Journal of Species Research 5(3):381-384, 2016

New Korean records of two amoeboid protozoa (Protist); *Vannella bursella* and *Pseudoparamoeba* sp.

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Two marine amoebae *Vannella bursella* (Page, 1974) Smirnov *et al*., 2007 and *Pseudoparamoeba* sp. were encountered from marine coastal waters of Masan Bay and Garorim Bay (Korea), respectively. 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. *Vannella bursella*: size in vivo, 17-29 μm long with flattened ovoid, semicircular locomotive forms. *Pseudoparamoeba* sp.: size in vivo, 10-15 μm long with elongated locomotive forms, producing a few short conical pseudopodia from anterior hyaline zone.

Keywords: Amoebozoa, Discosea, *Pseudoparamoeba*, *Vannella bursella*, *Vexillifera*

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DOI:10.12651/JSR.2016.5.3.381

Introduction

The genus *Vannella* belongs to the family Vannellidae, order Vannellida, subclass Flabellinia of the class Discosea (Smirnov *et al*., 2011). It unifies flattened fan-shaped amoebae with a large frontal area of hyaloplasm (Smirnov and Goodkov, 1999; Smirnov and Brown, 2004). Among the Flabellinia, the genus *Vannella* seems to be one of the most common and widely distributed genera (Smirnov, 2001; Smirnov *et al*., 2016), but species diversity within the genus requires further attention. Two species of *Vannella* [*V. mira* Page, 1988 and *V. placida* (Page, 1968) Smirnov *et al*., 2007] were previously recorded in Korea (Kim *et al*., 2012).

The genus *Pseudoparamoeba* belongs to the family Paramoebidae, order Dactylopodia and class Discosea, and includes only two species: *P. microlepis* Udalov, 2016 and *P. pagei* (Sawyer, 1975) Page, 1983. It is characterized by elongated locomotive form, producing a few short conical pseudopodia from anterior hyaline zone and one to three long narrowly conical pseudopodia, and surface covered with truncate glycostyles (Udalov, 2016). This genus is new to Korea.

This study reports for the first time the occurrences of *Vannella bursella* and *Pseudoparamoeba* sp. in South Korea.

Materials and Methods

Seawater samples were collected from marine water columns of Masan Bay (35°10′N, 128°34′E) and Garorim Bay (36°53′N, 126°21′E), Korea. The samples were processed as described elsewhere (Lee and Patterson, 2000): Briefly, water samples were collected, placed in layers 0.5 cm deep in trays and allowed to settle for several hours. The material was covered with a sheet of lens tissue upon which 22 × 22 mm No.1 coverslips were placed. After 12 hours the coverslips were removed and protists were observed with a Leica DMR microscope (Germany) equipped with a Zeiss Axiocam HR digital camera and its associated software (Axiovision 4.6). Single cells corresponding to the morphospecies *Pseudoparamoeba* and *Vannella* were isolated by micropipetting on the coverslips, inoculated into a well plate containing 1% v/v SL medium (Lee and Simpson, 2014), and maintained at 21°C. Upon growth, the cultures contained uncharacterised prokaryotes, which served as prey and were maintained at 21°C in 25 cm^2 culture flasks (10 mL media). Light micrographs were collected from cultures ~1 week old on the microscope and camera system described above using differential interference contrast optics.

The examined specimens were deposited in the National Institute of Biological Resources (NIBR), Korea.

Results and Discussion

Phylum Amoebozoa (Lühe, 1913) Corliss, 1984
Class Discosea Cavalier-Smith, 2004
Order Vannellida Smirnov *et al*., 2005
Family Vannellidae (Bovee, 1970) Page, 1987
Genus *Vannella* Bovee, 1965

Vannella bursella (Page, 1974) Smirnov *et al*., 2007 (Fig. 1)

Material examined. Korea: Kyungsangnam-do, Masan Bay (35°23′N, 128°68′E), 29 Aug. 2012, from marine water column, collected by Won Je Lee. Type strain, live cells are kept with the Culture Collection of Protists, Kyungnam University, Korea, reference "KM054".

Description. Locomotive form flattened ovoid, semi-circular. Frontal hyaloplasm occupies about half the body length; posterior end widely rounded, straight or (rarely) concave. Greatest dimension of locomotive form 17-29 μm (avg. 23 μm). Single vesicular nucleus with central nucleolus. Floating form a rounded mass, without pseudopodia or with few blunt hyaline pseudopodia, rarely exceeding in length the diameter of the central mass. Cell surface includes glycostyles. Cysts not seen.

Remarks. This species resembles *Vannella arabica* Page, 1980 in cell length, but its locomotive forms are rather different from those of *V. arabica*. *Vannella bursella* has clearly semicircular locomotive forms while *V. arabica* has generally fan-shaped forms. In the culture of *V. bursella*, most cells were semicircular forms, but few cells were fan-shaped forms. In addition, according to

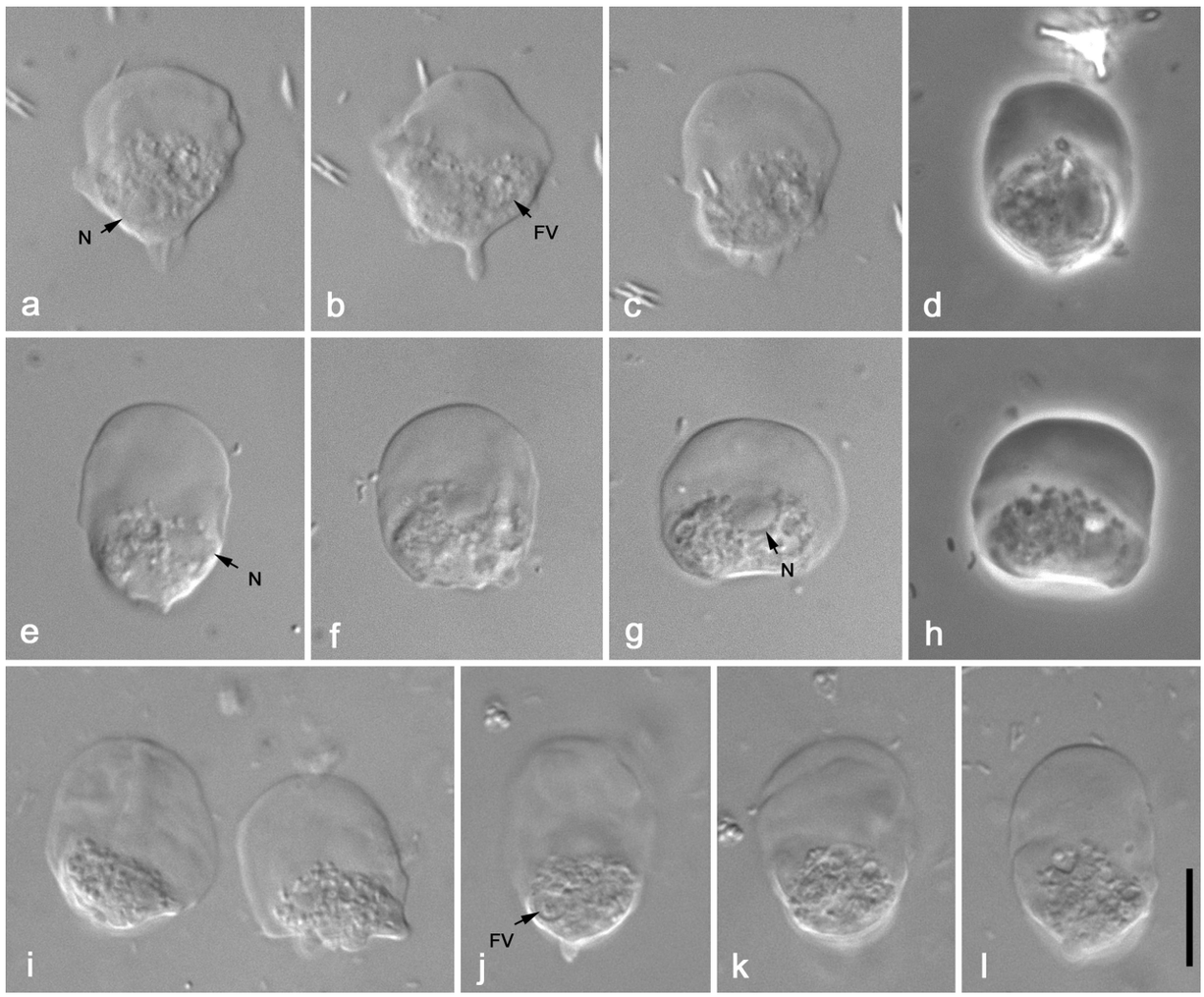


Fig. 1. *Vannella bursella*, isolate KM054, Locomotive forms of seven different cells. (a)-(c) Cell 1, (c) note pseudopodia drawn from posterior end of cell. (d) Cell 2. (e) Cell 3. (f)-(h) Cell 4. (i) Cells 5 & 6. (j)-(l) Cell 7. Nucleus (N), food vacuole (FV). All micrographs are DIC (differential interference contrast) images with the exceptions of (d) and (h) (phase contrast images). Scale bar in (l) = 10 μm for all images.

Smirnov *et al*. (2007), the species group of *V. arabica*, *V. bursella* and *V. calycinucleolus* has 100% support by three methods (NJ, PHYML and Bayesian trees), and only 5 bases differ between *V. arabica* and *V. bursella*; one at the beginning of the sequence, the rest in the most variable parts of the molecule. Further studies are needed to clarify these two species.

Habitat. Marine water.

World distribution. England, Korea.

Deposition. NIBRPR0000106579, NIBRPR0000106580.

Order Dactylopodida Smirnov *et al*., 2005
Family Paramoebidae (Poche, 1913) Kudryavtsev *et al*., 2011
Genus *Pseudoparamoeba* Page, 1979

***Pseudoparamoeba* sp. (Fig. 2)**

Material examined. Korea: Chungchungnam-do, Garorim Bay (36°78′N, 126°48′E), 31 Mar. 2015, from marine water columns, collected by Won Je Lee. Type strain, live cells are kept with the Culture Collection of Protists, Kyungnam University, Korea, reference "KM045".

Description. Locomotive form longer than broad, producing a few short subpseudopodia from anterior hyaline zone. Some may elongate to long, narrow pseudopodia. Length not including long pseudopodia 10-15 μm (avg. 12.5 μm). Floating form usually irregularly rounded with radiating hyaline pseudopodia tapering to very fine tip. Surface covered with truncate glycostyles. Cysts not seen.

Remarks. This species encountered here are assigned to *Pseudoparamoeba* because it has general appearances of the genus: elongated locomotive form, producing a few short pseudopodia from anterior hyaline zone, and surface covered with glycostyles. This genus includes only two species, *P. microlepsis* and *P. pagei*. *Pseudoparamoeba* sp. is different from these two species because *P. microlepsis* is from a freshwater habitat and has one contractile vacuole, and *P. pagei* often produces wide conical subpseudopodia, resembling in outline those of *Vexillifera* spp. This species does not have this kind of characters. Further studies such as electron microscopic and molecular works are needed to establish the identity of the species encountered here.

This genus is morphologically similar to *Vexillifera*,

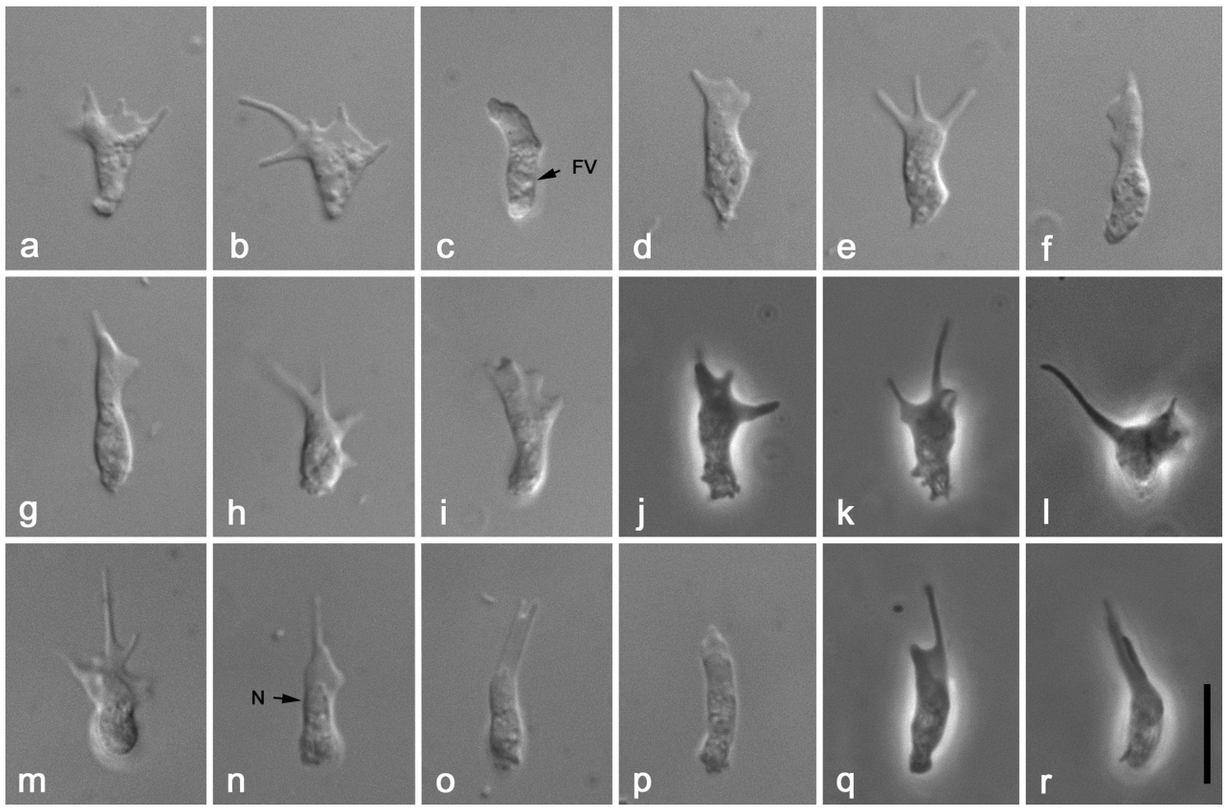


Fig. 2. *Pseudoparamoeba* sp, isolate KM045. Locomotive forms of two different cells. (a)-(l) Cell 1. (m)-(r) Cell 2. Nucleus (N), food vacuole (FV). All micrographs are DIC images with the exceptions of (j)-(l) and (q)-(r) (phase contrast images). Scale bar in (r) = 10 μm for all images.

belonging to the family Vexilliferidae but *Vexillifera* differs from five genera (*Pseudoparamoeba*, *Paramoeba*, *Neoparamoeba*, *Kornevella* and *Cunea*) of the family Paramoebidae within the order Dactylopodida by its remarkable locomotive form with long and slender dactylopodia in combination with an amorphous glycocalyx (Udalov, 2016).

Habitat. Marine water.

World distribution. Korea.

Deposition. NIBRPR0000106577, NIBRPR0000106578.

Acknowledgements

This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201501201), and by the project titled 'Long-term change of structure and function in marine ecosystems of Korea', funded by the Ministry of Oceans and Fisheries, Korea.

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Submitted: August 3, 2016
Revised: September 27, 2016
Accepted: October 13, 2016