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Morphology and Morphogenesis of Two Marine Ciliates, *Pseudokeronopsis pararubra* sp. n. and *Amphisiella annulata* from China and Japan (Protozoa: Ciliophora)

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Summary. The morphology and the morphogenesis of two hypotrich ciliates, *Pseudokeronopsis pararubra* sp. n. and *Amphisiella annulata*, collected from the coastal waters off Qingdao (Tsingtao), China and Nagasaki, Japan were studied using live observation and protargol impregnation. Based on current investigations, descriptions of these two species are provided and comparisons with their congeners are given. Diagnosis for *Pseudokeronopsis pararubra* sp. n.: Marine *Pseudokeronopsis*, long elliptical in outline, 180-350×50-90 μm *in vivo* and dark reddish in colour. Ciliature comprising: 64-92 adoral membranelles; bicorona of 15-26 frontal cirri; 1 buccal and 2 frontoterminal cirri; 7-11 transverse cirri; two midventral rows comprising 62-93 cirri and extending to transverse cirri; 48-79 left and 46-80 right marginal cirri; 5-8 dorsal kineties. Numerous (> 100) macronuclear segments. Two types of cortical granules: one orange-red pigment, mainly grouped around cirri and dorsal bristles; the other, colourless and blood-cell-shaped, lying just beneath the former and densely distributed. The morphogenetic process corresponds well with those of its congeners. The most notable point is that the fronto-midventral transverse cirral anlagen never develop in connection with new oral primordium at posterior portion during divisional process in the proter. Supplementary comparison of *Amphisiella annulata* with some marine congeners has been provided in this study. Some morphogenetic and reorganizational stages show that its stomatogenesis seems to be similar to that of its congeners, i.e. the oral primordium originates parakinetally from the amphisiellid median cirral row (ACR) in the opisthe, and the old adoral zone of membranelles seems to be retained completely by the proter. Usually only 6 cirral anlagen (including the anlage for undulating membrane) develop. In the proter, the undulating membranes, the buccal cirrus and cirri left of the ACR provides three streaks, other two anlagen derive from the ACR. In the opisthe, the oral primordium produces the anlage for the undulating membranes and very likely three cirral streaks; the other two anlagen also occur within ACR. The new ACR is formed by alignment of the two rightmost cirral anlagen.

Key words: *Amphisiella*, Hypotrichida, infraciliature, marine ciliates, morphogenesis, *Pseudokeronopsis pararubra* sp. n.

INTRODUCTION

Over the past 10 years, extensive ciliate surveys have been carried out along the coasts of North China, and many new or poorly known forms have been described using modern methods (Song and Wilbert 1997a, b; Song and Hu 1999; Song and Warren 2000; Hu and Song 2000a, b, 2001a, b, c, 2002, 2003; Hu *et al.* 2002; Song *et al.* 2002). Recent work further demonstrates that the biodiversity of marine hypotrichous ciliates is greater than previously supposed (Hu and Suzuki 2004). However, compared with those found in freshwater or terrestrial biotopes, marine forms are still insufficiently studied and many remain unknown regarding their infraciliature and morphogenetic events.

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In this paper we describe one new species and provide additional information on a recently redescribed marine hypotrich, collected from coastal waters in China and Japan.

MATERIALS AND METHODS

Sampling sites. Samples were collected from mariculture water near the coast of Qingdao (Tsingtao, 36°08'N; 120°43'E), China and in Mie Port (32°48'N; 129°46'E), Nagasaki, Japan. Two populations of *Amphisiella annnulata* were isolated from the same locality in January 1996 and November 2000 in Qingdao. *Pseudokeronopsis pararubra* was isolated from both Qingdao and Nagasaki. The Qingdao population of *P. pararubra* was found in the open scallop-culturing water on 20 October, 2000, while the Nagasaki population was collected from the fish-farming water on 17 June, 2003.

General methods. After collection and isolation, specimens were kept in the laboratory, either as pure or raw cultures in Petri dishes, in boiled seawater with squashed rice grains as a substrate for bacterial growth. Cells were observed in life using phase contrast and differential interference microscope. Mixtures of saturated mercury bichloride solution and Bouin's fluid were used to fix samples. Protargol silver impregnation according to Wilbert (1975) was applied to reveal the infraciliature. Measurements were performed at magnifications of 100-1250×. Drawings were carried out with the help of a camera lucida. Terminology and systematic arrangement are according to Borror and Wicklow (1983) and Eigner and Foissner (1994).

RESULTS AND DISCUSSION

Order Hypotrichida Stein, 1859

Family Pseudokeronopsidae Borror *et* Wicklow, 1983

Genus *Pseudokeronopsis* Borror *et* Wicklow, 1983

***Pseudokeronopsis pararubra* sp. n. (Figs 1-3, 6, 7; Tables 1, 2)**

Syn. *Pseudokeronopsis pulchra* Borror *et* Wicklow, 1983 Fig. 20

Diagnosis: Marine *Pseudokeronopsis*, long elliptical in outline, 180-350 × 50-90 μm *in vivo* and dark reddish in colour. Ciliature comprising: 64-92 adoral membranelles; bicorona of 15-26 frontal cirri; 1 buccal and 2 frontoterminal cirri; 7-11 transverse cirri; two midventral rows consisting of 62-93 cirri which extend to transverse cirri; 48-79 left and 46-80 right marginal cirri; 5-8 dorsal kineties. Numerous (>100) macronuclear segments. Two types of cortical granules: one orange-red pigment, mainly grouped around cirri and dorsal bristles; the other, colourless and blood-cell-shaped, lying just beneath the former and densely distributed.

Type location: Marine molluscs culturing waters off the coast of Qingdao (Tsingtao, 36° 08' N; 120° 43' E), China. Salinity 32-37 ‰; water temperature 16-24°C; pH 8.0-8.2.

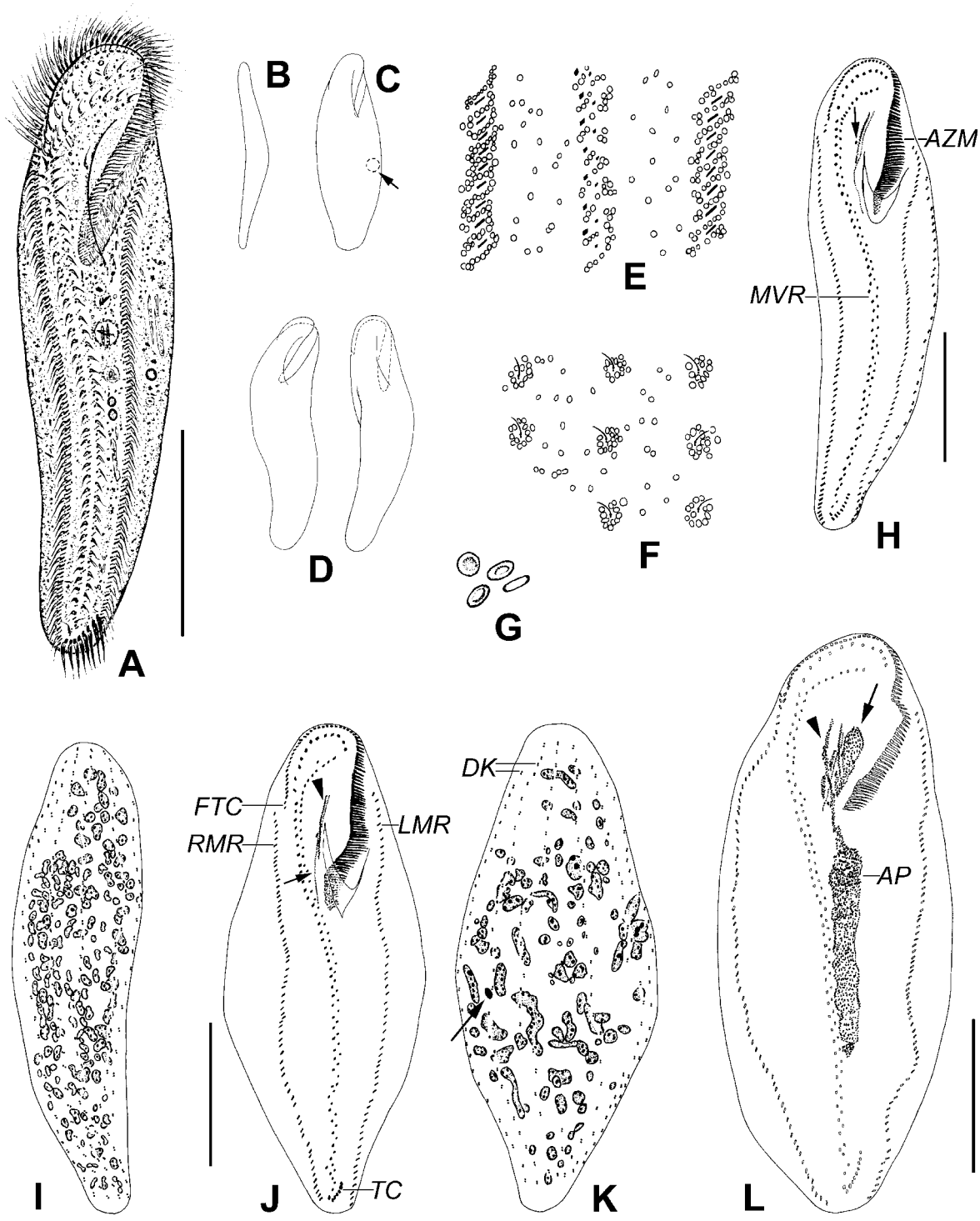
Type slides: One holotype slide has been deposited in the collections of the Natural History Museum, London, UK, with registration number 2004:6:2:1. One paratype slide of protargol-silver impregnated specimens has been deposited in the Laboratory of Protozoology, Ocean University of China (OUC), P. R. China with registration number: HD-2000102001. The Qingdao population is designated as the type population.

Morphology: The following descriptions are based on observations of two populations, one collected from Qingdao, China, the other from Nagasaki, Japan (Table 1). Body measures 180-350 × 50-90 μm *in vivo*, usually long elliptical in shape when viewed from ventral aspect with anterior end broadly rounded and posterior end narrowed, left margin conspicuously convex and right margin distinctly sigmoidal, widest at middle portion (Figs 1A, 6A); ratio of body length to width about 4-5:1; dorsoventrally flattened about 1: 2 (Fig. 1B). Body dark reddish at low magnification. Adoral zone of membranelles (AZM) about 1/3-1/4 of body length, with distal end bending posteriorly far onto right ventral side (Fig. 1A). Buccal field narrow, with cytostome deeply postioned and pharyngeal fibres conspicuous, *ca* 30-50 μm long in Nagasaki population after impregnation.

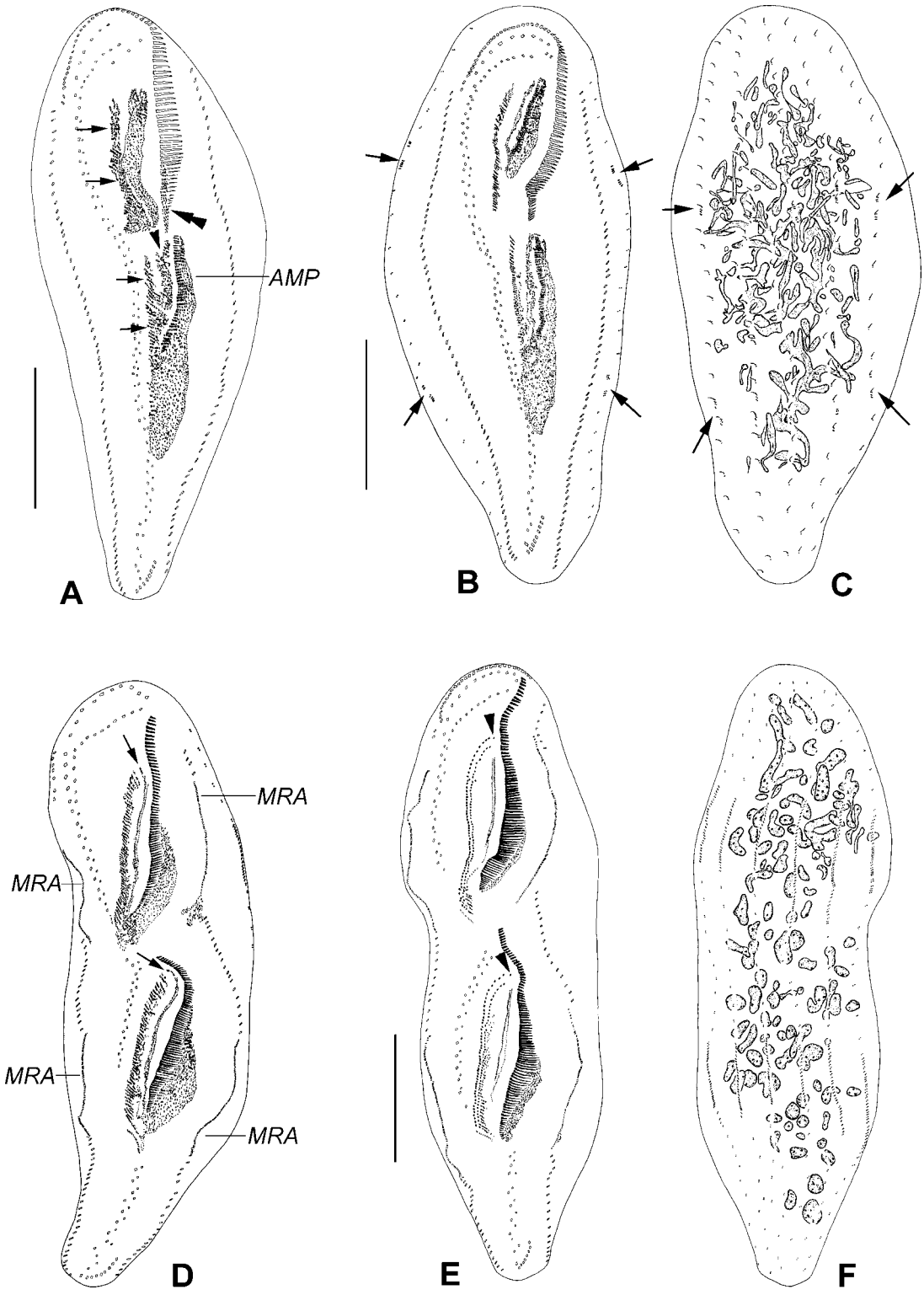
Pellicle comparatively thick, but flexible, thus variable in shape (Fig. 1D), with two kinds of cortical granules: one is orange-red pigment, spherical, 1-2 μm in diameter, mainly regularly grouped around ventral cirri and dorsal bristles (Figs 1E,F, 6D-F). These granules are thus distributed in belts or lines along cirral rows and dorsal kineties (Fig. 6B), which renders the whole cell dark reddish in colour. In addition, some were observed randomly positioned. The second type of cortical granules are red blood cell-shaped, about 2-3 μm in diameter, colourless, densely packed and positioned more deeply beneath cell surface than the former type (Figs 1G; 6E, arrows). Usually with several food vacuoles, about 6-12 μm across, and another vacuole (contractile vacuole?) sometimes observed below mid-body in Qingdao population (Fig. 1C, arrow).

Transverse cirri *ca* 15 μm long; other cirri comparatively fine, *ca* 10 μm in length, and motionless for most of the time. Locomotion by slowly crawling on substrate or rotating around main body axis when swimming.

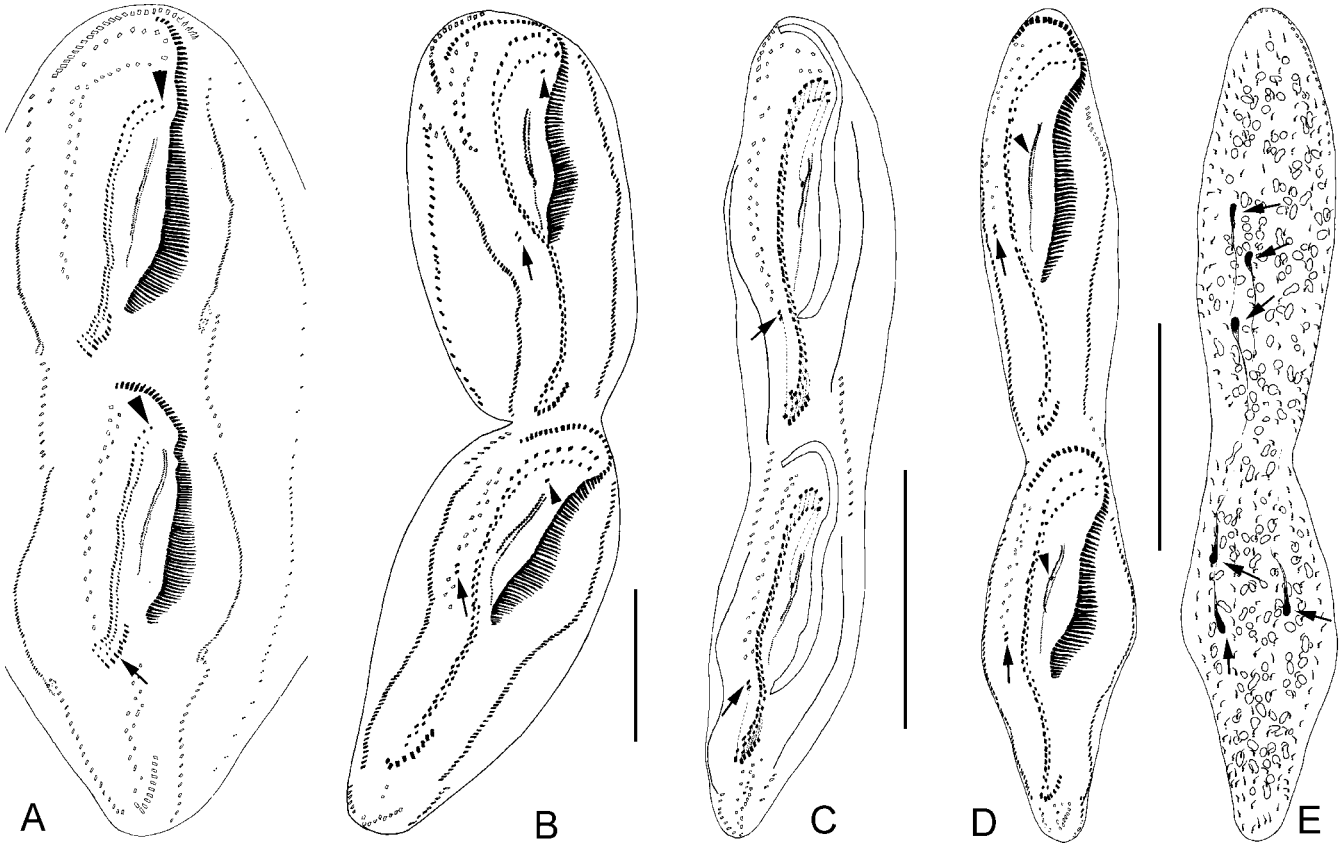
Infraciliature as shown in Figs 1H-K, 6G-K. Adoral zone of membranelles composed of 64-92 membranelles, its distal end extending to right margin of cell and bending posteriad (Fig. 6C, arrows). Paroral membrane short,



Figs 1A -L. Morphology and morphogenesis of *Pseudokeronopsis pararubra* sp. n. from life **(A-G)** and after protargol impregnation **(H-L)**. **A** - ventral view; **B** - left lateral view; **C** - plumper cell, arrow to show contractile vacuole (?); **D** - different body shapes; **E** - arrangement of pigment granules on ventral side; **F** - arrangement of pigment granules on dorsal side; **G** - red blood cell-shaped cortical granules; **H, I** - ventral and dorsal views of the same individual, showing the infraciliature and nuclear apparatus. Arrow indicates the buccal cirrus; **J, K** - ventral and dorsal views of the same cell, arrow in J to show endoral membrane, arrow in K to indicate micronucleus, arrowhead to mark paroral membrane; **L** - early stage of morphogenesis, arrow to show oral primordium of the proter, arrowhead to indicate the fronto-ventral transverse cirral anlage of the proter. AP - anarchic primordium; AZM - adoral zone of membranelles; DK - dorsal kineties; FTC - frontoterminal cirri; LMR - left marginal cirral row; MVR - midventral rows; RMR - right marginal cirral row; TC - transverse cirri. Scale bars 80 µm.



Figs 2A - F. Morphogenesis of *Pseudokeronopsis pararubra* sp. n. after protargol impregnation. **A** - ventral view, arrows to show fronto-ventral transverse cirral anlagen of both the proter and the opisthe, arrowhead to indicate undulating membranes anlagen of the opisthe, double-arrowhead to mark dedifferentiation in the posterior end of the old adoral zone of membranelles; **B, C** - ventral and dorsal views of the same individual, arrows to show dorsal kineties anlagen; **D** - ventral view, arrows to show small anlage separated from the anterior end of the undulating membranes anlagen in both the proter and the opisthe; **E, F** - ventral and dorsal views of the same cell, arrowheads to show frontal cirri derived from anterior end of undulating membranes. AMP - adoral membranelle primordium; MRA - marginal cirral row anlage. Scale bars 80 μm.



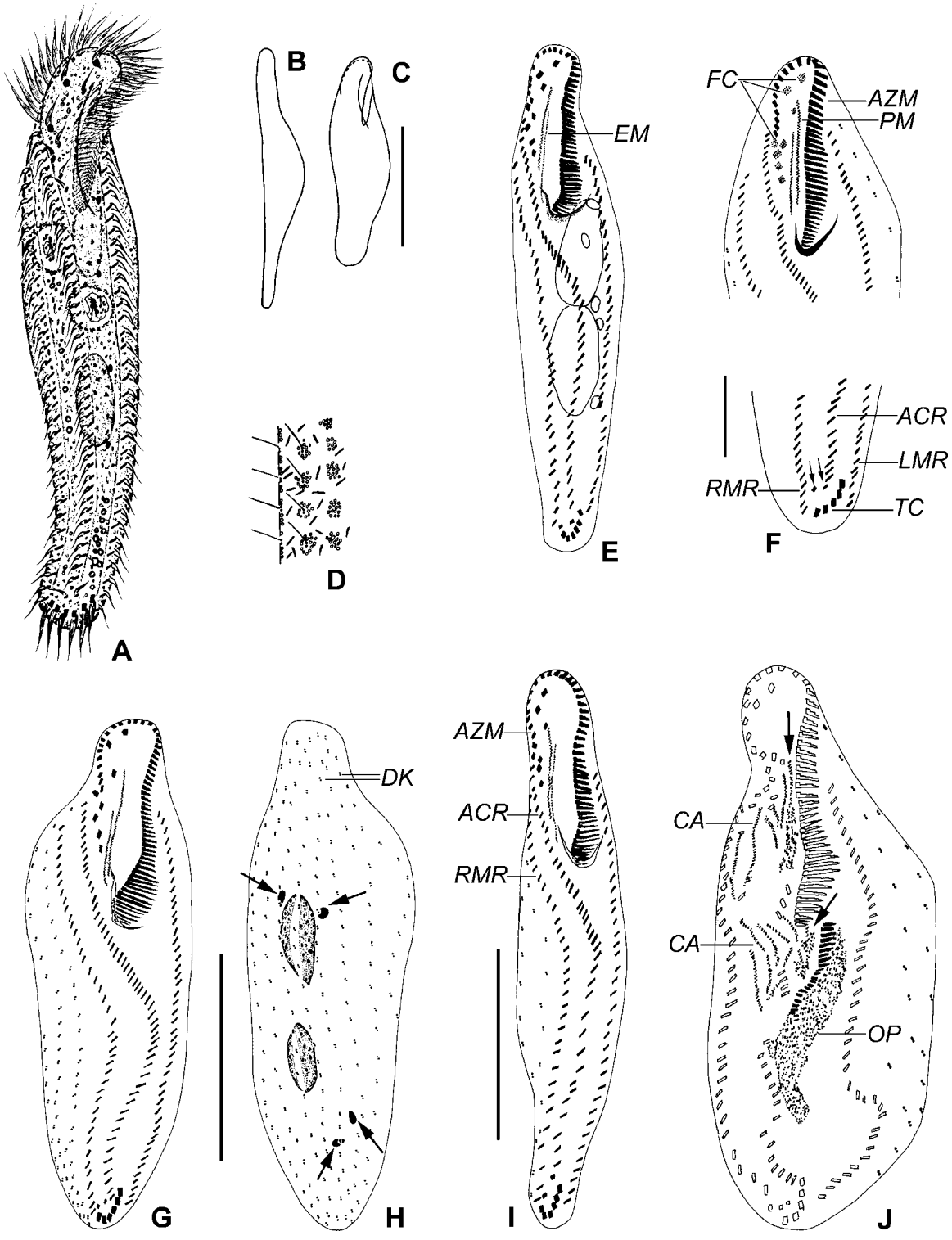
Figs 3A - E. Morphogenesis of *Pseudokeronopsis pararubra* sp. n. after protargol impregnation. **A** - ventral view, arrowheads to show frontal cirri derived from the anterior end of undulating membranes, arrow to mark the third anlage counted from posterior end in the opisthe, which produces four new cirri; **B, C** - ventral views of later dividers, arrows to show frontoterminal cirri moving anteriorly, arrowheads to mark buccal cirri in migration; cirri originating from the same anlage connected by broken lines; **D, E** - infraciliature of ventral and dorsal sides of the same later divider, arrows in D to show frontoterminal cirri moving anteriad, arrowheads to mark buccal cirri, arrows in E to indicate the dividing micronuclei. Scale bars 80 μm.

about half of endoral membrane in length (Figs 1J, 6I). Frontal cirri (FC, Fig. 6I) arranged in two arcs forming a "bicorona", which connect to midventral rows (MVR, Fig. 6J) comsisting of 62-93 cirri and extending posteriad to tranverse cirri (TC, Figs 1J, 6K), so that there is no gap between the posterior end of the midventral rows and the transverse cirri; 7-11 transverse cirri, distributed in J-shape (Figs 1H, J; 6K). Two frontoterminal cirri (FTC, Fig. 1J) between distal end of adoral zone of membranelles and anterior end of right marginal row (Figs 6C, arrowhead; 6H, arrow); single buccal cirrus lies close to paroral membrane (PM), positioned at level of posteior 1/3 (Figs 1H, arrow; 6H, arrowhead). One left and one right marginal cirral row (LMR, RMR) comprising 48-79 and 46-80 cirri, respectively (Figs 1J, 6K). Fibers connected to cirri highly developed (Fig. 6G). 5-8 dorsal kineties (DK) extending almost entire length of cell (Figs 1I, K); dorsal cilia about 5 μm long, easily recognizable *in vivo*.

Macronuclear segments numerous, more than 100 in number, each about 3-6 μm long. Occasionally, micronucleus can be recognized in daughter cells just after division (Fig. 1K, arrow).

Cell division: Since the main morphogenetic events correspond with those of other members in the genus *Pseudokeronopsis*, we here focus primarily on new discoveries for this species.

Stomatogenesis commences with the formation of small groups of basal bodies very close to several left midventral cirri (Fig. 7B, arrows). With the proliferation of basal bodies, these groups join to make a longish field, which is the anarchic primordium of the opisthe (AP, Fig. 1L). During this process, the left midventral cirri remain intact. As the new membranelles of the opisthe organize in a posteriad direction in the adoral membranelles primordia (AMP, Fig. 2A), the undulating membranes anlagen begin to separate to the right of it (Fig. 2A, arrowhead). Meanwhile, several oblique streaks appear



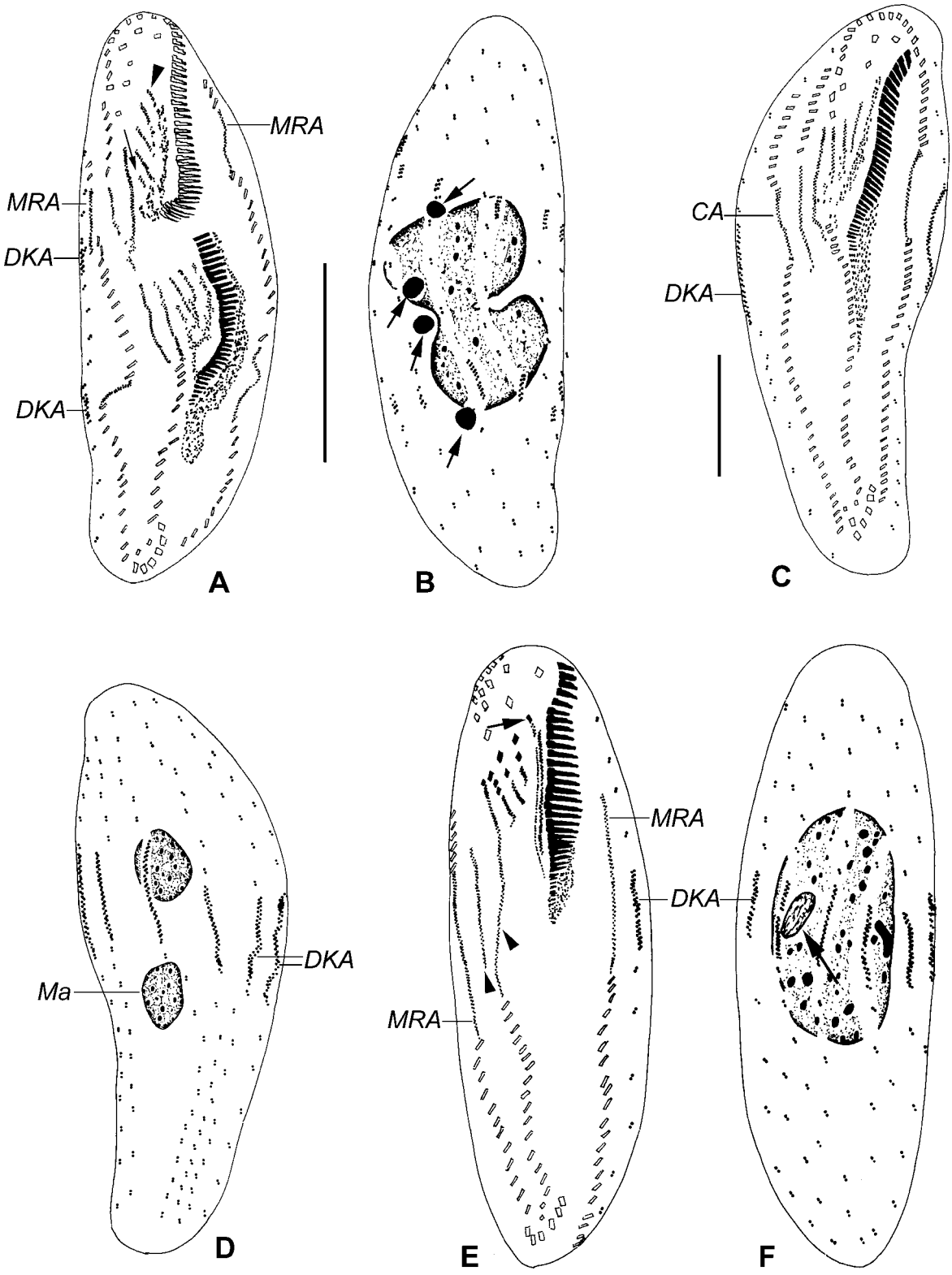
Figs 4A - J. Morphology and infraciliature of two populations of *Amphisiella annulata* from life **(A-D)** and after protargol impregnation **(E-J)**. **A** - ventral view; **B** - left lateral view; **C** - to show body in contracted form; **D** - note dorsal bristles and extrusomes around them, some extrusomes are ejected; **E, I** - infraciliature on ventral side from the population collected in November 2000; **F - H** - from the population collected in January 1996; **F** - infraciliature in anterior and posterior ventral portions, arrows to show pre-transverse cirri; **G, H** - ventral and dorsal views of the same individual, depicting infraciliature, arrows showing micronuclei; **J** - early-middle stage of morphogenesis, arrow to show undulating membranes anlagen. ACR - amphisiellid median cirral row; AZM - adoral zone of membranelles; CA - cirral anlage; DK - dorsal kineties, EM - endoral membrane; FC - frontal cirri; LMR- left marginal cirral row; MRA - marginal cirral row anlage; OP - oral primordium; PM - paroral membrane; RMR - right marginal cirral row; TC - transverse cirri. Scale bars 30 µm (F); 50 µm (I); 60 µm (C); 80 µm (G, H).

Table 1. Morphometric characterization of two populations of *Pseudokeronopsis pararubra* sp. n. All data based on protargol-impregnated specimens from the coastal waters of Nagasaki, Japan (upper line) and Qingdao, China (lower line). CV - coefficient of variation in %, Max - maximum, x - arithmetic mean, Min - minimum, n - number of specimens examined, SD - standard deviation, SE - standard error of the mean. Measurements in µm.

Character	Min	Max	x	SD	SE	CV	n
Body length	174	260	215.6	24.66	4.93	11.4	25
	238	336	288.4	28.68	7.40	9.9	15
Body width	41	66	55.6	7.13	1.43	12.8	25
	78	152	113.6	20.81	5.37	18.3	15
Adoral zone of membranelles, length	70	91	82.6	5.60	1.12	6.8	25
	88	104	95.8	4.68	1.21	4.9	15
Number of adoral membranelles	64	82	71.8	3.86	0.97	5.4	25
	68	92	77.9	6.50	1.74	8.4	14
Number of frontal cirri	15	23	18.4	2.08	0.42	11.3	25
	18	26	21.7	2.73	0.68	12.6	16
Number of midventral cirri	62	91	74.1	7.04	1.44	9.5	24
	65	93	79.0	9.40	2.35	11.9	16
Number of buccal cirri	1	1	1	0	0	0	25
	1	1	1	0	0	0	16
Number of frontoterminal cirri	2	2	2	0	0	0	25
	2	2	2	0	0	0	16
Number of transverse cirri	7	9	7.6	0.65	0.13	8.6	24
	7	11	9.4	1.53	0.29	12.2	16
Number of left marginal cirri	48	68	56.9	5.38	1.10	9.5	24
	57	79	68.5	6.66	1.72	9.7	15
Number of right marginal cirri	46	73	59.5	6.00	1.22	10.1	24
	59	80	69.6	6.66	1.78	9.6	14
Number of dorsal kineties	6	8	6.6	0.65	0.13	9.9	24
	5	7	6.4	0.62	0.15	9.7	16

at the right-anterior position of the anarchic primordium, which forms the fronto-ventral transverse cirral anlagen (Fig. 2A, arrows). At this stage the three parts are connected together at the posterior portion (Figs 2A, B). Later, they separate and further develop into new structures. As the number of adoral membranelles increases in the AMP, a small anlage separates from the anterior end of the undulating membranes anlagen in the opisthe (Fig. 2D, arrow), from which a single frontal cirrus is derived (Figs 2E, 3A, arrowhead). The remaining anlagen split longitudinally to form the paroral and endoral membranes (Figs 2E, 3A-D). During this process, each streak of cirral anlagen divides into 2 segments (cirri) except for the posterior 7-11 streaks, the posterior-most two of which usually generate 4 cirri each (Figs 2E, 3A; 7G, arrow; 7H) but occasionally 5 (Fig. 7G, double-arrowhead), and others form 3 cirri each. Very rarely, the third anlage counted from posterior end generates 4 (Fig. 3A, arrow). Among all these newly formed cirri, the posterior cirrus from the first streak moves to a position beside the paroral membrane and becomes the buccal cirrus (Figs 3B,D, arrowheads); the anterior-most two cirri from the last streak will migrate anteriad to form the frontoterminal cirri (Figs 3B-D, arrow; 7H, arrows); each of the posterior 7-11 streak contributes one transverse cirrus to the daughter cell (Figs 3A-D; 7M, arrows); the remaining new cirri become the frontal and midventral cirri. Marginal cirri and dorsal kineties develop in a usual way, that is, the anlagen appear within old structures and stretch toward both ends to form new ones (Figs 2B-F; 3A-E; 7F,G).

Just as the anarchic primordium in the opisthe appears, a kinetosomal field develops to the right of the buccal cavity and beneath the undulating membranes, which is the oral primordium of the proter (Figs 1L, arrow; 7A, arrow); at the same time the thread-like fronto-ventral transverse cirral anlage is formed independently on the surface between the buccal field and the midventral rows (Fig. 1L, arrowhead). Soon, the cirral anlagen (Figs 2A, arrows; 7K, arrow) and oral primordium (Fig. 7J, arrow) enlarge; meanwhile, the posterior end of the old oral apparatus begins to dedifferentiate (Fig. 2A, double-arrowhead). With the joining of the new basal bodies in the oral primordium, they



Figs 5A - F. Morphogenesis **(A, B)** and physiological regeneration **(C - F)** of *Amphisiella annulata* after protargol impregnation. **A, C, E** - ventral views of different individuals, arrowhead in A to show a small anlage separated from the anterior end of undulating membranes anlagen, arrow in **A** to mark additional small anlage between cirral anlagen IV and V, arrow in E to indicate frontal cirrus derived from the anterior end of undulating membranes anlagen, arrowheads in E to show the longer cirral anlagen V and VI; **B, D, F** - dorsal views of different cells, arrows to show micronucleus. CA - cirral anlage; DKA - dorsal kinety anlage; Ma - macronuclear nodule; MRA - marginal cirral row anlage. Scale bars 50 µm (A, B); 40 µm (C, D).

gradually organize in a posteriad direction (Figs 2B; 7L, double-arrowhead). Simultaneously the undulating membranes anlage separates and generates the new paroral and endoral membranes as well as the frontal cirrus at their anterior end (Figs 2B, D, E; 3A; 7L, arrow). As in the opisthe, cirral anlagen develop into frontal, buccal, midventral and transverse cirri (Figs 2B, D, E; 3A-D; 7C, arrows; 7L, arrowheads). The replication bands of the macronuclei appear at an early stage of morphogenesis (Fig. 7D, arrowheads). The macronuclear nodules divide without prior fusion (Fig. 7I). Micronuclei divide to be assigned to two daughter cells at late stages (Figs 3E, 7E, arrows).

Comparison with similar species and discussion: As widely noted, species identification in *Pseudokeronopsis*, especially those with pigmented granules, is often difficult because its members possess many overlapping morphological characters (e.g. body size and shape, and the infraciliature), and have very similar morphogenetic patterns (Kahl 1932, Borror and Wicklow 1983, Foissner 1984, Wirnsberger 1987, Wirnsberger *et al.* 1987, Hu and Song 2000b, Song *et al.* 2002). Prior to this investigation, at least five marine species have been described using modern methods, namely: *P. rubra*, *P. flava*, *P. flavicans*, *P. carnea* and *P. qingdaoensis* (Table 2). Of these, *P. qingdaoensis* can be easily separated from *P. pararubra* sp. n. by its body shape (wedge-shaped with posterior end tapered *vs.* long elliptical), higher numbers of transverse (27-45 *vs.* 7-11) and buccal (6-10 *vs.* 1) cirri, and fewer dorsal kineties (3 *vs.* 5-8). In addition these forms differ in the arrangement of their cortical granules (Hu and Song 2000b).

In terms of body size, the arrangement of the granules and the general ciliary pattern, *P. pararubra* sp. n. is very similar to *P. rubra* (Wirnsberger *et al*. 1987, Shi and Xu 2003). However, the former differs from the latter in the following features: body shape *in vivo*, which is often a useful character for species separation (Foissner 1982) (long elliptical *vs.* band-like); the position of buccal cirrus relative to the paroral membrane (i.e. at level of posterior 1/3 *vs.* anterior 1/3); orange-red pigment granules (*vs.* brick-red); the number of frontal cirri (18 and 22 on average, respectively *vs.* 14-16, data from Wirnsberger *et al.*, 1987 and 15 on average in Chinese population) and transverse cirri (7-11, 8 and 9 on average, respectively *vs.* 7 and 6 on average, respectively). Additionally, Wirnsberger *et al.* (1987) gave a revised diagnosis and detailed redescription of *Pseudokeronopsis rubra* and did not mention the red blood cell-shaped cortical granules that are present in *P. pararubra* sp. n. Shi and Xu (2003) redescribed *P. rubra* collected from the South China Sea and also failed to observe these granules suggesting that they are absent in this taxon.

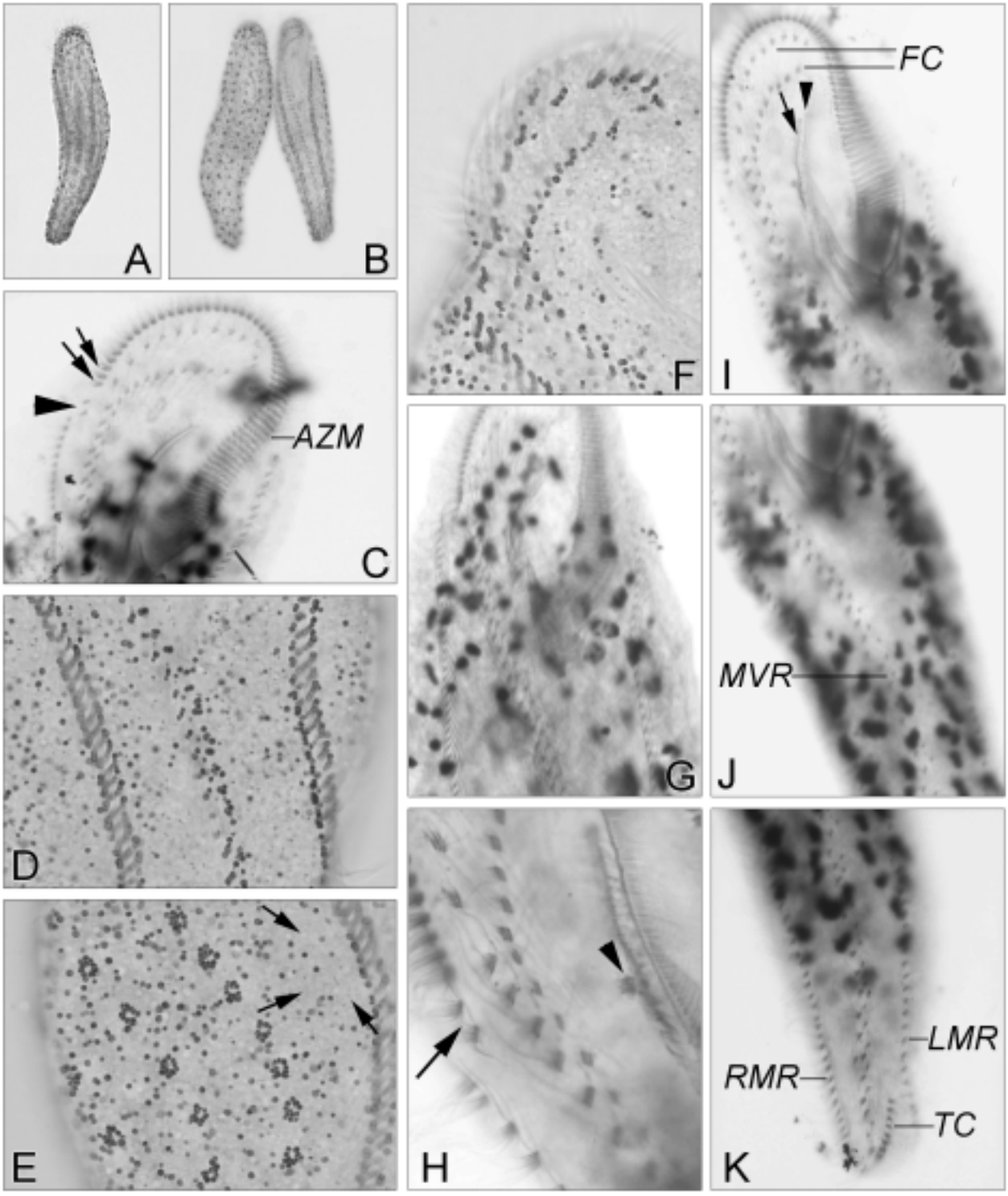
Pseudokeronopsis carnea resembles *P. pararubra* sp. n. in terms of its body size *in vivo*, the colour of the pigmented granules and most biometric characters (Wirnsberger *et al.* 1987). However, the former can be easily separated from the latter by its midventral rows terminating at different levels above the transverse cirri (*vs.* extending all the way to the transverse cirri), a plumper body shape with non-tapering posterior end (*vs.* long elliptical with a conspicuously narrowed caudal region), and having fewer frontal (10-14 *vs.* 15-26) and transverse cirri (6-7 *vs.* 7-11). Considering the strongly shortened midventral rows and orange-red coloured pigment granules present in the Mediterranean population of *Pseudokeronopsis rubra* (Foissner 1984), we agree with Wirnsberger *et al.* (1987) in regarding this as a population of *P. carnea*.

Compared with the new species, *P. flavicans* and *P. flava* are more slender (*vs.* plumper body shape in *P. pararubra* sp. n.), and have a yellow cell colour and yellow-brownish or yellow pigment granules (*vs.* dark red or orange-red), and fewer frontal cirri (*ca* 14 and 9 respectively *vs.* 15-26), transverse cirri (3-6 and 1-3 respectively *vs.* 7-11) and dorsal kineties (4-5 and *ca* 4 respectively *vs*. 5-8). Additionally, these species have a conspicuous gap between the posterior ends of the midventral rows and the transverse cirri (*vs.* no gap in *P. pararubra* sp. n.) (Wirnsberger *et al.* 1987, Song *et al.* 2002).

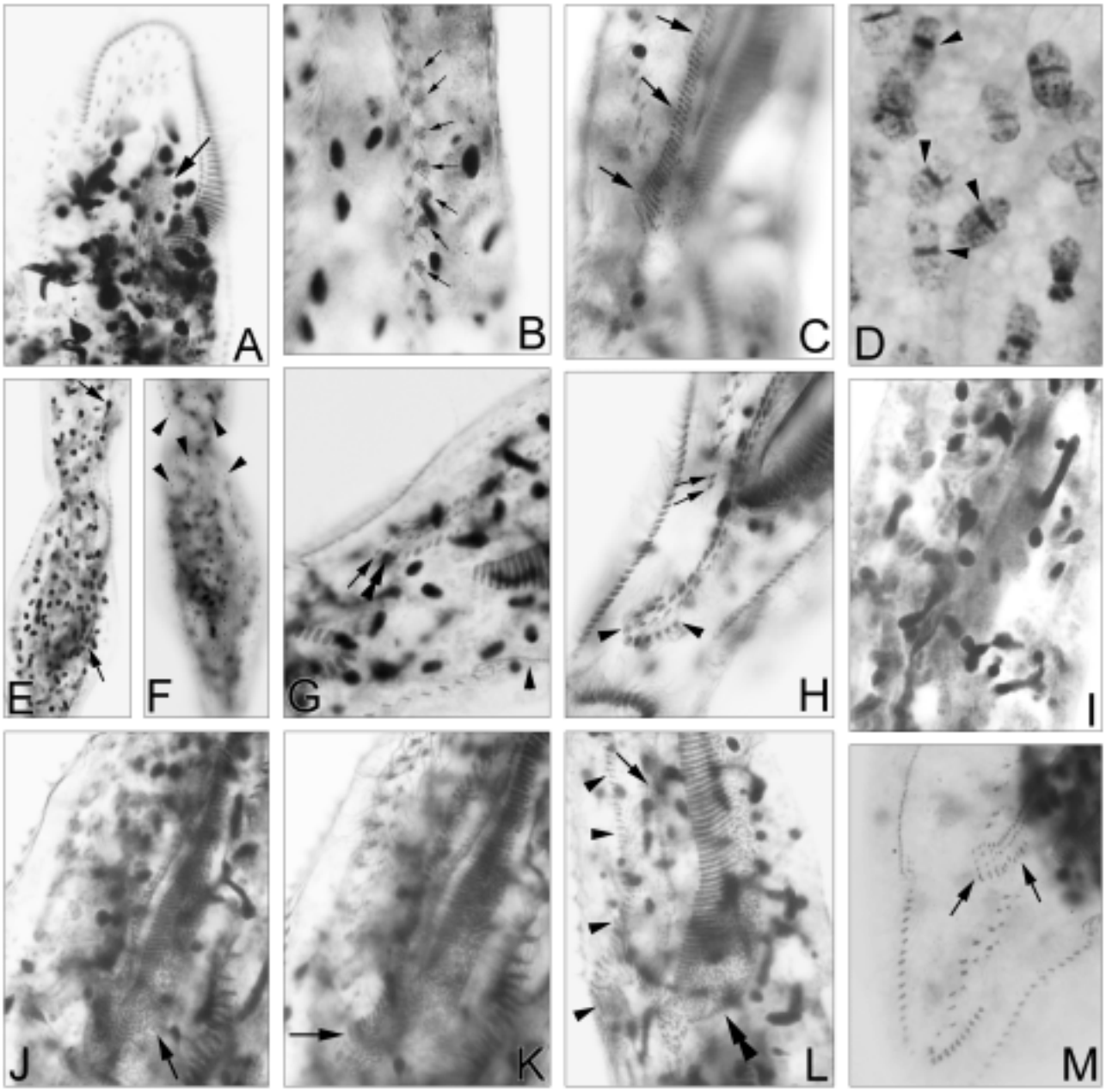
Hu and Song (2001a) gave a detailed description of a reddish *Pseudokeronopsis* species from Qingdao, China under the name of *P. rubra*. The shortened midventral rows of this population, the conspicuous specialization of cortical granules and the numbers of frontal cirri in the bicorona (11-14) and transverse cirri (2-4) suggest it might be a population of *P. flavicans*. However, some morphological characters are more similar to those of *P. rubra*, i.e. the brick-red pigment granules and the presence of median groove along the midventral rows.

Borror and Wicklow (1983) illustrated *Pseudokeronopsis pulchra in vivo* but did not give any description about its live features or infraciliature. Meanwhile, they just synomized *Holosticha (Keronopsis) pulchra* Kahl, 1932 and *Keronopsis pulchra* in Borror (1972) with their population but supplied no reasons.

However, the original description of this taxon (Kahl 1932, p. 573; Fig. 104_5 on p. 577; obviously incorrectly



Figs 6A - K. Photomicrographs of *Pseudokeronopsis pararubra* sp. n. from life **(A, B, D-F)** and after protargol impregnation **(C, G-K)**. **A** - ventral view; **B** - two cells, one (left) viewed from dorsal and one (right) from ventral aspect, to denote the arrangement of the pigment granules; **C** - buccal apparatus, arrows to show membranelles at distal end, arrowhead to indicate frontoterminal cirri; **D** - arrangement of pigment granules on ventral side; **E** - arrangement of pigment granules on dorsal side, arrows to show red blood-cell-shaped cortical granules; **F** - arrangement of pigment granules in frontal area; **G** - portion of anterior end of cell, ventral view, to show fibers; **H** - ventral view of part of cell, arrow to show frontoterminal cirri, arrowhead to indicate buccal cirrus; **I - K** - anterior (I), middle (J) and posterior (K) parts of the same cell, ventral view, arrowhead to show paroral membrane, arrow to indicate endoral membrane. AZM - adoral zone of membranelles; FC - frontal cirri; LMR - left marginal cirral row; MVR - midventral rows; RMR - right marginal cirral row; TC, transverse cirri.



Figs 7A - M. Photomicrographs of *Pseudokeronopsis pararubra* sp. n. after protargol impregnation. **A** - note the oral primordium (arrow) in the proter; **B** - arrows depict small groups of basal bodies very close to left midventral cirri; **C** - depicting fronto-midventral transverse cirral anlagen (arrows) of the proter**; D** - noting the replication bands of macronuclear segments (arrowheads). **E, F** - part of the same later divider, to show dividing micronuclei (arrows) and new dorsal kineties (arrowheads); **G** - ventral view of posterior portion of the proter, arrow to show the last streak producing four cirri, double-arrowhead to mark the next anlage forming five cirri, arrowhead to indicate the anlage for the left marginal cirral row; **H** - ventral view of the posterior part of the proter, arrows to show frontoterminal cirri moving anteriad, arrowheads to mark the new transverse cirri; **I** - noting the division of the macronuclear segments; **J, K** - ventral views of part of the same proter, arrow in **J** to show the oral primordium, arrow in K to mark the fronto-midventral transverse cirral anlagen; **L** - ventral view of part of the proter to show the fronto-midventral transverse cirral anlagen (arrowheads), undulating membranes anlagen (arrow) and oral primordium (double-arrowhead); M - ventral view of the posterior portion of the opisthe, arrows to show the new transverse cirri.

designated as *Keronopsis rubra* f. *heptasticha* in the legend) clearly shows that the anterior-most three cirri of *H. pulchra* are distinctly enlarged. Kahl (1933) provided an additional illustration, which also clearly shows that *H. pulchra* has only 3 frontal cirri. Therefore, the form described by Kahl should not be transferred to the genus *Pseudokeronopsis* since it lacks a distinct bicorona. Borror (1972) illustrated the infraciliature

Table 2. Comparison of the red, orange and other coloured taxa of marine *Pseudokeronopsis* reinvestigated using silver impregnation techniques. Measurement in µm. AM - adoral membranelles, BC - buccal cirrus, DK - dorsal kineties, FC - frontal cirri, MVC - midventral cirri, MVR - midventral rows, PM - paroral membrane, TC - transverse cirri

	P. flava	*P. flavicans*	*P. flavicans*[1]	*P. carnea*	*P. carnea*[2]	*P. rubra*	*P. rubra*	*P. qingdaoensis*	*P. pararubra* sp. n.
Cell size *in vivo*	140-260×40-57	200-300×40-55	160-200×24-40	140-200×28-40	200-320×30-50	170-290×30-70	165-230×54-90	130-240×50-70	180-350×50-90
Body shape	very slender, posteriorly tapered	belt-like, caudally narrowed	slender with posterior end spoon-like	belt-like, caudally slightly or not narrowed	slender with posterior end spoon-like	band-like with posterior end spoon-like	long elliptical with posterior end narrowed	elongated with tapering posterior end	long elliptical with posterior end narrowed
Colour of pigments	yellow brownish	yellow-	brick-red	orange-red	orange-red	brick-red	brick-red	reddish	orange-red
Position of single BC relative to PM	middle part* mid-point	beside	*ca* mid-point*	posterior 1/3*	posterior 1/3*	anterior 1/3	anterior 1/3	**	posterior 1/3
Number of AM	43-59	46-66	46-60	39-80	58-80	50-92	62-88	50-65	64-92
Number of FC	*ca* 9	*ca* 14	11-14	10-14	*ca* 14	14-16	12-18	21-35	15-26
Number of pairs of MVC	28-38	25-40	*ca* 24-38	22-44	*ca* 35	28-47	28-38	*ca* 40	*ca* 30-45
Number of TC	1-3	3-6	2-4	6-7	5-7	*ca* 7	5-8	27-45	7-11
Number of DK	*ca* 4	4-5	4-7	5-6	5	*ca* 6	6-7	3	5-8
Gap between posterior end of MVR and TC	always conspicuous	small and inconspicuous	small to conspicuous	small to conspicuous	conspicuous	absent	absent	absent	absent
References	Wirnsberger *et al.* (1987)	Song *et al.* (2002)	Hu and Song (2001a)	Wirnsberger *et al.* (1987)	Foissner (1984)	Wirnsberger *et al.* (1987)	Shi and Xu (2003)	Hu and Song (2000b)	present study

[1, 2] Both called *Pseudokeronopsis rubra* in these two papers; * based on the protargol illustrations; ** buccal cirri consisting of 6-10 cirri

of *Keronopsis pulchra*. The figure shows that this species has a more or less distinct bicorona suggesting that it should be assigned to the genus *Pseudokeronopsis* (Wirnsberger *et al.* 1987). In addition, the conspicuously shortened midventral rows and the lower numbers of frontal and transverse cirri clearly separate this taxon from *P. pararubra* sp. n. Consequently, the organisms observed by Borror (1972) and by Borror and Wicklow (1983) were very likely misidentified in both cases. Furthermore, the form illustrated by Borror and Wicklow (1983) is very similar to our new species in terms of its body shape, the general ciliary pattern and the number of frontal and transverse cirri. Therefore we consider it very likely a synonym of *P. pararubra* sp. n.

The morphogenesis of *Pseudokeronopsis* spp. has been reported on at least three occasions (Borror 1972, Wirnsberger 1987, Hu and Song 2001a). The new species corresponds well with its congeners in the origin and developmental pattern of the primordia and the anlagen. Only one point needs to be noted here, which is the connection of the oral primordium with the fronto-midventral transverse cirral anlagen in the proter. Based on our observations of this species and of *Pseudokeronopsis flavicans* (Hu and Song 2001a), these two structures appear in different places and never develop a connection at their posterior ends at any stage during the divisional process. However, this is in contrast with that in *P. carnea*, in which these two groups of primordium join posteriorly for a period (Borror 1972, Wirnsberger 1987). According to our studies, however, these two groups of primordia intersect each other at different depths within the cell (i.e. in different optical planes), so the apparently close contact between them, especially when viewed from the ventral side and at lower magnification, is likely an artefact.

Family Amphisiellidae Jankowski, 1979

Genus *Amphisiella* Gourret *et* Roeser, 1888

***Amphisiella annulata* (Kahl, 1928) Borror, 1972 (Figs 4, 5, 8; Tables 3, 4)**

Syn. *Holosticha annulata* Kahl, 1928

Holosticha (*Amphisiella*) *annulata* Kahl, 1928 in Kahl, 1932

Prior to this investigation, this species has not been reported from Yellow Sea, China although it has been noted elsewhere for a few times (Kahl 1932, Borror 1972, Aladro Lubel 1985, Alekperov and Asadullayeva 1999, Berger 2004). The description below is based on observations on the population found in mollusks-culturing waters off the coast of Qingdao, China.

Ecological features: For January 1996 and November 2000 respectively: water temperature about 4°C and 15°C, salinity 29‰ and 33‰, pH 8.0 and 8.3.

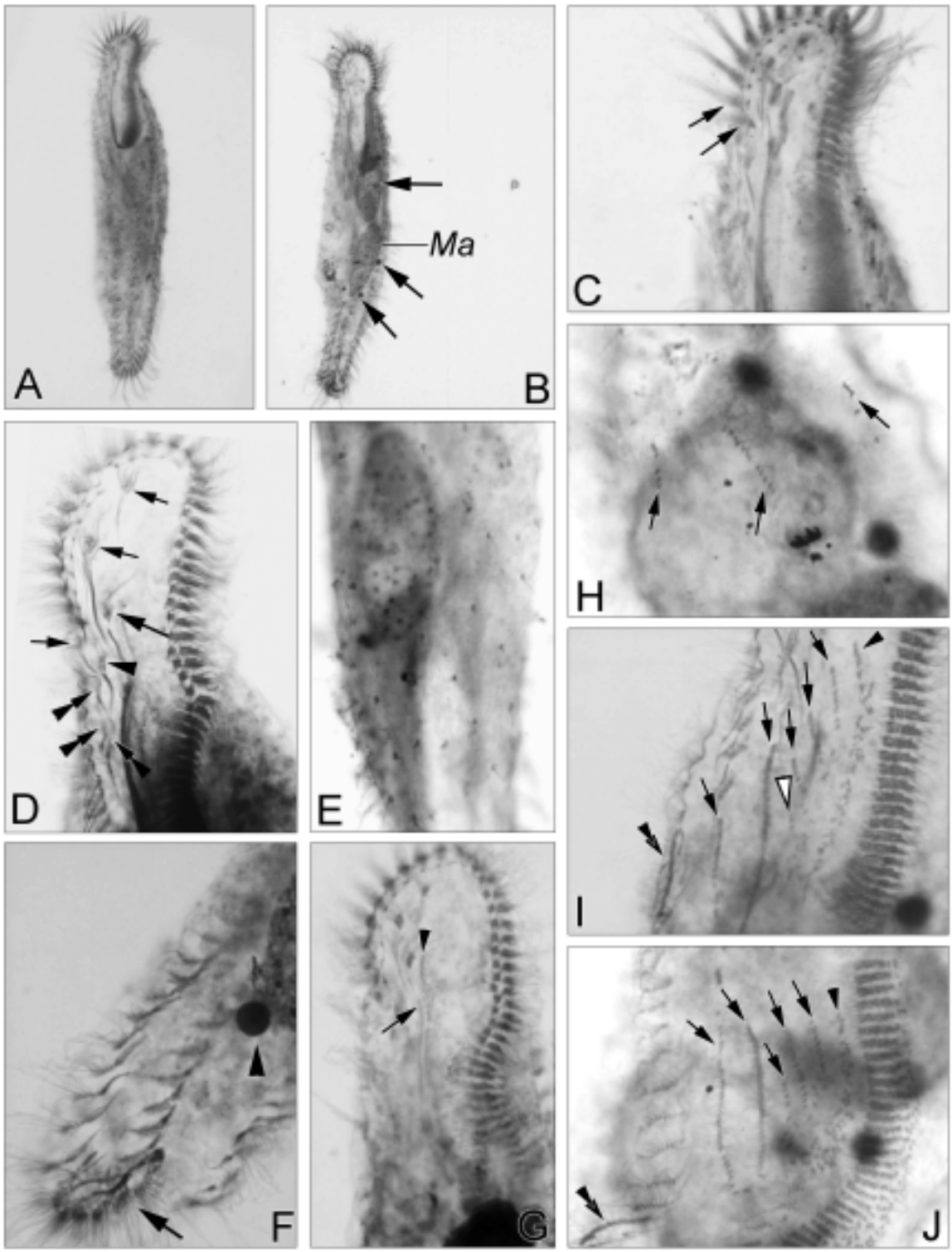
Morphology (Figs 4, 8; Table 3): Body measures 100-210 × 24-75 μm *in vivo*, usually elongated elliptical in shape with both ends rounded, left margin conspicuously convex at middle part and right one distinctly sigmoidal (Fig. 4A); ratio of body length to width about 3-5: 1. As in other hypotrichs, body variable in size depending on nutritious state. Dorsoventrally flattened, with ventral side even and dorsal side evidently convex at middle portion (Fig. 4B). Cell flexible and slightly contractile (Fig. 4C). Body colourless to grayish at low magnification. Adoral zone of membranelles about 25% - 33% of body length, with distal end bending posteriorly far onto right ventral side (Figs 4A; 8C, arrows).

Pellicle soft, with colourless extrusomes, less than 0.5 μm in diameter, grouped around dorsal bristles and arranged in lines along dorsal kineties; when ejected, 2-3 μm long (Fig. 4D). Cytoplasm transparent with several refractile granular inclusions, 2-5 μm long. Sometimes 1-2 food vacuoles recognized at mid-body; contractile vacuole not observed, probably absent. Two ellipsoid to long elliptical macronuclear nodules (Ma) positioned posterior to oral field and left of median (Figs 4H, 8B); 2-6 micronuclei (n=21), each about 4 μm long, and about 1-2 located adjacent to one macronuclear nodule (Figs 4A, E, H; 8B, arrows); sometimes micronuclei distantly located far from macronuclear nodules in impregnated cell (Fig. 4H).

Cilia of apical adoral membranelles *ca* 15 μm long. Enlarged frontal cirri about 15 μm in length; transverse cirri *ca* 16-20 μm long; other cirri slightly shorter, 10-12 μm long. Right marginal row located parallel to amphisiellid median cirral row, and cirri in both rows remain motionless for most of the time.

Movement without peculiarities, that is, by crawling on substrate or rotating around long body axis when swimming. Feeds mainly on bacteria.

Infraciliature as shown in Figs 4E-I, 8A-G. Adoral zone of membranelles composed of 33-62 membranelles. Paroral membrane composed of two rows of basal bodies in 'Zig-Zag' form parallel to single-rowed endoral membrane, and anteriorly positioned (Fig. 8G, arrowhead and arrow, respectively). Constantly three slightly enlarged frontal cirri (FC, Figs 4F; 8D, short arrows), one cirrus behind the right frontal cirrus (Fig. 8D, arrowhead), one buccal cirrus and three cirri left of amphisiellied median cirral row (Fig. 8D, long arrow and double-



Figs 8A - J. Photomicrographs of *Amphisiella annulata* after protargol impregnation. **A** - infraciliature on ventral side; **B** - macronuclear nodules and micronuclei (arrows); **C, D** - infraciliature in anterior ventral portion, arrows in C to show the membranelles at the distal end, short arrows in D to indicate the frontal cirri, long arrow to show the buccal cirrus, arrowhead to mark the cirrus behind the right frontal cirrus, double-arrowheads to note the cirri left of the amphisiellid median cirral row; **E** - dorsal part of the cell, to note the dorsal kineties; **F** - ventral view of the posterior portion of the cell, arrow to show the transverse cirri, arrowhead to indicate micronucleus; **G** - buccal apparatus, arrowhead to show the paroral membrane, arrow to indicate the endoral membrane; **H** - dorsal view of part of the cell, arrows to show the dorsal kineties anlagen; **I, J** - ventral views of the proter and the opisthe, arrows to show the cirral anlagen, solid arrowheads to indicate the anlagen separated from the anterior end of the undulating membranes anlagen, hollow arrowhead and double-arrowheads to mark additional anlage between cirral anlagen IV and V, and the marginal cirral anlagen, respectively.

Table 3. Morphometric characterizations of two populations of *Amphisiella annulata* (upper line, 1996 population; lower line, 2000 population). Data based on protargol impregnated specimens. Measurements in mm. ACR - amphisiellid median cirral row, CV - coefficient of variation in %, Max - maximum, x - arithmetic mean, Min - minimum, n - number of specimens examined, SD - standard deviation, SE - standard error of the mean. Measurements in µm.

Character	Min	Max	x	SD	SE	CV	n
Body length	120	203	167.3	24.69	6.37	14.8	15
	120	200	152.8	21.88	4.47	14.3	24
Body width	45	80	59.3	10.60	2.74	17.9	15
	28	64	42.1	9.74	2.08	23.1	22
Adoral zone of membranelles, length	48	78	65.7	8.63	2.23	13.1	15
	42	78	55.3	10.04	2.01	18.1	25
Number of adoral membranelles	41	62	52.2	5.70	1.52	10.9	15
	33	57	44.7	6.58	1.34	14.7	24
Number of frontal cirri	3	3	3	0	0	0	15
	3	3	3	0	0	0	25
Number of cirri behind of the right frontal cirrus	1	1	1	0	0	0	15
	1	1	1	0	0	0	25
Number of buccal cirri	1	1	1	0	0	0	15
	1	1	1	0	0	0	25
Number of cirri left of ACR	3	3	3	0	0	0	15
	3	3	3	0	0	0	25
Number of cirri in ACR	45	61	49.9	13.04	5.83	26.2	15
	33	56	42.5	5.98	1.34	14.1	20
Number of pre-transverse cirri	2	2	2	0	0	0	15
	2	2	2	0	0	0	25
Number of transverse cirri	5	7	5.9	0.46	0.12	7.7	15
	6	6	6	0	0	0	20
Number of left marginal cirri	36	46	40.9	3.50	0.93	8.5	15
	25	53	36.5	7.24	1.58	19.8	21
Number of right marginal cirri	36	49	42.4	4.29	1.15	10.1	15
	22	48	34.9	6.69	1.46	19.2	21
Number of dorsal kineties	8	10	8.2	0.53	0.13	6.5	15
	7	8	7.7	0.58	0.13	7.6	19
Number of macronuclear nodules	2	2	2	0	0	0	15
	2	2	2	0	0	0	25
Macronuclei length	16	35	24.3	8.47	2.19	34.8	15
	27	44	34.2	5.15	1.03	15.0	25
Macronuclei width	9	15	12.2	1.57	0.41	12.9	15
	12	28	17.8	4.85	0.97	0.27	25
Number of micronuclei	-	-	-	-	-	-	-
	2	6	3.5	0.98	0.21	28.2	21

- No data available

arrowheads, respectively). Five to seven (usually six) transverse cirri (TC) arranged in J-shape (Figs 4F, G; 8F, arrow). Amphisiellid median cirral row (ACR), of which the cirri are wide (up to 4-5 µm) and narrowly spaced, especially at middle portion (Figs 4E, G), is composed of 33-61 cirri, extending posterior to TC. Additionally 2 pre-transverse cirri located between the posterior end of ACR and TC (Figs 4F, arrows; 4G, I). One left and one right marginal cirral row with their posterior ends not confluent (Figs 4E-G, I), base of each cirrus consisting of two basal body rows. Seven to ten dorsal kineties, of which several rows are shortened at both ends (Figs 4H, 8E); dorsal cilia about 5 µm long, easily recognizable *in vivo*.

Morphogenesis (Figs 4J, 5): A detailed analysis of the morphogenetic process was not carried out because only a few dividers and reorganizers were found, mostly middle to late stages. Nevertheless, these allow us to deduce that stomatogenesis in *A. annulata* is similar to that in its congeners (Wicklow 1982): the oral primordium (OP) originates parakinetally from the ACR (Fig. 4J) in the opisthe, while the old adoral membranelles seems to be retained completely by the proter, and the old undulating membranes must be renewed (Figs 4J,

Table 4. Supplementary comparison of Qingdao populations of *Amphisiella annulata* with a few marine congeners. ACR - amphisiellid median cirral row, AM - adoral membranelles, BL - body length, DK - dorsal kineties, FC - frontal cirri, Ma - macronuclear nodules, TC - transverse cirri. Measurements in µm.

Species name	Body shape	BL	M	FC*	ACR	TC	Ma	DK	Data source
A. ovalis Fernandez-Leborans *et* Novillo, 2001	oval	50-63	16-19	4	18-22	6-7	32-45	4	Fernandez-Leborans and Novillo (1992, 2001)
A. capitata (Perejaslawzewa, 1885)	elongated, slightly cephalized	-	-	11	-	5	2	-	Kahl (1932)
A. oblonga (Schewiakoff, 1893)	elliptical	160	-	4	-	4	2	-	Kahl (1932)
A. thiophaga (Kahl, 1928)	long elliptical	70-100	-	5	-	7	2	-	Kahl (1928, 1932)
A. arenicola Fernandez-Leborans *et* Novillo, 2001	elongated oval	132-162	36-42	7	52-56	5-6	2	5-6	Fernandez-Leborans and Novillo (1992, 2001)
A. annulata (Kahl, 1928)	long elliptical	100-210	33-62	8	33-61	5-7	2	7-10	present study

* including other cirri left of ACR in frontal field

arrow; 5A). Six cirral anlagen (Figs 5A; 8I, J; arrows) eventually develop into the frontal, pre-transverse and transverse cirri and amphisiellid median cirral row plus undulating membranes (usually the anlage generating undulating membranes and one frontal cirrus at its anterior end also called undulating membrane anlage; Figs 5A, arrowhead; 5E, arrow; 8I, J, arrowhead). Rarely there is a small additional anlage between the normal anlagen IV and V (Fig. 5A, arrow). In both dividers, the rightmost two cirral anlagen are formed within the old amphisiellid median cirral row. In the proter, the undulating membranes, the buccal cirrus and some cirri left of the ACR provide three streaks, while in the opisthe, the oral primordium produces the anlage for the undulating membranes and very likely three cirral streaks (Figs 4J; 5A). The marginal row and dorsal kineties evolve as described by Berger (2004), that is, all anlagen occur within the old structures and develop to replace them (Figs 5; 8H-J, arrows and double-arrowhead). The division of the nuclear apparatus does not show a peculiarity. Two marconuclear nodules fuse to a single mass during the middle stages (Figs 5B, F), which subsequently divides twice to be assigned to the daughters.

Comparison: The Qingdao populations of *Amphisiella annulata* correspond well with the redescriptions by Kahl (1932) and Berger (2004) in terms of body shape and size, number of macronuclear nodules as well as ciliary pattern, especially the very narrowly spaced and rather wide cirri of the amphisiellid median cirral row. However, although ring-shaped structures (lithosomes?), the characteristic feature that Kahl (1928) and Berger (2004) described for *Amphisiella annulata*, could not be seen in the cytoplasm of our populations, this is not a proof of misidentification because Kahl (1932) also mentioned that the rings can be absent.

Since Berger (2004) very recently gave a detailed redescription and neotypication of *Amphisiella annulata*, we here provide only supplementary comparisons with some of its congeners (Table 4). Compared with *A. annulata*, *A. ovalis* is much smaller (49.5-63 µm long), has an oval body shape and fewer cirri as well as more macronuclear segments (32-45 *vs.* 2), thus the two can be easily separated (Fernandez-Leborans and Novillo 1992, 2001).

In terms of its body shape, *Amphisiella annulata* also resembles *A. thiophaga*, whose infaciliature is unknown. However, the latter is smaller (70-100 µm)

and has fewer frontal cirri (5 *vs.* 7-9) and sparsely arranged cirri in the amphisiellid median cirral row (Kahl 1932, Borror 1963, Aladro Lubel 1985, Alekperov and Asadullayeva 1999, Berger 2004). Likewise, the infraciliature of *Amphisiella capitata* and *A. oblonga* remain unknown although they differ from *A. annulata* in body shape and in the number of frontal and transverse cirri (Kahl 1932). Fernandez-Leborans and Novillo (1992, 2001) described a new form, *Amphisiella arenicola*, which is similar to *A. annulata* in terms of its body size and the number of adoral membranelles, cirri and macronuclear nodules. However, the former can be separated from the latter by its elongated oval body shape (*vs.* elongated elliptical) and the location of the amphisiellid median cirral row (in middle zone and with a distinct gap between its posterior end and transverse cirri *vs.* posteriorly located and with no gap between its posterior end and the transverse cirri).

As concerns morphogenesis and reorganization, the Qingdao populations lack the additional anlage between the ordinary anlagen IV and V described by Berger (2004) in the Adriatic population of *A. annulata*, so that usually only six ordinary anlagen (I-VI) are formed during division. According to Berger (2004) six transverse cirri in the Adriatic population was formed by the mode that six ordinary cirral anlagen plus an additional anlage contribute one transverse cirrus each at its posterior end except for undulating membrane anlage which does not generate transverse cirrus, 6 cirral anlagen present in Qingdao populations can only produce 5 transverse cirri. However, most cells have six transverse cirri at interphase in our studies. In order to elucidate this difference, we assume that the following possibilities exist: (1) in the instance of only 6 cirral anlagen (most common condition), if each anlage develops one transverse cirrus thus 5 transverse cirri (this number occurs very rarely in our population and the Adriatic population) are eventually formed; if the posterior-most anlage contributes two transverse cirri as demonstrated in *Pseudokeronopsis* spp. by Wirnsberger (1987), so that the filial cell will have 6 transverse cirri (this is very likely the case of Qingdao populations); (2) if an additional anlage occurs (rarely in Qingdao population), then the anlagen probably evolve in the way as shown by Berger (2004) and six transverse cirri are thus formed, one from each anlage.

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