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Characterizations of five heterotrophic nanoflagellates newly recorded in Korea

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Heterotrophic nanoflagellates (HNFs, 2–20 μm in size) are substantially capable of controlling bacterial abundance in aquatic environments, and microbial taxonomists have studied ecologically important and abundant HNFs for a long time. However, the classifications of HNFs have rarely been reported in Korea on the basis of morphology and 18S rDNA sequencing. Here, previously reported five HNFs from non-Korean habitats were isolated from Korean coastal seawater or intertidal sediments for the first time. Light microscopic observations and 18S rDNA phylogenetic trees revealed that the five isolated species were *Cafeteria burkhardae* strain PH003, *Cafeteria graefeae* strain UL001, *Aplanochytrium minuta* (formerly *Labyrinthuloides minuta*) strain PH004, *Neobodo curvifilus* strain KM017 (formerly *Procryptobia sorokini*), and *Ancyromonas micra* (formerly *Planomonas micra*) strain IG005. Being morphologically and phylogenetically indistinct from its closest species, all isolates from Korea were therefore regarded as identical species detected in other countries. Thus, this result indicates an expansion of known habitats that range from those of the five isolates in natural ecosystems on Earth.

Keywords: 18S rDNA, classification, heterotrophic nanoflagellates, morphology

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Introduction

Heterotrophic nanoflagellates (HNFs, 2–20 μm in size) are major components in freshwater, marine, and extreme ecosystems that play critical roles as prokaryotic grazers or prey to organisms at higher trophic levels (Sherr *et al*., 1989; Park *et al*., 2003; Fischer *et al*., 2006). The estimated number of marine species including HNFs was approximately 2.2 million in the ocean (Mora *et al*., 2011). It is speculated that the dominant marine HNFs are choanoflagellates and stramenopiles (Patterson *et al*., 1993; Arndt *et al*., 2000). Moreover, a diverse assemblage of HNFs also thrives in the marine habitats of Korea. Although many HNFs have been reported in Korea (Lee, 2002; Park *et al*., 2006; Park *et al*., 2007; Park and Simpson, 2010; Kim *et al*., 2012; Heiss *et al*., 2015; Lee, 2015; 2016; Lee and Park, 2016; Kang and Kim, 2018; Jhin and Park, 2019; Lax *et al*., 2019; Tikhonenkov *et al*., 2019; Lee, 2020), our knowledge about the biodiversity of autochthonous HNFs remains poor in the natural ecosystems of Korea.

The combined analyses of light microscopy, ultrastructure (not common), and molecular phylogeny have allowed for classifying HNFs. Especially, the molecular phylogenetic analyses of 18S rDNA sequences led to a substantial improvement in HNF classification (Cavalier-Smith, 1998). However, little is known about the modern phylogenetic relationships between 18S rDNA sequences of HNFs isolated from marine habitats in Korea. In this study, the morphological features of five marine HNFs (*Cafeteria burkhardae*, *Cafeteria graefeae*, *Aplanochytrium minuta* (formerly *Labyrinthuloides minuta*), *Neobodo curvifilus* (formerly *Procryptobia sorokini*), and *Ancyromonas micra* (formerly *Planomonas micra*)) were first recorded in Korea. Moreover, the molecular phylogenetic relationships between these 18S rDNA sequences of the isolated HNFs were reported for the first time.

Materials and Methods

Sampling, isolation, and cultivation

Five heterotrophic nanoflagellates (HNFs) were isolated from water/sediment interface samples collected from four locations (Yeongildae Beach, Gwangam Beach, Ilgwang Beach, and Ulleungdo) in Korea. The monoprotistan culture was established using the single-cell isolation method

(Guillard and Ryther, 1962). Briefly, a single cell was inoculated into a 24-well plate containing 0.5% Luria-Bertani (LB) broth (final concentration, Difco) in sterile seawater, then the inoculum was incubated at 25°C for 7 d. Subsequently, live flagellates were observed with phase-contrast microscopy (Nikon ECLIPSE Ts2, Tokyo, Japan). Furthermore, each isolate was acclimated in a 10 mL medium (0.5% LB broth in sterile seawater) and placed in a 50 mL tissue flask for enrichment. Live flagellates mounted on glass slides were then observed with differential interference microscopy using a Leica DM5500B microscope equipped with a DFC550 digital camera (Leica, Wetzlar, Germany). The dimensions of the live cells were measured from digital pictures.

DNA extraction and PCR amplification

DNA from the five isolates was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the supplied protocols. PCR amplification was used to obtain the 18S rRNA gene sequences were obtained by PCR amplification using two different primer sets; 528F 5′-CGGTAATTCCAGCTCC-3′/1209R 5′-GGG CATCACAGACCTG-3′ (Elwood *et al*., 1985; Giovannoni *et al*., 1988) for isolates PH004 and UL001, and EukA 5′-AACCTGGTTGATCCTGCCAGT-3′/ EukB 5′-TGATCCTTCTGCAGGTTCACCTAC-3′ (Medlin *et al*., 1988) for isolates KM017, PH003, and IG005. The 20 μL PCR reactions included 0.4 μL of 2.5 mM dNTP mixture, 2 μL of 10 × PCR buffer, 0.8 μL each of 10 μm primer stocks, 0.2 μL of *Taq* DNA polymerase (Takara, Shuzo, Japan), 2 μL of DNA template, and 13.8 μL of double distilled water. Also, the PCR cycling conditions for EukA and EukB were as follows: an initial denaturation step at 94°C for 5 min, 40 cycles of 45 s of denaturation at 94°C, 1 min of annealing at 55°C, and 3 min of extending at 72°C, with a final extension step for 20 min at 72°C. Furthermore, the PCR cycling conditions for 528F and 1209R were as follows: an initial denaturing step at 95°C for 5 min, 30 cycles of 45 s of denaturation at 95°C, 1 min of annealing at 55°C, and 3 min of extending at 72°C, with a final extension step for 10 min at 72°C. All amplicons were cloned into a pGEM-T Easy vector. At least five positive clones per sample were sequenced.

Phylogenetic analysis

The 18S rDNA sequences from the five isolates (PH003, UL001, PH004, KM017, and IG005) were compared to the sequences in the GenBank database, after which closely related sequences were retrieved from the database. Four 18S rDNA sequence datasets from the five isolates were then aligned using MAFFT program v. 7 (Katoh and Standley, 2013), and masked by eye. The seed alignments were originated from Schoenle *et al*., 2020 for strains PH003 and UL001 (1,637 unambiguously aligned sites in this study), Harel *et al*., 2008 for strain PH004 (1,763 unambiguously aligned sites in this study), Hirose *et al*., 2012 for strain KM017 (2,007 unambiguously aligned sites in this study), and Cavalier-Smith *et al*., 2008 for strain IG005 (1,429 unambiguously aligned sites in this study). Phylogenetic trees were inferred by maximum-likelihood (ML) and Bayesian analysis. ML trees were subsequently inferred using the IQ-tree; version of IQ-TREE 1.6.12 (Nguyen *et al*., 2015). TN + F + G4 for strains PH004 and IG005, TIM3e + I + G4 for strains PH003 and UL001, and TNe + G for strain KM017 were selected using the best-fit model test option. The statistical support was assessed using bootstrapping values with 1,000 replicates. The Bayesian analyses were inferred using MrBayes 3.2.7 (Ronquist *et al*., 2012) with two independent runs, each with four chains running for 5×10^6 generations. A burn-in of 1.5×10^6 generations (30%) was used. The average standard deviation of split frequencies for the last 75% of generations was < 0.05.

Results and Discussion

Phylum Bigyra Cavalier-Smith, 1998
(emend. Cavalier-Smith, 2006)
Class Bicoecea Cavalier-Smith, 1993
Order Bicosoecida Grasse, 1926 (emend. Karpov, 1998)
Family Cafeteriaceae Møestrup, 1995
Genus *Cafeteria* Fenchel and Patterson, 1988

1. *Cafeteria burkhardae* Schoenle and Arndt, 2020 (Fig. 1A, B)

Isolation. Dong Hyuk Jeong and Jong Soo Park conducted specimen collection on 27 February 2020, from the Yeongildae Beach (36°03′49.00″N, 129°23′12.12″E), Pohang, Korea. Temperature: 12°C, Salinity: 30.0 PSU, pH: 7.7.

Morphological description. The live strain PH003 was a D-shaped biflagellate (Fig. 1A, B). The average length and width of the cell body were 4.9 ± 0.6 μm (mean ± STD) and 4.6 ± 0.8 μm, respectively (n = 20). The curved channel on the surface of the cell was not observed. Sometimes, contractile and food vacuoles were presented within the cell body (Fig. 1A). The average length of the anterior and posterior flagellum was 5.8 ± 0.8 μm and 4.7 ± 1.3 μm, respectively (n = 20). When cells swam, the anterior flagellum was directed forwards, and beat with amplitude motion. Whereas, when the posterior flagellum was attached to a substrate, cells showed in the jerking movement, and the anterior flagellum created a feeding current.

Molecular phylogeny. The partial 18S rDNA sequence of the strain PH003 was 1,610 bp long (G + C content:

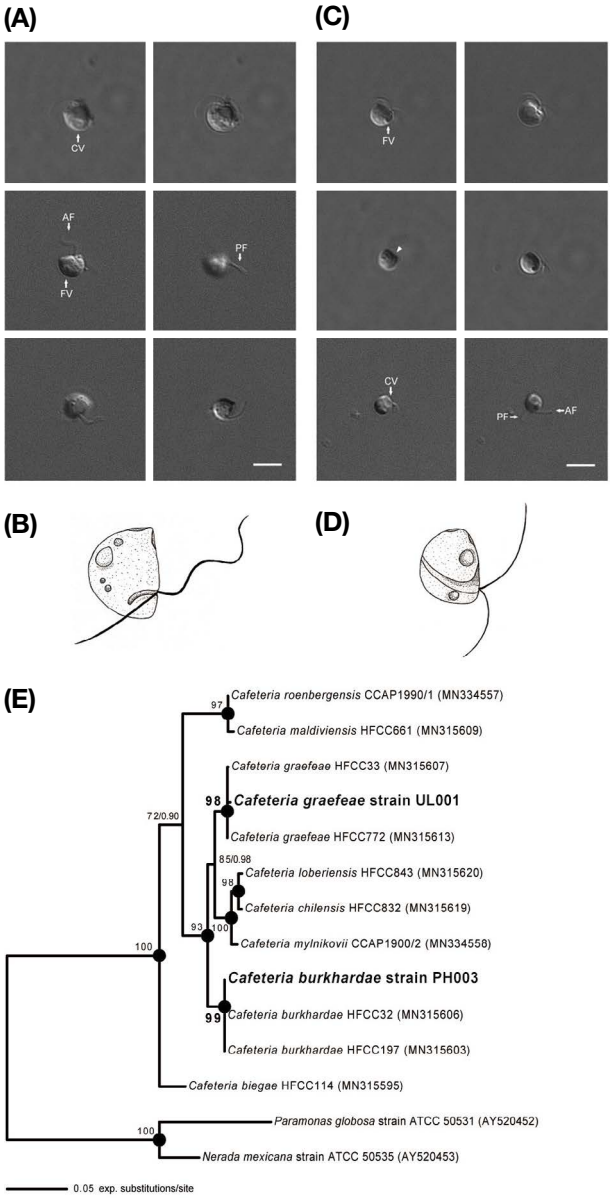

Fig. 1. (A, B) General light micrographs and sketch of *Cafeteria burkhardae* strain PH003. AF: anterior flagellum, PF: posterior flagellum, CV: contractile vacuole, FV: food vacuole, scale bar = 5 μm. Arrowhead represents curved channel. All micrographs are differential interference contrast (DIC) images. (C, D) General light micrographs and sketch of *Cafeteria graefeae* strain UL001. (E) Maximum likelihood phylogenetic tree inferred from the 18S rDNA sequences of *Cafeteria* (genus) species including *Cafeteria graefeae* strain UL001 and *Cafeteria burkhardae* strain PH003 and outgroup (*Paramonas globosa* strain ATCC 50531 and *Nerada mexicana* strain ATCC 50535). Bootstrap support values (>70%) are shown at the nodes. Solid circles indicate a Bayesian posterior probability of 1 (posterior probability < 0.95 not shown).

46%). The closest sequence returned by the BLASTn GenBank search was the 18S rDNA sequence of *Cafeteria burkhardae* strain HFCC187 (accession number; MN315602, 2,590 bp long, G + C content: 51%), with an identity of 100% (1610/1610 bp). Furthermore, the molecular phylogenetic tree of 18S rDNA sequences indicated that the strain PH003 belonged to the *C. burkhardae* clade with high statistical support (ML: 99%) and a posterior probability of 1 (Fig. 1E).

Remarks. The strain PH003 resembled *C. roenbergensis* in cell shape, and moving and feeding behaviors (Larsen and Patterson, 1990; Lee, 2006). However, the length of the posterior flagellum of the strain PH003 was shorter than those of *C. roenbergensis* CCAP 1900/1 (mean of 6.3 μm, Schoenle *et al*., 2020) and the original *C. burkhardae* (6.1−7.4 μm, Schoenle *et al*., 2020). Morphologically, the strain PH003 is very similar to *C. burkhardae* among *Cafeteria* species. The original *C. burkhardae* was D- or globular-shaped and ventrally flattened (Schoenle *et al*., 2020). The original cell body was 3.0−4.8 μm in length and 2.6−4.5 μm in width. The length of the two flagella of the original cells was also about 5−8 μm. When the posterior flagellum of the original cells was attached to a substrate, cells showed a jerking movement, whereas the anterior flagellum created a feeding current. Additionally, the curved channel on the original and strain PH003 cell surface was not observed. Consistently, the 18S rDNA sequence of the strain PH003 was 100% identical to those of *C. burkhardae* strains HFCC32 and HFCC197. Thus, we considered them the same species.

Habitat. Surface water/sediment interface of coastal areas.

Specimen deposition. National Institute of Biological Resources, Korea (KPZXPR0000000012−KPZXPR0000000016).

Identifiers. Dong Hyuk Jeong and Jong Soo Park.

2. *Cafeteria graefeae* Schoenle and Arndt, 2020 (Fig. 1C, D)

Isolation. Dong Hyuk Jeong and Jong Soo Park performed specimen collection on 13 October 2020, from Ulleungdo (37°28′49.06″N, 130°54″31.00″E), Korea. Temperature: 20.2°C, Salinity: 32.4 PSU, pH: 8.3.

Morphological description. The live strain UL001 was a biflagellate form and D- and glove-shaped (Fig. 1C, D). The average length and width of the cell body were 4.5 ± 0.6 μm (mean ± STD) and 3.7 ± 0.5 μm, respectively (n = 20). A slightly curved channel was observed on the dorsal side of the cell (Fig. 1C). Sometimes, contractile and food vacuoles showed within the cell body (Fig. 1C). The average length of the anterior and posterior flagellum was 5.0 ± 0.7 μm and 3.8 ± 0.7 μm, respectively (n = 20). When cells swam, the anterior flagellum was directed forwards, and the posterior flagellum trailed posteriorly. Also, when the posterior flagellum was attached to a substrate, cells showed a jerking motion, whereas the anterior flagellum beat for feeding.

Molecular phylogeny. The partial 18S rDNA sequence of the strain UL001 was 823 bp in length (G+C content: 46%). The 18S rDNA sequence of strain UL001 was 99.64% (822/825 bp) identical to that of *Cafeteria graefeae* strain HFCC33 (accession number; MN315607, 1,629 bp long, G+C content: 49%), suggesting identical species. The ML tree revealed that strain UL001 and *Cafeteria graefeae* formed a highly supported value clade (ML: 98%) and a posterior probability of 1 (Fig. 1E).

Remarks. The cell shape of the strain UL001 is very similar to a typical genus *Cafeteria*. The strain UL001 had a slightly curved channel, which was very similar to *C. biegae* (Schoenle *et al*., 2020). However, the original *C. biegae* (2.6−4.4 μm long and 2.1−3.4 μm wide) was smaller than the strain UL001, while the two flagella lengths of *C. biegae* (5.0−9.7 μm) was longer than the strain UL001. Furthermore, it seems that the length of the posterior flagellum of the strain UL001 was the shortest in *Cafeteria* groups. This character is similar to the original *C. graefeae*. However, the length of the anterior flagellum of the strain UL001 was longer than that of the original *C. graefeae* (2.3−5.5 μm long). It seems that the strain UL001 was identical to *C. graefeae* strain HFCC33 and HFCC772 based on the molecular phylogenetic analyses. Thus, the strain UL001 is proposed to be a member of *Cafeteria graefeae* strains.

Habitat. Surface water of coastal areas.

Specimen deposition. National Institute of Biological Resources, Korea (KPZXPR0000000017−KPZXPR0000000021).

Identifiers. Dong Hyuk Jeong and Jong Soo Park.

Phylum Bigyra Cavalier-Smith, 1998
(emend. Cavalier-Smith, 2006)
Class Labyrinthulomycetes Arx, 1970
(emend. Dick, 2001)
Order Labyrinthulida Doflein, 1901
Family Aplanochytriidae Leander ex Cavalier-Smith, 2012
Genus *Aplanochytrium* Bahnweg and Sparrow, 1972

3. *Aplanochytrium minuta* (Watson and Raper, 1957) Leander and Porter, 2000 (Fig. 2A, B)

Synonym: *Labyrinthuloides minuta* Watson and Raper, 1957.

Isolation. Dong Hyuk Jeong and Jong Soo Park performed specimen collection on 15 September 2020, from the Yeongildae Beach (36°03′49.00″N, 129°23′12.12″E), Pohang, Korea. Temperature: 24.9°C, Salinity: 29.9 PSU, pH: 7.7.

Morphological description. The live strain PH004 was ovoid to elliptical-shaped, but with the posterior end of the cell was more pointed than the anterior end (Fig. 2A, B). The average length and width of the cell body were 4.3 ± 1.2 μm (mean ± STD) and 2.9 ± 1.1 μm, respectively (n = 20). Cells had spindle forms during actively gliding motility (Fig. 2A). Cells also had two unequal flagella. The average length of the anterior and posterior flagellum was 1.9 ± 1.3 μm and 5.1 ± 0.7 μm, respectively (n = 20). Furthermore, the anterior flagellum created the movement of a stiff flicker, while the posterior flagellum trailed posteriorly.

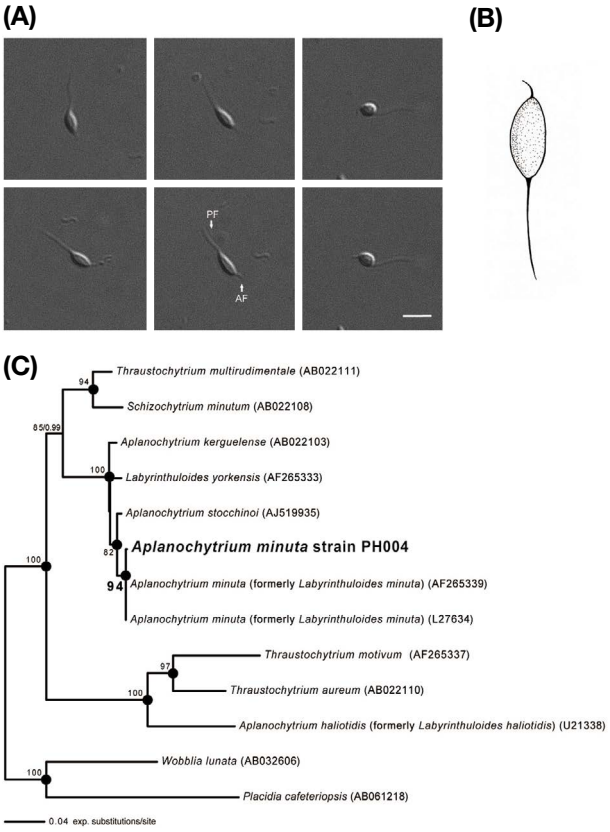

Fig. 2. (A, B) General light micrographs and sketch of *Aplanochytrium minuta* (formerly *Labyrinthuloides minuta*) strain PH004. AF: anterior flagellum, PF: posterior flagellum, scale bar = 5 μm. All micrographs are differential interference contrast (DIC) images. (C) Maximum likelihood phylogenetic tree inferred from the 18S rDNA sequences of Labyrinthulomycetes (class) species including *Aplanochytrium minuta* strain PH004 and outgroup (*Wobblia lunata* and *Placidia cafeteriopsis*). Bootstrap support values (>80%) are shown at the nodes. Solid circles indicate a Bayesian posterior probability of 1 (posterior probability < 0.95 not shown).

Molecular phylogeny. The partial 18S rDNA sequence of the strain PH004 was 873 bp long (G+C content: 41%). The closest sequence retrieved by BLASTn search from GenBank was that of 18S rDNA sequence of *Aplanochytrium minuta* (formerly *Labyrinthuloides minuta*, accession number; L27634, 1,802 bp long, G+C content: 43%), with a high identity of 99.89% (872/873 bp). Additionally, the molecular phylogenetic tree of the 18S rDNA

sequences placed the strain PH004 within the *A. minuta* clade with a high bootstrap value (ML: 94%) and a posterior probability of 1 (Fig. 2C).

Remarks. The strain PH004 is morphologically indistinguishable from the original *A. minuta*. The original cells were oval, cuneiform, or spherical-shaped (Perkins, 1974). Also, the original cells were 5−10 μm in length, and 3−5 μm in width. It seems that the cell size of our isolate is smaller than that of the original *A. minuta*. Furthermore, like the strain PH004, the original biflagellate cells displayed gliding motility, and sometimes the spindle-shaped cells formed a sorus (Watson and Raper, 1957; Raghukumar, 2002). The original *A. minuta* was spread as a monolayer on the agar surfaces, although we did not observe this monolayer. Phylogenetic trees of the 18S rDNA sequences showed clearly that the strain PH004 was a member of previously known *A. minuta*. Overall, the strain PH004 is speculated to be among the *A. minuta* strains in terms of morphology and molecular sequencing.

Habitat. Surface water/sediment interface of coastal areas.

Specimen deposition. National Institute of Biological Resources, Korea (KPZXPR0000000022−KPZXPR0000000026).

Identifiers. Dong Hyuk Jeong and Jong Soo Park.

Phylum Euglenozoa Cavalier-Smith, 1981
(emend. Simpson, 1997)
Class Kinetoplastea Honigberg, 1963
(emend. Vickerman, 1976)
Order Neobodonida Vickerman in Moreira *et al*., 2004
Family Neobodonidae Cavalier-Smith, 2016
Genus *Neobodo* Vickerman, 2004

4. *Neobodo curvifilus* (Griessmann, 1913) Moreira *et al*., 2004 (Fig. 3A, B)

Synonym: *Procryptobia sorokini* (Zhukov, 1975) Frolov *et al*., 2001.

Isolation. Dong Hyuk Jeong and Won Je Lee conducted specimen collection on 13 January 2020, from the Gwangam Beach (35°06′08.01″N, 128°30′00.09″E), Changwon, Korea. Temperature: 14.1°C, Salinity: 34.0 PSU, pH: 8.3.

Morphological description. The live strain KM017 was a biflagellate form and usually ovoid or bean-shaped (Fig. 3A, B). The average length and width of the cell body were 7.2 ± 0.6 μm (mean ± STD) and 4.2 ± 0.7 μm, respectively (n = 20). Sometimes, the granules were observed within its cell body (Fig. 3A). Cells displayed a rapid squirming movement. Furthermore, the two flagella beat heterodynamically, and had a hair-like tip. Also, the average length of the anterior and posterior flagellum was 8.6 ± 0.9 μm and 16.5 ± 1.8 μm, respectively (n = 20). The anterior flagellum showed quickly paddling motion, while the posterior flagellum trailed posteriorly.

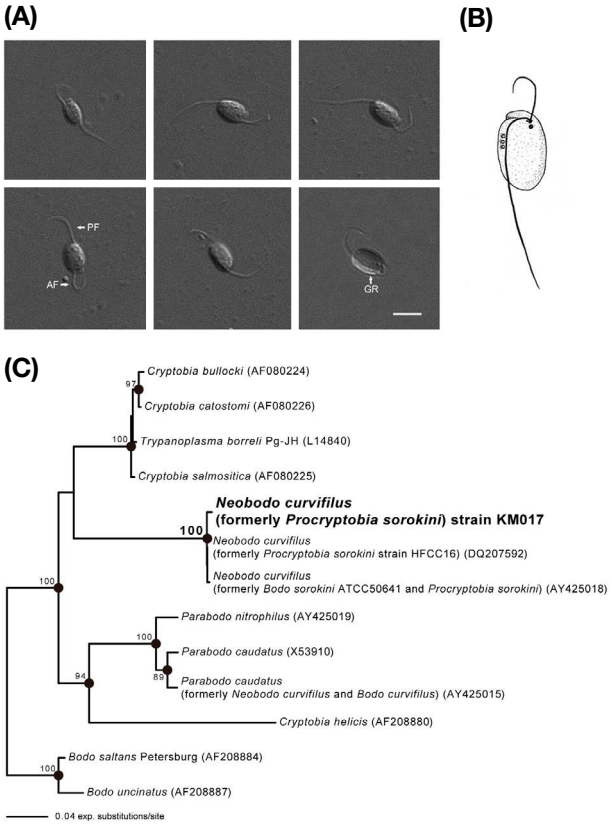

Fig. 3. (A, B) General light micrographs and sketch of *Neobodo curvifilus* (formerly *Procryptobia sorokini*) strain KM017. AF: anterior flagellum, PF: posterior flagellum, GR: granules, scale bar = 5 μm. All micrographs are differential interference contrast (DIC) images. (C) Maximum likelihood phylogenetic tree inferred from the 18S rDNA sequences of Neobodonida (order) species including *Neobodo curvifilus* strain KM017 and outgroup (*Bodo saltans* and *Bodo uncinatus*). Bootstrap support values (>80%) are shown at the nodes. Solid circles indicate a Bayesian posterior probability of 1 (posterior probability < 0.95 not shown).

Molecular phylogeny. The partial 18S rDNA sequence of the strain KM017 was 2,066 bp long (G + C content: 50%). The closest sequence retrieved by BLASTn GenBank search was the 18S rDNA sequence of *Procryptobia sorokini* isolate FB (accession number; KF479401, 2,053 bp long, G + C content: 50%), with a high identity of 99.66% (2047/2054 bp). Moreover, this study has shown that strain KM017 was a member of *P. sorokini* with a maximum bootstrap value (ML: 100%) and a posterior probability of 1 (Fig. 3C).

Remarks. The strain KM017 is morphologically similar to the previously reported *Neobodo curvifilus*. The original biflagellate cells were oval- or pear-shaped (Frolov *et al*., 2001). The original cell body was 6.6−8.3 μm in length and 3.0−4.5 μm in width. The width of cell body of the strain KM017 was slightly larger than the original species. Like our isolate, granules of the original cells

were also observed along the ventral side of the cells. The length of the anterior flagellum of the original cells was similar to the cell body length, whereas the length of the posterior flagellum of the original cells was 1.5 times longer than the cell body length. The anterior flagellum of the original cells often displayed a hook motion. Moreover, the length of a hair-like tip on the posterior flagellum was longer than that on the anterior flagellum (Kim *et al*., 2014). The flagellar length and motion of the strain KM017 are consistent with the original cells. Furthermore, phylogenetic trees of the 18S rDNA sequences showed that the strain KM017 was a member of *Neobodo curvifilus*. *Procryptobia sorokini* in GenBank is a junior synonym of *Neobodo curvifilus* (W.J. Lee, personal communication).

Habitat. Surface water/sediment interface of coastal areas.

Specimen deposition. National Institute of Biological Resources, Korea (KPZXPR0000000007–KPZXPR0000000011).

Identifiers. Won Je Lee.

Phylum Not assigned
Class Not assigned
Order Ancyromonadida Cavalier-Smith, 1998
Family Ancyromonadidae Cavalier-Smith, 1993
Genus *Ancyromonas* Kent, 1880

5. *Ancyromonas micra* (Cavalier-Smith, 2008) Heiss *et al*., 2010 (Fig. 4A, B)

Synonym: *Planomonas micra* Cavalier-Smith, 2008.

Isolation. Dong Hyuk Jeong and Jong Soo Park conducted specimen collection on 16 March 2021, from the Ilgwang Beach (35°19′12.06″N, 129°15′57.08″E), Busan, Korea. Temperature: 17.7°C, Salinity: 34.0 PSU, pH: 8.3.

Morphological description. The live strain IG005 was oval and flatten-shaped (Fig. 4A, B). Also, the average length and width of the cell body were 4.9 ± 1.2 μm (mean ± STD) and 3.5 ± 0.6 μm, respectively (n = 20). Cells had a shallow groove in the body's anterior and a reflected rostrum between the flagellum (Fig. 4A) Cells displayed gliding movement, and the two flagella showed acronematic form. The average length of the anterior and posterior flagellum was 3.6 ± 0.6 μm and 8.6 ± 1.6 μm, respectively (n = 20). The anterior flagellum swayed from side to side, while the posterior flagellum trailed posteriorly.

Molecular phylogeny. The partial 18S rDNA sequence of the strain IG005 was 1,689 bp long (G + C content: 42%). The 18S rDNA sequence of strain IG005 was 99.88% (1688/1690 bp), which was identical to that of *Ancyromonas micra* (formerly *Planomonas micra* ATCC 50267, accession number; EF455780, 1,753 bp long, G + C content: 43%), suggesting identical species. The ML

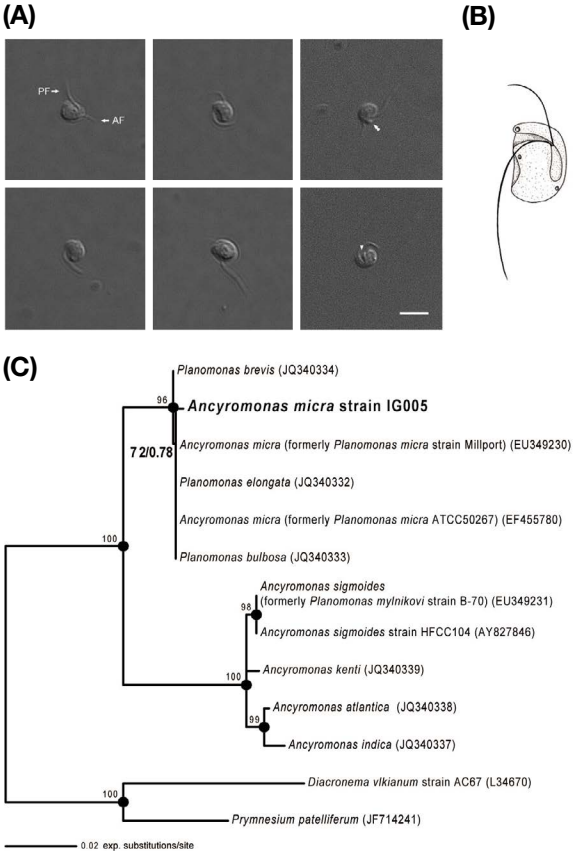

Fig. 4. (A, B) General light micrographs and sketch of *Ancyromonas micra* strain IG005. AF: anterior flagellum, PF: posterior flagellum, scale bar = 5 μm. Arrowhead and double arrowhead represent a shallow groove and rostrum, respectively. All micrographs are differential interference contrast (DIC) images. (C) Maximum likelihood phylogenetic tree inferred from the 18S rDNA sequences of Ancyromonadida (order) species including *Ancyromonas micra* strain IG005 and outgroup (*Diacronema vlkiauum* strain AC67 and *Prymnesium patelliferum*). Bootstrap support values (>70%) are shown at the nodes. Solid circles indicate a Bayesian posterior probability of 1 (posterior probability <0.75 not shown).

tree revealed that the strain IG005, two *A. micra* strains Millport and ATCC50267, *Planomonas elongata* (JQ340 332), and *P. bulbosa* (JQ340333), formed a clade with a moderate bootstrap value (ML: 72%) and a posterior probability of 0.78 (Fig. 4C).

Remarks. The strain IG005 resembled the original cells, which were a flat disc-shaped (Cavalier-Smith *et al*., 2008). The cell body of the original cells was about 4 μm in length, which is slightly shorter than our isolate. Also, the ventral surface behind the flagellar pocket of the original cells showed a concave depression. A broad and curved snout projected between the flagellum. Those features are also examined in the strain IG005. Contractile vacuole and cysts of the original cells were also absent. Like the strain IG005, the original cells displayed a slowly gliding motility. The anterior flagellum beats stiffly, while

the posterior flagellum displayed a gliding motion on the substrata. Furthermore, the anterior flagellum was usually shorter than the cell body. The flagellar length and motion, and moving behavior of the cells are consistent with the original cells. It is likely that 18S rDNA sequence of the strain IG005 formed a clade with *Ancyromonas micra* strains Millport and ATCC50267, *Planomonas elongata*, and *Planomonas bulbosa*, although the statistical supports were moderate. Moreover, the 18S rDNA sequence of the strain IG005 was closest to *A. micra* strains ATCC50267 and Millport (99.88%), rather than *P. elongata* (99.70%) and *P. bulbosa* (99.53%). In fact, *A. micra*, *P. elongata*, and *P. bulbosa* were morphologically similar to each other (Glücksman *et al*., 2013). However, the length of the posterior flagellum of *P. elongata* (11−13 μm) was longer than *A. micra* including our isolate. *P. bulbosa* had more bulbous rostrum than *A. micra* strains. Thus, we did not exclude the possibility that our isolate is identical to previous *A. micra*. However, further sequencing of various genes is needed to assess the monophyletic relationship of *A. micra*, *P. elongata*, and *P. bulbosa*.

Habitat. Surface water/sediment interface of coastal areas.

Specimen deposition. National Institute of Biological Resources, Korea (KPZXPR0000000027−KPZXPR0000000031).

Identifiers. Dong Hyuk Jeong and Jong Soo Park.

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