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Morphogenesis of the Euplotid Ciliate *Uronychia binucleata* Young, 1922 (Ciliophora, Hypotrichia)

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Abstract Morphogenesis of a population of the marine euplotid ciliate, *Uronychia binucleata*, which was found in Yellow Sea coastal waters next to a sewage outfall at a beach near Zhanqiao Pier, Qingdao, China, was investigated using protargol staining. The main pattern of morphogenesis is typical for the genus and can be summarized as follows: 1) the oral primordium in both proter and opisthe develops *de novo* in a subcortical pouch. In each daughter cell, the developing adoral zone of membranelles divides into two parts. The new membranelles formed in the proter's oral primordium will replace the leftmost five parental ones; six parental membranelles are retained by the proter; 2) the undulating membranes anlage is formed and develops independently from the oral primordium within the same subcortical pouch; 3) five primary front oventral-transverse cirral anlagen appear *de novo* on the cell surface; 4) the marginal cirral anlagen are formed *de novo*; 5) the leftmost frontal cirrus develops *de novo* on the cell surface; 6) two caudal cirri are formed at the posterior end of the rightmost anlage while the second primordium from the right gives rise to the third caudal cirrus. In contrast to its congeners, the anlage of the leftmost frontal cirrus is formed to the right of the undulating membranes anlage and before the formation of the latter.

Key words Euplotida; hypotrichs; ontogenesis; *Uronychia*

1 Introduction

The ciliate order Euplotida Small & Lynn, 1985 is characterized by its sparse but well-developed ventral cirri arranged in frontal, ventral, and transverse groups (Fan *et al*., 2013; Jiang *et al*., 2013; Gao *et al*., 2017; Huang *et al*., 2011, 2012, 2018; Lian *et al*., 2018). Eupotids have been the subject of numerous recent investigations of their morphology, morphogenesis and systematics (Shao *et al*., 2010a, b; Yi *et al*., 2012; Liu *et al*., 2017; Luo *et al*., 2014, 2018; Wang *et al*., 2017a, b; Yan *et al*., 2018; Zhao *et al*., 2017, 2018). Uronychidae is a distinctive family in the Euplotida that is characterized by its highly developed paroral. *Uronychia binucleata* was first reported by Young (1922). It is characterized by its medium-sized, oval to almost rectangular body, two macronuclear nodules, high numbers of basal bodies in its dorsal kineties and characteristic buccal apparatus (Song and Wilbert, 1997).

Divisional morphogenesis of *Uronychia* has been studied for several species and appears to be highly conserved among all congeners for which data are available (Taylor, 1928; Wilbert and Kahan, 1981; Hill, 1990; Wilbert, 1995; Song, 1996; Song *et al*., 2004; Shen *et al*., 2009; Shi *et al*., 2017; Song and Shao, 2017). However, the development of the leftmost frontal cirrus and paroral in *Uronychia* has not been described in detail.

Uronychia binucleata is a rare species, and its morphogenesis was partially described by Hill (1990) (misidentified as *U. transfuga*) and Song (1996) (misidentified as *U. uncinata*). The rediscovery of *U. binucleata*in in coastal waters of northern China provided the opportunity to investigate its morphogenetic process in detail, including, for the first time in *Uronychia*, the formation of the leftmost frontal cirrus and the paroral.

2 Materials and Methods

2.1 Sampling, Culturing, and Isolation

Uronychia binucleata was collected from Yellow Sea coastal waters next to a sewage outfall at a beach near Zhanqiao Pier, (120°32′E, 36°06′N), Qingdao, China, on December 6, 2014. The water temperature was about

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6.5℃ and the salinity was 25%–28%. Individual *U. binucleata* cells were isolated in the laboratory using micropipettes. Cultures were established at 10–15℃ using boiled sea water and adding small scuticociliates as the food source (Shao *et al*., 2007).

2.2 Morphogenesis

Protargol staining (Wilbert, 1975) was used to reveal the ciliature and nuclear apparatus. Counts and measurements of stained specimens were performed at a magnification of 1000×. Drawings of protargol-stained cells were made with the help of a camera lucida (Yan *et al*., 2016, 2017). To illustrate the changes occurring during morphogenetic processes, old (parental) ciliary structures are depicted by contour whereas new structures are shaded black (Chen *et al.*, 2018; Lu *et al*., 2018).

2.3 Terminology and Systematics

Terminology is mainly according to Song *et al*. (2004). Systematics is according to Lynn (2008), Gao *et al.* (2016) and Lyu *et al*. (2018).

3 Results and Discussion

The morphology of the present population matches almost exactly with that described by Song and Wilbert (1997), hence no further details are documented here (Figs.1A–C; 4A, B; Table 1).

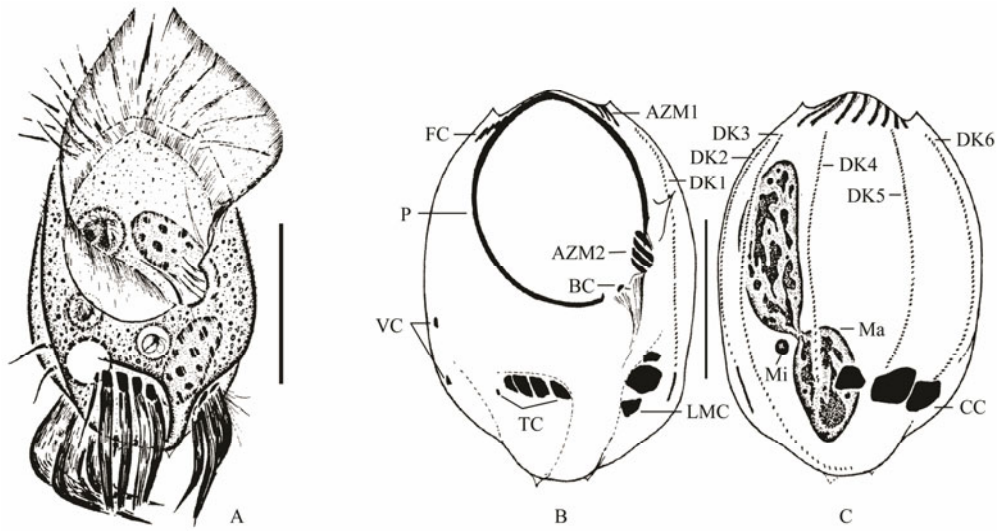

Fig.1 *Uronychia binucleata in vivo* (A) and after protargol staining (B, C) (modified from Song and Wilbert, 1997). A, Ventral view of a representative individual. B and C, Ventral (B) and dorsal (C) views of the general infraciliature. AZM1–2 = adoral zone of membranelles 1, 2; BC = buccal cirrus; CC = caudal cirri; DK1–6 = dorsal kineties 1–6; FC = frontal cirri; LMC = left marginal cirri; VC = ventral cirri; Ma = macronuclei; Mi = micronucleus; TC = transverse cirri; P = paroral. Scale bar = 50 μm.

Table 1 Morphometric characterization of *Uronychia binucleata*

Character†	Min	Max	Mean	M	SD	CV	*n*
Body length	73	115	97.2	98	11.1	11.4	15
Body width	55	93	76.3	78	11.1	14.5	15
Body length: width, ratio	0.91	1.48	1.3	1.33	0.2	12.7	15
Adoral zone, length	43	70	55.5	55	8.0	14.5	15
Adoral zone length: body length, ratio	0.45	0.71	0.6	0.57	0.1	14.6	15
Adoral membranelles 1, no.	11	11	11.0	11	0	0	15
Adoral membranelles 2, no.	4	4	4.0	4	0	0	15
Anterior Ma, length	23	53	40.9	39	8.3	20.4	15
Anterior Ma, width	12	23	17.5	18	2.8	15.8	15
Ma, no.	2	2	2.0	2	0	0	15
Mi, no.	1	1	1.0	1	0	0	15
Buccal cirri, no.	1	1	1.0	1	0	0	15
Frontal cirri, no.	4	4	4.0	4	0	0	15
Ventral cirri, no.	2	2	2.0	2	0	0	15
Left marginal cirri, no.	3	3	3.0	3	0	0	15
Transverse, no.	5	5	5.0	5	0	0	15
Caudal cirri, no.	3	3	3.0	3	0	0	15
Dorsal kineties, no.	6	6	6.0	6	0	0	15

Notes: CV, coefficient of variation in %; M, median; Max, maximum; Mean, arithmetic mean; Min, minimum; *n*, sample size; no., number; SD, standard deviation. † All data are based on protargol-stained specimens, measurements in μm.

3.1 Morphogenesis During Binary Fission in *Uronychia binucleata* (Figs.2–4)

Stomatogenesis Morphogenesis starts with the appearance of a small patch of kinetosomes, the opisthe' soral primordium (OOP) within a subsurface or subcortical pouch positioned between cytostome and left marginal cirri (Fig.2A). At the same time, the proter's oral primordium (POP) appears, also subcortically, slightly anterior of the parental adoral zone of membranelles (AZM2) (Figs.2A, B). Both the OOP and POP develop by rapid proliferation of kinetosomes (Fig.2D). Subsequently, two groups in the OOP, with eleven and five membranelles respectively, and two groups in the POP, each with five membranelles, differentiate and migrate to the surface of the cell (Figs.2G, J; 3A, E, G, I).

Meanwhile, an additional anlage (anlage for leftmost frontal cirrus, LFCA) appears within the subcortical pouch adjacent to the posterior end of the oral primordium in each divider (Figs.2D, E). The undulating membranes anlage (UM-anlage) then appears to the left of LFCA and locates between the oral primordium and LFCA in each divider (Figs.2G, H; 5A). Because we cannot observe the earliest stages of morphogenesis, we were unable to determine whether the UM-anlage and LFCA develop from the same anlage. Later, the UM-anlage and the LFCA begin to lengthen and further develop along the right edge of the pouch opening (Figs.2J, K; 5B). The anterior portion of the LFCA gradually widens (Figs.3A, B; 5C–H). Finally the leftmost frontal cirrus develops from the LFCA and the paroral is formed from the UM-anlage (Figs.3D, E, G; 5I, J).

During the late stages, the two groups of membranelles in both oral primordia separate. In the proter, the anterior group migrates anteriorly. As in its congeners, the anterior group will replace the posterior five membranelles of the adoral zone of the proter (AZM1), while the parental six membranelles of the adoral zone are retained (Fig.3I). In the opisthe, the anterior group migrates anteriorly and forms 11 membranelles in AZM1 (Fig.3I). In both daughter cells, the posterior groups form the membranelles in AZM2. In the posterior group of new membranelles, the most posterior one (which is also the smallest) moves apart from the other four and reaches its final position as the 'buccal cirrus' (Figs.3I; 4L).

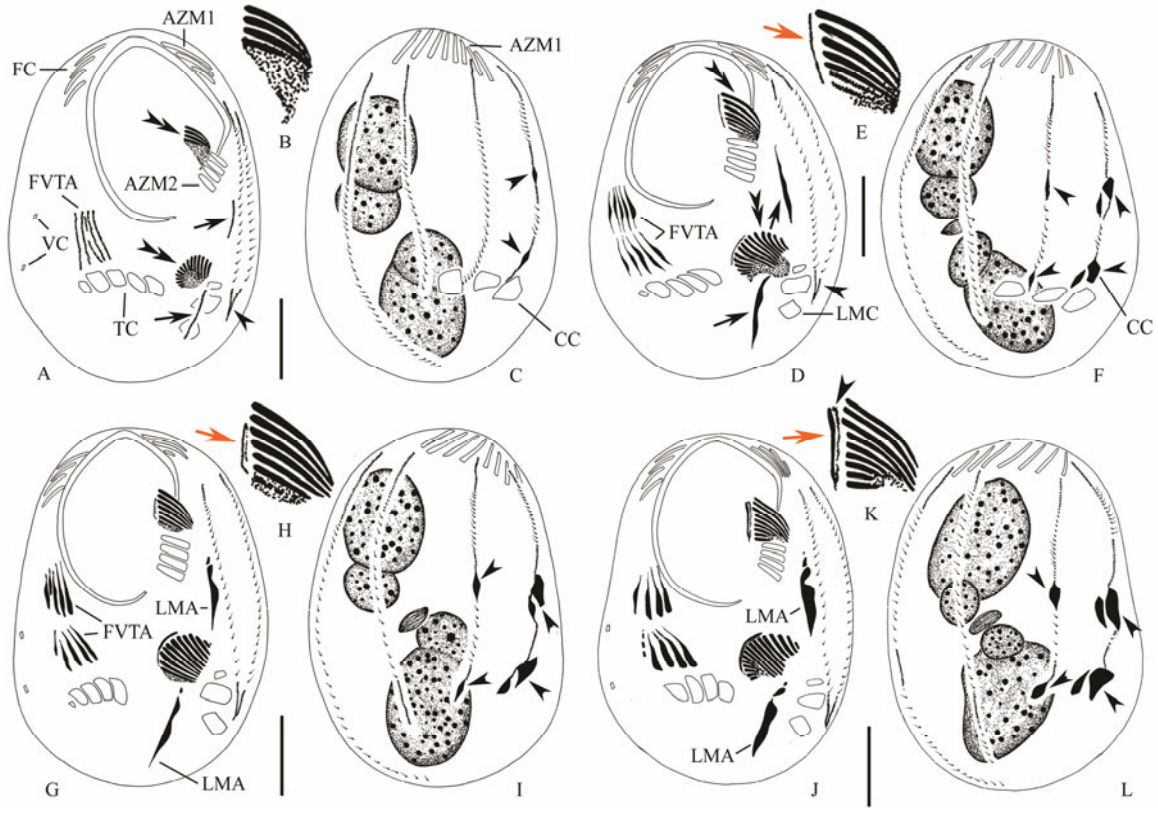

Fig.2 Morphogenesis of *Uronychia binucleata* during cell division. A–C, Ventral (A, B) and dorsal (C) views to show the formation of the oral primordia (double-arrowheads), the frontoventral-transverse cirral anlagen, anlagen for left marginal cirri (arrows) and caudal cirri (arrowheads). D–F, Ventral (D, E) and dorsal (F) views to mark the appearance of the anlagen for leftmost frontal cirri (arrow in E) and development of the frontoventral-transverse cirral anlagen and oral primordia (double-arrowheads), as well as development of anlagen for left marginal cirri (arrows) and caudal cirri (arrowheads in D, F). G–I, Ventral (G, H) and dorsal (I) view to demonstrate the appearance of the undulating membranes anlagen (arrow) and the development of the caudal cirri (arrowheads). J–L, Ventral (J, K) and dorsal (L) views showing the development of the undulating membranes anlagen (arrowhead), the anlagen for leftmost frontal cirri (arrow) and the anlagen for the caudal cirri (arrowheads). AZM1–2 = adoral zone of membranelles 1, 2; CC = caudal cirri; FC = frontal cirri; FVTA = frontoventral transverse cirral anlagen; LMA = left marginal cirral anlagen; VC = ventral cirri; TC = transverse cirri. Scale bars = 30 μm.

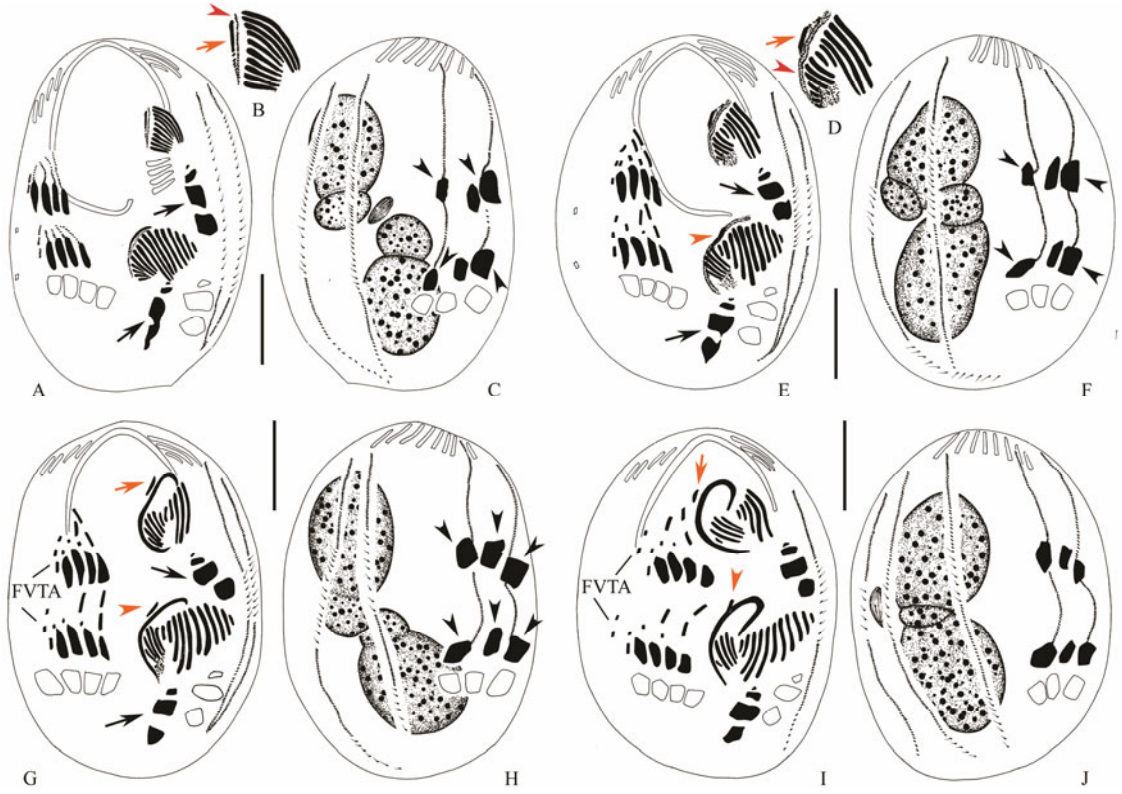

Fig.3 Morphogenesis of *Uronychia binucleata* during cell division. A–C, Ventral (A, B) and dorsal (C) views showing the development of the undulating membranes anlagen (arrowhead in B), the anlagen for leftmost frontal cirri (arrow in B), left marginal cirri (arrows in A) and caudal cirri (arrowheads in C). D–F, Ventral (D, E) and dorsal (F) views to denote the formation of leftmost frontal cirri in proter (arrow) and opisthe (arrowheads in E), as well as the undulating membranes anlage (arrowhead in D). Note the development of the anlagen for the left marginal cirri (arrows in E) and caudal cirri (arrowheads in F). G and H, Ventral (G) and dorsal (H) views to mark the development of the frontoventral-transverse cirral anlagen and the leftmost frontal cirri in proter (arrow) and opisthe (arrowhead). Note the development of the anlagen for the left marginal cirri (arrows in G) and caudal cirri (arrowheads in H). I and J, Ventral (I) and dorsal (J) views of a middle divider, demonstrating the development of the undulating membranes and the leftmost frontal cirri in proter (arrow) and opisthe (arrowhead). FVTA = frontoventral transverse cirral anlagen. Scale bars = 30 μm.

Development of the somatic ciliature in proter and opisthe At about the same time as the oral primordia are formed, basal bodies for FVT-cirral primordia (CA) develop on the cell surface firstly as four, and then five, streaks (anlagen I–V) anterior of the transverse cirri (Figs.2A; 4C). No parental ciliary organelles are involved in the formation of these new primordia. Each of the five anlagen extends to its maximum length before they divide into two sets (Figs.2A, D; 4D). Subsequently the streaks broaden, break apart and develop into distinct cirri as they migrate to their final positions (Figs.2G, J; 3A, E; 4F). According to their segmentation, cirral anlagen I–V give rise to cirri in the pattern 3:3:2:2:3, respectively, while the leftmost cirrus is formed near the UM-anlage as described above (Figs.3G, I; 4H).

The anlagen of the left marginal cirri are formed *de novo* and separately on the cell surface near the old AZM2 and the marginal cirri (Figs.2A; 4E). These anlagen then enlarge and segment to form the new left marginal cirri (Figs.2D, G, J; 3A, E, G, I). On the dorsal side, the proliferation of new basal bodies occurs at two levels within each of the six old kineties. The development of these anlagen seems to occur progressively from right to left (Figs.2A, C, D, F, G, I; 4M). Two caudal cirri are formed at the posterior end of the rightmost anlage while the second primordium from the right gives rise to the third caudal cirrus (Figs.2I, L; 3C, F, H, J; 4G, I).

3.2 Noteworthy Morphogenetic Features in *Uronychia*

Morphogenesis in five *Uronychia* species, including *U. xinjiangensis*, *U. multicirrus*, *U. transfuga*, *U. binucleata*, and *U. setigera*, has been investigated using silvers staining methods (Dembowska, 1926; Taylor, 1928; Fauré-Fremiet 1964; Wilbert and Kahan, 1981; Hill, 1990; Wilbert, 1995; Song, 1996; Song *et al*., 2004; Shen *et al*., 2009; Chen *et al*., 2017; Shi *et al*., 2017). The pattern of morphogenesis is highly conserved among these congeners and the same general pattern was also observed for *U. binucleata* in the present study. The mode of development of leftmost frontal cirrus and paroral, however, was unknown in *Uronychia* and is described here for the first time. In *U. binucleata*, the anlage for the leftmost frontal cirrus is formed to the right of the UM-anlage and is earlier than the formation of the UM-anlage. This contrasts with the process in *U. multicirrus,* in which this anlage

develops anterior of the UM-anlage after the formation of the latter (Shen *et al*., 2009).

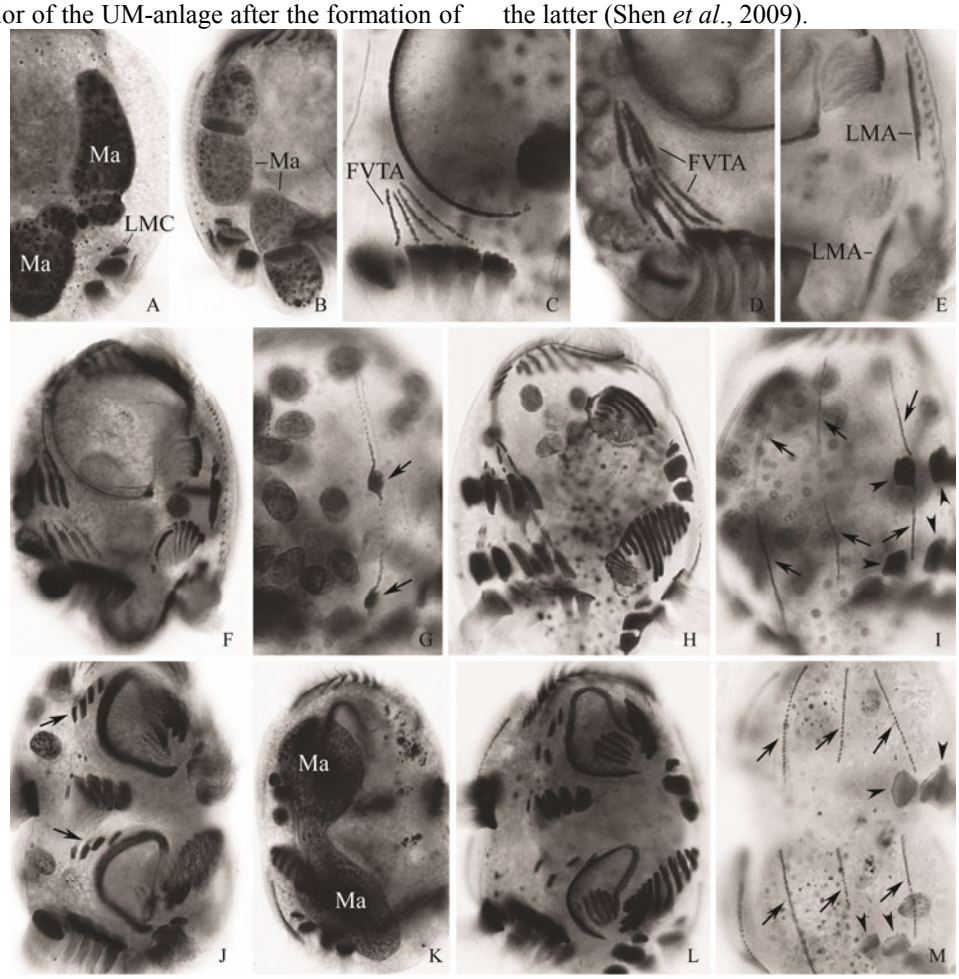

Fig.4 Morphogenesis of *Uronychia binucleata* during cell division after protargol staining. A and B, Ventral (A) and dorsal (B) views of same cell in interphase, marking the marginal cirri and macronuclear nodules. C, Ventral view of an early divider, to show the formation of the frontoventral-transverse cirral anlagen. D, Ventral view of an early divider, to show the development of the frontoventral-transverse cirral anlagen. E, Ventral view to show the appearance of the marginal anlagen. F and H, Ventral views of early dividers to show the development of the frontoventral-transverse cirral anlagen. G and I, Dorsal views, to denote the formation of the caudal cirri (arrows in G, arrowheads in I) and development of dorsal kineties anlagen (arrows in I). J, Ventral view to show the migration of the frontal cirri (arrows). K, Dorsal view to demonstrate the macronuclear nodules. L, Ventral view showing the development of the frontoventral-transverse cirri, undulating membranes, and adoral zone of membranelles. M, Dorsal view to mark the formation of the caudal cirri (arrowheads) and development of dorsal kineties anlagen (arrows). FVTA = frontoventral transverse cirral anlagen; LMA = left marginal cirral anlagen; LMC = left marginal cirri; Ma = macronuclear nodules.

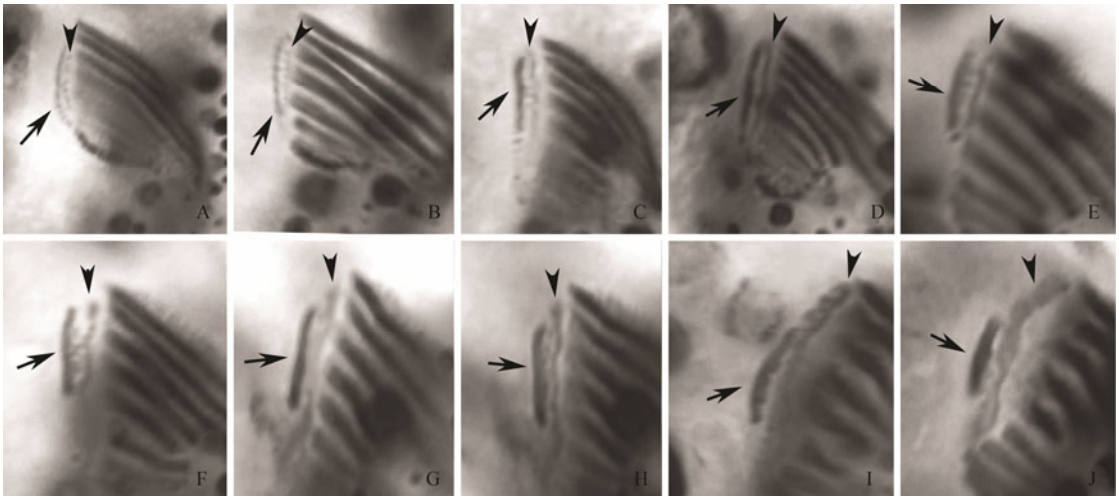

Fig.5 Development of leftmost frontal cirrus and paroral in *Uronychia binucleata* during cell division after protargol staining. A–H. Ventral views to show the formation of the undulating membranes anlagen (arrowheads) and the anlagen

for leftmost frontal cirri (arrow). I and J, Ventral views to show the formation of the leftmost frontal cirri.

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