
Wenping Chen,¹ Xumiao Chen,² Lifang Li,³ Alan Warren⁴ and Xiaofeng Lin¹

¹Laboratory of Protozoology, Key Laboratory of Ecology and Environment Science in Guangdong Higher Education, College of Life Science, South China Normal University, Guangzhou 510631, PR China
²Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, PR China
³Marine College, Shandong University, Weihai 264209, PR China
⁴Department of Life Sciences, Natural History Museum, London SW7 5BD, UK

The morphology and morphogenesis of an oxytrichid ciliate, *Rubrioxytricha haematoplasma* (Blatterer & Foissner, 1990) Berger, 1999, collected from brackish and marine waters in China, were investigated using live observation and the protargol staining method. The main features of the morphogenetic process are: (i) the parental adoral zone of membranelles is retained completely in the proter and the anlage of undulating membranes originates from dedifferentiation of the old structures; (ii) three frontal, four frontoventral, one buccal, five ventral and five transverse cirri are derived from the anlagen of the undulating membranes and the five streaks of frontal-ventral-transverse anlagen in the pattern of 1 : 3 : 3 : 3 : 4 : 4 from left to right; (iii) the morphogenesis of the dorsal kineties is simpler than the *Oxytricha* pattern, i.e. without fragmentation of the dorsal kinety 3 anlagen; (iv) the single caudal cirrus originates from the dorsal kinety 3 anlage on the right side; (v) the two macronuclear nodules fuse into a single mass during the mid-stage of morphogenesis. These features correspond well with *Rubrioxytricha indica*, indicating that the morphogenetic pattern of *Rubrioxytricha* is stable. Phylogenetic analysis based on small-subunit rRNA gene sequence data supports the monophyly of the genus *Rubrioxytricha*, which is nested within the non-Stylonychinae clade.

INTRODUCTION

The hypotrichs are one of the most speciose groups of ciliates and are highly divergent, in terms not only of their morphology but also of their morphogenesis (Berger, 1999, 2006, 2008, 2011; Song et al., 2009, 2011; Shao et al., 2011; Chen et al., 2013a, b, c; Jiang et al., 2013; Küppers & Claps, 2013; Pan et al., 2013; Park et al., 2013; Fan et al., 2014). Morphogenesis can provide valuable information for understanding the taxonomy and evolutionary relationships among hypotrichs. However, morphogenetic data are still limited for hypotrichs, considering their high diversity (Foissner, 1996; Berger, 1999, 2006, 2008, 2011). Although members of the large hypotrich family Oxytrichiidae have broadly similar patterns of morphogenesis (Foissner, 1996; Berger, 1999), there are a number of significant differences in detail among them (Berger, 1999, 2011; Chen et al., 2013b; Shao et al., 2013; Singh & Kamra, 2013; Singh et al., 2013).

Berger (1999) established the genus *Rubrioxytricha* for two species of *Oxytricha* that possess one or two caudal cirri and a homogeneously coloured cytoplasm. The morphogenetic process in *Rubrioxytricha* is simpler than the typical *Oxytricha* pattern in terms of the formation of the dorsal kineties (Berger & Foissner, 1997; Naqvi et al., 2006). Hitherto, morphogenesis in the type species, *Rubrioxytricha haematoplasma*, was incompletely known (Blatterer & Foissner, 1990), and its small-subunit (SSU) rRNA gene sequence was not available.

In the present study, we provide a detailed account of morphogenesis in *Rubrioxytricha haematoplasma* based on...
a population isolated from aquaculture water collected in southern China. We also investigate its molecular phylogeny based on SSU rRNA gene sequence data.

**METHODS**

**Sample collection, isolation and identification.** *Rubrioxytricha haematoplasma* was isolated from water samples collected on 27 December 2008 from two shrimp-farming ponds on the campus of the South China Normal University, Guangzhou, China (23° 08′ 19″ N 113° 21′ 22″ E), one brackish pond (salinity 8.6) and the other marine (34.4). During the aquaculture process, shrimps are moved between the two ponds in order to control disease. Cells of *R. haematoplasma* from the two ponds were morphologically identical and were assumed to belong to a single population. The ciliates were maintained and cultured for several days at room temperature in Petri dishes containing water from the collecting site with rice grains added to promote the growth of bacterial food organisms. Isolated cells were observed in vivo using bright-field and differential interference contrast microscopy. The protargol staining method of Wilbert (1975) was used to reveal the nuclear apparatus and the oral and somatic ciliature. Counts and measurements of stained specimens were performed at a magnification of ×1000. Drawings of stained cells were made with the aid of a camera lucida. Terminology and systematics mainly follow Berger (1999).

**DNA extraction, PCR amplification, sequencing and phylogenetic analyses.** Genomic DNA from several cleaned cells isolated from the brackish pond (salinity 8.6) was extracted using a REDExtract-N-Amp Tissue PCR kit (Sigma). The small-subunit (SSU) rRNA gene was amplified with universal primers 82 Forward and Euk B (Elwood et al., 1985; Medlin et al., 1988). Cloning and sequencing were performed according to Gao et al. (2013). Using CLUSTAL W implemented in BioEdit 7.0 (Hall, 1999), the SSU rRNA gene sequence was aligned with those of 49 other taxa downloaded from the GenBank database (see Fig. 6 for accession numbers). Highly variable regions in which alignments could not be determined unambiguously were excluded prior to phylogenetic analysis, leaving 1694 bp. Novistrombidium testaceum, Strombidinopsis acuminata, Strombidium purpureum and Tintinnidium mucicola were selected as the outgroup taxa. A Bayesian inference (BI) analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) with the Akaike information criterion GTR + I + G as the best model selected by the program MrModeltest 2.2 (Nylander, 2004). The program was run for 1 000 000 generations with a sample frequency of 100 and a burn-in of 2500. The maximum-likelihood (ML) tree was reconstructed online on the CIPRES Science Gateway version 3.3 (CIPRES Portals: http://www.phylo.org/sub_sections/portal) using RAxML-HPC2 on XSEDE (Miller et al., 2010) with default parameters and bootstrapping with 1000 replicates. The topologies of all derived trees were merged into a single consensus tree for the purposes of illustration. In order to visualize tree topologies, TreeView version 1.6.6 (Page, 1996) and MEGA 4.0 (Tamura et al., 2007) were used.

**RESULTS**

**Morphology of Chinese population of Rubrioxytricha haematoplasma** (Blatterer & Foissner, 1990) Berger, 1999

Body 90–180 μm × 30–70 μm in vivo, slightly red to ash-black in colour, flexible (Figs 1b and 2d–f). Elongate oval and somewhat sigmoid in shape (Fig. 2d, e) with both ends rounded and both margins slightly convex (Figs 1a and 2a, b); ratio of length to width 3–4:1 and dorsosventrally flattened about 1.5:1 (Fig. 2c). Adoral zone approximately 30% of body length and buccal field rather small but deep with transparent frontal scutum (about 10 μm long) (Figs 1b and 2b–g). Two types of cortical granules: type I smaller, approximately 0.8 μm in diameter, orange–red, spherical, arranged in lines or irregularly throughout cortex close to cell surface (Fig. 2g–j); type II larger, 3–4 μm in diameter, colourless and spherical, densely packed underneath cell surface (Fig. 2h–j). Cytoplasm slightly red with numerous irregular globules, 3–5 μm across (Figs 1b and 2d–f, k). Food vacuoles approximately 5 μm in diameter, containing diatoms and bacteria. Single contractile vacuole approximately 10 μm in diameter, located at mid-region of cell near left margin (Fig. 2a), pulsating at intervals of 2 min. Two ellipsoidal macronuclear nodules, 26–50 μm × 20–30 μm after protargol staining (Figs 1d and 2l–n; Table 1), sometimes with replication band in non-dividing cells (Fig. 2m). One or two spherical micronuclei located near macronuclear nodules, 4–10 μm in diameter after protargol staining (Fig. 1d; Table 1). Locomotion by slow crawling on substrate or by swimming while rotating about longitudinal axis of body.

Infraciliature as shown in Figs 1(c, d) and 2(l, n). Adoral zone comprises 33–45 membranelles (Figs 1c and 2n; Table 1), cilia of membranelles about 15 μm long in vivo (Fig. 2g). Undulating membranes not in typical Oxytricha pattern, rather paroral and endoral membranes almost equal in length, distinctly curved, and intersect in mid-region (Figs 1c and 2g, n). Cirral pattern with 18 frontal-ventral-transverse (FVT) cirri (Figs 1c and 2n): single buccal cirrus located slightly ahead of intersection of undulating membranes; three strong frontal cirri that are continuous with frontoventral cirri; four frontoventral cirri forming V-shaped pattern; five ventral cirri in two groups: three postoral and two pretransverse ventral cirri; five enlarged transverse cirri arranged in a hook-shaped row, with cilia 20–25 μm long in vivo. One left and one right marginal cirral row, with 27–41 and 31–41 cirri, respectively (Table 1), extending to posterior end of cell; posterior ends of marginal rows separated by a small gap (Figs 1c and 2n).

Three bipolar dorsal kineties (Figs 1d and 2l) and one slightly shorter dorsomarginal (Figs 1c, d and 2l, n) with cilia 5 μm long in vivo. Single caudal cirrus at end of dorsal kinety 3 (Figs 1d and 2n), with cilia about 10 μm long in vivo.

**Morphogenesis of Chinese population**

Based on the information we obtained, stomatogenesis starts with the appearance of the oral primordium in the opisthe as an elongated field of densely arranged basal bodies that extends from just below the parental adoral zone of membranelles almost to the anterior-most transverse cirrus (Figs 3a and 4a). At this stage, the four frontoventral cirri, undulating membranes and three postoral ventral cirri
remain intact (Figs 3a and 4a). With the proliferation of basal bodies, the oral primordium develops and differentiates new membranelles posteriori from the anterior end (Figs 3b and 4c). Simultaneously, the old undulating membranes dedifferentiate anteriorly and the FVT-anlagen begin to form (Figs 3b and 4b, c).

In mid-dividers, the old paroral and endoral membranes dedifferentiate to form the undulating membranes anlage of the proter (Figs 3c, e and 4d, e). Meanwhile, in the opisthe, the oral primordium forms new membranelles posteriori (Figs 3c, e and 4d, e); the anlagen of both the undulating membranes and the FVT are generated simultaneously as streaks (Figs 3c and 4d) that later elongate and broaden (Figs 3e and 4e). As shown in Table 2, in the proter, anlage I originates from the parental undulating membranes (Figs 3b and 4d, f), anlage II develops by dedifferentiation of the parental buccal cirrus (II/2; Figs 3c and 4d), frontoventral cirri III/2 and IV/3 dedifferentiate into anlagen III and IV, respectively (Figs 3b, c and 4d, f), and anlagen V and VI derive from streaks V and VI of the opisthe (Fig. 3b). In the opisthe, the FVT-anlagen originate from the oral primordium and the three postoral ventral cirri (Figs 3c and 4d), but we can only be certain that postoral ventral cirrus V/3 participates in the formation of streak VI based on our observation (Fig. 3b).

Later, when the differentiation of the oral primordium is almost complete, the anterior part of the new adoral zone of membranelles stretches and the anterior portion curves rightwards in the opisthe (Figs 3g and 4g). The FVT-anlagen differentiate into new cirri (Figs 3g and 4f, g), and the left frontal cirrus is generated from the undulating membranes anlage in both dividers (Figs 3g and 4f, g).

During the late stage, the undulating membranes anlage in both the proter and the opisthe divides to form the paroral and endoral membranes (Figs 4i and 5a, c). The five streaks of FVT-anlagen differentiate into new cirri in the pattern of 3:3:3:4:4 from left to right (Fig. 4i). The parental adoral zone of membranelles is retained completely in the proter, and the new one in the opisthe bends further towards the right (Fig. 5c). The buccal cirrus migrate to a position near the undulating membranes, and the other cirri migrate to their final positions in both dividers (Fig. 5c).

The marginal rows anlagen develop intrakinetally during the mid-stage by dedifferentiation of the old structures. The anlagen of the left marginal row appear somewhat later than those of the right marginal row (Figs 3c, e and 4e), and then lengthen towards both ends of the dividing cell (Figs 3g and 4f). Finally, all marginal row anlagen...
Fig. 2. Photomicrographs of *Rubrioxytricha haematoplasma* in vivo (a–k) and after protargol impregnation (l–n). (a, b) Ventral views of typical individuals; arrows mark the contractile vacuole (a) and the transparent membrane-like structure at the anterior part of the adoral zone of membranelles (b). (c–f) Lateral (c) and ventral (d–f) views of bending cells to demonstrate the flexibility of the body; arrows mark the transparent membrane-like structure at the anterior part of membranelles. (g) Ventral view of buccal field; the arrow shows the transparent membrane-like structure at the anterior part of the adoral zone of membranelles; double arrowheads mark the orange–red, spherical, type-I cortical granules; arrowheads indicate the dorsal bristles. (h–j) Dorsal views of the middle (h), anterior (i) and posterior (j) cell parts; arrows demonstrate the orange–red, spherical, type-I cortical granules arranged in lines; arrowheads mark the colourless, densely distributed, type-II cortical granules. (k) To show the inclusions within the cytoplasm. (l) Dorsal view of the infraciliature to mark the macronuclear nodules and dorsal kineties. (m) Non-dividing cell with replication band on the macronuclear nodule. (n) Ventral view of the infraciliature; arrowheads, double arrowheads and arrow indicate the three frontal cirri, one buccal cirrus and one caudal cirrus, respectively; the dashed circle depicts the three postoral ventral cirri. AZM, adoral zone of membranelles; DK1–4, dorsal kineties 1–4; e, endoral membrane; FVC, frontoventral cirri; LMR, row of left marginal cirri; Ma, macronuclear nodules; p, paroral membrane; PTVC, pretransverse ventral cirri; RMR, row of right marginal cirri; TC, transverse cirri; UM, undulating membranes. Bars, 60 μm.
generate new cirri that replace the old structures (Figs 4i and 5a, c).

On the dorsal surface, there are two sets of dorsal primordia: one set appears in the three dorsal kineties from the left of the parental cell during the middle-division stage (Fig. 3c, d), which replaces the parental kineties at the late stage (Fig. 5c, d) by the intrakinetal proliferation of basal bodies and its strengthening at both ends in each daughter cell (Figs 3e, f, h, 4e, i and 5a, b). One caudal cirrus is generated from the posterior end of dorsal kinety 3 in both the proter and opisthe (Fig. 5d). The other set of primordia develops de novo to the right of the right marginal row during the late stage of morphogenesis, and becomes the dorsomarginal kinety in each daughter cell (Fig. 5a–c).

The macronuclear nodules expand conspicuously during the middle stage (Figs 3d and 4d, e) and then fuse into a single mass (Figs 3h, 4h and 5g) that divides into two during the late stage, one for each filial cell (Figs 4j and 5d). Subsequent division of the macronuclear apparatus was not observed. The micronuclei divide separately during the final stages of morphogenesis (Figs 4j and 5d).

Notes on physiological reorganization

Three cells were observed undergoing reorganization (Figs 4k–n and 5e–i). The new oral primordium originates from the dedifferentiation of the posterior part of the parental adoral zone (Figs 4l and 5e, g), but it is unclear whether the adoral zone of membranelles is completely renewed. The anlage of the undulating membranes is generated by the dedifferentiation of the old structures (Figs 4k and 5e) and gives rise to the left frontal cirrus (Figs 4l and 5g, h, n).

This anlage subsequently splits longitudinally to form the paroral and endoral membrane (Figs 4n and 5h). The FVT-anlagen develop as five oblique streaks (Figs 4k and 5e) that differentiate into new cirri in the pattern of 3 : 3 : 3 : 4 : 4 from left to right (Figs 4l, n and 5g, h). The anlagen of the marginal rows (Figs 4k and 5e) and of the three left dorsal kineties (Fig. 5f) are derived from dedifferentiation of the old structures (Fig. 5g–i). The formation of the dorsomarginal kinety commences apokinetally on the right side of the right marginal primordium (Fig. 5h).

Molecular data and phylogenetic analyses

The length and DNA G+C content of the SSU rRNA gene of *R. haematoplasma* were 1648 bp and 45.51 mol%, respectively. A broad selection of SSU rRNA gene sequences (50 species in total) was included in the phylogenetic analyses. *R. haematoplasma* clustered with its congener *R. ferruginea* (99 % ML, 1.00 BI) to form a clade that nested within the oxytrichid assemblage (Fig. 6).

**DISCUSSION**

Identification of the Chinese population of *R. haematoplasma*

The genus *Rubrioxytricha* comprises three morphospecies, namely *R. haematoplasma* (Blatterer & Foissner, 1990) Berger, 1999, *R. ferruginea* (Stein, 1859) Berger, 1999 and *R. indica* Naqvi et al., 2006 (Berger, 1999; Naqvi et al., 2006). *R. haematoplasma*, the type species of the genus, was first reported in Germany, with a partial description of its
Fig. 3. Infraciliature and nuclear apparatus of Rubrioxytricha haematoplasma during morphogenesis (after protargol staining). Parental cirri are shown in outline, whereas new ones are shown in solid black. (a, b) Ventral views of early dividers; note the basal bodies in the OP forming an elongated field (a), micronuclei (double arrowheads in a) and the three postoral ventral cirri, which remain intact (arrowheads in a); later, the OP develops and differentiates into new membranelles posteriad from the anterior end (arrow in b), the old cirri are involved in the formation of FVT-anlagen (arrowheads in b) and the old undulating membranes dedifferentiate (double arrowheads in b). (c, d) Ventral (c) and dorsal (d) views of the same specimen; arrows, arrowheads and double arrowheads demonstrate the newly formed adoral membranelles in the opisthe, the FVT-anlagen streaks in both proter and opisthe, and the DKA, respectively. (e–h) Ventral (e, g) and dorsal (f, h) views of dividers in middle stage; arrows mark the new adoral membranelles stretching anteriad (e, g), micronuclei (f) and DKA (h); arrowheads in (g) show the FVT-anlagen developing into cirri; double arrowheads indicate the forming of the leftmost frontal cirri (g) and the micronuclei (h). Occasionally more than six anlagen are formed in the opisthe (g). DK, dorsal kineties; DKA, anlagen of dorsal kineties; FVT-anlagen, frontal-ventral-transverse cirral anlagen; LMA, anlagen of left marginal row; Ma, macronuclear nodules; OP, oral primordium; RMA, anlagen of right marginal row; UMA, anlagen of undulating membranes. Bars, 60 μm.
Fig. 4. Photomicrographs of *R. haematoplasma* during morphogenesis (after protargol staining). (a) Ventral view of an early divider; arrowheads indicate the three postoral ventral cirri that remain intact. (b, c) Ventral views of proter (b) and opisthe (c); double arrowheads, arrows and arrowheads demonstrate the dedifferentiating undulating membranes, the OP forming new adoral membranelles, and old cirri involved in forming the FVTA, respectively. (d, e) Ventral views of two middle dividers; arrowheads mark the developing FVTA (d) and DKA (e); arrows show the OP differentiating to form the membranelles. (f, g) Ventral views of proter (f) and opisthe (g); arrowheads, double arrowheads and arrow indicate the FVTA differentiating to form cirri, the formation of the left frontal cirri, and the adoral membranelles of the opisthe, respectively. (h) Middle divider, to note the fusing macronuclear nodules. (i, j) Ventral (i) and dorsal (j) views of a late divider; double arrowhead marks the UMA splitting to form the paroral and endoral membranes; arrowheads show the new DK (i) and the dividing micronuclei (j); dashed lines
connect cirri that differentiate from the same anlage streak. (k, l) Ventral views of early (k) and middle (l) reorganizers; arrowheads, double arrowheads and arrow indicate the FVTA, formation of the leftmost frontal cirrus, and the OP, respectively. (m, n) Ventral views of same late reorganizer; arrow shows the dorsomarginal kineties; the arrowhead indicates the UMA splitting to form the paroral and endoral membranes; dashed lines connect cirri that differentiate from the same anlage streak. DK, dorsal kineties; DKA, anlagen of dorsal kineties; FVTA, frontal-ventral-transverse cirral anlagen; LMA, anlagen of left marginal row; Ma, macronuclear nodules; OP, oral primordium; RMA, anlagen of right marginal row; UMA, undulating membrane anlagen.

morphogenesis (Blatterer & Foissner, 1990). Subsequently, a population was reported from Korea (Shin & Kim, 1993). The Chinese population of *R. haematoplasma* corresponds closely with the original description, although the degree of curvature of the undulating membranes is much more distinct than in the type material (Berger & Foissner, 1997).

In their description of the Korean population of *Rubrioxytricha haematoplasma*, Shin & Kim (1993) did not mention the cortical granules or the colour of the cytoplasm. Furthermore, this population has distinctly fewer adoral membranelles (26 vs 38 in the German population and 37 in the Chinese population) and minor differences in the dorsal ciliature, i.e. dorsal kinety 1 shortened anteriorly and dorsal kinety 4 interrupted (Shin & Kim, 1993). Berger (1999) concluded that these differences were possibly due to geographical variation. In the absence of other data (e.g. SSU rRNA gene sequence), we also interpret these differences as variations among different populations.

The German and Korean populations of *R. haematoplasma* were both collected from freshwater habitats. By contrast, the Chinese population came from two shrimp-farming ponds, one brackish (salinity 8.6) and other marine (34.4). However, the brackish water was created artificially by mixing together marine and freshwater, and material was regularly transferred between the two ponds, so it is unclear whether the Chinese population of *R. haematoplasma* was originally from a marine or a freshwater habitat.

**Morphogenetic comparison with related taxa**

In the present study, we provide supplementary information on morphogenesis in the type species, *R. haematoplasma*.

**Table 2. Parental structures associated with the origin of FVT anlagen during morphogenesis of *Rubrioxytricha haematoplasma***

<table>
<thead>
<tr>
<th>Anlage</th>
<th>Parental structure</th>
<th>Proter</th>
<th>Opisthe</th>
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<tbody>
<tr>
<td>I</td>
<td>Parental undulating membranes</td>
<td>Oral primordium</td>
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<tr>
<td>II</td>
<td>II/2</td>
<td>Oral primordium</td>
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<td>III</td>
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<td>IV</td>
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<td>V</td>
<td>Anlage V of opisthe</td>
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<tr>
<td>VI</td>
<td>Anlage VI of opisthe</td>
<td>V/3</td>
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*Naqvi et al. (2006)* reported the morphogenetic process of *R. indica*, which is very similar to that of *R. haematoplasma*, the only difference of note being that the dedifferentiation of the undulating membranes occurs slightly later than the splitting of the FVT cirral anlage. This indicates that morphogenesis in the genus *Rubrioxytricha* is relatively stable and conservative, and is mainly characterized by the involvement of parental structures (i.e. the two posterior frontoventral cirri and the three postoral ventral cirri) in the formation of the new FVT cirri, which clearly separates it from the Stylonychinae.

In most oxytrichids, the new dorsal kineties originate from two groups of primordia; the first group develops intrakinetically within the old structures, whereas the other group originates de novo near the right marginal row(s) to form the dorsomarginal kineties. *R. haematoplasma* and *R. indica* (*Naqvi et al., 2006*) also have a *Urosomoida* pattern of dorsal kinety development (Berger & Foissner, 1997; Berger, 1999; Küppers *et al.*, 2011), i.e. each row of the first group of primordia forms one new dorsal kinety (vs the rightmost streak fragments in the posterior region to form two anlagen in the typical *Oxytricha* pattern).

**Phylogenetic position of Rubrioxytricha**

The molecular and morphogenetic data reported here and elsewhere support the monophyly of the genus *Rubrioxytricha* (Blatterer & Foissner, 1990; Berger, 1999; *Naqvi et al.*, 2006). Berger (1999, 2006, 2008) hypothesized that the 18-cirri pattern is the major morphological apomorphy of the hypotrichs, and defined the family Oxytrichidae mainly on the basis of the oxytrichid dorsal kinety fragmentation. *Rubrioxytricha* shows a simplified pattern of dorsal morphogenesis (i.e. without the fragmentation of dorsal kinety 3) compared with the typical *Oxytricha* pattern. Furthermore, the participation of postoral ventral cirrus V/3 in primordium formation excludes the possibility that *Rubrioxytricha* might belong to the subfamily Stylonchinae. In addition, *Rubrioxytricha* has a flexible body and cortical granules, in contrast to the stylonychids.

Based on the SSU rRNA gene sequences, the Chinese population of *Rubrioxytricha haematoplasma* (brackish habitat) and *R. ferruginea* (freshwater habitat; Chen & Song, 2002) form a clearly outlined clade with high support in the phylogenetic trees (99% ML, 1.00 BI). As in previous molecular phylogenetic analyses (e.g. Bernhard *et al.*, 2001; Foissner *et al.*, 2004; Schmidt *et al.*, 2007; Chen *et al.*, 2013b, c;
Fig. 5. Infraciliature of R. haematoplasma during divisional morphogenesis (a–d) and reorganization (e–i) processes (after protargol staining). Parental cirri are shown in outline, whereas new ones are shown in solid black. (a, b) Ventral (a) and dorsal (b) views of the same specimen in late stage; arrows mark the formation of the dorsomarginal kinetics; arrowheads show DKA stretching anteriad and posteriad; double arrowheads indicate the UMA splitting to form the paroral and endoral membranes. (c, d) Ventral (c) and dorsal (d) views of a late divider; arrows demonstrate the buccal cirri (c) and the third dorsal kinetics forming the caudal cirri (d); arrowheads mark the dividing micronuclei. (e, f) Ventral (e) and dorsal (f) views of an early reorganizer; arrows show the oral primordium formed near the posterior end of the adoral zone (e) and the DKA (f), arrowheads mark the FVTA streaks; double arrowheads indicate the micronuclei. (g) Ventral view of a reorganizer in middle stage; arrows, arrowheads, and double arrowheads demonstrate the posterior part of the adoral zone dedifferentiating, FVTA developing into cirri, and UMA giving rise to the leftmost frontal cirrus, respectively. (h, i) Ventral (h) and dorsal (i) views of a reorganizer in late stage; arrows show the dorsomarginal kinetics (h) and DK (i); the arrowhead indicates the UMA splitting to form the paroral and endoral membranes; double arrowheads mark the micronuclei; dashed lines connect cirri that differentiate from the same anlage streak. AZM, adoral zone of membranelles; DK, dorsal kinetics; FVTA, frontal-ventral-transverse cirral anlagen; LMA, anlagen of left marginal row; LMR, row of left marginal cirri; Ma, macronuclear nodules; RMA, anlagen of right marginal row; RMR, row of right marginal cirri; UMA, anlagen of undulating membranes. Bars, 60 μm.
Gao & Katz, 2014; Huang et al., 2014), our results recovered the subfamily Stylonychinae as a well-supported monophyletic group, and revealed the subfamily Oxytrichinae to be paraphyletic, thus supporting the decision of Berger (2008) to submerge it. However, considering the high level of undersampling within the family Oxytrichidae, more data, both morphogenetic and molecular, are needed in order to gain a better understanding of the evolutionary relationships and systematics of the non-Stylonychinae oxytrichids.

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