Taxonomy, ontogeny and molecular phylogeny of *Anteholosticha marimonilata* spec. nov. (Ciliophora, Hypotrichida) from the Yellow Sea, China

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The morphology, morphogenesis and molecular phylogeny of the marine ciliated protozoan *Anteholosticha marimonilata* spec. nov., isolated from mollusc-farming waters of the Yellow Sea, Qingdao, PR China, were investigated using microscopic observations of live and protargol-impregnated specimens and by small subunit rRNA gene sequence analysis. The novel species could be distinguished by the following features: an elongated elliptical body, in vivo size 80–160 μm × 30–50 μm; an adoral zone consisting of about 36 membranelles; three frontal, one parabuccal, one buccal, two frontoterminal and usually two pretransverse ventral cirri; 10–13 transverse cirri; a midventral complex composed of 12–17 pairs of cirri only, terminating in posterior 1/5; four or five dorsal kineties; two types of colourless cortical granules; four to nine moniliform macronuclear nodules and one to three micronuclei, and a contractile vacuole positioned at mid-body. Hitherto, the ontogenesis of the genus *Anteholosticha* has been regarded as rather diverse, which was confirmed by the morphogenetic processes of this novel species. The most noteworthy feature of *A. marimonilata* was that the proter retained almost the entire parental adoral zone except for a few proximal membranelles that were renewed in situ. The SSU rRNA gene sequence information clearly discriminated this isolate from its congeners. Molecular phylogenetic analyses demonstrated, with high statistical support, that *A. marimonilata* branched as a sister lineage to the *Nothoholosticha*–*Pseudokeronopsis* clade and hence belongs to the core part of the order Urostylida.

**INTRODUCTION**

Recent studies have shown that there is high diversity of marine ciliates in Chinese coastal waters (Chen et al., 2010; Fan et al., 2009; Jiang et al., 2010; Shao et al., 2010). This paper describes a novel species of the genus *Anteholosticha*, a group extracted from the *Holosticha* by Berger (2003) that is characterized as follows: AZM continuous; rearmost membranelles not wider than the remaining membranelles of the proximal portion; three enlarged frontal cirri; buccal cirrus/cirri right of paroral; frontoterminal cirrus present; midventral complex composed of midventral pairs only; number of transverse cirri usually distinctly lower than the number of midventral pairs; one left and one right marginal row; anterior end of left marginal row either straight or curved, commences left of adoral zone; caudal cirri absent; nuclear apparatus left of midline or scattered.

To date, about 38 species of the genus *Anteholosticha* have been described (Alekperov & Asadullayeva, 1999; Berger, 2006, 2008; Blatterer & Foissner, 1988; Borror & Wicklow, 1983; Buitkamp, 1977; Dragesco, 1966, 1970; Dragesco & Dragesco-Kerner, 1986; Foissner, 1982, 1987, 2000; Foissner et al., 2002; Gellért, 1956; Grolière, 1975; Hemberger, 1985; Kahl, 1928, 1932; Von Mereschkowsky, 1877; Song & Wilbert, 1997; Wirnsberger & Foissner, 1987). Of these, only six species have been described in detail in terms of their morphogenetic processes and likewise SSU rRNA gene sequence data are also only available for six species (Berger, 2006; Chen et al., 2010). Based on this limited morphogenetic and molecular information, the genus *Anteholosticha* does not seem to
be monophyletic (Berger, 2006; Chen et al., 2010; Yi et al., 2010; Schmidt et al., 2007). In order to verify this conclusion, and to increase our knowledge and understanding of the systematics and biodiversity of the genus *Anteholosticha*, morphogenetic and molecular studies of a wider range of species are needed.

In 2008, a previously unknown hypotrichous ciliate was found in the coastal waters of Qingdao, northern China, and pure cultures were maintained successfully. This enabled a detailed study of morphology and morphogenesis of the new isolate to be made. The results of our observations demonstrate that it represents a novel species within the genus *Anteholosticha*. The SSU rRNA gene of the new isolate was sequenced and analysed in order to assess the systematic positions of the novel species and its congeners within the stichotrichs.

**METHODS**

Samples were collected on 18th November 2008 from a sandy beach near a sewage outfall on the Yellow Sea coast at Qingdao (36° 08’ N; 120° 43’ E), China. The water temperature was ~14 °C and the salinity was ~18‰. The sampling method was mainly as described by Wang et al. (2009). Water samples with some sand were collected from a 15 cm deep hole dug in the sand and were processed within two hours of collection. Specimens were isolated using a micropipette and maintained in the laboratory for about two weeks at room temperature (20–23 °C).

Individuals were observed *in vivo* using differential interference contrast microscopy. The infraciliature was revealed with the protargol impregnation method according to Wilbert (1975).

Drawings of impregnated specimens were made with the help of a camera lucida at a magnification of ×1250. Measurements were performed with an ocular micrometer. To distinguish the changes that occurred during morphogenesis and reorganization, parental cirri are depicted in outline, whereas new ones are shaded black. Drawings of live specimens are based on live observations and photomicrographs. The terminology and systematic arrangement employed are mainly according to Eigner and Foissner (1994), Berger (2006) and Lynn (2008).

Genomic DNA extraction, PCR amplification and SSU rRNA gene cloning and sequencing were performed according to the methods described by Gong et al. (2009) and Yi et al. (2009).

The SSU rRNA gene sequences used for analyses were obtained from GenBank (for accession numbers, see Fig. 8). The sequences were aligned with CLUSTAL W as implemented in BioEdit 7.0 (Hall, 1999). The alignment was then modified by removing both termini of the alignment and highly variable regions. Preliminary neighbour-joining (NJ) analysis performed with MEGA 4 software (Tamura et al., 2007) showed that different selections of representative taxa (e.g. 41, 48, 51 and 52 species) generally resulted in trees with similar topologies for the taxa studied. Based on preliminary analyses, the exclusion of choerotrichs and oligotrichs improved the bootstrap values and made the trees more stable. A set of 41 SSU rRNA gene sequences was selected for further phylogenetic analyses. *Protocozelia contrax* was used as the outgroup. Using the AIC criterion, Modeltest 3.7 (Posada & Crandall, 1998) and MrModeltest v.2.0 (Nylander, 2004) both selected the GTR + I (0.5125) + G (0.5053) model, which was then used for maximum-likelihood (ML) and Bayesian inference (BI) analyses. A ML tree was constructed using the PhyML v.2.4.4 program (Guindon & Gascuel, 2003). The reliability of the internal branches was assessed using a non-parametric bootstrap method with 1000 replicates. BI analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) using the Markov chain Monte Carlo algorithm. The program was run for 3,000,000 generations with a sample frequency of 100 and a burn-in of 7500. A maximum-parsimony (MP) tree was obtained via random addition and swapped using the tree-bisection-reconnection (TBR) algorithm. Gaps were treated as missing data. MP analysis was performed with the PAUP* 4.0b10 software package (Swofford, 2002) and the reliability of the internal branches was estimated by using the bootstrap method with 1000 replicates. NJ analysis was conducted using the MEGA 4 program package (Tamura et al., 2007). Distances were estimated by the NJ method using the Maximum Composite Likelihood model of substitution with 1000 replication steps.

**RESULTS**

*Anteholosticha marimonilata* spec. nov. (Figs 1–3; Table 1)

**Diagnosis.** Marine *Anteholosticha*, 80–160 μm × 30–50 μm *in vivo*, body elongate and flexible; about 36 membranelles; one buccal and one parabuccal cirrus; three frontal, two frontoterminal, usually two pretransverse ventral and 10–13 transverse cirri; long midventral complex composed of 12–17 pairs of cirri only, extending to posterior 1/5 of body; left and right marginal rows with about 28 and 29 cirri respectively; four or five dorsal kinetics. Two types of colourless cortical granules: one large, mostly grouped and arranged in longitudinal rows; the other tiny, sparsely distributed beneath the cell surface. Moniliform macronucleus composed of four to nine nodules; one to three micronuclei. Single contractile vacuole positioned at mid-body.

**Type locality.** A sandy beach at Qingdao (36° 08’ N 120° 43’ E), China.

**Type specimens.** One holotype slide (no. xy-2008-11-18-01) with protargol-impregnated specimens is deposited in the Laboratory of Protozoology, Ocean University of China, China. One paratype slide is deposited in the Natural History Museum, London, UK, with registration no. 2010:6:21:1.

**Etymology.** The species-group name *marimonilata* is a composite of the Latin prefix mari- (marine habitat) and the species name *monilata* which comes from the morphologically similar freshwater species *Anteholosticha monilata* and refers to the moniliform macronucleus of this species.

**Description.** Cell *in vivo* about 80–160 μm × 30–50 μm, length : width ratio about 3.5 : 1; body shape basically constant, elongate elliptical when viewed from dorsoventral aspect with both ends narrowly rounded (Figs 1a and 2a–d, f); dorsoventrally flattened about 2 : 1 (Fig. 2e). Buccal field about 1/3 to 2/5 of body length. Pellicle thin and flexible.
Two types of colourless cortical granules: the larger one spherical and about 1 μm in diameter, mostly grouped along the cirral rows and between dorsal kineties (Figs 1c, d and 2h–j, l, m; arrows); the smaller one tiny (around 0.3 μm across), distributed sparsely and located deeper in the cortex (Figs 1c, d and 2i; arrowheads). Cytoplasm appears greyish to dark at low magnifications, colourless at high magnification, usually containing several colourless light-reflecting globules (1–3 μm across) in the anterior and posterior portions (Figs 1a and 2f, 2d, arrowheads). Contractile vacuole (CV) about 10 μm in diameter, positioned near left margin in the mid-body region (Figs 1a, b and 2c, d, g, arrow); accompanying it, a granular belt (possibly comprised of mitochondria, each around 2 μm long) can be clearly seen extending from the buccal area to the posterior portion of the cell (Figs 1b, arrow; 2g, arrowheads). Food vacuoles difficult to observe. Macronucleus left of cell median, moniliform composed of four to nine globular to elliptical nodules, each 8–12 μm in length (Figs 1e, f and 2f, k, arrowheads; Fig. 3a, b, e, g); one to three micronuclei (Mi), ovoid in shape, adjacent to macronuclear nodules (Figs 1e, f; 2k; 3a, g, h; 3e, arrow). Locomotion by slowly swimming while rotating about main body axis, or crawling on the bottom of a Petri dish or on debris with occasional pauses and then changing the direction of movement.

Adoral zone of membranelles (AZM) extending to about 35% of body length in fixed specimens. Distal end of AZM terminating at right margin of cell and bending slightly posterior (Figs 1e and 3a, c). Paroral and endoral (P, E; both constitute the undulating membranes) almost equal in length, intersecting each other above mid-point and terminating anteriorly in upper 1/3 of buccal field (Figs 1e and 3c). Single buccal cirrus (BC) situated near undulating membranes (Figs 1e and 3c, double arrowhead). Three enlarged frontal cirri (FC) lying in anterior frontal area (Figs 1e and 3c). Invariably one parabuccal cirrus present at lower left side of rightmost frontal cirrus (Figs 1e and 3c, arrowhead). Midventral complex (MC) composed of 12–17 pairs of cirri arranged in typical zigzag pattern and terminates in posterior 1/5 of cell (Figs 1e and 3a, f). Two frontoterminal cirri (FT) present close to the distal end of AZM (Figs 1e and 3c). Ten to 13 transverse cirri (TC) arranged in J-shaped row (Figs 1e and 3a, h; 3f, arrow), cilia of cirri strong, about 18 μm long. Usually two pretransverse ventral (PT) cirri located close to the posterior transverse cirri (Figs 1e, encircled; 3f, arrowhead; 3h, arrow). Out of 30 specimens observed, one had a single PT cirrus and another had three PT cirri. One right and one left marginal row, clearly separated posteriorly (Figs 1e and 3a, f, h); 23–36 cirri in left row (LMR), 26–35 in right marginal row.
(RMR); cilia of marginal cirri around 10 μm long; two basal body pairs located anterior to RMR (Figs 1f, arrowheads; 3c, d, arrow). Usually four or five complete dorsal kineties with dorsal cilia about 3 μm long (Figs 1f, 3d, arrowheads).

**Divisional morphogenesis (Figs 4–7)**

**Stomatogenesis.** In the opisthe: stomatogenesis commences with the apokinetal proliferation of closely spaced basal bodies near the anterior two or three...
Fig. 3. Photomicrographs of *Anteholosticha marimonilata* spec. nov. after protargol impregnation. (a, b) Ventral and dorsal views of the same specimen, to show the general ciliature and nuclear apparatus. (c, d) Ventral and dorsal views of the anterior portion respectively; arrows in (c) and (d) show basal body pairs at the anterior end of right marginal row; arrowhead in (c) indicates the parabuccal cirrus; double arrowhead in (c) marks the buccal cirrus; arrowheads in (d) show the dorsal kineties. (e, g) Posterior views, showing macronuclear nodules and micronucleus (arrow in e). (f, h) Ventral views of the posterior portion; arrow in (f) indicates the transverse cirri, arrowhead in (f) shows usual two pretransverse ventral cirri and arrow in (h) marks three pretransverse ventral cirri; broken line in (h) marks two redundant cirri migrating anterior. (i–l) Ventral views of reorganizers in early stages (j, k) and later stages (i, l); arrowhead in (i), (k) and (l) shows buccal cirrus; double arrowhead in (i) and (k) indicates the newly built frontoterminal cirri, while in (i) marks the dedifferentiation of old proximal membranelles; arrow in (i) marks a redundant cirrus migrating anterior, while in (j) shows the fragmentation of FVTA; three newly formed frontal cirri are enclosed by broken line in (i, l). LMA, left marginal row anlage; RMA, right marginal anlage; other abbreviations as in Fig. 1. Bar, 70 μm.
transverse cirri, which is oral primordium (OP; Figs 4a; 6a, arrow). As the number of basal bodies increases, a longish anarchic field is formed anterior to the transverse cirral row (Figs 4b; 6b, arrow). It is apparent that no parental structures join in the formation of the OP as the midventral and transverse cirri nearby remain unchanged. The OP develops further and becomes elongated and extends anteriorly (Figs 4c and 6d; arrow). The anterior portion of the OP then gradually differentiates into new adoral membranelles (Figs 4d; 6f). At the same time, a streak [undulating membranes anlagen (UMA)=fron- tal-midventral-transverse (FVT) anlage I] is formed to the right of the anterior end of the OP (Figs 4d, f and 6k, arrowhead). Slightly later, the leftmost frontal cirrus is produced from the anterior end of the UMA (Figs 5a, c; 7a, arrow). Meanwhile the parental structures are resorbed; the cytostome is completed and the daughter cells begin to separate with the formation of an equatorial furrow (Figs 4d, 5b; 7c, d, e, f, g, arrowheads). The cirri from anlage III become the right frontal cirrus and parabuccal cirrus, respectively (Figs 5c, 6g, 7f, h, i, arrowheads). The cirri from anlage II become the middle frontal cirrus and one buccal cirrus (Figs 5a, b, 7d, arrowheads). The anterior two cirri from the posteriormost streak migrate anteriorly to become the frontterminal cirri (Figs 5b, 7d, arrowheads). Two third cirri from two posteriormost streaks become the pretransverse cirri (Figs 5b, 7d, encircled). The last cirrus of each of the last nine to twelve streaks usually produce three cirri each except for the posteriormost two, each of which forms four cirri; other streaks invariably generate two cirri each (Figs 5b; 7d). The cirri from anlage II become the middle frontal cirrus and one buccal cirrus (Figs 5a, b, 7d, arrowheads). The cirri from anlage III become the right frontal cirrus and parabuccal cirrus, respectively (Figs 5c, 6g, 7f, h, i, arrowheads). The anterior two cirri from the posteriormost streak migrate anteriorly to become the frontterminal cirri (Figs 5b, 7d, arrowheads). Two third cirri from two posteriormost streaks become the pretransverse cirri (Figs 5b, 7d, encircled). The last cirrus of each of the last nine to twelve streaks becomes a transverse cirrus. Finally, most of the remaining cirri are rearranged in a ‘zig-zag’ pattern (Figs 5b, c and 7f, g, h–j).

Finally, the divider begins to elongate and the new ciliary structures move further apart as they migrate towards their final positions. Note that the two frontterminal cirri will migrate anteriorly (Figs 5c and 7f, arrow; 7g, arrow). Meanwhile the parental structures are resorbed; the cytostome is completed and the daughter cells begin to separate with the formation of an equatorial furrow (Figs 5c and 7f).

Marginal cirri: shortly after the appearance of the FVT-anlagen, a few cirri within the parental marginal rows, i.e. near the anterior end in the left row, and below the mid-body in the right row, are dedifferentiated to form the marginal anlagen for proter (Figs 4d, arrows; 6a, arrow; 4f, 6i, arrowhead). The anlagen in opisthe appear later than dedifferentiate (Figs 4d, double arrowhead; 6e; arrow). As this process continues, the UMA is formed (Figs 4f; 6g, arrowhead). Then the posterior end of the parental AZM begins to disorganize (Figs 4g, 6i, double arrowhead). Later several proximal membranelles are replaced by new ones in situ (Figs 5a, c; 5b, double arrowhead; 7a, d; 7b, arrow). The basic development of the UMA follows a similar pattern to that in the opisthe: the leftmost frontal cirrus is produced from the anterior end of the UMA (Figs 5a, arrow; 7a, arrowhead); the endoral and paroral are derived from longitudinal splitting of the UMA (Figs 4g, arrowhead; 5a–c; 7a, arrow).

Development of somatic ciliation. FVT-cirral anlagen: initially several midventral cirri behind mid-body are dedifferentiated into loosely arranged basal bodies, which are the frontal-midventral-transverse cirral anlagen (FVTA, Figs 4c and 6d, arrowheads). With more midventral and buccal cirri joining in the formation of these anlagen, about 15–20 oblique streaks are formed as a ladder-like structure (Figs 4d and 6e, arrowhead). Each streak then divides at its mid-point forming two groups, the anterior group for the proter and the posterior group for the opisthe (Figs 4d; 4f, arrows; 6e, g, double arrowhead; 6i, m). Subsequently, these cirral anlagen will develop independently in both dividing parts. Each streak begins to segment into new cirri (Figs 4g, arrows; 5a, 7a–c): the last nine to twelve streaks usually produce three cirri each except for the posteriormost two, each of which forms four cirri; other streaks invariably generate two cirri each (Figs 5b; 7d). The cirri from anlage II become the middle frontal cirrus and one buccal cirrus (Figs 5a, b, 7d, arrowheads). The cirri from anlage III become the right frontal cirrus and parabuccal cirrus, respectively (Figs 5c, 6g, 7f, arrowheads). The anterior two cirri from the posteriormost streak migrate anteriorly to become the frontterminal cirri (Figs 5b, 7d, arrows). Two third cirri from two posteriormost streaks become the pretransverse cirri (Figs 5b, 7d, encircled). The last cirrus of each of the last nine to twelve streaks becomes a transverse cirrus. Finally, most of the remaining cirri are rearranged in a ‘zig-zag’ pattern (Figs 5b, c and 7f, g, h–j).

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Table 1. Morphometric data on Anteholosticha marimonilata spec. nov. based on protargol-impregnated specimens

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*Parabuccal cirrus not included.
those of the proter (Figs 4g and 6n, arrowheads). All these marginal row anlagen then generate new cirri to replace the old structures (Figs 5a–c and 7a, d, f, h–j).

Dorsal ciliature: new dorsal kineties develop from two separate anlagen within each parental kinety (Fig. 4e, f, arrowheads). These anlagen stretch continuously in both
directions (Figs 4h and 7e, arrowheads) and eventually replace the parental kineties.

**Nuclear division.** The nuclear apparatus divides in the usual way for urostylids, i.e. all macronuclear nodules fuse to form a single mass during the middle stage (Figs 4e; 6l, arrow) which divides several times during the later stages (Figs 4f, inset; 6h, arrowheads; 7h–j, arrows). The divisional process of micronuclei is unclear though they can be recognized in some dividers (Figs 4e, f, inset; 6h, arrows; 7h–j, arrowheads).

**Reorganization.** Only a few physiological regeneration stages were observed for this species. From the data available, the process in reorganizers appears to be very similar to those in cell division and can be summarized as follows. Several proximal membranelles are replaced by new ones *in situ* (Fig. 3j, double arrowhead). All FVT-cirri derive from 17 or 18 cirral streaks (Fig. 3j, arrow; 3i, k, l). Anlagen I–III provide one frontal cirrus each (Fig. 3i, l, encircled) and anlage II generates the buccal cirrus (Fig. 3i, k, l, arrowhead). The two newly built frontoterminal cirri will migrate anteriorly (Fig. 3i, k, double arrowhead). Both left and right marginal anlagen occur within the parental marginal rows and generate new cirri to replace the old structures (Fig. 3j, k).

**SSU rRNA gene sequences and phylogenetic analyses (Fig. 8)**

The 1772 bp SSU rRNA gene sequence of *Anteholosticha marimonilata* was deposited in the GenBank database with accession no. FJ870075. Among the morphologically similar species for which SSU rRNA gene sequences are available, *A. marimonilata* differs most from *Anteholosticha manca* (by 130 nt) and least from *Nothoholosticha fasciola* (by 76 nt).

*Protocruzia contrax* was chosen as the outgroup to test the systematic position of the novel species among the stichotrichine species. The trees constructed under ML criteria using either BI or non-parametric bootstraps had

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**Fig. 5.** Late morphogenetic stages of *Anteholosticha marimonilata* spec. nov. after protargol impregnation. (a) Ventral view, showing the leftmost frontal cirrus derived from anterior end of undulating membranes anlagen (arrows), and buccal cirrus from cirral anlage II (arrowheads) in both proter and opisthe, the lengthening of the marginal rows anlagen and the fragmentation of the FVTA. (b) Ventral view, indicating the development of FVTA; arrows and arrowheads show frontoterminal cirri and buccal cirrus respectively; double arrowhead marks the reorganization of proximal membranelles in the proter; new pretransverse ventral cirri are encircled by broken lines. (c) Ventral view, showing the completion of new cirri and oral apparatus development; arrows indicate parabuccal cirri coming from anlage III; cirri which originate from the same anlage are connected by broken lines. Abbreviations as in Figs 1, 3 and 4. Bars, 60 μm.
Fig. 6. Photomicrographs of Anteholosticha marimonilata spec. nov. after protargol impregnation. (a, b) Ventral views of two early dividers; arrows mark the opisthe’s oral primordium. (c, d) Ventral views of the same early divider, showing dedifferentiation of endoral at its anterior end (arrow in c) and midventral cirri (arrowheads in d), and lengthening of the opisthe’s oral primordium (arrow in d). (e, f, j) Ventral views of the same early divider, showing dedifferentiation of the paroral (arrow in e), the endoral (arrow in j) and the buccal cirrus (arrowhead), and the division of the streaks of the FVTA (double arrowheads). (g, h, k) Ventral views of the same early middle divider, indicating the formation of two groups of the FVTA (double arrowhead in g and k), the undulating membranes anlagen (arrowhead in g and k), the left marginal row anlagen in the proter (arrow in g) and the new membranelles in the opisthe’s oral primordium (arrow in k); also showing the macronuclear nodules (arrowheads in h) and micronuclei (arrows in h). (i, m, n) Ventral views of the same middle divider, showing fragmentation of FVTA, splitting of undulating membranes anlagen (arrow in i and m), marginal rows anlagen (arrowheads) and dedifferentiation of proximal membranelles in the proter (double arrowhead in i). (l, o) Ventral views of the same middle divider, marking the fused macronucleus (arrow) and micronucleus (arrowhead).
similar topology. *Anteholosticha marimonilata* formed a strongly supported clade with *Nothoholosticha fasciola* and *Pseudokeronopsis* spp. (ML/BI/MP/NJ, 99/1.00/97/89). In accordance with previously published results (Yi et al., 2008; Chen et al., 2010), the genus *Anteholosticha* is not monophyletic: its congeners are distributed in five clades, while three *Holosticha* species form one clade with maximum support (ML/BI/MP/NJ, 100/1.00/100/100).

**Fig. 7.** Photomicrographs of *Anteholosticha marimonilata* spec. nov. after protargol impregnation. (a–c) Ventral views of middle-late divider, showing fragmentation of FVTA, the leftmost frontal cirrus generated from the anterior end of undulating membranes anlagen (arrowhead in a), splitting of undulating membranes anlagen (arrow in a and arrowhead in c), reorganization of proximal membranelles in the proter (arrow in b). (d, f, g) Ventral views of late dividers, showing the near completion of morphogenesis; arrows mark the migrating frontoterminal cirri; arrowheads in (d) indicate buccal cirrus in proter and opisthe; arrowheads in (f) and (g) show parabuccal cirrus; pretransverse ventral cirri are encircled by broken line in (d), and frontal cirri are encircled by broken line in (f) and (g). (e) Dorsal view, showing dorsal kineties anlagen (arrowheads). (h) Ventral view of the anterior daughter cell just after division, showing the old frontoterminal cirri (double arrowhead), macronuclear nodules (arrows), and micronucleus (arrowhead); new frontal cirri are encircled by broken line. (i, j) Ventral views of the posterior daughter cells just after division, indicating macronuclear nodules (arrows) and micronucleus (arrowhead).
DISCUSSION

Comparison of *A. marimonilata* spec. nov. with similar taxa (Table 2)

Considering its body size and shape, moniliform macronucleus and basic ciliary pattern, *Anteholosticha marimonilata* should be compared with five similar congeners, i.e. *A. monilata*, *A. xanthichroma*, *A. sigmoidea*, *A. distyla* and *A. australis* (Augustin & Foissner, 1992; Blatterer & Foissner, 1988; Buitkamp, 1977; Foissner, 1982, 1984; Foissner & Didier, 1981; Song & Wilbert, 1989; Wirnsberger & Foissner, 1987). Among these, *A. monilata* is most similar to the novel species. However, the former is a freshwater (vs marine) form with conspicuously more midventral cirral pairs (18–27 compared with 12–17). In addition, *A. monilata* has only one kind of cortical granule, i.e. rod-shaped ones (about 2–3 × 1–1.5 μm), which cluster in 30 rows under the pellicle and can be ejected and stain heavily with protargol, whereas the novel species possesses two kinds of cortical granule, both of which are spherical and neither of which can be ejected (Augustin & Foissner, 1992; Foissner & Didier, 1981; Song & Wilbert, 1989; Table 2).

*Anteholosticha xanthichroma* differs from *A. marimonilata* in having more midventral pairs (around 40 vs 12–17), fewer transverse cirri (0–6 vs 10–13) and a freshwater (vs marine) habitat (Wirnsberger & Foissner, 1987; Table 2).

In addition to its moniliform macronucleus and elongate body shape, *Anteholosticha sigmoidea* resembles the novel species in also having four dorsal kineties. However, the former can be easily separated by having fewer adoral membranelles (16–28 compared with 33–43) and transverse cirri (3–6 vs 10–13), only one (vs two) type of cortical granule and by its terrestrial (versus marine) habitat (Foissner, 1982, 1984; Table 2).

*Anteholosticha australis* can be distinguished from *A. marimonilata* in having fewer transverse cirri (3–6 compared with 10–13), ellipsoidal cortical granules, which can be impregnated heavily with protargol, and ejected (vs spherical cortical granules that cannot be ejected) and its terrestrial (vs marine) habitat (Blatterer & Foissner, 1988; Table 2).

*Anteholosticha distyla* has more macronuclear nodules (16 compared with 4–9) and fewer transverse cirri (2 vs 10–13),
Table 2. Comparison of some closely related congeners with Anteholosticha marimonilata spec. nov.

Taxa: 1, Anteholosticha marimonilata; 2, A. xanthichroma; 3, A. signoida; 4, A. monilata; 5, A. warreni; 6, A. pulchra; 7, A. australis; 8, A. distyla. CV, contractile vacuole; —, data not available.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4*</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tr>
<td>Body length in vivo</td>
<td>80–160 μm</td>
<td>110–220 μm</td>
<td>90–130 μm</td>
<td>90–160 μm</td>
<td>80–120 μm</td>
<td>120–360 μm</td>
<td>130–190 μm</td>
<td>150–180 μm</td>
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<tr>
<td>Body shape</td>
<td>Elongated elliptical and both ends rounded</td>
<td>Long belt-like, both ends rounded</td>
<td>Elongated elliptical and both ends narrowly rounded</td>
<td>Elongated elliptical and both ends widely rounded</td>
<td>Elliptical</td>
<td>Long belt-like, both ends rounded</td>
<td>Long belt-like, both ends broadly rounded</td>
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<td>Position of CV</td>
<td>Mid-body</td>
<td>Above mid-body</td>
<td>—</td>
<td>Mid-body</td>
<td>—</td>
<td>Posterior 1/4 to 1/5</td>
<td>Above mid-body</td>
<td>Above mid-body</td>
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<tr>
<td>Colour of cortical granules</td>
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<td>—</td>
<td>Colourless</td>
<td>—</td>
<td>Colourless</td>
<td>Brick-reddish</td>
<td>Colourless</td>
<td>—</td>
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<td>Feature of cortical granules/extrusomes</td>
<td>Globular, 1 μm</td>
<td>—</td>
<td>Globular, 0.5–1 μm</td>
<td>Rod-shaped, 2–3 × 1–1.5 μm</td>
<td>Elliptical, 2 μm</td>
<td>Spherical, 0.8 μm</td>
<td>Elliptical, 2.5 × 1.5 μm</td>
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<td>Midventral pairs</td>
<td>12–17</td>
<td>—</td>
<td>40</td>
<td>—</td>
<td>18–27</td>
<td>7–9</td>
<td>21–38</td>
<td>—</td>
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<td>Transverse cirri</td>
<td>10–13</td>
<td>0–6</td>
<td>3–6</td>
<td>7–12</td>
<td>10–12</td>
<td>5–8</td>
<td>3–6</td>
<td>2</td>
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<td>Macronuclear nodules</td>
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<td>Moniliform</td>
<td>Moniliform</td>
<td>Moniliform</td>
<td>Scattered</td>
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<td>No.</td>
<td>4–9</td>
<td>4–13</td>
<td>5–12</td>
<td>4–23</td>
<td>50</td>
<td>31–47</td>
<td>10–16</td>
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<td>Freshwater</td>
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<td>Freshwater</td>
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<td>Marine</td>
<td>Soil</td>
<td>Soil</td>
</tr>
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</table>

*Formerly called Holosticha similis (Stokes, 1886) Foissner & Didier, 1981, which was regarded as a junior synonym of H. monilata by Foissner (1991) and then transferred to the genus Anteholosticha by Berger (2003).

‡According to Berger (2006).
and is found in a terrestrial (vs marine water) biotope, and so cannot be confused with the novel species (Buitkamp, 1977; Table 2).

**Morphogenesis**

Since the genus *Anteholosticha* was erected by Berger (2003), about 38 morphospecies have been included in the genus. Among these, morphogenetic processes have been investigated for only six species, namely *A. monilata*, *A. multistilata*, *A. warreni*, *A. pulchra*, *A. heterocirrata* and *A. manca* (Hemberger, 1982, 1985; Hu et al., 2000; Berger, 2006, 2008; Li et al., 2007; Li et al., 2008). Based on the data available, *A. marimonilata* shares several common features with its congeners including the intrakinetal mode, i.e. ‘within row proliferation’, of generation of marginal rows and dorsal kinetics. However, in terms of the formation of the proter’s AZM, members of this genus differ significantly from one to another: in *A. monilata* only a few proximal membranelles of the parental adoral zone are replaced by new ones which originate in situ, while the majority are retained; in *A. multistilata*, *A. warreni*, *A. pulchra* and *A. manca*, the parental zone is completely resorbed; and in *A. heterocirrata* the entire parental adoral zone is inherited by the proter (Hemberger, 1982; Hu et al., 2000; Berger, 2006, 2008; Li et al., 2007; Li et al., 2008). *A. marimonilata* also partially renews its old AZM but has fewer proximal membranelles to be rebuilt than in *A. monilata*, indicating its intermediate relationship between *A. monilata* and *A. heterocirrata*. In addition, the FVT-anlagen in the proter and opisthe are generated in one of several separate clades which is consistent with Berger’s hypothesis (Berger, 2003) and with previous molecular studies (Schmidt et al., 2007; Yi et al., 2008; Chen et al., 2010). However, gene sequence data are available for only six out of nearly 40 species of the genus *Anteholosticha*. Therefore, both gene sequence and morphogenetic data for more species are required in order to fully determine the phylogeny of this group.

**Molecular comparison with congeners**

Berger (2003) redefined the genus *Holosticha sensu lato* as the type species. The separation of the new isolate *A. marimonilata* from *N. fasciola* and other species of the genus *Anteholosticha* is supported by differences of 76 to 130 bp in their SSU rRNA gene sequences and by its distinct position in the phylogenetic trees (Fig. 8). Furthermore, pairwise sequence similarities between *A. marimonilata* and its congeners range from 91.8 % to 95.6 %, further supporting the validity of *A. marimonilata* as a separate species.

The SSU rRNA gene sequence data presented here support the clear separation of the genus *Anteholosticha* from the genus *Holosticha*, although the former is recovered as a conspicuously divergent group with its congeners distributing in several separate clades which is consistent with Berger’s hypothesis (Berger, 2003) and with previous molecular studies (Schmidt et al., 2007; Yi et al., 2008; Chen et al., 2010). However, gene sequence data are available for only six out of nearly 40 species of the genus *Anteholosticha*. Therefore, both gene sequence and morphogenetic data for more species are required in order to fully determine the phylogeny of this group.

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