

Redescription of Two Marine Ciliates (Ciliophora: Urostylida: Pseudokeronopsidae), *Pseudokeronopsis carnea* and *Uroleptopsis citrina*, from Korea

Ye-Seul Baek^{1,2}, Jae-Ho Jung¹, Gi-Sik Min^{1,*}

¹Department of Biological Sciences, Inha University, Incheon 402-751, Korea

²Division of Life Sciences, Korea Polar Research Institute, KORDI, Incheon 406-840, Korea

ABSTRACT

The morphology of the two marine urostyloid ciliates, *Pseudokeronopsis carnea* (Cohn, 1866) and *Uroleptopsis citrina* Kahl, 1932, in the family Pseudokeronopsidae, collected from the Yellow Sea, and the East Sea, Korea, respectively, were studied using live observation and protargol impregnation. Additionally, the small subunit ribosomal RNA (SSU rRNA) gene was sequenced. These two species are firstly recorded in Korea. The main diagnostic key is as follows. *Pseudokeronopsis carnea*: body outline elongate-elliptical, brown-reddish or orange-red in colour *in vivo*; bicorona of 16-24 frontal cirri; one buccal and two frontoterminal cirri; 7-10 transverse cirri; 5-7 dorsal kineties; two types of cortical granules (one orange-red pigment, mainly grouped around cirri and dorsal bristles, arranged in typical *rubra*-pattern; the other, colourless and blood-cell-shaped, and densely distributed); contractile vacuole in the posterior half of the cell on the left side, usually in posterior 1/3-2/5. *Uroleptopsis citrina*: body outline elongate-elliptical, lemon-yellow in colour *in vivo*; two types of cortical granules (one yellow pigment; the other, blood-cell-shaped, densely distributed); bicorona of 12-18 frontal cirri; 2-3 frontoterminal cirri; two midventral rows comprising 26-35 cirri (consisting of anterior paired cirri, non-paired single cirri, and posterior paired cirri); three dorsal kineties. In addition, the SSU rRNA sequences of the two species were compared with public database of these species and consequently, showed high similarity.

Keywords: *Pseudokeronopsis carnea*, *Uroleptopsis citrina*, marine ciliate, morphology, SSU rRNA gene, Korea

INTRODUCTION

The genera *Pseudokeronopsis* and *Uroleptopsis* are included in the family Pseudokeronopsidae which was established by Borror and Wicklow (1983).

The *Pseudokeronopsis* consists of 10 species and all members have frontal cirri arranged as a bicorona, which continue posteriorly to two midventral rows and marginal cirri on each side of the body (Borror and Wicklow, 1983; Berger, 2006; Song et al., 2006). Identification of species in the *Pseudokeronopsis*, however, is somewhat difficult because the diagnostic keys such as body shape, body colour, body size and ciliary pattern either are overlapped or similar among congeners (Song et al., 2006).

Kahl (1932) established the genus *Uroleptopsis* due to the lack of transverse cirri in some species on classification in *Holosticha* (*Keronopsis*). Later, Berger (2004) redescribed *Uroleptopsis citrina* by its morphology and morphogenesis, and divided *Uroleptopsis* into the two subgenus, *Uroleptopsis* (*Uroleptopsis*) and *Uroleptopsis* (*Plesiouroleptopsis*), through the presence of cirrus II/2 in the ordinary position, right of the undulating membranes.

In this study, we described two marine ciliates new to Korea, *P. carnea* and *U. citrina*, based on live and protargol-impregnated specimens. Moreover, the sequences of the small subunit ribosomal RNA (SSU rRNA) gene from two species were determined and compared with those of known sequences obtained from the NCBI website.

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

***To whom correspondence should be addressed**
Tel: 82-32-860-7692, Fax: 82-32-874-6737
E-mail: mingisik@inha.ac.kr

MATERIALS AND METHODS

Sample collection and identification

The specimens of *Pseudokeronopsis carnea* were collected from Incheon harbor in the Yellow Sea (salinity, 28.5‰; temperature, 15°C; 37°26'N, 126°35'E), Korea, in November 2010, and those of *Uroleptopsis citrina* were collected from Guryongpo, Pohang in the East Sea (salinity, 32.3‰; temperature, 24.1°C; 35°59'N, 129°33'E), Korea, in September 2008.

After collection and isolation, specimens were maintained in the laboratory, either as pure or raw cultures in Petri dishes and 50 mL tissue culture flasks (Greiner Bio-one, Frickenhausen, Germany). Autoclaved seawater was supplied with putting rice grains as a substrate for bacterial growth (Jung et al., 2011). The living specimens were observed under a light microscope (Leica DM2500; Leica Microsystems, Wetzlar, Germany) at 50-1,000 magnification. Protargol impregnation was applied according to Foissner (1991) to reveal the infraciliature.

Terminology and classification are mostly according to Berger (2006) and Lynn (2008).

DNA sequence determination

A cell (single specimens of each species) was transferred to a 1.5 mL microtube with a minimum volume of water. Genomic DNAs were extracted using a RED-Extract-N-Amp Tissue PCR kit (Sigma, St. Louis, MO, USA), according to the manufacturer's protocol. The nearly complete SSU rRNA genes were amplified by polymerase chain reaction (PCR) with the universal eukaryotic primers: New EukA (5'-CTG GTT GAT YCT GCC AGT-3'), modified from Medlin et al. (1988), and LSU rev3 (Sonnenberg et al., 2007) primers. The optimized conditions for this process were as follows: Denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 95°C for 15 sec, annealing at 58°C for 30 sec, extension at 72°C for 4 min, and then a final extension step at 72°C for 7 min. The PCR products were purified with the QIAquick® PCR Purification kit (Qiagen, Valencia, CA, USA). Three internal primers were used for sequencing: 18S+810 (5'-GCC GGA ATA CAT TAG CAT GG-3') and 18S-300 (5'-CAT GGT AGT CCA ATA CAC TAC-3') and 18S+1470 (5'-TCT GTG ATG CCC TTA GAT GTC-3'). Sequencing in both directions was conducted by an ABI 3700 Sequencer (Applied Biosystems, Foster City, CA, USA).

The sequencing fragments of the SSU rRNA gene were combined via BioEdit (Hall, 1999) and were aligned using Clustal X 1.81 (Jeanmougin et al., 1998). Mega 4.0 (Tamura et al., 2007) was used to calculate genetic distance by applying the Kimura two-parameter distance method (Kimura, 1980).

SYSTEMATIC ACCOUNTS

Phylum Ciliophora Doflein, 1901

Class Spirotrichea Bütschli, 1889

Order Urostylida Jankowski, 1979

Family Pseudokeronopsidae Borror and Wicklow, 1983

Genus *Pseudokeronopsis* Borror and Wicklow, 1983

¹**Pseudokeronopsis carnea* (Cohn, 1866)

Wirnsberger et al., 1987 (Table 1, Figs. 1A-D, 2)

Oxytricha flava var. *carnea* Cohn, 1866: 288, 300.

Holosticha (*Keronopsis*) *rubra* var. *carnea* (Cohn, 1866) Kahl, 1932: 573.

Pseudokeronopsis carnea (Cohn, 1866) nov. comb. Wirnsberger et al., 1987: 79, fig. 9, tables 1-3.

Pseudokeronopsis rubra sensu Shi and Xu, 2003: 23-30.

Pseudokeronopsis pararubra Hu, Warren and Suzuki, 2004: 351-368.

Pseudokeronopsis carnea (Cohn, 1866) Wirnsberger et al.,

Table 1. Morphometric characterization of *Pseudokeronopsis carnea*

	Min	Max	Mean	SD	SE	CV	n
Body length (µm)	162.5	220	194	17.0	3.8	8.8	20
	208	288	249.6	24.8	6.2	9.9	16
Body width (µm)	30	55	38.8	5.6	1.3	14.4	20
	56	96	76.3	10.3	2.6	13.4	16
Length of buccal field (µm)	55	75	65	6.2	1.4	9.4	20
	80	94	87.6	3.3	0.8	3.8	16
No. of membranelles	56	78	69	6.6	1.5	9.7	20
	69	79	73.1	3.0	0.8	4.1	16
No. of cirral pairs in bicorona	8	12	10	1.2	0.3	11.8	20
	9	11	10.1	0.8	0.2	7.6	16
No. of buccal cirri	1	1	1	0	0	0	18
	1	1	1	0	0	0	14
No. of frontoterminal cirri	2	2	2	0	0	0	20
	2	2	2	0	0	0	16
No. of transverse cirri	7	10	8	0.9	0.2	10.5	20
	8	9	8.6	0.5	0.2	6.0	12
No. of cirri pairs in MVR	30	46	36	4.7	1.0	12.4	20
	38	43	40.3	1.7	0.4	4.2	16
No. of cirri in left marginal row	56	87	69	8.9	2.0	13.1	20
	58	79	66.4	5.7	1.4	8.6	16
No. of cirri in right marginal row	53	86	67	9.8	2.2	14.1	20
	63	72	67.5	2.8	0.7	4.2	16
No. of dorsal kineties	5	7	6	0.6	0.1	10.4	19
	7	8	7.5	1.8	0.5	24.1	15

All data, including the Korean population (first line) and the Chinese population (second line), are based on protargol-impregnated specimens. The data of the Chinese population is cited from Song et al. (2006). Min, minimum; Max, maximum; Mean, arithmetic mean; SD, standard deviation; SE, standard error of the mean; CV, coefficient of variation in %; n, number of individuals examined; MVR, midventral row.

Korean name: ¹*똥똥이홍색위각모충 (신칭)

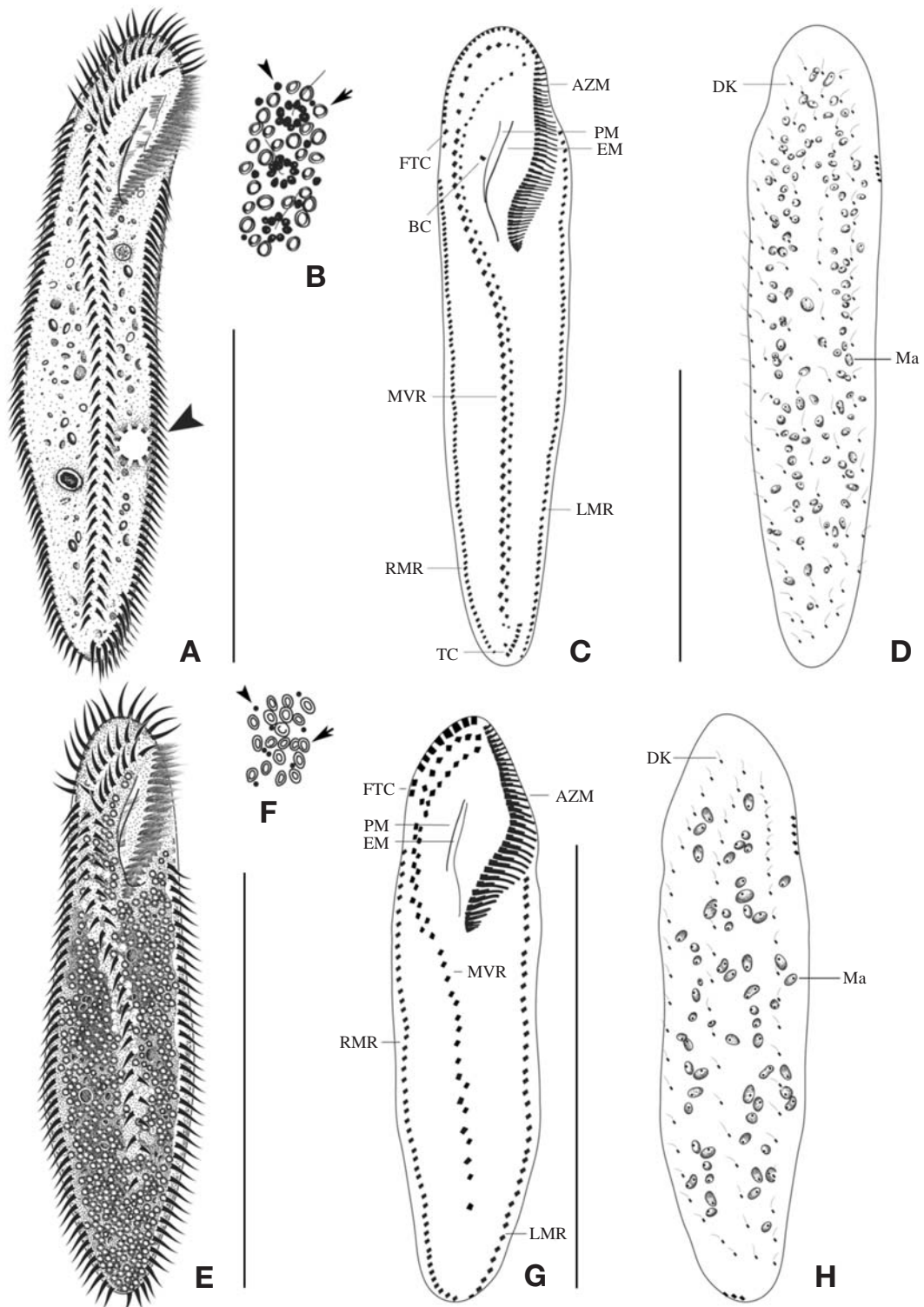


Fig. 1. Morphology and infraciliature of *Pseudokeronopsis carnea* and *Uroleptopsis citrina* from live specimens (A, B, E, F) and after protargol impregnation (C, D, G, H). A-D, *Pseudokeronopsis carnea*: A, Ventral view of live specimen, arrowhead in (A) denotes CV; B, Two types of granules; infraciliature of the ventral (C) and dorsal (D) sides. E-H, *Uroleptopsis citrina*: E, Ventral view of live specimen; F, Two types of granules; infraciliature of the ventral (G) and dorsal (H) sides. AZM, adoral zone of membranelles; BC, buccal cirri; CV, contractile vacuule; DK, dorsal kineties; EM, endoral membrane; FTC, frontoterminal cirri; LMR, left marginal row; Ma, macronuclei; MVR, midventral row; PM, paroral membrane; RMR, right marginal row; TC, transverse cirri. Scale bars=100 μ m.

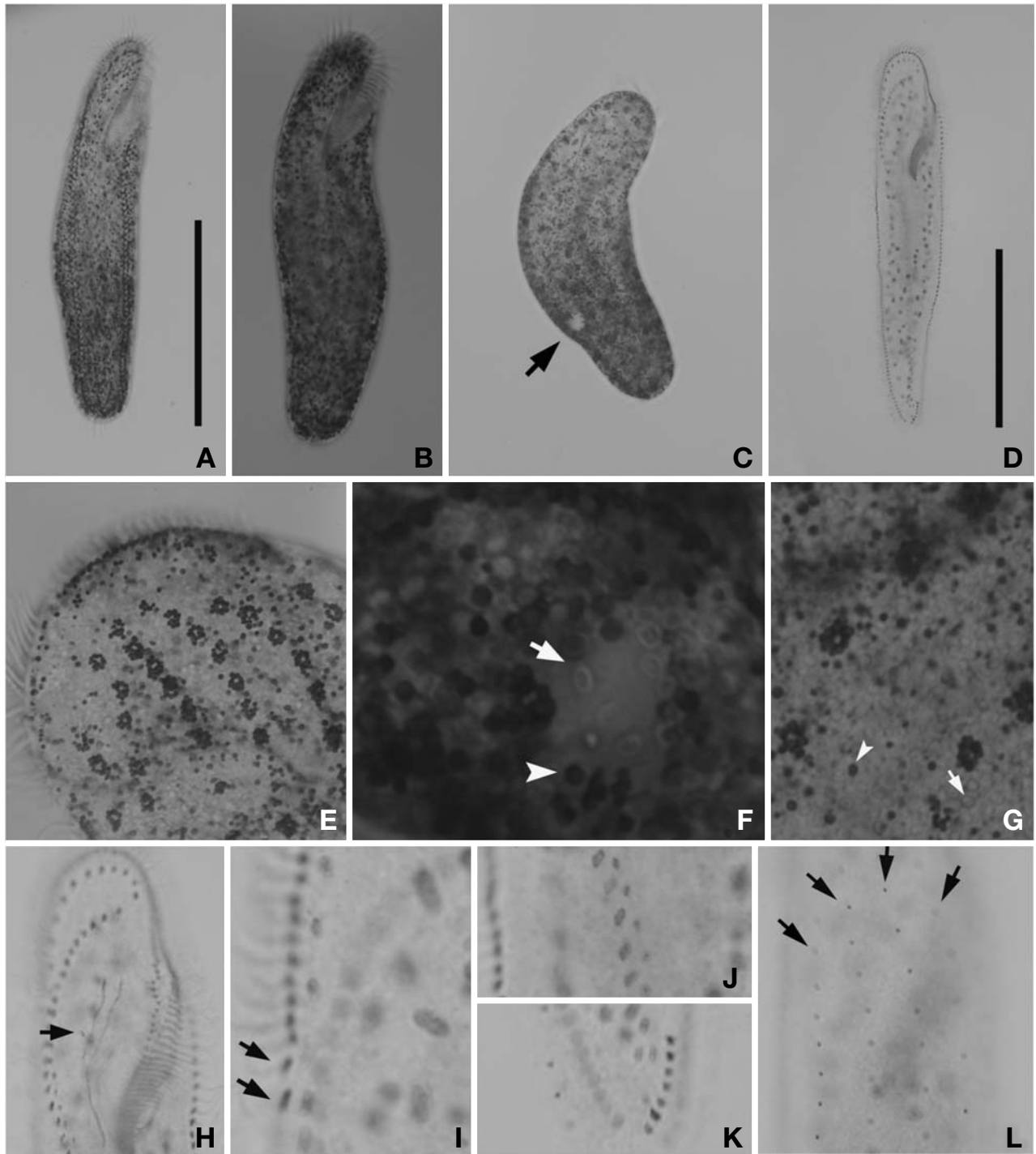


Fig. 2. Morphology and infraciliature of *Pseudokeronopsis carnea* from live specimens (A-C, E-G) and after protargol impregnation (D, H-L). A, B, Ventral views of live specimen; C, Dorsal views of live specimen, arrow marks a contractile vacuole; E, Cortical granules around dorsal kineties; Arrows in (F, G) indicate ring-shaped hollow structures, arrowheads show cortical granules; D, H-K, Ventral and (L) dorsal views of protargol-impregnated specimen; D, General ciliature of the specimen; H, Frontal (bicorona), arrow indicates the buccal cirrus; I, Two frontoterminal cirri, arrows indicate the cirri near the distal end of adoral zone; J, Two midventral rows; K, Denotes transverse ventral cirri; Arrows in (L) show the dorsal kineties. Scale bars=100 μ m.

1987, Song et al., 2006: 271-287, figs. 1A-G, 2, 3, 9C, tables 1-3.

Material examined. One population was obtained from Incheon harbor on November 2, 2010.

Description. Cell *in vivo* slender shape, 190-255 × 55-70 μm, usually 225 × 61.3 μm (Figs. 1A, 2A); anterior end bluntly rounded (Fig. 2B); posterior end inconspicuously narrowed; both anterior and posterior ends round; dorsoventrally flattened. Contractile vacuole located on the left side usually in posterior 1/3-2/5 (Figs. 1A, arrowhead; 2C, arrow); reddish cortex due to underlying reddish-brown or orange-red in colour cortical granules, which are around both dorsal kineties and cirri (Fig. 2E; F, G, arrowhead); cortical granules colourless, blood cell shaped, scattered throughout the cell body (Fig. 2F, G, arrow).

The adoral zone of membranelles distinct, approximately 1/3 of the cell length, and composed of about 69 membranelles (Fig. 2D, H). Bicornia of frontal cirri slightly enlarged, composed of about 8-12 cirral pairs, extending as a midventral complex consecutively. One buccal cirrus near the paroral membrane (Fig. 2H, arrow), whereas two frontoterminal cirri behind the distal end of the adoral zone (Fig. 2I, arrows); midventral complex distinctly separated rows (Fig. 2J), composed of 30-46 cirral pairs, terminating near transverse cirri; both posterior ends of marginal cirral rows not overlapped; 7-10 transverse cirri located between both posterior ends of the left and right marginal cirral rows (Fig. 2K). Almost no gap found between the midventral rows and the transverse cirri; from five to seven dorsal kineties (Figs. 1D; 2L, arrows).

Distribution. North Sea, German, Denmark, Mediterranean, Yugoslavia, China and Korea (this study).

Remarks. Cohn (1866) published *Oxytricha flava* var. *carnea* without any illustration. As the former species is almost identical to *P. flava*, he classified it as a variety of *Oxytricha flava*. The derivation of the name was not given in the original description of *P. carnea*. The meaning of *carnea* in Latin is "fleshy." In 1882, Kent transferred *Oxytricha rubra* to the genus *Holosticha*. Kahl (1932) classified *Keronopsis* as a subgenus of *Holosticha*. Then, Kahl named it *Holosticha (Keronopsis) rubra* var. *carnea*. Even after several taxonomists recorded this species, they were considered it *Pseudokeronopsis rubra* or *P. flava* in confusion. Entz (1884) considered *P. carnea* as a transitional form between *P. rubra* and *P. flava*. The neotype of *P. carnea* was fixed by Wirnberger et al. (1987) and until now, a Chinese population of *P. carnea* has been redescribed solely (Song et al., 2006).

Eight species among the genus *Pseudokeronopsis* live in marine habitats. Because *Pseudokeronopsis* species are

somewhat difficult to classify and identify among congeners, the colour as main diagnostic key is the critical factor distinguishing *P. carnea* from the other congeners (Hu and Song, 2001). The orange-red colour of cortical granules is essential for identifying *P. carnea* (vs. colourless, *P. decolor* and *P. ovalis*; yellow, *P. flavicans* and *P. flava*; brick-red, *P. rubra*; yellow-greenish, *P. sepetibensis*; brick-red and yellow, *P. multinucleata*). Moreover, with the exception of the colour of the cortical granules, the ciliary pattern and position of the contractile vacuole support species separation (Song et al., 2002; Berger, 2006). Like the name suggests, this species has the most plump body shape among the congeners. Although the anterior end is bluntly rounded, the posterior end is inconspicuously narrowed. This species can be separated from the other congeners by having: more cirral pairs in both the bicorona and the midventral rows; more transverse cirri; more dorsal kineties; a contractile vacuole in the posterior half of the cell, usually in the posterior 1/3-2/5; more conspicuous pigment granules, always dark red or orange-red. The number of adoral membranelles in this organism is also conspicuously more than that of other congeners. In addition, the adoral zone of membranelles is relatively long compared to body length (ratio, 1 : 3), and almost no gap exists between the midventral rows and the transverse cirri.

The Korean population, *Pseudokeronopsis carnea*, has a few differences from the Chinese population of *P. carnea* (Song et al., 2006) as follows: (1) dorsal kineties (5-7 vs. 7-8); and (2) transverse cirri (on average 8 vs. 8.6). In addition, we ascertained that the sequence was successfully amplified on the partial region of the SSU rRNA gene and the amplified sequence length is 1,756 bp (GenBank accession no: JN714476) and shows 99.89% similarity with the Chinese population (GenBank accession no: AY881633).

¹*Genus *Uroleptopsis (Uroleptopsis)* Kahl, 1932

²**Uroleptopsis citrina* Kahl, 1932 (Table 2, Figs. 1E-H, 3)
Uroleptopsis citrina Kahl, 1932: 543, fig. 87; Kahl, 1933: 107, fig. 16.12; Kudo, 1950: 672; Borrer, 1972: 11; Berger, 2004: 99-121, figs. 5-28, 35-42, table 1.

Material examined. One population was obtained from Guryongpo, Pohang in September 2008.

Description. Cell *in vivo* slender shape, 118-165 × 45-55 μm, usually 130.2 × 50 μm (Figs. 1E, 3A, B); body shape elongate-elliptical; both anterior and posterior ends round and dorsoventrally flattened. Contractile vacuole difficult to recognize, located on the left side of usually slightly squeezed cells. Body colour is lemon-yellow due to cortical granules,

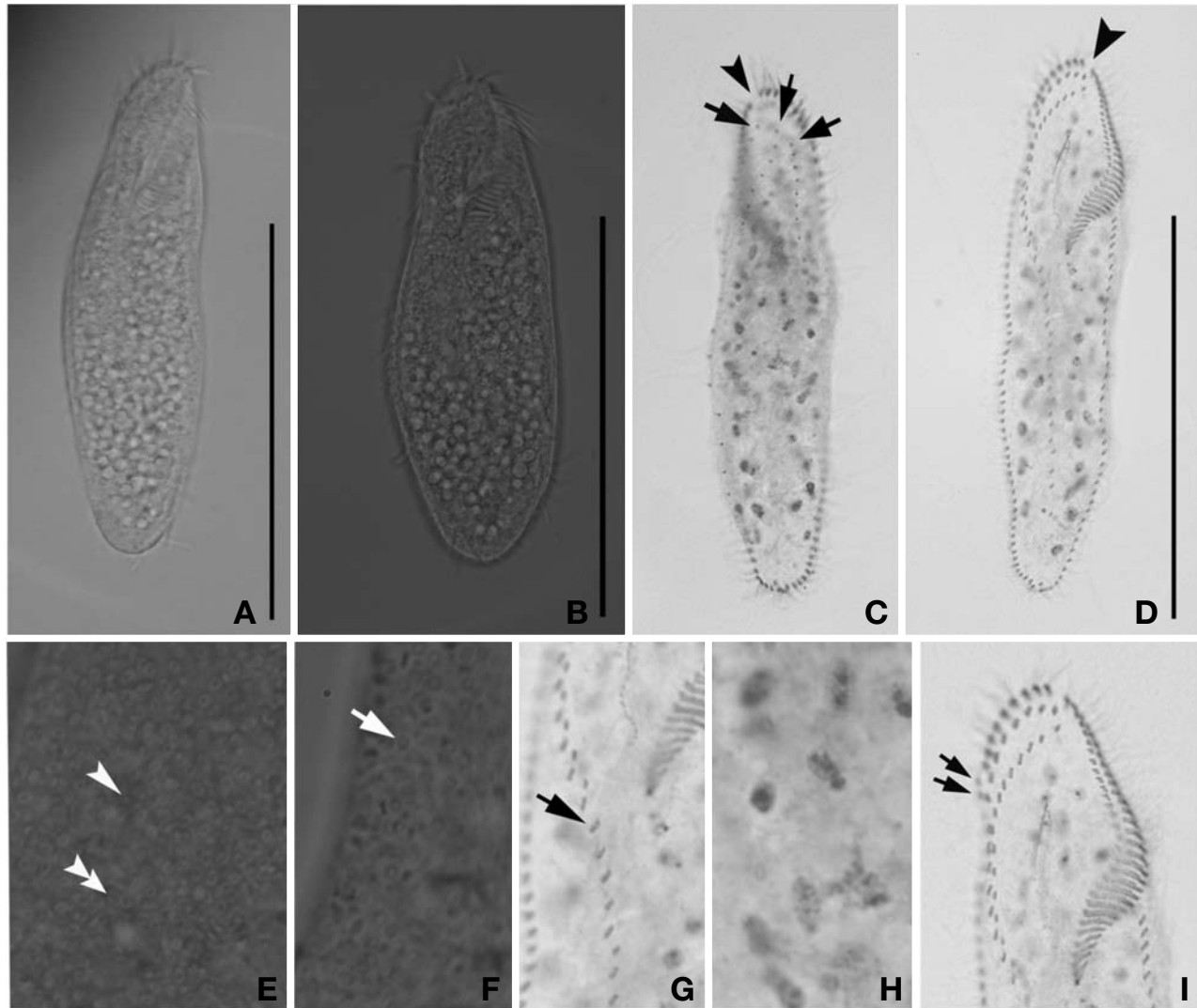


Fig. 3. Morphology and infraciliature of *Uroleptopsis citrina* from live specimens (A, B, E, F) and after protargol impregnation (C, D, G-I). A, B, Ventral views of live specimen; C, Dorsal and (D, G-I) ventral views of protargol-impregnated specimen; C, Arrows mark the invariable three dorsal kineties; C, D, Arrowheads point to gap in adoral zone; D, General ciliature of the specimen; E, F, Arrow and arrowheads indicate the two kinds of granules, respectively; G, Anterior pairs and single cirri (arrow mark) on the midventral complex; H, Indicates macronucleus; I, Frontal cirri (bicornate); arrows show two frontoterminal cirri. Scale bars=100 μ m.

which are around both dorsal kineties and cirri; cortical granules colourless, blood cell shaped, scattered throughout the cell body (Figs. 1F, 3E, F).

The adoral zone of membranelles distinct; about 1/3 of cell length, and composed of about 40 membranelles (Fig. 3D, I), left anterior corner a minute process causing a break (Fig. 3C, D, arrowhead). Bicornate of frontal cirri slightly enlarged, composed of about 6-9 cirral pairs, extending as a midventral complex consecutively (Fig. 3I). Midventral complex distinctly separated rows, composed of 26-35 cirri containing anterior, single cirri (Fig. 3G, arrow) in middle portion, posterior portion. Two or three frontoterminal cirri behind

the distal end of the adoral zone (Fig. 3I, arrows); invariably three dorsal kineties (Figs. 1H; 3C, arrows); of particular interest, there is no buccal cirrus and transverse cirri.

Distribution. Adriatic Sea, and Korea (this study).

Remarks. Kahl (1932) established the genus *Uroleptopsis* and described firstly *U. citrina*. Later, Berger (2004) re-described *U. citrina* of the Adriatic Sea by its morphology and morphogenesis. *Uroleptopsis citrina* has a gap in the adoral zone and lacks transverse cirri. The loss of the transverse cirri is the main diagnostic character to separate *U. citrina* from other Pseudokeronopsidae species. This species has conspicuous differences from the congener *U. ignea* as fol-

Table 2. Morphometric characterization of *Uroleptopsis citrina*

	Min	Max	Mean	SD	SE	CV	n
Body length (µm)	95	155	122.5	14.4	3.2	11.7	20
	99	188	154	19.9	3.6	12.9	31
Body width (µm)	20	45	30	5.9	1.3	19.4	20
	26	57	42	7.2	1.3	17.5	31
Length of buccal field (µm)	37.5	50	42.5	3.3	0.7	7.8	20
	32	54	46	5.0	0.9	10.9	31
No. of membranelles	35	43	40	2.3	0.5	5.9	20
	29	47	39	4.0	0.7	10.2	31
No. of cirral pairs in bicorona	6	9	8	0.9	0.2	12.2	20
	6	10	7	1.2	0.2	15.3	31
No. of frontoterminal cirri	2	3	2	0.4	0.1	19.0	19
	2	3	2	0.5	0.1	20.5	31
No. of midventral pairs in anterior portion of MVC	5	9	7	1.1	0.2	15.1	19
	4	15	7	2.0	0.4	27.6	31
No. of non-paired midventral cirri	3	15	8	3.5	0.8	41.0	19
	4	18	11	4.5	0.8	43.5	31
No. of midventral pairs in posterior portion of MVC	2.5	8.5	3.5	2.0	0.5	43.0	19
	2.5	14	5.5	3.3	0.6	48.2	31
No. of total Midventral cirri	26	41	32	3.9	0.9	12.3	19
	26	53	38	6.4	1.2	16.4	31
No. of cirri in left marginal row	26	41	31	3.9	0.9	12.3	20
	28	49	38	6.1	1.1	15.7	31
No. of cirri in right marginal row	29	53	37	6.1	1.4	16.2	20
	34	63	48	6.3	1.1	13.3	31
No. of dorsal kineties	3	3	3	0	0	0	20
	3	3	3	0	0	0	31

All data, including the Korean population (first line) and the Adriatic Sea population (second line), are based on protargol-impregnated specimens. The Data of the Adriatic Sea population is cited from Berger et al. (2004). Min, minimum; Max, maximum; Mean, arithmetic mean; SD, standard deviation; SE, standard error of the mean; CV, coefficient of variation in %; n, number of individuals examined; MVC, midventral complex.

lows: presence of a buccal cirrus and the pattern of the midventral complex. Circumstantially, *U. citrina* lacks a buccal cirrus in the ordinary position, right of the paroral, whereas is present in *U. ignea*. Also, the anterior and posterior portion of the midventral complex in this species primarily consist of ordinary midventral pairs; the middle portion is composed only of the right cirri of the cirral pairs, whereas the anterior portion of the midventral complex in *U. ignea* is composed of paired cirri, and the middle and posterior portion consist of non-paired cirri (Mihailowitsch and Wilbert, 1990). Yellow cortical granules and ring-shaped structures are underneath the cell surface. Consequently, *U. ignea* is transferred to the subgenus *Uroleptopsis* (*Plesiouroleptopsis*) by Berger (2004).

Also, *U. citrina* is a little different from *Pseudokeronopsis flava* in that the cell colour is yellow. However, *P. flava* has one buccal cirrus in the ordinary position, 2-4 transverse cirri, 3-4 dorsal kineties, and lacks a break in the adoral zone (Song et al., 2004).

The Korean population, *U. citrina*, has a few differences

from the Adriatic Sea population of *U. citrina* (Berger, 2004) as follows: (1) left marginal cirri (26-41 vs. 28-49); (2) right marginal cirri (29-53 vs. 34-63); and (3) single midventral cirri (on average 8 vs. 11). Additionally, we ascertained that the sequence was successfully amplified on the partial region of the SSU rRNA gene and the amplified sequence length is 1,754 bp (GenBank accession no: JN714477) and shows 99.88% similarity with that of Chinese population (GenBank accession no: GU437211). Unfortunately, no Adriatic Sea population sequence is available in GenBank.

ACKNOWLEDGEMENTS

This study was supported by the Invasive Species Management Program in Marine Ecosystem, Korean Ministry of Land, Transport & Maritime Affairs of Korean Government, and also funded by the National Fisheries Research & Development Institute (NFRDI) of Korea and Polar Academic Program (PAP), KOPRI.

REFERENCES

- Berger H, 2004. *Uroleptopsis* Kahl, 1932 (Ciliophora : Hypotricha): morphology and cell division of type species, redefinition, and phylogenetic relationships. *Acta Protozoologica*, 43:99-121.
- Berger H, 2006. Monograph of the Urostyleloidea (Ciliophora, Hypotricha). Springer, Dordrecht, pp. 832-1001.
- Borror AC, 1972. Revision of the order Hypotrichida (Ciliophora, Protozoa). *Journal of Eukaryotic Microbiology*, 19:1-23.
- Borror AC, Wicklow BJ, 1983. The suborder Urostylelina Jankowski (Ciliophora, Hypotrichida): morphology, systematics and identification of species. *Acta Protozoologica*, 22:97-126.
- Cohn F, 1866. Neue Infusorien im Seeaquarium. *Zeitschrift für Wissenschaftliche Zoologie*, 16:253-302.
- Entz G, 1884. Über Infusorien des Golfes von Neapel. *Mitteilungen aus der Zoologischen Station zu Neapel*, 5:359-364.
- Foissner W, 1991. Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. *European Journal of Protistology*, 27:313-330.
- Hall TA, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41:95-98.
- Hu X, Song W, 2001. Morphological redescription and morphogenesis of the marine ciliate, *Pseudokeronopsis rubra* (Ciliophora: Hypotrichida). *Acta Protozoologica*, 40:107-115.
- Hu X, Warren A, Suzuki T, 2004. Morphology and morphogenesis of two marine ciliates, *Pseudokeronopsis pararubra* sp. n. and *Amphisiella annulata* from China and Japan (Protozoa: Ciliophora). *Acta Protozoologica*, 43:351-368.

- Jeanmougin F, Thompson JD, Gouy M, Higgins DG, Gibson TJ, 1998. Multiple sequence alignment with Clustal X. *Trends in Biochemical Sciences*, 23:403-405.
- Jung JH, Baek YS, Min GS, 2011. New record of two *Apokeronopsis* species (Ciliophora: Urostylida: Pseudokeronopsidae) from Korea. *Korean Journal of Systematic Zoology*, 27:115-122.
- Kahl A, 1932. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria), 3. Spirotricha. *Tierwelt Deutschlands*, 25:399-650.
- Kahl A, 1933. Ciliata libera et ectocommensalia. *Die Tierwelt der Nord- und Ostsee*, 23:29-146.
- Kimura M, 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16:111-120.
- Kudo R, 1950. *Protozoology*. Charles C Thomas Publisher, Springfield, IL, p. 672.
- Lynn D, 2008. *The ciliated protozoa: characterization, classification, and guide to the literature*. Springer, New York, pp. 1-605.
- Medlin L, Elwood HJ, Stickel S, Sogin ML, 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene*, 71:491-499.
- Mihailowitsch B, Wilbert N, 1990. *Bakuella salinarum* nov. spec. und *Pseudokeronopsis ignea* nov. spec. (Ciliata, Hypotrichida) aus einem solebelasteten Fließgewässer des östlichen Münsterlandes, BRD. *Archiv für Protistenkunde*, 138: 207-219.
- Shi X, Xu R, 2003. Morphology and infraciliature of *Pseudokeronopsis rubra* in Jieshi waters of south China sea. *Journal of Tropical Oceanography*, 22:23-30 (in Chinese with English summary).
- Song W, Sun P, Ji D, 2004. Redefinition of the yellow hypotrichous ciliate, *Pseudokeronopsis flava* (Hypotrichida: Ciliophora). *Journal of the Marine Biological Association of the United Kingdom*, 84:1137-1142.
- Song W, Warren A, Roberts D, Wilbert N, Li L, Sun P, Hu X, Ma H, 2006. Comparison and redefinition of four marine, coloured *Pseudokeronopsis* spp. (Ciliophora: Hypotrichida), with emphasis on their living morphology. *Acta Protozoologica*, 45:271-287.
- Song W, Wilbert N, Warren A, 2002. New contribution to the morphology and taxonomy of four marine hypotrichous ciliates from Qingdao, China (Protozoa: Ciliophora). *Acta Protozoologica*, 41:145-162.
- Sonnenberg R, Nolte AW, Tautz D, 2007. An evaluation of LSU rDNA D1-D2 sequences for their use in species identification. *Frontiers in Zoology*, 4:6.
- Tamura K, Dudley J, Nei M, Kumar S, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24:1596-1599.
- Wirnsberger E, Larsen HF, Uhlig G, 1987. Rediagnoses of closely related pigmented marine species of the genus *Pseudokeronopsis* (Ciliophora, Hypotrichida). *European Journal of Protistology*, 23:76-88.

Received September 16, 2011
 Revised November 8, 2011
 Accepted November 16, 2011