

Description of a New Testate Amoebae Genus *Meisterfeldia* with Notes on the Systematics of the Suborder Phryganellina (Amebozoa; Tubulinea; Arcellinida)

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Abstract. The review on the systematics of the suborder Phryganellina is presented. The diagnosis of newly erected genus *Meisterfeldia* is provided. Three new species, namely *Meisterfeldia chibisovi*, *Meisterfeldia wegneri* and *Meisterfeldia polygonia*, of testate amoeba family Cryptodiffugiidae are described. Two species, namely *Meisterfeldia vanhoornei* and *Meisterfeldia turfacea* are transferred from the genus *Cryptodiffugia*. The specimens of new genus are characterised by ovoid shell, which is bilaterally symmetrical and laterally compressed, composed of proteinaceous material without mineral particles; circular subterminal aperture placed on ventrally and obliquely cut apertural end, or it is situated on a well developed or poorly expressed neck inclined ventrally.

Key words: Testate amoebae, Cryptodiffugiidae, taxonomy, new genus *Meisterfeldia*, three new species, tundra, subarctica, Russia.

INTRODUCTION

Suborder Phryganellina Bovee, 1985 comprises two families – Cryptodiffugiidae Jung, 1942 with the genera *Cryptodiffugia*, *Diffugiella*, *Wailesella* and Phryganellidae Jung, 1942 with a genus *Phryganella* (Bovee, 1985; Meisterfeld, 2001; Adl *et al.*, 2012). According to original species description (see details below), the genus *Cryptodiffugia* includes 15 species and subspecies taxa, the genus *Diffugiella* – 17 taxa, the genus *Wailesella* – 1 taxon, the genus *Phryganella* – 13 taxa. The genera *Cryptodiffugia* and *Diffugiella* seems

to be most interesting to analyse in terms of systematics, ecology and bioindication.

The taxonomic position of the testate amoeba genera *Cryptodiffugia* (Penard 1890) and *Diffugiella* (Cash 1904) has been uncertain for a long time. From one point of view, both genera are considered as being part of the family Cryptodiffugiidae Jung, 1942, together with the genera *Phryganella* and *Wailesella* (Deflandre 1953, Grospietsch 1964). However, Loeblich and Tappan (1964) assigned *Cryptodiffugia*, *Diffugiella*, *Wailesella* and *Petalopella* to Cryptodiffugiidae, whereas *Phryganella* to Phryganellidae Jung, 1942. We do not discuss here the genus *Petalopella* due to both insufficient information available on the type species *P. diffluens* and high polymorphism of its pseudopods (Loeblich, Tappan 1964). The shape of ectoplasmic anastomosing pseudopods (reticulobopods)

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unites testate amoebae of both families within the suborder Phryganellina (Meisterfeld 2001). The shells with agglutinated mineral particles distinguish the genus *Phryganella*. Other genera are characterized by chitinous shells with mineral particles rarely observed in some species, e.g. *Diffugiella sacculus*, *D. patinata*, and *D. voighti*.

The first described species of *Cryptodiffugia*, namely *Cryptodiffugia oviformis* (Penard, 1890), possesses a shell with axial symmetry and circular aperture. Later, Penard (1902) described another species, *C. compressa*, with a shell compressed along the longitudinal axis and narrowly elliptical aperture. Cash (1904) introduced a new genus *Diffugiella* with the type species *Diffugiella apiculata* Cash, 1904, which is similar to *C. oviformis* in the main morphological characters (particularly, the axial symmetry), but considerably different in size. The description of this new genus has caused major taxonomic uncertainties. According to Cash (1904) the genus *Cryptodiffugia* includes species with shells compressed laterally with the type species *C. compressa* Penard, 1902, despite the fact that first described species, *C. oviformis* Penard, 1890, is characterized by the shell with axial symmetry and circular aperture.

This classification has been unfortunately followed by successive authors. Representatives of the genus *Cryptodiffugia* are characterized by elliptical section of the shell, whereas the shells of genus *Diffugiella* are circular in transverse section (Deflandre 1953, Schönborn 1965). At the same time, this character is stable and reliable only for *C. compressa*. In *C. oviformis*, the lateral compression is inconspicuous, even when it sometimes occurs. It is more clearly expressed in *C. angulostoma*. Application of this character as significant would lead to unjustified monotypy of genus *Cryptodiffugia* and would violate the priority of Penard (1890). Page (1966) resolved this problem, having stated that the genus *Diffugiella* is a synonym of the genus *Cryptodiffugia*. However, both generic names are still applied in routine ecological and geographical works. The best solution, therefore, seems to be the final synonymization of the genera *Diffugiella* and *Cryptodiffugia*. The composition of the shell surface is another important character of the genera discussed. It is known that the shell composition of *C. oviformis* includes amorphous calcium phosphate (Hedley *et al.* 1977), but, regrettably, this remains the only study on the shell composition of *Cryptodiffugia* and *Diffugiella*. Therefore, it is still unknown whether this character is valid for all other species of genus *Cryptodiffugia*.

The very small size of most species and their low incidence in samples hamper their study with SEM and with molecular genetic analysis. Many species were described on the basis of morphology of the empty shells only, without an analysis of the cell structure or of the shape of pseudopodia. Such data are only available for the type species *C. oviformis* (Hedley *et al.* 1977) and *C. leachi* (Nicholls 2006). *C. oviformis* is perhaps the easiest to grow Arcellinida species. They also can be isolated using soil serial dilutions. The first results of molecular genetic studies were published recently on *C. operculata* (Lahr 2011; Lahr *et al.* 2011, 2013).

An additional evidence of insufficient examination of the genus is the lack of morphometric analysis of species morphological variability. This hampers a comparison of closely related species and intraspecific taxa. There are groups of related species in this genus, e.g. *C. oviformis*–*C. operculata*–*C. leachi*. A statistical analysis of morphological characters was performed only for some species, e.g. *C. bassini* (Bobrov 2001), *Cryptodiffugia leachi* (Nicholls 2006), *C. patinata* and *C. sacculus* (Davidova 2012).

Presently, *Cryptodiffugia* seems to be one of the least studied testate amoeba genera. The aim of this study is to describe three new species and one genus from the family Cryptodiffugiidae basing on the morphological approach and to discuss the systematics of the suborder Phryganellina.

MATERIALS AND METHODS

The study was conducted in subarctic tundra within the floodplain and thermokarst depressions along the Berelek river, 28 km north-west from the Chokurdakh settlement (Russia, Yakutia, Fig. 1). The predominant vegetation is represented by polygonal tundra with grasses (*Carex stans*, *Eriophorum scheuchzeri*) on the polygons and *Salix-Carex*-green moss tundra (*Salix glauca*, *Carex stans*) on the ridges (Beermann and Kokhanova 2012).

Testate amoebae were isolated from litter and soil (taking the uppermost 5 cm). The cells were studied using Motic BA300 (China) and Axioplan 2 (Carl Zeiss, Germany) light microscopes under x 200 and x 400 magnifications. The following measurements were taken for the shell characters from 20 cells of each taxon: Min – minimum; Max – maximum; Mean – arithmetic mean; SD – standard deviation; M – median; CV – coefficient of variation. Measurements are in μm . Four shell characters have been measured, e.g. 1 – length of shell, 2 – breadth of shell; 3 – depth of shell; 4 – axis of aperture. To estimate the relative abundance of new species in the samples we counted 200–300 specimens in each sample in total. Statistical analysis was performed using Statistica 6.0.

RESULTS AND DISCUSSION

Genus *Meisterfeldia* gen. nov.

Description: Test ovoid; bilaterally symmetrical, more or less laterally compressed; colorless, yellow or brown; composed of proteinaceous material without mineral particles; aperture circular, subterminal as seen on ventrally and obliquely cut apertural end, or it is situated on a well developed or poorly expressed neck inclined ventrally; sometimes aperture border shows a slight swelling.

Etymology: The genus is named after the German protozoologist Ralf Meisterfeld.

Type species: *Meisterfeldia chibisovi* sp. nov.: Shell small, bilaterally symmetrical, elongated-ovoid, almost not flattened, colorless to light-brown. Dorsal side convex in profile, posterior shell end smoothly curved, anterior end with aperture bent towards ventral side. Aperture circular, subterminal, shifted to ventral side. Aperture, unlike *Wailesella eboracensis*, at the end of a short neck. Shell surface smooth, without mineral inclusions.

Meisterfeldia chibisovi sp. nov.

Description: Shell small, bilaterally symmetrical, elongated-ovate, almost not compressed, colorless to light-brown. Dorsal part convex, posterior end evenly curved, anterior end with aperture curved towards ventral side. Aperture circular, subterminal, shifted to ventral side. Aperture, unlike that in *Wailesella eboracensis*, situated at the end of a short neck. Shell surface smooth, without mineral inclusions. Nuclei have been detected in several specimens (Figs 2, 3).



Fig. 1. Location of the Kytalyk study site in the northeastern Siberian lowland. Digital Elevation Model compiled by G. Grosse (AWI Potsdam).

Measurements (Table 1): Shell length 9.50–10.75 μm ; shell breadth 4.5–5.5 μm ; shell depth 4.5–5.5 μm ; axis of aperture 2.25–3.00 μm .

List of associated testate amoebae: The species was found in associations with the following species (occurrence, %): *Cryptodiffugia oviformis* f. *fusca* (26.70); *Trinema lineare* (10.86); *Centropyxis sylvatica* (9.50); *Trinema complanatum* v. *platystoma* (8.60); *Centropyxis aerophila* (4.52); *Cryptodiffugia bassini* (4.07); *Wailesella eboracensis* (4.07); *W.* sp. (4.07); *Trinema complanatum* (3.62); *Schoenbornia humicola* (3.17);

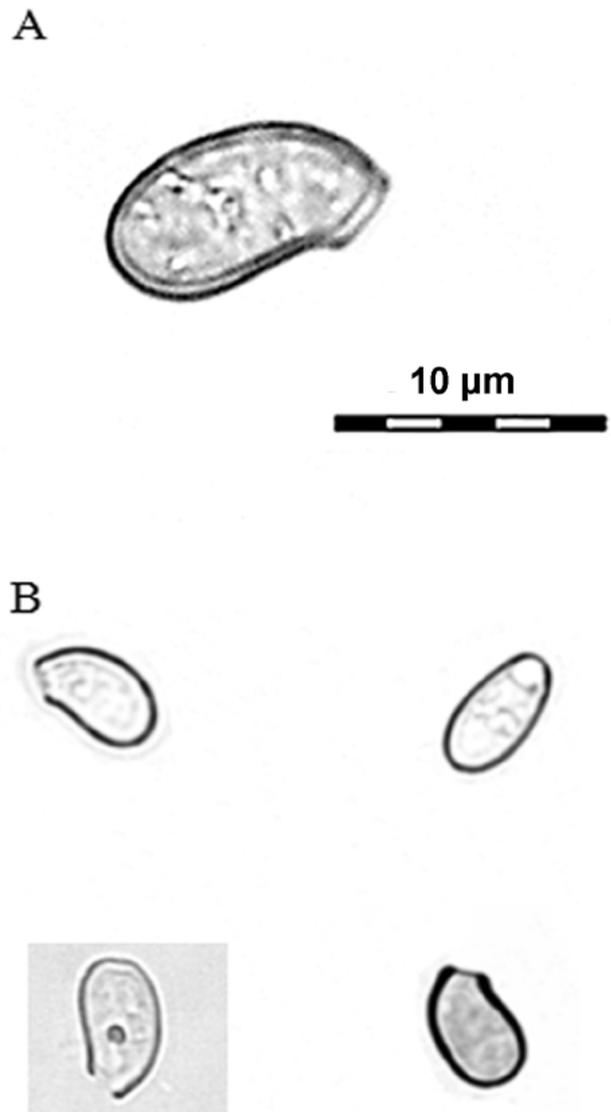


Fig. 2. Light microscopical image of *Meisterfeldia chibisovi* in lateral view. A – typical specimen, B – morphological variability.

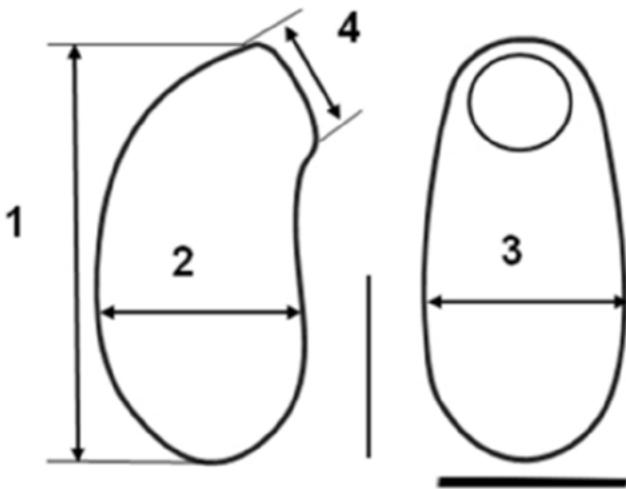


Fig. 3. Outline of *Meisterfeldia chibisovi*: lateral (left) and ventral (right) views. 1–4 – characters of the test measured. Scale bar: 5 μm .

Euglypha laevis (3.17); *E. cristata* (2.71); *Heleopera petricola* v. *amethystea* (1.36); *Assulina muscorum* (1.36); *Meisterfeldia chibisovi* sp. nov. (0.91); *Nebela tinctoria* (1.36); *N. militaris* (0.90); *Centropyxis constricta* (0.90); *Cyclopyxis eurystoma* (0.90); *Meisterfeldia wegneri* sp. nov. (0.90); *Bullinularia gracilis* (0.45); *Heleopera rosea* (0.45); *Hyalosphenia minuta* (0.45); *H. subflava* (0.45); *Argygnia dentistoma* (0.45); *N. parvula* (0.45); *Valkanovia elegans* (0.45); *Corythion dubium* v. *minima* (0.45); *Cryptodiffugia minuta* (0.45); *Archerella flavum* (0.45); *Meisterfeldia polygonia* sp. nov. (0.45); *Meisterfeldia vanhoornei* comb. nov. (0.45); *Euglypha filifera* v. *magna* (0.45); *Centropyxis* cf. *aculeata* (0.45).

This species, as well as the following species, represent a rare component of the community inhabited the *Sphagnum* layer of tundra soil. Low occurrence and abundance along with the tiny size normally complicate detection of these organisms in the samples.

Type locality: Polygonal tundra, 28 km north-westward of Chokurdakh settlement (Yakutia). 70°49'55.4"N 147°28'39.1"E. Biotope: *Sphagnum*, peat tussock, microelevation. Vegetation: *Carex chordeorrhiza*, *Andromeda polifolia*, *Salix myrtilloides*, *Ledum decumbens*, *Carex stans*, *Polytrichum commune*, *Sphagnum* sp. Soil – Sphagnic Fibristel (US ST).

Type specimen: Laboratory of Soil Bioindication, Department of Soil Geography, Faculty of Soil Science, Lomonosov Moscow State University, slide No. 1-2015.

Etymology: The species was named in honor of Olga Chibisova, a Russian protozoologist.

***Meisterfeldia wegneri* sp. nov.**

Description: Shell small, bilaterally symmetrical, ovate, rather wide in plane, flattened, colorless, transparent. Dorsal side convex, posterior end shifted to ventral side. Aperture elliptical, subterminal, anterior end with the aperture curved towards ventral side. Shell surface smooth, without mineral inclusions (Figs 4, 5).

Measurements (Table 1): Shell length 6.00–10.50 μm ; shell breadth 4.00–7.75 μm ; shell depth 4.00–5.00 μm ; axis of aperture 3.25–4.25 μm .

List of associated testate amoebae. The species was found in associations with the following species (occurrence, %): *Cryptodiffugia oviformis* f. *fusca* (26.70); *Trinema lineare* (10.86); *Centropyxis sylvatica* (9.50); *Trinema complanatum* v. *platystoma* (8.60); *Centropyxis aerophila* (4.52); *Cryptodiffugia bassini* (4.07); *Wailesella eboracensis* (4.07); *W. sp.* (4.07); *Trinema complanatum* (3.62); *Schoenbornia humicola* (3.17);

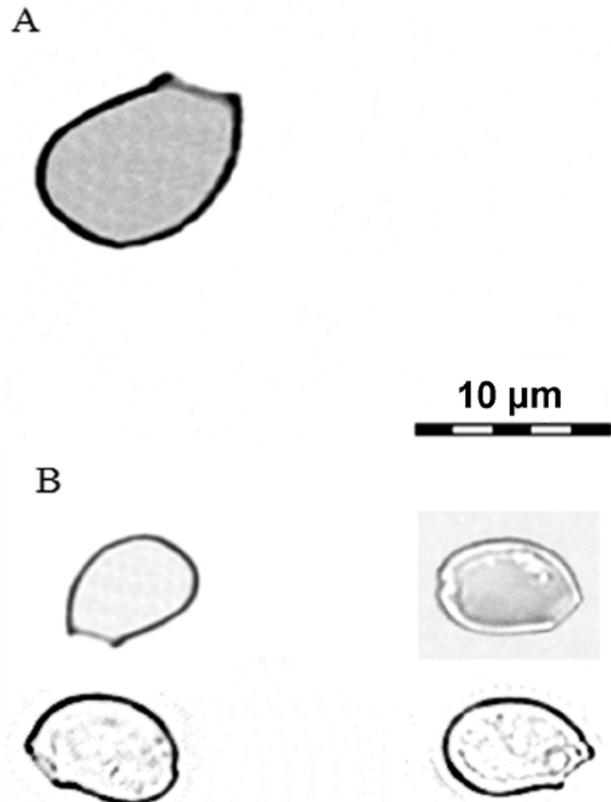


Fig. 4. Light microscopical image of *Meisterfeldia wegneri* in lateral view. A – typical specimen, B – morphological variability.

Table 1. Biometric characterization of *Meisterfeldia chibisovi* sp. nov., *Meisterfeldia wegeneri* sp. nov. and *Meisterfeldia polygonia* sp. nov. based on 20 measured specimens. 1 – shell length, 2 – breadth of the test, 3 – shell depth, 4 – axis of aperture. Mean – arithmetic mean; M – median; SD – standard deviation; Min – minimum; Max – maximum. Measurements in μm .*

N = 20	<i>Meisterfeldia chibisovi</i> sp. nov.				<i>Meisterfeldia wegeneri</i> sp. nov.				<i>Meisterfeldia polygonia</i> sp. nov.			
	1	2	3	4	1	2	3	4	1	2	3	4
Min	9.50	4.50	4.50	2.25	6.00	4.00	4.00	3.25	6.75	3.75	3.75	2.50
Max	10.75	5.50	5.50	3.00	10.50	7.75	5.00	4.25	7.50	4.50	4.00	3.00
Mean	10.01	5.05	4.93	2.72	9.40	6.85	4.55	3.80	7.25	4.18	3.88	2.65
SD	0.14	0.12	0.11	0.10	0.55	0.45	0.14	0.10	0.12	0.07	0.05	0.08
M	10.00	5.00	5.00	2.75	10.00	7.38	4.63	3.75	7.38	4.25	3.87	2.50
CV	3.54	5.70	5.78	8.88	14.65	16.25	7.48	6.86	4.26	3.93	3.31	7.11

* – These measurements are shown in Figures 3, 5, 7.

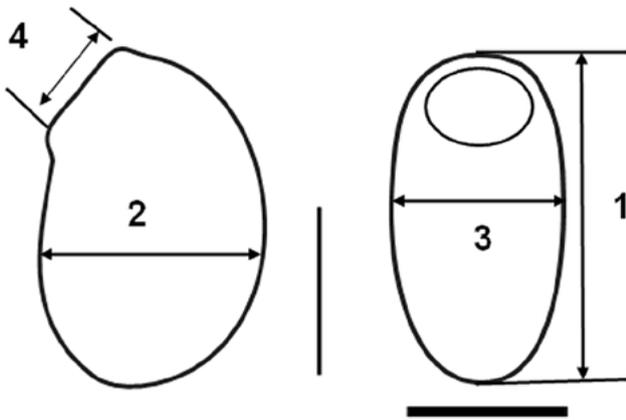


Fig. 5. Outline of *Meisterfeldia wegeneri*: lateral (left) and ventral (right) views. 1–4 – characters of the test measured. Scale bar: 5 μm .

Euglypha laevis (3.17); *E. cristata* (2.71); *Heleopera petricola* v. *amethystea* (1.36); *Assulina muscorum* (1.36); *Meisterfeldia chibisovi* sp. nov. (0.91); *Nebela tinctoria* (1.36); *N. militaris* (0.90); *Centropyxis constricta* (0.90); *Cyclopyxis eurytoma* (0.90); *Meisterfeldia wegeneri* sp. nov. (0.90); *Bullinularia gracilis* (0.45); *Heleopera rosea* (0.45); *Hyalosphenia minuta* (0.45); *H. subflava* (0.45); *Argygnia dentistoma* (0.45); *N. parvula* (0.45); *Valkanovia elegans* (0.45); *Corythion dubium* v. *minima* (0.45); *Cryptodiffugia minuta* (0.45); *Archerella flavum* (0.45); *Meisterfeldia polygonia* sp. nov. (0.45); *Meisterfeldia vanhoornei* comb. nov. (0.45); *Euglypha filifera* v. *magna* (0.45); *Centropyxis* cf. *aculeata* (0.45).

Type locality: Polygonal tundra, 28 km north-westward of Chokurdakh settlement (Yakutia). 70°49'55.4"N 147°28'39.1"E. Biotope: *Sphagnum*, peat tussock, microelevation in the center of the testing area. Vegetation: *Carex chordorrhiza*, *Andromeda polifolia*, *Salix myrtilloides*, *Ledum decumbens*, *Carex stans*, *Polytrichum commune*, *Sphagnum* sp. Soil – Sphagnic Fibristel (US ST).

Type specimen: Laboratory of Soil Bioindication, Department of Soil Geography, Faculty of Soil Science, Lomonosov Moscow State University, slide No. 2-2015.

Etymology: The species was named in honor of Alfred Wegener, a German geophysicist who developed the theory of continental drift, an investigator of the Arctic. The Institute for Polar and Marine Research in Bremerhaven, Germany, was named in his honor.

Related species – similarities and differences: The species differs from *Meisterfeldia chibisovi* in the elliptical shape of pseudostom, in a wider shell (the length/width ratio is 1.98 in *Meisterfeldia chibisovi* and 2.47 in *Meisterfeldia wegeneri*), in the lateral compression (1.02 in *Meisterfeldia chibisovi* and 1.51 in *Meisterfeldia wegeneri*), and in the absence of pseudostomal neck.

***Meisterfeldia polygonia* sp. nov.**

Description: Shell is very small, bilaterally symmetrical, narrowly ovate, slightly compressed, almost circular in a transverse section, brown, non-transparent. Dorsal side weakly convex, posterior end evenly curved, anterior end with aperture slightly cut obliquely towards

ventral side. Aperture elliptical, subterminal. Shell surface smooth, without mineral inclusions (Figs 6, 7).

Measurements (Table 1): shell length 6.75–7.50 μm ; shell breadth 3.75–4.50 μm ; shell depth 3.75–4.00 μm ; axis of aperture 2.50–3.00 μm .

List of associated testate amoebae: The species was found in associations with the following species (occurrence, %): *Cryptodiffugia oviformis* f. *fusca* (26.70); *Trinema lineare* (10.86); *Centropyxis sylvatica* (9.50); *Trinema complanatum* v. *platystoma* (8.60); *Centropyxis aerophila* (4.52); *Cryptodiffugia bassini* (4.07); *Waillesella eboracensis* (4.07); *W. sp.* (4.07); *Trinema complanatum* (3.62); *Schoenbornia humicola* (3.17); *Euglypha laevis* (3.17); *E. cristata* (2.71); *Heleopera petricola* v. *amethystea* (1.36); *Assulina muscorum* (1.36); *Meisterfeldia chibisovi* sp. nov. (0.91); *Nebela tinctoria* (1.36); *N. militaris* (0.90); *Centropyxis constricta* (0.90); *Cyclopyxis eurytoma* (0.90); *Meisterfeldia wegneri* sp. nov. (0.90); *Bullinularia gracilis* (0.45); *Heleopera rosea* (0.45); *Hyalosphenia minuta* (0.45);

H. subflava (0.45); *Argygnia dentistoma* (0.45); *N. parvula* (0.45); *Valkanovia elegans* (0.45); *Corythion dubium* v. *minima* (0.45); *Cryptodiffugia minuta* (0.45); *Archerella flavum* (0.45); *Meisterfeldia polygonia* sp. nov. (0.45); *Meisterfeldia vanhoornei* comb. nov. (0.45); *Euglypha filifera* v. *magna* (0.45); *Centropyxis* cf. *aculeata* (0.45).

Type locality: Polygonal tundra, 28 km north-westward of Chokurdakh settlement (Yakutia). 70°49'55.4"N 147°28'39.1"E. Biotope: *Sphagnum*, peat tussock, microelevation in the center of the testing area. Vegetation: *Carex chordorrhiza*, *Andromeda polifolia*, *Salix myrtilloides*, *Ledum decumbens*, *Carex stans*, *Polytrichum commune*, *Sphagnum* sp. Soil – Sphagnic Fibristel (US ST).

Type specimen: Laboratory of Soil Bioindication, Department of Soil Geography, Faculty of Soil Science, Lomonosov Moscow State University, slide No. 3-2015.

Etymology: The species was named after the abbreviation of the Joint German-Russian DFG – RFBR (Russian Foundation for Basic Research) Research Project “Polygons in tundra wetlands: dynamics and response to climate variability in Polar Regions (POLYGON)”. The field and laboratory protozoological studies were carried out within the framework of the latter project.

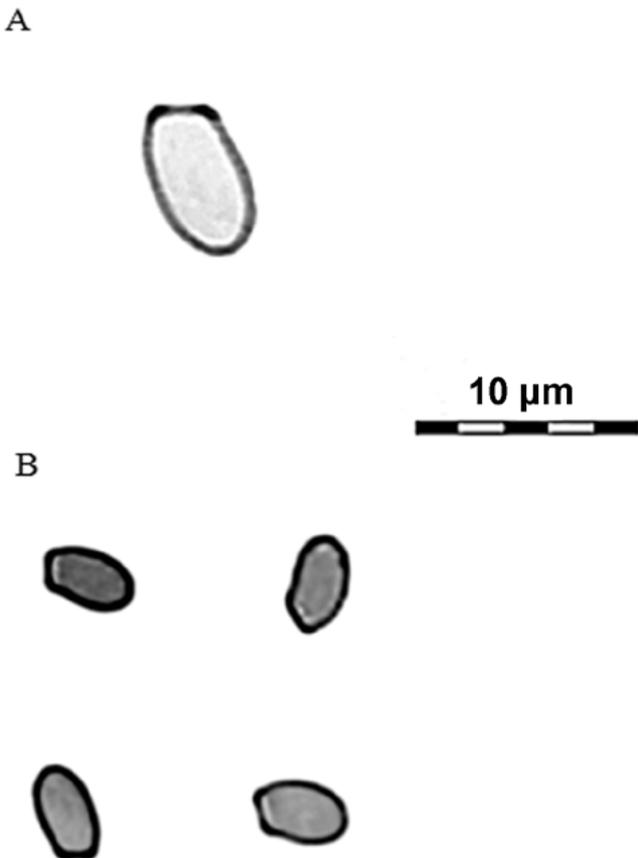


Fig. 6. Light microscopical image of *Meisterfeldia polygonia* in lateral view. A – typical specimen, B – morphological variability.

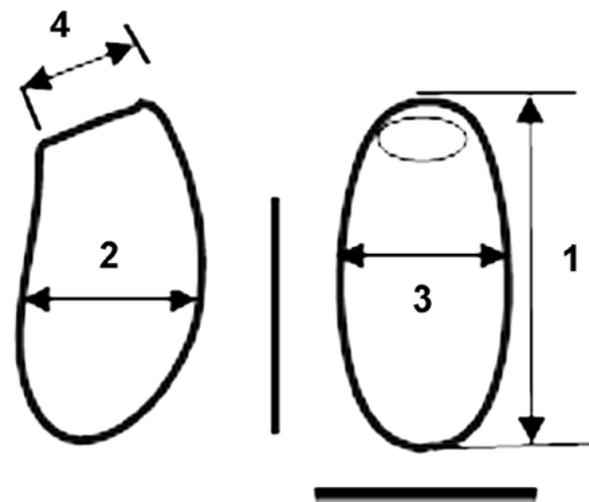


Fig. 7. Outline of *Meisterfeldia polygonia*: lateral (left) and ventral (right) views. 1–4 – characters of the test measured. Scale bar: 5 μm .

Related species – similarities and differences:

The species differs morphologically from *Cryptodiffugia vanhoornei* by its smaller size: the shell is nearly twice smaller, it is more graceful and always stained with brown.

***Meisterfeldia vanhoornei* (Beyens et Chardez, 1986) comb. nov.**

Basionym: *Diffugiella vanhoornei* Beyens et Chardez, 1986

Description: The shell is ovoid, with a circular cross-section. It is entirely chitinoid, with a yellowish hyaline tint. The aperture is circular, obliquely truncated, and the border shows a slight swelling (Fig. 8).

Measurements: shell length 13–15.5 μm ; shell depth 8–8.5 μm ; diameter of aperture 3.8–4.5 μm (n = 5).

Habitat: moss (*Sphagnum girgensohnii*) from steep bank a brook.

***Meisterfeldia turfacea* (Zacharias, 1903) comb. nov.**

Basionym: *Cryptodiffugia turfacea* Zacharias, 1903

Description: Shell is looking like curved bowl with short neck. The aperture is circular, surrounded by well developed broad collar. Shell surface is smooth, without extraneous mineral particles (Fig. 9).

Measurements: shell length 14–18 μm ; shell breadth 10–16 μm ; diameter of aperture 6 μm .

Habitat: wet peat mosses.

CONCLUSIONS

All five species described above are different both morphologically and morphometrically. Each species represents distinct size group within length-breadth axes (Fig. 10). Unfortunately, I did not find any living organism and, therefore, I could not describe the cytoplasm, nor obtain any molecular data.

The main reason for the description of new genus derives from the fact that, within the family Cryptodiffugiidae, this genus represents a transition from species of *Cryptodiffugia* with axial symmetry (*Cryptodiffugia angusta*, *Cryptodiffugia apiculata*, *Cryptodiffugia bassini*, *Cryptodiffugia oviformis*, etc.) to the bilaterally symmetrical species *Wailesella eboracensis*. This is reflected in gradual formation of dorsal and ventral sides, with lateral compression and gradual shift of aperture to the ventral side: from *Meisterfeldia polygonia* sp. nov. to *Meisterfeldia wegneri* sp. nov. and to

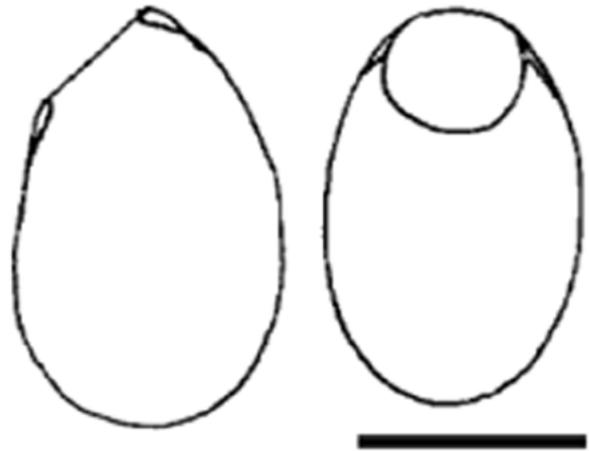


Fig. 8. *Meisterfeldia vanhoornei* (after: Beyens et al., 1986). Scale bar: 5 μm .



Fig. 9. *Meisterfeldia turfacea* (after Zacharias, 1903). Scale bar: 5 μm .

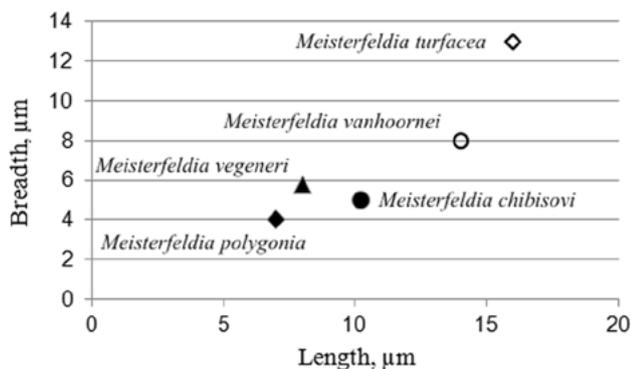


Fig. 10. Schematic demonstration of relationship of *Meisterfeldia chibisovi* sp. nov., *M. vegeneri* sp. nov., *M. polygonia* sp. nov., *M. vanhoornei* comb. nov. and *M. turfacea* comb. nov. based on morphological characters (average morphometric characteristics of species based on literature data and personal observations).

Meisterfeldia chibisovi sp. nov. This trend results in bilaterally symmetrical species with a dorso-ventral compression and an aperture on the ventral side – *Wailesella eboracensis*. For the time being, we can only suggest that species of these four genera form an adaptive evolutionary lineage in testate amoebae of the family Cryptodiffugiidae.

In general, it is possible to assume the increasing of species diversity within the families Cryptodiffugiidae and Phryganellidae in case of increasing the geographical range of investigations. However, the task is quite hard due to tiny size of the taxa and low abundances.

In my current practice I have detected the specimens of *Wailesella* sp. with highly deviated shell and aperture size from the type species *Wailesella eboracensis*. This species sometimes present in high abundances, which make it possible to obtain molecular data in near future. Thus, the systematics of suborder Phryganellina seem to be rather dynamic, as well as the systematics of other testate amoebae.

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