

Three Newly Recorded Marine Heterotrophic Flagellates (Protist), *Neometanema parovale*, *Stephanopogon pattersoni* and *Thaumatomastix* sp. from South Korea

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Abstract - Three marine heterotrophic flagellates from intertidal sediments of Gwang-Am beach and Garorim Bay, Korea were identified as *Neometanema parovale* Lee and Patterson 2014, *Stephanopogon pattersoni* Lee et al. 2014 and *Thaumatomastix* sp. These species are reported taxonomically for the first time from Korea, and are described with illustrations and micrographs. Diagnostics of these species are as follows. *Neometanema parovale* (Euglenozoa): size in vivo, 10~23 μm long with 22 pellicular strips, ingestion apparatus barely visible by light microscopy and two flagella pointed in different directions when moving. *Stephanopogon pattersoni* (Percolozoa): size in vivo, 20~33 μm long, with 6 ventral and 1 ventro-lateral ciliary rows, and three barbs. *Thaumatomastix* sp. (Cercozoa): size in vivo, 14~17 μm with body scales and spines, and two flagella with one naked and one scaled.

Key words: Heterotrophic flagellates, *Neometanema parovale*, *Stephanopogon pattersoni*, *Thaumatomastix*

INTRODUCTION

Heterotrophic flagellates are important numerically and ecologically in aquatic ecosystems both in the water column and in the sediments (e.g., Azam *et al.* 1983; Sherr and Sherr 1988; Lee and Patterson 2002). Despite their importance, their taxonomy has until recently been little studied. Previous taxonomical studies of heterotrophic flagellates in Korea are Lee (2002) and Park *et al.* (2006, 2007).

The genus *Neometanema* Lee and Simpson 2014 (Euglenozoa: Euglenozoa) is one of heterotrophic euglenoid genera and composed of 11 species (Lee and Simpson 2014). Two species of *Neometanema* (*N. exaratum* and *N. ovale*) were previously recorded under the genus *Heteronema* in Korea (Lee 2002). This genus is new to Korea.

The genus *Stephanopogon* Entz 1884 (Pseudociliata: Percolata: Percolozoa) is one of 12 genera belonging to the

family Stephanopogonidae Corliss 1961. Seven species have been recorded in the genus *Stephanopogon* (Lee 2001; Lee *et al.* 2014). *Stephanopogon colpoda* was previously recorded in Korea (Lee 2002).

The genus *Thaumatomastix* Lauterborn 1899 (Thaumatomonadida: Imbricatea: Cercozoa) is one of four genera belonging to the family Thaumatomonadidae Hollande 1952 and is currently composed of 17 species (Howe *et al.* 2010; Zolotarev *et al.* 2011). This genus is new to Korea.

This study reports for the first time the occurrences of *Neometanema parovale*, *Stephanopogon pattersoni* and *Thaumatomastix* sp. in South Korea.

MATERIALS AND METHODS

1. Isolation, cultivation and light microscopy

Samples were collected from intertidal marine sediments to a depth of about 1 cm from a 1 m² quadrat at the Gwang-

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Am beach (35°06'N, 128°30'E) and Garorim Bay (36°53'N, 126°21'E), Korea. 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 containing sterile 'SL medium' (1% v/v Luria-Bertani (LB) media in seawater). Upon growth, the *Stephanopogon* and *Neometanema* cultures also contained an ancyromonad (*Ancyromonas sigmoides*), which served as prey, as well as uncharacterised prokaryotes. The *Thaumatostix* culture contained uncharacterized prokaryotes. The cultures were maintained at 21°C in 25 cm² culture flasks (10 mL media). Light micrographs were collected from cultures ~2 weeks old on the microscope and camera system described above using differential interference contrast optics.

The examined specimens, except for *Thaumatostix* sp. were deposited in the National Institute of Biological Resources (NIBR), Korea.

2. Scanning Electron Microscopy

A culture sample ~2 weeks old was fixed with 4% (w/v) OsO₄ for 30 min at room temperature and after that, one drop of 4% (w/v) OsO₄ was added to the sample. Cells were transferred onto a 5µm polycarbonate membrane filter (Corning Separations Div., Acton, MA), washed with sterile distilled water, dehydrated with a graded series of ethanol [30%-50%-70%-80%-90%-95% (2)-100% (3)] and critical-point-dried with CO₂. Filters were mounted on stubs, sputter-coated with gold and viewed under a Hitachi S4700 Scanning Electron Microscope (SEM).

RESULTS AND DISCUSSION

Phylum Euglenozoa Cavalier-Smith 1981 유글레나문
Class Euglenoidea Bütschli 1884 유글레나강
Genus *Neometanema* Lee and Simpson 2014
신메타네마속 (신칭)

1. *Neometanema parovale* Lee and Simpson 2014

스키신메타네마충 (신칭) (Fig. 1A-D)

Material examined: Korea, Kyungsangnam-do, Changwon, Gwang-Am beach (35°06'N, 128°30'E), 20 Aug 2013, collected by WJ Lee.

Diagnosis: Cells 10~23 µm (usually 15~20 µm) with 22 pellicular strips total, similar on dorsal and ventral sides. Two flagella pointed in different directions when moving. With knob on posterior flagellum, but this displaced 2.5~3 µm from base of flagellum and difficult to observe by light microscopy. Feeding apparatus visible by light microscopy in most cells, with difficulty.

Remarks: Present observations are in good agreement with the previous observation of Lee and Simpson (2014). *Neometanema parovale* is most similar to *Neometanema ovale* and *Neometanema exaratum*. These are flattened and ovoid, and have a skidding movement and a feeding apparatus visible by light microscopy. *Neometanema larseni* is notably larger than these three species, and the feeding apparatus is conspicuous. Although *Neometanema parovale* appears most similar to *N. ovale*, there are some differences between *N. parovale* and *N. ovale*. *Neometanema parovale* often have a posterior point, whereas this is not recorded in *N. ovale*. In fact, this feature supposedly distinguishes *N. ovale* and *N. exaratum* from *N. larseni*. *Neometanema parovale* does not have a knob at the base of the posterior flagellum as in *N. ovale* (Kahl 1928; Lee and Patterson 2000), but has the knob displaced 2.5~3 µm from the base of the flagellum (Lee and Patterson 2014). It is very difficult to see by light microscopy.

Neometanema is easily distinguished from other heterotrophic euglenids by the skidding movement and two flagella pointed in different directions when moving; the anterior flagellum points to the right, the posterior flagellum to the left.

Habitat: This species collected from the South Korea on marine intertidal sediments.

World distribution: Canada, Korea.

Deposition: NIBR No. KOSPPR0000106330.

Identifiers: Won Je Lee.

2. *Neometanema exaratum* (Larsen and Patterson

1990) Lee and Simpson 2014 타원신메타네마충 (신칭)

Heteronema exaratum Larsen and Patterson 1990: 844, figs.

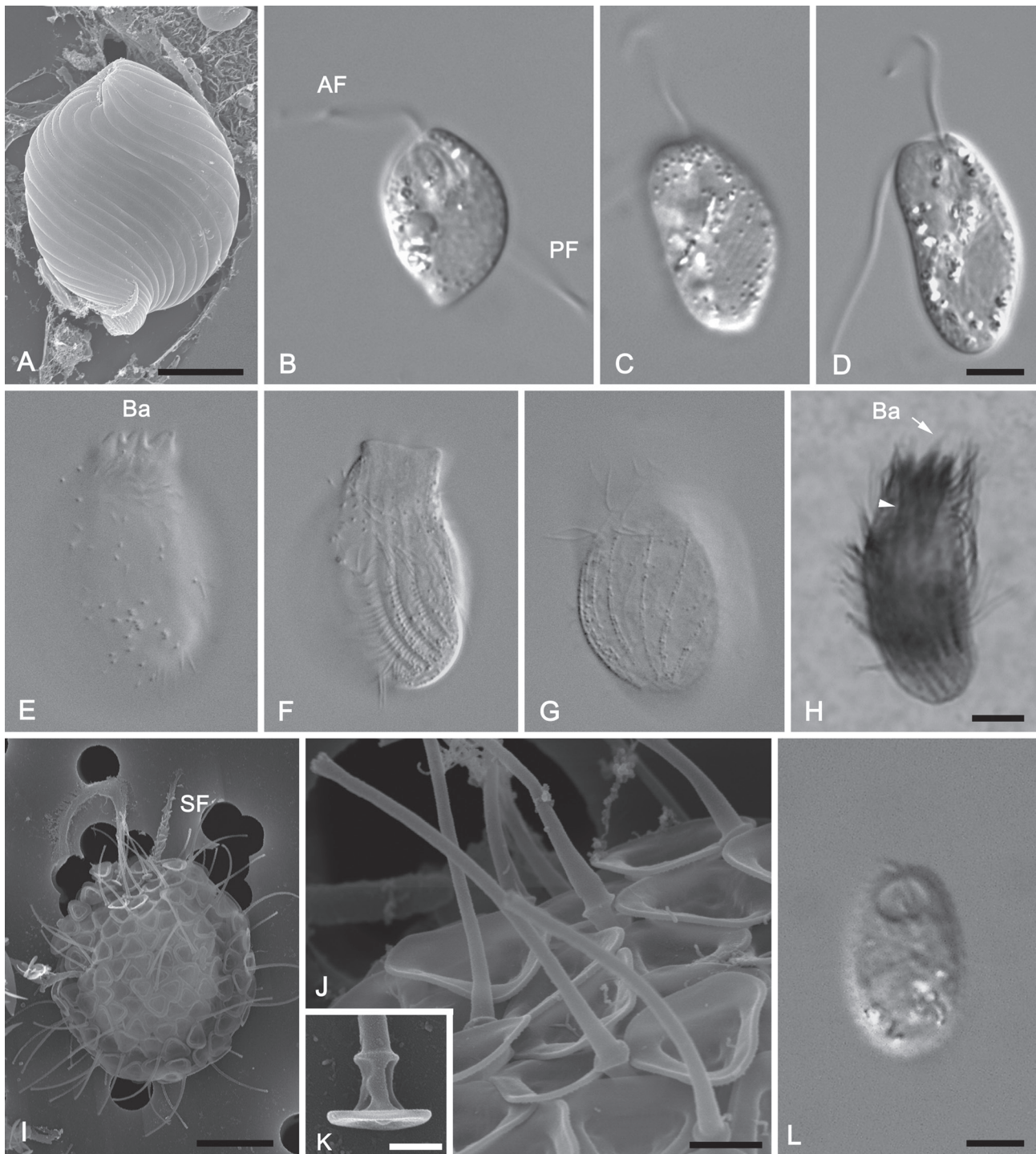


Fig. 1. A-D: *Neometanema parovale*. A: SEM image, showing ventral side of cell. B-D: DIC light microscopy images. B: General appearance of cell, showing two flagella pointed in different directions (AF: anterior flagellum, PF: posterior flagellum). C, D: elongated profiles of cell. E-H: *Stephanopogon pattersoni*. E-G: DIC light microscopy images. E, F: ventral side, showing barbs (Ba) and ciliary rows. G: dorsal side, showing rows of granules. H: Fixed, protagol-impregnated cell, showing feeding apparatus (arrowhead) and barbs (Ba). I-L: *Thaumatomastix* sp. I-K: SEM image, showing scales and spines. I: General appearance of cell, showing short flagellum (SF) with scales. J: Body scales and spines, K: Base of spine. L: DIC microscopy image, showing general appearance of cell. Scale bars: 5 μm in (A), 5 μm in (D) for Fig (B-D), 5 μm in (H) for Fig (E-H), 5 μm in (I), 0.5 μm in (J), 0.2 μm in (K) and 5 μm in (L).

15a-c; Lee 2002: 129, fig. 2g.

3. *Neometanema ovale* (Kahl 1929) Lee and Simpson

2014 꿈틀신메타네마충 (신칭)

Heteronema ovale Kahl 1928: 221, fig. 49g; Lee 2002: 129, figs. 2i-j.

Phylum Percolozoa Cavalier-Smith 1993 퍼콜로충문 (신칭)

Class Percolatea Cavalier-Smith 2003 퍼콜라타강 (신칭)

Order Pseudociliata Corliss and Lipscomb 1982

거짓섬모충목 (신칭)

Family Stephanopogonidae Corliss 1961 스테파노포과 (신칭)

Genus *Stephanopogon* Entz 1884 스테파노속 (신칭)

4. *Stephanopogon pattersoni* Lee, Miller and Simpson

2014 스테파노패터슨충 (신칭) (Fig. 1E-H)

Material examined: Korea, Kyungsangnam-do, Changwon, Gwang-Am beach (35°06'N, 128°30'E), 20 Aug 2013, collected by WJ Lee.

Diagnosis: *Stephanopogon* cells, 20~33 µm long, dorso-ventrally flattened, vase-shaped in profile, with neck and dorsal hump; 6 ventral and 1 ventro-lateral ciliary rows and 3 very short dorsal rows of cilia on left side at base of neck, 6~10 dorsal rows of granules on hump, with 3 barbs on ventral side; two isomorphic nuclei, each with central nucleolus; moves by crawling and swimming via cilia beating; reproduction occurred in cystic form.

Remarks: Generally, the observations of the species encountered here agree with that of Lee *et al.* (2014). *Stephanopogon pattersoni* differs from *Stephanopogon apogon* because *S. apogon* lacks ventral barbs, and also differs from *Stephanopogon mobilensis* because *S. mobilensis* has a total of five dorsal and ventral barbs (Jones and Owen 1974; Patterson and Brugerolle 1988). *Stephanopogon pattersoni* has less ciliary rows than *S. colpoda*, *S. mesnili* and *S. paramesnili*, and *S. pattersoni* is substantially smaller. By contrast, *S. pattersoni* resembles *S. minuta* in overall appearance, the cell length, the number of barbs, and the approximate number of ventral ciliary rows (Lei *et al.* 1999; Yubuki and Leander 2008; Lee *et al.* 2014). However, according to Lee *et al.* (2014), the SSU rDNA sequence of *Stephanopogon pattersoni* is substantially different to that of an isolate identified as *S. minuta* by Yubuki and Leander (2008).

Stephanopogon is distinguished from other heterotrophic flagellates by the cell appearance-like ciliate, and normally having barbs.

Habitat: This species collected from the South Korea on marine intertidal sediments.

World distribution: Canada, Korea.

Deposition: NIBR No. KOSPPR0000106330.

Identifiers: Won Je Lee.

5. *Stephanopogon colpoda* Entz 1884 스테파노콜포다충 (신칭)

Stephanopogon colpoda Entz 1884: 326, figs. 16-19; Lee 2002: 131, fig. 4a.

Phylum Cercozoa Cavalier-Smith 1998 아메바성편모충문

Class Imbrictea Cavalier-Smith 2003 임브리카타강 (신칭)

Order Thaumatomonadida (Shirkina 1987) Karpov 1990

싸우마토모나드목 (신칭)

Family Thaumatomonadidae Hollande 1952

싸우마토모나드과 (신칭)

Genus *Thaumatomastix* Lauterborn 1899

싸우마토마스틱속 (신칭)

6. *Thaumatomastix* sp. (Fig. 1I-L)

Material examined: Korea, Chungchungnam-do, Garorim Bay (36°53'N, 126°21'E), 31 Mar. 2015, collected by WJ Lee.

Diagnosis: Solitary, cells 14~17 µm long, oval or ovoid shaped with ventral groove, with layer of visible scales and delicate spines. With two unequal flagella loosely emerging from shallow apical depression. Long flagellum 1.2~1.6 times cell length, naked and directed posteriorly. Short flagellum 0.6~1 times cell length and covered by minute oval scales. Glide slowly. Food materials seen throughout cell.

Remarks: This species is assigned to *Thaumatomastix* because it is a gliding flagellate, with two flagella inserting into a subapical depression, and has a layer of visible scales and spines. *Thaumatomastix* Lauterborn 1899, *Thaumatomonas* de Saedeleer 1931 and *Protaspa* (Skuja 1939) Cavalier-Smith 2011 have similar characters; a similar shape, a ventral groove, anteriorly located nucleus. They glide, produce pseudopodia and have two flagella. *Thaumatomastix* and *Thaumatomonas* have been known to have scales visible by

electron microscopy or light microscopy, but *Protaspa* has not been known whether it has scales or not. Further studies are needed to establish the identities of these genera and also the species encountered here.

Thaumatostix can be distinguished from other heterotrophic flagellates by having body scales and spines, and the short flagellum with scales.

Habitat: This species collected from the South Korea on marine sediments.

World distribution: Korea.

Identifiers: Won Je Lee.

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