

First Record of Three *Uronychia* Species (Ciliophora: Spirotrichea: Euplotida) from Korea

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ABSTRACT

Three morphospecies of the genus *Uronychia*, i.e. *U. setigera* Calkins, 1902, *U. binucleata* Young, 1922, and *U. multicirrus* Song, 1997, were collected from the coastal waters of Gumjin-ri on the East Sea and the public waterfront of Incheon on the Yellow Sea in Korea, respectively. These species are described based on live observation, protargol impregnation, silver nitrate impregnation, and their morphometrics. Diagnostic keys for these species are also provided. In addition, their small subunit ribosomal DNA sequences were compared with previously known sequences of *Uronychia* species. Diagnostics of three *Uronychia* species are as follows: *U. setigera*: 50-80 μm long *in vivo*, oval-shaped, 2 macronuclear nodules (Ma), 1 spur on the left margin, 11 adoral membranelles (AM) 1, 4 AM2, 1 buccal cirrus (BC), 4 frontal cirri (FC), 3 left marginal cirri (LMC), 2 ventral cirri (VC), 5 transverse cirri (TC), 3 caudal cirri (CC), 6 dorsal kineties (DK), and approximately 23 cilia in the leftmost kinety. *U. binucleata*: 70-110 μm long *in vivo*, oval to slightly rectangular shaped, 2 Ma, 1 micronucleus (Mi), 2 spurs on the posterior region, 11 AM1, 4 AM2, 1 BC, 4 FC, 3 LMC, 2 VC, 5 TC, 3 CC, 6 DK, and approximately 37 cilia in the leftmost kinety. *U. multicirrus*: 140-200 μm long *in vivo*, oval to slightly rectangular shaped, ca. 7 Ma, 1 Mi, 11 AM1, 4 AM2, 1 BC, 4 FC, 3 LMC, approximately 8 VC, 5 TC, 3 CC, and 6 DK. This study presents the first record of this genus in Korea.

Keywords: *Uronychia setigera*, *U. binucleata*, *U. multicirrus*, SSU rDNA, Korea

INTRODUCTION

Members of the genus *Uronychia* Stein, 1859, are euplotid ciliates and are known as being among the most cosmopolitan ciliates in marine ecosystems (Kahl, 1932; Foissner, 1984; Song, 1997; Song and Wilbert, 1997). *Uronychia* can be distinguished from other euplotid ciliates by the horseshoe-shaped hypertrophied paroral ciliature around its oral cavity, and well-developed caudal cirri, left marginal cirri, and transverse cirri (Song, 1997; Lynn, 2008; Song et al., 2009). This name originated from the ponytail-shaped cirri on the posterior region. Monophyly of *Uronychia* and its primitive position within euplotids were confirmed by phylogenetic studies based on comparison of small subunit ribosomal DNA (SSU rDNA) sequences (Song et al., 2004; Jiang et al., 2010).

The number of morphospecies in *Uronychia* has been changed several times. Eight species have traditionally been recognized based on the shape of the nucleus and body, as well as cell size (Müller, 1786; Claparède and Lachmann,

1858; Wallengren, 1900; Pierantoni, 1909; Buddenbrock, 1920; Young, 1922; Taylor, 1928; Kahl, 1932; Bullington, 1940; Fenchel, 1965). However, this number changed to 5 based on redefinition by Curds and Wu (1983) and Song (1997), and, finally, four valid morphospecies were recently confirmed (Song, 1999): *U. transfuga* (type species), *U. setigera*, *U. binucleata*, and *U. multicirrus*.

In this study, we describe for the first time three *Uronychia* species in Korea. In addition, the SSU rDNA sequences of these species were determined and compared with previously known sequences of *Uronychia* species.

MATERIALS AND METHODS

Morphological taxonomy processes

Samples were collected from coastal areas in Korea using a polyurethane foam enveloped slide system (Xu et al., 2009) and then transferred to a laboratory. In successive steps,

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cultures were maintained at 17°C in a chamber that was adjusted with a light and dark photoperiod of 12:12 hr. Rice grains were used as a food source for enrichment of bacterial growth in cultures. Living specimens were observed using phase contrast and a differential interference microscope at different magnifications. Protargol and silver nitrate impregnation (Foissner, 1991) were applied in order to reveal the cirri pattern and infraciliature. Enumeration and measurement of stained specimens were performed under $\times 1,000$ magnification (Leica DM2500; Leica Microsystems, Germany) and specimens were drawn with a camera lucida. The classification scheme used here was based on that of Lynn (2008) and terminologies follow those of Corliss (1979) and Lynn (2008). Abbreviations are as follows: AM, adoral membranelles; AZM, adoral zone of membranelles; BC, buccal cirrus; CC, caudal cirri; CV, contractile vacuole; DK, dorsol kineties; FC, frontal cirri; FV, food vacuole; LMC, left marginal cirri; Ma, macronuclear nodule; Mi, micronucleus; PM, paroral membrane; TC, transverse cirri; and VC, ventral cirri.

Molecular taxonomy processes

Isolation of each living individual ciliate was performed under a dissecting microscope using a micropipette (Leica MZ 12.5; Leica Microsystems). DNA extraction, amplification of SSU rDNA, and sequencing were performed according to Kim and Min (2009). Sequenced strands were aligned using ClustalX (Thompson et al., 1997) and BioEdit 7.0.9.0 (Hall, 1999). Nucleotide diversity was calculated by MEGA 4.0 (Tamura et al., 2007) using the *p*-distance value.

RESULTS AND DISCUSSION

Phylum Ciliophora Doflein, 1901
 Class Spirotrichea Bütschli, 1889
 Subclass Hypotrichia Stein, 1859
 Order Euplotida Small and Lynn, 1985
 Family Uronychiidae Jankowski, 1979
¹*Genus *Uronychia* Stein, 1859

²**Uronychia setigera* Calkins, 1902 (Table 1, Fig. 1)

Uronychia uncinata sensu Kahl, 1932: 627, fig. S. 622, 28.
Uronychia setigera Song and Wilbert, 1997: 424, fig. 7;
 Song et al., 2004: 315, figs. 1-3.

Material examined. Specimens collected from the coastal water of Gumjin-ri (36°22'N, 129°23'E), Gyeongsangbuk-do in the East Sea of Korea on 6 Oct 2006. The raw culture

maintained for up to ten days.

Diagnosis. Size about 50-80 μm long *in vivo*, oval-shaped, 2 Ma, 1 spur on left margin, 11 AM1, 4 AM2, 1 BC, 4 FC, 3 LMC, 2 VC, 5 TC, 3 CC, 6 DK, approximately 23 cilia in the leftmost kinety.

Description. The smallest *Uronychia*, size 50-80 \times 35-55 μm *in vivo*; oval-shaped, slightly convex right margin region. Dorso-ventrally flattened, ratio of about 2:3; buccal area deeply caved oral area in the ventral region, extended about 70% of body length (Fig. 1A, B, D-F). Two distinctly apical spurs, each on left and right of anterior portion; one lateral spur on left margin, very conspicuous (Fig. 1B, E, F). On posterior portion, with 2 depressions on ventral side, each TC and LMC emerged; 1 depression on dorsal side, CC appeared (Fig. 1A-D). Cytoplasm colorless to grayish colored, with numerous granules.

Two Ma connected by funiculus, located on the left side of body; each nodule ellipsoid-shaped, with spherical nucleoli; Mi not recognized (Fig. 1C, D). CV recognized near the right of TC; several FV recognized after protargol impregnation. Movement typically rapid jumping aside or backwards, swimming with very fast rotation against the longitudinal axis.

AZM pattern stable in this genus; 11 AM1 on apical area of anterior region, extended dorsal surface, each membranelle consists of 3 kineties; 4 AM2 on the left of middle region, each membranelle consists of roughly 6-8 kineties; 1 BC, small cirrus-like membranelle, apart from AZM2, inconspicuous, about 2-3 μm long (Fig. 1A-C, G-I). Buccal field surrounded by a horseshoe-shaped robust PM (Fig. 1B, E, F).

FC 4 in number, narrow, positioned at the most anterior area on ventral surface; 2 small VC, on right margin; 5 TC, rightmost cirrus rather small; 3 enlarged LMC, positioned on left of ventral region; 3 enormous CC emerged on dorsal surface (Fig. 1A-D, G, H). Six DK composed of dikinetids on dorsal surface; 2 DK dorsolaterally located on left margin of ventral surface, densely packed near posterior region, contained in an average of 23 cilia in the leftmost kinety; other 3 DK positioned on dorsal surface, extended to CC (Fig. 1B-D).

Three SSU rDNA sequences of Korean population were deposited in Genbank under the accession number HQ380021-HQ380023. They are 1,625 bp in length and show 99.45-99.69% similarity between Korean population and Chinese population (Genbank accession number: EF198669) of *U. setigera*.

Remarks. *Uronychia setigera* can be clearly distinguished from other congeners by new diagnostic characteristics proposed by Song and Wilbert (1997) and Song (1999); i.e. the number of dikinetids in kineties and the existence of spur(s).

Korean name: ¹*말총충속 (신칭), ²*강모말총충 (신칭)

Table 1. Morphometric characteristics of *Uronychia setigera* (1st line), *U. binucleata* (2nd line), and *U. multicirrus* (3rd line) from protargol impregnated specimens

Character	Min.	Max.	Mean	SD	SE	CV	n
Body length	50	82.5	62.63	8.64	1.93	13.80	20
	75	112.5	99.50	9.72	2.17	9.77	20
	80	140	110.5	13.76	3.08	12.45	20
Body width	35	57.5	42.75	6.17	1.38	14.44	20
	52.5	75	63.75	6.46	1.45	10.14	20
	55	105	76.75	13.40	3.00	17.46	20
Length of buccal field	35	55	43.63	5.65	1.26	12.94	20
	47.5	87.5	68.63	8.17	1.83	11.91	20
	60	120	93	11.40	2.55	12.26	20
No. of macronucleus nodules	2	2	2	0	0	0	20
	2	2	2	0	0	0	20
	6	9	7.15	0.75	0.17	10.42	20
No. of micronuclei	–	–	–	–	–	–	–
	1	1	1	0	0	0	20
	1	1	1	0	0	0	11
No. of membranelles in AZM1	11	11	11	0	0	0	20
	11	11	11	0	0	0	20
	11	11	11	0	0	0	20
No. of membranelles in AZM2	4	4	4	0	0	0	20
	4	4	4	0	0	0	20
	4	4	4	0	0	0	20
No. of frontal cirri	4	4	4	0	0	0	20
	4	4	4	0	0	0	20
	4	4	4	0	0	0	20
No. of transverse cirri	5	5	5	0	0	0	20
	5	5	5	0	0	0	20
	5	5	5	0	0	0	20
No. of left marginal cirri	3	3	3	0	0	0	20
	3	3	3	0	0	0	20
	3	3	3	0	0	0	20
No. of ventral cirri	2	2	2	0	0	0	20
	2	2	2	0	0	0	20
	6	9	8.1	0.91	0.20	11.26	20
No. of caudal cirri	3	3	3	0	0	0	20
	3	3	3	0	0	0	20
	3	3	3	0	0	0	20
No. of dorsal kineties	6	6	6	0	0	0	20
	6	6	6	0	0	0	20
	6	6	6	0	0	0	20
No. of cilia in left marginal kinety	18	26	23.14	2.28	0.61	9.86	14
	33	40	37	2	0.53	5.41	14
	–	–	–	–	–	–	–

All measurements in μm .

Min., minimum; Max., maximum; Mean, arithmetic mean; SD, standard deviation; SE, standard error of mean; CV, coefficient of variation in %; n, number of individuals examined; AZM, adoral zone of membranelles.

The Korean population is congruent with the Chinese population (Song and Wilbert, 1997; Song et al., 2004) in most respects, including body shape and size, the number of dikinetids in the leftmost kinety and nuclei, and a spur on the left margin with the population by Song and Wilbert (1997) and Song et al. (2004) (Table 2).

Uronychia binucleata is the species most similar to *U. setigera* in morphology. *U. setigera* can be distinguished

from *U. binucleata* by the following features: body size (ca. 60 vs. 100 μm), the number of dikinetids in the leftmost kinety (ca. 23 vs. 37), and the presence of a lateral spur on the left margin in only *U. binucleata*.

Distribution. Germany, China, and Korea (this study).

¹*Uronychia binucleata* Young, 1922 (Table 1, Fig. 2)

Uronychia binucleata Young, 1922: 360, figs. 4–6; Song and

Korean name: ¹*쌍핵말총충 (신칭)

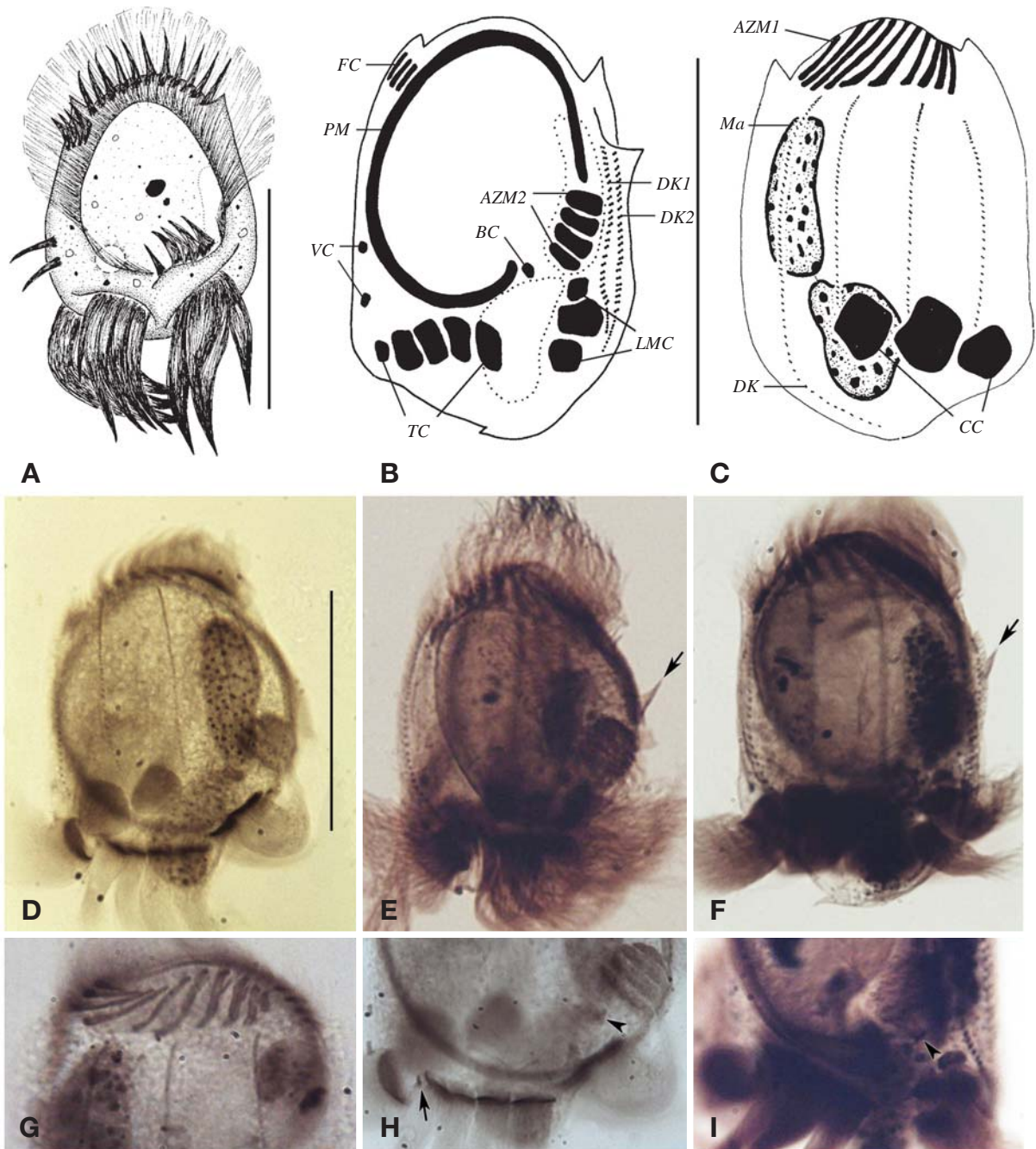


Fig. 1. Morphological characteristics of *Uronychia setigera* from life (A) and after protargol impregnation (B-I). A, Ventral view of a typical individual; B, E, F, The cirri pattern of the ventral region, arrows indicate a lateral spur on the left margin; C, D, Dorsal side, views CC, DK, and nuclei; G, Anterior portion, shows AZM1 and FC; H, I, The rightmost TC (arrow) and BC (arrowhead) in the posterior region. AZM, adoral zone of membranelles; BC, buccal cirrus; CC, caudal cirri; DK, dorsol kineties; FC, frontal cirri; LMC, left marginal cirri; Ma, macronuclear nodules; PM, paroral membrane; TC, transverse cirri; VC, ventral cirri. Scale bars: 40 μ m.

Wilbert, 1997: 427, fig. 8.

Uronychia uncinata Taylor, 1928: 3, fig. 1.

Material examined. Specimens collected from the public

waterfront (37°26'N, 126°35'E), Incheon in the Yellow Sea of Korea on 19-26 Jun 2006. The raw culture maintained for up to one week.

Diagnosis. Size about 70-110 μ m long *in vivo*, oval to slight-

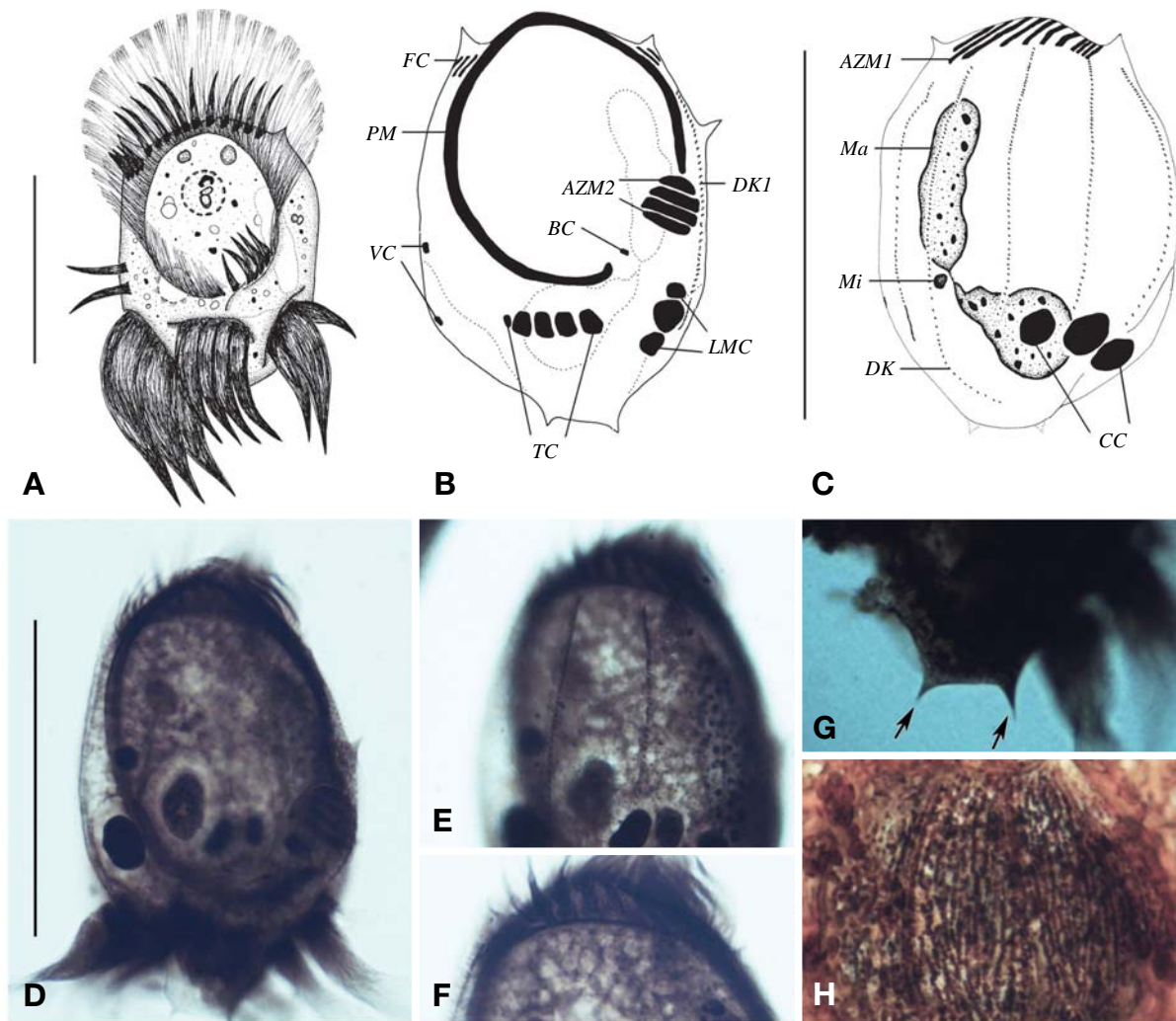


Fig. 2. Morphological characteristics of *Uronychia binucleata* from life (A), after protargol impregnation (B-G) and silver nitrate impregnation (H). A, D, Ventral view of a typical individual; B, The cirri pattern of the ventral region; C, E, H, Dorsal side, views DK (C, E) and silverline system (H); F, Anterior portion, shows AZM1; G, Posterior region, arrows indicate posterior spurs. AZM, adoral zone of membranelles; BC, buccal cirrus; CC, caudal cirri; DK, dorsol kineties; FC, frontal cirri; LMC, left marginal cirri; Ma, macronuclear nodules; Mi, micronucleus; PM, paroral membrane; TC, transverse cirri; VC, ventral cirri. Scale bars: 70 μ m.

ly rectangular shaped, 2 Ma, 1 Mi, 2 spurs on the posterior region, 11 AM1, 4 AM2, 1 BC, 4 FC, 3 LMC, 2 VC, 5 TC, 3 CC, 6 DK, approximately 37 cilia in the leftmost kinety.

Description. Body size about 70-110 \times 55-75 μ m *in vivo*, oval to slightly rectangular shaped; slightly convex both right and left margins. Dorso-ventrally flattened, ratio of about 2 : 3; buccal area deeply caved, extended about 70% of body length (Fig. 2A, B, D). Two conspicuous spurs appeared on posterior region, visible after protargol staining (Fig. 2B, G); lateral spurs inconspicuous. On posterior portion, 2 depressions on ventral side, TC and LMC emerged; 1 depression on dorsal side, CC appeared (Fig. 2A-C). Cytoplasm grayish to dark gray colored, with numerous granules and large par-

ticles.

Two Ma connected by funiculus, located on left of body; each nodule irregular sausage-shaped, with spherical nucleoli; Mi located between Ma nodules, globular form (Fig. 2C). CV recognized near the right of TC; several FV recognized after protargol impregnation (Fig. 2D). Movement usually genus typical, like *U. setigera*.

AZM pattern stable in this genus; 11 AM1 on apical area of anterior region, extended dorsal surface, each membranelle contained with 3 kineties; 4 AM2 on left of middle region, each membranelle consists of about 6-8 kineties; 1 BC, small cirrus-like membranelle, aperted from AZM2, about 2-3 μ m long (Fig. 2A-C, F). An oral area surrounded by horseshoe-

Table 2. Comparison of *Uronychia* species in present and previous specimens

Species	Body length <i>in vivo</i> (μm)	No. of macronucleus nodules	No. of ventral cirri	No. of cilia in left marginal kinety	Buccal field length in body (%)	Reference
<i>U. setigera</i>	40-70	2	2	ca. 15	54-69	Song and Wilbert (1997)
	50-90	2	2	17-25	60 \leq	Song et al. (2004)
	50-80	2	2	18-26	ca. 70	This study
<i>U. binucleata</i>	70-120	2	2	ca. 30	47-56	Song and Wilbert (1997)
	70-110	2	2	33-40	ca. 70	This study
<i>U. multicirrus</i>	120-200	5-9	6-8	–	ca. 75	Song (1997)
	120-200	5-8	6-8	–	60-70 \leq	Shen et al. (2009)
	140-200	6-9	6-9	–	ca. 80	This study
<i>U. transfuga</i>	110-250	7-10	2	ca. 50	64-78	Song and Wilbert (1997)

–, data not described.

shaped PM (Fig. 2B, D).

FC 4 in number, positioned at the most anterior area on ventral surface; 2 small VC, on right margin; 5 TC, rightmost cirrus rather small; 3 enlarged LMC, positioned on left side of ventral region; 3 strong CC emerged on right margin of dorsal side (Fig. 2A-C). Six DK composed of dikinetids on cell surface, genus-typical; 2 DK dorsolaterally located on left margin of ventral surface, densely packed on posterior region, included in an average of 37 cilia in the leftmost kinety (Fig. 2B, C, E, F). Silverline system on dorsal surface formed net-type (Fig. 2H).

The SSU rDNA sequence of the Korean population was deposited in Genbank under the accession number HQ380024. The sequence is 1,625 bp in length and shows 99.82% similarity between the Korean population and the Chinese population (Genbank accession number: EF198667) of *U. binucleata*.

Remarks. *Uronychia binucleata* was originally described by Young (1922) and is commonly found in the Yellow Sea of Korea. This species was characterized through comparison with its congeners with respect to body shape, medium-sized body, number of basal bodies in dorsal kineties and nuclei, and the existence of spur(s) on the posterior region (Song and Wilbert, 1997; Song, 1999). In most respects, the Korean population showed close correspondence with the Chinese population, characterized by Song and Wilbert (1997), except that buccal field length in body is longer than that of Chinese (Table 2).

However, *U. binucleata* is most similar to *U. setigera*, based on morphological characteristics, and can be distinguished by the following features: body size (ca. 100 vs. 60 μm), the number of dikinetids in the leftmost kinety (ca. 37 vs. 23), and spurs on the posterior region (present vs. absent).

Distribution. China and Korea (this study).

¹**Uronychia multicirrus* Song, 1997 (Table 1, Fig. 3)

Uronychia multicirrus Song, 1997: 279, figs. 1-5; Shen et al.,

2009: 296, figs. 1-3.

Material examined. Specimens collected from the public waterfront (37°26'N, 126°35'E), Incheon in the Yellow Sea of Korea on 6-13 Aug 2007. The raw culture maintained for up to ten days.

Diagnosis. Size about 140-200 μm long *in vivo*, oval to slightly rectangular shaped, ca. 7 Ma, 1 Mi, 11 AM1, 4 AM2, 1 BC, 4 FC, 3 LMC, approximately 8 VC, 5 TC, 3 CC, 6 DK.

Description. Body size about 140-200 \times 70-120 μm *in vivo*, rectangular body shape; slightly convex in left margin. Dorsoventrally flattened, ratio of about 1:2; buccal area deeply caved, extended about 80% of body length (Fig. 3A, B, F). Usually, spur is inconspicuous. On posterior portion, 2 depressions on ventral side, TC and LMC inserted; 1 depression on dorsal side, CC positioned (Fig. 3A-E, H, I). Cytoplasm grayish to dark gray colored, with numerous granules and large crystal-like particles.

Usually moniliform Ma connected with 6-9 nodules by funiculus, C-shaped; each nodule ellipsoidal to elongated shape, with spherical nucleoli; Mi located at the lower left corner of Ma, spherical form (Fig. 3C, G). CV recognized near the right of TC; several FV recognized after protargol impregnation (Fig. 3G). Movement usually genus typical, as *U. setigera*.

AZM pattern genus-typical; 11 AM1 on apical area of anterior region, extended dorsal surface, each membranelle consists of 3 kineties; 4 AM2 on left side of middle region; 1 BC, small cirrus-like membranelle, separated from AZM2, base about 4-5 μm long (Fig. 3A, B, H). A buccal field surrounded by horseshoe-shaped mighty PM (Fig. 3A, G).

FC 4 in number, positioned at the most frontal area on ventral surface; 6-9 VC, on right border of cell; 5 TC, rightmost cirrus rather small; 3 enlarged LMC, positioned on left of ventral region; 3 strong CC emerged on right margin of dorsal side (Fig. 3A-C, G-I). Six DK on cell surface, genus-typical, composed of dikinetids; 2 DK dorsolaterally located on

Korean name: ¹*다극모말총충 (신칭)

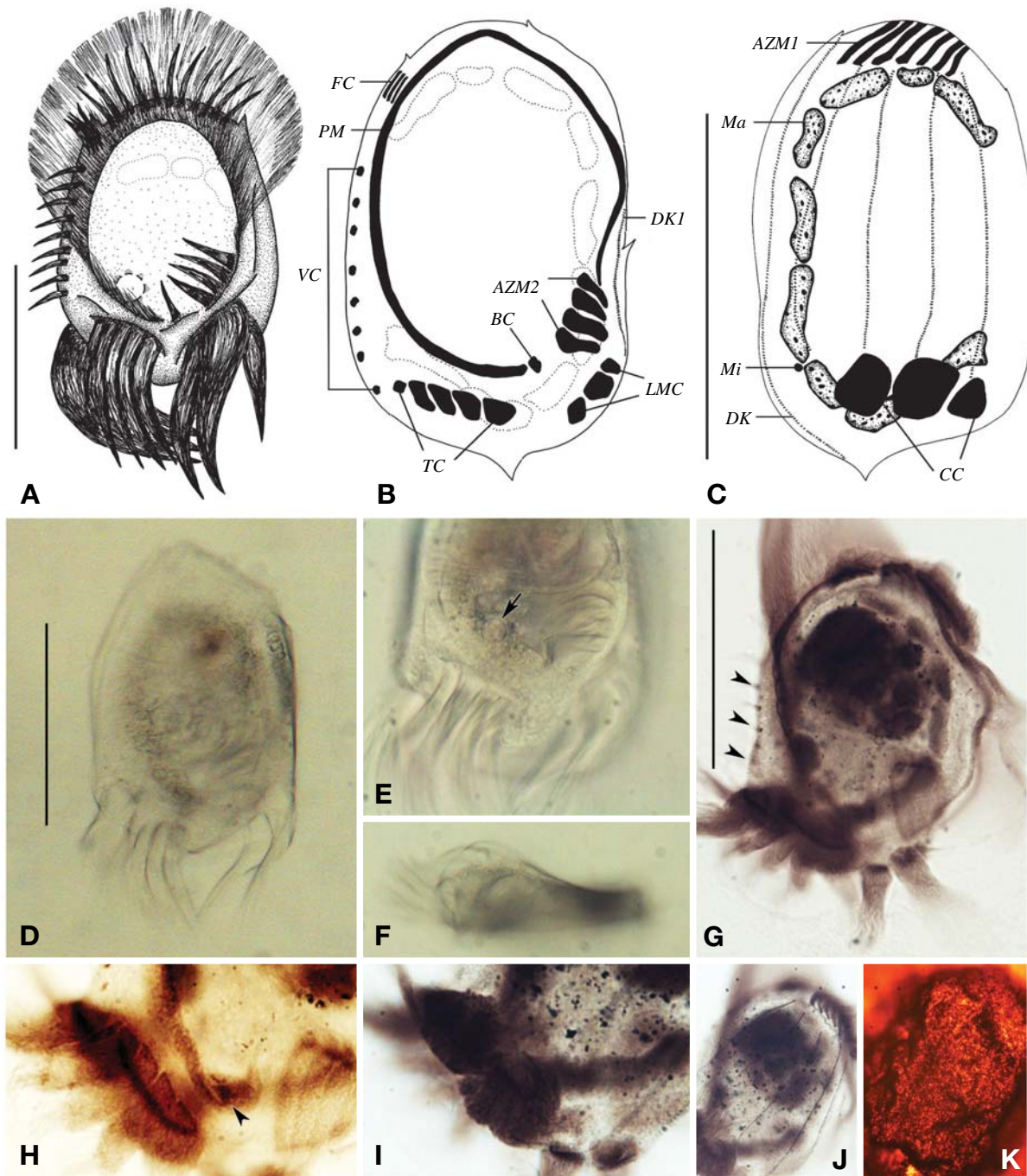


Fig. 3. Morphological characteristics of *Uronychia multicirrus*. A, Ventral view of a typical individual; B, C, The cirri pattern on the ventral (B) and dorsal (C) side drawn from protargol stained specimens; D-F, Living specimens, shows CV (arrow in E) and lateral side (F); G, Ventral region, views Ma, several VC (arrowheads) and PM; H, I, Posterior region, shows AZM2, BC (arrowhead in H), CC, DK, and TC; J, K, Dorsal side, views DK (J) and silverline system (K). AZM, adoral zone of membranelles; BC, buccal cirrus; CC, caudal cirri; DK, dorsol kineties; FC, frontal cirri; LMC, left marginal cirri; Ma, macronuclear nodule; Mi, micronucleus; PM, paroral membrane; TC, transverse cirri; VC, ventral cirri. Scale bars: 100 μ m.

left margin of ventral surface (Fig. 3B, C, J). Silverline system on dorsal side formed net-type (Fig. 3K).

The SSU rDNA sequence of the Korean population was deposited in Genbank under the accession number HQ380025.

The sequence is 1,624 bp in length and shows 99.88% similarity between the Korean population and the Chinese population (Genbank accession number: EU267929) of *U. multicirrus*.

Remarks. *Uronychia multicirrus* was originally reported by Song (1997) and then redescribed by Shen et al. (2009). This species is unusually composed of a large number of ventral cirri and characterized by its congeners, including body shape and size, number of nuclei (Song, 1997; Shen et al., 2009). The Korean population is clearly congruent with the Chinese population in most respects (Table 2).

U. multicirrus is most similar to *U. transfuga* (Song, 1997, 1999; Song and Wilbert, 1997). These species can be clearly distinguished by the following features: the number of ventral cirri (ca. 8 vs. 2) and macronuclear nodules (ca. 7 vs. ca. 11).

Distribution. China and Korea (this study).

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