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Pseudodifflugia klarae nov. spec., *Bereczkya minuta* nov. gen. nov. spec. and *Paramphitrema muelleri* nov. spec.: Three New Filose Testate Amoebae from the Plankton of the River Danube

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Summary. Three new, minute testate amoeban species smaller than 15 µm, including one new genus, are described from the plankton of the River Danube (Hungary) using high resolution video micrography. *Pseudodifflugia klarae* nov. spec. is characterised by an oval/pyriform, rigid, slightly compressed, scarcely or densely agglutinated test with a length of 8–14 µm. Its aperture is irregular in outline and inconspicuous; the nucleus contains one nucleolus and a few bent rods in the nucleoplasm. *Bereczkya* nov. gen., an incertae sedis cercozoan, has a minute spherical cell enclosed in a thin, rigid, more or less agglutinated organic test that is filled entirely by the cytoplasm. Its test bears an irregular and inconspicuous aperture. A collar-like ectoplasmic rim is situated in the aperture, from which a pseudopodial stem with filopodia is erected. The nucleus is slightly irregular, without a central nucleolus, but it contains rod-shaped granules in the nucleoplasm. *Bereczkya minuta* nov. spec. (test length: 3.5–8 µm, the diameter of the ectoplasmic rim: 0.8–2.3 µm) has a spherical test with asymmetric swellings and depressions, agglutinated with refractile mineral and other flat or irregularly-shaped xenosomes that may sometimes be almost entirely absent. *Paramphitrema muelleri* nov. spec. has a tubular or lemon-like test (length: 13–17 µm), which tapers towards the rigid apertures. The test is rigid, agglutinated and is circular in cross section. The nucleus is vesicular. Thin filopodia, as well as 1–2 thick, straight, unbranched, tubular pseudopodia are produced.

Key words: *Pseudodifflugia klarae*, *Bereczkya*, *Paramphitrema muelleri*, testate amoebae, river plankton.

Abbreviations: a – aperture; cg – refractile cytoplasmic granules; er – ectoplasmic rim around the pseudopodial stem; cv – contractile vacuole; fp – ingested plastids/endosymbionts; fv – food vacuole; mx – mineral xenosome; ne – nuclear envelope; ng – granules in the nucleoplasm; nu – nucleolus; pm – plasmamembrane; po – pores in the organic cement matrix; ps – pseudopodial stem in the aperture; tw – the organic layer of the test wall; xl – layer of xenosomes.

INTRODUCTION

A considerable number of new testacean species has been recently described from intensively studied habitats, like marine littoral psammon (Golemansky and Todorov 2004), or the benthos of small freshwa-

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ter bodies (e.g., Nicholls 2007). However, apart from some ecological surveys (Bini *et al*. 2003), the testacean community of river plankton has received little attention. The few exceptions are restricted to faunistic lists (Alves *et al*. 2007). The riverine plankton, nevertheless, comprises a high diversity of protists, partially because species are collected from a large area. There is a number of small sized or light shelled testacean species in the plankton of rivers, which are mainly drifted from the benthos (Bereczky 1979, Schönborn 1981). In this paper we describe three new testacean species, including one new genus, from the plankton of the River Danube (Hungary). They all have an extremely small size (under 15 µm), thus they fall out of the size range of the ordinary testacean surveys. We use high resolution video micrography to study these species. This method has been technically available for a long time but has not been widely used by taxonomists for the observation and description of species. We prefer this method to traditional photomicrography for its benefits in documenting the movements of the organisms and a number of minute structures and processes, which can be reconstructed from the different focal plains in three dimensions.

MATERIALS AND METHODS

These species have been found during a diversity survey that focused on heterotrophic flagellates and other nanoeukaryotes. A single plankton sample was taken on 16 March 2008 from the east bank of the River Danube, at Göd, 20 km north from Budapest (river km 1668, Hungary). The depth of the water was 1.5 m and the current of the water was about 1.5 ms^{-1} at the sampling point. The water level was moderately high (333 cm at Budapest) and slowly subsiding at the sampling time. Approximately 50 liters of river water (5 sequential dippings by one bucket) were filtered through a 10 µm mesh plankton net. The concentrated plankton sample with a 150 ml final volume was transported immediately to the laboratory at *in situ* temperature. The concentrated sample was transferred into a round glass vessel with a flat bottom of 15 cm diameter, and covered by a large Petri dish lid. Afterwards it was left undisturbed for 37 days at room temperature for the microbial succession. Exposure to direct sunlight was avoided. All sampling equipment was sterile; plastic components were disinfected with ethanol. This unenriched bulk culture was subsampled and investigated daily using an Olympus IX-70 inverted microscope, equipped with a 100×HI (NA 1.3) objective, standard Köhler illumination and a Normanski DIC system, which was adjusted to strong, close-to-darkfield contrast. The observed material was put in a microaquarium, with a coverslip bottom and a plastic frame border. The condenser was pushed directly into the water sample. Video micrography was performed with an analogous 3 CCD camera (JVC KY-F30B, 720 × 576 pixels). The signal of the camera was digitalized by an A/D converter external hardware (Dazzle Hollywood DV-Bridge), and was recorded without compression on the hard disk of a computer. Videos were later analyzed in detail, often frame by frame. Some further digital contrasting was also used during the analysis. Pictures were cut out from the videos and improved by some digital editing (Adobe Photoshop) to decrease the black-noise and increase the contrast. Some drawings were drawn precisely from the videos by combining frames of different focal plains and reconstructing the structures three dimensionally.

RESULTS AND DISCUSSION

Pseudodifflugia klarae nov. spec. Kiss and Török (Fig. 1)

Rhizaria, Cercozoa incertae sedis, Family: Pseudodifflugiidae, Genus: *Pseudodifflugia*

Diagnosis: *Pseudodifflugia* with oval or pyriform test; the length of the test is between 8–14 µm. The test is rigid, slightly compressed and scarcely or densely agglutinated with small mineral particles. The aperture is irregular in outline and inconspicuous, it is not encrusted with xenosomes, and it has no organic rim. The nucleus contains one eccentric nucleolus and a few bent rods in the nucleoplasm.

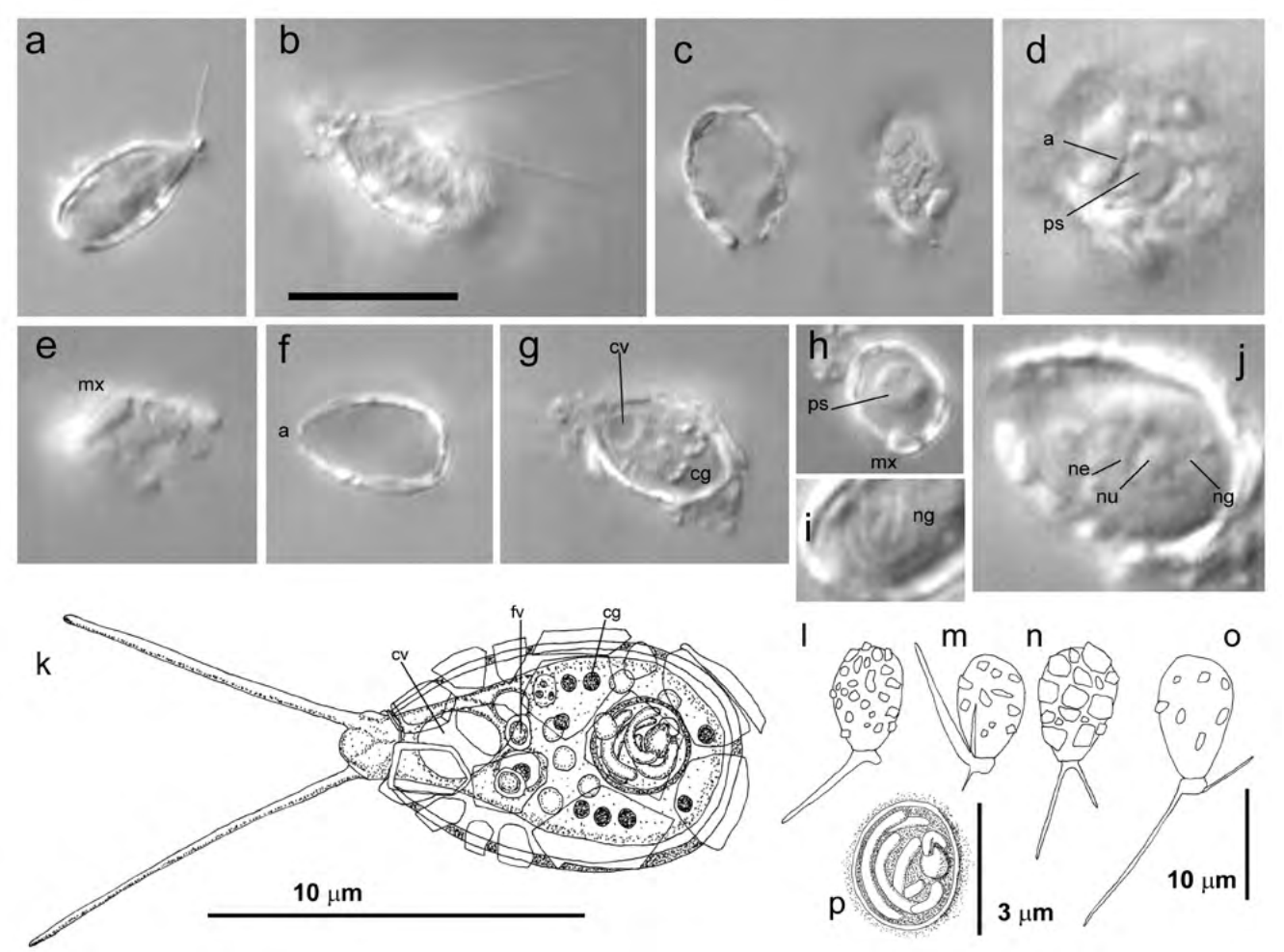
The morphology of the test: This minute *Pseudodifflugia* species has a rigid hyaline test, which is agglutinated with mineral particles. The size of the test is variable with a length of 8–14 µm (Table 1). The shape of the test also varies considerably: it may be oval, equally rounded on both ends (Fig. 1c), elliptical (Fig. 1a), or pyriform with a tapered aperture region. The posterior pole of the test is nearly circular, or it may be a little bit pointed (Fig. 1g) or truncated (Fig. 1f). The test is compressed slightly (Fig. 1h), with about a 0.8 depth/width

▸▸

Figs 1a–p. *Pseudodifflugia klarae* nov. spec. **a–b** – specimens with filopodia; **c**, **f** – empty tests: **d** – the shape of the aperture (top view), **e** – xenosomes on the surface of the test; **g** – the structure of the cytoplasm; **h** – optical cross section of the test posterior to the aperture; **i–j** – the structure of the nucleus; **i** – optical cross section with the rods in the nucleoplasm, **j** – optical cross section with the nucleolus; **k** – a combined drawing with the test and the components of the cytoplasm; **l–o** – specimens with different test forms and pseudopodia: **l–m** – thicker conical pseudopodia, **n–o** – typical thin filopodia; **p** – the structure of the nucleus showing the nucleolus and the rods. Scale bars for the photos: a–c, e–h = 10 µm, d, i–j = 5 µm. Scale bars for the drawings: k, l–o = 10 µm, p = 3 µm.

Table 1. Morphometric data on 34 specimens of *Pseudodifflugia klarae* nov. spec. Contractile vacuoles are measured before systole, pseudopodia at their maximal lengths. All measurements are in µm.

Pseudodifflugia klarae n. sp.	Mean	Median	Min	Max	SD	SE	CV %	n
Test length	9.5	9.1	8.0	14.0	1.4	0.24	14	32
Test width	6.7	6.3	5.1	10.7	1.4	0.24	21	34
Test depth	6.6		5.5	8.6				4
Test depth/width	0.81		0.85	0.77				4
Diameter of aperture	1.3	1.2	0.4	3.0	0.54	0.10	40	27
Diameter of nucleus	3.1	2.9	2.7	4.3	0.51	0.17	16	9
Diameter of nucleolus	1.2	1.2	1.1	1.3				5
Diameter of contractile vacuoles	1.9	1.8	1.1	2.8	0.49	0.16	26	9
Length of filopodia	12.6	12	6.4	22	4.3	1.1	34	15
Length of ingested bacteria	0.84	0.9	0.3	2	0.35	0.077	42	21



ratio. The test is organic, hyaline and highly refractile; it may be colourless, or yellowish. It is agglutinated, but the number and size of the attached particles is variable. Some tests are almost bare with very few particles (Figs 1a, f, o). Others are densely agglutinated, where the xenosomes cover the organic test layer without free gaps (Figs 1c, n). The xenosomes are angular quartz particles. The larger xenosomes are usually more or less flat, some of them may be as long as one third the test length (Fig. 1e). The xenosomes are attached on the surface, or embedded into the organic cement material. The aperture is a nearly circular, irregular, terminal hole (Fig. 1d). It is not encrusted regularly with xenosomes and not surrounded by an organic rim.

The morphology of the cell: In some specimens the cytoplasm fills the test entirely (Figs 1a, g), while in others the posterior part of the fundus is free of the cytoplasm. The cytoplasm is usually separated from the test near the aperture (Fig. 1g; optical transverse section in, 1h). About 3–10 contractile vacuoles are present in the cytoplasm near to the aperture (Fig. 1g). The food vacuoles are situated in the anterior and the middle parts of the cytoplasm. The nucleus is in the posterior part of the cell. It is spherical or oval, but it may be also reniform. A single spherical, eccentric nucleolus is situated in every nucleus (Figs 1j, 2p). There are 5–8 pieces of sausage shaped, usually curved rods in the nucleoplasm (Figs 1i, p). About 10–20 moderately refractile, spherical granules are located in the middle and the posterior parts of the cytoplasm (Figs 1g, k). The pseudopodia originate from a blunt pseudopodial stem, which is extended out of the aperture. The pseudopodia are mostly long, thin, tubular, parallel sided filopodia (Figs 1a–b, k, n–o). Their tips are pointed or rounded. 1–4 filopodia are produced simultaneously, they may reach twice the test length. Sometimes conical pseudopodia are also produced with thicker proximal and continuously tapering distal parts (Figs 1l–m).

Movement and feeding: The organism can glide on the attached tips and sides of the rigid extended filopodia. The gliding is discontinuous and slow. The angle of the test to the surface may change rapidly. As the test is rigid, the aperture limits the size of the ingestible food particles to a maximum diameter of 2–3 µm. Accordingly, the observed size of the ingested food particles falls between 0.3–1.9 µm (Table 1). The food vacuoles may remain single, or they may fuse to form larger collective food vacuoles. Mainly coccoid and bacilliform bacteria were ingested, algal cells have not been found in the food vacuoles.

Occurrence: This species formed an abundant living population in the culture. It persisted from the 5th to the 19th days of the succession experiment. Living specimens of this species have also been found in other occasions (Nov. 2007, Jun. 2008) in fresh, living plankton samples from the Danube. The description is based on 34 specimens, of which 9 were living.

Etymology: We dedicate this species to the 95th anniversary of Klára Gelei, who is the eldest daughter of Prof. József Gelei, the great Hungarian zoologist, cytologist and ciliatologist working in the early 20th century.

Type figures: Figs 1a, c, e.

Type locality: The plankton of the main arm of the River Danube at Göd, 20 km north from Budapest, Hungary (47°40′44,2″N, 19°07′29,9″E).

Material from the type locality: The sample with this species was preserved and retained by the first author under sample number KD 080308-1.

Discussion: Species of the genus *Pseudodifflugia* Schlumberger, 1845 are filose testate amoebae with a *Difflugia*-like rigid test, which is chitinoid, or completely covered with xenosomes (De Saedeleer 1934, Meisterfeld 2002). So far 20 species and two additional subspecies have been described under the generic name of *Pseudodifflugia*. The variation among the described *Pseudodifflugia* species is high in terms of the shape, size and rigidity of the test, the arrangement and nature of the agglutinated particles, as well as the size and the shape of the aperture and the type of the nucleus. Accordingly, this is a problematic and heterogeneous collective genus, which comprises filoseans with an agglutinated oval or pyriform test, which has no apertural collar. There are a number of incomplete species descriptions; some of them rely only on empty tests. Unfortunately, the type species, *Pseudodifflugia gracilis* Schlumberger, 1845 is described superficially without any illustrations and thus it is not identifiable to species level. The great variety of the organisms described under the name *Pseudodifflugia*, and the lack of an adequate type species description and generic diagnosis makes the description of a new species somewhat problematic.

The genus *Pseudodifflugia sensu stricto* can be characterised by the followings (Ogden and Hedley 1980, Meisterfeld 2002): the species have an oval or pyriform, rigid test without collar. The test is not filled entirely by the cytoplasm. It is densely or sparsely agglutinated with mineral xenosomes. The xenosomes attach to the surface of the test, or they are embedded into and sur-

rounded by a structureless, sheath like organic cement matrix. The aperture may be circular and regularly agglutinated with xenosomes; it may have an organic rim, or it may be narrow and irregular.

We assume that *Pseudodifflugia klarae* belongs to the genus *Pseudodifflugia sensu stricto*, because of its rigid, agglutinated, oval or pyriform test and the presence of filopodia. It differs substantially from all the described species by its minute size. The vesicular nucleus with the bent rods also seems to be a unique feature. From the 20 species of *Pseudodifflugia*, three are described from marine psammon, other two from soil, while 15 are from freshwater environments. The smallest *Pseudodifflugia* species reported from freshwater are *Pseudodifflugia fulva* (Archer 1870) Penard, 1902 (size range according to Penard: 15–23 μm), *Pseudodifflugia fascicularis* Penard, 1902 (size range according to Penard: 17–71 μm) and *Pseudodifflugia gracilis* Schlumberger, 1845 (size range according to Penard 1902: 20–65 μm). Besides its larger size, *Pseudodifflugia fulva* differs from *P. klarae* in the circular cross section of its test, the larger, more conspicuous circular aperture that sometimes has a delicate organic rim, and the frequent presence of larger, rough xenosome particles. *Pseudodifflugia fascicularis* differs in the presence of a more or less regular, tapering, then widening neck, and the apertural collar with the agglutinated particles. *Pseudodifflugia gracilis* (*sensu* Penard 1902) has a broadly ovoid or subspherical test with a narrow or wide circular aperture. Two slightly larger, but somewhat similar species are *Pseudodifflugia microstoma* Playfair, 1917 and *Pseudodifflugia procera* Badewitz, 2003. *P. microstoma* is similar to *P. klarae* in its narrow and undefined aperture, but it is larger than *P. klarae* (30–31 μm), and has a wider and broadly ovate test (Playfair 1917). *P. procera* is larger (42–55 μm), and its test is narrower with almost straight sides tapering towards a wide circular aperture (Badewitz 2003).

The test of *P. klarae* shows considerable variations concerning length, general shape and the density of agglutination. The variability in the shape of the test in *P. klarae* (from rounded oval to pyriform with concave tapering 'neck') occur in other *Pseudodifflugia* species, like *P. fulva* (straight sided tapering vs. rounded apertural pole) or *P. fascicularis sensu* Penard (regularly arched long neck vs. dumpy subspherical forms with tapering pointed aperture region). The density of the agglutination is highly variable in the population of *P. klarae* (from almost bare tests to densely covered ones). Similarly, only partial xenosome covering is reported in *P. fulva* (Penard 1902). The fragility and high transparency of the test in *P. klarae* is considered to be an effect of the small size, and consequently the presence of smaller and thinner xenosomes and thinner organic layer.

The type of the nucleus is variable within the genus *Pseudodifflugia sensu stricto*: vesicular type is reported for '*P. gracilis'* (De Saedeleer 1934, Hoogenraad and De Groot 1940) and *P. fulva* (Penard 1902, Cash *et al*. 1915), ovular is for *P. fascicularis sensu* Penard (Penard 1902, Cash *et al*. 1915). The unusual nuclear structure of *P. klarae* cannot be classified unambiguously into vesicular or ovular types according to the simple scheme of Raikov (1982). As this nuclear structure was found in all living specimens, even from different sampling occasions, this seems to be a stable character of this species.

Although most *Pseudodifflugia* species are reported to be herbivorous (Meisterfeld 2002), *P. klarae* consumes primarily bacteria. Its narrow, rigid aperture does not allow the ingestion of particles larger than 2–3 μm. The mean length of consumed bacteria (0.8 μm) is rather small, even when compared to some smaller interception feeding nanoflagellates (Boenigk and Arndt 2000). By its minute size and straight filopodia, *P. klarae* is able to glide on rough aggregate surfaces, graze bacteria on them efficiently, and grow actively in the plankton of rivers.

Bereczkya nov. gen. Kiss and Török

Rhizaria, Cercozoa incertae sedis

Diagnosis: Minute spherical cells enclosed in a thin, rigid, often asymmetric, more or less agglutinated organic test, which has an irregular and inconspicuous apertural hole. The cytoplasm fills the test entirely. A collar-like ectoplasmic rim is situated within the aperture, from which a pseudopodial stem is erected with occasionally branching filopodia. The nucleus is slightly irregular with rod shaped granules in the nucleoplasm and without a central nucleolus.

Etymology: This genus is dedicated to the 70th birthday of Dr. Magdolna Csutorné Bereczky, who worked for more than 30 years at the Hungarian Danube Research Station, HAS, exploring the protists of the River Danube with particular emphasis on ciliates.

Type species: *Bereczkya minuta* nov. spec.

***Bereczkya minuta* nov. spec. Kiss and Török (Fig. 2)**

Rhizaria, Cercozoa incertae sedis, Genus: *Bereczkya*

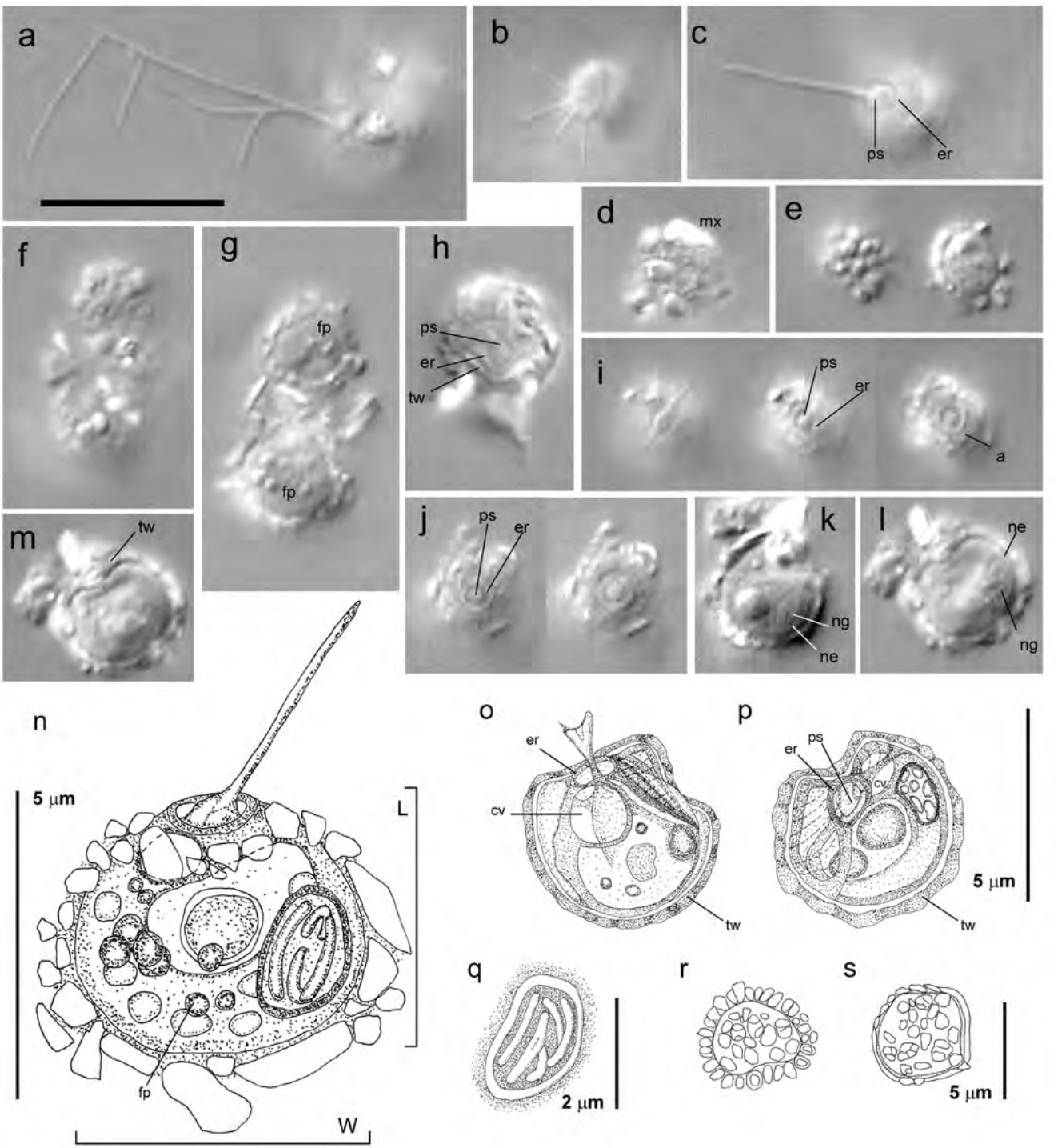
Diagnosis: *Bereczkya* with a 3.5–8 µm long test, and an ectoplasmic rim with about 0.8–2.3 µm diameter. The test is spherical, but often asymmetric because of swellings and depressions. The test is agglutinated with refractile mineral xenosomes and other flat or irregular particles. The xenosomes cover the test typically in a single layer, but the covering may be thick and irregular, or absent almost completely. With the characters of the genus.

The morphology of the test and the cell: The minute spherical cells are enclosed in a thin, rigid organic test with agglutinated particles. The length of the cell (marked on Fig. 2n) varies between 2.7–6.2 µm, the whole organism with the test is 3.5–7.8 µm (morphometries are in Table 2). The shape of the cell is nearly spherical (Figs 2k, n), but in most specimens it is somewhat asymmetric: Swellings and depressions alter the shape to irregular or reniform (Figs 2h, m, l, p). The cytoplasm fills the test entirely in all specimens. The test has a colourless inner organic layer (Figs 2h, k, m), which usually becomes thinner towards the aperture. The xenosomes are quartz particles with various shapes, mainly flattened plates (Figs 2d, f), or small spheres (Fig. 2g). A particle type of relatively large (up to 1 µm), wrinkled, ovoid knobs is also characteristic in some specimens (Figs 2e, r). The xenosomes attach only superficially to the organic layer (Fig. 2e). They are arranged rather irregularly. They usually form a single layer, but thicker aggregation of particles is also observed (Figs 2g, m). Sometimes the xenosomes are nearly completely absent (Fig. 2s). All the cells have a collar-like ectoplasmic rim (0.8–2.3 µm diameter) with a central depression, from the middle of which the pseudopodial stem originates (Figs 2c, i, j). This ectoplasmic rim is well visible, and thus it might be easy to confuse with the unpronounced aperture of the test, which surrounds the rim. The aperture is a wider irregular rigid hole on the thin test wall (Fig. 2i). In the anterior part of the cytoplasm, adjacent to the ectoplasmic rim, one-two larger, and a few smaller contractile vacuoles are present (Fig. 2o). The nucleus is peripheral or eccentric; it is situated in the middle or posterior region of the cell. Its shape is ovoid (Fig. 2k), reniform (Fig. 2l), or somewhat flattened with acute edges. The nucleoplasm is filled with about 4–8 straight or curved rods (Figs 2k, l, q), there is no central nucleolus.

Usually 1–4 typical filopodia are produced from the pseudopodial stem. The filopodia may be straight or

Table 2. Morphometric data on 21 specimens of *Bereczkya minuta* nov. gen. nov. spec. Contractile vacuoles are measured before systole, pseudopodia at their maximal lengths. All measurements are in µm.

Bereczkya minuta n. gen. n. sp.	Mean	Median	Min	Max	SD	SE	CV %	n
Test length	5.9	5.9	3.5	7.8	1.1	0.29	18	14
Cell length	4.2	4.0	2.7	6.2	0.94	0.25	23	14
Test width	5.7	5.7	3.0	7.7	1.1	0.25	20	20
Cell width	3.9	3.6	2.0	5.9	1.0	0.23	26	20
Test depth	5.2	5.1	4.3	6.1	0.74	0.28	14	7
Cell depth	4.1	3.5	3.1	6.5	1.2	0.45	29	7
Test length/width	1.05	1.07	0.90	1.18	0.097	0.026	9.2	14
Diameter of the ectoplasmic rim	1.5	1.4	0.8	2.3	0.43	0.12	28	13
Length of nucleus	1.9	1.8	1.3	2.7	0.49	0.13	26	14
Diameter of contractile vacuoles	1.1	0.9	0.7	1.9				5
Length of filopodia	12	9.6	1.6	26	8.2	2.1	69	15
Ingested plastid remnants	0.67	0.6	0.4	2.6	0.36	0.056	54	42
Length of ingested bacteria	1.16		0.7	2.2				6



Figs 2a–s. *Bereczkya minuta* nov. gen. et nov. spec. **a–c** – specimens with different forms of filopodia; **c** – the filopodium originates from the pseudopodial stem; **d–f** – the agglutinated surface of the test; **f** – the test surface of two attached specimens; **g** – ingested plastids/endosymbionts (fp) in the cytoplasm of two specimens; **h–j** – the organisation of the aperture; **h** – oblique optical cross section through the aperture; **i–j** – views from the aperture; **k–l** – the structure of the nucleus; **m** – an irregular depression in the test wall; **n** – a combined drawing with the components of the cytoplasm; **o–p** – drawings focusing on the dense components of the cytoplasm; **o** – specimen is almost in profile; **p** – specimen is from top view; **q** – the structure of the nucleus, showing the thick nuclear envelope and the rods in the nucleoplasm; **r–s** – variations in the shape of the tests and in the agglutinated particles. Scale bar for photos: a–m = 10 µm. L – length of the cell, W – width of the cell. Scale bars for drawings: n = 5 µm (bar is left to n), o–p = 5 µm (bar is right to p), q = 2 µm (bar is right to q), r–s = 5 µm (bar is right to s).

curved (Figs 2a, c), and they may branch perpendicularly or at an acute angle to each other (Fig. 2a). Their tips are rounded. Their length may reach up to 6 × cell length. A flattened lamellipodium-like cytoplasmic extrusion may also be produced with thin acute subpseudopodia on its edge (Fig. 2b). Sometimes specimens attach to each other (Fig. 2g).

Movement and feeding: The cells can move very slowly by gliding on the surfaces of extended filopodia. A relatively faster, but temporary movement is achieved, when the cell retracts a filopodium, which is attached to the surface on its distal part. In this case, the whole cell is pulled along by the filopodium. The longitudinal cell axis is more or less perpendicular to the surface during the movement (Figs 2a, c, i).

The egestion of a big food particle through the ectoplasmic rim was observed, during which process the ectoplasmic rim had to dilate considerably. Food vacuoles were found in every cell. The inner endoplasmic region may contain food vacuoles anywhere. Food vacuoles remain solitary, or they fuse to larger collective vacuoles. Spherical or ovoid bright green bodies with a 0.4–2.6 μm size are found in some cells. Their number may vary from 1 to 23 per cell (Fig. 2g). Coccoid or rod shaped bacteria are ingested frequently. The length of the food bacteria are between 0.8–2.2 μm. As the cytoplasmic rim can dilate, this does not limit the size of the ingested food particles. While the vast majority of ingested particles fall under 1 μm, the biggest food particles (e.g., 2.6 μm) are rather large when compared to the cells that ingested them (length: 5.2 μm).

Occurrence: This species formed an abundant population in the culture. It was present from the 2nd to the 15th day of the succession experiment. The species has been also found at other times (Jun. 2008) in a living plankton sample from the River Danube. The description is based on 21 living specimens.

Etymology: The specific epithet (*minuta*) refers to the minute size of the organism.

Type figures: Figs 2a, i, k.

Type locality: The plankton of the main arm of the River Danube at Göd, 20 km north from Budapest, Hungary (47°40′44,2″N, 19°07′29,9″E).

Material from the type locality: The sample with the species was preserved and retained by the first author under sample number KD 080308-1.

Discussion: Although *Bereczkya* does not have a conspicuous appearance, the combination of some characters differentiates it from other testacean genera. Differential characters are the very minute size, the thin, rigid, spherical, often asymmetric, sparsely or densely, but superficially agglutinated test, the cytoplasm, which fills the test entirely, the very inconspicuous irregular aperture, the distinct ectoplasmic collar-like rim in the aperture, and probably the slightly irregular nucleus with the rods in the nucleoplasm. The test of *Bereczkya* posseses the following characters that are infrequent among testaceans: The size range of the tests is considerably high (2.2 max/min length ratio), the test is often irregular, the xenosomes are superficially embedded and the test is filled entirely by the cytoplasm. Although the size of the cytoplasm depends on the physiological state and the growth cycle of the cells, and specimens with almost complete cytoplasm filling appear in many genera, there are few testaceans, where the test is filled entirely by the cytoplasm in every specimen. These are mainly filoseans with more or less flexible test (*Lecythium*, *Plagiophrys*, *Clypeolina* – inner test, *Penardeugenia*, *Rhogostoma*, *Capsellina*, *Frenzelina* – inner sac). *Bereczkya* resembles mostly the genus *Pseudodifflugia*; a comparison between the two genera is given in Table 4. The most important differences are as follows: the test of *Bereczkya* is often asymmetric, bearing depressions and swellings, and often lacking a rotational symmetry. In contrast, most *Pseudodifflugia* specimens have a more regular, radially symmetrical test shape. The organic layer of *Bereczkya* is very thin and delicate, in which the xenosomes are agglutinated superficially. On the contrary, even the smallest *Pseudodifflugia* species have a conspicuous organic layer, and the xenosomes are embedded into a sheath-like organic cement (Ogden and Hedley 1980). Finally, the test of *Bereczkya* is filled entirely by the cytoplasm, while the test of *Pseudodifflugia* is not (Meisterfeld 2002). Since *Bereczkya minuta* has a combination of characters that are not present in other genera, and its minute test differs seemingly from other testaceans, we erect a new genus and place it among Cercozoa incertae sedis.

This organism is smaller than almost all reported testate amoeban species (test length: 3.5–7.8 μm). The smallest sizes of some very minute testaceans are: *Apogromia* spp.: 8.5 μm, *Heterogromia intermedia*: 9.3 μm, *Pseudoditrema microus*: 9.3 μm, *Microcometes paludosa*: 6.8 μm (a heterotrophic flagellate), *Diplophrys archeri*: 4 μm (a labyrinthulid; all measurements according to De Saedeleer 1934) and *Cryptodifflugia leachi*: 10 μm (Nicholls 2006).

The collar-like ectoplasmic rim, which was found in every specimen, is not reported for other testaceans but seems to be a stable structure in this species. *Bereczkya* is a minute voracious predator, being able to ingest food particles up to the half length of the cell. The green coccoid algal bodies in the cytoplasm mostly seem to be endosymbiotic algal cells. They are often in collective vacuoles, which phenomenon has been quite rarely found among other organisms (Tchistyakova *et al*. 1997). The very small size of this species undoubtedly allows a successful grazing on the surface of riverine aggregates, and active growing in the plankton.

***Paramphitrema muelleri* nov. spec. Kiss and Török (Fig. 3)**

Rhizaria, Cercozoa incertae sedis, Family: Amphitremidae, Genus: *Paramphitrema*

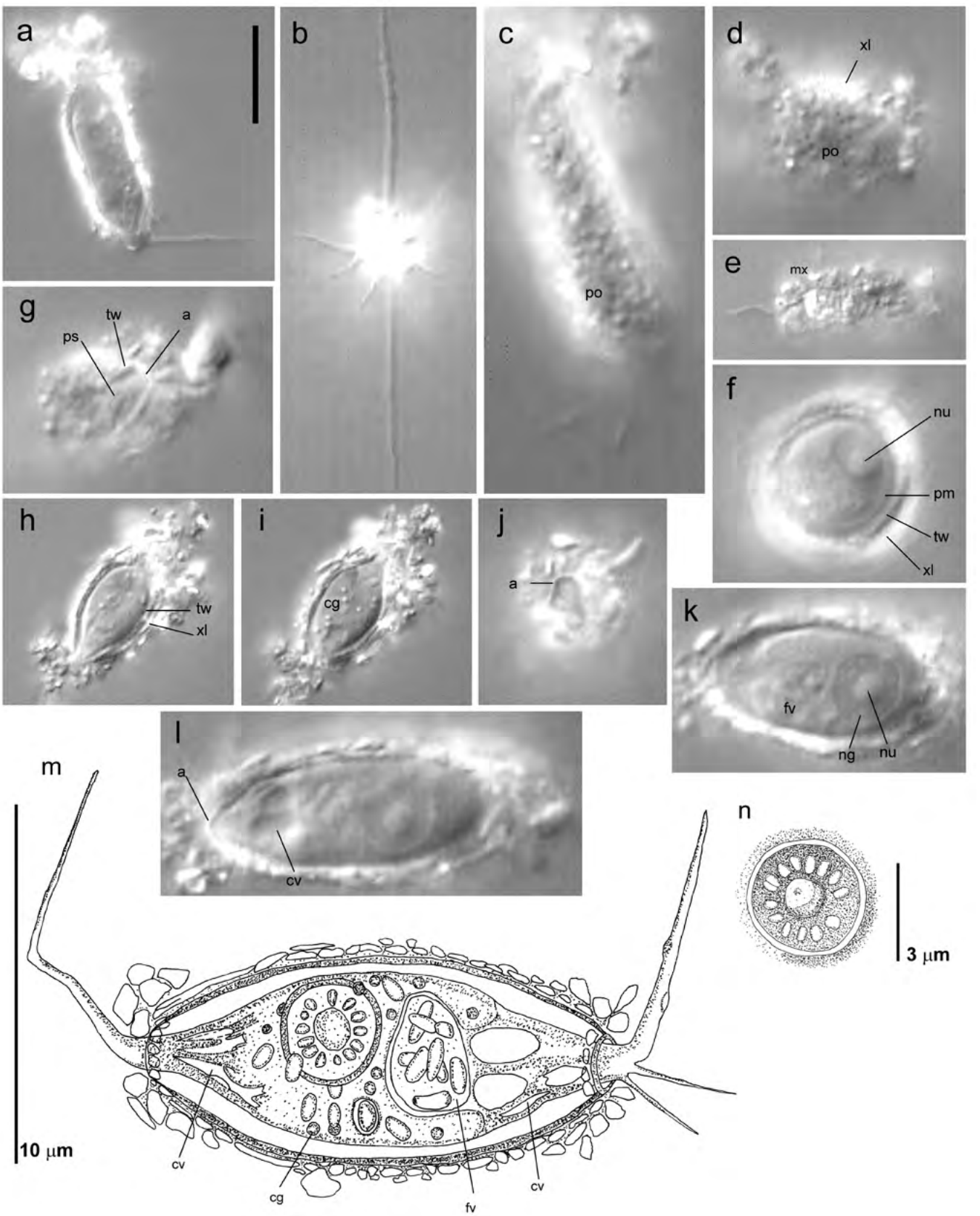
Diagnosis: *Paramphitrema* with tubular or lemon–like fusiform rigid test, which is circular in cross section. The test tapers towards the rigid apertures. The length of the test is about 13–17 µm. The nucleus is vesicular. Besides thin filopodia, 1–2 thick, straight, unbranched, tubular pseudopodia are also produced.

The morphology of the test: The minute cells are enclosed in a rigid, fusiform, agglutinated organic test that tapers towards the apertures. The length of the test is between 13–17 µm (morphometries are in Table 3). The shape of the test may be lemon-like, with a wide convex middle region (Fig. 3i), or tube-like with parallel sides (Fig. 3a). The test is composed of a thick, conspicuous, refractile, colourless or yellowish organic inner layer, which is covered by xenosomes (Figs 3a, f, h). The xenosomes are mineral quartz particles, the larger ones of which are usually flat plates (Fig. 3e). There is a quite regular pattern of dark circular holes and separating ribs among the xenosomes in the organic layer (the holes are about 0.2–0.3 µm diameter; Figs 3c, d). In some specimens, a thick, irregularly arranged xenosome covering is present near the apertures (Figs 3a, h). The apertures are rigid, oval and slightly irregular, their diameter is 1–1.6 µm (Figs 3g–i: longitudinal optical cross sections; Fig. 3j: top view). A delicate organic rim may be present at the ends of the apertures (Fig. 3j).

The morphology of the cell: The cytoplasm fills the test nearly entirely (Figs 3a, l). It extrudes through the apertures, and forms pseudopodial stems from which the pseudopodia are produced. The pseudopodial stems fill the apertures entirely (Figs 3h, i). Three regions in the cytoplasm can be differentiated. The two opposite regions near the apertures contain the contractile vacuoles, while the inner part of the endoplasm contains the nucleus, the food vacuoles and some cytoplasmic gran-

Table 3. Morphometric data on 3 specimens of *Paramphitrema muelleri* nov. spec. Pseudopodial stems are measured in the aperture, contractile vacuoles before systole, pseudopodia at their maximal lengths. All measurements are in µm.

Paramphitrema muelleri n. sp.	Mean	Median	Min	Max	SD	SE	CV %	n
Test length	15	16	13	17				3
Test width	7.9	8.0	7.0	8.7				3
Cell width	5.5	5.8	4.8	6.0				3
Test length/width	1.97	1.85	1.64	2.42				3
Aperture 1 (smaller)	1.18	1.1	1.0	1.5				3
Aperture 1 pseudopodial stem diameter	1.03	1.1	0.9	1.1				3
Aperture 2 (larger)	1.32	1.3	1.1	1.6				3
Aperture 2 pseudopodial stem diameter	1.03	1.0	0.9	1.2				3
Diameter of nucleus	3.7	3.7	3.5	3.7				3
Diameter of nucleolus	1.25	1.3	1.0	1.5				3
Diameter of contractile vacuoles	2.5	2.6	1.9	2.9				3
Length of filopodia	16	15	3.9	29	8.1	2.02	50	16
Length of ingested bacteria	1.18	1.2	0.6	1.9	0.28	0.060	24	22
Width of ingested bacteria	0.71	0.7	0.4	1.1	0.19	0.039	26	22



ules. The two contractile vacuole systems are working alternately; each contains 1–5 contractile vacuoles (Fig. 3l). The vesicular nucleus is central, eccentric or peripheral. Its shape is spherical (Fig. 3k), or slightly compressed (Fig. 3f). It contains one spherical nucleolus (Figs 3k, l, n). There are up to 30 spherical, endogenous, bright granules in the cytoplasm (Fig. 3i).

Two types of pseudopodia can be distinguished. One is a rather thick, long, straight, tubular, unbranching pseudopodium, which tapers only at the distal region (Fig. 3b). The other type is a much thinner, parallel sided or tapering filopodium (Figs 3b, e, m). The thick pseudopodia may be as long as 20–25 µm. They may bend slowly in the surrounding water, or they may break (Fig. 3m) and become zigzag. One or two (Figs 3a and b) thick pseudopodia are produced per aperture. When two are produced, they usually branch immediately at the aperture, and they are directed oppositely and perpendicularly to the longitudinal axis of the cell (Fig. 3b). The thinner filopodia may reach lengths similar to the thick ones (up to 28 µm).

Movement, feeding: No gliding movement on the surfaces of the filopodia could be observed. The cell changes its position by changing the angles of filopodia. A slow movement is achieved, when the cell retracts back a thick pseudopodium, which is attached distally to the surface. When the two thick pseudopodia are oriented oppositely, the cell may glide back and forth by this track. The pseudopodia do not glide even in these situations.

The rigid and narrow aperture strictly limits the size of the ingestible food particles. The length of ingested bacteria exceed the diameter of the aperture, but their widths are certainly lower (Table 3). Only bacteria (mostly bacilli) have been found in the food vacuoles. Food vacuoles may remain solitary, but they often fuse to large collective food vacuoles (Fig. 3k). Only one collective vacuole per cell was observed, it was situated next to one of the contractile vacuole systems.

Occurrence: This species was rare and sporadic in the culture, however, it persisted for a long time (from the 6th to the 21st days of the experiment). The description is based on 3 living specimens.

Etymology: We dedicate this species to the honour of Prof. Miklós Müller, the greatest living Hungarian protistologist working in the U.S., whose contribution is primary to our understanding on the function and the origin of the hydrogenosomes and other endosymbiotic bacteria derived organelles in the eukaryotic cell.

Type figures: Figs 3a, h, l.

Type locality: The plankton of the main arm of the River Danube at Göd, 20 km north from Budapest, Hungary (47°40′44,2″N, 19°07′29,9″E).

Material from the type locality: The sample with the species was preserved and retained by the first author under sample number KD 080308-1.

Discussion: Although, unfortunately we found very few specimens from this species, even too few to make adequate statistics from its morphometries, we argue, that excluding the natural variability of the shape and the size of the test, we have all substantial information to describe the qualitative characters of this species, with a detailed description and a differential diagnosis. The genus *Paramphitrema* contains two species described in detail: *Paramphitrema lemanense* (Penard, 1903) Valkanov, 1970 and *Paramphitrema pontica* Valkanov, 1970. A third one, *Paramphitrema rhenanum* (Lauterborn, 1895) Valkanov, 1970 is a doubtful species, which is described without figures. It differs from *P. lemanense* only in the neck region of the test that tapers irregularly into a collar towards the aperture, and the numerous attached particles around the aperture. A comparison of *Paramphitrema lemanense*, *P. pontica* and *P. muelleri*, as well as the two other genera of Amphitremidae (*Amphitrema* and *Archerella*) is given in Table 5 and not listed here in details.

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Figs 3a–n. *Paramphitrema muelleri* nov. spec. **a**–**b** – specimens with different forms of pseudopodia; **b** – the long cell axis is perpendicular to the plane of the picture, two thick pseudopodia are produced oppositely; **c**–**e** – the surface structure of the test with the xenosomes, and the pores in the organic layer; **f** – the optical cross section of the test in the plane of the nucleus; **g**–**j** – the structure of the aperture; **g** – a longitudinal optical section of the aperture with the pseudopodial stem; **h**–**i** – longitudinal optical cross sections from the same specimen in the plains of the lower and the upper apertures; **j** – a top view of the aperture showing the delicate organic rim; **k**–**l** – the structure of the cytoplasm; **k** – a plane including the nucleus and a collective food vacuole; **l** – the contractile vacuole region; **m** – a combined drawing with the components of the cytoplasm; **n** – the structure of the nucleus with the nucleolus and the minute granules in the nucleoplasm. Scale bars for photos: a–b, e, h–i = 10 µm; c–d, f–g, j–l = 5 µm. Scale bars for drawings: m = 10 µm, n = 3 µm.

Table 4. Comparison of the genera *Bereczkya* and *Pseudodifflugia.*

	Bereczkya	*Pseudodifflugia*
Test shape	spherical	ovoid, spherical in some forms of *P. gracilis*
Symmetry	often asymmetric with conspicuous depressions	usually symmetric, sometimes irregular
Organic layer	very thin and delicate	thicker, conspicuous
Agglutination	superficial	xenosomes are embedded into a sheath-like cement
Aperture	irregular, inconspicuous	wide or narrow, circular or irregular
Cytoplasm fills the test entirely	yes	no
Ectoplasmic rim at the aperture	present	absent
Origin of pseudopodia	from a central pseudopodial stem in the esctoplasmic rim	separately from aperture or from a cytoplasmic mass outside the aperture

Table 5. Comparison of the three *Paramphitrema* species, and the genera *Amphitrema* and *Archerella*.

	Paramphitrema lemanense	*Paramphitrema pontica*	*Paramphitrema muelleri*	*Amphitrema*	*Archerella*
Size	20–35 µm	35–56 µm	13–17 µm		
Cross section of the test	compressed	compressed	circular	compressed	compressed
Cytoplasm fills the test	entirely	entirely	entirely	partially	partially
Xenosome coverage	moderately dense with free surfaces, small mineral particles	moderately dense with free surfaces, small mineral particles	dense, small mineral particles	dense, large mineral particles, diatom frustules	absent
Layers of the test	thin inner organic test, xenosomes	thin inner organic test, xenosomes	thin inner organic test, xenosomes	thin inner organic test, xenosomes	thick organic test (3 layers)
Organic cement network	unknown	unknown	rather regular net of ridges with enclosed circular pores	irregular net of ridges with enclosed oval meshes	absent
Apertural pole	tapered, concave	tapered, concave	tapered	rounded, convex	rounded, convex
Neck or rim	a short neck occasionally present	long tubular neck with terminal collar	very short terminal rim at the aperture	a short neck with parallel sides may be present	back-turning rim at the aperture
Aperture flexibility	flexible	flexible	rigid	rigid	rigid
Nucleus	vesicular or ovular	presumably ovular	vesicular	ovular	vesicular
Contractile vacuoles	1, drawn to be central or close to aperture	absent	bipolar, 1–5 per aperture	1–2 central, near the nucleus	1–3, dispersed in cytoplasm
Zoochlorellae	absent	absent	absent	present	present
Types of pseudopodia	1 long, thick, unbranched or more thin branching per aperture	1 long, thick, unbranched per aperture	1–2 long, thick, unbranched or more thin branching per aperture	thin branching filopodia	thin branching filopodia

We placed *P. muelleri* in the genus *Paramphitrema* because of its thick, tubular, unbranched type of pseudopodia and the tapering neck region. Two characters differ from the generic diagnosis of Valkanov (1970): the test of *P. muelleri* is not compressed, but circular in cross section and the apertures are not flexible, but

rigid. However, the compression of the test in the genus *Paramphitrema* seems to be a variable character. The *P. lemanense* specimens found by De Groot (1979) were much narrower, and consequently less compressed than the population described by Penard (1903). Valkanov (1970) also mentions that some *P. pontica* specimens may have a fusiform test with circular cross section. The flexibility of the aperture may be somewhat also variable, and does not emphasise the erection of a new genus. The differential characters of *P. muelleri* are the small size, the circular cross section of the test, the rigidity of the test and the apertures, and the vesicular nucleus. Some features of the *Paramphitrema* species are compared below, with references to the genera *Amphitrema* and *Archerella*, too.

The type of the nucleus is variable among the genera of Amphitremidae. The nucleus of *P. muelleri* is vesicular, while *P. pontica* presumably has an ovular nucleus (structureless, large; Valkanov 1970). *P. lemanense* has a vesicular nucleus (Penard 1903), but a specimen with ovular nucleus have also been found in the Danube (pers. obs.) *Archerella flavum* has a vesicular nucleus with one large central nucleolus (Penard 1902, Bonnet *et al*. 1981), or sometimes with smaller, spherical nucleoli (Meisterfeld 2002, p. 1064, Fig. 19). The type of the nucleus within the genera and species is more or less stable, but transitions may noticeably occur.

Paramphitrema pontica have thick, tubular, non-branching, bending, flagellum-like pseudopodia with plain surface, only one is produced per aperture (Valkanov 1970). *P. lemanense* may similarly have this type of pseudopodium, or may produce two or more long, thinner, occasionally branching filopodia (Penard 1903, De Groot 1979). *P. muelleri* also has both of these two types of pseudopodia. The thick pseudopodia of all the three *Paramphitrema* species may break, bend, and perform flagellum-like squirming movements. In contrast, the species of *Amphitrema* and *Archerella* have long, thin, typical filopodia that emerge separately from the apertural hole.

P. lemanense and *P. pontica* can move by gliding on the thick anterior pseudopodium, which pulls the cell forwards. The posterior pseudopodium may squirm in the water body, or in *P. lemanense*, it may transform to 2–3 thinner branching filopodia (De Groot 1979). The cell may change the direction of movement by gliding backwards on the posterior pseudopodium (Valkanov 1970). The cell may also lift to become perpendicular to the surface. *P. muelleri* was not observed to glide on the surfaces, but it similarly changes the angles of the pseudopodia frequently, and the test often emerges to become perpendicular to the attachment surface. With its small size, *P. muelleri* may be able to graze on the surfaces of aggregates, and grow in the plankton of rivers.

The formerly described two *Paramphitrema* species are voracious nanophagous grazers with flexible apertures. On the contrary, the minute size of the rigid apertures in *Paramphitrema muelleri* (max 1–1.6 µm) severely restricts the size of the ingested food particles. Consequently, this organism is a picophagous bacterivor with a 1.2 µm mean length of the ingested bacteria.

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REFERENCES

Alves G. M., Lansac-Tôha F. A., Velho L. F. M., Joko C. Y., Costa D. M. (2007) New records of testate lobose amoebae (Protozoa, Arcellinida) for the Upper-Paraná River floodplain. *Acta Limnol. Bras*. **19:** 175–195

Badewitz H.-J. (2003) Testacea (Rhizopoda, Protozoa) des Flusses in Mecklenburg-Vorpommern. *Lauterbornia* **46:** 11–42

Bereczky Cs. M. (1979) Vergleichende Untersuchungen über die Gestaltung der im Plankton vorkommenden Testaceen im Haupt- und Nebenarm der Donau bei Göd. *Danubialia Hungarica* 90. *Ann. Univ. Sci. Budapest Sect. Biol*. **20–21:** 229–236

Bini L. M., Velho L. F. M., Lansac-Tôha F. A. (2003) The effect of connectivity on the relationship between local and regional species richness of testate amoebae (Protozoa, Rhizopoda) in floodplain lagoons of the Upper Paraná River, Brazil. *Acta Oecol*. **24:** 145–151

Boenigk J., Arndt H. (2000) Particle Handling during Interception Feeding by Four Species of Heterotrophic Nanoflagellates. *J. Eukaryot. Microbiol*. **47:** 350–358

Bonnet L., Brabet J., Comoy N., Guitard J. (1981) Nouvelles données sur le thécamoebien filosia *Amphitrema flavum* (Archer 1877) Penard, 1902. *Protistologica* **18:** 225–233

Cash J., Wailes G. H., Hopkinson J. (1915) The British Freshwater Rhizopoda and Heliozoa III. Ray. Soc., London, 156 pp.

De Groot A. A. (1979) Über einige wenig bekannten Rhizopoden. *Aquat. Ecol*. **13:** 34–49

De Saedeleer H. (1934) Beitrag zur Kenntnis der Rhizopoden: morphologische und systematische Untersuchungen und ein Klassifikationversuch. *Mem. Mus. R. Hist. Nat. Belg*. **60:** 1–112

Golemansky V., Todorov M. (2004) Shell Morphology, Biometry and Distribution of Some Marine Interstitial Testate Amoebae (Sarcodina: Rhizopoda). *Acta Protozool*. **43:** 147–162

Hoogenraad H. R., De Groot A. A. (1940) Zoetwaterrhizopoden en Heliozoen. In: Fauna van Nederland 9, (Eds. H. Boschma, L. F. Beaufort, H. C. Redeke, W. Roepke), Leiden, 302 pp.

Meisterfeld R. (2002) Testate amoebae with filopodia. In: An Illustrated Guide to the Protozoa, 2nd edition, (Eds. J. J. Lee, G. F. Leedale, P. Bradbury). Society of Protozoologists, Lawrence, Kansas, 1054–1084

Nicholls K. H. (2006) *Cryptodifflugia leachi* n. sp., a Minute New Testate Rhizopod Species (Rhizopoda: Phryganellina). *Acta Protozool*. **45:** 295–299

Nicholls K. H. (2007) Descriptions of *Phryganella laurentiana* n. sp. and *Difflugia yorkui* n. sp. two new species of testate amoebae from boreal forest wetlands in Ontario, Canada. *Acta Protozool*. **46:** 65–72

Ogden C. G., Hedley R. H. (1980) An atlas of freshwater testate amoebae. Oxford Univ. Press., Oxford, 222 pp.

Penard E. (1902) Faune Rhizopodique du Bassin du Lèman. Kündig, Genève, 714 pp.

Penard E. (1903) Sur quelques Protistes voisin des Héliozoaires ou des Flagellates. *Arch. Protistenk.* **2:** 283–304

Playfair G. I. (1917) Rhizopods of Sydney and Lismore. *Proc. Linn. Soc. New South Wales* **42:** 633–675

Raikov I. B. (1982) The Protozoan Nucleus. Morphology and Evolution. *Cell Biol. Monogr.* **9**. Springer Verlag, Berlin, New York, 221–242

Schönborn W. (1981) Population dynamics and production of Testacea (Protozoa: Rhizopoda) in the river Saale. *Zool. Jahrb. Syst.* **108:** 301–313

Tchistyakova L., Karpov A. S. Goodkov D. V. (1997) Experimentally induced endocytobiosys of *Amoeba amazonas* with different *Chlorella vulgaris* strains: fine structural investigations. *Endocytob. Cell Res.* **12:** 57–63

Valkanov A. (1970) Beitrag zur Kenntnis der Protozoen des Schwarzen Meeres. *Zool. Anz.* **184:** 241–290

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