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Morphological Redescription and Morphogenesis of the Marine Ciliate, *Pseudokeronopsis rubra* (Ciliophora: Hypotrichida)

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Summary. The morphology and morphogenesis of the marine ciliate, *Pseudokeronopsis rubra* (Ehrenberg, 1838) isolated from shrimp culturing water of Qingdao (Tsingtao, China), are investigated using protargol silver impregnation. The Qingdao population presents the following characteristics: body slim and reddish, size in vivo 160-200 x 24-40 μm, with longitudinal furrow on the ventral side; brick-red pigments constituting rosettes on dorsal side and ventral arrays along the base of cirri; blood cell-like cortical granules throughout the endoplasm. AZM consisting of 46-60 membranelles; long midventral row with 30 pairs of cirri on average; 2-4 transverse cirri and 4-6 dorsal kineties. The overall pattern of the morphogenetic events resembles the other populations described previously: in the opisthe new basal bodies originate close to intact left midventral cirri; the adoral membranelles primordia (AMP), undulating membranes anlagen (UMA) and fronto-midventral-cirral anlagen (FMA) develop from the growing anarchic primordium. In the proter, the AMP and UMA generate de novo on the surface of the buccal cavity. The FMA originate apokinetally to the right of the parental paroral membrane. The UMA generate the first frontal cirrus, PM and EM in both daughter cells as in other hypotrichs. The AMP, UMA and FMA develop in a usual way as in other urostylids. Marginal cirral rows and dorsal kineties occur within the parental structures. The numerous macronuclear segments divide without prior fusion. Reorganization corresponds to the divisional processes in the opisthe. The most remarkable feature is that the old structures do not participate in the formation of the new organelles.

Key words: Hypotrichida, marine ciliate, morphology, morphogenesis, *Pseudokeronopsis rubra.*

INTRODUCTION

The genus *Pseudokeronopsis* is commonly found in all different habitats with mostly overlapping characters (cell color, size and infraciliature), which gives rise to great confusion for species identification. Among this genus, the marine form, *P. rubra* is likely one of the most debatable species though many studies using modern methods have been carried out (Ehrenberg 1835 a, b; Borror 1972, 1979; Borror and Wicklow 1983; Foissner 1984; Wirnsberger *et al.* 1987). Moreover, according to the data obtained, all known *Pseudokeronopsis* studied share similar morphogenetic characters (Wallengren 1901, Morgan 1926, Rühmekorf 1935, Borror 1972, Ruthmann 1972, Wirnsberger 1987, Mihailowitsch and Wilbert 1990). However, the complete morphogenetic process including the development of new ciliature in daughters and the evolution of macronuclear segments is still lacking.

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The present study gives the results based on the Qingdao population of *P. rubra*, in order to provide some new characters for a solid redefinition of these "well-known" ciliates at morphological and morphogenetic level.

MATERIALS AND METHODS

Samples were isolated from a shrimp culturing water in Qingdao (Tsingtao, 36°08'N; 120°43'E), China. Water salinity was about 12‰, water temperature about 20°C and pH *ca* 8.0. Specimens were cultured in boiled seawater to which squeezed rice grains were added.

The infraciliature was revealed with the protargol staining method according to Wilbert (1975). All drawings were made at a magnification of x125 with the help of camera Lucida. For clarity, in the morphogenetic stages, the parental structures were showed by outline, whereas the new ones were shaded. The terminology was according to Hemberger (1982) and Wirnsberger (1987).

RESULTS

Morphological redescription of the Qingdao population of *Pseudokeronopsis rubra* (Figs.1a-i, 4-10; Table 1)

Body slender, intensively contractile and highly flexible, size *in vivo* about 160-200 x 24-40 μm. Right and left edges are slightly convex, anterior end bluntly round, while posterior end often spoon-like. One conspicuous longitudinal furrow present along the middle of the cell at the ventral side. Dorsoventrally flattened (Fig.1c). Cortex reddish (under low magnification), with underlying brick-red pigment (<0.5 μm in diameter), which are grouped along the cirral rows (Fig. 1b) and dorsal kineties (about 6-10 granules comprise one rosette around the cilia) (Figs. 4-6); cortical granules about 1-2 μm in diameter, blood cell shaped (Fig. 1d, arrowheads). Numerous macronuclear segments, spherical to ovoid, distributed throughout the whole cell. Cytoplasm relatively transparent, containing several food vacuoles. Movement slowly. Conjugation was frequently observed under cultural condition.

Adoral zone of membranelles (AZM) consisting of 46-60 membranelles, usually like a question mark extending onto right-ventral side, about 1/3-1/4 of body length, apical membranelles *ca* 15 μm long. Buccal field narrowed and strongly deepened; pharyngeal fibers conspicuous; paroral membrane rather shorter than endoral one. Bicorona consists of about 7 anterior, slightly enlarged frontal cirri and 5-6 posterior ones. Constantly one buccal and two frontoterminal cirri (Fig.1e, arrowhead); midventral rows consisting of 49-77 cirri, located at ventral concaved furrow (Fig. 1c), cirri about 7-8 μm long; 2-4 transverse cirri about 15 μm in length each, protruding out of posterior edge.

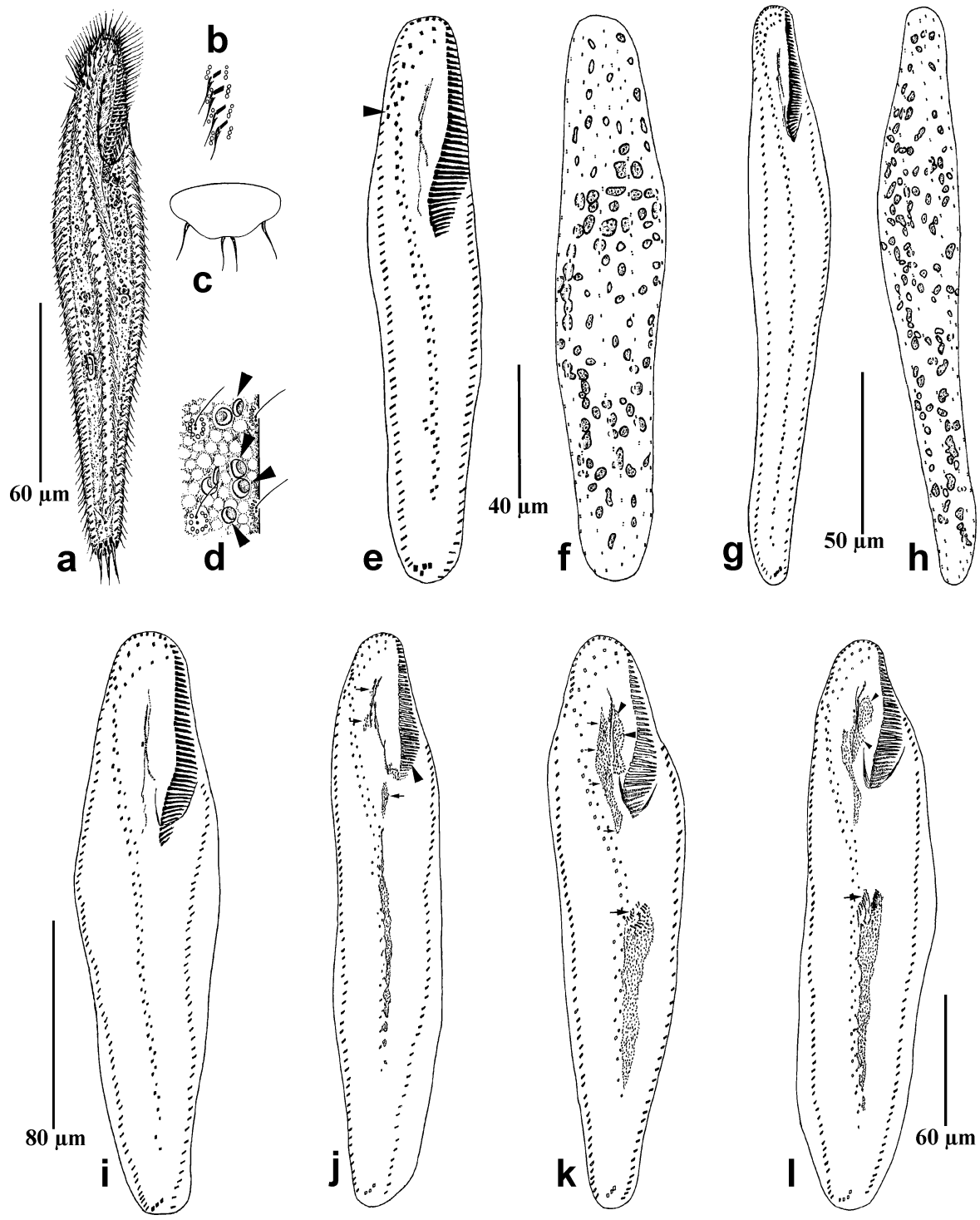
Marginal rows posteriorly unconfluent, cirri 8 μm long, bases composed of 2 basal body rows; 4-6 dorsal kineties, each as long as the body; dorsal cilia about 3 μm long.

Divisional morphogenesis of *Pseudokeronopsis rubra* (Figs.1j-l; 2a-h; 3a, b; 11; 14-16)

Marginal cirri and dorsal kineties develop in a usual way, of which the anlagen appear within old structures and stretch toward both sides to replace the old structures (Figs. 2b-h; 3a, b)

1. Stomatogenesis and development of the somatic ciliature in the opisthe

Stomatogenesis commences with the forming of small groups of basal bodies very close to several left midventral cirri. With the proliferation of basal bodies, these groups join to make a longish field, which is the anarchic primordium (AP) of the opisthe (Fig.1j). During the process, the left midventral cirri remain intact. When the adoral membranelles of the opisthe organize in a posteriad direction in adoral membranelles primordia (AMP, as shown in Figs.1k, l), several oblique streaks appear at the right-anterior position of AP, which is fronto-midventral cirral anlagen (FMA, arrows; Figs. 1k, l; 2a); later, undulating membranes anlagen (UMA) separate to the right of the remaining primordia (Fig. 2a, arrow). At the present three parts join together at the posterior portion (Figs. 2a, b). Soon, they depart one another and develop into new structures. With the number of adoral membranelles increasing in AMP, a single frontal cirrus derives from UMA and the remaining of UMA splits longitudinally to make undulating membranes (PM, EM) (Figs. 2b, d; arrows); meanwhile, each streak of FMA divides into 2 segments (cirri) except for the posterior 4 streaks (3 or 4 segments/cirri) (Figs. 2d, e): the posterior cirrus from the first streak move to PM to be buccal cirrus (Figs. 2e, g; long arrows); the anterior two cirri from the last streak will migrate anteriad to be FTC (Figs. 2g, arrowheads; 3a, arrow); each of the posterior 2-4 streak contributes one cirrus (TC) to the daughter cell; the remaining new cirri are to be FC and MVC.



Figs. 1 a-l. Morphology and early stages of morphogenesis in *Pseudokeronopsis rubra* from living observation (a-d) and after protargol impregnated specimens (e-l); **a** -ventral view *in vivo*; **b** - showing the arrangement of pigments near cirri; **c** - transverse section; **d** - portion of dorsal view, indicating dorsal cilia, pigments and cortical granules (arrowheads); **e, f** - ventral and dorsal views of the same individual, arrowhead showing FTC; **g, h** - ventral and dorsal views of the same cell; **i** - ventral view; **j** - ventral view; arrowhead indicating dedifferentiating of old AZM, short arrows marking FMA in the proter; **k** - ventral view, long arrow showing FMA in the opisthe, short arrows marking FMA in the proter, arrowheads indicating AMP in the proter; **l** - ventral view, long arrow showing FMA in the opisthe, arrowheads indicating AMP in the proter, AM - adoral membranelles, AMP - adoral membranelles primordium, AP - anarchic primordium, AZM - adoral zone of membranelles, FMA - frontomidventral cirral anlagen, FTC - frontoterminal cirri

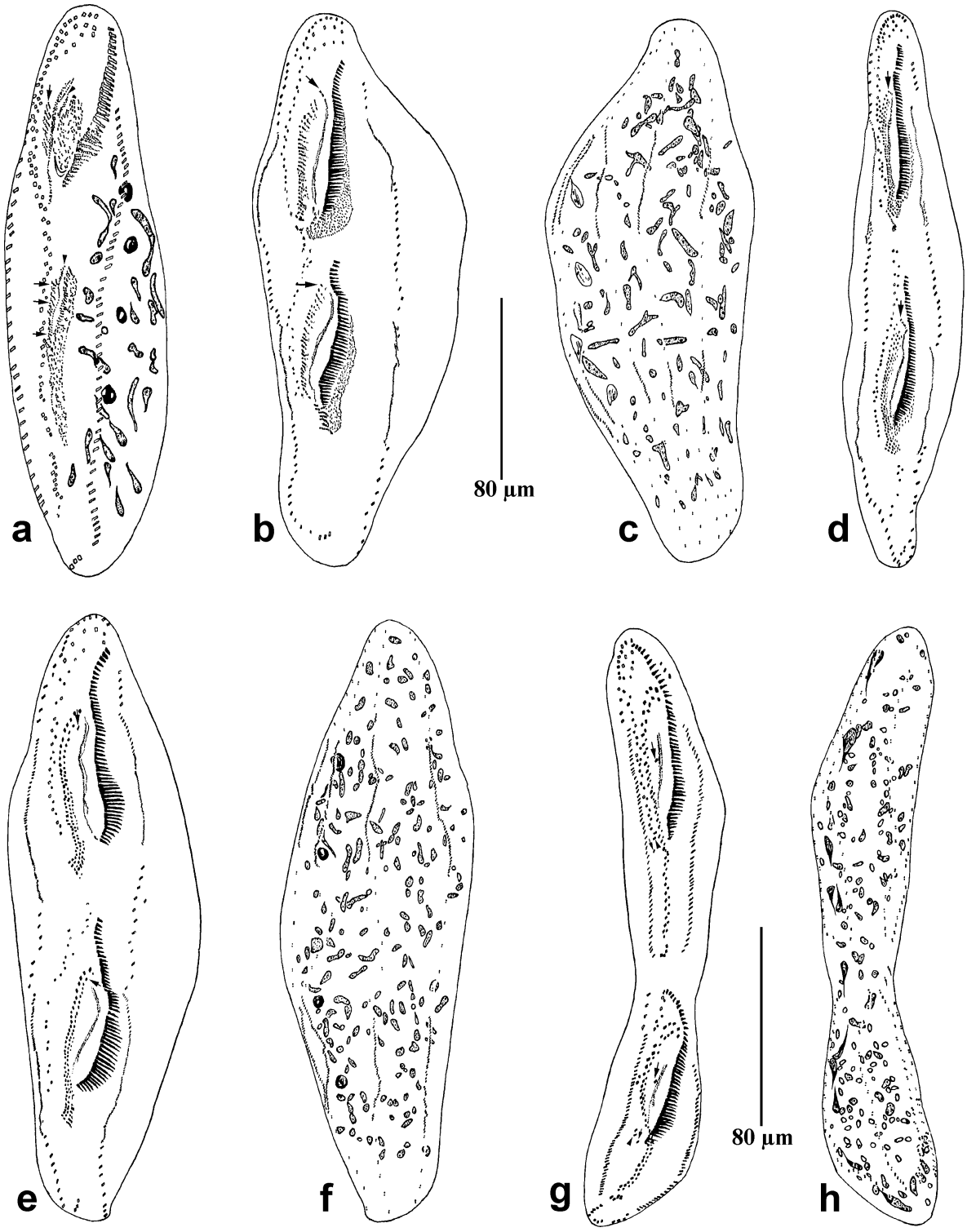
Table 1. Morphometrical data of *Pseudokeronopsis rubra*. Three populations are respectively according to author (first line) and Wirnsberger *et. al.* (1986) (second and third line). Data are based on protargol-impregnated specimens. Measurements in μm. CV - coefficient of variation in %; Max - maximum; Min - minimum; n - number of individuals examined; SD - standard deviation; SE - standard error of mean; Mean - arithmetic mean

Character	Min	Max	Mean	SD	SE	CV	n
Body length	124	264	180.7	37.51	8.19	20.8	21
	140	189	171.8	12.50	3.20	7.3	15
	159	220	189.5	17.10	4.40	9.4	15
Body width	32	64	50.8	8.15	1.86	16.0	19
	24	41	30.5	4.40	1.10	14.4	15
	37	63	50.4	8.40	2.20	16.7	15
Buccal field length	51	76	62.2	7.23	1.62	11.6	20
	48	57	52.0	2.90	0.70	5.6	15
	57	80	62.7	6.20	1.60	9.9	15
Adoral membranelles, No.	46	60	51.6	3.72	0.83	7.2	20
	50	65	58.0	3.90	1.00	6.7	15
	66	92	78.1	7.10	1.80	9.1	15
Anterior frontal cirri, No.	11*	14*	12.6*	1.07*	0.24*	8.4*	19*
	6	10	8.1	1.00	0.30	12.8	15
	5	12	8.5	2.60	0.70	31.2	15
Posterior frontal cirri, No.	-	-	-	-	-	-	-
	4	7	5.6	1.00	0.20	17.5	15
	5	13	7.7	2.10	0.60	27.9	15
Frontoterminal cirri, No.	2	2	2	0	0	0	20
	-	-	-	-	-	-	-
	-	-	-	-	-	-	-
Midventral cirri, No.	49**	77**	61.6**	8.28**	2.07**	13.4**	16**
	30***	43***	38.0***	3.50***	0.90***	9.2***	15***
	28***	47***	36.7***	5.20***	1.30***	14.2***	15***
Buccal cirrus, No.	1	1	1	0	0	0	21
	-	-	-	-	-	-	-
	-	-	-	-	-	-	-
Left marginal cirri, No.	45	62	52.7	5.19	1.26	9.9	17
	57	82	72.0	7.10	1.80	9.8	15
	48	71	62.0	6.40	1.60	10.2	15
Right marginal cirri, No.	48	67	57.9	4.64	1.13	8.0	17
	59	95	79.0	9.10	2.30	11.5	15
	45	73	63.6	7.80	2.00	12.2	15
Transverse cirri, No.	2	4	3.1	0.62	0.15	19.8	16
	6	9	7.2	0.90	0.20	13.0	15
	4	9	7.1	1.50	0.40	21.7	15
Dorsal kineties, No.	4	7	5.1	0.72	0.18	14.0	16
	5	8	6.3	-	-	-	15
	5	8	6.4	-	-	-	15

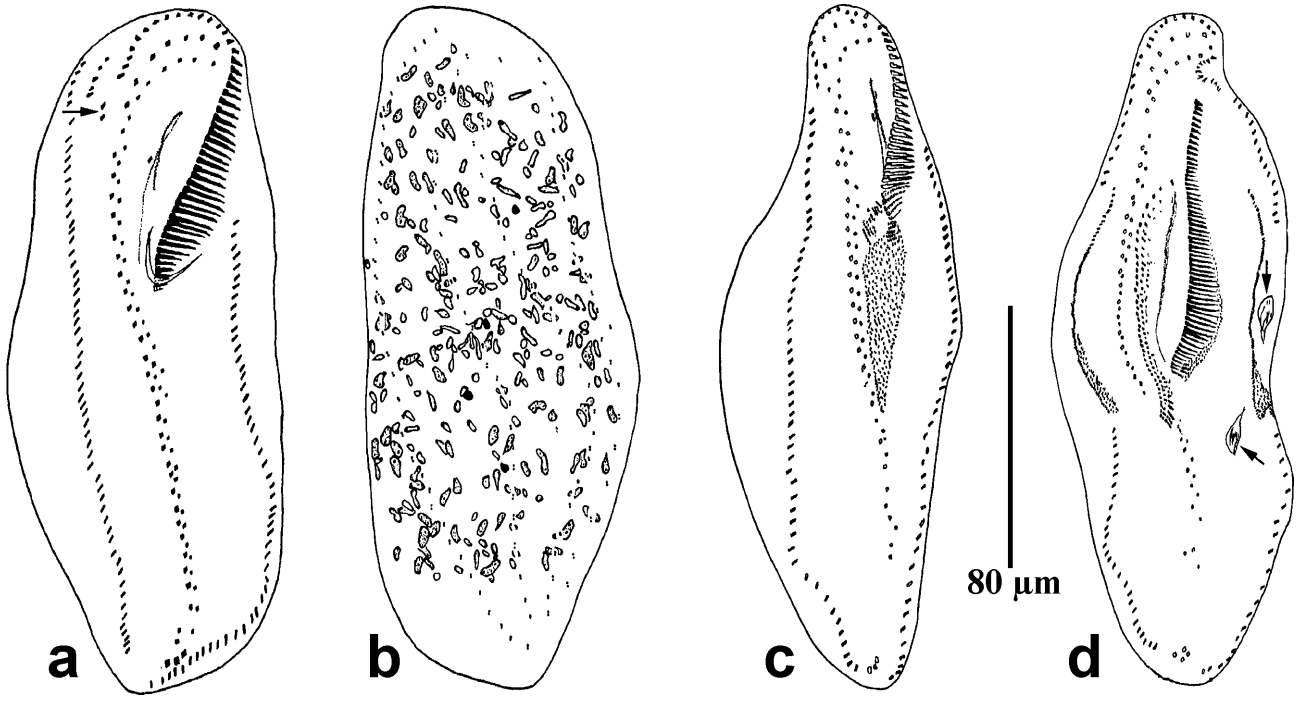
* including posterior frontal cirri
** including right midventral cirri
*** referring to number of pairs of midventral cirri
- not available

2. Stomatogenesis and development of the somatic ciliature in the proter

Just as appearing of AP in the opisthe, groups of basal bodies developed independently on the surface and to the right of the buccal cavity and behind buccal fields (Figs. 1j, k; short arrows); meanwhile, the posterior adoral membranelles begin to dedifferentiate in an anteriad direction (Fig. 1j, arrowhead). Lately, with the proliferation of basal bodies, a longish field forms, which will develop into FMA (Fig. 2a); at the same time, an anarchic field occur on the surface of buccal cavity and to the left of the endoral membrane (Figs.1k, l, arrowheads; 2a). With the joining of new basal bodies in the



Figs. 2a-h. Middle and late stages of morphogenesis in *Pseudokeronopsis rubra* from protargol impregnated specimens; **a** - ventral view with partial macronuclear segments, arrows showing FMA, arrowheads indicating UMA; **b, c** - ventral and dorsal views of the same individual, arrows showing the first frontal cirrus from UMA; **d** - ventral view, arrows showing the first frontal cirrus from UMA; **e, f** - ventral and dorsal views of the same cell, arrows showing buccal cirri in both dividers; **g, h** - ventral and dorsal views of the same individual, long arrows indicating buccal cirrus, arrowheads showing the migration of cirri, short arrows marking additional cirri (probably to be absorbed lately). FMA - frontoterminal cirral anlagen, UMA - undulating membranes anlagen



Figs. 3a-d. The daughter cell and regenerative stages of *Pseudokeronopsis rubra*. **a, b** - ventral and dorsal views of the same daughter cell, arrow showing FTC; **c, d** - different regenerative stages, arrows showing micronuclei. FTC - frontoterminal cirri

anarchic field, the new adoral membranelles gradually organize in a posteriad direction (Figs. 2b, d); simultaneously, UMA detaches and generates frontal cirrus at its anterior end (Figs. 2b, d; short arrow). As in the opisthe, FMA develop into FC, BC, MVC and TC (Figs. 2b, d, e, g).

3. Evolution of nuclear apparatus

In the process of evolution of macronuclei, a striking feature was apparently observed that the numerous (more than 100) macronuclear segments divide without prior fusion as in the description of Wirnsberger (1987). The micronuclei behave like those of other hypotrichs.

Reorganization morphogenesis (Figs. 3c, d)

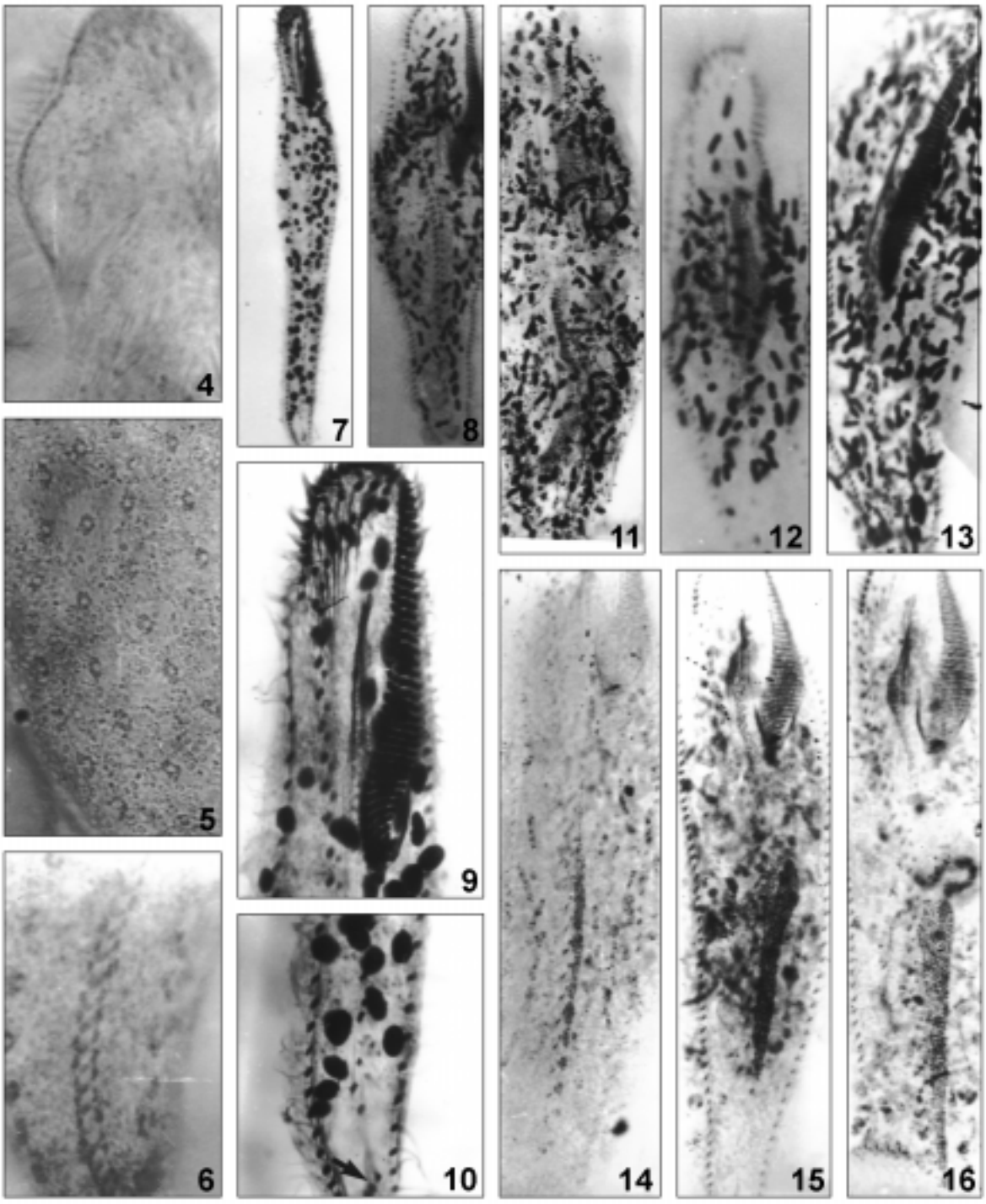
Physiological regeneration normally takes place in well-fed cultures. Regenerator possesses only one set of primordium, which develops in the same way as in the opisthe, as showing in Figs. 3c, d and Figs. 12, 13. However, the old adoral membranelles dedifferentiate and the old undulating membranes are reabsorbed. Now, it is uncertain that the basal bodies from the old AZM join in the forming of new adoral membranelles in reorganization, but the new adoral membranelles replace the old AZM completely.

DISCUSSION AND CONCLUSION

Pseudokeronopsis remains one of the most compound taxa because of the overlapping of many morphological characters, including body form, cell color and the wide range of variation of the dorsal kineties (Foissner 1982, Wirnsberger *et al.* 1987).

According to the descriptions by Foissner (1984), Borror and Wicklow (1983) and Wirnsberger *et al.* (1987), we identified our form mainly because of slender body form, brick-red pigment and long midventral row. But Qingdao-population differs from other populations described previously in the presence of blood cell-like cortical granules and the low number of TC and DK (Table 1).

The color of the cell is still an arguable feature: e.g., at low magnification, cells seem dark red (or red-brown?), but the cortical granules at high magnification are only reddish. So it is evidently a character which is



Figs. 4-16. Micrographs of morphology, morphogenesis and reorganization in *Pseudokeronopsis rubra*. **4** - anterior view, showing undulating membranes; **5** - portion of dorsal view, marking rosettes and cortical granules; **6** - posterior view, indicating pigments arrays along the base of cirri; **7, 8** - ventral views of different cells, showing infraciliature; **9** - ventral view in the anterior area of the same individual as in Fig. 7, showing oral apparatus; **10** - ventral view in the posterior region of the same individual as in Fig. 7, arrow marking transverse cirri; **11** - ventral view of a middle stage in morphogenesis, showing the forming of both AZM and new cirri; **12, 13** - showing ventral views of two reorganization stages; **14-16** - early stages of morphogenesis, indicating the development of AP and the dedifferentiating of the old AZM

highly dependent on the author's observations and description, i.e. it is likely less reliable than respected for species identifications.

Another thing resulting in confusion is the number of DK. In most hypotrichs, the number of DK is rather constant but *Pseudokeronopsis* seems to be very variable even within the same population (Table 1), which probably indicates a strong generic and ecological radiation of this taxon (Wirnsberger *et al*. 1987). We agree with Wirnsberger *et al.* (1987) that the population studied by Foissner (1984) could be *P. carnea* rather than *P. rubra* because of low number of midventral cirri and orange-red color.

While in divisional morphogenesis, the Qingdao population of *P. rubra* exhibits the following characteristics:

(1) Complete replacement of the old AZM in the proter by newly-built structures, as described in *P. carnea*, *P. rubra*, *P. ignea* by Wallengren (1901), Wirnsberger (1987) and Mihailowitsch and Wilbert (1990) respectively.

(2) Independent occurring of FMA and AMP (Wirnsberger 1987), unlike that in *P. carnea*, *P. pulchra* and *P. ignea*, in which FMA and AMP join together posteriorly.

(3) The joined sets of primordia in the opisthe, which is in accordance with observation in the opisthe of *P. rubra* by Wirnsberger (1987).

(4) A peculiar dividing behavior of the macronuclei, like in other congeners, a few haptorids, a scuticociliate and the heterotrich genus *Protocruzia* (Raikov 1982), in which the macronuclear segments will not confuse into a spherical macronucleus before asexual division. According to Raikov (1982), this character is probably very important in evolution (Wirnsberger 1987). However, the whole morphogenetic process distinctly shows the partial confusion of macronuclear segments before cytogenesis, which seems to signify some kind of phylogenetic relationship among urostylid genus.

(5) The parental basal bodies do not participate in the forming of ciliary structures of daughter cells, which is confirmed by Wirnsberger (1987) and different from other urostylids, in which fronto-ventral-transverse cirri originate from left or right midventral cirri or from buccal and/or frontal cirri (Jerka-Dziadosz 1972, Jerka-Dziadosz and Janus 1972, Borror 1972, Wicklow 1981, Martin *et al*. 1981, Hemberger 1982, Wiąckowski 1985, Hu *et al*. 2000). The phenomenon of the non-involvement of the buccal cirrus is also known in the *Stylonychia mytilus* complex and *Kerona polymorphum* (Hemberger 1982, Hemberger and Wilbert 1982, Wirnsberger *et al*. 1986).

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