Ontogeny and phylogeny of *Metaurostylopsis cheni* sp. n. (Protozoa, Ciliophora), with estimating the systematic position of *Metaurostylopsis*

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The ciliate genus *Metaurostylopsis* seems to be a highly divergent marine-habiting group, of which neither systematic position nor the variation of their ontogeny has been critically checked. In the present work, the morphology and morphogenesis during asexual division of a new form, *Metaurostylopsis cheni* sp. n., isolated from the Yellow Sea, China, were investigated and comparison among known congeners was performed. The new species has two types of cortical granules, the larger ones of which are flattened and oval or circular in outline with a longitudinal groove, yellow–green in colour, and arranged along the cirral rows and dorsal kineties, whereas the smaller ones are colourless or grayish and sparsely distributed. The main morphogenetic features are: (i) the entire parental ciliature, including the old oral apparatus, is renewed, (ii) the oral primordium of the proter originates *de novo* and beneath the surface of the buccal cavity, that is, sub-apokinetally, (iii) the anlagen of the marginal rows and of the dorsal kineties are formed intrakinetally and (iv) fusion of the macronuclear nodules results in an irregular mass with only few branches. The small subunit ribosomal RNA (SSU rRNA) gene of *M. cheni* was sequenced. Phylogenetic analysis based on SSU rDNA gene sequence data shows that *M. cheni* clusters with all other *Metaurostylopsis* spp. sequenced to date indicating that the genus is monophyletic and is probably closely related to the *Apokeronopsis–Thigmokeronopsis*-group, within the order Urostylida.

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**Introduction**

The urostylid genus *Metaurostylopsis* was established by Song *et al.* (2001) with *M. marina* as the type species by original designation. Five other species have as been described: *M. rubra*, *M. songi*, *M. salina*, *M. struederkypkeae* and *M. sinica* (Song & Wilbert 2002; Lei *et al.* 2005; Shao *et al.* 2008b,c). Morphogenesis has been reported for *M. marina*, *M. rubra*, *M. sinica* and *M. struederkypkeae* (Song *et al.* 2001; Wilbert & Song 2005; Shao *et al.* 2008b; Li 2009, dissertation, unpublished). It was found that the main morphogenetic features of *Metaurostylopsis* species are consistent with those of most other urostylids, but with three notable exceptions: (i) the macronuclear segments do not form the usual rounded mass prior to division, but rather an irregular, branched complex, (ii) the short row of unpaired ventral cirri posterior to the midventral rows originates from FVT-anlage n-1 and (iii) the oral apparatus in the proter originates from an oral primordium that is located beneath the cortex within the buccal field and which forms *de novo*.

In this paper, we describe the morphology and morphogenesis of *Metaurostylopsis cheni* spec. nov. The phylogeny of *Metaurostylopsis*, based on small subunit ribosomal RNA (SSU rDNA) gene sequence data, is also investigated and discussed.

**Materials and methods**

*Morphological and morphogenetic studies*

*Metaurostylopsis cheni* sp. n. was collected from the surface of sandy sediments of a beach at Qingdao (Tsingtao),
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China (120°24'E; 36°03'N) on 9 June 2008. The water temperature was ca. 18 °C and salinity was ca. 30‰.

Specimens were maintained in Petri dishes for several weeks at room temperature with added rice grains to enrich bacteria as food for the ciliates. Isolated cells were observed in vivo using bright field and differential interference contrast microscopy at x100 to x1000 magnifications. Protargol impregnation (Wilbert 1975; Liu et al. 2009) was used to reveal the infraciliature and nuclear apparatus. Counts and measurements of stained specimens were carried out with an ocular micrometre. Drawings were made with the aid of a drawing device (Chen et al. 2010). To illustrate the changes during morphogenetic processes, parental cirri are depicted by contour whereas new ones are shaded black (Li et al. 2010). Terminology and systematics are mainly according to Song et al. (2001) and Berger (2006).

DNA extraction, PCR amplification and sequencing
Genomic DNA extraction, PCR amplification and sequencing of the SSU rRNA gene were performed according to Gong et al. (2009). In brief, around 10 cells were isolated and transferred to sterilized seawater (0.22 μm filtered). In order to minimize contamination, the isolates were washed by transferring cells to successive drops of sterilized water and/or by removing excessive water using a micropipette and replenishing with sterilized water. The washed cells were transferred to a PCR microfuge tube with a minimum volume of water.

Genomic DNA was extracted with REDExtract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO, USA) according to the manufacturer’s protocol, with the modification suggested by Gong et al. (2009). The SSU rRNA gene was amplified by PCR with primers Euk A (5'-AACCTG GTTGATCCTGCGCAGT-3') and Euk B (5'-TGAATCC TTCTGCAAGTTACCTAC-3') (Medlin et al. 1988). The PCR amplification cycle was as follows: pre-run of 5 min at 94 °C; 35 cycles of 30 s at 94 °C, 1 min at 60 °C and 3 min at 72 °C; and one cycle of 7 min at 72 °C. After confirmation of the appropriate size of the amplified fragments on an agarose gel, the PCR product was purified with UNIQ-5 DNA Cleaning Kit (Sangon Bio. Co., Shanghai, China), inserted into the pUCm-T vector (Sangon Bio. Co.), and transformed into Escherichia coli DH5α cells. Sequencing in both directions was carried out on an ABI 3700 sequencer.

The new sequence of *M. cheni* sp. n. has been deposited in the GenBank database with accession number GU170204.

**Phylogenetic analyses**
The SSU rRNA gene sequence of the new species, together with sequences of 52 other taxa downloaded from GenBank database (see Fig. 7 for GenBank accession numbers), were aligned using Clustal W implemented in Bioedit 7.0 (Hall 1999). Ambiguously aligned regions and gaps were excluded prior to phylogenetic analyses. *Protocruzia adherens* (AY217727) was selected as the out-group species. The program MrModeltest v. 2.0 (Nylander 2004) was implemented to select the GTR + I + C model with AIC criterion. Using these parameter values, a maximum likelihood (ML) tree was constructed with the PhyML V2.4.4 program (Guindon & Gascuel 2003). The reliability of internal branches was assessed using a nonparametric bootstrap method with 1000 replicates. A maximum parsimony (MP) tree was obtained via random addition and swapped using the tree-bisection-reconnection (TBR) algorithm (Yi et al. 2009). Gaps were treated as missing data. MP analysis was performed with the software package PAUP* 4.0b10 (Swofford 2002), and the support for the internal branches was estimated using the bootstrap method with 1000 replicates (Gao et al. 2009). The topologies of the ML and MP trees were almost identical therefore; they were merged into a single tree for purposes of illustration. TREEVIEW v1.6.6 (Page 1996) and MEGA 4.0 (Tamura et al. 2007) were used to visualize tree topologies.

**Results**

*Morphology of M. cheni sp. n.* (Figs 1, 5A–D; Table 1)
Deposition of type slides. The holotype slide with protargol-impregnated specimens is deposited in the Laboratory of Protozoology, Ocean University of China, Qingdao, China (no. CXM08060901). One paratype slide with protargol-impregnated specimens is deposited in the Natural History Museum, London, UK (no. 2010.2.3.1).

**Etymology.** This species is named in honour of Prof. Yiyu Chen, Director of the Natural Science Foundation of China and academician of CAS, in recognition of his significant contributions to many fields in zoology and for his support of protozoological research in China.

**Type locality.** A sandy beach at Qingdao (120°24'E; 36°03'N), China.

**Diagnosis.** Small-sized marine *Metaurostylopsis*, about 90–140 × 40–60 μm in vivo. Two types of cortical granules: larger ones flattened and oval or circular in outline with a longitudinal groove, yellow–green in colour, and distributed along the cirral rows and dorsal kineties; smaller ones colourless and sparsely arranged. About 21–26 adoral membranelles; four frontal, four frontoterminal, one buccal and five to eight transverse cirri; five to nine midven-
tral cirral pairs and about four unpaired ventral cirri; three or four left and three right marginal rows; invariably three complete dorsal kinetics; ca. 40 macronuclear nodules; contractile vacuole positioned in equatorial region near left margin of cell.

Description. Body flexible but not contractile, ellipsoid to pyriform, mostly about 90–140 × 40–60 μm in vivo, dorso-ventrally flattened ca. 3:1 (Fig. 1A, H, I). Buccal cavity prominent, about 1/3 body length (Fig. 1A). Pellicle thin, with two types of cortical granules on both ventral and dorsal sides: larger ones about 2 × 1.5 μm in size and 1 μm thick, flattened and oval or circular in outline with a longitudinal groove, green to yellow–green, arranged in lines alongside the midventral and marginal cirral rows (Fig. 1B, C; K, arrows) and along the dorsal kinetics (Fig. 1D, E; J, arrows); smaller ones colourless or grayish, about 0.2 μm in diameter, scattered and sparsely arranged (Fig. 1B–E; J, K, arrowheads). Cytoplasm colourless, usually with many lipid droplets (ca. 2–4 μm across) and food vacuoles that typically contain flagellates, small ciliates and bacteria in environmental isolates. Contractile vacuole about 10–20 μm across, located in equatorial region near left body margin, contracting at intervals of about 5–10 mins (Fig. 1I). Numerous (ca. 40) macronuclear nodules, oval to elongate in outline, 3–9 μm long, scattered and usually difficult to observe in vivo (Fig. 1G, Ma). Micronuclei not observed.

Locomotion with no specialities, crawling moderately quickly on substrate.
Table 1 Morphometric characterization of *Metaurostylopsis cheni* sp. n. All data are based on protargol-impregnated specimens. Measurements in μm.

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CV, coefficient of variation in %; DK, dorsal kinetics; FTC, frontoterminal cirri; LMR, left marginal rows; Ma, macronuclear nodules; Max, maximum; Mean, arithmetic mean; Min, minimum; MP, midventral cirral pairs; n, sample size; RMR, right marginal rows; SD, standard deviation; TC, transverse cirri; VC, unpaired ventral cirri. *Numbered from inside to outside. **The 2-3 dikinetids anterior of the rightmost marginal ciral row not included.

Adoral zone of membranelles (AZM) about 1/3 of body length, cilia of membranelles ca. 12 μm long *in vivo*. Distal end of AZM bending only slightly towards the right. Both paroral and endoral membranes long and slightly curved, almost parallel to each other (Figs 1F, 5A). Pharyngeal fibres about 30 μm long, conspicuous after protargol impregnation (Fig. 5C, arrows).

Most cirri (except for the transverse cirri) relatively fine with cilia about 7 μm long. Distal end of AZM bending only slightly to the right. Both paroral and endoral membranes long and slightly curved, almost parallel to each other (Figs 1F, 5A). Pharyngeal fibres about 30 μm long, conspicuous after protargol impregnation (Fig. 5C, arrows).

Stomatogenesis and cirral development. Division commences with the appearance of a single anarchic field consisting of closely spaced basal bodies (the oral primordium for the opisthe, OP) adjacent to the unpaired ventral cirri. The AO subsequently becomes longer and wider by further proliferation of basal bodies (Figs 2A, C, 5E, H). Simultaneously a small elliptical field of basal bodies, the proter’s oral primordium (POP), appears *de novo* beneath the surface of the buccal field between the parental undulating membranes (UMs) and the adoral zone of membranelles (AZM) (Figs 2C, 5G). The POP seems to develop within a pouch, the margin of which is always clearly outlined.

In the following stage both oral primordia continue to develop and the new membranelles start to differentiate in a posteriad direction (Figs 2D, E, 5I, J). The anlagen for the new UM start anteriorly that will become the leftmost frontal cirrhus (Fig. 2D, arrow, E, arrowheads).

At about the same time, basal bodies appear to the right of the parental UM and form several narrow, longitudinal streaks which later become the proter’s fronto-ventral-transverse cirral anlagen (FVT-anlagen). In the opisthe, the FVT-anlagen are formed in a similar way to the right of the OP (Figs 2E, 5I, J). The FVT-anlagen then grow by increasing the number of basal bodies and organize into about 10 oblique streaks posteriad in both dividers (Figs 2F, H, 5J, arrows). During this stage, the parental midventral complex probably do not contribute to the formation of the new anlagen.

Immediately, organization of the adoral membranelles proceeds simultaneously in both dividers (Fig. 2F, H). The old UM and buccal cirrus are already resorbed, while the development of the FVT-anlagen and the UM-anlagen continues (Fig. 2H).

In the next stage, the differentiation of membranelles and formation of the new oral apparatus in each are almost complete. The UM-anlagen in both dividers split longitudinally and give rise to the paroral and endoral membranes (Figs 3A, B, 6B, double-arrowheads). The FVT-anlagen begin to differentiate. Each FVT-streak, apart from the right marginal rows (RMR), anterior portion of rightmost RMR and posterior portion of leftmost LMR extending onto dorsal side (Figs 1F, G, 5D).

Three complete dorsal kinetics. Dorsal cilia 3–5 μm long. On right margin, two or three pairs of basal bodies anterior to rightmost marginal row (Figs 1G, DK; 5B, arrowheads).
rightmost two, develops into two or three cirri (Figs 3A, B, D, 6A, B, D, arrows). Four frontoterminal cirri are then formed as seen in most other urostylids, that is from the last FVT-streak and moving anteriorly (Figs 3D, E, 6A, D, arrowheads). Streak I (numbered from left to right) provides the second frontal cirrus in each divider (arrow in d and arrowheads in E); in both dividers, old midventral rows do not contribute to the construction of the FVT-anlagen (arrows); note the anlagen of the marginal rows (LMA, RMA) generated intrakinetally. 

In the late stage, the anterior ends of the two new AZMs arch to the right, each divider begins to elongate and the new ciliary structures move further apart as they migrate towards their final positions (Fig. 4A). The anterior four (frontoterminal) cirri from the last streak will migrate anteriad (Figs 4A, C, 6F, G, arrowheads). Finally, the

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**Fig. 2** Morphogenesis of *Metaurostylopsis cheni* sp. n. at early to middle stages after protargol impregnation. —A, B. Ventral (A) and dorsal (B) views of an early divider. —C. Ventral view of a slightly later divider, to show the formation of oral primordia in the proter and the opisthe. —D. Proter’s oral primordium. —E. Ventral view of the same specimen in (D), to demonstrate the undulating membranes anlage will give the leftmost frontal cirrus in each divider (arrow in d and arrowheads in E); in both dividers, old midventral rows do not contribute to the construction of the FVT-anlagen (arrows); note the anlagen of the marginal rows (LMA, RMA) generated intrakinetally. —F, G. Ventral (F) and dorsal (G) views of the same specimen, arrows show the FVT-anlagen organizing into oblique streaks in both dividers. Note the anlagen of marginal rows (LMA, RMA) and dorsal kineties (DKA). —H, I. Ventral (H) and dorsal (I) views of the same middle stage specimen, arrowheads indicate the first frontal cirri arise from the undulating membranes anlage, and arrows show the development of the FVT-anlagen. Note all macronuclear nodules fuse to form a slightly branched complex. DKA, dorsal kineties anlage; FVT-anlagen, fronto-ventral-transverse cirral anlage; LMA, left marginal anlage; Ma, macronuclear nodules; OP, opisthe’s oral primordium; POP, proter’s oral primordium; RMA, right marginal anlage. Scale bars—40 μm.
parental structures are resorbed, the cytostome is generated and the daughters begin to separate (Figs 4C, 6G).

Marginal and dorsal structures. Shortly after the beginning of morphogenesis, within each parental marginal row a few cirri near the anterior end, and a few others below the mid-body, de-differentiate to form two separate anlagen (Figs 2E, F, LMA, RMA; 5I, double-arrowheads, arrowheads). These anlagen then stretch and begin to segments posteriad to form the new cirri which will completely replace the parental rows (Figs 3A–C, arrowheads; 5L, RMA; 6B, arrowheads).

The dorsal kineties anlagen (DKA) develop by intrakinetal proliferation, with two anlagen forming within each parental row (Figs 2G, I, 5M, DKA). The anlagen subsequently elongate and the parental structures are

Fig. 3 Middle and late stages of Metaurostylopsis cheni sp. n. after protargol impregnation. —A–C. Ventral (A, B) and dorsal (C) views of middle stage dividers, arrows show the FVT-anlagen beginning to differentiate, double-arrowheads indicate the undulating membrane anlagen splitting to give rise to the paroral and endoral membranes, and arrowheads mark the marginal rows anlagen. —D–F. Ventral (D, E) and dorsal (F) views of late stage dividers, arrows indicate the FVT-streaks differentiating into cirri, arrowheads show the last FVT-streaks forming the frontoterminal cirri and double-arrowheads mark the buccal cirri provided by FVT-streak I (numbered from left to right) in both dividers. DKA, dorsal kineties anlagen; FVT-anlagen, fronto-ventral-transverse cirral anlagen; Ma, macronuclear nodules. Scale bars—40 μm.
either incorporated or resorbed (Figs 3C, 6H, DKA). During the division, neither surplus anlagen nor caudal cirri are formed. The extra dorsal bristles (usually only two pairs) anterior to the rightmost marginal row are very likely formed from the anterior end of the rightmost marginal anlage (Fig. 4D, arrowheads).

Division of nuclear apparatus. Nuclear division proceeds in the usual way for Metaurostylopsis. Briefly, during the mid-divisional stage all macronuclear nodules fuse to form a single, somewhat branched complex which then divides into bar-shaped segments or nodules (Figs 2B, I, 3C, 5F, 6E, Ma; 3F, 4D). Division of the micronuclei was not observed.

Phylogenetic analyses (Fig. 7)
In addition to Metaurostylopsis cheni sp. n., SSU rRNA gene sequences are available for three congeners, namely M. struederkypkeae, M. salina and M. sinica, all of which were included in the phylogenetic analyses. As shown in Fig. 7, the four Metaurostylopsis species form a monophyletic assemblage albeit with weak statistical support (ML/MP, 52/55). Metaurostylopsis cheni sp. n. clusters with M. salina in a strongly supported clade (ML/MP, 100/100).

Discussion
Comparison with congeners
Morphology (Table 2). The genus Metaurostylopsis is characterized by having several marginal rows, urostylic-type midventral complex, clearly differentiated frontal cirri and a marine habitat (Song et al. 2001). In addition to M. cheni, six species have been reported: M. marina (the type species), M. rubra, M. songi, M. salina, M. struederkypkeae and M. sinica (Song et al. 2001; Song & Wilbert 2002; Lei et al. 2005; Shao et al. 2008b, c).

Metaurostylopsis cheni differs from M. marina (Kahl, 1932) Song et al. 2001; in its body shape (elliptical vs. oval) and in having two (vs. one) types of cortical granules, fewer membranelles (21–26 vs. 27–30) and invariably 4 (vs. 3–6) frontoterminal cirri and 3 (vs. 3–5) right marginal rows (Song et al. 2001).

Metaurostylopsis rubra Song & Wilbert 2002 is unique and can be easily distinguished from M. cheni, and indeed all other congeners, by its distinctly larger body size, reddish cytoplasm, and higher numbers of membranelles and cirri (Song & Wilbert 2002).

Metaurostylopsis songi Lei et al. 2005 can also be readily separated from M. cheni in having only one (vs. two) type(s) of cortical granules, more membranelles (28–47 vs. 21–26) and midventral cirri pairs (9–12 vs. 5–9), fewer frontoterminal cirri (2–3 vs. invariably 4), and may have fewer (invariably 3 vs. 3–4) left marginal rows (Lei et al. 2005).

Metaurostylopsis salina Lei et al. 2005 resembles M. cheni most closely. However, the former differs from the latter in having only one (vs. two) type(s) of cortical granules, 3–5 (vs. invariably 4) frontoterminal cirri and 2–5 (vs. 5–8) transverse cirri (Lei et al. 2005; Shao et al. 2008b).
Metaurostylopsis struederkypkeae Shao et al., 2008 resembles *M. cheni* in terms of its body size and shape and most aspects of its infraciliature (Shao et al. 2008c). However, these two taxa can be separated by a combination of the following features (data for *M. struederkypkeae* given first): (i) cell colouration (rose–reddish vs. colourless), (ii) the number of transverse cirri (ca. 3 vs. 6), (iii) shape of type I (large) cortical granules (without a longitudinal groove vs. with a longitudinal groove), (iv) the distribution of the smaller, type II cortical granules (in irregular rows vs. evenly distributed), (v) the position of the contractile vacuole (anterior 2/5 vs. in equatorial region). Thus, based on its morphology, *M. cheni* is more closely related to *M. salina* than to *M. struederkypkeae*. This finding is also supported by SSU rRNA gene sequence data which reveals that *M. cheni* differs from *M. struederkypkeae* in 45 nucleo-

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**Fig. 5** Photomicrographs of *Metaurostylopsis cheni* sp. n. during interphase and morphogenesis after protargol impregnation. —A–D. Ventral (A, C, D) and dorsal (B) views, to show the frontal cirri (A), frontoterminal cirri (A), buccal cirrus (arrows in A and D), dorsal kineties (arrowheads in B), pharyngeal fibers (C, arrows), midventral cirral pairs (D), unpaired ventral cirri (arrowheads in D), and the left and right marginal rows (D). —E, F. Ventral views of an early divider, to show the oral primordium in the opisthe (E) and macronuclear nodules (F). —G, H. Ventral views of the same specimen, note the oral primordium in the proter (G) and opisthe (H). —I, J. Ventral views of a divider in middle stage, to indicate the FVT-anlagen (arrows), left (double-arrowheads) and right (arrowheads) marginal anlagen. —K–M. Ventral (K, L) and dorsal (M) views of the same divider, to show the proter’s oral primordium, FVT-anlagen (arrows), undulating membranes anlagen (arrowheads), right marginal and the dorsal kineties anlagen. DKA, dorsal kineties anlagen; FC, frontal cirri; FTC, frontoterminal cirri; FVT-anlagen, fronto-ventral-transverse cirral anlagen; LMR, left marginal rows; Ma, macronuclear nodules; MP, midventral cirral pairs; OP, opisthe’s oral primordium; POP, proter’s oral primordium; RMA, right marginal anlagen; RMR, right marginal rows. Scale bars—20 μm.
tides compared to only 18 nucleotide differences between M. cheni and M. salina. *Metaurostylopsis sinica* Shao et al., 2008 differs from our new species *M. cheni* by its yellow–brownish (vs. colourless) body and in having 2 pre-transverse cirri (vs. pre-transverse cirri absent), no (vs. 4 or 5) unpaired ventral cirri and 2 (vs. 4) frontoterminal cirri (Shao et al. 2008b).

**Morphogenesis.** Four *Metaurostylopsis* congeners, i.e. *M. marina*, *M. rubra*, *M. struederkypkeae* and *M. sinica*, have been morphogenetically investigated (Song et al. 2001; Wilbert & Song 2005; Berger 2006; Shao et al. 2008b; Li 2009, dissertation, unpublished). Compared with these, the morphogenetic events in *M. cheni* share similarities in three aspects: (i) the entire parental ciliature, including the oral apparatus, is renewed, (ii) the oral primordium of the proter originates *de novo* and probably within a pouch beneath the parental buccal cavity, that is, sub-apokinetally and while the oral primordium of the opisthe is formed epi-apokinetally and (iii) the marginal cirral rows and dorsal kineties each develop intrakinetically within the parental structures.

There are, however, some differences in morphogenesis among the species of *Metaurostylopsis*, particularly between *M. sinica* and its congeners. For example: (i) in *M. marina*, *M. rubra* and *M. cheni*, FVT-anlage n-1 forms a row of unpaired ventral cirri which migrates together with the midventral pairs, whereas in *M. sinica* an extra ventral cirrus is generated that migrates towards the transverse cirri, which migrates together with the midventral pairs, whereas in *M. sinica* an extra ventral cirrus is generated that migrates towards the transverse cirri.

**Fig. 6** Photomicrographs of *Metaurostylopsis cheni* spec. nov. during morphogenesis after protargol impregnation. —B. Ventral view of a middle stage divider, arrows show the FVT-anlagen beginning to differentiate, double-arrowheads indicate the undulating membrane anlagen splitting to give rise to the paroral and endoral membranes, and arrowheads mark the marginal rows anlagen. —A, D. Ventral views of late stage dividers, arrows indicate the FVT-streaks differentiating into cirri, arrowheads show the last FVT-streaks forming the frontoterminal cirri in both dividers. —C, H. Ventral (C) and dorsal (H) views of the same divider, double-arrowheads mark the buccal cirri provided by the FVT-streaks I in both dividers (numbered from left to right); note the dorsal kineties anlagen (H). E–G. Ventral (F, G) and dorsal (E) views of late dividers, to show the migration of the newly formed frontoterminal cirri (arrowheads) and division of the macronuclear nodules (E). DKA, dorsal kineties anlagen; FVT-anlagen, fronto-ventral-transverse cirral anlagen; Ma, macronuclear nodules; TC, transverse cirri. Scale bars—20 μm.
the frontoterminal cirri (i.e. typical Metaurostylopsis mode) whereas in *M. sinica* the FVT-anlage streak forms two frontoterminal cirri, an extra ventral cirrus and a transverse cirrus (i.e. typical urostylid mode) and (iii) in most *Metaurostylopsis* spp. the macronuclear nodules fuse into a branched structure prior to division whereas in *M. sinica* they form an unbranched sausage-like mass. The latter is possibly a primitive character indicating that *M. sinica* might be an ancestral form in the diversification of *Metaurostylopsis*. These divergencies indicate the heterogeneity of the genus and may account for the low support for the grouping of *M. sinica* with its congeners in the phylogenetic trees (Fig. 7).

Although *Metaurostylopsis struederkypkeae* and *M. cheni* share many similarities in morphogenesis as mentioned above, two subtle differences are noted: (i) the oral primordium of both proter and opisthe appear simultaneously in *M. struederkypkeae* whereas in *M. cheni* the opisthe’s OP appears before that of the proter and (ii) prior to division the macronuclear nodules of *M. struederkypkeae* fuse into a branched structure and then generate many sub-branches whereas in *M. cheni* the macronuclear nodules fuse into a less conspicuously branched structure that then fragments quickly. This latter feature, however, needs to be confirmed by observation of more continuous stages in the morphogenetic process. As these two species form a well-supported clade together with *M. salina* (Fig. 7), we assume that the timing of the appearance of the oral primordium in the proter and opisthe, and the extent of branching of the macronuclear primordium (originally from a single mass) dur-
Phylogenetic position of the genus Metaurostylopsis

(Fig. 7)

Morphogenetic (Song et al. 2001; Wilbert & Song 2005; Berger 2006; Shao et al. 2008b) and molecular analyses (Yi et al. 2008; Hu et al. 2009; Li et al. 2009) both indicate that the genus Metaurostylopsis is a clearly outlined and monophyletic assemblage, although the support values based on SSU rRNA gene sequences data are not very high.

In the molecular trees, Metaurostylopsis is always sister to the Thigmokeronopsis–Apokeronopsis clade while the latter are clearly separated from the morphologically similar genus Pseudokeronopsis. Nevertheless, based on morphological and morphogenetic features, Thigmokeronopsis and Apokeronopsis appear to be more closely related to Pseudokeronopsis than to Metaurostylopsis. For example, both Thigmokeronopsis and Apokeronopsis have a Pseudokeronopsis-like ciliature pattern including a bicorona of frontal cirri and only one marginal row on each side (vs. distinctly differentiated frontal cirri and several marginal rows on each side in Metaurostylopsis). Furthermore, during morphogenesis, the FVT-anlagen in Apokeronopsis, Thigmokeronopsis and Pseudokeronopsis develop considerably more streaks than those in Metaurostylopsis. On the other hand, Metaurostylopsis and Pseudokeronopsis share features which are lacking in Thigmokeronopsis and Apokeronopsis.

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Table 2 Comparison of seven species (eight populations) of Metaurostylopsis. Measurements in μm

<table>
<thead>
<tr>
<th>Characters</th>
<th>M. marina</th>
<th>M. rubra</th>
<th>M. songi</th>
<th>M. salina*</th>
<th>M. salina**</th>
<th>M. struederkypkeae</th>
<th>M. sinica</th>
<th>M. cheni spec. nov.</th>
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<tbody>
<tr>
<td>Cell colour***</td>
<td>colourless to grayish</td>
<td>brick reddish</td>
<td>colourless</td>
<td>somewhat russet</td>
<td>colourless to grayish</td>
<td>colourless</td>
<td>type I: yellow-green</td>
<td>type I: yellow-green</td>
</tr>
<tr>
<td>Colour of cortical granules</td>
<td>colourless</td>
<td>colourless</td>
<td>colourless</td>
<td>colourless</td>
<td>colourless</td>
<td>type II: wine reddish</td>
<td>type II: colourless equatorial level</td>
<td></td>
</tr>
<tr>
<td>Position of CV</td>
<td>above mid-body</td>
<td>anterior 2/5</td>
<td>anterior 2/5</td>
<td>equatorial level</td>
<td>anterior 2/5</td>
<td>ca. 23 (21–25)</td>
<td>ca. 23 (20–25)</td>
<td>ca. 27 (25–29)</td>
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<td>No., membranelles</td>
<td>ca. 28 (27–30)</td>
<td>ca. 40 (35–46)</td>
<td>ca. 34 (28–47)</td>
<td>ca. 20 (18–23)</td>
<td>ca. 23 (21–25)</td>
<td>ca. 23 (20–25)</td>
<td>ca. 27 (25–29)</td>
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<tr>
<td>No., FC</td>
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<td>4</td>
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<td>3</td>
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<tr>
<td>No., FTC</td>
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<td>ca. 2 (2–3)</td>
<td>ca. 3 (5–4)</td>
<td>ca. 5 (4–5)</td>
<td>ca. 5 (4–6)</td>
<td>2</td>
<td>4</td>
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<td>No., MP</td>
<td>ca. 10 (7–11)</td>
<td>ca. 11 (9–12)</td>
<td>ca. 5 (4–5)</td>
<td>6–7</td>
<td>4–7</td>
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<tr>
<td>No., VC</td>
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<td>5–7</td>
<td>5–8</td>
<td>4–8</td>
<td>4–8</td>
<td>4–5</td>
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<tr>
<td>No., PTC</td>
<td>–</td>
<td>–</td>
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<td>–</td>
<td>–</td>
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<tr>
<td>No., LMR</td>
<td>ca. 8 (6–9)</td>
<td>ca. 3 (2–4)</td>
<td>ca. 3 (2–4)</td>
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<td>ca. 3 (3–4)</td>
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<tr>
<td>No., RMR</td>
<td>ca. 6 (7–7)</td>
<td>ca. 3 (3)</td>
<td>ca. 3 (3)</td>
<td>ca. 3 (3)</td>
<td>ca. 3 (3–4)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>No., TC</td>
<td>ca. 7 (5–9)</td>
<td>ca. 6 (7–7)</td>
<td>ca. 4 (2–5)</td>
<td>ca. 4 (3–5)</td>
<td>ca. 3 (2–5)</td>
<td>ca. 3 (2–5)</td>
<td>ca. 3 (2–5)</td>
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</table>

CV, contractile vacuole; FC, frontal cirri; FTC, frontoterminal cirri; LMR, left marginal rows; MP, midventral cirral pairs; PTC, pre-transverse cirri; RMR, right marginal rows; TC, transverse cirri; VC, unpaired ventral cirri; –, absent. *Korean population. **Qingdao population. ***Colour or general appearance of living cells when observed at low magnifications.
Protozoology, OUC, for proofreading and for sample collection.

References


