

Redescription of Two Urostylid Ciliates (Ciliophora: Urostylida), *Anteholosticha pulchra* and *Metaurostylopsis struederkypkeae* from Korea

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ABSTRACT

Two urostylid ciliates, *Anteholosticha pulchra* (Kahl, 1932) Berger, 2003 and *Metaurostylopsis struederkypkeae* Shao et al., 2008, new to Korea, were collected from the Yellow Sea and the East Sea, Korea, respectively. They were identified based on live observation and protargol impregnation. Taxonomical characters of *A. pulchra* are as follows: 190-300 × 30-55 µm size *in vivo*; contractile vacuole located on the left side of the posterior 1/4 of the cell; spherical-reddish granules at cirral bases and around dorsal bristles, somewhat sparsely distributed throughout the cell surface; four frontal and two frontoterminal cirri; four dorsal kineties; caudal cirri absent. *Metaurostylopsis struederkypkeae* is characterized as follows: 80-110 × 40-50 µm size *in vivo*; caudal cirri absent; two types of cortical granules: type 1, yellow-green arranged along the ventral cirral rows and dorsal kineties; type 2, small and reddish, with an irregular arrangement; four frontal, four to eight frontoterminal, and two to six transverse cirri; five to seven left and three to five right marginal rows. Sequences of small subunit ribosomal DNA were determined from both species, and pairwise distances with their relatives were analyzed.

Keywords: *Anteholosticha pulchra*, *Metaurostylopsis struederkypkeae*, urostylid ciliate, marine, Korea

INTRODUCTION

Genera *Anteholosticha* Berger, 2003 and *Metaurostylopsis* Song et al., 2001 belong to the family Urostylidae Bütschli, 1889. This family is characterized by having one or more marginal rows of left and right marginal cirri and the midventral cirri which arranged as a “zig-zag” file (Borror, 1979; Borror and Wicklow, 1983; Lynn, 2008).

Diverse and polyphyletic species in the *Holosticha* complex (Gao et al., 2010) were split up into four genera, *Holosticha*, *Anteholosticha*, *Caudiholosticha* and *Biholosticha* by Berger (2003). Recently Li et al. (2009) added another genus *Nothoholosticha* to this complex, and therefore there are five genera in the *Holosticha* complex at present. The genus *Anteholosticha* can be distinguished from other genera in *Holosticha* complex by its lack of caudal cirri and several other apomorphic characters, e.g., anterior end of left marginal row curved rightwards, proximalmost membranelles widened (Berger, 2003). Recently a Chinese population of *A. pulchra*

was reported by Li et al. (2007).

The genus *Metaurostylopsis* was established by Song et al. (2001). The characteristics of this genus are as follows: clearly differentiated frontal, buccal, transverse cirri and frontoterminal cirral row present; caudal cirri absent; more than one left and right marginal row (Song et al., 2001). *Metaurostylopsis struederkypkeae* was newly established by Shao et al. (2008).

Here, we redescribe two urostylid ciliates, *A. pulchra* and *M. struederkypkeae*, collected from Korea. For precise identification and description, we conducted live observation, protargol impregnation and small subunit ribosomal DNA (SSU rDNA) sequence analysis.

MATERIALS AND METHODS

Anteholosticha pulchra was isolated from Incheon Harbor (salinity, 30.6‰; temperature, -0.6°C; 37° 26'N, 126° 35'E),

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Incheon, Korea at January of 2011. *Metaurostyloopsis struederkypkeae* was collected from the lagoon of Youngsang (salinity, 14.8‰; temperature, 18.3°C; 38° 13'N, 128° 35'E), Sokcho, Korea at June of 2011. These two populations were maintained in petri dishes with rice grains to enrich bacteria growth as a food resource. The specimens were observed *in vivo* under a light microscope (Leica DM2500, Wetzlar, Germany) at a magnification of 50× to 1,000×. Protargol impregnation was performed to reveal the infraciliatures (Foissner, 1991). Drawing was performed under a light microscope at a magnification of 1,000×. Terminology and classification were mainly followed according to Lynn (2008).

DNA extraction, amplification and sequencing of nearly complete SSU rDNA were performed according to Jung et al. (2011). Our sequences and retrieved sequences from GenBank were aligned using BioEdit (Hall, 1999), and pairwise genetic distance based on Kimura two-parameter inference was obtained using MEGA 4.0 (Kumar et al., 2004).

RESULTS

Phylum Ciliophora Doflein, 1901
Class Spirotrichea Bütschli, 1889
Order Urostyleida Jankowski, 1979
Family Urostyleidae Bütschli, 1889
Genus *Anteolosticha* Berger, 2003

¹*Anteolosticha pulchra* (Kahl, 1932) Berger, 2003 (Table 1, Figs. 1A-E, 2)

Keronopsis pulchra Kahl, 1932: 573, fig. 104; Kahl, 1933: 109, fig. 16.

Holosticha (*Keronopsis*) *pulchra*: Berger, 2001: 36.

Anteolosticha pulchra: Berger, 2003: 377; Berger, 2006: 435, fig. 90; Li et al., 2007: 113, figs. 4-7.

Description. Live cell size usually 190-300 × 30-55 μm, slender in shape (Figs. 1A, 2A, B), flexible but not contractile, anterior and posterior ends rounded, contractile vacuole approxi-

Table 1. Comparison of morphometric data from *Anteolosticha pulchra* with Li et al. (2007)

	P	Min	Max	Mean	SD	CV	n
Length of body (μm)	Ko	150	220	184.9	16.9	9.1	21
	Ch	172	352	250.0	45.1	18.0	25
Width of body (μm)	Ko	37.5	57.5	48.7	5.6	11.6	21
	Ch	48	100	74.8	12.5	16.7	25
Length of buccal field (μm)	Ko	45	57.5	51.1	3.8	7.4	21
	Ch	46	100	70.1	10.0	14.3	25
No. of membranelles	Ko	46	60	51	3.6	7.1	21
	Ch	38	57	47.7	4.2	8.8	25
No. of buccal cirri	Ko	1	1	1	0	0	21
	Ch	1	1	1	0	0	25
No. of frontal cirri	Ko	4	4	4	0	0	21
	Ch	4	4	4	0	0	25
No. of transverse cirri	Ko	7	9	7.8	0.7	9.6	21
	Ch	5	8	6.4	0.7	10.9	25
No. of frontoterminal cirri	Ko	2	2	2	0	0	21
	Ch	2	2	2	0	0	25
No. of pairs of midventral cirri	Ko	27	34	32	2.1	6.5	21
	Ch	21	38	28.4	4.9	17.3	25
No. of left marginal row	Ko	51	68	56.1	4.1	7.3	21
	Ch	40	70	52.8	8.2	15.5	25
No. of right marginal row	Ko	54	73	61.6	4.3	7.0	21
	Ch	49	75	60.3	7.9	13.1	25
No. of macronuclear nodules	Ko	38	71	49.1	7.4	15.1	21
	Ch	31	47	40.9	4.8	11.7	8
No. of dorsal kineties	Ko	4	4	4	0	0	21
	Ch	4	4	4	0	0	25

All data compared protargol-impregnated specimens of Korean population (Ko) and Chinese population (Ch).

Frontal cirri including parabuccal cirrus (III/2).

P, population; Min, minimum; Max, maximum; Mean, arithmetic mean; CV, coefficient of variation in %; n, number of individuals examined.

Korean name: ¹*홍색엿전열하모충 (신칭)

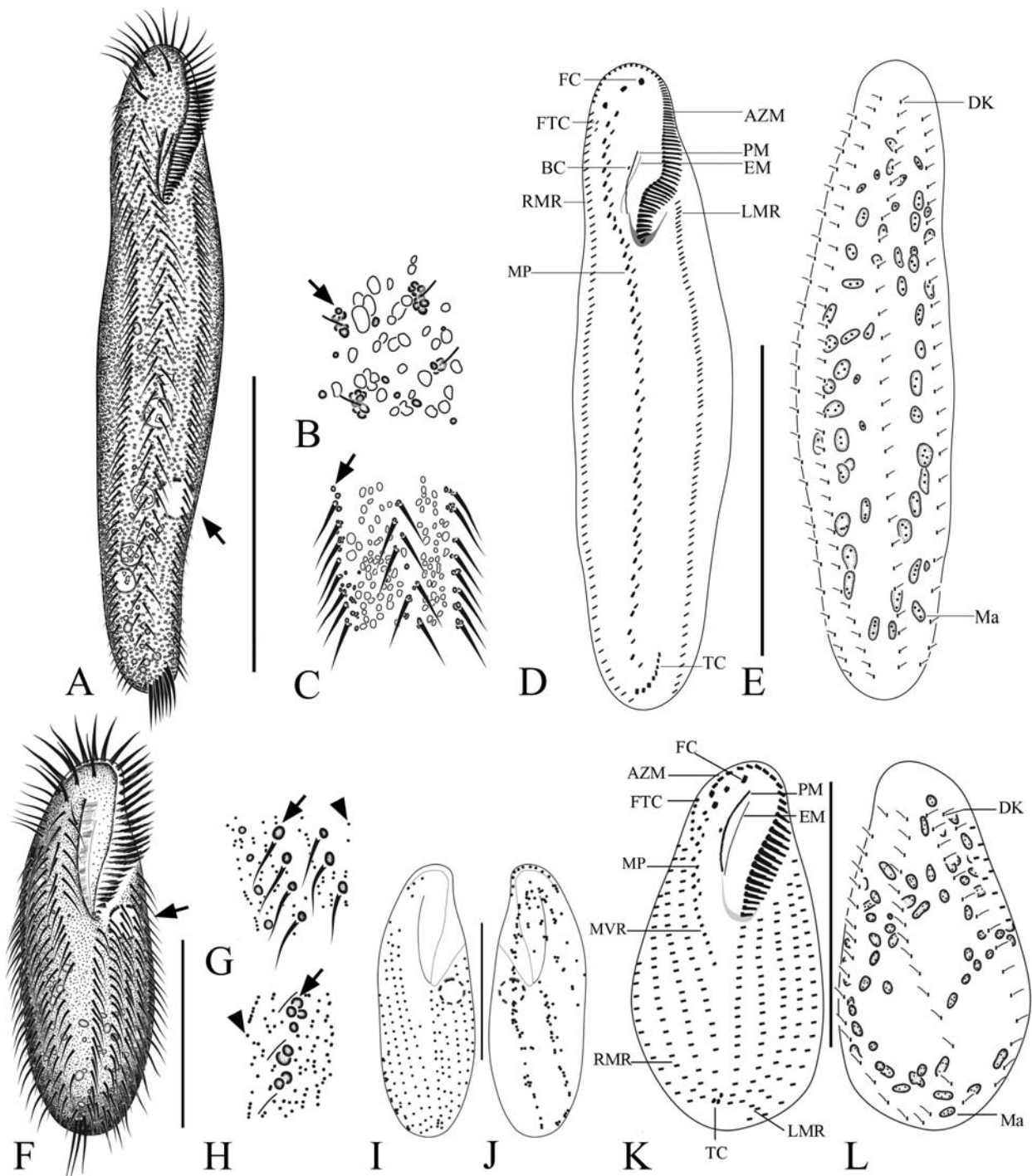


Fig. 1. Morphology of *Anteholosticha pulchra* and *Metaurostylopsis struederkypkeae* from live (A-C, F-J) and protargol impregnated (D, E, K, L) specimens. A-E, *Anteholosticha pulchra*: A, Ventral view of live, contractile vacuole (arrow); B, C, Pattern of cortical granules in dorsal and ventral views, reddish cortical granules (arrow); D, E, Cirral base in ventral and dorsal views from protargol impregnation; F-L, *Metaurostylopsis struederkypkeae*: F, Ventral view of live, contractile vacuole (arrow); G, H, Ventral and dorsal views, yellow-green cortical granules (arrow), small reddish cortical granules (arrowhead); I, J, Ventral and dorsal views which show the arrangement of yellow-green cortical granules; K, L, Cirral base on ventral and dorsal views with protargol impregnation. AZM, adoral zone of membranelles; BC, buccal cirrus; DK, dorsal kineties; EM, endoral membrane; FC, frontal cirri; FTC, frontoterminal cirri; LMR, left marginal row; Ma, macronuclear nodules; MP, midventral pairs; MVR, midventral row; PM, paroral membrane; RMR, right marginal row; TC, transverse cirri. Scale bars: A-E=100 μ m, F-L=50 μ m.

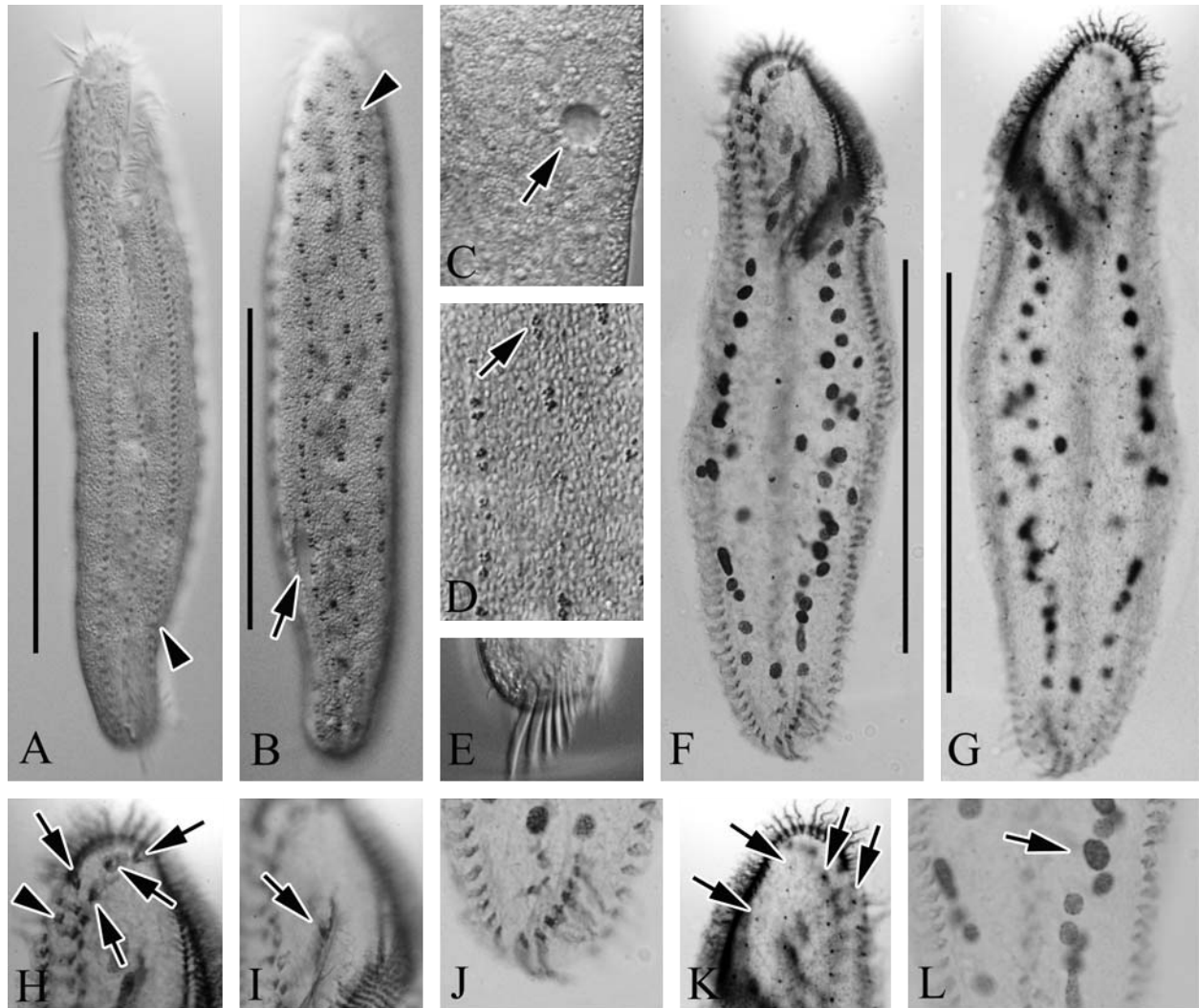


Fig. 2. Micrographs of *Anteholosticha pulchra* from live (A-E) and protargol impregnated (F-L) specimens. A, B, Live ventral (A) and dorsal (B) view, arrangement of cortical granules (arrowhead); C, Ventral view, contractile vacuole (arrow); D, Dorsal view, reddish cortical granules (arrow); E, Live ventral view of transverse cirri; F, G, Dorsal and ventral views of protargol-impregnated specimen; H, Ventral view, the frontal (arrows) and frontoterminal (arrowhead) cirri; I, Buccal cirri (arrow); J, Ventral view, the transverse cirri; K, Dorsal view, the dorsal kineties, typically four; L, Macronuclear nodules (arrow). Scale bars: A, B, F, G=100 μ m.

mately 10 μ m in diameter, positioned on left side of posterior 1/4 of cell (Figs. 1A, 2B, C, arrow). Spherical-reddish cortical granules at cirral bases and around dorsal bristles, ca. 1 μ m across, some sparsely distributed throughout cell surface (Figs. 1B, C, 2D, arrow). Four frontal cirri each located in anterior ventral area, ca. 18 μ m (Fig. 2H, arrows), and two frontoterminal located near anterior end of right marginal row (Fig. 2H, arrowhead) and seven to nine transverse cirri (Fig. 2E, J), respectively ca. 10 and 20 μ m in length, with one left and right marginal row located on left and right side (left marginal cirri ca. 56, right marginal cirri ca. 61). Buccal cirrus positioned near paroral membrane (Fig. 2I, arrow), cau-

dal cirri absent. Four dorsal kineties extended from anterior to posterior area (Fig. 2G, K, arrows) and approximately 49 macronuclear nodules scattered in body (Figs. 1E, 2L).

46-60 membranelles in adoral zone, ca. 25-35% of body length, and thick buccal lip. Long midventral pairs extending to transverse cirri, in a "zig-zag" pattern, 27-34 pairs of cirri. Food vacuoles colorless and sparsely located on cell.

Distribution. Germany (Kiel, the Baltic Sea), China (Laizhou, the Yellow Sea), and Korea (this study).

Remarks. Polyphyly of genus *Anteholosticha* was shown by molecular phylogenetic analysis based on the SSU rDNA. Therefore this genus should be divided into subgroups acc-

Table 2. Comparison of morphometric data from *Metaurostyloopsis struederkypkeae* with Shao et al. (2008)

	P	Min	Max	Mean	SD	CV	n
Length of body (μm)	Ko	65	98	82.7	11.17	13.5	16
	Ch	45	75	64.3	7.81	12.1	20
Width of body (μm)	Ko	25	56	35.2	7.41	21.0	16
	Ch	18	36	25.8	5.66	21.9	20
Length of buccal field (μm)	Ko	25	43	33.7	4.85	14.4	16
	Ch	16	40	25.1	5.08	20.3	20
No. of membranelles	Ko	25	36	30.8	3.27	10.6	16
	Ch	20	25	22.7	1.45	6.4	19
No. of frontal cirri	Ko	4	4	4.0	0	0	16
	Ch	4	4	4.0	0	0	19
No. of frontoterminal cirri	Ko	4	8	5.8	0.91	15.7	16
	Ch	4	6	4.6	0.69	15.1	19
No. of buccal cirri	Ko	1	1	1.0	0	0	16
	Ch	1	1	1.0	0	0	20
No. of cirri in midventral pairs	Ko	12	20	15.6	2.66	17.0	16
	Ch	8	14	10.6	1.66	15.7	18
No. of cirri in midventral row	Ko	6	9	7.3	0.95	12.9	16
	Ch	4	8	5.4	1.25	22.9	18
No. of transverse cirri	Ko	2	6	3.7	1.14	30.9	16
	Ch	2	5	3.2	0.75	23.3	17
No. of left marginal rows	Ko	5	7	5.9	0.50	8.5	16
	Ch	4	5	4.1	0.32	7.7	19
No. of cirri in left marginal row 1	Ko	12	20	16.0	1.79	11.2	16
	Ch	10	16	11.7	1.88	16.0	19
No. of cirri in left marginal row 2	Ko	12	20	16.8	1.94	11.5	16
	Ch	11	17	13.8	1.80	13.0	19
No. of cirri in left marginal row 3	Ko	15	26	19.1	2.83	14.8	16
	Ch	12	19	15.6	2.22	14.2	19
No. of cirri in left marginal row 4	Ko	16	24	20.1	2.02	10.0	16
	Ch	13	22	16.3	2.29	14.0	19
No. of cirri in left marginal row 5	Ko	16	25	20.6	2.85	13.9	16
	Ch	11	16	—	—	—	2
No. of cirri in left marginal row 6	Ko	16	25	19.7	2.56	13.0	13
	Ch	—	—	—	—	—	—
No. of cirri in left marginal row 7	Ko	23	23	23	—	—	1
	Ch	—	—	—	—	—	—
No. of right marginal rows	Ko	3	5	4.1	0.50	12.1	16
	Ch	3	3	3.0	0	0	20
No. of cirri in right marginal row 1	Ko	16	29	23.5	3.76	16.0	16
	Ch	12	22	17.5	2.74	15.7	19
No. of cirri in right marginal row 2	Ko	21	34	26.4	3.16	12.0	16
	Ch	14	27	21.9	4.00	18.3	19
No. of cirri in right marginal row 3	Ko	20	34	26.6	4.18	15.7	16
	Ch	11	20	16.5	2.59	15.7	19
No. of cirri in right marginal row 4	Ko	16	35	23.5	5.00	21.2	15
	Ch	—	—	—	—	—	—
No. of cirri in right marginal row 5	Ko	22	32	26.0	5.29	20.4	3
	Ch	—	—	—	—	—	—
No. of dorsal kineties	Ko	3	3	3.0	0	0	16
	Ch	3	3	3.0	0	0	17
No. of macronuclear nodules	Ko	62	111	81.9	18.15	22.2	9
	Ch	50	71	61.4	6.71	10.9	16

All data compared protargol-impregnated specimens of Korean population (Ko) and Chinese population (Ch).

P, population; Min, minimum; Max, maximum; Mean, arithmetic mean; CV, coefficient of variation in %; n, number of individuals examined.

ording to the relativeness of both morphology and phylogeny (Berger, 2006; Gao et al., 2010; Yi and Song, 2011). Among ca. 40 species known from this genus, following five species showed closest relationship with *Anteholosticha pulchra* in morphology: *A. gracilis*, *A. xanthichroma*, *A. australis*, *A. sigmoidea* and *A. monilata*.

Anteholosticha gracilis is most similar to *A. pulchra* because both species have four frontal and two frontoterminal cirri as marine ciliates. However *Anteholosticha pulchra* differs from *A. gracilis* mainly in size (190-300 × 30-55 μm vs. 100-150 × 30-40 μm *in vivo*), position of contractile vacuole (posterior 1/4 vs. anterior 1/3), color of cortical granules (reddish vs. yellow-greenish), number of dorsal kineties (four vs. three), adoral membranelles (46-60 vs. 24-30) and pair of midventral cirri (27-34 vs. 17-24) (Hu and Suzuki, 2004). *Anteholosticha pulchra* can be distinguished from other species by the position of contractile vacuoles: it locates around 1/4 region from the posterior margin in *A. pulchra*; around 1/3 from the anterior margin in *A. gracilis*; ahead of mid-body in *A. xanthichroma*, *A. australis*, *A. sigmoidea* and *A. monilata* (Foissner and Didier, 1981; Foissner, 1982; Wirnsberger and Foissner, 1987; Blatterer and Foissner, 1988; Hu and Suzuki, 2004).

Nothoholosticha fasciola transferred from the genus *Anteholosticha* by Li et al. (2009) because this species has no frontoterminal cirri. However two species are similar in following characteristics, e.g., one buccal cirrus and long belt-like body shape. However, *A. pulchra* differs from *N. fasciola* in having frontoterminal cirri (vs. absent), four frontal cirri (vs. six), four dorsal kineties (vs. three), reddish cortical granules (vs. bright orange) and the contractile vacuole position (located around 1/5 region from the posterior margin in *N. fasciola*) (Li et al., 2009).

The Korean population corresponds well with Chinese population reported by Li et al. (2007), and showed high similarity each other in morphometric characteristics (Table 1).

The SSU rDNA sequence of Korean *A. pulchra* is 1,650 bp in length (GenBank accession no: JN880476). Inter-specific genetic variation among five species (*A. pulchra*, *A. gracilis*, *A. monilata*, *A. pseudomonilata*, and *N. fasciola*) are ranged from 3.3% to 6.6% (Table 3).

¹*Genus *Metaurostyloopsis* Song, Petz and Warren, 2001

²**Metaurostyloopsis struederkypkeae* Shao et al., 2008
(Table 2, Figs. 1F-L, 3)

Metaurostyloopsis struederkypkeae Shao et al., 2008: 289, figs. 1-20.

Description. Live cell size usually 80-110 × 40-50 μm (Figs. 1F, 3A-C), slender in shape and slightly contractile, with anterior and posterior ends rounded, and contractile vacuole approximately 10 μm in diameter located on left side of equatorial level of cell (Fig. 3C, arrow). Two types of cortical granules: one small and reddish, approximately 0.5 μm across, spread irregularly on surface (Figs. 1G, H, 3D, arrowhead), and other one yellow-green, approximately 1.5 μm across, situated at base of cirri, with some cortical granules clustered along cirral rows and dorsal kineties (Figs. 1G, H, 3D, arrow). Color of cells appeared reddish under low magnification due to numerous small cortical granules. Lengths of transverse and frontal cirri 15 μm and 13 μm, respectively. Other somatic cirri, midventral, left and right marginal cirri, ca. 10 μm in length. Four frontal cirri and four to eight frontoterminal cirri located near distal portion of adoral membranelles (Fig. 3H, arrow). Two to six transverse cirri, and one buccal cirrus located in middle of next paroral membrane (Fig. 3H, arrowhead). Left and right marginal rows composed of five to seven and three to five, respectively (Fig. 3F). 12-20 midventral pairs, and six to nine unpaired midventral cirri between midventral pairs and transverse cirri, three dorsal kineties (Fig. 3G, arrows), and separately two dikinetids (Fig. 3G, arrowheads) on anterior dorsal side. Macronuclear nodules, ovoid and ellipsoid shape, ca. 82. Membranelles in adoral zone consisted of 25-36 membranelles, and portion of adoral zone is ca. 35-45% in length. Paroral and endoral membrane are equal level in length.

Distribution. China (Qingdao, the Yellow Sea) and Korea (this study).

Remarks. There are six species in this genus: *Metaurostyloopsis marina* Song et al., 2001, *M. rubra* Song and Wilbert, 2002, *M. songi* Lei et al., 2005, *M. salina* Lei et al., 2005, *M. struederkypkeae* Shao et al., 2008, and *M. cheni* Chen et al., 2011.

Metaurostyloopsis struederkypkeae and *M. rubra* have reddish cell color. However *M. struederkypkeae* is distinguished from *M. rubra* by the number of right marginal rows (3-5 vs. 6-7) and the type of cortical granules (two vs. one) and size (80-110 × 40-50 μm vs. 150-300 × 50-90 μm *in vivo*) (Song and Wilbert, 2002). *Metaurostyloopsis struederkypkeae* is distinguished from *M. marina* by the body shape (slender vs. oval), the cell color (reddish vs. colorless) and the type of cortical granules (two vs. one) (Song et al., 2001).

The Korean population of *M. struederkypkeae* corresponds with the original description by Shao et al. (2008) and morphometric comparisons highly overlap (Table 2); however, there are a few differences. The Korean population has more

Korean name: ¹*사미주하모충 (신칭), ²*작은홍색사미주하모충 (신칭)

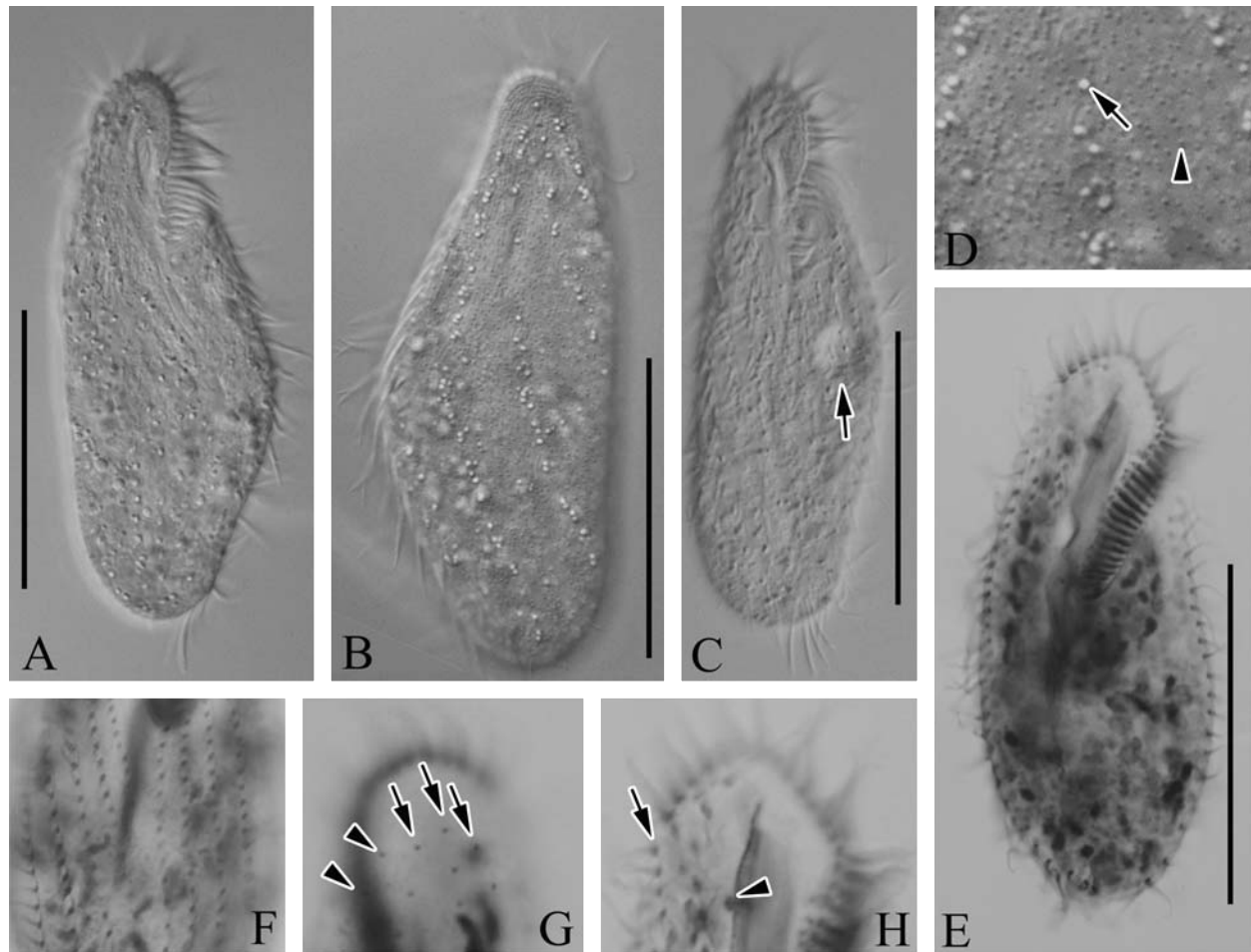


Fig. 3. Micrographs of *Metaurostyloopsis struederkypkeae* from live (A-D) and protargol impregnated (E-H) specimen. A, B, Live ventral and dorsal views; C, Contractile vacuole (arrow); D, Yellow-green cortical granules (arrow), small reddish cortical granules (arrow-head); E, Ventral view of protargol-impregnated specimen; F, Ventral view, left and right marginal rows; G, Dorsal view, typically three dorsal kineties (arrows) and two dikinetids (arrowheads); H, Frontoterminal (arrow) and buccal (arrowhead) cirri. Scale bars: A-C, E=50 μ m.

Table 3. Kimura two-parameter pairwise distances (%) among the five species in *Anteholosticha*-*Nothoholosticha* assemblage based on small subunit ribosomal DNA sequences

	<i>A. pulchra</i>	<i>A. gracilis</i>	<i>A. monilata</i>	<i>A. pseudomonilata</i>	<i>N. fasciola</i>
<i>A. pulchra</i> (this study)	—				
<i>A. gracilis</i> (FJ775713)	6.58	—			
<i>A. monilata</i> (GU942567)	5.04	4.10	—		
<i>A. pseudomonilata</i> (HM568416)	3.96	5.19	3.29	—	
<i>N. fasciola</i> (FJ377548)	3.62	6.72	5.18	3.89	—

number of left and right marginal rows, midventral pairs, and macronuclear nodules than those of the Chinese population (Shao et al., 2008).

The SSU rDNA sequence of *M. struederkypkeae* is 1,652 bp in length (GenBank accession no: JN880477). The inter-

specific pairwise distances between *M. struederkypkeae* and its relatives (*M. cheni* and *M. salina*) are more than 2%, while intra-specific variation are less than 0.3% among two Korean and one Chinese *M. struederkypkeae* populations (Table 4).

Table 4. Kimura two-parameter pairwise distance (%) based on small subunit ribosomal DNA sequences

	<i>Metaurostyloopsis struederkypkeae</i> ^a	<i>M. struederkypkeae</i> ^b	<i>M. struederkypkeae</i> ^c	<i>M. cheni</i>	<i>M. salina</i>
<i>M. struederkypkeae</i> ^a (this study)	–				
<i>M. struederkypkeae</i> ^b (GU942568)	0.18	–			
<i>M. struederkypkeae</i> ^c (EU220228)	0.12	0.30	–		
<i>M. cheni</i> (HM623916)	2.22	2.28	2.34	–	
<i>M. salina</i> (EU220229)	2.22	2.28	2.34	0.61	–

^aKorean population from Youngrang lagoon (this study), ^bKorean population from Taehwagang river (Li et al., 2011), ^cChinese population (Shao et al., 2008).

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