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Three newly recorded heterotrophic euglenids (Protist), *Entosiphon oblongum*, *Euglena longa* and *Keelungia pulex* from South Korea

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Three heterotrophic euglenids from marine water column (Seodo port, Yeosu) and freshwater sediment (Seodong-chun, Incheon), Korea were identified as *Entosiphon oblongum* Cavalier-Smith and Vickerman, 2016; *Euglena longa* (Pringsheim, 1936) Marin and Melkonian, 2003; and *Keelungia pulex* Chan and Moestrup, 2013 based on morphological characters and 18S rDNA sequence analysis. These species are reported taxonomically for the first time from Korea and are described with micrographs. Diagnoses of these species are as follows. *Entosiphon oblongum*: phagotrophic, gliding, size in vivo, 23.1−29.3 μm (Avg. 26.5 μm, n = 30) long, ovate with a protrusive feeding siphon (apparatus), several deep grooves and two heterodynamic flagella. *Euglena longa*: osmotrophic, swimming, size in vivo, 32.3−52.2 μm (Avg. 42.2 μm, n = 26) long, elongated with many paramylum granules and two flagellar. *Keelungia pulex*: phagotrophic, gliding, size in vivo, 13.5−19.7 μm (Avg. 16.4 μm, n = 97) long, oblong to ovoid with a hook-shaped ingestion apparatus, several dorsal ridges and two flagella.

Keywords: *Astasia longa*, *Entosiphon oblongum*, *Euglena longa*, Heterotrophic euglenids, *Keelungia pulex*

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Introduction

The phylum Euglenozoa is represented by three major subgroups; kinetoplastids, diplonemids and euglenids. Euglenids are abundant in freshwater, soil and marine habitats, and can be osmotrophic, phagotrophic and photosynthetic, or depend on ectosymbiotic bacteria.

The genus *Entosiphon* Stein, 1878 is phagotrophic. The characteristic feature of the genus is the long feeding siphon, which can make slight pumping movements. The most commonly reported species is *E. sulcatum*, which was previously recorded in Korea (Lee, 2015a). The genus *Euglena* Ehrenberg, 1830 contains photosynthetic euglenid species, except for *Eu. longa* and *Eu. quartana*, which are osmotrophs. Thirty-nine photosynthetic species were previously recorded in Korea (NIBR, 2019). The genus *Keelungia* created by Chan *et al.* (2013) contains two species, *K. nitschei* and *K. pulex*, which are phagotrophic with a hook-shaped ingestion apparatus. This genus is new to Korea.

Previous taxonomical studies of heterotrophic euglenids in Korea are Lee (2002; 2015a; 2015b; 2020) and Lax *et al.* (2019; 2021). This study reports for the first time the occurrences of *E. oblongum*, *Eu. longa* and *K. pulex* in South Korea, with small subunit ribosomal DNA (18S rDNA) sequence analysis.

Materials and Methods

Isolation, cultivation and light microscopy

Samples were collected from the marine water column and freshwater sediments, and the samples were placed in a Petri dish in ~0.3 cm deep layer. The materials were covered with a lens tissue. After 18−24 hours, euglenids were observed with a Nikon Eclipse TS100 inverted micro-scope (Japan). A single cell of each species was isolated by micro-pipetting from the Petri dish. The cells were inoculated into a well plate containing sterile 'SL medium' or 'FL medium [1% v/v Luria-Bertani (LB) media in seawater or freshwater]. Upon growth, the cultures also included uncharacterized prokaryotes, which served as prey. The cultures were maintained at 21°C in 25 cm^2 culture flasks (7 mL media). Light micrographs were collected from cultures ~one week old on a Leica DMR microscope (Germany) equipped with a Zeiss Axiocam HR digital

camera and its associated software (Axiovision 4.6).

The examined specimens, *E. oblongum* and *Eu. longa* were deposited in the National Institute of Biological Resources (NIBR), and *K. pulex* deposited in the Honam National Institute of Biological Resources (HNIBR), Korea.

DNA extraction, PCR amplification and sequencing

A 25-mL sample of about one-week-old culture was centrifuged at 8,000 rpm for 10 min, and the supernatant carefully removed by pipetting. The pellet was transferred to 2 mL Eppendorf tube and DNA was extracted using a Qiagen Blood & Cell Culture Extraction Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. 18S ribosomal DNA (18S rDNA) sequences were obtained by PCR amplification using combinations of the eukaryote primers [EukA (5′-AACCTGGTTGATCCT GCCAGT-3′) and EukB (5′-TGATCCTTCTGCAGGT TCACCTAC-3′), Medlin *et al*., 1988] for the strains *E. oblongum* (KF030) and *K. pulex* (KM080) and the euglenid specific primers [EukA and EzoaR (5′-GGRGCATCA CAGACCTGC-3′), Lax *et al*., 2019] for the strain *Eu. longa* (KF072). For the PCR amplification, a reaction volume of about 20 μL was used that included 1.5 μL of 10 μM stocks of the primers EukA and EukB (or EukA and EzoaR), 2 μL of 0.25 mM dNTP-mix, 1.8 μL of 25 mM $MgCl_2$, 0.8 μL total of 5 U/μL *Taq* DNA polymerase (Bioneer, Korea) and 3−5 μL of DNA template. The cycling conditions started with a denaturing step at 94°C for 5 min, followed by 35 cycles of 30 s at 94°C, 1 min of annealing at 55°C, and extension at 72°C for 2 min (10 min at 72°C for the final cycle only). A PCR product corresponding to the expected size was gel-isolated, directly purified, and then sequenced by Sanger sequencing using PCR primers along with internal sequencing primers (514F, 5′-GGTGCCAGCASCCGCGGTAA-3′ and Euk1209R 5′-GGGCATCACAGACCTG-3′). Individual reads were assembled using Geneious ver. 8.1.5 (Kearse *et al*., 2012).

The species identities were confirmed by NCBI BLASTN search results as well as by morphological features.

Results and Discussion

Phylum Euglenozoa Cavalier-Smith, 1981
Subphylum Euglenoida Bütschli, 1884
Class Entosiphonea Cavalier-Smith, 2016
Order Entosiphonida Cavalier-Smith, 2016
Family Entosiphonidae Cavalier-Smith, 2016
Genus *Entosiphon* Stein, 1878

1. *Entosiphon oblongum* Cavalier-Smith and Vickerman, 2016 (Figs. A−D)

Material examined. Korea, Incheon, Seo-gu, Yeonhui-dong, Shimgok-chun, 37°31′37.10″N, 126°39′36.81″E, 25 November 2019, collected by Won Je Lee. Type strain, live cells are kept with the Korean Culture Collection of Protists, Kyungnam University, Korea, reference 'KF030'.
Description. Phagotrophs and gliding euglenid. Cells are oblong and 23.1−29.3 μm (Avg. 26.5 μm, n = 30) long. The cells are usually slightly dorso-ventrally flattened and not metabolic or plastic. Two unequal heterodynamic flagella. The anterior flagellum is about the cell length and beats actively with a sweeping motion. The thicker posterior flagellum is 1−1.5 times the cell length, is deflected posteriorly and trails along the substrate. The chisel-shaped feeding siphon with two rods is moved in and out. Pellicle with several deep grooves. The cells may detach and swim with a trembling motion. The nucleus is located near the equator and to the right and is granular.

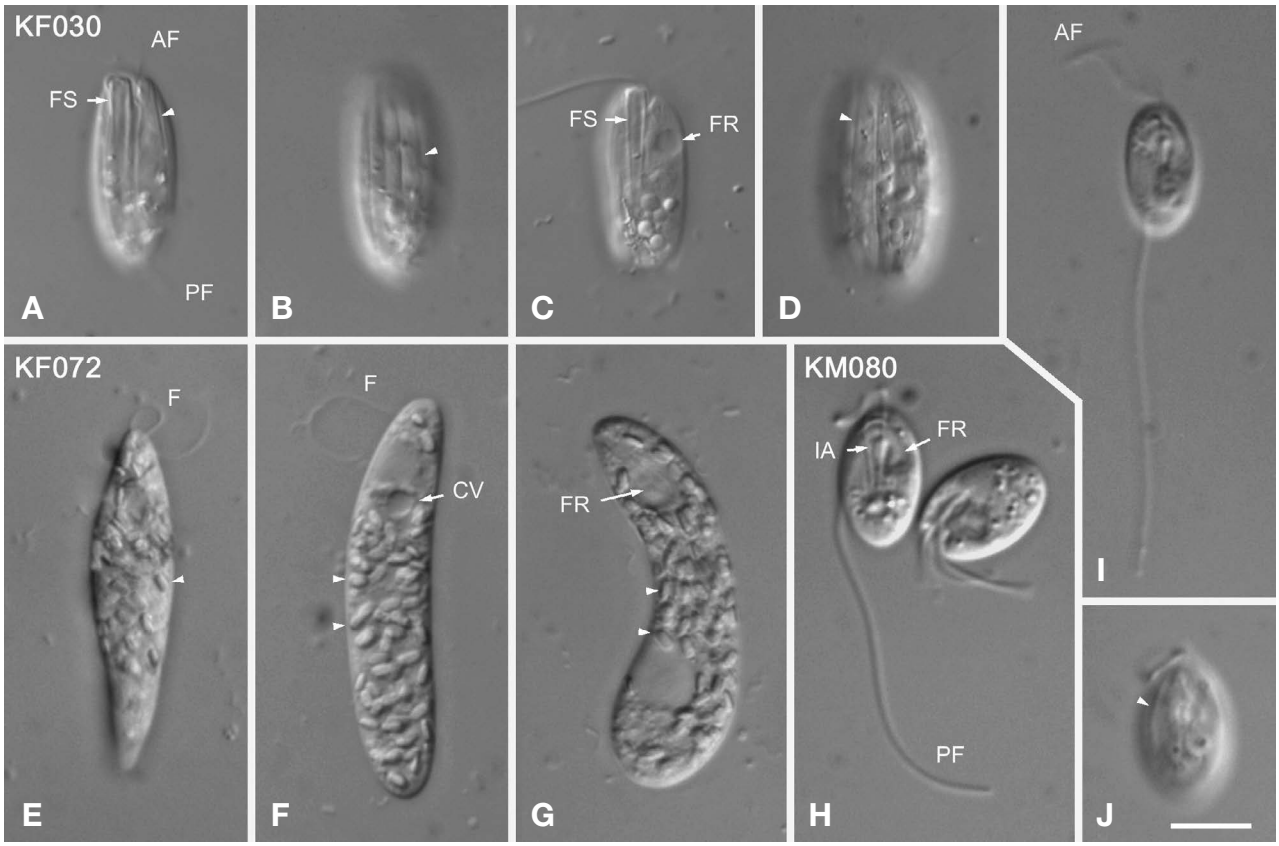
The closest sequence retrieved by NCBI BLASTN search was that of 18S rDNA sequence of *E. oblongum* CCAP 1220/2 (accession number; KP306754) with a high identity of 99.83%. The 18S rDNA sequence of the stain is 1,761 bp in length.
Remarks. The most similar genera are *Ploeotia* and *Serpenomonas*, but *Entosiphon* can be distinguished because its siphon is extensible, and because of ultrastructural differences in the construction of the feeding siphon (apparatus) and pellicle (Triemer, 1986; Triemer and Farmer, 1991). Among the genus, *E. oblongum* is closely related to *E. sulcatum*, but differing in shape and RNA and Hsp90 sequences (see Cavalier-Smith *et al*., 2016).
Habitat. Soil, Freshwater sediment.
World distribution. UK, Korea.
Deposition. NIBREG0000000195.
NCBI GenBank Accession Number. OM177657.
Identifiers. Won Je Lee.

Class Euglenoidea Lankester, 1885
Order Euglenida Stein, 1878
Family Euglenaceae Dujardin, 1841 emend. Klebs, 1883
Genus *Euglena* Ehrenberg, 1830 emend. Marin and Melkonian, 2003

2. *Euglena longa* (Pringsheim, 1936) Marine and Melkonian, 2003 (Figs. E−G)

Basionym: *Astasia longa* Pringsheim, 1936

Material examined. Korea, Incheon, Seo-gu, Yeonhui-dong, Shimgok-chun, 37°31′37.10″N, 126°39′36.81″E, 25 November 2019, collected by Won Je Lee. Type strain, live cells are kept with the Korean Culture Collection of Protists, Kyungnam University, Korea, reference 'KF072'.
Description. Osmotrophic euglenid. Cells are 32.3−52.2 μm (Avg. 42.2 μm, n = 26) long and elongated; narrowed posteriorly ending into a short tail piece; anteriorly bilabiate. Pellicle is spirally striated. Cytoplasm is slightly granular and have many small oval, paramylum granules, scat-



Figs. A–D: *Entosiphon oblongum* (KF030). A: General appearance of cell 1, showing ventral view. B: Dorsal view of cell 1. C: Ventral view of cell 2. D: Dorsal view of cell 3; Arrowheads show grooves. E–G: *Euglena longa* (KF072). E: General appearance of cell 1. F: Cell 2, showing CV. G: Cell 3 showing FR. Arrow heads show paramylum granules. H–J: *Keelungia pulex* (KM080). H: Cell 1 and cell 2. I: General appearance of gliding cell 3. J: Cell 4 showing dorsal view and ridge (arrow head). FS: feeding siphon, AF: anterior flagellum, PF: posterior flagellum, FR: flagellar reservoir, F: flagellum, CV: contractile vacuole, IA: ingestion apparatus. All micrographs are DIC (differential interference contrast) images. Scale bar: 10 μm in (J).

tered throughout the cell. The nucleus is nearly central. The cells swim with rotation.

The closest sequence retrieved by NCBI BLASTN search was that of 18S rDNA sequence of *Euglena longa* SAG: 1204-17a (accession number; AJ532428) with a high identity of 99.31%. The 18S rDNA sequence of the stain is 2,166 bp in length.

Remarks. Based on SSU rDNA analysis, a colorless species, *Astasia longa* Pringsheim, 1936 formed a clade with phototrophic species (*Eu. gracilis*, *Eu. clara*, *Eu. agilis*, strain M 1856) and a colorless species, *Eu. quartana* (Marin *et al*., 2003). Therefore, *Astasia longa* was transferred to the genus *Euglena* by Marin *et al*. (2003). Now the genus *Euglena* contains at least two secondary heterotrophs (*Eu. longa*, *Eu. quartana*). *Khawkinea* is a colorless genus with an eyespot and may have to be completely incorporated in *Euglena* following further study (Marin *et al*., 2003).

Habitat. Freshwater sediment.

World distribution. Canada, Korea.

Deposition. NIBREG0000000194.

NCBI GenBank Accession Number. OM177659.

Identifiers. Won Je Lee.

Class Stavomonadea Cavalier-Smith, 2016
Order Decastavida Cavalier-Smith, 2016
Family Keelungiidae Cavalier-Smith, 2016
Genus *Keelungia* Chan and Moestrup, 2013

3. *Keelungia pulex* Chan and Moestrup, 2013 (Figs. H–J)

Material examined. Korea, Chunnam, Yeosu-si, Samsan-myeon, Seodo-ri, Seodo Port, 34°3′18.26″N, 127°17′54.33″E, 6 May 2021, collected by Miran Kim. Type strain, live cells are kept with the Korean Culture Collection of Protists, Kyungnam University, Korea, reference 'KM080'.

Description. Phagotrophs and gliding euglenid with two emergent flagella. Cells are oblong to ovoid and 13.5–

19.7 μm (Avg. 16.4 μm, n = 97) long with two flagella. The anterior flagellum is directed forward and about the cell length, and the posterior flagellum is 2−4 times the cell length. The anterior flagellum commonly keeps in an S-shape and beats rapidly and is used for change of direction. The posterior flagellum is trailing. The hook-shaped ingestion apparatus with two rods is visible by light microscopy and reach almost to the posterior end of the cell. The flagellar reservoir extended for about half the cell length. The cells have several longitudinal ridges, which are generally difficult to see in the light microscope.

The closest sequence retrieved by NCBI BLASTN search was that of 18S rDNA sequence of *K. pulex* (accession number; HM044218) with a high identity of 100%. The 18S rDNA sequence of the stain is 1,576 bp in length.

Remarks. Present observations are in agreement with the previous observation (Chan *et al*., 2013), although the cell length ranges do not overlap (this population, 13.5−19.7 μm long; Chan and Moestrup's population, 8−11 μm). *Keelungia pulex* shows a high similarity in morphological characters with *K. nitschei* Arndt *et al*., 2019 (8.3−11.6 μm), but the anterior end of *K. nitscheri* is truncated. This is the main morphological character distinguishing *K. pulex* from *K. nitschei* (Schoenle *et al*., 2019). *Keelungia pulex* can be distinguished from a small ploeotid species, *Ploeotia azurina* (10−16 μm), because *P. azurina* is ovate with pointed posterior end. It resembles *P. longifilum* (12−20 μm) and *Keelungia* sp. (KM082) (9.3−13 μm) reported by Lax *et al*. (2019) in cell shape, cell length and long posterior flagellum. Further studies are needed to establish the identities of these species.

Habitat. Marine water column.

World distribution. Taiwan, Korea.

Deposition. HnibrEG000001-EG000005.

NCBI GenBank Accession Number. OM177658.

Identifiers. Won Je Lee.

Acknowledgements

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