesis, *Pseudoamphisiella alveolata.*

INTRODUCTION

Ontogenetic data have been widely used in the reconstruction of ciliate phylogenies (Corliss 1968). These data might be employed to separate morphologically similar taxa at genus or species levels (Foissner 1996). Recently studies showed that species and genera are often separated by minor - but none the less important - ontogenetic details (Wirnsberger *et al.* 1985; Hu and Song 2001a, b; Hu *et al.* 2000, 2003, 2004a, b; Blatterer and Foissner 2003).

The genus *Pseudoamphisiella* has been included in the recently erected family Pseudoamphisiellidae due to its unique cortical pattern in non-dividers and dividers (Song 1996, Song *et al.* 1997, Song and Warren 2000). Up to date, it comprises two forms: *P. lacazei*, the type species and *P. alveolata*. Hitherto, morphogenetic events have only been known for *P. lacazei*.

Address for correspondence: Xiaozhong Hu, Laboratory of Protozoology, KLM, Ocean University of China, Qingdao 266003, China; E-mail: xiaozhonghu@ouc.edu.cn

In the present paper, new data on the morphology and morphogenesis of a Japanese population of *Pseudoamphisiella alveolata* are provided. These observations are compared with those from its congener described by Song *et al.* (1997). This study shows how morphogenetic data might be useful in separating genera or species with very similar morphologies.

MATERIALS AND METHODS

Samples were collected from the coastal waters in the New Fishing Port of Nagasaki (32° 48'N; 129° 46'), Japan. Water salinity was about 34‰, water temperature 21-23°C and pH 8.1-8.2. After isolation, specimens were cultured in boiled seawater to which squeezed rice grains were added to enrich bacteria. Cells were observed in life using differential interference contrast microscope. Mainly saturated mercury bichloride solution was used to fix organisms. To reveal the infraciliature, the protargol silver staining method according to Wilbert (1975) was applied. Body shape of live cells was drawn from slides without coverslips. Drawings of stained specimens were carried out with the help of a camera lucida. To make plain the changes during morphogenetic processes, old cirri are depicted only by contour whereas the new ones are blocked. Measurements were performed at magnifications of 100-1250×.

Terminology and systematic arrangements are according to Corliss (1979), Foissner (1982) and Borror and Wicklow (1983).

RESULTS

Morphology of the non-divider

Prior to this investigation, *Pseudoamphisiella alveolata* has not been reported from East China Sea, Japan, although it has previously been isolated at least three times (Kahl 1932, Borror 1963, Song and Warren 2000).

Description (Figs 1-3, 19-33, 38; Table 1): Cell *in vivo* 100-200 × 40-80 µm, usually 150 × 40 µm, length to width ratio 2.5-4.5:1. Body generally elongated, rather fragile and contractile, thus variable in shape; when extended during motion, both ends rounded, and left and right margins distinctly sigmoidal (Figs 19, 25), when stationary cell outline oval. Dorsoventrally flattened about 2:1. Adoral zone of membranelles conspicuous, about 1/3 of cell length, with distal end bending posteriorly far onto right side and towards posterior of cell (Figs 3, arrows; 21, double-arrowheads; 24, arrowheads).

Cell surface covered by conspicuous, hyaline alveolar layer, about 3 µm thick and visible even under low magnification (Fig. 25, arrows). When viewed dorsally, this alveolar layer is seen to have irregular polygonal structure (Fig. 27). Within alveolar layer are sparsely arranged extrusomes which are bar-like, about 1.5-2 µm long (Fig. 20, arrows).

Cytoplasm usually grayish to dark grey when examined under dissecting microscope, due to presence of numerous granular inclusions 5 to 10 µm in size. Neither contractile vacuoles nor food vacuoles observed.

Cilia of adoral membranelles *ca* 13 µm long. Frontal and transverse cirri strong, about 15-20 µm in length. Other cirri *ca* 12-14 µm long. Marginal cirri bending towards ventral side or lying along the ventral grooves, not protruding from outline and not discernible when observed from dorsal aspect, except in the posterior region. Dorsal cilia about 3 µm long, derived from the base of the alveolar layer (Fig. 26, arrows). Caudal cirri (CC) close-set, normally lying along posterior margin (Figs 1, 3; 31, arrowheads).

Locomotion typified by continuous crawling on substrate or on bottom of Petri dish, reacting quickly when disturbed by contracting and remaining motionless for a short while.

Usually two (rarely 3) ellipsoidal macronuclear nodules positioned in center of body (Figs 2, 21; 30, arrows). One to six micronuclei, about 1-2 µm long, often within indentations of macronuclear nodules (Figs 2, arrows; 30, arrowheads).

Infraciliature Figs 1-3, 21, 28, 29, 31-33: Adoral zone of membranelles (AZM) composed of about 40 membranelles. Paroral membrane (PM, Figs 3; 28, arrowhead; 29, arrow; 33) conspicuously short, almost parallel to endoral membrane (EM, Figs 3; 28, double-arrowhead; 29, arrowhead). Constantly three large frontal cirri (FC; Figs 1; 21, arrowheads; 33, 38, arrows), of which the rightmost one is located posterior to the distal end of the AZM, and two buccal cirri (BC) separated from each other near mid-body of endoral membrane (Figs 3; 28, arrows; 33, arrowheads). Ventral row 1 (VR1) relatively short, terminating anteriorly at the rightmost frontal cirrus; anterior end of ventral row 2 (VR2) extending to buccal cirri. Highly developed, close-set transverse cirri (TC) arranged in a J-shaped row, terminating near the posterior end of body (Figs 1, 3, 21; 22, arrow; 29, 32). Posterior end of left marginal row (LMR) continues with row of caudal cirri (CC), which renders it difficult to determine where LMR terminates during interphase (Figs 1; 31, arrowheads). Right marginal row toward the center of the cell and parallel to ventral row 1, thus not convergent with row of

Table 1. Morphometrical characteristics of *Pseudoamphisiella alveolata* (Kahl, 1932) Song *et* Warren, 2000 from Nagasaki New Fishing Port. Data are based on protargol-impregnated specimens. Measurements in µm. CV - coefficient of variation in %, Max - maximum, Mean - arithmetic mean, Min - minimum, n - number of specimens examined, SD - standard deviation, SE - standard error of the mean.

Character	Min	Max	Mean	SD	SE	CV	n
Body length	93	150	113.7	12.95	2.59	11.4	25
Body width	45	96	68.2	15.01	4.75	22.0	10
Length of adoral zone of membranelles	32	43	37.3	3.44	0.69	9.2	25
Number of adoral membranelles	37	43	40.2	1.83	0.37	4.6	25
Number of frontal cirri,	3	3	3	0	0	0	25
Number of buccal cirri	2	2	2	0	0	0	25
Number of cirri in ventral row 1	11	15	12.7	0.79	0.16	6.2	25
Number of cirri in ventral row 2	10	15	12.0	1.10	0.22	9.2	25
Number of cirri in left marginal row	15	30	20.5	3.14	0.50	15.3	39
Number of cirri in right marginal row	12	16	13.0	0.89	0.18	6.8	25
Number of extra right marginal cirri	2	4	3.4	0.90	0.14	2.3	40
Number of caudal cirri	8	12	10.1	0.98	0.16	2.5	40
Number of transverse cirri	13	17	14.3	0.95	0.19	6.6	25
Number of dorsal kineties	9	12	10.8	1.09	0.36	10.1	9
Number of macronuclear nodules	2	3	2.1	0.27	0.04	0.7	40
Length of macronuclear nodule	14	30	20.0	4.13	0.83	20.7	25
Width of macronuclear nodule	7	25	13.6	3.95	0.79	29.0	25
Number of micronuclei	1	6	3.1	1.18	0.19	3.0	40

CC posteriorly. Nevertheless, a few extra, sparsely distributed right marginal cirri located nearby the rightmost caudal cirrus (Figs 1; 3, arrowheads; 21, arrows; 38, arrowheads). Fibrils from TC are highly developed and associated with those of ventral cirri (Fig. 29). Mostly dorsal kineties extending over entire length of body, with 2 or 3 kineties on lateral sides (Figs 1-3; 31, 32 arrows).

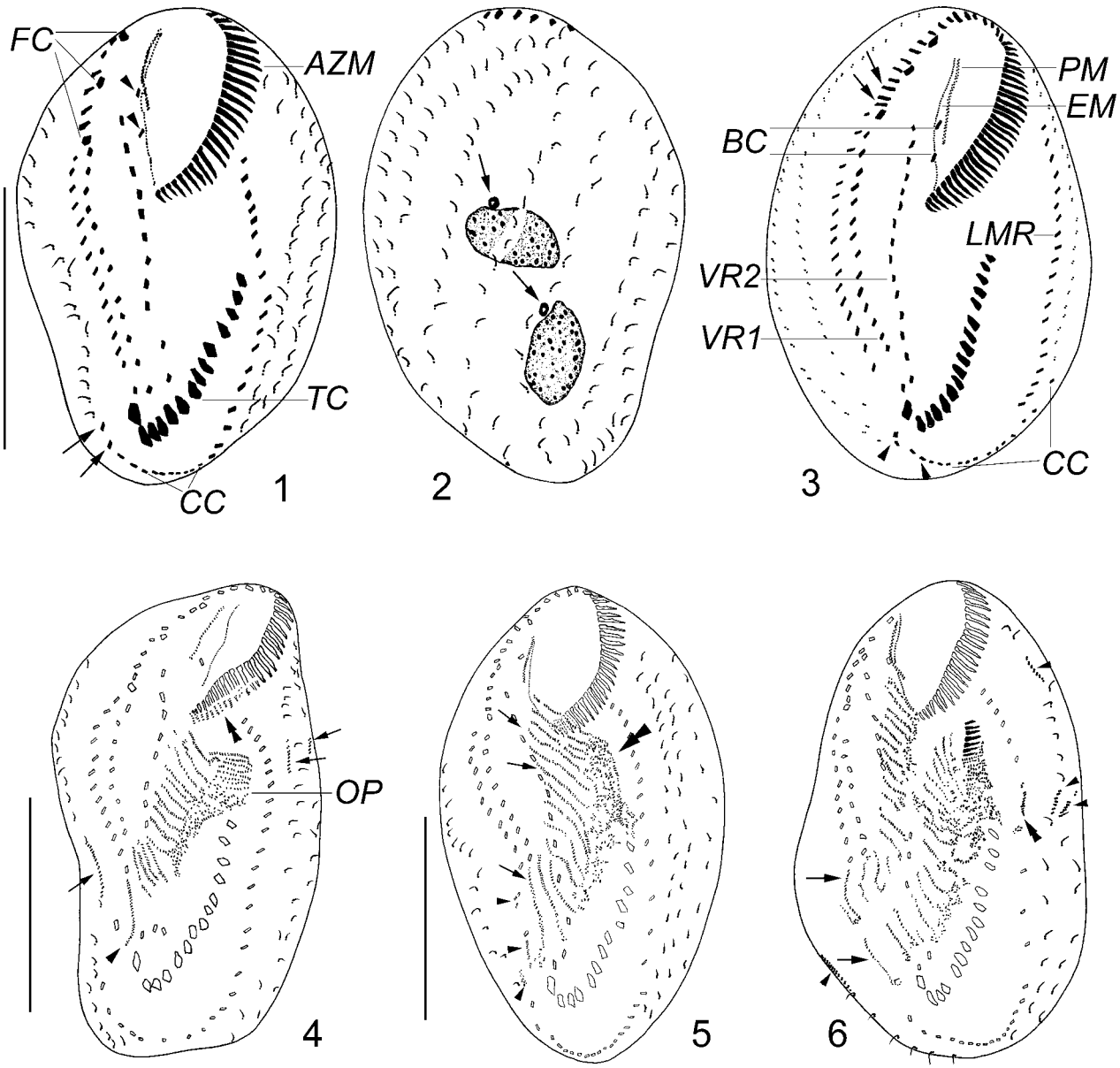
Divisional morphogenesis (Figs 4-18, 34-37, 39-49)

The first stage of morphogenesis was not observed. However, observations on an early stage of recognization (Figs 4, 35) allows us to deduce that morphogenesis seems to commence apokinetally with the appearance of loosely arranged basal bodies behind buccal cavity and between rows of left marginal cirri and ventral cirri. Furthermore, with the proliferation of these basal bodies this anarchic field is enlarged, later gradually splits into two parts, one is oral primordiun (OP) of the opisthe and the other is the cirral anlagen (FVT-anlagen, Figs 4; 35, arrows). During this process, most old cirri appear to remain intact and thus do not contribute to the formation of these anlagen.

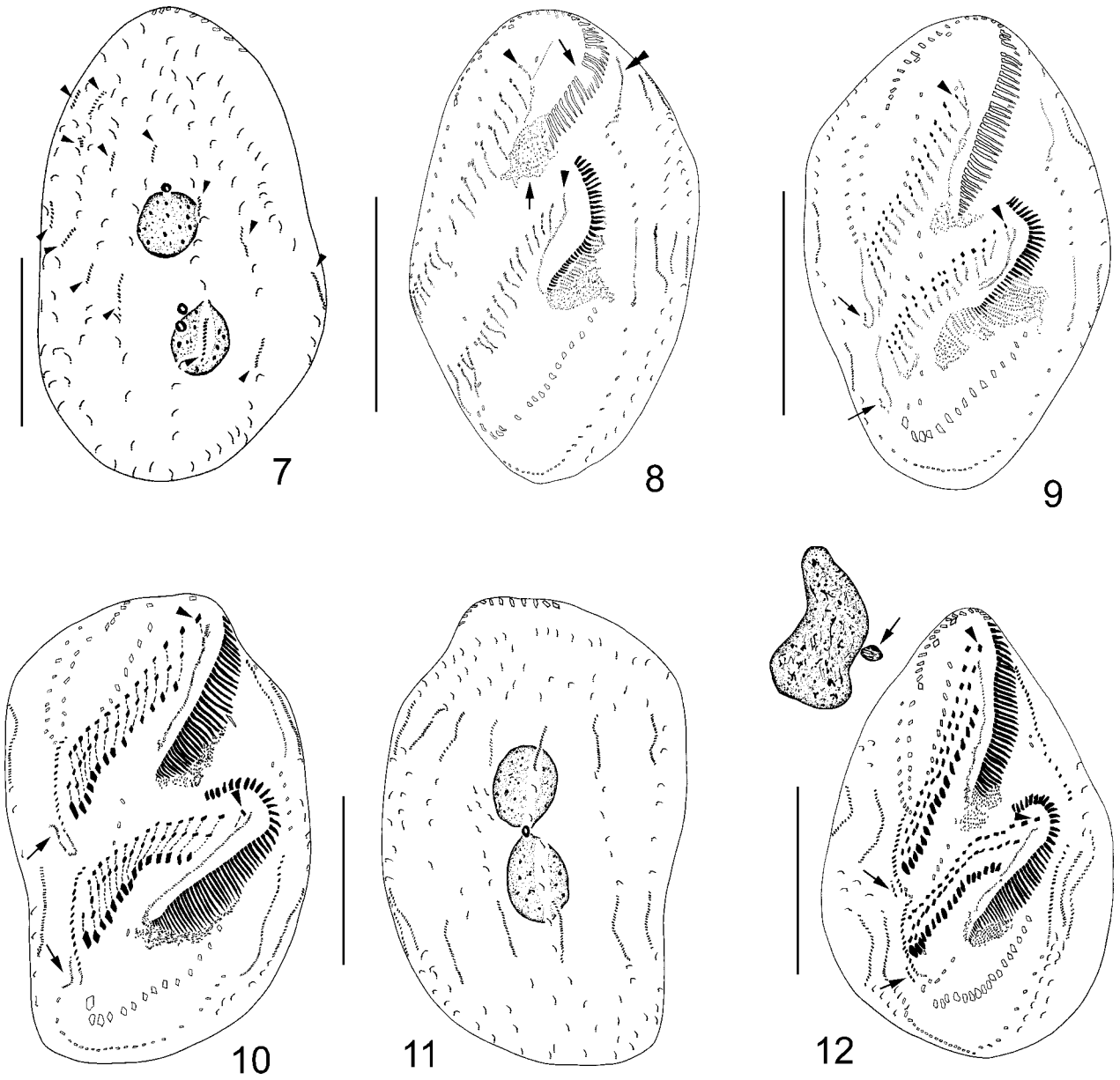
Then, the FVT-anlagen become larger with further joining of more basal bodies, that is, the number of the cirral steaks increases, and each steak is lengthened. Some steaks begin to divide at their mid-point (Figs 5, 36, arrows). Posterior to and right of them, a small anlage begin to develop intrakinetally in the posterior portion of the right marginal row (Fig. 5, arrowheads).

In the next stage, each streak of FVT-anlagen divide into two parts, thus two groups of FVT-anlagen, each of which has about 13-19 oblique streaks, are formed for the proter and the opisthe respectively (Fig. 39, arrows). The anlage for right marginal row is formed parallel to FVT anlagen and then splits into two (Figs 6, arrows; 39, arrowheads), each part accompanied by several loosely arranged kinetosomes. Meanwhile, new membranelles differentiate at the left anterior end of the OP. At the same time, the anlage for the left marginal row of the opisthe begins to appear in the middle port of the parental row (Fig. 6, double-arrowhead), and two separate anlagen occur in each old dorsal kinety (Figs 6, 7, arrowheads; 37, arrows), which develop to replace the old structures (Figs 8-18; 34, 42, arrows). One caudal cirrus will be derived from the posterior end of each dorsal kinety (Figs 13, 14, arrowheads; 45, 47, arrows).

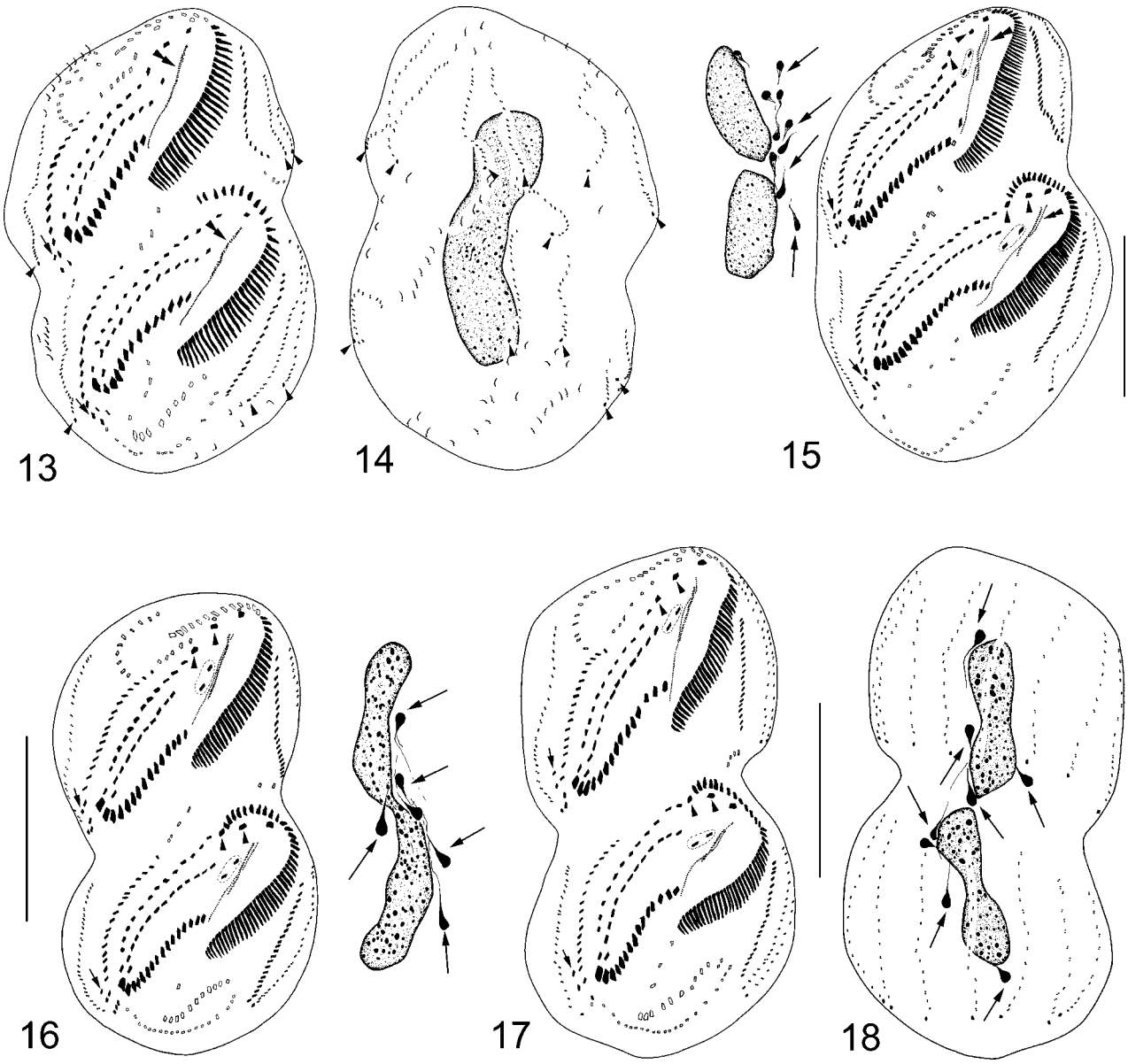
Slightly later, all cirral anlagen start to divide into segments (Figs 8; 41, arrows). In the proter, the parental paroral and endoral membranes begin to dissolve and participate in the formation of the undulating membrane



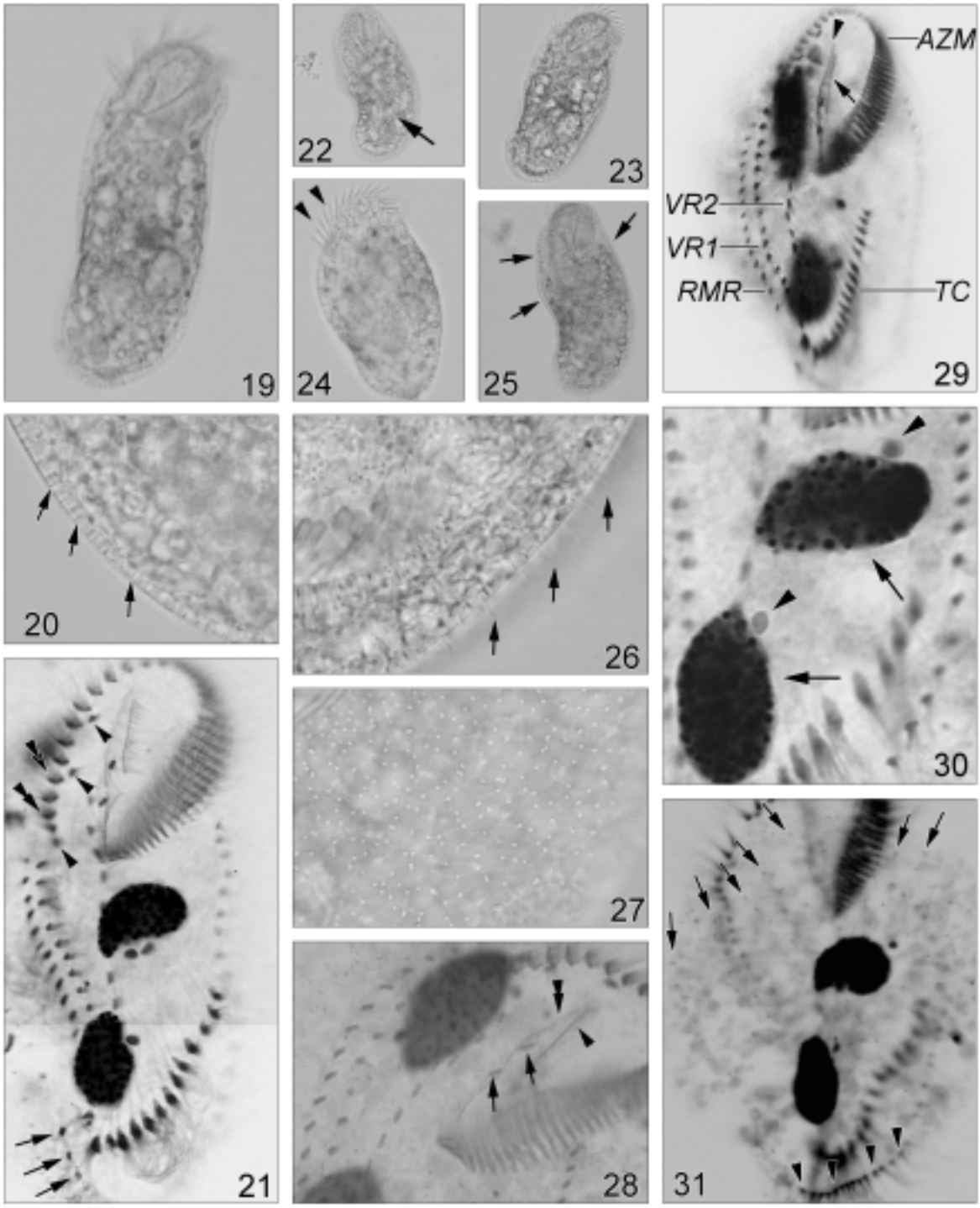
Figs 1-6. *Pseudoamphisiella alveolata* after protargol impregnation. **1, 2** - infraciliature of the ventral and dorsal side and nuclear apparatus in the same specimen. Arrows in Fig. 1 mark the extra right marginal cirri, while in Fig. 2 denote micronuclei. Arrowheads indicate buccal cirri. **3** - infraciliature of ventral side in the cell just after division. Arrows mark the bases of the membranelles at the distal end of the adoral zone. Arrowheads denote extra right marginal cirri. **4-6** - infraciliature of the ventral side of an early recognizer or divider. Arrows in Figs 4-6 mark the anlagen for the dorsal kineties (4), fronto-ventral transverse cirri (5) and the right marginal row (6). Arrowheads in Figs 4, 5 mark the anlagen for right marginal row, while in Fig. 6 denote dorsal kineties anlagen. Double-arrowheads in Figs 4-6 marks the disaggregated the adoral zone of membranelles at its posterior part (4), the oral primordium of the opisthe (5) and the anlage for the left marginal row of the opisthe (6). AZM - adoral zone of membranelles; BC - buccal cirri; CC - caudal cirri; EM - endoral membrane; FC - frontal cirri; LMR - left marginal row; OP - oral primordium; PM - paroral membrane; TC - transverse cirri; VR1, 2 - ventral row 1, 2. Scale bars: 50 μm.



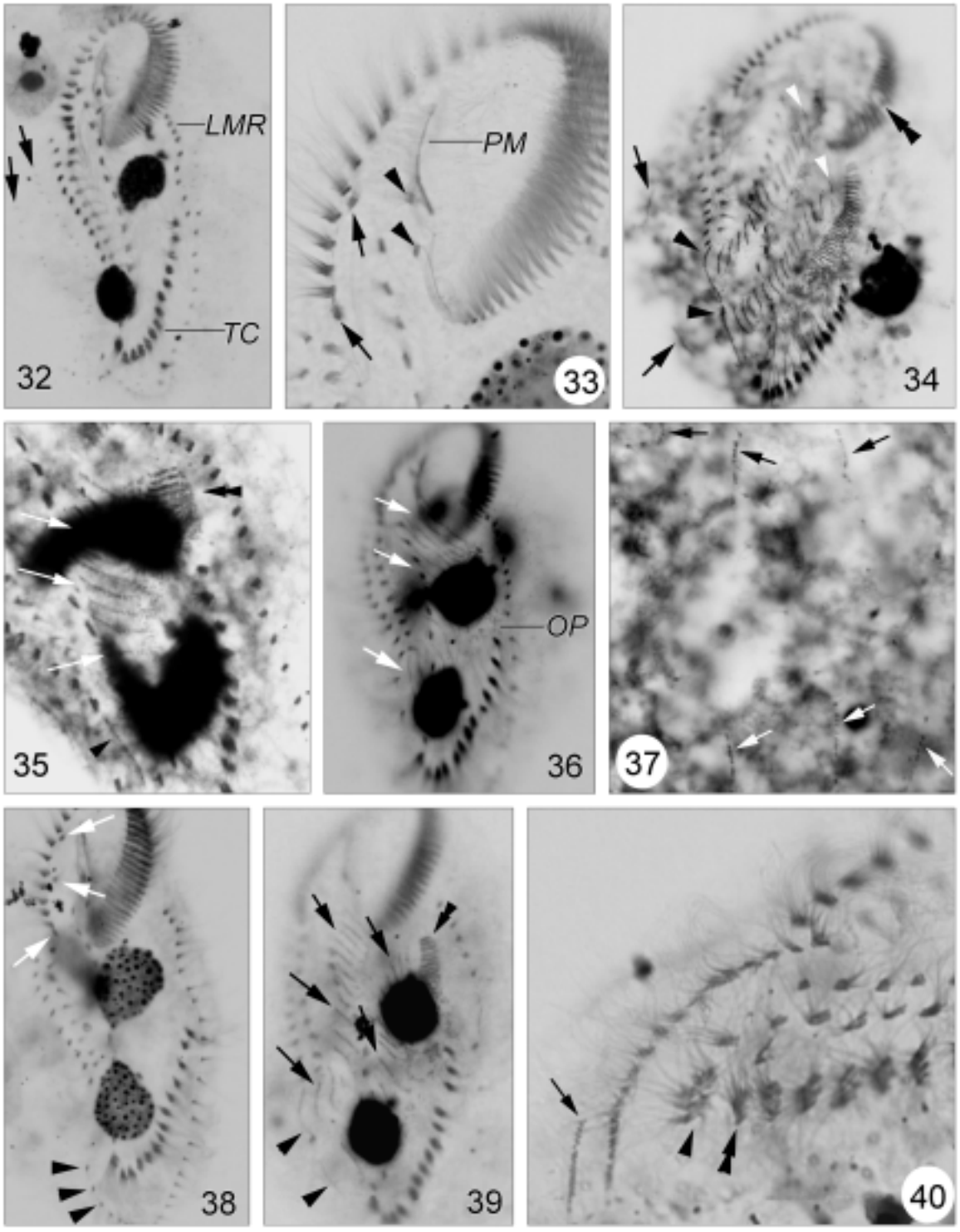
Figs 7-12. *Pseudoamphisiella alveolata* after protargol impregnation. Infraciliature of ventral (8-10, 12) and dorsal (7, 11) sides. Parental structures in white, new structures in black. **7** - the same cell as that in Fig. 6. Arrowheads mark the dorsal kineties anlagen. **8** - early divider, which has two sets of anlagen for the cirri and the dorsal kineties. Arrows mark dedifferentiation of the parental proximal portion. Arrowheads mark the anlagen for the undulating membranes, and show the deorganization of the posterior buccal cirrus. Double-arrowhead denotes the anlage for the left marginal row of the proter. **9** - early to middle divider. Note that fronto-ventral transverse cirral anlagen begins to generate new cirri. Arrows mark extra small anlagen beside the posterior end of the anlagen for the right marginal rows. Arrowheads denote the leftmost frontal cirri derived from the anterior end of the undulating membranes anlage. **10, 11** - middle divider. Note that almost all fronto-ventral transverse cirral anlagen have produce three cirri each except for the posterior two anlagen, which form 4 and 2 cirri respectively. The cirri originating from the same anlage are connected by a broken line. Arrows mark lengthened extra anlagen. Arrowheads show the anteriormost frontal cirri. Two separate anlagen occur within each parental dorsal kinety, and two macronuclear nodules move together. **12** - middle divider. Note that cirri are formed from the extra anlagen (arrow) and that the new cirri are beginning to move. Arrowheads mark the anteriormost frontal cirri. Inset: fused macronuclear mass and micronucleus (arrow). Scale bars: 40 μm (7), 80 μm (8, 9) and 50 μm (10-12).



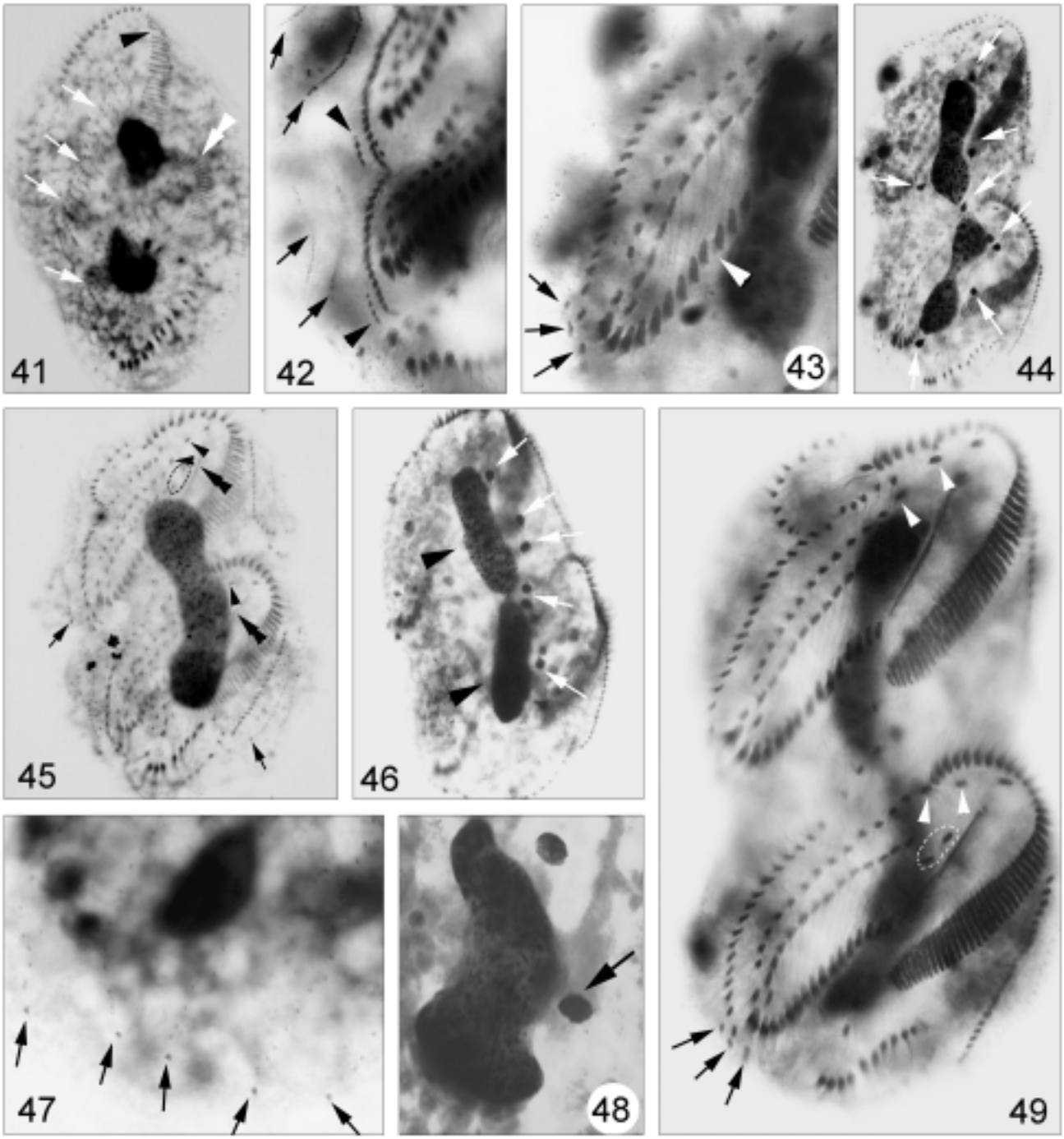
Figs 13-18. *Pseudoamphisiella alveolata* after protargol impregnation. Infraciliature of ventral (13, 15-17) and dorsal sides (14, 18). Parental structures in white, new structures in black. **13, 14** - middle to late divider. Note the formation of the new oral and somatic ciliature. Small arrows in Figs 13, 15-17 mark the extra right marginal cirri which start to move posteriorly. Arrowheads denote caudal cirri originated from the posterior end of the dorsal kineties anlagen. Double-arrowheads mark the streak-like undulating membranes anlagen composed of disorganized kinetosomes. **15-18** - very late divider to note the migration of all new structures and the formation of paroral and endoral membranes (double-arrowheads). The two buccal cirri encircled by a broken line. Arrowheads mark the other two frontal cirri. Insets: division of macronucleus and micronuclei (long arrows). Scale bars: 50 µm



Figs 19-31. Photomicrographs of *Pseudoamphisiella alveolata* from life (19, 20, 22-27) and after protargol impregnation (21, 28-31). Ventral (19-30) and dorsal (31) views. **19** - extended body with anterior part cephalized. **20, 26, 27** - portion of cortex in side (20, 26) and top (27) views to show extrusomes (arrows in 20), dorsal bristles (arrows in 26) and the polygonal-shaped alveolar layer. **21, 29** - infraciliature. Arrows, arrowheads and double-arrowheads in Fig. 21 mark the extra marginal cirri, frontal cirri and the bases of adoral membranelles at distal end respectively. Arrow and arrowhead in Fig. 29 indicate endoral and paroral membranes respectively. **22-25** - different cells in contracted state. Arrows mark highly developed transverse cirri (22) and hyaline alveolar layer covering cell surface (25). Arrowheads denote the adoral membranelles at distal end. **28** - portion of infraciliature, note paroral (arrowhead) and endoral (double-arrowahead) membranes and buccal cirri (arrows). **30** - note macronuclear nodules (arrows) and micronuclei (arrowheads). **31** - note dorsal kineties (arrows) and caudal cirri (arrowheads). AZM - adoral zone of membranelles; RMR - right marginal row; TC - transverse cirri; VR1, 2 - ventral row 1, 2.



Figs 32-40. Photomicrographs of *Pseudoamphisiella alveolata* after protargol impregnation. Infraciliature of ventral (32-36, 38 - 40) and dorsal (37) sides. **32** - arrows mark dorsal kineties. **33, 38** - note frontal cirri (arrows), buccal cirri (arrowheads in 33) and the extra marginal cirri (arrowheads in 38). **34, 37** - note the anlagen for the right marginal rows (black arrowheads), dorsal kineties anlagen (arrows), anlagen for undulating membranes (white arrowheads) and dedifferentiation of the parental oral apparatus (double-arrowhead). **35, 36, 39** - note oral primordium (double-arrowheads), fronto-ventral transverse cirral anlagen (FVT-anlagen, arrows) and the anlagen for right marginal rows (arrowheads). **40** - note the extra anlage beside the posterior end of the anlage for the right marginal row (arrow), and the posterior two FVT-anlagen which have produced two and four cirri respectively (arrowhead and double-arrowhead). LMR - left marginal row; OP - oral primordium; PM - paroral membrane; TC - transverse cirri.



Figs 41-49. Photomicrographs of *Pseudoamphisiella alveolata* after protargol impregnation. Infraciliature of ventral (41-46, 48, 49) and dorsal (47) sides. **41** - note FVT-anlagen which start to develop into new cirri (arrows), disaggregation of the parental adoral membranelles (arrowhead) and the new membranelles curving to the right (double-arrowhead). **42, 48 -** the same cell. Note the dorsal kineties anlagen (arrows in 42), the newly formed extra marginal cirri (arrowheads), fused macronuclear mass and micronucleus (arrow in 48). **43** - note the newly-formed extra marginal cirri (arrows) and transverse cirri (arrowhead). **44, 47** - the same cell. Note the dividing of macronuclear nodules and micronuclei (arrows in 44) and caudal cirri derived from the posterior end of the dorsal kineties (arrows in 47). **45** - middle divider. Note that the development of the oral and somatic ciliature is nearly completed. Arrows mark the new caudal cirri at the posterior end of the dorsal kineties. Arrowheads mark the frontal cirri from the anterior two streaks of fronto-ventral transverse cirral anlagen. Double-arrowheads denote the streak-like undulating membranes anlagen. Two buccal cirri are encircled by a broken line in the proter. **46** - late divider. Note division of macronuclear nodules (arrowheads) and micronuclei (arrows). **49** - very late divider. Note that all new cirri continue to migrate. Arrows mark the extra marginal cirri. Arrowheads mark new frontal cirri from the anterior two streaks of the fronto-ventral transverse cirral anlagen. Two buccal cirri are encircled by a broken line in the opisthe.

Table 2. Morphological and morphometrical comparison of *Pseudoamphisiella alveolata* and *P. lacazei*. Measurements in µm.

Character	*Pseudoamphisiella alveolata*	*P. alveolata*	*P. lacazei*	*P. lacazei*
Body size *in vivo*	120-200 × 50-70	120-240 × 50-80	120-300 × 40-80	200-300 × 70-100
Number of adoral membranelles	37-43 (n=25)	47-59 (n=9)	39-49 (n=25)	50-56
Number of buccal cirri	2 (n=25)	2 (n=18)	2 (n=25)	2
Number of frontal cirri	3 (n=25)	3 (n=18)	3 (n=25)	3
Number of cirri in ventral row 1	11-15 (n=25)	10-14 (n=11)	11-15 (n=26)	14-16
Number of cirri in ventral row 2	10-15 (n=25)	11-15 (n=11)	16-23 (n=26)	16-21
Number of cirri in left marginal row	15-30 (n=39)	14-20 (n=11)	21-31 (n=16)	66-73*
Number of cirri in right marginal row	12-16 (n=25)	12-14 (n=11)	20-29 (n=16)	-
Number of transverse cirri	13-17 (n=25)	12-16 (n=11)	16-23 (n=26)	*ca* 20
Number of caudal cirri **	11-16 (n=40)	11-16 (n=9)	9-11 (n=5)	-
Number of dorsal kineties	9-12 (n=9)	10-12 (n=16)	8-11 (n=25)	9-11
Number of macronuclei	2-3 (n=40)	2 (n=16)	24-57 (n=20)	50-60
Number of micronuclei	1-6 (n=40)	2-5 (n=11)	7-10 (n=6)	-
Alveolar covering	present	present	absent	absent
Data source	This study	Song and Warren (2000)	Song *et al*. (1997)	Song (1996)

* including caudal, left and right marginal cirri; ** including the extra right marginal cirri

anlage (UM-anlage; Fig. 8, anterior arrowhead); the posterior part of the old adoral zone of membranelles becomes dedifferentiated and the middle membranelles get resorbed (Figs 8, arrows; 41, arrowhead). While in the opisthe, the undulating membranes anlage (Fig. 8, posterior arrowhead) detaches from the main body of the oral primordum, which extends anteriorly and curves to right with the increase in the number of membranelles (Fig. 41, double-arrowhead). Meanwhile another anlage occurs at the anterior of the parental left marginal row (Fig. 8, double-arrowhead). Subsequently, new cirri are gradually formed in the FVT-anlagen (Fig. 9). A small anlage is derived from the anterior end of the undulating membranes anlage in both dividers (Fig. 9, arrowheads), which eventually forms the anteriormost frontal cirrus (Figs 10, 12, arrowheads). Other anlagen develop further. Near the posterior part of the right marginal row anlagen, two small extra anlagen appear (Figs 9, arrows; 40, arrow).

Thereafter, three new cirri are formed in each streak of the FVT anlagen except the posteriormost two, which produce four and two cirri respectively (Figs 10, 12; 40, double-arrowhead and arrowhead). The extra anlagen lengthen and fragment into 2-4 cirri (Figs 10, 12, 13, 15-17, 40, arrow; 42, arrowheads; 43, 49, arrows), which will move posteriorly to the newly formed caudal cirri in the interphase cell (Fig. 3, arrowheads). As the cell elongates in the next stages, all these new cirri migrate and are accurately positioned to form the mature cortical pattern: the first cirrus from the two anterior streaks develop into two frontal cirri (Figs 15-17, 45, arrowheads), while the second cirrus develop into two buccal cirri (encircled in Figs 15-17, 45, 49); each streak of FVT-anlagen contributes the last cirrus to form the row of transverse cirri (Fig. 43, arrowhead) the remainder will constitute the two ventral rows (Figs 15-17, 45, 49). At this time, new cirri begin to organize proteriad in the anlagen of the marginal rows (Figs 10, 12, 13). In oral primordium, new adoral membranelles continue to be arched in the opisthe, while in the old oral appratus the posterior part is gradually renewed *in situ* (Figs 10, 12), and the re-built membranelles combine with the anterior old ones to generate the new adoral zone (Fig. 13). Then the undulating membranes anlagen split longitudinally to form paroral and endoral membranes (Fig. 15, double-arrowheads). Next, the daughter dividers start to separate (Figs 16, 17, 45, 49) and with the completion of the cytostome become trophic cells once again.

Nuclear apparatus evolves in the usual way: the fused macronuclear mass (Figs 12, inset; 48) can be observed with an enlarged micronucleus (Figs 12, inset; 48, arrow)

in the middle stage. Both divide and be assigned to two daughters at late stages (Figs 14, 15, 16, insets; 18; 44, 46, arrows and arrowheads).

DISCUSSION

Within the genus *Pseudoamphisiella*, only two species have been described using silver staining method. Based on the data from this study and previous descriptions, the most readily observed differences (Table 2) between the two are: the number of macronuclei (2-3 in *P. alveolata vs.* 24-60 in *P. lacazei*) and the conspicuous alveolar layer (present in *P. alveolata vs.* absent *P. lacazei*).

The Japanese population of *Pseudoamphisiella alveolata* has relatively stable morphological characters as indicated by the low coefficients of variation for the morphometrical data (Table 1). Our population corresponds well with the descriptions by Kahl (1932), Borror (1963) and Song and Warren (2000) in terms of body shape and size, characteristic cortical structure (e.g. cell surface covered by alveolar layer), nuclear apparatus and the cirral pattern, especially the widely separated ventral rows, all of which indicate that these four forms are conspecific. Nevertheless, minor differences exist in morphometrical data, e.g. number of adoral membranelles (Table 2, Kahl 1932, Borror 1963), which can be population dependent. Compared with the Qingdao population, Japanese population has relatively fewer caudal cirri because, due to a lack of morphogenetic data, Song and Warren (2000) misidentified the extra right marginal cirri at the posterior end of body as caudal cirri.

In terms of morphogenesis, *Pseudoamphsiella* is very similar to the genera of the suborder Urostylina, e.g. *Holosticha*, *Pseudokeronopsis*, *Thigmokeronopsis*, in having only one marginal cirral anlage on each side and numerous oblique cirral anlagen (Hu and Song 2001a, b; Hu *et al.* 2000, 2003, 2004a, b). However, these anlagen do not develop into frontoterminal cirri and a zig-zag structured midventral row but rather into two widely separated ventral rows. Within this genus, *P. alveolata* resembles *P. lacazei* in the combination of the following features: the oral primordium of the opisthe and FVT-anlagen originate *de novo*, and most ventral cirri do not contribute to the formation of these primordia; the two buccal cirri are derived from the anterior two streaks of the FVT-anlagen; the left marginal row and dorsal kineties develop by "within-proliferation"; one caudal cirrus is derived from the posterior end of each dorsal kinety anlage; the anlage for the right marginal row develops simulatenously and parallel to the posterior streaks of the FVT-anlagen; the macronuclear nodules evolve in the usual way as in other hypotrichs (Song and Wilbert 1994, Eigner 1995). However, difference between *P. alveolata* and *P. lacazei* include: (1) the parental adoral zone of membranelles is partly renewed in *P. alveolata*, that is, its posterior portion is rebuilt *in situ* whilst the anterior part is retained by the proter (vs. completely replaced in *P. lacazei*); (2) the presence of extra migratory marginal cirri in *P. alveolata* (vs. absence in *P. lacazei*); (3) joining of the old undulating membranes and (possibly) a few posterior right marginal cirri in the formation of new anlagen in *P. alveolata* (vs. not involvement of these structures in *P. lacazei*). Additionally, the oral primordium of the opisthe and the primary primordium of the fronto-ventral transverse cirri are possibly derived from an anarchic field of kinetosomes on the cell surface, and the FVT-anlagen of both dividers are formed by the separation of the primary primordium, which was not confirmed by the work of Song *et al.* (1997). Therefore, the present study further demonstrates the value of morphogenetic data in discriminating between species and genera with similar morphologies.

According to Borror and Wicklow (1983) the suborder Urostylina includes genera where "frontal ciliature differentiate from the longitudinal field of more than 5 oblique ciliary streaks". In a sense, *Pseudoamphisiella* undoubtedly has the same developmental pattern as the urostyline genera, e.g. *Holosticha* (Hu and Song 2001a, Hu *et al.* 2003) despite it lacks typical midventral rows; therefore it is reasonable to assign *Pseudoamphisiella* to the suborder Urostylina (Shi 1999). Furthermore, absence of frontoterminal (migratory) cirri in *Pseudoamphisiella* also exists in several urostyline genera such as *Urostyla* and *Australothrix* (Song *et al.* 1997).

Considering the similarity of the general developmental process of ciliary primordia between *Pseudoamphisiella alveolata* and *Psammocephalus faurei* (Wicklow 1982), it is possible that the family Pseudoamphisiellidae should belong to the suborder Discocephalina Wicklow, 1982, despite the fact that the infraciliature of *Pseudoamphiella* is very similar to that of *Pseudouroleptus* Hemberger, 1985 which Lynn and Small (2000) assigned to the order Stichotrichida (formerly Stichotrichina).

As mentioned above, the systematic assignment of *Pseudoamphisiella*/Pseudoamphisiellidae depends on which one is adopted among criteria such as similarity of infraciliature and/or morphogenetic pattern. However, the question about the significance of these criteria at the suborder level has not yet solved to date and thus we cannot assign the family Pseudoamphisiellidae to any suborder at present (Song *et al.* 1997).

Acknowledgements. This work was supported by the "Natural Science Foundation of China (project number: 40206021, 30570236)" and "JSPS Postdoctoral Fellowship for Foreign Researcher".

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Received on 22nd September, 2005; revised version on 30th November, 2005; accepted on January 3rd, 2006