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

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**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.
MATERIALS AND METHODS

Sample collection and identification
The specimens of *Pseudokeronopsis carnea* were collected from Incheon harbor in the Yellow Sea (salinity, 28.5±0.5; 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 GTT 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).

Table 1. Morphometric characterization of *Pseudokeronopsis carnea*  

<table>
<thead>
<tr>
<th>Characteristic (μm)</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
<th>CV</th>
<th>n</th>
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<tr>
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<td>220</td>
<td>194</td>
<td>17.0</td>
<td>3.8</td>
<td>8.8</td>
<td>20</td>
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<tr>
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<td>24.8</td>
<td>6.2</td>
<td>9.9</td>
<td>16</td>
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<tr>
<td>Length of buccal field</td>
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<td>75</td>
<td>65</td>
<td>6.2</td>
<td>1.4</td>
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<td>20</td>
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<tr>
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<td>94</td>
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<td>3.3</td>
<td>0.8</td>
<td>3.8</td>
<td>16</td>
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<tr>
<td>No. of cirral pairs in bicornon</td>
<td>56</td>
<td>78</td>
<td>69</td>
<td>6.6</td>
<td>1.5</td>
<td>9.7</td>
<td>20</td>
</tr>
<tr>
<td>No. of buccal cirri</td>
<td>69</td>
<td>79</td>
<td>73.1</td>
<td>3.0</td>
<td>0.8</td>
<td>4.1</td>
<td>16</td>
</tr>
<tr>
<td>No. of frontoterminal cirri</td>
<td>8</td>
<td>12</td>
<td>10</td>
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<td>0.3</td>
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<tr>
<td>No. of transverse cirri</td>
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<td>11</td>
<td>10.1</td>
<td>0.8</td>
<td>0.2</td>
<td>7.6</td>
<td>16</td>
</tr>
</tbody>
</table>

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*동둥이홍색위각모충 (신청)
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 vacuole; 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 μm.
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 μm.
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). Bicorona 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 about 8-12 cirral pairs, extending as a midventral rows; more transverse cirri; from five to seven dorsal kineties (Figs. 1D; 2L, arrows). Material examined. One population was obtained from Incheon harbor on November 2, 2010.

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. flavus, 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 Pseudokeronopsis rubra or P. flavus in confusion. Entz (1884) considered P. carnea as a transitional form between P. rubra and P. flavus. The neotype of P. carnea was fixed by Wimberger 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. decolar and P. ovalis; yellow, P. flavicans and P. flavus; 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).

Genus Uroleptopsis (Uroleptopsis) Kahl, 1932

Uroleptopsis citrina Kahl, 1932 (Table 2, Figs. 1E-H, 3)


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,
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). Bicorona 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) redescribed *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-

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**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 (bicorona); arrows show two frontoterminal cirri. Scale bars=100 μm.
Table 2. Morphometric characterization of *Uroleptopsis citrina*

<table>
<thead>
<tr>
<th></th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
<th>CV</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length (μm)</td>
<td>95</td>
<td>155</td>
<td>122.5</td>
<td>14.4</td>
<td>3.2</td>
<td>11.7</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>188</td>
<td>154</td>
<td>19.9</td>
<td>3.6</td>
<td>12.9</td>
<td>31</td>
</tr>
<tr>
<td>Body width (μm)</td>
<td>26</td>
<td>57</td>
<td>42</td>
<td>7.2</td>
<td>1.3</td>
<td>17.5</td>
<td>31</td>
</tr>
<tr>
<td>Length of buccal field</td>
<td>37.5</td>
<td>50</td>
<td>42.5</td>
<td>3.3</td>
<td>0.7</td>
<td>7.8</td>
<td>20</td>
</tr>
<tr>
<td>(μm)</td>
<td>32</td>
<td>54</td>
<td>46</td>
<td>5.0</td>
<td>0.9</td>
<td>10.9</td>
<td>31</td>
</tr>
<tr>
<td>No. of membranelles</td>
<td>35</td>
<td>43</td>
<td>40</td>
<td>2.3</td>
<td>0.5</td>
<td>5.9</td>
<td>20</td>
</tr>
<tr>
<td>No. of midventral pairs</td>
<td>6</td>
<td>9</td>
<td>8</td>
<td>0.9</td>
<td>0.2</td>
<td>12.2</td>
<td>20</td>
</tr>
<tr>
<td>in anterior portion of MVC</td>
<td>6</td>
<td>10</td>
<td>7</td>
<td>1.2</td>
<td>0.2</td>
<td>15.3</td>
<td>31</td>
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<td>No. of non-paired midventral cirri</td>
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<td>15</td>
<td>7</td>
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<td>0.4</td>
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<tr>
<td>No. of midventral pairs in posterior portion of MVC</td>
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<td>8.5</td>
<td>3.5</td>
<td>2.0</td>
<td>0.5</td>
<td>41.0</td>
<td>19</td>
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<td>No. of total Midventral cirri</td>
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<td>3.3</td>
<td>0.6</td>
<td>48.2</td>
<td>31</td>
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<tr>
<td>No. of cirri in left marginal row</td>
<td>26</td>
<td>41</td>
<td>32</td>
<td>3.9</td>
<td>0.9</td>
<td>12.3</td>
<td>31</td>
</tr>
<tr>
<td>No. of cirri in right marginal row</td>
<td>26</td>
<td>41</td>
<td>32</td>
<td>3.9</td>
<td>0.9</td>
<td>12.3</td>
<td>31</td>
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<tr>
<td>No. of dorsal kineties</td>
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<td>31</td>
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</table>

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.

Also: 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* (*Plesiourroleptopsis*) 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.

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REFERENCES


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