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First records of nine free-living heterotrophic flagellates from South Korea

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Nine free-living heterotrophic flagellates were cultured from marine intertidal sediments and freshwater sediments from Korea. These species are described with uninterpreted records based on light microscopy of living cells and reported taxonomically for the first time from Korea. Diagnostics of these species are as follows; *Notosolenus hemicircularis*: 9−11.8 μm long with flagellar reservoir, ventrally flattened and dorsally convex with hyaline semicircular collar around short anterior neck, and 8 ridges on cell surface. *Thecamonas tranhens*: 4.5−7.1 μm long, plastic with proboscis comprising an anterior flagellum surrounded by membranous sleeve. *Bodomorpha minima*: 4.5−7.0 μm long, rigid with small rostrum in anterior end and active anterior flagellum. *Cercomonas hiberna*: 5.6−10.9 μm long, very plastic with pseudopodia, cytoplasmic strand and 1 or 2 contractile vacuoles. *Cercomonas pellucida*: 7.5−13 μm long, plastic with pseudopodia, cytoplasmic strand and single contractile vacuole. With nucleus closely connected to basal bodies. *Eocercomonas echina*: 4.7−6.5 μm long, plastic with pseudopodia, cytoplasmic strand and 1 or 2 contractile vacuoles. *Paracercomonas astra*: 5.7−7.3 μm long, moderately metabolic with pseudopodia, cytoplasmic strand and 1 or 2 contractile vacuoles. *Paracercomonas minima*: 5−9 μm long, metabolic with pseudopodia, cytoplasmic strand and single contractile vacuole. *Paracercomonas producta*: 6.1−9.9 μm long, very metabolic with pseudopodia, long cytoplasmic strand and single contractile vacuole.

Keywords: *Bodomorpha*, *Cercomonas*, *Eocercomonas*, heterotrophic flagellates, *Notosolenus*, *Paracercomonas*, *Thecamonas*

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Introduction

Heterotrophic flagellates are important numerically and ecologically in aquatic ecosystems (Azam *et al*., 1983; Sherr and Sherr, 1988; Lee and Patterson, 2002). Despite their importance, their taxonomy has been little studied. Previous taxonomical studies of heterotrophic flagellates (excluding dinoflagellates and haptophytes) in Korea are Lee (2002; 2015a; 2015b), Park *et al*. (2006; 2007), Heiss *et al*. (2015), Lee and Park (2016), Jhin and Park (2019), and Lax *et al*. (2019). These studies have revealed 108 species to date (NIBR, 2012; NMBIK, 2018). Most of these studies were carried out in the marine habitats. Studies in the freshwater habitats are needed to reveal the flagellate diversity in Korea.

This study reports for the first time the occurrences of *Notosolenus hemicircularis*, *Thecamonas tranhens*, *Bodomorpha minima*, *Cercomonas hiberna*, *C. pellucida*, *Eocercomonas echina*, *Paracercomonas astra*, *P. minima* and *P. producta* in Korea. These genera, with the exceptions of *Cercomonas* and *Notosolenus*, are new to Korea.

Materials and Methods

Isolation, cultivation and light microscopy

Samples were collected from intertidal marine sediments and freshwater sediments to a depth of about 1 cm from a 0.25 m^2 quadrat. To remove macrofauna, the sediment was sieved and then placed in a plastic tray in 1 cm deep layers. The sediment materials were covered with lens tissue and coverslips were placed on the lens tissue. After 12 hours the coverslips were removed and flagellates were observed with a Leica DMR microscope (Germany) equipped with a Zeiss Axiocam HR digital camera and its associated software (Axiovision 4.6). A single cell of each species was isolated by micro-pipetting from a coverslip. The cells were inoculated into a well plate con-

taining sterile medium [0.5−1% v/v Lysogeny-Broth (LB) media in seawater or freshwater]. The cultures were maintained at 21°C in 15 mL conical tubes (5 mL media). Light micrographs were collected from cultures ~1 week old on the microscope and camera system described above using differential interference contrast (DIC) optics.

The examined specimens were deposited in the National Institute of Biological Resources (NIBR), Korea. The cultures are kept with the Korean Culture Collection of Protists at Kyungnam University (Korea): KM065 (*Notosolenus hemicircularis*), KM100 (*Thecamonas tranhens*), KF005 (*Eocercomonas echina*), KF017 (*Cercomonas pellucida*), KF019 (*Cercomonas hiberna*), KF023 (*Paracercomonas astra*), KF086 (*Paracercomonas minima*), KF088 (*Bodomorpha minima*), and KF093 (*Paracercomonas producta*).

Results and Discussion

Phylum Euglenozoa Cavalier-Smith, 1981
Class Euglenoidea Bütschli, 1884
Order Petalomonadida Cavalier-Smith, 1993
Family Scytomonadidae Stein, 1878
Genus *Notosolenus* Stokes, 1884

1. *Notosolenus hemicircularis* Lee & Patterson, 2000 (Fig. 1a–f)

Material examined. Korea, Gangwon-do, Uljin-gun, Bongpyung Beach (37°02′37″N, 129°24′47″E), 22 Oct 2017, collected by Won Je Lee.
Description. Phagotrophic euglenid. Cells are 9−11.8 μm long, ventrally flattened and dorsally convex. There is a hyaline semicircular collar (Fig. 1a, arrowhead) around the short anterior neck (Fig. 1c, 1e, arrowheads). The cells have five dorsal ridges running along the cell (Fig. 1b, 1d). On the ventral side there are three fine ridges. The right and left ventral ridges arise at the neck. The left ventral ridge curves slightly from the anterior to the posterior forming an arc. The mid-ventral ridge arises from a small protrusion near the anterior end of the cell. Two flagella are unequal in length and are slightly thickened. The anterior flagellum is 1.2−1.3 times the cell length and the recurrent posterior flagellum is 0.6−1.0 times the cell length (Fig. 1e). The flagellar reservoir lies anteriorly in the right hand side of the cell (Fig. 1f, arrow) and the nucleus in the left hand side (Fig. 1a). The cells move by gliding.
Previously reported cell length. 9−10 μm (Lee and Patterson, 2000).
Habitat. Marine intertidal sediments.
World distribution. Australia (Lee and Patterson, 2000), Korea.
Deposition. National Institute of Biological Resources, Korea (ZCIVEG0000000002).
Identifiers. Won Je Lee.

Phylum Apusozoa Cavalier-Smith, 1997
Class Thecamonadea Cavalier-Smith, 1993
Order Apusomonadida Karpov & Mylnikov, 1989
Family Apusomonadidae Karpov & Mylnikov, 1989
Genus *Thecamonas* Larsen & Patterson, 1990

2. *Thecamonas trahens* Larsen & Patterson, 1990 (Fig. 1g–l)

Synonym. *Amastigomonas trahens* (Larsen & Patterson, 1990) Molina & Nerad, 1991

Material examined. Korea, Gangwon-do, Gangneung-si, Sachun Beach (37°49′51″N, 128°52′42″E), 21 Oct 2017, collected by Won Je Lee.
Description. Cells are ovate to elliptical and 4.5−7.1 (avg. 5.5) μm long. The proboscis (see Heiss *et al*., 2015) with a slow to-and-fro motion emerges from the anterior right margin of the cell, and comprises the anterior flagellum surrounded by a membranous sleeve (Fig. 1i, 1j, 1l, arrowheads). The anterior flagellum is only visible as a distinct structure within the sleeve (Fig. 1j, 1k). A posterior flagellum trails under the left margin, and sometimes is long enough to extend beyond the back of the cell (Fig. 1g−1k). It moves by a slow, even gliding. The cell body is plastic but not amoeboid, and is deformed during turning. Swimming is not seen. Strands of cytoplasm may be trailed from behind the cell (Fig. 1h, 1k, arrows). The nucleus is in the anterior left part of the cell. Food vacuoles contain bacteria and detritus.
Previously reported cell length. 4.5−7 μm (Larsen and Patterson, 1990).
Habitat. Marine intertidal sediments.
World distribution. Australia, Hawaii, Panama, Brazil (Larsen and Patterson, 1990), Korea.
Deposition. National Institute of Biological Resources, Korea (NIBRPR0000109148).
Identifiers. Won Je Lee.

Phylum Cercozoa Cavalier-Smith, 1998
Class Sarcomonadea Cavalier-Smith, 1995
Order Glissomonadida Howe *et al*., 2009
Family Sandonidae Howe *et al*., 2009
Genus *Bodomorpha* Hollande, 1942

3. *Bodomorpha minima* (Hollande, 1942) Hollande, 1956 (Fig 1m–r)

Basionym. *Pseudobodo minima* Hollande, 1942

Material examined. Korea, Gangwon-do, Yangyang-gun, Namdaecheon Stream (38°04′39″N, 128°37′55″E), 21 Oct 2017, collected by Won Je Lee.

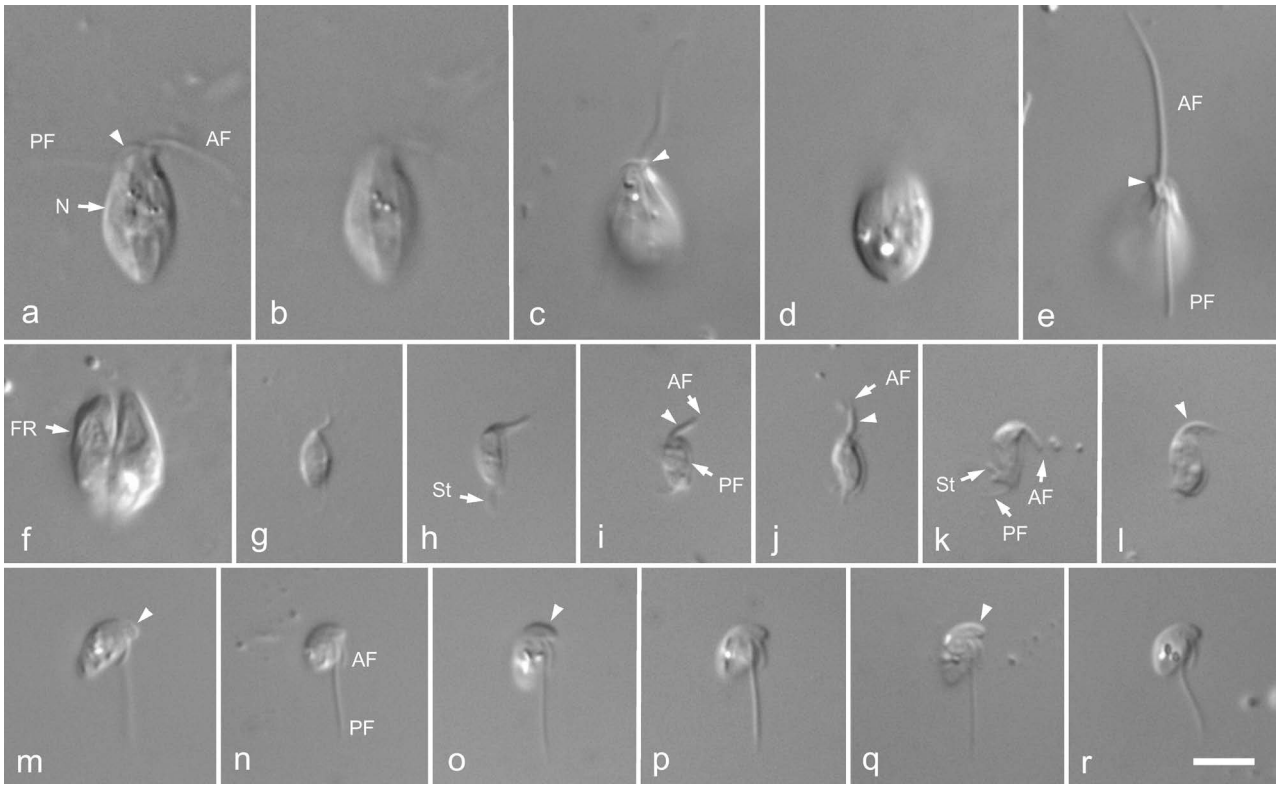


Fig. 1. (a)–(f) *Notosolenus hemicircularis*. (a), (b) cell 1 showing general appearance, (a) note the hyaline semicircular collar (arrowhead), (b) dorsal view showing dorsal ridges, (c)–(e) cell 2 showing a short anterior neck (arrowheads) and dorsal ridges (d), (f) dividing cell. (g)–(l) *Thecamonas trahens*, showing general appearance, nucleus (N), sleeve (arrowheads) and cytoplasmic strand (St), and note the anterior flagellum (AF). (g) cell 1, (h) cell 2, (i)–(l) cell 3. (m)–(r) *Bodomorpha minima*, showing general appearance and a rostrum (arrowheads). (m) cell 1, (n) cell 2, (o) cell 3, (p)–(r) cell 4. AF: anterior flagellum, PF: posterior flagellum, FR: flagellar reservoir. All micrographs are DIC images. Scale bar = 5 μm for all figures.

Description. Cells are oval or globular in shape, 4.5–7.0 (avg. 5.7) μm long and rigid. The cells have a small rostrum in the anterior end (Fig. 1m, 1o, 1q, arrowheads), behind which two flagella insert. The active anterior flagellum is short and is 0.3–0.4 times the cell length, and bends slightly ventrally. The longer posterior flagellum is 1.3–1.8 times the cell length and trails posteriorly. This species is common in freshwater habitats. This genus is morphologically similar to the genera *Heteromita* and *Neoheteromita*.

Previously reported cell length. 4–5 μm (Hollande, 1942; Howe *et al*., 2009).

Habitat. Freshwater sediments, soils.

World distribution. France (Hollande, 1942), USA (Howe *et al*., 2009), Korea.

Deposition. National Institute of Biological Resources, Korea (NIBRPR0000109168).

Identifiers. Won Je Lee.

Order Cercomonadida Poche, 1913
Family Cercomonadidae Kent, 1880
Genus *Cercomonas* Dujardin, 1841

4. *Cercomonas hiberna* Howe & Cavalier-Smith, 2009 (Fig. 2a–d)

Material examined. Korea, Gyeongsangbuk-do, Bongwha-gun, Sukpo-myeon, Nakdonggang River (37°02′30″ N, 129°03′04″E), 21 Jul 2015, collected by Won Je Lee.

Description. Cells are 5.6–10.9 (avg. 8.4) μm and very plastic. The anterior flagellum is 1.8–2.3 times the cell length and sweeps gently. The posterior flagellum is 1.7–2.2 times the cell length and may attach to the cell body. Lamellar, finger-like and filose cytoplasmic strand is drawn from the posterior end of the cell (Fig. 2d). Lamellar and finger-like pseudopodia are all over the cell, but predominantly on the left side of the cell (Fig. 2d, arrowheads). 1–2 contractile vacuoles are seen; usually on the right side of cell, and the other in posterior half (Fig. 2a, 2c). The cells glide with the flagella in contact with the substrate.

Previously reported cell length. 7–13 μm (Bass *et al*., 2009).

Habitat. Freshwater sediments, soils.

World distribution. UK (Bass *et al*., 2009), Korea.

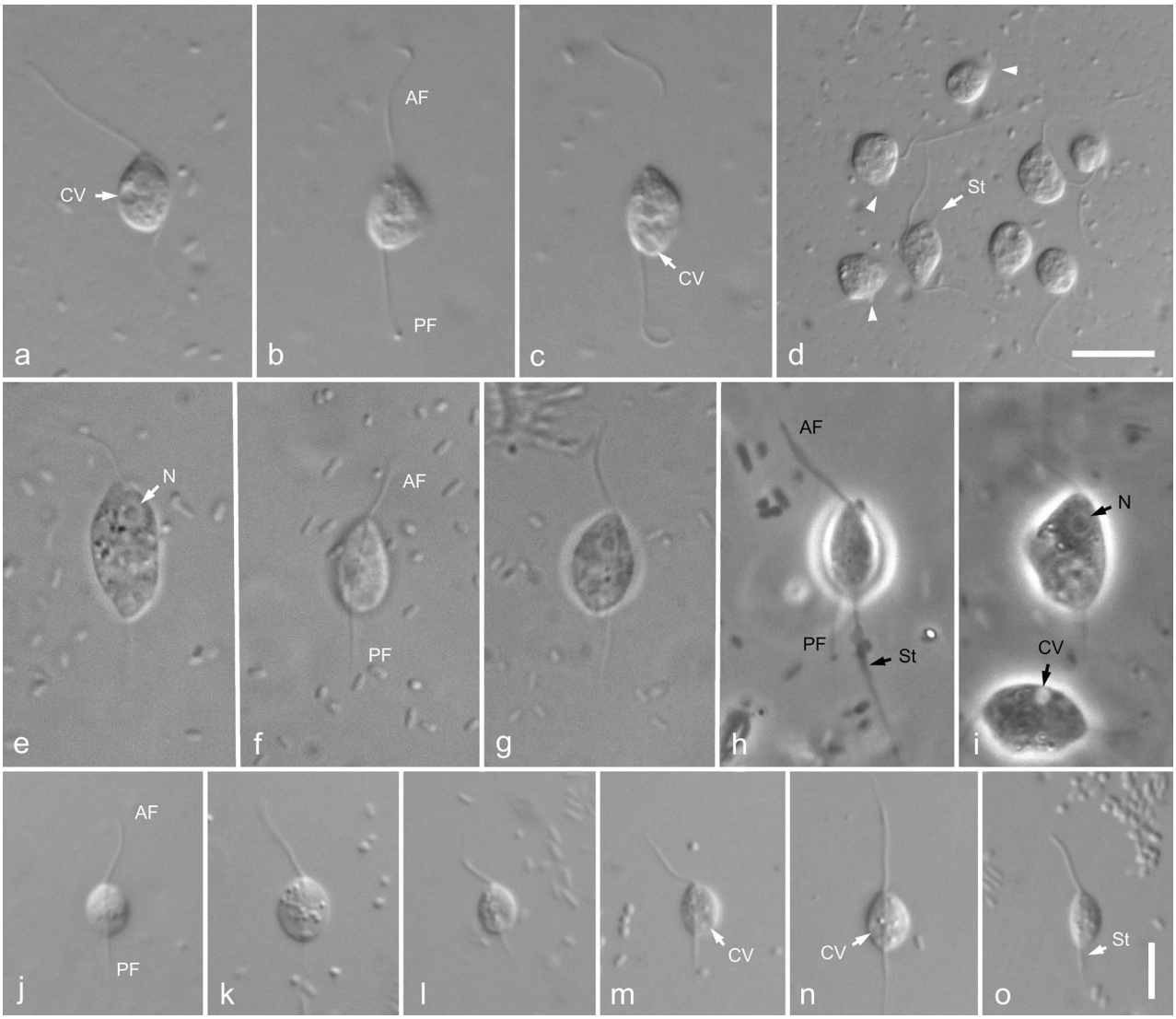


Fig. 2. (a)−(d) *Cercomonas hiberna*. (a) cell 1 showing contractile vacuole, (b), (c) cell 2 showing general appearance and contractile vacuole, (d) several cells showing cytoplasmic strand (St) and pseudopodia (arrowheads). (e)−(i) *Cercomonas pellucida*, showing general appearance, contractile vacuole (CV), cytoplasmic strand and nucleus (N) closely connected to the basal body. (e) cell 1, (f) cell 2, (g) cell 3, (h) cell 4, (i) cell 5 & 6. (j)−(o) *Eocercomonas echina*, showing general appearance, contractile vacuole and cytoplasmic strand. (j) cell 1, (k) cell 2, (l), (m) cell 3, (n) cell 4, (o) cell 5. AF: anterior flagellum, PF: posterior flagellum. All micrographs are DIC images with the exceptions of (h), (i) which are phase contrast images. Scale bar in (d) = 10 μm for (d), and in (o) = 5 μm for other figures.

Deposition. National Institute of Biological Resources, Korea (NIBRPR0000109188).
Identifiers. Won Je Lee.

5. *Cercomonas pellucida* Brabender, Domonell, Kiss, Nitsche & Arndt, 2012 (Fig. 2e−i)

Material examined. Korea, Gyeongsangbuk-do, Bongwha-gun, Sukpo-myeon, Nakdonggang River (37°02′30″ N, 129°03′04″E), 21 Jul 2015, collected by Eun Hee Kim.
Description. Cells are 7.5−13 μm long and plastic. The anterior flagellum is 1.3−1.5 times the cell length and the posterior flagellum is 1.3−1.5 times the cell length. The cells rarely form finger-like and bulbous pseudopodia at the posterior end. The nucleus has a coarse appearance; the nuclear envelope is very thin but many microbodies attach to it, the nucleolus is slightly irregular, there are many granules and rods in the nucleoplasm. The nucleus is closely connected to the basal bodies (Fig. 2e, 2i). Cytoplasmic strand is drawn out from the posterior end of the cell, but may not be seen (Fig. 2h). The single large contractile vacuole is located in the posterior or lateral part of the cell (Fig. 2i). The cells move by gliding and consume bacteria.

Previously reported cell length. 9.1−17.2 μm (Brabender *et al*., 2012).
Habitat. Freshwater sediments, soils.
World distribution. Germany (Brabender *et al*., 2012), Korea.
Deposition. National Institute of Biological Resources, Korea (NIBRPR0000107970).
Identifiers. Won Je Lee.

Genus *Eocercomonas* Karpov *et al*., 2006

6. *Eocercomonas echina* Howe & Cavalier-Smith, 2009 (Fig. 2j−o)

Material examined. Korea, Gyeongsangnam-do, Changwon, Gwangnyeocheon Stream (35°14′50″N, 128°30′36″E), 27 Oct 2017, collected by Won Je Lee.
Description. Cells are 4.7−6.5 (avg. 5.3) μm long and plastic. The anterior flagellum is 1.2−1.8 times the cell length and beats slowly. The posterior flagellum is 1.3−1.8 times the cell length and attaches to the cell body. Cytoplasmic strand is drawn out from the posterior end of the cell, but may not be seen (Fig. 2o). Numerous finger-like pseudopodia are seen all around the cell. 1−2 contractile vacuoles are posterio-centrally located (Fig. 2m, 2n). The cells glide slowly by gliding.
Previously reported cell length. 6−10 μm (Bass *et al*., 2009).
Habitat. Freshwater sediments, soils.
World distribution. UK (Bass *et al*., 2009), Korea.
Deposition. National Institute of Biological Resources, Korea (NIBRPR0000109208).
Identifiers. Won Je Lee.

Order Paracercomonadida Cavalier-Smith, 2018
Family Paracercomonadidae Cavalier-Smith in Cavalier-Smith & Karpov, 2012
Genus *Paracercomonas* Karpov *et al*., 2006

7. *Paracercomonas astra* Howe & Cavalier-Smith, 2009 (Fig. 3a−f)

Material examined. Korea, Gyeongsangnam-do, Changwon, Jinjuncheon Stream (35°06′02″N, 128°26′04″E), 4 Apr 2012, collected by Won Je Lee.
Description. Cells are 5.7−7.3 (avg. 6.6) μm long and moderately metabolic. The anterior flagellum is about the cell length, tapered or acronematic, and the posterior flagellum is about the cell length, difficult to see and attached. Sporadic finger-like or filose cytoplasmic strand is tailed (Fig. 3c−3f, arrowheads). Lamellar and/or branching pseudopodia are seen at the posterior part of the cell and finger-like pseudopodia are seen most often at the anterior part. 1−2 contractile vacuoles lie in the middle of the cell (Fig. 3a, 3d). The cells move by slow gliding, but sometimes move quite rapidly.
Previously reported cell length. 6−13 μm (Bass *et al*., 2009).
Habitat. Freshwater sediments, soils.
World distribution. UK, Sweden, Panama (Bass *et al*., 2009), Korea.
Deposition. National Institute of Biological Resources, Korea (NIBRPR0000109228).
Identifiers. Won Je Lee.

8. *Paracercomonas minima* (Mylnikov, 1985) Bass & Cavalier-Smith, 2009 (Fig. 3g−i)

Basionym. *Cercobodo minima* Mylnikov, 1985

Material examined. Korea, Gangwon-do, Yangyang-gun, Ssangcheon Stream (38°09′33.6″N, 128°36′27.3″E), 21 Oct 2017, collected by Won Je Lee.
Description. Cells are 5−9 μm, often still and metabolic. The anterior flagellum is about the cell length and acronematic, and waves quite slowly along the entire length of the flagellum. The posterior flagellum is 1−1.5 times the cell length. Branched, finger-like or filose cytoplasmic strand is always present. Finger-like, bulbous, lamellar, filose-branching pseudopodia are almost always present all around the cell. One contractile vacuole is laterally positioned (Fig. 3g). Cysts are spherical (Fig. 3i). This species is slightly smaller than *P. marina*.
Previously reported cell length. 5−9 μm (Mylnikov, 1985; Bass *et al*., 2009).
Habitat. Freshwater sediments, soils.
World distribution. UK, Sweden, Chile, New Zealand, Argentina, Panama (Bass *et al*., 2009), Russia (Mylnikov, 1985), Korea.
Deposition. National Institute of Biological Resources, Korea (NIBRPR0000109248).
Identifiers. Won Je Lee.

9. *Paracercomonas producta* Howe & Cavalier-Smith, 2009 (Fig. 3j−o)

Material examined. Korea, Gangwon-do, Yangyang-gun, Namdaechun-river (38°04′39″N, 128°37′55″E), 21 Oct 2017, collected by Won Je Lee.
Description. Cells are 6.1−9.9 (avg. 7.6) μm long and very metabolic. The anterior flagellum is slightly longer than the cell and the posterior flagellum is 1−1.5 times the cell length. Cytoplasmic strand is long and very often present (Fig. 3j−3o, arrowheads). Lamellar, bulbous, finger-like, filose and branched pseudopodia are seen all around the cell, almost always present. The nucleus is located in the anterior part of the cell. One contractile vacu-

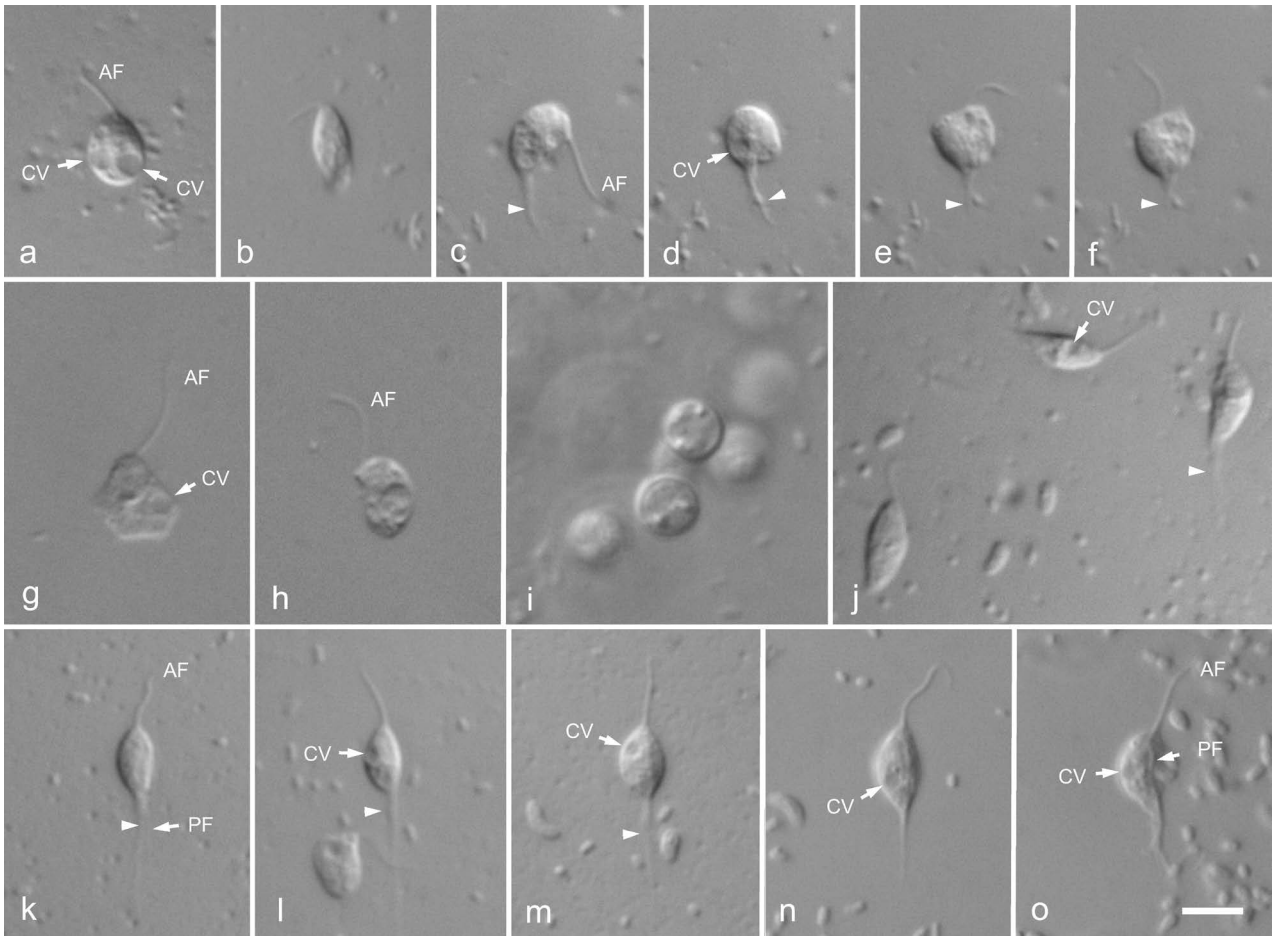


Fig. 3. (a)–(f) *Paracercomonas astra*, showing general appearance, contractile vacuole (CV) and cytoplasmic strand (arrowheads). (a) cell 1, (b) cell 2, (c)–(f) cell 3. (g)–(i) *Paracercomonas minima*, (g) cell 1 showing CV, (h) cell 2, (i) cysts. (j)–(o) *Paracercomonas producta*, showing general appearance, contractile vacuole and cytoplasmic strand (arrowheads). (j) three different cells, (k) cell 1, (l) cell 2, (m) cell 3, (n), (o) cell 4. AF: anterior flagellum, PF: posterior flagellum. All micrographs are DIC images. Scale bar = 5 μm for all figures.

ole is near the middle of the cell, but often near the left or right side of the cell (Fig. 3j, 3l–3o). The cells glide slowly on the substrate.

Previously reported cell length. 4–9 μm (Lara *et al*., 2007; Bass *et al*., 2009).

Habitat. Freshwater sediments, soils.

World distribution. UK (Bass *et al*., 2009), Spain (Lara *et al*., 2007), Korea.

Deposition. National Institute of Biological Resources, Korea (NIBRPR0000109268).

Identifiers. Won Je Lee.

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