# Morphological redescriptions of three *Cyrtohymena* ciliates (Ciliophora: Sporadotrichida: Oxytrichidae) new to Korea

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Three ciliate species of genus *Cyrtohymena, C. australis* Foissner, 1995, *C. citrina* (Berger & Foissner, 1987) and *C. muscorum* (Kahl, 1932) new to Korea, were collected from semi-terrestrial habitats. The description was based on the observation of live and silver stained specimens. Diagnostic characteristics of these species are as follows. *C. autralis*: size about  $275 \times 100 \,\mu\text{m}$  in live specimens. Cortical granules yellowish. 18-31 right and 31-40 left marginal cirri in number. Dorsal kineties eight rows. *C. citrina*: size about  $180 \times 50 \,\mu\text{m}$  in live specimens. Cortical granules yellowish.  $26-32 \,\text{right}$  and  $23-27 \,\text{left}$  marginal cirri in number. Dorsal kineties five to six rows. *C. muscorum*: size about  $180 \times 60 \,\mu\text{m}$  in live specimens. Cortical granules reddish.  $31-34 \,\text{right}$  and  $33-36 \,\text{left}$  marginal cirri in number. Dorsal kineties six rows. Three species of *Cyrtohymena* have been added to Korean ciliate fauna from this study. Including previous one species of *C. quadrinucleata*, four species of this genus in total have been recorded so far in this country.

Keywords: Cyrtohymena, freshwater, morphology, soil, taxonomy, terrestrial

#### **INTRODUCTION**

The oxytrichid genus *Cyrtohymena* Foissner, 1989 is highly specialized group of hypotrichous ciliates which are characterized by the *Cyrtohymena* pattern of undulating membrane, i.e. specific arrangement of paroral and endoral. The distal portion of the paroral is semicircularly curved and the straight anterior half of the endoral extends diagonally on the bottom of the buccal cavitiy, while the posterior half of the endoral is curved and at the right buccal wall, thus intersected optically with the posterior half of the paroral. In addition, this genus usually has six dorsal kineties of *Oxytricha* like dorsal kinety fragmentation, broad and deep buccal cavity, and colorless cytoplasm (Hemberger, 1982; Voss, 1991; Berger and Foissner, 1989; 1997; Berger, 1999; 2011; Song *et al.*, 2002; Song, 2004).

This genus was originated and separated from the genus *Steinia* Diesing, 1866 and/or subgenus *Oxytricha* (*Steinia*) Kahl, 1932 by Foissner (1989), on the base of pattern of undulating membrane which has a fragmented endoral and a conspicuous cavity (pit) in the anterior end of buccal area. So far, 15 *Cyrtohymena* species have been investigated worldwide, while only one species, *C. quadrinucleata*, of them has been taxonomically investigated in Korea, while it was redescribed under the name of *Steinia quadrinucleata* (Shin and Kim, 1996). They are mostly

collected from semi-terrestrial and rarely from marine habitats (Kahl, 1932; Gellért, 1956; Foissner, 1997; 1999; Berger, 1999; Song *et al.*, 2002; Çapar, 2007).

In this study, we described three *Cyrtohymena* species which are new to Korea, *C. australis, C. citrina* and *C. muscorum*, based on live and protargol-impregnated specimens to elucidate Korean ciliate diversity.

# **MATERIALS AND METHODS**

*Cyrtohymena australis* was collected from the freshwater of farming land in Beonam-myeon, Jangsu-gun, Jeollabuk-do, Korea (127° 32′20″E and 35° 30′58″N), on 23 December 2006. *C. citrina* was collected from fallen leaves and soil under false acacia tree (*Robinia pseudoacacia*) in Jeokseong-myeon, Danyang-gun, Chungcheongbuk-do, Korea (128° 18′31″E and 37° 01′07″N), on 17 November 2005. *C. muscorum* was collected from fallen leaves and moss soil in valley of the Mt. Munsusan in Mugeo-dong, Nam-gu, Ulsan, Korea (129° 14′ 50″E and 35° 32′33″N), on 14 March 2005.

Laboratory cultures were maintained in a commercial mineral water with dry wheat grain at room temperature. The living cell on slides was studied using a high-power oil immersion objective and differential interference contrast microscopy. The infraciliature was observed by using the protargol impregnation method (Wilbert, 1975). All counts and measurements were undertaken at the magnifications of 400-1,000X. Biometrical analysis was performed using the methods described in Foissner (1982). The classification scheme used in accordance with that of Lynn (2008).

## **RESULT AND DISCUSSION**

Phylum Ciliophora Doflein, 1901 Subclass Stichotrichia Small and Lynn, 1985 Order Sporadotrichida Fauré-Fremiet, 1961 Family Oxytrichidae Ehrenberg, 1838 Genus *Cyrtohymena* Foissner, 1989 관막하모충 속 (신칭)

#### 1. Cyrtohymena australis Foissner, 1995 호주관막하모충(신칭) (Fig. 1, Table 1)

*Cyrtohymena australis* Foissner, 1995, p. 70, figs. 110-119; Berger, 1999, p. 296, fig. 97.

General morphology and behavior: Body size in live specimens  $250-288 \times 85-115 \,\mu\text{m}$ , usually  $275 \times 100 \,\mu\text{m}$ , length: width ratio about 3:1 on average. Body shape generally elongated elliptical to slightly oval, anterior end broadly rounded, posterior end narrowly rounded, ventral side flatted, dorsal side convex in mid-region (Fig. 1A). Body very flexible and slightly contractile (Fig. 1E). Contractile vacuole located in mid-region near left margin of cell, approximately 13-16 µm in diameter during diastole, with inconspicuous collecting canals (Fig. 1D). Cytoplasm colorless, with ellipsoidal fat globules 2-3 µm long. Cell yellowish green at low magnification due to yellow cortical granules, 0.5-1 µm in across, mainly in groups of two to five in anterior and posterior ends and grouped around bases of cirri and dorsal bristles (Fig. 1F). Locomotion by gliding or crawling on substratum, or freely swimming.

Buccal field and oral infraciliature: Adoral zone of membranelles shaped like a question mark, about 42% of cell length with 55-60 adoral membranelles. Buccal area large and deep. Undulating membranes in typical *Cyrtohymena*-pattern; paroral bent in a broad arc along anterior margin of oral cavity; endoral sharply bent to left in mid-region; membranes optically intersect near mid-region of paroral. Pharyngeal fiber about 35 µm long (Fig. 1G).

Somatic infraciliature: Three enlarged frontal cirri. Buccal cirrus located to right of paroral. Four frontoventral cirri arranged in a V-shape. Three, rarely two postoral ventral cirri just below buccal vertex. Two pre-transverse cirri. Five prominent transverse cirri at about level of posteriormost right marginal cirri. Right marginal row with 18-31 cirri, left marginal row with 31-40 cirri and extends around posterior end of cell onto right side; posterior ends of marginal rows distinctly separated. Eight dorsal kineties, dorsal bristles short (Fig. 1B, G). Three caudal cirri located slightly right of centre (Fig. 1C).

Nuclear apparatus: Two ellipsoidal macronuclear nodules,  $38-40 \times 23-25 \,\mu\text{m}$  in protargol-impregnated specimens, nodules slightly left of cell median, anterior nodule located just below buccal vertex, posterior nodule slightly below equatorial region. Six spherical micronuclei,  $2-5 \,\mu\text{m}$ , closely associated with macronuclear nodules (Fig. 1B, G).

**Microhabitats.** Freshwater of farmland in Korea, soil from the Amazon rain forest in Peru, soil from a dry forest in Costa Rica, and saline soil in Venezuela.

**Distribution.** South and Central America (Csta Rica, Peru and Venezuela) and Asia (Korea).

**Remarks.** The Korean population of *Cyrtohymena australis* Foissner, 1995 is characterized by 2 or more micronuclei, yellowish cortical granules, freshwater habitat and large body size about  $275 \times 130 \,\mu\text{m}$ . The Korean population resembles those from Peru and Costa Rica, however it differs slightly from both populations in having: a slightly larger body size ( $150 \,\mu\text{m}$  vs.  $97 \,\mu\text{m}$  in Peru, 68  $\mu\text{m}$  in Costa Rica), fewer right marginal cirri (28 vs. 36 in Peru and 33 in Costa Rica populations); more dorsal kineties (5-9 vs. 5-7 in both populations); no fan-shaped fibers attached at anterior portion of the paroral membrane (vs. fan-shaped fibers present in both populations) (Foissner, 1995, 1997).

This species is similar to *Cyrtohymena citrina* (Berger and Foissner, 1987) and *C. primicirrata* (Berger and Foissner, 1987) in having yellowish cortical granules. *C. australis*, however, has a larger body size in live specimens (250-288  $\mu$ m vs. 75-112  $\mu$ m in *C. citrina*, vs. 74-102  $\mu$ m in *C. primicirrata*).

## 2. Cyrtohymena citrina (Berger and Foissner, 1987) Foissner, 1989 노랑관막하모충 (신칭) (Fig. 2, Table 1)

Steinia citrina Berger and Foissner, 1987, p. 225-228, figs. 81-84, table 11.

*Cyrtohymena citrina*: Berger, 1999, p. 293, figs. 96a-e, table 20; Çapar, 2007, p. 47.

General morphology and behavior: Body size in live specimens  $145-210 \times 30-55 \mu m$ , about  $180 \times 50 \mu m$  on average, length: width ratio 4:1 on average. Body shape long ellipsoid, anterior and posterior ends broadly rounded, left and right margin slightly convex, dorsoventrally flattened about 2:1 (Fig. 2A). Body very flexible and rather contractile. Contractile vacuole located in mid-region near left margin of cell, spherical during diastole, approximately 10-15  $\mu m$  in diameter, with inconspicuous collecting canals. Cytoplasm contains yellowish crystals, 2-5  $\mu m$  long, distributed throughout cell. Cell yellowish



**Fig. 1.** *Cyrtohymena australis* Foissner, 1995 from live (A, D, E, F) and protargol-impregnated specimens (B, C, G). A, D. Ventral view of a typical individual. B, G. Ventral view of general infraciliature and nuclear apparatus. C. Showing dorsal kineties and caudal cirri. E. Flexible body. F. Cortical granules on dorsal side. Scale bar= $100 \mu m$ .



**Fig. 2.** *Cyrtohymena citrina* (Berger and Foissner, 1987) from live (A, D, E, F) and protargol-impregnated specimens (B, C, G, H, I). A and D. Ventral view of a typical individual. B, G and I. Ventral view of general infraciliature and nuclear apparatus. C. Dorsal kineties. E and F. Cortical granules on dorsal side. Scale bar=50 µm.

at low magnification due to yellow cortical granules,  $1-2\,\mu$ m across, mainly in groups of two to five in anterior and posterior ends and grouped around bases of cirri and dorsal bristles (Fig. 2E). Locomotion moderately rapid.

Buccal field and oral infraciliature: Buccal area large and deep. Adoral zone of membranelles shaped like a question mark, about 40% of cell length, with 38-49 adoral membranelles, widest membranelle about 18 µm wide (Fig. 2A, B). Undulating membranes in typical *Cyrtohymena*-pattern, paroral bent in a broad arc along anterior margin of oral cavity; endoral sharply bent to left in midregion; membranes optically intersect near mid-region of paroral (Fig. 2B). Pharyngeal fiber about 20-28 µm long.

Somatic infraciliature: Three enlarged frontal cirri. Buccal cirrus located to right of paroral. Four frontoventral cirri arranged in a V-shape. Three postoral ventral cirri just below buccal vertex. Two pre-transverse cirri. Five prominent transverse cirri at about level of posteriormost right marginal cirri (Fig. 2B). Right marginal row with 26-32 cirri, left marginal row with 23-27 cirri and extends around posterior end of cell; posterior ends of marginal rows distinctly separated (Fig. 2B). Five or six dorsal kineties, dorsal bristles short (Fig. 2C). Three caudal cirri located right of centre (Fig. 2C).

Nuclear apparatus: Two ellipsoidal macronuclear nodules,  $19-42 \times 11-20 \,\mu\text{m}$  in protargol-impregnated specimens; nodules slightly left of cell median, anterior nodule located just below buccal vertex, posterior nodule slightly below equatorial region. Three to eight, usually five ellipsoidal micronuclei close to macronuclear nodules (Fig. 2B).

#### Microhabitats. Terristrial.

**Distribution.** Europe (Germany, Austria, Greece), Asia (Korea), Central and South America (Costa Rica and Peru). **Remarks.** The Korean population of *C. citrina* (Berger and Foissner, 1987) closely matches the type population from European population in terms of the body shape, number of macronuclei, color of cortical granules, number of dorsal kineties and location of transverse cirri (Berger and Foissner, 1987). There are, however, a few differences between them, for example the Korean population has a larger body size, more cirri in both marginal rows and the number of micronuclei is more variable.

*Cyrtohymena citrina* is most similar to *C. primicirrata* (Berger and Foissner, 1987) although the former has more micronuclei and the transverse cirri are located in a more posterior position than the latter. *C. citrina* also resembles *C. muscorum* (Kahl, 1932) although the former can be separated by its yellowish (vs. reddish in the latter) cortical granules.

# 3. Cyrtohymena muscorum (Kahl, 1932) Foissner, 1989 이끼관막하모충 (신칭) (Fig. 3, Table 1)

Steinia muscorum Kahl, 1932, p. 613, fig. 94a; Foissner,

1982, p. 105-107, figs. 29a-e, table. 24; Berger and Foissner, 1989, p. 41, figs. 79-82, table. 20.

Steinia dubia Gellért, 1956, p. 349-350, fig. 94a.

Oxytricha muscorum: Borror, 1972, p. 14.

Tachysoma dubia Borror, 1972, p. 15.

Oxytricha rubra Buitkamp, 1977, p. 122-123, add. 5.

Oxytricha germanica Foissner, 1987, p. 224.

*Cyrtohymena muscorum*: Foissner, 1989, p. 239; Berger, 1999, p. 281-293, figs. 94, table 20.

General morphology and behavior: Body size in live specimens  $160-200 \times 50-70 \,\mu\text{m}$ , about  $180 \times 60 \,\mu\text{m}$  on average, length: width ratio 3:1 on average. Body shape long ellipsoid, anterior and posterior ends broadly rounded, dorsoventrally flattened about 2:1 (Fig. 3A). Body rather flexible. Contractile vacuole located in mid-region of cell near left margin, about 10-15 µm in diameter during diastole, with inconspicuous collecting canals. Cytoplasm colorless and contains some crystals; sized 2-5 µm long, yellowish and drumstick in shape. Cortical granules reddish, 1-1.5 µm across, located mainly in anterior and posterior ends of cell and in groups of two to five around bases of cirri and dorsal bristles; after death granules lose color very rapidly. Locomotion by moderately rapid crawling, sometimes slowly gliding, on bottom of Petri dish.

Buccal field and oral infraciliature: Buccal area long and deep. Adoral zone of membranelles shaped like a question mark, about 30% of cell length, with 44-49 adoral membranelles the widest of which is about 15 µm wide (Fig. 3A, B). Undulating membranes in typical *Cyrtohymena*-pattern; paroral bent in a broad arc along anterior margin of oral cavity; endoral sharply bent in lower third; membranes optically intersect near mid-region of endoral (Fig. 3B). Pharyngeal fiber about 25 µm long.

Somatic infraciliature: Three enlarged frontal cirri. Buccal cirrus located right of paroral membrane. Four frontoventral cirri arranged in a V-shape. Three postoral ventral cirri. Two pre-transverse cirri. Five transverse cirri in a J-shaped row, posteriormost cirrus located about level with the posteriormost right marginal cirrus. Right marginal row with 31-34 cirri, left marginal row with 33-36 cirri and extends around posterior end of cell; posterior ends of marginal rows distinctly separated (Fig. 3B). Six dorsal kineties (Fig. 3C). Three caudal cirri located slightly right of centre (Fig. 3C).

Nuclear apparatus: Two ellipsoidal macronuclear nodules  $30-43 \times 15-28 \,\mu\text{m}$  in protargol-stained specimens; both nodules left of cell median; anterior nodule located below buccal vertex, posterior nodule located just below mid-body region (Fig. 3B). Three to six ellipsoidal micronuclei close to macronuclear nodules.

Microhabitats. Terrestrial and freshwater.

Distribution. Europe (Norway, Germany, Austria, Czech



**Fig. 3.** *Cyrtohymena muscorum* (Kahl, 1932) from live (A, D, E, F) and protargol-impregnated specimens (B, C, G). A and D. Ventral view of a typical individual. B and G. Ventral view of general infraciliature. C and E. Dorsal kineties. F. Red cortical granules. Scale bar=50  $\mu$ m.

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Characteristics	Species	Method	Mean	Med.	Min.	Max.	SD	SE	CV	n
Body, length	C.a.	L	275.0	275	250	288	11.8	3.7	4.3	10
	C.a.	S	252.3	250	238	263	8.0	2.5	3.2	10
	C.c.	L	180.4	182	145	208	15.6	3.1	8.7	25
	C.c.	S	175.4	175	150	200	21.3	4.3	10.2	25
	C.m.	L	180.5	180	160	265	15.9	5.0	6.5	10
	C.m.	S	175.9	175	150	200	30.1	9.5	13.7	10
Body, width	C.a.	L	100.0	100	85	115	5.8	1.8	4.4	10
	C.a.	S	102.5	105	85	120	5.5.	1.8	3.6	10
	C.c.	L	46.2	47	30	55	4.8	1.0	10.4	25
	C.c.	S	94.1	91	77	124	13.7	2.7	14.6	25
	C.m.	L	60.0	60	50	70	6.6	2.1	8.1	10
	C.m.	S	62.5	62	50	75	21.4	6.8	22.9	10
Body length/width, ratio	C.a.	L	2.8	2.8	2	3	0.1	0.0	4.0	10
	C.c.	L	4.0	4	3	7	0.7	0.1	17.7	25
	C.m.	S	3.0	3	3	3	0.2	0.1	5.3	10
AZM. length	C.a.	L	100.0	100	90	115	11.5	3.6	10.0	10
, . 8	C.a.	S	102.5	100	100	108	3.3	1.1	3.3	10
	C.c.	L	46.6	49	32	61	8.9	1.8	19.2	25
	C.c.	s	72.8	76	53	88	10.1	2.0	13.9	25
	C.m.	S	55.6	55	50	60	8.6	2.7	10.4	10
AM number	C.a.	S	57.1	58	55	60	2.0	0.6	3.5	10
,	C.c.	S	43.0	43	38	49	3.2	0.6	7.5	25
	C.m.	S	46.3	46	44	49	1.7	0.5	3.7	10
Ma, number	Ca	S	2.0	2	2	2	0	0	0	10
	C.c.	ŝ	2.0	2	2	2	Ő	Ő	Ő	25
	Cm	Š	2.0	2	2	2	Ő	0	Ő	10
Anterior Ma, length	Сс	Š	27.4	27	19	42	53	12	194	20
	C m	Š	39.1	40	30	43	3.2	0.7	8.1	20
Anterior Ma, width	C c	S	15.4	15	11	19	2.2	0.5	14.3	20
	C m	S	22.3	22	16	28	1	0.9	17.9	20
Distance between macronuclear nodules	C.m.	S	22.5	30	20	20 45	65	17	23.2	15
	C m	S	20.1	25	20	35	8	1.7	35.3	20
Posterior Ma, length	C.m.	2	25.0	25	19	31	33	0.8	13.1	15
	C.c.	S	34.8	36	30	38	2.5	0.0	73	20
Destarion Ma width	C.m.	2	16.4	17	12	20	2.5	0.0	16.2	15
Posterior Ma, widui	C.c. C.m.	S	18.9	17	12	20	2.7	0.7	10.2	20
Mi number	C	S	5.1	5	3	8	15	0.3	20.5	25
wii, number	C.e.	S	4.4	5	3	6	0.9	0.3	29.5	20
Mi_number (adjacent to anterior Ma)	Ca	S	29	3	2	3	03	0.1	10.9	10
Mi_number (adjacent to nosterior Ma)	C a	S	3.0	3	3	3	0.0	0.1	0.0	10
Mi diameter	C.a.	S	5.0	5	3	8	1.5	0.0	20.5	25
ואוו, עומוווכוכו	C.m.	S	4.4	5	3	6	1.0	0.3	22.0	10
FC, number	Ca	S	3.0	3	3	3	0.0	0.0	0.0	10
	C.a.	S	3.0	3	3	3	0.0	0.0	0.0	25
	C.m.	S	3.0	3	3	3	0.0	0.0	0.0	10
FVC, number	Ca	S	4.0	4	4	4	0.0	0.0	0.0	10
	C.a.	S	4.0				0.0	0.0	0.0	25
	C.m.	S	4.0	4	4	4	0.0	0.0	0.0	10
BC, number	C a	ç	1.0	1	1	1	0.0	0.0	0.0	10
	C.a.	2	1.0	1	1	1	0.0	0.0	0.0	25
	C.c. C.m.	S	1.0	1	1	1	0.0	0.0	0.0	10
POVC. number	C.c	S	3.0	3	3	3	0.0	0.0	0.0	25
DTVC number	C.c.	c	1 6	2	1	2	0.5	0.0	22.2	10
PTVC, number	C.a.	2	1.0	2	1	2	0.5	0.2	32.3	10
	C.C.	2	2.0	2	2	2	0.0	0.0	0.0	2J 10
	U.III.	د	2.0	2	2	2	0.0	0.0	0.0	10

**Table 1.** Morphometricalal characterization of *Cyrtohymena australis* Foissner, 1995 (C.a.), *C. citrina* (Berger and Foissner, 1987) (C.c), and *C. muscorum* (Kahl, 1932) (C.m.). Data based on live (L) and protargol-impregnated specimens (S). Measurements in micrometers.

Table 1. Continued.

Characteristics	Species	Method	Mean	Med.	Min.	Max.	SD	SE	CV	n
LMC, number	C.a.	S	35.2	34	31	40	3.3	1.0	9.3	10
	C.c.	S	24.8	25	23	27	1.4	0.3	5.5	25
	C.m.	S	34.8	35	33	36	0.9	0.3	2.6	10
RMC, number	C.a.	S	27.5	30	18	31	4.8	1.5	17.4	10
	C.c.	S	29.3	30	26	32	1.6	0.3	5.5	25
	C.m.	S	32.7	33	31	34	0.9	0.3	2.9	10
TC, number	C.a.	S	5.0	5	5	5	0.0	0.0	0.0	10
	C.c.	S	5.1	5	5	6	0.3	0.1	6.5	25
	C.m.	S	5.1	5	5	6	0.2	0	4.4	10
CC, number	C.a.	S	3.0	3	3	3	0.0	0.0	0.0	10
	C.c.	S	3.0	3	3	3	0.0	0.0	0.0	19
	C.m.	S	3	3	3	3	0.0	0.0	0.0	10
DK, number	C.a.	S	7.5	8	5	9	1.3	0.4	16.9	10
	C.c.	S	6.0	6	5	6	0.2	0.0	3.6	22
	C.m.	S	6.0	6	6	6	0.0	0.0	0.0	10

Abbreviations: AM=adoral membranelles; AZM=adoral zone of membranelles; CV=coefficient of variation in %; DK=dorsal kineties; FC=frontal cirri; Ma=macronuclear; Max.=maximum; Med.=median; Mi=micronuclear; Min.=minimum; POVC=postoral ventral cirri; PTVC=pretransverse cirri; RMC and LMC=right and left marginal cirri; SD=standard deviation; SE=standard error; TC=transverse cirri; n=population size, L=live specimens, S=impregnanted specimens.

Republic and Hungary), Africa (Cameroon), Asia (China, Korea and Japan), North America (U.S.A.) and Antarctica.

**Remarks.** This species was identified as *Cyrtohymena muscorum* (Kahl, 1932) by its flexible body, six dorsal kineties, reddish cortical granules and terrestrial habitat. It does however, differ slightly from the original and subsequent descriptions of populations of *C. muscorum* from Europe in having a larger body size  $(210-265 \times 70-90 \,\mu\text{m} \text{ vs. } 105-155 \times 36-56 \,\mu\text{m})$ , more adoral membranelles (24-40 vs. 43-49) and more micronuclei (3-6 vs.2-5) (Kahl, 1932; Berger and Foissner, 1989). Superficially, *C. australis* Foissner, 1995 bears the closest resemblance to *C. muscorum*. However, the former can be separated from the latter by having 2-8 (vs. 3-6) micronuclei, 5-7 (vs. invariably 6) dorsal kineties and yellowish (vs. reddish) cortical granules (Berger, 1999).

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