Ontogeny and Phylogeny of a New Hypotrichous Ciliate (Protista, Ciliophora), *Metaurostylopsis alrasheidi* n. sp., With Establishment of a New Genus *Monourostylopsis* n. gen.

Wenya Song1,2, Yu Qiao1, Jingyi Dong1, William A. Bourland3, Tengteng Zhang1* and Xiaotian Luo2*

1 Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao, China, 2 Key Laboratory of Aquatic Biodiversity and Conservation of Chinese Academy of Sciences, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China, 3 Department of Biological Sciences, Boise State University, Boise, ID, United States

In the present study, based on both morphologic and phylogenetic analyses, a new genus, *Monourostylopsis* n. gen., and new species, *Metaurostylopsis alrasheidi* n. sp. as well as a new combination, *Metaurostylopsis antarctica* (Jung et al., 2011) n. comb. (original combination: *Metaurostylopsis antarctica* Jung et al., 2011), are suggested. The new genus is diagnosed mainly by having three or more frontoterminal cirri, a midventral complex with midventral pairs and a single midventral row, one right marginal row and two or more left marginal rows. The new genus can be easily separated from the morphologically similar genera mainly by having single right marginal row (vs. two or more right marginal rows). Based on live observation and protargol staining, the morphology and morphogenesis of a new species, *M. alrasheidi* n. sp. isolated from China, were investigated. The new species can be characterized by: two types of cortical granules; about 22 adoral membranelles; three or four frontoterminal, four or five transverse cirri; about eight midventral pairs and a midventral row of three or four unpaired midventral cirri; three or four left and right marginal rows. The main morphogenetic features of *Metaurostylopsis alrasheidi* n. sp. can be summarized as: (1) the entire parental ciliature, including the oral apparatus, is renewed; (2) the oral primordium of the proter probably originates within a pouch; (3) the oral primordium of the opisthe forms *de novo* on the cell surface; (4) the anlagen of marginal rows and dorsal kineties are formed intrakinetally, and (5) the fusion of macronuclear nodules results in an irregular branched mass prior to karyokinesis. In the phylogenetic trees, all the available *Metaurostylopsis* sequences cluster together in a clade with full support (ML/BI: 100/1.00) revealing that the genus is monophyletic within the large group of core urostyliids.

**Keywords:** *Metaurostylopsis*, *Monourostylopsis* n. gen., ontogenesis, 18S rRNA gene, systematics, taxonomy
INTRODUCTION

Hypotrichs have been widely regarded as the most complex and highly differentiated ciliate group (Foissner, 1982, 2016; Tuffrau and Fleury, 1994; Berger, 1999, 2006, 2008, 2011; Song et al., 2009; Li et al., 2018). Recent faunistic studies have revealed numerous new hypotrichous taxa, suggesting that the diversity of this group is still underestimated (Kaur et al., 2019; Luo et al., 2019; Dong et al., 2020; Lu et al., 2020; Ma et al., 2020; Wang et al., 2020a). Furthermore, the extensive studies on morphogenesis and molecular phylogeny of hypotrichs have led to a better understanding of their systematics and evolutionary relationships (Song and Shao, 2017; Lyu et al., 2018; Kim and Min, 2019; Shao et al., 2019; Wang et al., 2020b; Zhang et al., 2020).

Urostylids, with a diversity of more than 200 species, are one of the largest groups in hypotrichs, and are commonly found in marine, soil and freshwater habitats. Morphologically, they are characterized by a midventral complex composed of zigzag-patterned midventral cirral pairs (Berger, 2006; Kumar et al., 2010; Chen et al., 2011; Pan et al., 2016; Kim et al., 2017; Hu et al., 2019; Jung and Berger, 2019). They are characterized by a midventral complex composed of zigzag-patterned midventral cirral pairs (Berger, 2006; Kumar et al., 2010; Chen et al., 2011; Pan et al., 2016; Kim et al., 2017; Hu et al., 2019; Jung and Berger, 2019). The clearly differentiated frontal cirri and several morphogenetic features, Song et al. (2001) transferred *Urostyla marina* Kahl, 1932 to a newly established urostyloid genus, *Metaurostylopsis*, with *M. marina* (Kahl, 1932) Song et al., 2001 as the type species. In the following decades, more species of this genus have been identified and described (Song and Wilbert, 2002; Lei et al., 2005; Shao et al., 2008a,b; Chen et al., 2011, 2013; Jung et al., 2011; Lu et al., 2016). Nevertheless, detailed ontogenetic and molecular information has consistently questioned the assignments of some *Metaurostylopsis* species and further revisions of the genus have been made. Based on comprehensive analyses, Song et al. (2011) recognized five species in the genus *Metaurostylopsis* (*M. marina*, *M. rubra* Song and Wilbert, 2002, *M. salina* Lei et al., 2005, *M. struederkypkeae* Shao et al., 2008, and *M. cheni* Chen et al., 2011) and reassigned *M. sinica* Shao et al., 2008 into a new genus *Apouroystylopsis*. While *M. flavicans* Wang et al., 2011 and *M. songi* Lei et al., 2005 were treated as incertae sedis. In the same year, a new *Metaurostylopsis* species, *M. antarctica* Jung et al., 2011 was reported. Subsequently, Chen et al. (2013) transferred the two incertae sedis, *M. flavicans* and *M. songi*, into a new genus *Neouroystylopsis* and provided improved diagnoses for both the genus *Metaurostylopsis* and *Apouroystylopsis*. Most recently, Lu et al. (2016) added a new species, *Metaurostylopsis parastruederkypkeae* to the genus. As of this writing, seven species are included in *Metaurostylopsis*.

In the present work, morphological, ontogenetic, and molecular data for a new urostyloid species, *Metaurostylopsis alrasheidi* n. sp., are provided. In addition, morphological, morphogenetic, and phylogenetic analyses comparing *Metaurostylopsis antarctica* and other *Metaurostylopsis* species are carried out, leading to the establishment of a new genus for *Metaurostylopsis antarctica*.
The 66 18S rRNA gene sequences were aligned using the MUSCLE algorithm on the webserver GUIDANCE 21 (Sela et al., 2015). Both primers were removed using BioEdit 7.0 (Hall, 1999). The final alignment of 1,709 positions was used to construct phylogenetic trees. Maximum likelihood (ML) analysis with 1,000 bootstrap replicates was performed using RAxML-HPC2 on XSEDE 8.2.12 on the online server CIPRES Science Gateway (Stamatakis, 2014), with the GTRGAMMA model as the optimal choice. Bayesian inference (BI) analysis was carried out using MrBayes on XSEDE 3.2.6 (Ronquist et al., 2012) on CIPRES Science Gateway with the GTR + I + G model selected under Akaike Information Criterion (AIC) by jModelTest 2 (Darriba et al., 2012). Four Markov chain Monte Carlo (MCMC) chains were run for 1,000,000 generations, with sampling every 100 generations, the first 2,500 trees as burn-in. SeaView 4.6.1 (Gouy et al., 2010) and MEGA X (Kumar et al., 2018) were used to adjust the tree topologies.

RESULTS

ZooBank Registration
Present work: LSIDurn:lsid:zoobank.org:pub:0085C8A1-6EE5-4E85-A245-D30CF9E18FC2.

Monourostylopsis n. gen.: LSIDurn:lsid:zoobank.org:act:CF7BB200-33C6-4BBE-8998-2299FA70A0E7.

Metaurostylopsis alrasheidi n. sp.: LSIDurn:lsid:zoobank.org:act:077DAC39-8F8B-4BCD-849A-D07BE1C0C790.

Taxonomy and Morphological Description of the New Species
Subclass Hypotrichia Stein, 1859.
Order Urostylida Jankowski, 1979.
Family Urostylidae Bütschli, 1889.
Genus Metaurostylopsis Song et al., 2001

Metaurostylopsis alrasheidi n. sp. (Figures 2–5 and Table 1).

Diagnosis
Body 60–115 × 20–60 μm in vivo; two types of cortical granules: larger yellow-greenish ones and smaller colorless ones; about 22 adoral membranelles; three frontal, three or four frontoterminal, one buccal, four or five transverse cirri; 5–13 midventral pairs and a midventral row of three or four unpaired ventral cirri; three or four left and right marginal rows respectively; invariably three dorsal kineties; over 40 macronuclear nodules; freshwater habitat.

Type Locality
Lake Weishan (34°46′14″N, 117°12′56″E), Shandong Province, China, a freshwater lake crowded with water plants, algae and decaying plant matter.

Material Deposited
The protargol slide (accession no. SWY201906301-1) containing the holotype and several paratype slides (accession...
FIGURE 2 | Morphology of Metaurostylopsis alrasheidi n. sp. from life (A,B,E,F,H,I, bright field; G,J, differential interference contrast) and after protargol preparation (C,D,K–M). (A,B) Ventral views showing representative individuals. (C,D) Ventral (C) and dorsal (D) views, arrowhead in (C) indicates the buccal cirrus, arrowheads in (D) mark the extra dorsal bristles. (E–H) Arrangement of cortical granules on ventral (E,G) and dorsal (F,H) side, arrowheads in (H) indicate small cortical granules. (I,J) Ventral views of different individuals, arrow in (J) shows the contractile vacuole. (K) Ventral view of the holotype specimen to show the ciliature and nuclear apparatus. (L) Showing the J-shaped transverse cirri. (M) Details of the undulating membranes, arrowhead indicates the buccal cirrus. FC, frontal cirri; FTC, frontoterminal cirri; PBC, parabuccal cirrus; TC, transverse cirri. Scale bars = 40 µm.

no. SWY2019060301–2–9) are deposited in the Laboratory of Protozoology, Ocean University of China.

Dedication
We dedicate this new species to our eminent colleague, Prof. Khaled A.S. Al-Rasheid, King Saud University, Saudi Arabia, in recognition of his contributions to ciliatology.

Description
Cell size about 60–115 × 20–60 µm in vivo, with ratio of length to width about 2–3:1, body outline broad to elongated elliptical (Figures 2A,B,I,J). Cortex rather flexible, but not contractile. Two types of cortical granules on both ventral and dorsal sides (Figures 2E–H): larger ones about 1.0 µm in size, yellow-greenish, wheat-grain shaped along the cirral rows, dorsal kineties and between dorsal dikinetids, giving cells a yellow-greenish appearance; smaller ones colorless, about 0.4–0.5 µm in diameter, relatively densely arranged. Cytoplasm colorless, usually with many tiny lipid droplets (about 0.6–1.0 µm across) and food vacuoles containing algae. Contractile vacuole about 15–18 µm across, located at two-fifth of body length, behind buccal vertex, near left body margin, collecting canals not observed (Figures 2A,B,J). On average 67 (46–80) spherical to elliptical macronuclear nodules (Figure 2K). Micronuclei not easily distinguished from macronuclear nodules in stained individuals. Locomotion unremarkable, by moderately fast crawling on bottom of Petri dishes and debris.

Adoral zone extending about 38% of body length, with cilia up to 10 µm long in vivo, consisting of 19–30 (on average 22) membranelles (Table 1), distal end of adoral zone of membranelles (AZM) bending only slightly to the right of the midline in protargol preparations (Figures 2C,K). Paroral and endoral of nearly equal length, slightly curved and optically intersect at about posterior quarter of the paroral (Figures 2C,K,M). Three clearly differentiated frontal cirri (Figures 2C,K and Table 1). Single buccal cirrus located at level of anterior one-third of paroral (Figures 2C,K,M and Table 1). Three or four frontoterminal cirri behind the distal end of adoral zone (Figures 2C,K and Table 1). Midventral complex extending to about half of cell length, composed of five to thirteen pairs of cirri arranged in typical zigzag pattern and a midventral row of three or four unpaired ventral cirri posteriorly (Figures 2A–C,K and Table 1). Four or five transverse cirri in J-shaped row (Figures 2A–C,K,L and Table 1). Both right and left marginal cirri composed of three to four rows (Figures 2A–C,K and Table 1). Three complete dorsal kineties and two or three dorsal bristles to right of dorsal
FIGURE 3 | Morphogenesis of Metaurostylopsis alrasheidi n. sp. from early to middle stages after protargol impregnation. (A) Newly formed oral primordium in proter (arrowhead) and opisthe (arrow) at very early stage. (B,C) Ventral (B) and dorsal (C) views of an early divider to show the development of oral primordia for proter and opisthe (arrowheads), and the unchanged dorsal ciliation (arrowheads). (D) Ventral view of an early-middle divider, showing the streak-like frontoventral transverse cirral anlagen (arrows) and the intrakinetally formed marginal anlagen (arrowheads). (E,F) Ventral (E) and dorsal (F) views of the same early-middle divider, to show the newly formed adoral membranelles (arrows), and dorsal kinety anlagen (shaded in light-blue). (G,H) Ventral (G) and dorsal (H) views of a middle divider, showing the leftmost frontal cirrus (arrowheads) forming from the undulating membrane anlagen, dorsal kinety anlagen (shaded in light-blue) and macronuclear nodules fusing into a mass with few branches in (H). Scale bars = 50 µm.

FIGURE 4 | Morphogenesis of Metaurostylopsis alrasheidi n. sp. from middle-late to late stages after protargol impregnation, hatched lines show cirri originated from the same cirral anlage. (A,B) Ventral (A) and dorsal (B) views of the same middle-late divider, arrows indicate the leftmost frontal cirrus. (C) Ventral view of a divider, arrows show the migration of newly formed frontoterminal cirri. (D,E) Ventral (D) and dorsal (E) views of the same divider, arrows mark the frontoterminal cirri migrating toward their final positions. Scale bars = 50 µm.
kinety 3 anteriorly (Figure 2D, arrowheads), with cilia 2–3 µm long.

**Divisional Ontogeny**

**Stomatogenesis and Development of Frontal-Ventral-Transverse Cirral Anlagen**

Stomatogenesis commences with the proliferation of densely arranged basal bodies forming a slender anarchic field, that is, the opisthe’s oral primordium, which occurs de novo to the left of posterior midventral pairs (Figures 3A, 5A). Simultaneously, a small field of basal bodies, the proter’s oral primordium, appears de novo in a subsurface pouch beneath the buccal field and near the parental undulating membranes (UM). Subsequently, the opisthe’s oral primordium widens and lengthens between the midventral complex and the inner left marginal row during which time the parental structures remain unchanged. At the same time, the proter’s oral primordium continues to develop within the pouch, which is clearly outlined (Figures 3B, 5B,C). The oral primordium
then differentiates into new adoral membranelles, and the UM anlage appears to the right of the oral primordium in both proter and opisthe (Figures 3D,E,G). During this time, the parental oral structures remain unchanged, while the frontal-ventral-transverse cirral anlagen (FVTA) appear as several streak-like collections of basal bodies to the right of the UM anlage (Figures 3D,E,G). Subsequently, the UM anlagen split and give rise to the paroral endoral membranes and the leftmost frontal cirrus is generated from the anterior end of the UM anlage (=FVTA n). The UM anlagen split and give rise to the paroral and endoral membranes and the leftmost frontal cirrus is generated from the anterior end of the UM anlage (=FVTA n). In the same way, the dorsal kineties develop by intrakinetal basal body proliferation. During cell division, no caudal cirri are formed. As some key stages were not observed, the origins of the extra dorsal bristles (usually only two) are not clear (Figures 3C,F,H, 4B,E, 5H,L).

Development of Marginal Rows and Dorsal Kineties
At the early-to-middle stages, the marginal anlagen are formed intrakinetically both in proter and opisthe. Subsequently, these anlagen elongate toward both ends and generate new cirri and gradually replace the parental structures (Figures 3D,E,G, 4A,C,D, 5E,K,M,N). In the same way, the dorsal kineties develop by intrakinetal basal body proliferation. During cell division, no caudal cirri are formed. As some key stages were not observed, the origins of the extra dorsal bristles (usually only two) are not clear (Figures 3C,F,H, 4B,E, 5H,L).

Division of Nuclear Apparatus
Although macronuclear replication bands were not definitely identified in M. alrasheidi n. sp., they have been identified (but seen with some difficulty) in M. rubra and M. marina which, otherwise, have the same macronuclear morphology as M. alrasheidi n. sp. during division and replication bands were very possibly overlooked in the new species (Song et al., 2001; Song and Wilbert, 2002). During the middle stage, all macronuclear nodules fuse into a single somewhat branched complex which then divides into numerous nodules (Figures 3C,F,H, 4B,E, 5I,J,N). Division of the micronuclei was not observed.

Phylogeny Analyses Based on 18S rRNA Gene Sequence
The partial 18S rRNA gene sequence of Metaurostylopsis alrasheidi n. sp. has been submitted into GenBank database (acc. no. MT911525), with 1,643 bp long and G + C content of 44.92%.

According to sequence comparisons of the 18S rRNA gene, the sequence of Metaurostylopsis alrasheidi n. sp. differs by 8 bp from an unidentified Metaurostylopsis sequence (FJ870098) (from a taxonomic research lab), 13–14 bp from five populations of M. cheni, 16 bp from M. salina, 34–44 bp from four M. struenderkykeae sequences, and 73 bp from Monourostylopsis antarctica (Jung et al., 2011) n. comb (Figure 6).

The topologies of the ML and BI trees are mostly congruent. As a result, only the ML tree with nodal supports inferred from both algorithms is shown (Figure 7). According to our 18S rRNA gene tree, M. alrasheidi n. sp. clusters with Metaurostylopsis sp. (FJ870098), M. salina (EU220229) and five M. cheni populations (GU170204, FJ775719, HM623916, MG63611, FJ775720) with full support, forming a sister clade of four M. struenderkykeae populations (JF424832, GU942568, EU220228, JN880477). Monourostylopsis antarctica (Jung et al., 2011) n. comb. (JF906730) is sister to all Metaurostylopsis species with strong to full support (ML/BI: 97/1.00).

Geographic Distribution
Metaurostylopsis alrasheidi n. sp. was isolated from freshwater habitat. All the other Metaurostylopsis species have been reported in marine or brackish water (Song et al., 2001; Lei et al., 2005; Berger, 2006; Shao et al., 2008a,b; Chen et al., 2011; Lu et al., 2016). These studies have shown that Metaurostylopsis species can be found in a variety of habitats (Figure 8). Metaurostylopsis marina, has been reported in Mauritania, Poland, China, Korea, and United States, and is probably a cosmopolitan species. While most of the other Metaurostylopsis species have been found in one place only, this likely reflects undersampling.

**Table 1: Morphometric characterization of Metaurostylopsis alrasheidi n. sp.**

| Character                  | Min | Max | Mean | SD  | CV% | n  |
|----------------------------|-----|-----|------|-----|-----|----|
| Body, length               | 65  | 120 | 91.5 | 90  | 11.5 | 12.6 | 25 |
| Body, width                | 34  | 86  | 45.2 | 44  | 10.5 | 23.3 | 25 |
| Adoral zone, length        | 22  | 42  | 32.5 | 42  | 4.7  | 14.2 | 25 |
| Adoral membranelles, number| 19  | 30  | 22.1 | 27  | 2.5  | 11.5 | 25 |
| Frontal cirri, number      | 3   | 3   | 3.0  | 3   | 0    | 0   | 25 |
| Buccal cirrus, number      | 1   | 1   | 1.0  | 1   | 0    | 0   | 25 |
| Frontoterminal cirri, number| 3  | 4   | 3.0  | 3   | 0.2  | 6.4  | 25 |
| Midventral pairs, number   | 5   | 13  | 8.1  | 8   | 2.0  | 25.0 | 25 |
| Ventral cirri, number      | 3   | 4   | 3.5  | 3   | 0.5  | 14.4 | 25 |
| Transverse cirri, number   | 4   | 5   | 4.4  | 4   | 0.5  | 11.0 | 22 |
| Left marginal row, number  | 3   | 4   | 3.0  | 3   | 0.7  | 21.8 | 25 |
| Right marginal row, number | 3   | 4   | 3.3  | 3   | 0.4  | 13.7 | 25 |
| Cirri in left marginal row 1, number| 10 | 19 | 13.7 | 14 | 2.0 | 14.9 | 25 |
| Cirri in left marginal row 2, number| 11 | 23 | 15.0 | 14 | 3.0 | 20.2 | 25 |
| Cirri in left marginal row 3, number| 13 | 24 | 16.2 | 15 | 3.0 | 18.6 | 25 |
| Cirri in left marginal row 4, number| 14 | 24 | 18.4 | 18 | 3.5 | 19.0 | 9 | 25 |
| Cirri in right marginal row 1, number| 13 | 23 | 17.7 | 18 | 2.4 | 13.5 | 25 |
| Cirri in right marginal row 2, number| 13 | 23 | 18.4 | 18 | 2.3 | 12.5 | 25 |
| Cirri in right marginal row 3, number| 11 | 21 | 14.8 | 14 | 2.9 | 16.6 | 25 |
| Cirri in right marginal row 4, number| 8  | 15 | 12.3 | 13.0| 2.7 | 21.9 | 4  |
| Dorsal kineties, number    | 3   | 3   | 3.0  | 3   | 0    | 0   | 25 |
| Macronuclear nodules, number| 46 | 80 | 66.8 | 67 | 10.0 | 14.9 | 21 |

CV, coefficient of variation in %; M, median; Max, maximum; Mean, arithmetic mean; Min, minimum; n, number of cells measured; SD, standard deviation.
DISCUSSION

Comparison of *Metaurostylopsis alrasheidi* n. sp. With Closely Related Species (Table 2)

With respect to the ciliature, that is, frontal and transverse cirri clearly differentiated, buccal cirri present, more than two frontoterminal cirri, midventral complex with midventral pairs and a single short midventral row, more than two marginal rows on each body side; pretransverse cirri and caudal cirri absent, the new isolate corresponds well with the diagnosed characteristics of the genus *Metaurostylopsis* (Chen et al., 2013).

*Metaurostylopsis alrasheidi* n. sp. is most similar to *M. cheni*, especially in body shape, yellow-greenish cell color, two types of cortical granules, and most aspects of the ciliature, but differs from the latter by having a relatively smaller body size (<60–115 µm vs. 90–140 × 40–60 µm), a dense distribution pattern of the smaller type of cortical granules on dorsal side (vs. sparse), contractile vacuole located anteriorly (2/5 of body length vs. equatorial region), relatively more midventral pairs (5–13 vs. 5–9) and less transverse cirri (4–5 vs. 5–8) (Chen et al., 2011). The 18S rRNA gene sequence of the newly isolated organism differs from that of *M. cheni* (GU170204) by 13 nucleotides (Figure 6), which also reveals these two species are not conspecific.

*Metaurostylopsis alrasheidi* n. sp. differs from *M. marina* in body shape (broad to elongated elliptical vs. oval), two (vs. one) types of cortical granules, relatively fewer frontoterminal cirri (3–4 vs. 3–6), frontal cirri (3 vs. 4), transverse cirri (4–5 vs. 5–9), and different habitat (freshwater vs. marine) (Song et al., 2001).

*Metaurostylopsis parastruederkypkeae* is distinguished from *M. alrasheidi* n. sp. by having reddish body (vs. yellow-greenish), reddish cortical granules (vs. yellow-greenish), more frontoterminal cirri (5–7 vs. 3–4), more adoral membranelles (28–47 vs. 19–30), and more cirri in midventral row (5–9 vs. 3–4) (Lu et al., 2016).

*Metaurostylopsis rubra* can be easily distinguished from *M. alrasheidi* n. sp., by its distinctly larger body size (150–300 × 50–90 vs. 60–115 × 20–60 µm), brick-red body color (vs. yellow-greenish), one (vs. two) type of cortical granules, and higher numbers of membranelles (35–46 vs. 19–30) (Song and Wilbert, 2002).

*Metaurostylopsis salina* differs from *M. alrasheidi* n. sp. mainly in terms of having only one (vs. two) type of cortical granules and relatively more cirri in the midventral row 5–8 (vs. 3–4) (Shao et al., 2008a).
Metaurostylopsis struederkypkeae can be easily separated from M. alrasheidi n. sp. by its cell coloration (rose-reddish vs. yellow-green), the number of frontal cirri (4 vs. 3), frontoterminal cirri (4–6 vs. 3–4), and the distribution of the smaller cortical granules (in irregular rows vs. evenly distributed) (Shao et al., 2008b).

Metaurostylopsis antarctica resembles M. alrasheidi n. sp. closely in terms of body size, number of membranelles and types of cortical granules, but can be separated from the new species by having fewer midventral pairs (2–5 vs. 5–13), and fewer left and right marginal rows (2 vs. 3–4, 1 vs. 3–4 respectively) and transverse cirri (2 vs. 4–5) (Jung et al., 2011).

The character of cortical granules, that is, two types of cortical granules, of the new species is distinguishable from some of the congeners with only one type of cortical granules present (Table 2). However, it is possible that earlier researchers overlooked the tiny type of cortical granules. Considering the habitat, Metaurostylopsis alrasheidi n. sp. can be separated from all the other congeners (freshwater vs. marine or brackish, Table 2).

All the Neouroystylopsis species can be separated from M. alrasheidi n. sp. by having fewer frontoterminal cirri (2 vs. 3–4), lack of a midventral row (vs. midventral row present) (Lei et al., 2005; Wang et al., 2011; Chen et al., 2013; Pan et al., 2016; Zhang et al., 2018).

Apoaurostylopsis sinica (Shao et al., 2008) Song et al., 2011 differs from our new species in its more transverse cirri (5–8 vs. 4–5), pretransverse cirri present (vs. absent), and midventral row absent (vs. present) (Shao et al., 2008a).
Emended Diagnosis of Genus 
*Metaurostylopsis* and Reassignment of 
the Species, *M. antarctica* Jung et al., 2011

In the most recent revision of the genus *Metaurostylopsis*, Chen et al. (2013) provided an improved diagnosis for the genus *Metaurostylopsis*, which were defined as marine urostylids with characteristics mentioned in the above section. However, the new species in the present study, *Metaurostylopsis alrasheidi* n. sp., which was isolated from a freshwater lake, fits the morphological characteristics of the genus *Metaurostylopsis* very well. Moreover, our new species clusters in a clade together with all the other *Metaurostylopsis* sequences, indicating the close relationship among them and supporting the classification of the new species in the genus *Metaurostylopsis*. Thus, an emended diagnosis including marine, brackish and freshwater habitats is supplied.

As discussed above, the genus *Metaurostylopsis* is characterized by having more than one marginal row on each body side. However, *M. antarctica* was characterized by having only one right marginal row, which does not fit the diagnosis of *Metaurostylopsis*. Also, in the phylogenetic trees, *M. antarctica* is placed as sister to the clade including all the other *Metaurostylopsis* sequences with strong to full support. Therefore, we propose that *M. antarctica* should be excluded from the genus *Metaurostylopsis*. As *M. antarctica* also can be separated from other morphologically similar genera, i.e., *Neourostylopsis* and *Apourostylopsis*, by having only one right marginal row (vs. two or more right marginal rows) (Table 2), this suggests that *M. antarctica* represents a new genus. Hence, an emended diagnosis of the genus *Metaurostylopsis* and diagnosis of a new genus, *Monourostylopsis* n. gen., are supplied here.

**Metaurostylopsis Song et al., 2001**

**Improved Diagnosis**

Marine, brackish or freshwater urostylids with frontal and transverse cirri clearly differentiated; three or more frontoterminal cirri; midventral complex with midventral pairs extending to the level of the buccal vertex and a single midventral row; buccal and transverse cirri present; pretransverse ventral and caudal cirri absent; more than two marginal rows on each body side.

**Type Species**

*Urostyla marina* Kahl, 1932.

**Species Assignable**

*Metaurostylopsis marina* (Kahl, 1932) Song et al., 2001 (type species); *Metaurostylopsis rubra* Song and Wilbert, 2002; *Metaurostylopsis salina* Lei et al., 2005; *Metaurostylopsis struederkypkeae* Shao et al., 2008; *Metaurostylopsis cheni* Chen
### TABLE 2 | Morphometric comparison of Metaurostylopsis alrasheidi n. sp. with closely related species.

| Characters          | M. alrasheidi n. sp. | M. cheni | M. marina | M. parastrue dyrkykeae | M. rubra | M. salina | M. strueder kypkeae | Mo. antarctica | N. flava | N. flavicona | N. flava paraflava | N. orientalis | N. songi | A. sinica |
|---------------------|----------------------|---------|-----------|------------------------|---------|-----------|----------------------|----------------|---------|-------------|--------------------|--------------|---------|-----------|
| Body size           | 60–115 × 20–65       | 90–140 × 40–60 | 80–120 × 50–80 | 165–200 × 45–60 | 150–300 × 20–30 | 70–120 × 20–30 | 70–120 × 20–30 | 150–220 × 50–75 | 130–200 × 30–60 | 150–220 × 45–80 | 120–200 × 45–75 | 90–150 × 20–35 | 100–120 × 25–35 |
| Cell color          | Yellow-greenish      | Yellow-greenish | Colorless to grayish | Brown to reddish | Brick-reddish | Colorless to grayish | Rose-reddish | Grayish to colorless | Yellowish | Yellowish | Reddish brown | Slightly yellow-brown | Colorless | Yellow-brown |
| Cortical granules   | Type I: yellow-green; type II: colorless | Type I: yellow-green; type II: colorless | Colorless or slightly greenish | Type I: reddish | Colorless | Type I: yellow-green; type II: colorless | Type I: yellow-green; type II: colorless | Bright yellow to yellow-brownish | Bright-yellow | Bright-yellow | Yellow-brown | Colorless | Type I yellow; type II: colorless |
| AZM/BL (%)          | 38                   | 33                   | 40           | 35–40               | 33                   | 30                   | 33                   | 35                | 35       | 35          | 25–33              | 35          |
| Position of CV      | Anterior 2/5         | Equatorial level    | Above mid-body | Anterior 2/5 | Above mid-body | Anterior 2/5 | Anterior 2/5 | 33% of the body length | Anterior 2/5 | 35% of the body length | Anterior 2/5 | Anterior 2/5 | Anterior 2/5 |
| No. AZM             | ca. 22               | ca. 23               | ca. 28       | ca. 33               | ca. 39               | ca. 23               | ca. 23               | ca. 22             | 40–55    | 33–45        | 33–72              | 25–31        | ca. 34              | ca. 27             |
| No. FC              | 4                   | 3                   | 3                   | 3                   | 3                   | 4                   | 4                   | 4                 | 6–8     | 4–8          | 8–15               | 5            | 5           | 4                  |
| No. FTC             | ca. 3 (3–4)         | 3                   | ca. 4 (3–6)     | ca. 6 (5–7)        | ca. 6 (5–8)        | ca. 5 (4–5)        | ca. 5 (4–6)        | ca. 4 (2–5)       | 2       | 2            | 2                  | 2            | 2          | 2                  |
| No. MP              | ca. 8 (6–13)       | ca. 7 (5–9)         | ca. 9 (7–11)    | ca. 9 (7–13)       | ca. 10 (8–11)      | ca. 6 (7–6)        | ca. 11 (8–14)      | ca. 4 (3–5)       | 13–20   | 13–17        | 17–29              | 8–10         | ca. 11              | ca. 13              |
| No. VC              | ca. 3 (2–4)        | ca. 4 (4–5)         | ca. 7 (5–9)    | ca. 7 (5–9)        | ca. 10 (8–13)      | ca. 6 (5–8)        | ca. 5 (4–8)        | ca. 12 (8–13)     | 0       | 0            | 0                  | 0            | 0          | 0                  |
| No. LMR             | 3–4                 | 3                   | 3                   | 3                   | 3                   | 4                   | 4                   | 4                 | 6–9     | 3            | 3                  | 2            |
| No. RMR             | 3–4                 | 3                   | 3                   | 3                   | 3                   | 3                   | 1                   | 4                 | 3        | 4–6          | 3                  | 3            | 3          | 3                  |
| No. TC              | 3 (4–5)             | 3                   | 3                   | 3                   | 3                   | 2                   | 7–9                 | 7–10              | 8–13    | 5–7          | 7 (5–8)            | Ca. 7        |
| No. Ma              | ca. 67 (46–80)      | ca. 40 (30–65)      | ca. 50         | ca. 75              | ca. 100             | ca. 29 (17–36)    | ca. 61 (50–71)     | ca. 43 (38–50)    | 49–98   | 62–97        | 110–190            | Ca. 61       | 54         | Ca. 70              |
| Salinity            | 0‰                  | 30‰                 | 32‰           | 24‰                 | 33‰                 | Saline             | Saline             | 31‰              | 34.9‰   | 0‰          | 20‰                | 6‰          | 9‰         | Saline 30‰          |
| Ref. Original       | Chen et al., 2011   | Song et al., 2001   | Song and Wilbert, 2002 | Lu et al., 2016 | Song and Wilbert, 2005 | Lei et al., 2005 | Shao et al., 2008b | Jung et al., 2011 | Pan et al., 2016 | Wang et al., 2011 | Zhang et al., 2018 | Chen et al., 2013 | Lei et al., 2005 | Shao et al., 2008a |

AZM, Adoral zone of membranelles; BL, Body length; CV, Contractile vacuole; FC, Frontal cirri; FTC, Frontoterminal cirri; MP, Midventral pair; VC, Unpaired ventral cirri; LMR, Left marginal row; RMR, Right marginal row; TC, Transverse cirri; Ma, Macronuclear nodules; Ref, Reference; A, Apourostylopsis; M, Metaurostylopsis; Mo., Monourostylopsis; N, Neourostylopsis.
Metaurostylopsis parastruederkypkeae et al., 2011; Song et al., 2011; Metaurostylopsis alrasheidi n. sp.

**Monourostylopsis n. gen.**

**Diagnosis**
Urostylids with frontal and transverse cirri clearly differentiated; three or more frontoterminal cirri; midventral complex with midventral pairs extending to the level of the buccal vertex and a single midventral row; buccal and transverse cirri present; pretransverse ventral and caudal cirri absent; one right marginal row and two or more left marginal rows.

**Type Species**
*Monourostylopsis antarctica* (Jung et al., 2011) n. comb. (original combination: *Metaurostylopsis antarctica* Jung et al., 2011).

**Species Assignable**
*Metaurostylopsis antarctica* (Jung et al., 2011) n. comb. (type species).

**Etymology**
The generic name is a composite of the Greek mono- (single), referring to the single right marginal row, and the posterior part (urostylopsis) of the genus-group name *Metaurostylopsis*.

**Morphogenetic Comparison**
As of this writing, the morphogenetic process of four *Metaurostylopsis* species, i.e., *M. alrasheidi* n. sp., *M. cheni*, *M. marina*, and *M. rubra* has been investigated (Wilbert and Song, 2005; Chen et al., 2011; Song et al., 2011). The morphogenetic pattern of the genus *Metaurostylopsis* shows high stability, and shares similar morphogenetic features with *Apourostylopsis*, *Monourostylopsis*, and *Neourostylopsis* as follows: (1) in the proter, the oral primordium (OP) is formed de novo in a subcortical pouch beneath (i.e., dorsal to) the buccal field, the new undulating membranes (UM) anlage is generated near the OP, and the parental oral structures are completely replaced; (2) in the opisthe, both the OP and the UM anlage develop apokinetally on the cell surface; (3) the FVT cirral anlagen are formed apokinetally, and none of the old ciliature seems to be involved in the development of the new primordia; (4) marginal anlagen and dorsal anlagen develop intrakinetally; (5) all macronuclear nodules fuse to a single branching mass during the middle divisional stage (Wilbert and Song, 2005; Shao et al., 2008a; Chen et al., 2011; Jung et al., 2011; Song et al., 2011; Zhang et al., 2018).

Ontogenetic features of *Metaurostylopsis* show striking differences when compared with genera *Apourostylopsis* and *Neourostylopsis*, namely: (1) FVTA n-1 forms a row of unpaired ventral cirri which migrates along with the midventral pairs, whereas in *Apourostylopsis* and *Neourostylopsis*, no unpaired ventral cirri are generated; (2) FVTA n formed a transverse cirrus and more than two frontoterminal cirri in *Metaurostylopsis* (i.e., typical *Metaurostylopsis* mode), whereas the FVTA n forms two frontoterminal cirri, a pretransverse and a transverse cirrus in *Apourostylopsis* (i.e., typical urostylid mode), and whether FVTA n forms pretransverse cirrus in *Neourostylopsis* is variable (Wilbert and Song, 2005; Shao et al., 2008a; Chen et al., 2011; Jung et al., 2011; Song et al., 2011; Song and Shao, 2017; Zhang et al., 2018).

**Phylogenetic Analyses**
Consistent with previous studies (Shao et al., 2008a; Chen et al., 2011; Song et al., 2011; Lu et al., 2016), we confirm the monophyly of genus *Metaurostylopsis* based on the phylogenetic analyses in present work.

In the 18S rRNA gene tree, our new taxon, *Metaurostylopsis parastruederkypkeae* n. sp., clusters with *Metaurostylopsis* species and falls into the monophyletic clade of *Metaurostylopsis*, which can also be supported by the shared morphological features, such as, similar body shape and size, as well as semblable ciliature. Additionally, 8–44 different nucleotides of 18S rRNA gene are discovered between *M. alrasheidi* n. sp. and its congeners, further indicating the validity of our isolate as a distinct species. It is worth noting that the genus *Metaurostylopsis* forms a sister clade to *Monourostylopsis* n. gen., with which it shares some similar characteristics: (1) three or more frontoterminal cirri; (2) midventral complex with midventral pairs and a single midventral row; (3) buccal and transverse cirri present; (4) pretransverse ventral and caudal cirri absent. However, in addition to its molecular phylogenetic position, *Metaurostylopsis* can be clearly separated from *Monourostylopsis* n. gen. morphologically by having more than two marginal rows on each body side (vs. one left marginal row and two right marginal rows) (Jung et al., 2011).

**DATA AVAILABILITY STATEMENT**

The datasets generated for this study can be found in the online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI GenBank (accession: MT911525).

**AUTHOR CONTRIBUTIONS**

XL and TZ conceived the study. WS carried out the live observation, protargol staining, DNA extraction, and data analyses. All authors contributed to the manuscript and approved the final version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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