Divisional morphogenesis in the marine ciliate *Anteholosticha manca* (Kahl, 1932) Berger, 2003 (Ciliophora: Urostylida)

LI Liqiong¹, HU Xiaozhong¹*, WARREN Alan², AL – RASHEID Khaled A S³, AL – FARRAJ Saleh A¹, SHAO Chen¹, SONG Weibo¹

1. Laboratory of Protozoology, Key Laboratory of Mariculture, Ocean University of China, Qingdao 266003, China  
2. Department of Zoology, Natural History Museum, London SW7 5BD, UK  
3. Zoology Department, King Saud University, Riyadh 11451, Saudi Arabia

Received 11 January 2007; accepted 18 July 2007

Abstract

The cortical development and macronuclear evolution of the marine hypotrichous ciliate *Anteholosticha manca*, collected from the coastal waters near Qingdao, China, were investigated using the protargol impregnation method. The morphogenesis of *A. manca* is generally typical for the genus, and can be characterized as the following features; (1) in the proter, a new oral primordium is formed on the bottom (beneath the pellicle) of the buccal cavity, disorganization of the parental undulating membranes do not contribute to the formation of this primordium; (2) FVT-cirral anlagen in both dividers develop independently, and very likely only a few midventral cirri join in the formation of these primordia; (3) both marginal row and dorsal kinety anlagen develop intrakinetically within the parental structure; (4) at the middle stage, macronuclear segments fuse together to form a single mass as observed in most of its related forms.

Key words: hypotrichs, marine ciliate, morphogenesis, *Anteholosticha manca*

1 Introduction

The genus *Holosticha* Wrzesniowski, 1877 was recently redefined by Berger (2003). *Anteholosticha* is one of the four newly erected genera, and it is comprised of ca. 30 species from different habitats (Song and Wilbert, 1997a, b; Wirnberger and Foissner, 1987; Borror and Wicklow, 1983; Foissner, 1982; Hemberger, 1982; Borror, 1972; Kahl, 1932). Most congeners of the newly defined *Anteholosticha* share the similar or the same ciliary patterns; with continuous adoral zone and the rearmost membranelles not wider than remaining ones of proximal portion, frontoterminal, midventral and three enlarged frontal cirri present, buccal cirrus/cirri right of paroral, one left and one right marginal row, anterior end of left marginal row straight, and lack of caudal cirri. However, in terms of morphogenesis, only two studies have so far been conducted on *Anteholosticha multistilata* and...
A. warreni (Hu, Song et al., 2000; Song and Wilbert, 1997a; Hemberger, 1982).

In order to get a deeper understanding of morphogenetic patterns in the genus Anteholosticha and test the rationality of Berger's system on the genus Holosticha, the present study showed a detailed morphogenetical process of Anteholosticha manca (Kahl, 1932) Berger, 2003 during asexual reproduction.

2 Materials and methods

In January 2005, a population of Anteholosticha manca was isolated from the Jiaozhou Bay in China. The salinity was about 33, water temperature 11°C and pH ca. 8.4.

After isolation and investigation on both live and silver samples (Wilbert, 1975), a pure culture was set up at room temperature in boiled seawater with crushed rice grains for enriching bacteria. Cells in different divisional stages were picked and impregnated using the protargol method (Wilbert, 1975) in order to reveal the infraciliature.

All drawings were performed under 1 250 by magnification with the help of a camera lucida. Measurements were carried out with an ocular micrometer. To distinguish the changes during morphogenesis, parental cirri is depicted by outlines, whereas new ones are shaded black. The terminology and abbreviations are mainly according to Hemberger (1982) and Foissner et al. (1991).

3 Results

Stages of divisional morphogenesis in Anteholosticha manca are shown in Figs 1—4.

3.1 Stomatogenesis

Stomatogenesis commences with the formation of small groups of basal bodies very close to several left midventral cirri (see Fig. 1a, arrowheads and Fig. 4b, arrows) which develop subsequently to form the oral primordium of the opisthe (see Fig. 1b, arrowheads; Fig. 1c, OOP; Fig. 4d, arrows). Apparently, no midventral cirri contribute to the formation of this primordium, as all the cirri nearby remain intact. At this stage, this primordium anteriorly extends to the posterior end of the parental adoral zone of membranelles. With the number of the basal bodies increasing, the new membranelles initially appear in the anterior of the field and proceed in a posterior direction and to the left (see Fig. 1d, arrowhead). Meanwhile, a thread-like kinetosomal field (undulating membranes anlage, UMA; Figs 1d and 4f, arrow), is separated from the main part of OOP, which then aggregates into undulating membranes (PM, EM). The UMA gives rise to two cirri originally, one is finally resorbed while the other becomes the leftmost frontal cirrus (see Fig. 1e, arrowhead).

In the proter, the oral primordium appears as some anarchic basal bodies at the bottom of the buccal field adjacent to the endoral membrane without the disorganization of parental adoral membranelles and the undulating membranes (see Fig. 1a, double arrowheads and inset, arrows). By proliferation of basal bodies, the elliptical primordium field is formed (see Fig. 1c, POP; Fig. 4c, arrow) and then performs as in the opisthe. Subsequently, the parental oral apparatus begins to disaggregate, and then UM-anlage will be formed (see Fig. 1d, arrow). Lately, two cirri were derived from the anterior part of the UM-anlage, then will perform the same as in the opisthe (see Fig. 1e, arrowhead; Figs 2a, c, e, f; Fig. 4g, arrow), while the remaining UM-anlage develops into paroral and endoral membranes by splitting longitudinally.

At late stages, the anterior portion of the new AZM in the opisthe bends to the right of the cytostome, and the parental AZM will be replaced completely by the new structure (see Figs 2e, f; Fig. 3a—c).
3.2 Development of somatic ciliature

Along with the splitting of the oral primordium in the opisthe (OOP), a ladder-like structure (FVT-cirral anlagen) appears in the middle position (Figs 1d, e and 4e, arrowheads). During this process, most midventral cirri remain intact. Then, within the cirral anlagen, each streak seems to break into two segments (cirri), except for the last four to five streaks which divide into more segments each, from the last anlage, two to three frontterminal cirri are formed and move anteriorly (see Figs 2a, c, e, arrows and Fig. 4k, arrowhead). The cirri from the first two streaks turn to be enlarged frontal cirri (FC) and one buccal cirrus. Each of the last four to five anlagen contributes the last cirrus to be transverse cirrus. Finally, most of the remaining cirri are then arranged in a “zig-zag” pattern (see Figs 2e and 4l).

**Fig. 1.** Morphogenesis of *Anteholosticha manca* after protargol impregnation. Ventral views (a, b, c) at the early stage of morphogenesis, showing appearance of oral primordium; arrowheads indicate groups of basal bodies commencing close to the left midventral row, arrows and double arrowheads show the anarchic basal bodies at the bottom of the buccal field adjacent to the endoral membrane; ventral view (d), depicting frontoventral-transverse anlagen, double arrowheads indicate the replication bands of macronuclear nodules; arrowhead marks the macronucleus; ventral and dorsal views (e, f) of the same cell showing the marginal row anlagen and dorsal kinetics anlagen, arrowheads indicate the leftmost cirrus derived from undulating membranes anlage in both divides. CA is short for cirral anlagen, DKA dorsal kinetics anlagen, MRA marginal row anlagen, OOP opisthe’s oral primordium, POP proter’s oral primordium. The length of scale bars represent 50 μm.
Fig. 2. Morphogenesis of *Anteholosticha manca* after protargol impregnation. Ventral and dorsal views (a, b) of the same specimen showing the fusing macronuclear nodules; ventral and dorsal views (c, d) of the same cell, arrow marks the fused macronuclear nodules; ventral view (e) and nodules of the same cell, arrows indicate the frontoterminal cirri of the daughter cells in the migration; inset: a single macronuclei mass and several micronuclei, arrowhead marks the dividing of the single mass; ventral and dorsal views (f, g) of the same specimen to show infraciliature. The lengths of scale bars represent 50 μm.

Fig. 3. Morphogenesis of *Anteholosticha manca* after protargol impregnation. Ventral view (a), inset; division of macronuclear nodules; ventral views (b, c) of the daughter cells to show infraciliature and division of macronuclear nodules. The lengths of scale bars represent 50 μm.
Fig. 4. Photomicrographs of morphogenesis in *Anteholosticha manca* after protargol impregnation. Ventral view (a) of the early stage individual in anterior portion, arrow indicates oral primordium in the proter; ventral view (b) of the same specimen as in a, arrowheads mark groups of basal bodies commencing close to the left midventral row; arrow in c indicates oral primordium in the proter; ventral view (d) of the same cell as in c, arrowheads mark the oral primordium in the opisthe; ventral view (e), arrowheads indicate fronto-ventral transverse cirral anlagen in the proter, double arrowheads indicate dedifferentiation of the old undulating membranes, arrow indicates oral primordium deep down the parental buccal field; ventral view (f) of the same specimen as in e, arrow indicates the posterior joining of the primordium in the opisthe; ventral view (g), arrow indicates the cirri derived from undulating membranes anlage in the proter and arrowhead marks the dedifferentiation of the posterior portion of parental adoral zone of membranelles; ventral views (h, i) to show the fission of macronuclear nodules, arrow indicates the single mass; dorsal view (j), arrows indicate the dorsal kinetics anlagen; ventral view (k) of the proter, arrowhead marks the migration of the frontal terminal cirri and arrow indicates the splitting of undulating membranes; ventral view (l) of the daughter cell to show infraciliature and division of macronuclear nodules. The lengths of scale bars represent 30 μm.
New marginal row anlagen (MRA) develop within the old structures on each side corresponding to the FVT at almost the same time as FVT-cirral anlagen appear (see Fig. 1e). Then all those anlagen stretch in both directions to replace the parental structures (see Figs 2a—f).

The formation style of dorsal kineties performs in the same way as the marginal cirri (see Figs 1f; 2b, d, g; Fig. 4j, arrows).

3.3 Nuclear evolution

The nuclear apparatus develops in a usual way and thus no further description will be given (see Figs 2b, d, e, g; Figs 3a, 4h, i). Yet, it should be noticed that the replication bands of macronuclear nodules are apparently observed in the early stages of morphogenesis (see Fig. 1d, inset; double arrowheads). As in many other multinucleate-having hypotrichs, the division of macronuclear segments proceeds long after the cytokinesis (see Figs 3b and c).

4 Discussion and conclusions

Morphogenesis in Anteholosticha manca can be characterized as follows:

1) In the proter, the adoral zone of membranelles is generated from a newly-built oral primordium formed de novo beneath the parental endoral membrane; and the parental structure is completely replaced;

2) The frontoventral-transverse cirral anlagen in the proter and opisthe generate separately and formed close to the left midventral row; transverse cirri seem not to contribute to the building of the primordia;

3) Only the posterior several FVT-cirral streaks give rise to the transverse cirri;

4) The UM-anlage forms two cirri originally, one of which finally resorbed and the other one becoming the frontal cirrus;

5) Marginal cirral and dorsal kinetics anlagen are formed intrakinetally;

6) Macronuclear nodules fuse into a single mass before cell division as usual.

As for morphogenesis, Anteholosticha manca corresponds very well to its congeners. For A. warreni, minor differences only lie in that A. manca generates oral primordium of the opisthe much earlier and only the posterior several FVT-cirral streaks give rise to the transverse cirri (all the FVT-cirral anlagen contribute one each) (Hu, Song et al., 2000). Another congener A. multistilata shares almost the same morphogenetic progress with A. manca except that several streaks of the FVT-cirral anlagen give rise to a buccal cirral row and more frontal cirri than the latter (Hemberger, 1982).

Compared with some closely related forms in Holosticha, the parental structure of Anteholosticha manca is completely replaced, which is remarkably different from H. diademata, H. heterofoissneri (completely retained) and H. bradburyae (partly renewed) (Hu et al., 2003; Hu and Song, 2001; Hu, Wang et al., 2000).

Considering most of the morphogenetic features, Anteholosticha manca is also very similar to that in some Pseudokeronopsis-species (Wirmberger, 1987; Hu et al., 2004). However, the great difference is macronuclear nodules of the latter never fuse into a single mass.

In conclusion, the present investigation on the morphogenesis of Anteholosticha manca contributes to demonstrating that most hypotrichs at generic level possess the same or very similar morphogenetic mode, and ontogenetic data are of great value for species separation among members of the most related genera. It also partly supports the transfer of A. manca, A. warreni, and A. multistilata from the genus Holosticha Wrzesniowski, 1877 to the genus Anteholosticha Berger, 2003.

However, since the evolutionary significance of
different morphogenetic patterns (i.e., whether they are of apomorphic or plesiomorphic characters) and morphogenetic data for a wider range of species remain unclear, extensive investigations using morphogenetical as well as molecular methods will be necessary in order to confirm Berger’s hypothesis.

Acknowledgements

Our thanks are due to Miss Jin Caixia, postgraduate of Ocean University of China, for her kind help during the sampling.

References


Foisner W. 1982. Ökologie und Taxonomie der Hypotrichida (Protozoa; Ciliophora) einiger österreichischer Böden. Arch Protistenkd, 126; 19 – 143


Hu Xiaozhong, Song Weibo. 2001. Morphology and morphogenesis of Holosticha heterofoisneri nov. spec. from the Yellow Sea, China (Ciliophora, Hypotrichida). Hydrobiologia, 448; 171 – 179


Wirmberger E. 1987. Division and reorganization in the genus Pseudokeronopsis and relationships between urostyliids and oxytrichids (Ciliophora, Hypotrichida). Arch Protistenkd, 134; 149 – 160