

Redescription of two soil ciliates, *Anteholosticha bergeri* and *Bakuella granulifera*, from South Korea

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Anteholosticha bergeri and *Bakuella granulifera* were isolated from soil samples collected from Muui-dong and Songdo-dong, Incheon and confirmed new to South Korea. Including these two newly recorded species, 11 species of *Anteholosticha* and four species of *Bakuella* have been recorded in South Korea to date. *Anteholosticha bergeri* was discriminated from congeners by following characters: cortical granules, 12–16 macronuclei, 5–8 midventral pairs, 2–3 pretransverse cirri, 4–6 transverse cirri, and three dorsal kineties. *Bakuella granulifera* was identified by cortical granules, 5–11 buccal cirri, 2–5 frontoterminal cirri, 2–5 midventral cirri rows, and 8–12 transverse cirri. The Korean *A. bergeri* population corresponds to the Austrian population, except for the number of marginal and transverse cirri, and the Korean *B. granulifera* population corresponds to the Namibian population, except for body size. In addition, small subunit ribosomal RNA (18S rRNA) gene sequences from both species were determined.

Keywords: *Anteholosticha*, *Bakuella*, Ciliophora, South Korea, Soil, 18S rRNA gene

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INTRODUCTION

Soil ciliates were first reported in South Korea by Shin (1993), and currently, more than 100 species are known (Jung *et al.*, 2017; Kwon *et al.*, 2019). In the plant rhizosphere, soil ciliates mineralize nutrients and play an important role in plant nutrition (Ekelund and Rønn, 1994; Li *et al.*, 2010). They also regulate soil microbial biomass and community composition (Lousier and Bamforth, 1988; Gel'tser, 1991; Acosta-Mercado and Lynn, 2004), making them a reliable soil environmental biomarker (Foissner, 1987a; 1997; 1999; Li *et al.*, 2010).

The genus *Anteholosticha* Berger, 2003 is characterized by a continuous adoral zone of membranelles, three enlarged frontal cirri, buccal cirrus or cirri, frontoterminal cirri, midventral pairs only, more midventral pairs than number of transverse cirri, one left and one right marginal rows, and caudal cirri lacking. Ten *Anteholosticha* species have been recorded in South Korea (Jung *et al.*, 2017; Moon *et al.*, 2017; Kim and Min, 2018).

The genus *Bakuella* Agamaliyev & Alekperov, 1976 is characterized by a continuous adoral zone of membranelles, three frontal cirri, one or more buccal cirri, two or more frontoterminal cirri, midventral complex composed

of midventral pairs and midventral rows, one left and one right marginal row, and transverse cirri (Berger, 2006). Three *Bakuella* species have been recorded in Korea (Jo *et al.*, 2015; Kim *et al.*, 2018): *Bakuella (Bakuella) incheonensis* Jo *et al.*, 2015, *Bakuella (Psuedobakuella) litoralis* Jo *et al.*, 2015, and *Bakuella marina* Agamaliyev and Alekperov, 1976.

Herein, we report two soil ciliate species new to Korean and provide morphological descriptions and the small subunit ribosomal RNA (18S rRNA) gene sequences.

MATERIALS AND METHODS

Anteholosticha bergeri and *Bakuella granulifera* were isolated from soil samples in Muui-dong (37°23'45"N 126°25'32"E) and Songdo-dong (37°23'27"N 126°40'00"E), Incheon, Korea, respectively. The soil samples were placed in Petri dishes, mixed with tap water, and incubated at room temperature (Foissner *et al.*, 2002). Raw cultures were microscopically observed *in vivo* (Leica DM2500; Wetzlar, Germany) at ×50 to ×1000 magnification. Cell staining was performed as described by Foissner (2014) (Procedure A). Classification and terminology follow

Table 1. Morphometric data for *Anteholosticha bergeri* (upper line) and *Bakuella granulifera* (lower line).

Characteristic ^a	Mean	SD	SE	CV	Min	Max	n
Body length, μm	73.2	9.3	1.5	9.3	62	86	17
	156.3	14.2	3.2	9.1	128	185	20
Body width, μm	21.4	3.6	0.8	16.8	15	30	17
	55.1	6.4	1.4	11.6	40	65	20
Adoral zone length, μm	18.1	1.3	0.3	7.3	16	20	17
	64	7.2	1.6	11.3	52	82	20
AM, number	15.1	1.1	0.2	7.4	13	17	17
	41.6	3.8	0.8	9.1	36	50	20
BC, number	1	0.0	0.0	0.0	1	1	17
	7.5	1.5	0.3	20.2	5	11	20
FC, number	3	0.0	0.0	0.0	3	3	17
	3	0.0	0.0	0.0	3	3	20
FTC, number	2	0.0	0.0	0.0	2	2	17
	2.4	0.5	0.1	20.8	2	3	20
MC, number	13.4	1.9	0.4	14.2	11	16	17
	–	–	–	–	–	–	–
MP, number	6.5	1.1	0.2	16.4	5	8	17
	14.6	3.1	0.7	21.1	9	19	20
MV, number	–	–	–	–	–	–	–
	4.0	0.8	20.9	0.2	2	5	20
MV 1, number of cirri	–	–	–	–	–	–	–
	4.4	1.2	0.3	27.2	3	7	20
MV 2, number of cirri	–	–	–	–	–	–	–
	5.9	1.9	0.4	32.9	4	10	20
MV 3, number of cirri	–	–	–	–	–	–	–
	7.9	1.5	0.3	18.5	4	10	19
MV 4, number of cirri	–	–	–	–	–	–	–
	7.7	2.0	0.5	26.4	3	10	15
MV 5, number of cirri	–	–	–	–	–	–	–
	7.8	1.5	0.3	19.4	6	9	4
PTC, number	2.1	0.2	0.1	11.8	2	3	17
	–	–	–	–	–	–	–
TC, number	5.2	0.7	0.2	14.1	4	6	17
	9.4	1.2	0.3	12.6	8	12	20
LMC, number	22.4	2.4	0.5	10.6	17	26	17
	49.6	7.1	1.6	14.2	39	67	20
RMC, number	22.5	2.3	0.5	10.1	18	27	17
	52.2	7.0	1.6	13.4	42	71	20
DK, number	3	0.0	0.0	0.0	3	3	17
	3	0.0	0.0	0.0	3	3	20
Ma, number	13.8	1.4	0.3	10.3	12	16	17
	145.2	17.3	3.9	11.9	109	180	20
Mi, number	2.6	1.1	0.3	43.3	1	6	17
	–	–	–	–	–	–	–

AM, adoral membranelles; BC, buccal cirrus; CV, coefficient of variation in %; DB, dorsal bristles; DK, dorsal kineties; FC, frontal cirri; FTC, frontoterminal cirri; LMC, left marginal cirri; Ma, macronuclear nodules; Max, maximum; MC, midventral cirri; Mi, micronuclei; Min, minimum; MP, midventral pairs; MV, midventral rows; n, number of specimens examined; PTC, pretransverse cirrus; RMC, right marginal cirri; SD, standard deviation; SE, standard error of the arithmetic mean; TC, transverse cirri.

^aData based on randomly selected, protargol-stained specimens.

Berger (2006) and Foissner (2016). Gene amplification and sequencing were performed according to the methods of Jung *et al.* (2012). The 18S rRNA gene sequences of 11 *Anteholosticha* species and five *Bakuella* species

used for calculating the pairwise distances were retrieved from GenBank. Pairwise distances were calculated using MEGA 5.0 (Tamura *et al.*, 2011), based on the Kimura-2 parameter model.

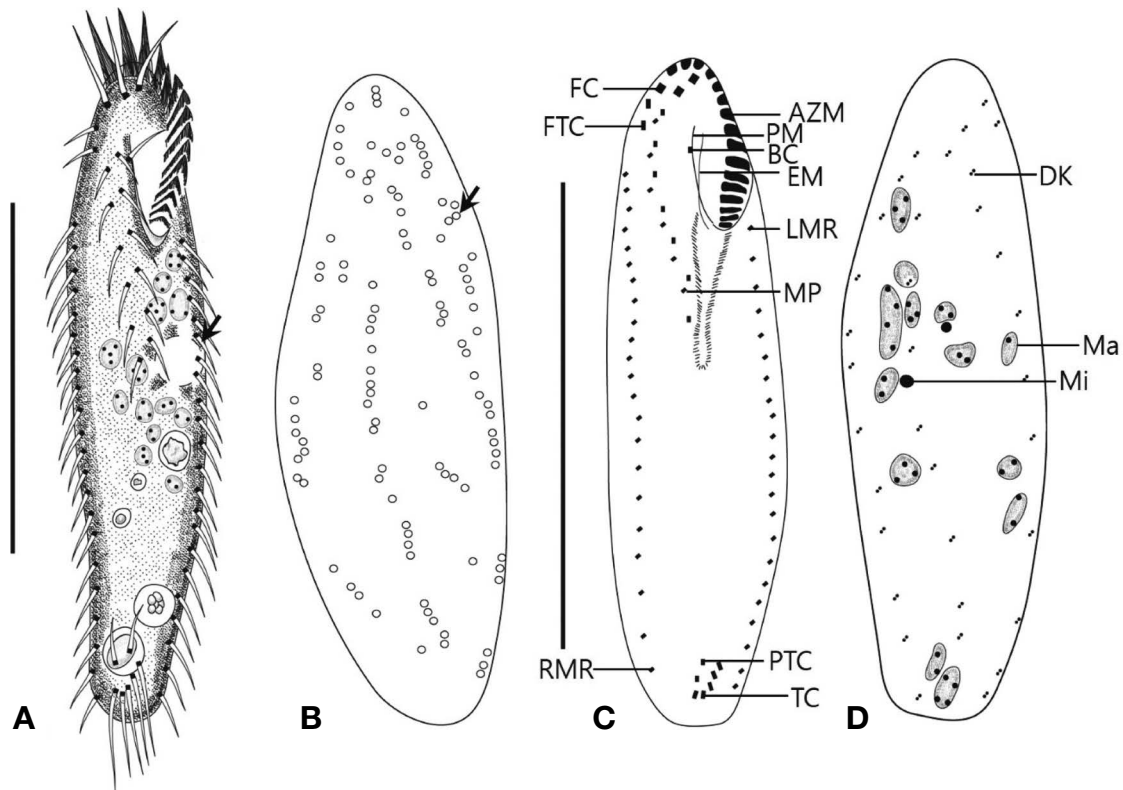


Fig. 1. Morphology of *Anteholosticha bergeri* from live (A, B) and protargol-impregnated (C, D) specimens. A, Ventral view arrow denotes a contractile vacuole; B, Pattern of cortical granules (arrow) on dorsal side; C, D, Ventral and dorsal view of typical specimen; AZM, adoral zone of membranelles; BC, buccal cirrus; DK, dorsal kinety; EM, endoral membrane; FC, frontal cirri; FTC, frontoterminal cirri; LMR, left marginal cirral row; Ma, macronuclear nodules; Mi, micronuclei; MP, midventral pair; PM, paroral membrane; PTC, pretransverse cirri; RMR, right marginal cirral row; TC, transverse cirri; Scale bars: 50 μm (A, C).

SYSTEMATIC ACCOUNTS

Phylum Ciliophora Doflein, 1901
 Class Spirotrichea Bütschli, 1889
 Order Urostylida Jankowski, 1979
 Family Urostylidae Bütschli, 1889
 Genus *Anteholosticha* Berger, 2003

1. *Anteholosticha bergeri* (Foissner, 1987b) Berger, 2003 (Figs. 1, 2)

Holosticha bergeri Foissner, 1987b: p. 197; Blatterer & Foissner, 1988: p. 50; Berger, 2001: p. 33.
Anteholosticha bergeri Berger, 2003: p. 377.

Korean name. 인두원전열하모충

Diagnosis of the Korean population. Size *in vivo* about $90 \times 20 \mu\text{m}$, ellipsoidal body shape; 12–16 macronuclear nodules and 1–6 micronuclei; contractile vacuole at left of mid-body; 13–17 adoral membranelles; cortical granules present; three frontal cirri; two frontoterminal cirri; one buccal cirrus; 5–8 midventral pairs; 2–3 pretransverse and 4–6 transverse cirri; 17–26 left and 18–27 right

marginal cirri; 3 dorsal kineties; caudal cirri lacking.

Description. Body size $80\text{--}100 \times 10\text{--}20 \mu\text{m}$ *in vivo*, $60\text{--}85 \times 15\text{--}30 \mu\text{m}$ after protargol preparations (Table 1). Body outline usually elongated elliptical, both ends widely rounded and flexible (Figs. 1C, 2F). 12–16 macronuclear nodules scattered irregularly, with a size of $4 \times 2.2 \mu\text{m}$ after protargol preparation (Fig. 2F, arrow). 1–6 micronuclei, size about $1.2 \mu\text{m}$ diameter after protargol preparation (Fig. 2F, arrowhead). Contractile vacuole in left mid-body margin, about $7.5 \mu\text{m}$ in diameter (Fig. 2B, arrow). Yellowish cortical granules usually arranged in longitudinal rows along with dorsal kineties (Figs. 1B, 2C, 2D). Cytoplasm colorless (Fig. 2C).

Adoral zone of membranelles approximately 25% of body length and composed of 13–17 membranelles. Pharynx conspicuous in protargol preparation with short rods in the wall (Fig. 2H, arrow). Three frontal cirri length $13 \mu\text{m}$ long *in vivo* (Fig. 1A, 1C); two frontoterminal cirri; one buccal cirrus about $10 \mu\text{m}$ long in life (Fig. 1A, 1C). Midventral complex with zigzagging pattern consisting of 5–8 pairs and terminates slightly ahead of middle of the body (Figs. 1C, 2F). Two to three pretransverse and 4–6

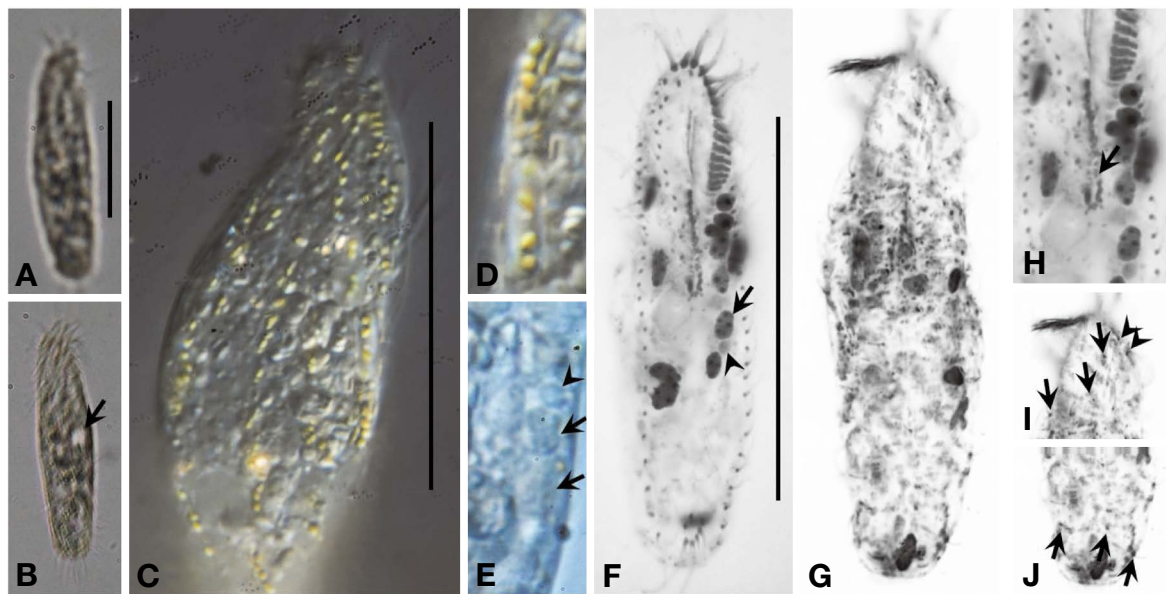


Fig. 2. Photomicrographs of *Anteholosticha bergeri* from live (A–E) and after protargol impregnation (F–J). A, B, Ventral view showing contractile vacuole (arrow); C, D, Dorsal view showing yellowish cortical granules; E, Ventral view showing macronuclear nodules (arrows) and micronuclei (arrowhead) from live; F, G, Ventral and dorsal view of typical specimen showing macronuclear nodules (arrow) and micronuclei (arrowhead); H, Ventral view of midbody showing pharyngeal fibres (arrow); I, J, Dorsal views showing three dorsal kineties (arrows), two dikinetids above right marginal row (arrowhead); Scale bars: 50 µm (A, C, F).

Table 2. The number of nucleotides difference (above the diagonal) and pairwise distance (below the diagonal) between selected genus *Anteholosticha* 18S rRNA sequence.

	1	2	3	4	5	6	7	8	9	10	11	12
1. <i>Anteholosticha bergeri</i> MT809479	–	16	51	52	53	57	65	72	69	70	72	93
2. <i>Anteholosticha gracilis</i> KF306397	0.010	–	56	56	58	64	75	80	78	75	79	96
3. <i>Anteholosticha paramanca</i> KF806443	0.033	0.036	–	69	19	78	90	92	92	37	93	100
4. <i>Anteholosticha intermedia</i> AJ277876	0.034	0.036	0.045	–	71	71	74	80	71	84	71	87
5. <i>Anteholosticha multicirrata</i> KC307773	0.034	0.038	0.010	0.046	–	81	90	92	93	35	94	101
6. <i>Anteholosticha monilata</i> GU942567	0.037	0.042	0.051	0.046	0.053	–	68	63	48	97	47	75
7. <i>Anteholosticha randani</i> KU234524	0.043	0.049	0.059	0.048	0.059	0.044	–	81	71	106	72	76
8. <i>Anteholosticha pseudomonilata</i> HM568416	0.045	0.051	0.061	0.046	0.061	0.031	0.046	–	65	112	67	83
9. <i>Anteholosticha rectangula</i> KU175624	0.047	0.053	0.061	0.052	0.061	0.041	0.053	0.042	–	112	5	61
10. <i>Anteholosticha manca</i> DQ503578	0.046	0.049	0.024	0.055	0.022	0.064	0.071	0.075	0.075	–	113	118
11. <i>Anteholosticha marimonilata</i> FJ870075	0.047	0.052	0.061	0.047	0.062	0.030	0.047	0.003	0.044	0.075	–	62
12. <i>Anteholosticha pulchra</i> JN880476	0.061	0.064	0.066	0.057	0.067	0.049	0.050	0.040	0.055	0.079	0.040	–

The bold letters indicate the differences between *Anteholosticha bergeri* and *Anteholosticha gracilis*.

transverse cirri (Figs. 1C, 2F). One left (17–26 cirri) and right (18–27 cirri) marginal row, both marginal rows non-confluent posteriorly (Fig. 1C). Three dorsal kineties (Figs. 1D, 2I, 2J) and two additional basal bodies at right-ahead of dikinetids 3 (Fig. 2I, arrowhead). Caudal cirri lacking (Fig. 1D).

Distribution. Antarctica, Austria, Australia, and South Korea (This study).

Remarks. There are more than 40 species in the genus

Anteholosticha (Berger, 2003; 2006; 2008; Li *et al.*, 2007; 2008; 2011; Kumar *et al.*, 2010; Xu *et al.*, 2011; Park *et al.*, 2012; 2013; Fan *et al.*, 2014; 2016; Jung *et al.*, 2016; Chen *et al.*, 2018; 2020). Of these, *A. sigmoidata* (Foissner, 1982) Berger, 2003 and *A. brachysticha* (Foissner, 2002) Berger, 2003 are the most similar to *A. bergeri* based on their elliptical cell shape, cortical granules present and more than two macronuclear nodules. *Anteholosticha bergeri* is very similar to *A. sigmoidata* in elliptical cell shape

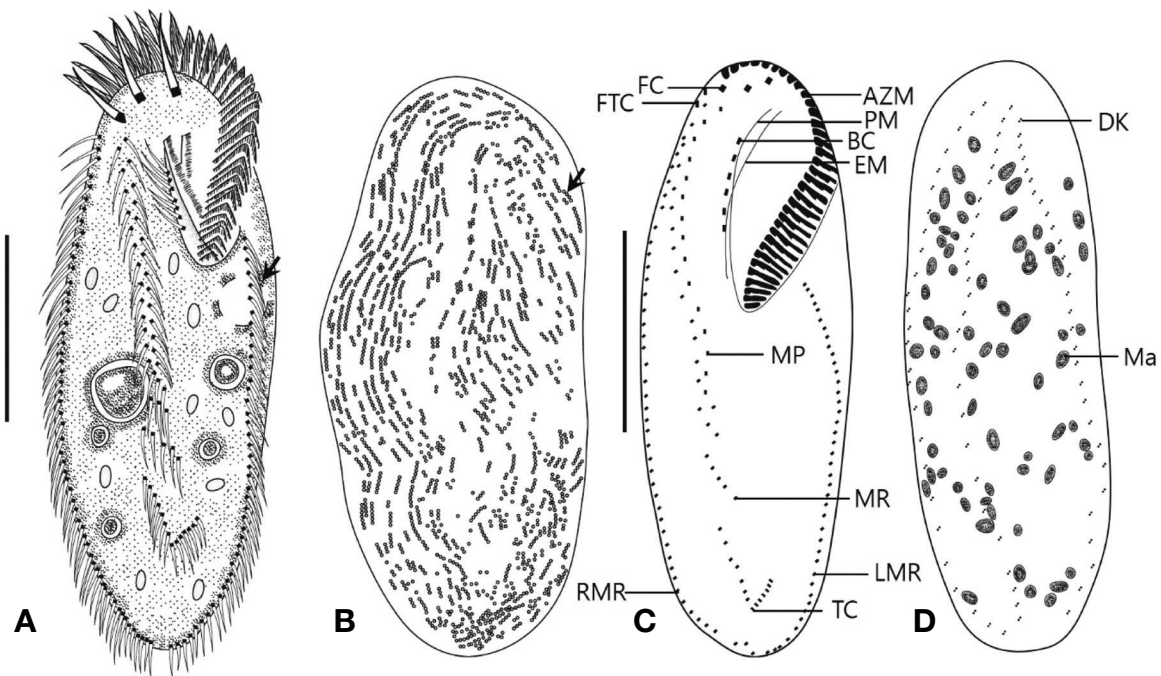


Fig. 3. Morphology of *Bakuella granulifera* from live (A, B) and protargol-impregnated (C, D) specimens. A, Ventral view of typical specimen live arrow denotes contractile vacuole; B, Pattern of cortical granules on dorsal side (arrow); C, D, Ventral and dorsal view of typical specimen; AZM, adoral zone of membranelles; BC, buccal cirri; DK, dorsal kinety; EM, endoral membrane; FC, frontal cirri; FTC, fronto-terminal cirri; LMR, left marginal cirral row; Ma, macronuclear nodules; Mi, micronuclei; MP, midventral pair; MR, midventral row; PM, paroral membrane; RMR, right marginal cirral row; TC, transverse cirri; Scale bars: 50 μ m (A, C).

and cortical granules present. However, *A. bergeri* differs from *A. sigmoida* in the number of adoral zone membranelles (13–17 vs. 16–28), midventral cirri (11–16 vs. 15–36), macronuclei (12–16 vs. 6–12), and dorsal kineties (three vs. four) (Foissner, 1982). *Anteholosticha bergeri* and *A. brachysticha* have yellowish cortical granules, but *A. bergeri* differs in the number of pretransverse cirri (two vs. one), transverse cirri (4–6 vs. 2–3), and macronuclei (12–16 vs. 28–38) (Foissner, 2002).

The Korean population of *A. bergeri* corresponds to type population from Austria based on body size, number of midventral cirri pairs, and the number of macronuclear nodules. However, they differ in the number of left and right marginal cirri (17–26 and 18–27 vs. 13–16 and 10–15) and transverse cirri (4–6 vs. 3–4) (Foissner, 1987a).

In addition, the cytopharynx structure of the Korean and Australian population (distinguishable) differ from that of original population (indistinguishable). The cytopharynx structure can be important feature to classify hypotrich species (Kim *et al.*, 2016; Jung *et al.*, 2019). Thus, they can be identified possibility as new population or subspecies. However, further studies are needed to confirm the cytopharynx structure pattern of the three population.

The 18S rRNA gene sequence of *A. bergeri* was 1663 bp in length (GenBank accession number: MT809479).

The closest species to *A. bergeri* in the genus *Anteholosticha* based on the pairwise distance of the 18S rRNA sequences is *A. gracilis*. The pairwise distance between *A. bergeri* and *A. gracilis* was very low at 0.01 (Table 2).

Deposition. The voucher slide with protargol-impregnated specimens is deposited in the National Institute of Biological Resources in Korea (NIBRPR0000110227).

Family Bakuellidae Jankowski, 1979

Genus *Bakuella* Agamaliev & Alekperov, 1976

2. *Bakuella granulifera* Foissner *et al.*, 2002 (Figs. 3, 4)

Bakuella granulifera Foissner *et al.*, 2002: p. 561, figs.

129a–k, 380a–c, f–h, table. 111; Berger, 2006: p. 569.

Korean name. 과립바쿠하모충

Diagnosis of the Korean population. Size *in vivo* about 160 \times 55 μ m, elongate and ellipsoidal body shape; 109–180 macronuclear nodules; contractile vacuole at left of mid-body; 36–50 adoral membranelles; cortical granules present; three frontal cirri; 2–3 frontoterminal cirri; 5–11 buccal cirrus; 9–19 midventral pairs; 2–5 midventral rows; 8–12 transverse cirri; 39–67 left and 42–71 right marginal cirri; three dorsal kineties.

Description. Body size of specimens *in vivo* 140–

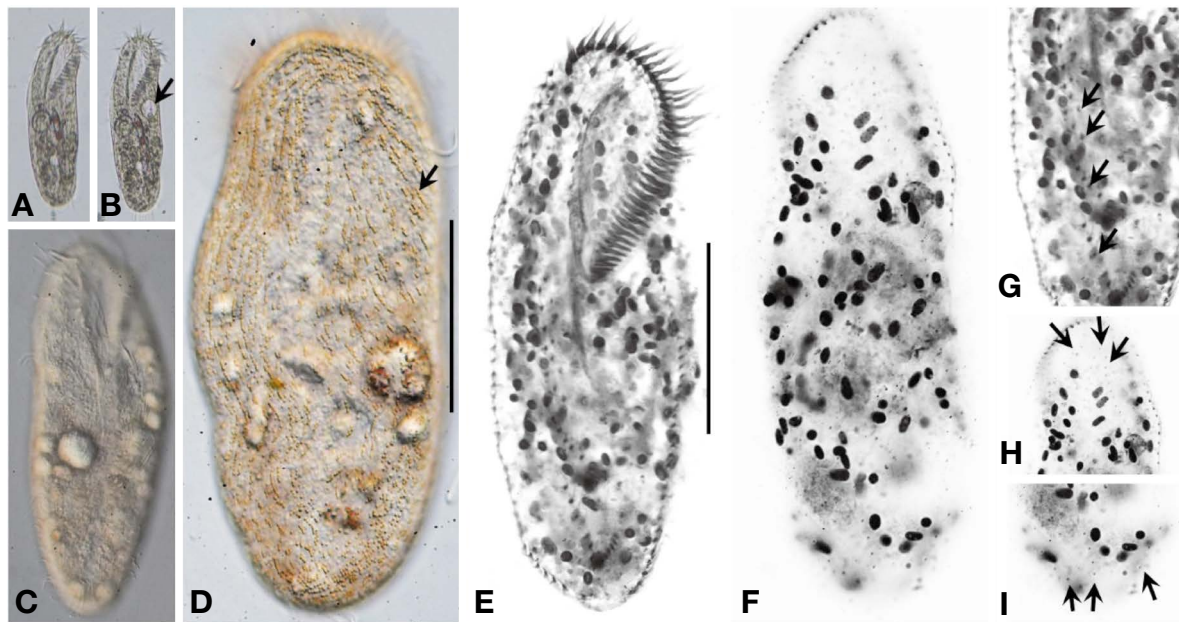


Fig. 4. Photomicrographs of *Bakuella granulifera* from live (A–D) and after protargol impregnation (E–I). A, B, Ventral views arrow denotes contractile vacuole; C, Ventral view showing elliptical body shape; D, Dorsal view showing pattern of cortical granules (arrow); E, F, Ventral and dorsal views of typical specimen; G, Ventral view of midbody showing midventral rows (arrows); H, I, Three dorsal kineties (arrows); Scale bars: 50 μm (D, E).

200 \times 50–80 μm , 130–185 \times 40–65 μm after protargol impregnation (Table 1). Body shape elongated elliptical, both ends of cell rounded and cortex flexible (Figs. 3C, 3D, 4E, 4F). Contractile vacuole on the left side of mid body, about 14 μm in diameter (Figs. 2A, 4B, arrow). On average 145 macronuclear nodules scattered irregularly, size in about 5–7 \times 3–4 μm after protargol preparations (Figs. 3D, 4E, 4F). Yellow cortical granules arranged in short series (Figs. 3B, 4D, arrow), cytoplasm colorless (Fig. 4C, 4D).

Adoral zone of membranelles conspicuous, about 41% of the body length, and composed of 36–50 membranelles (Fig. 3A, 3C). Both paroral and endoral membranes curved leftward anteriorly (Fig. 3C). Three enlarged frontal cirri about 25 μm long *in vivo* (Fig. 3A, 3C); 2–3 frontoterminal cirri about 13 μm long *in vivo* located near distal end of adoral zone (Fig. 3A, 3C). Buccal cirral row composed of 5–11 cirri about 12 μm long *in vivo* (Fig. 3A, 3C). Midventral complex composed of 9–19 midventral pairs and 2–5 midventral rows (Figs. 3C, 4G, arrows). 8–12 transverse cirri at posterior end of rightmost midventral row (Fig. 3C). One left marginal row and one right marginal row composed of 39–67 and 42–71 cirri, respectively (Fig. 3C). Three dorsal kineties arranged on the dorsal side of the specimens (Figs. 3D, 4H, 4I, arrows). Caudal cirri lacking (Fig. 3D).

Distribution. Austria, China, Namibia, and South Korea (This study).

Remarks. A total of 12 species belongs to the genus *Bakuella* (Agamaliev and Alekperov, 1976; Borror and Wicklow, 1983; Mihailowitsch and Wilbert, 1990; Eigner and Foissner, 1992; Song *et al.*, 1992; Foissner *et al.*, 2002; Foissner, 2004; Chen *et al.*, 2013; Jo *et al.*, 2015). Of these, *B. pampinaria pampinaria* Eigner & Foissner, 1992 and *B. edaphoni* Song, Wilbert & Berger, 1992 are most similar to *B. granulifera* based on their multiple buccal cirri, multiple midventral rows, and three dorsal kineties (Eigner and Foissner, 1992; Song *et al.*, 1992).

Considering the body shape, midventral row and yellowish cortical granules, *B. granulifera* is the most similar to *B. pampinaria pampinaria* Eigner & Foissner, 1992. However, the former differs from the latter in the number of buccal cirri (5–11 vs. 3–6), frontoterminal cirri (2–3 vs. 5–8), and transverse cirri (8–12 vs. 2–5) (Eigner and Foissner, 1992). *Bakuella granulifera* differs from *B. edaphoni* Song, Wilbert & Berger, 1992 in the number of midventral cirri rows (2–5 vs. 5–10) (Song *et al.*, 1992).

The morphology of the Korean population corresponds to the type population from Namibia, but the former population differs from the type population in body size (128–185 \times 40–65 μm vs. 274–396 \times 77–144 μm) and the number of cirri of rearmost midventral row (6–9 vs. 9–14) (Foissner *et al.*, 2002). The Korean population morphology corresponds to the Chinese population, but the former population differ in number of cirri of rearmost midventral row (6–9 vs. 8–18) (Chen *et al.*, 2020b).

Table 3. The number of nucleotides difference (above the diagonal) and pairwise distance (below the diagonal) between selected genus *Bakuella* 18S rRNA sequence.

	1	2	3	4	5	6
1. <i>Bakuella granulifera</i> MT800874	–	1	6	40	41	42
2. <i>Bakuella granulifera</i> KY873998	0.001	–	7	39	40	41
3. <i>Bakuella granulifera</i> KJ958489	0.004	0.004	–	37	38	39
4. <i>Bakuella incheonensis</i> KR024011	0.026	0.025	0.024	–	10	2
5. <i>Bakuella litoralis</i> KR024010	0.026	0.026	0.024	0.006	–	12
6. <i>Bakuella subtropica</i> KC631826	0.027	0.026	0.025	0.001	0.008	–

The bold letters indicate the differences between the population of *Bakuella granulifera*.

The 18S rRNA gene sequence of *B. granulifera* was 1749 bp in length (GenBank accession number: MT800874). The closest species to *B. granulifera* in the genus *Bakuella* based on the pairwise distance of the 18S rRNA sequences is *B. incheonensis*. The intraspecific variation of *B. granulifera* was 0.001–0.004 and the pairwise distance between *B. granulifera* and *B. incheonensis* was 0.026 (Table 3).

As mentioned above, the Korean population can be distinguished from the type population in terms of body size and the number of cirri of rearmost midventral row. These differences can be discussed at the subspecies level rather than the same species. However, unfortunately, the 18S rRNA gene sequences of the type population has not been reported. In addition, the Chinese population lacks morphological information. Thus, in order to clarify, information of the morphology and gene sequences of other populations of *B. granulifera* is needed.

Deposition. The voucher slide with protargol-impregnated specimens is deposited in the National Institute of Biological Resources in Korea (NIBRPR0000110228).

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