

Two New Species of Testate Amoebae from Mountain Forest Soils of Japan and Redescription of the Genus *Deharvengia* Bonnet, 1979

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Summary. Two new species of *Deharvengia* and *Assulina* are described from samples in the mountain forests of Honshu Is. (Japan). Morphometric analysis showed low variability of their morphological characteristics with coefficients of variation ranging between 1.4 and 9.1% for the two new species. An amended description of the genus *Deharvengia* is provided.

Key words: Testate amoebae, *Deharvengia japonica*, *Assulina discoides*, taxonomy, mountain soils, Honshu, Japan.

INTRODUCTION

Testate amoebae from terrestrial habitats in Japan, especially in mountain soils, have been inadequately studied. The few existing publications on testate amoebae in Japan are of special interest for biogeography and ecology. Soil protists of Japan have not been reviewed. Publications dealing with specific aspects of morphology and biology of soil protists are scarce (Aoki 2003, Aoki *et al.* 2007, Shimano and Miyoshi 2008). Cousteaux (1978) described several new species and one new genus found on the island of Honshu, in the valley of Shiga, 200 kilometers northwest of Tokyo. In the study area, only one prominent testate amoebae species *Nebe-la* (*Pterygia*) *carinata* was recorded in *Sphagnum* pond

of Chubu Mountains National Park located in Toyama, Nagano, Gifu, and Niigata prefectures (Okada 1965). The geological history and climate in Japan, as well as its relative remoteness from the Eurasian continent may facilitate endemism among animals and plants. Arguably, this also applies to some testate amoeba species, which are in general a cosmopolitan group of protists.

MATERIAL AND METHODS

Samples were taken on the slopes of the mountain massif Tateyama (Fig. 1), Honshu Is. at stations Murodo (2,412 m ASL), Midagahara (1,898 m ASL), Bijyodaira (997 m ASL), Tateyama fenicular station (484 m ASL), and Shraine Oyama (177 m ASL). Honshu Is. is the largest island of Japan. In the Sea of Japan side of Japan, the climate is characterized by the northwesterly winter winds off the Asian continent bringing regular, heavy snows that make this one of the snowiest regions on Earth. The amount of precipitation during September is typically about 260 mm in Murodo, 180 mm in

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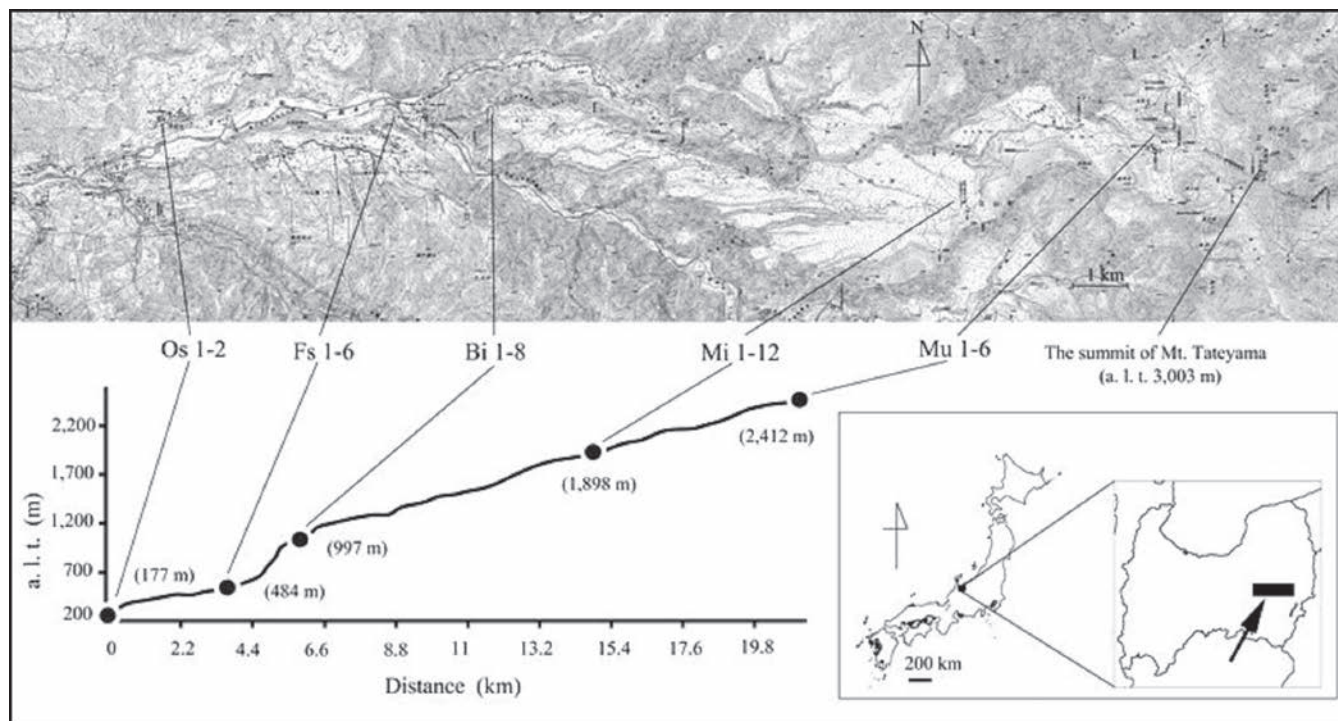


Fig. 1. Location map of study site.

Midagahara, and 165 mm in Bijyodaira. Average of snow depth in Murodo in April is 6–9 m (Ichikawa 1976).

The three upper plots sampled for testate amoebae (Murodo, Midagahara and Bijyodaira) were located on the Quaternary volcanic rocks of the macro-Tateyama (Harayama *et al.* 2000). Other habitats (Tateyama funicular station and Shraime Oyama) were located on river terraces. Soils in the area are formed in the old alluvial Quaternary sediments. The study area falls into the biogeographic province of Japanese evergreen forests. At the top of the mountain massif within the Murodo plot the vegetation is dominated by mountain pine (*Pinus pumila*) in the mountain gravelly soils with high moisture coming from melting snow and rainfall. Plot Midagahara is water-logged and covered by a few coniferous trees, birch (*Betula emanii*) and almost continuous cover of bushy bamboo (*Sasa* sp.) on the wetland soils. At altitudes below 1000 m ASL (Bijyodaira, Tateyama funicular station, and Shraime Oyama) mixed broadleaved-coniferous (*Cryptomeria japonica*, *Fagus crenata*, *F. japonica*) and deciduous forests (*Quercus dentata*, *Q. grosserrata*, *Q. crispula*, *Acer pictum*, *A. japonicum*, *Fraxinus japonica*) cover the mountain brown earth.

For testate amoebae sampling, both litter and soil of the uppermost 5 cm were sampled from all existing microhabitats in each sampling point. Testate amoebae were extracted from samples in accordance with the method described in Mazei and Chernyshov (2011). The samples were studied using Motic BA300 (China) light microscope. Scanning micrographs were made according to the technique described in Ogden (1979a) using Jeol 6060A (Japan) scanning electron microscope.

RESULTS AND DISCUSSION

Deharvengia japonica sp. nov. (Figs 2–10; Table 1)

Description: Test large, colorless, transparent, ovoid to broadly ovoid (Figs 3, 4, 7, 8), tapering to the front end, dorso-ventrally flattened (Fig. 10), narrowly elliptic in cross-section (Fig. 9). Aperture narrowly elliptic (Fig. 9), with the ventral side cut at about 6/7 of the length of the entire test (Fig. 10), and surrounded by a thin organic lip. There is a small cap on the dorsal side of the shell (Figs 5, 7, 10), which is surrounded by a narrow organic lip (Fig. 5). Shell is covered by elliptical flat silica plates (idiosomes), about $7 \times 11 \mu\text{m}$ in size and arranged regularly in up to 18 transverse and 13–15 longitudinal rows (Figs 3, 4). On the dorsal side of the cap the direction of the rows follows the outline of the shell (Fig. 5). Flat transparent clavate spines of about $10 \mu\text{m}$ in length are located on each side of the test between the posterior end and the middle of the test (Figs 6–8).

Shell is large (Table 1), slightly less than $100 \mu\text{m}$ in length, which is almost twice the average size of soil testate amoebae. The population is monomorphic. Vari-

Table 1. Biometric characterization of *Deharvengia japonica* sp. nov. based on 30 measured specimens. 1–7 – shell features (Fig. 2): 1 – shell length, 2 – shell breadth, 3 – fundus length, 4 – shell depth, 5 – cap length, 6 – cap width, 7 – recessed depth of the pseudostome. Mean – arithmetic mean, M – median, SD – standard deviation, CV – variation coefficient (%), Min – minimum, Max – maximum. Measurements in μm .

	1	2	3	4	5	6	7
Mean	93.6	71.2	79.6	36	45.2	22	10.3
M	94	70	79	36	45	22	10
SD	3.5	2.8	2.4	2.2	2.3	2.0	0.9
CV	3.7	4.0	3.0	6.1	5.1	9.1	9.0
Min	86	69	76	32	41	18	9
Max	97	79	83	39	48	24	12

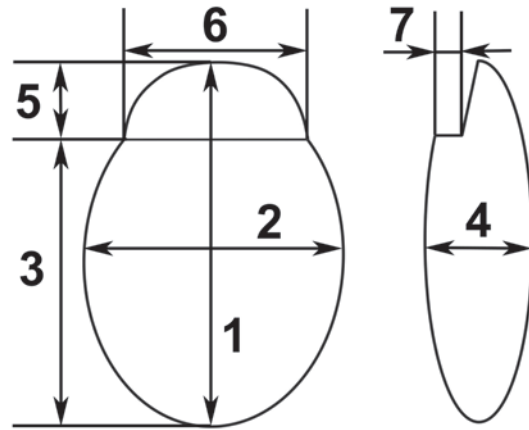
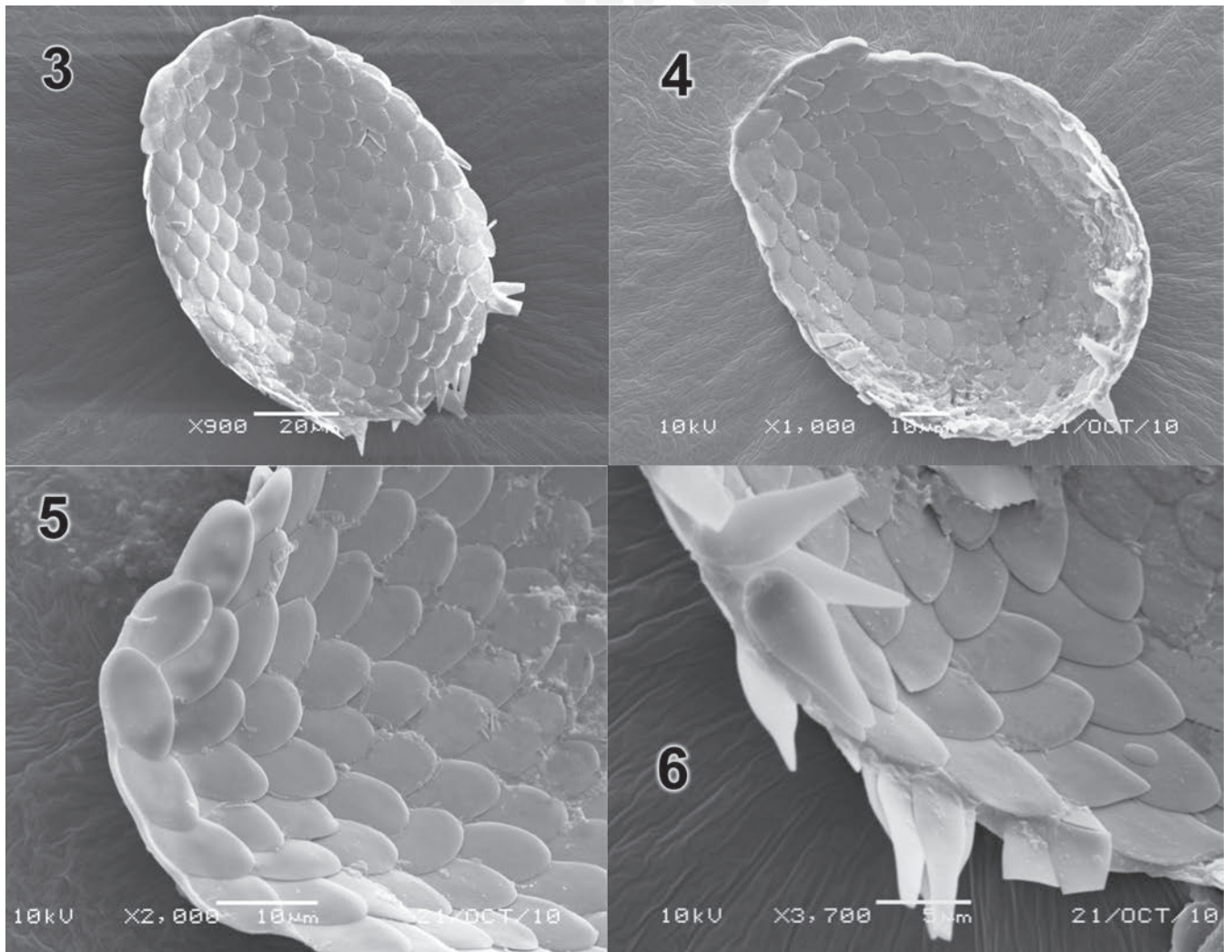
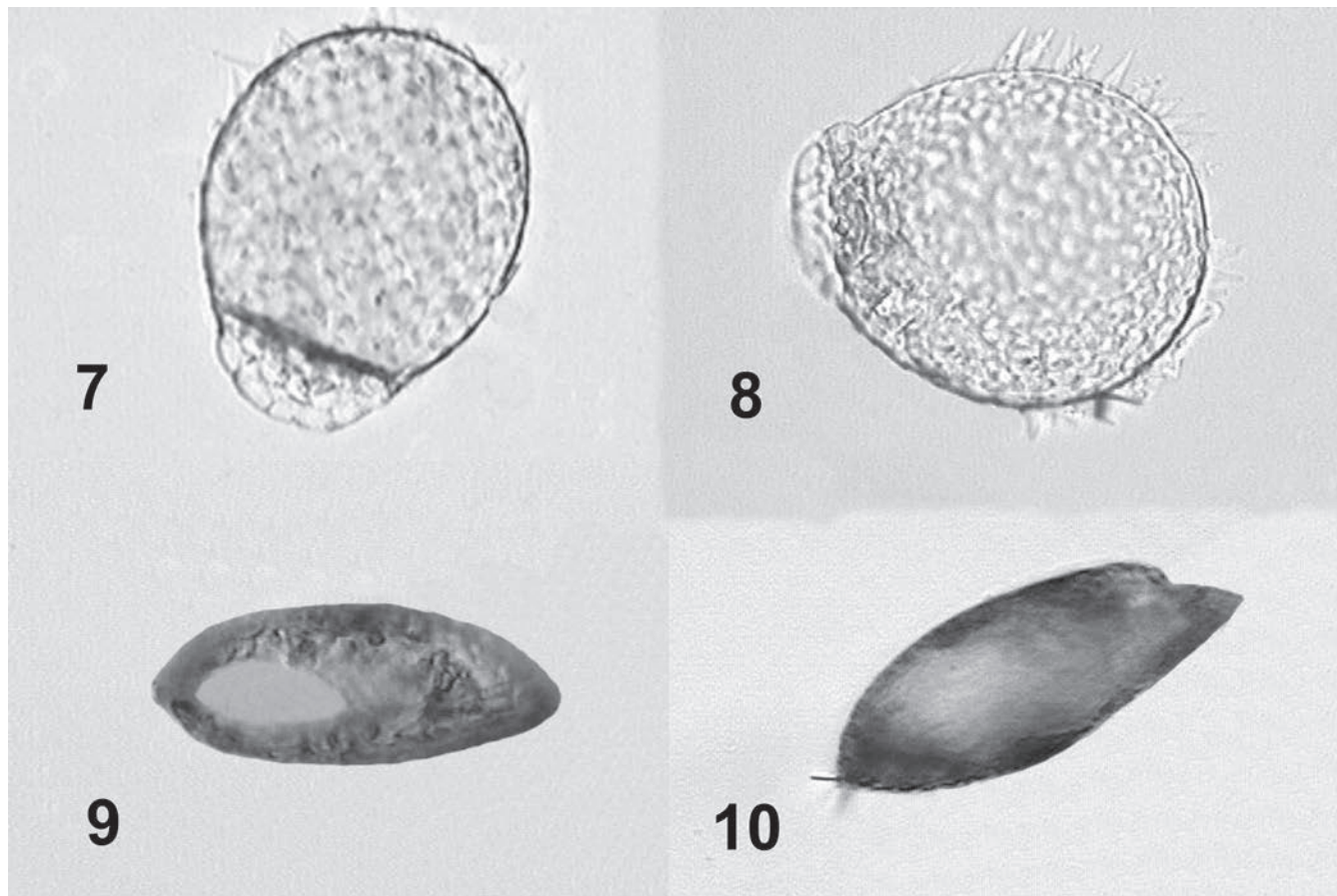


Fig. 2. Outline of *Deharvengia japonica*: ventral (left) and lateral (right) views. 1–7 – characters of the test measured (see Table 1).



Figs 3–6. Scanning electron microscopical (SEM) micrographs of *Deharvengia japonica*. 3–4 – dorsal view; 5 – dorsal view of the apertural cap; 6 – flat clavate spines on the side of the test.



Figs 7–10. Light microscopical (LM) micrographs of *Deharvengia japonica*. 7 – ventral view; 8 – ventro-lateral view; 9 – apertural view; 10 – lateral view.

ation coefficients of all the morphological characteristics are small, ranging between 3.0 and 9.1% for the six morphological variables under investigation (Table 1).

List of associated testate amoebae. The species was found in associations with the following species: *Arcella arenaria* Greeff, 1866, *A. arenaria compressa* Chardez, 1974, *Centropyxis constricta* (Ehrenberg, 1841) Deflandre, 1929, *C. sylvatica* (Deflandre, 1929) Bonnet et Thomas, 1955, *Cyclopyxis eurytoma* Deflandre, 1929, *Nebela tinctoria* (Leidy, 1879) Awerintzew, 1906, *Phryganella acropodia* (Hertwig et Lesser, 1874) Hopkinson, 1909, *Valkanovia elegans* Schönborn, 1964, *Assulina discoides* sp. n., *A. muscorum* Greeff, 1888, *Euglypha compressa* Carter, 1864, *E. compressa glabra* Wailes, 1915, *E. laevis* (Ehrenberg, 1832) Perty, 1849, *Corythion dubium* Taránek, 1881, *C. dubium minima* Chardez, 1969, *Trinema complanatum* Penard,

1890, *T. lineare* Penard, 1890, *Cryptodiffugia oviformis fusca* Penard, 1890.

Type locality: Mikuriga-pond, Murodo, 36°34'46.98"N 137°35'44.97"E; altitude 2,412 m ASL among the mountain pine (*Pinus pumila*) vegetation in the organic surface layer of soil consisting mainly of fallen needles (coniferous semi-decomposed litter – organic surface soils). Mountain gravelly seasonally freezing soils with high moisture content formed on volcanic rocks. This species was found also in another microhabitat at the same location in tall grasses.

Type specimen: Laboratory of Soil Bioindication, Department of Soil Geography, Faculty of Soil Science, Lomonosov Moscow State University, slide No. 1-2010.

Etymology: By analogy with the type species *Deharvengia papuensis*, the new species is named after the country where it was found (Japan).

Related species – similarities and differences: In comparison with the type species *Deharvengia papuensis* (Fig. 11) the new species (Fig. 2) is more dorso-ventrally flattened with an elliptical cross section (axial ratio 2:1); it is broadly ovoid in the broad view; the shell is covered by smaller idiosomes, which are arranged in more than 2 times the number of transverse and longitudinal rows found in the type species. The structure of the dorsal cap differs from those of *Deharvengia papuensis*; it is surrounded by a thin organic lip. There are numerous siliceous spines on the sides of the new species, which are absent in type species. Morphology of spines in *Deharvengia japonica* is unique and different from the spines of all other known species of testate amoebae.

Comments: Both *D. japonica* and *D. papuensis* are characterized by a similar structure of their apertural regions (Figs 7–11). This is the main morphological feature which allows uniting them into one genus. Among other morphological characteristics, there are differences, which are listed in the differential diagnosis. According to Bonnet (1979) *Deharvengia papuensis* with circular cross-section and large idiosomes belongs to the group of species within the genera *Sphenoderia* and *Tracheleuglypha*. A new species *Deharvengia japonica* seems to be morphologically closer to the group of species within the genera *Assulina* and *Placocista*, because the shell is laterally flattened, covered with medium-sized idiosomes and has spines. The nature of phylogenetic relations of the genus *Deharvengia* with these four genera can be determined only by DNA analysis and it is possible that these two species can be separated into two monotypic genera. The degree of morphological differences between these species is

as substantial as that between *Hoogenraadia* and *Planhoogenraadia* or *Ellipsopyxis* and *Ellipsopixella*.

Both species share apparent preferences for similar levels of acidity and moisture of their habitats. They were found in the soils of coniferous forests, which are characterized by pH 4–5 in the soil solution. The new species, unlike *Deharvengia papuensis*, was found in seasonally freezing soils. In contrast, the type species was described from the soils of the tropical rain forests of conifers *Araucaria cunninghami* and *A. hunsteinii* in New Guinea. Araucarias in the New Guinea forests occur in the lower mountain zone up to 1,500–2,000 m ASL at constant above-zero temperatures. The fact that representatives of this genus (*D. papuensis* and *D. japonica*) were found exclusively in the mountain soils with precipitation of up to 2,000 mm per year and average temperatures around 20°C (Balik 1995, Womersley 1995), indicates their affinity to hygrophilous mountain species.

Deharvengia papuensis was viewed as a species with limited geographical distribution (Foissner 2006). However, the expanding geography of protistological investigations casts new light on the ranges of rare species. Data on the distribution of *Deharvengia papuensis* are limited, and include the publication of Balik (1995) who discovered this species in the primary mountain rainforest in the Tam-Dao region, in northern Vietnam.

The area of possible distribution of the species from the genus *Deharvengia* includes Australian and Oriental biogeographic regions (based on classification of Udvardy, 1975). The southern half of the Honshu Is. is in the Japanese evergreen forest province of the Palaearctic biogeographical realm (Udvardy 1975). It borders subtropical forests, which stretch along the Pa-

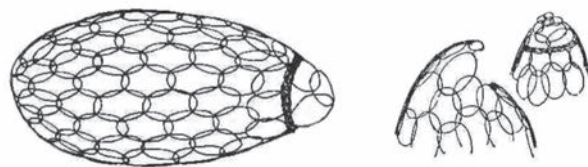


Fig. 11. *Deharvengia papuensis* (after Bonnet 1979): ventral view of the shell (left) and details of the aperture (right).

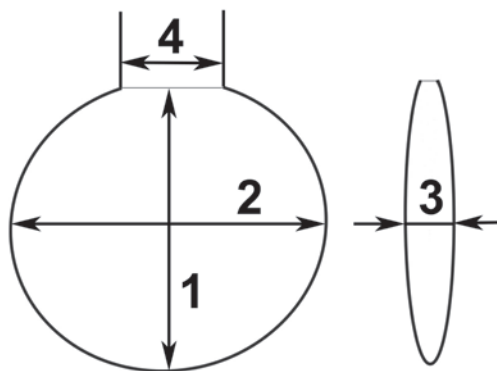
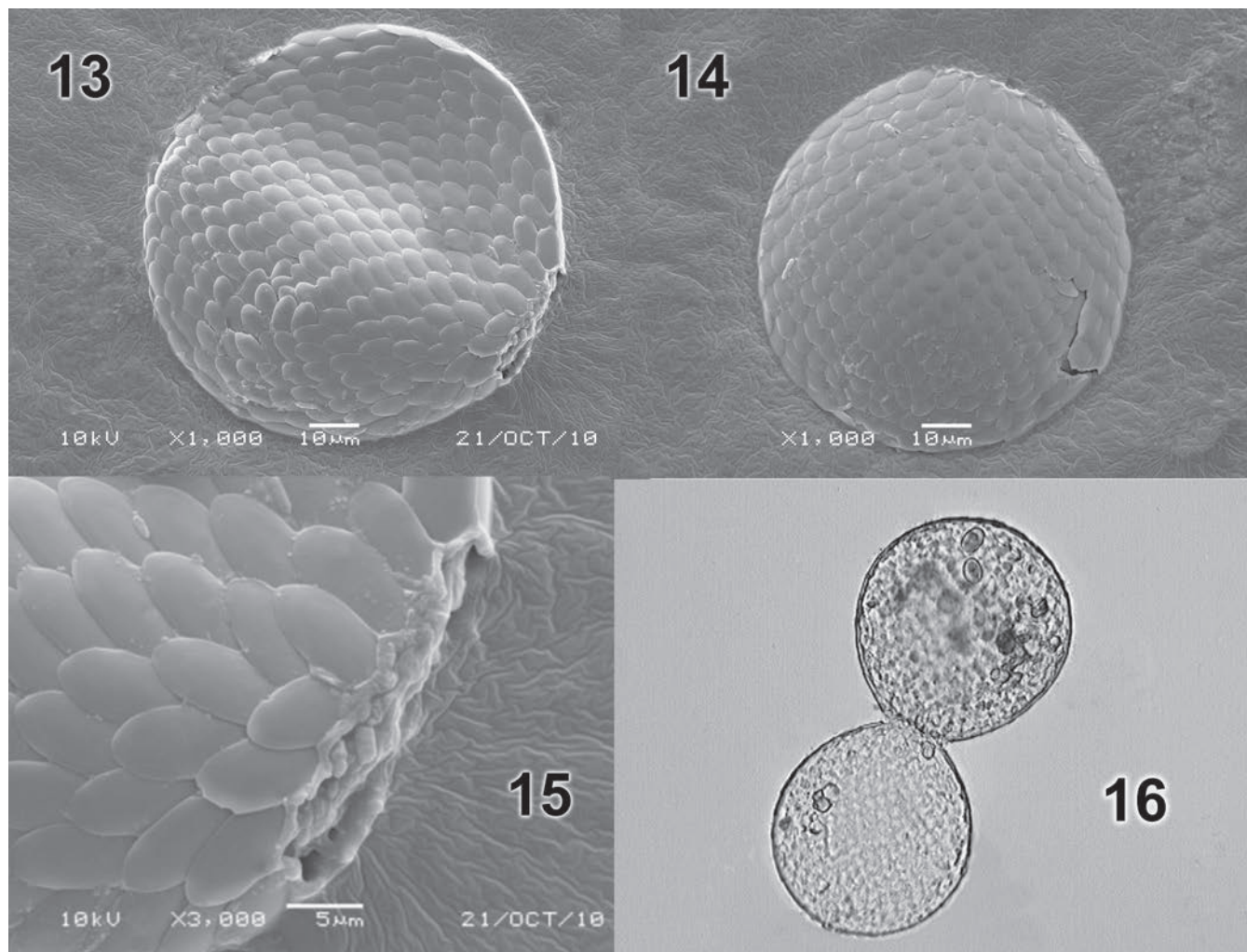


Fig. 12. *Assulina discoides*: broad (left) and lateral (right) views. 1–4 – characters of the test measured (see Table 2).



Figs 13–16. SEM (13–15) and LM (16) micrographs of *Assulina discoides*. **13–14** – broad views; **15** – details of the aperture; **16** – dividing cells.

cific coast of Indomalaya on the territory of China (Chinese subtropical forest province). This region, according to Hugget (1998) was excluded from the Palearctic and placed into the Oriental region. This viewpoint was supported by Bobrov (2001) based on Gondwanian tropical species from the genera *Hoogenraadia* and *Planhoogenraadia*. The finding of a new species provides an additional argument for this pattern and emphasizes the complex Quaternary history of modern ecosystems of this region.

Redescription of the genus *Deharvengia*

The appearance of the new morphological characters in *Deharvengia japonica*, compared with the type

species is the reason for amending the description of the genus *Deharvengia*.

***Deharvengia* Bonnet, 1979 emend.**

Test is transparent, oval or ovoid in the broad view, circular to narrow elliptical in the cross-section. Aperture elliptic or narrow elliptic, the ventral side is cut at about 6/7 of the length of the entire test and is surrounded by a thin organic lip. On the dorsal side the shell forms a small cap over the pseudostome, sometimes with a narrow organic lip along the edge. Shell is covered by elongate-elliptic idiosomes varying in size in different species. Transparent clavate spines, may be present on each side of the test.

***Assulina discoides* sp.nov. (Figs 12–17, Tables 2–3)**

Description: Shell large in size, disc shaped (Figs 13, 14, 16), colorless, transparent, narrow-elliptical in cross-section. The breadth of the shell usually exceeds the length (Table 2). Aperture is narrow-elliptical with an uneven narrow organic lip (Fig. 15). Aperture diameter is about 1/3 of the shell breadth. Surface is covered by large narrow elliptical idiosomes (Figs 13–15) up to 10 µm in length and 5–6 µm in breadth.

The population is monomorphic. Variation coefficients of all the morphological characteristics are small (Table 2).

Table 2. Biometric characterization of *Assulina discoides* sp. nov. based on 20 measured specimens. 1–4 – shell features (Fig. 12): 1 – shell length, 2 – shell breadth, 3 – shell depth, 4 – aperture width. Measurements in µm.

	1	2	3	4
Mean	86.7	85.5	24.45	27.1
M	87	85.5	24	27
SD	1.2	1.4	1.3	1.2
CV	1.4	1.6	5.4	4.4
Min	84.0	83.0	23.0	26.0
Max	88.0	88.0	27.0	30.0

List of associated testate amoebae. It was found in associations with the following species: *Arcella arena-ria* Greeff, 1866, *A. arenaria compressa* Chardez, 1974, *Centropyxis constricta* (Ehrenberg, 1841) Deflandre, 1929, *C. sylvatica* (Deflandre, 1929) Bonnet et Thomas, 1955, *Cyclopyxis eurystoma* Deflandre, 1929, *Nebela tincta* (Leidy, 1879) Awerintzew, 1906, *Phryganella acropodia* (Hertwig et Lesser, 1874) Hopkinson, 1909, *Valkanovia elegans* Schönborn, 1964, *Assulina discoides* sp. n., *A. muscorum* Greef, 1888, *Euglypha compressa* Carter, 1864, *E. compressa glabra* Wailes, 1915, *E. laevis* (Ehrenberg, 1832) Perty, 1849, *Deharvengia japonica* sp. n., *Corythion dubium* Taránek, 1881, *C. dubium minima* Chardez, 1969, *Trinema complanatum* Penard, 1890, *T. lineare* Penard, 1890, *Cryptodiffugia oviformis fusca* Penard, 1890.

Type locality: Mikuriga-pond, Murodo, 36°34'46.98"N 137°35'44.97"E; altitude 2,412 m ASL; mountain pine (*Pinus pumila*) vegetation; organic surface layer of soil consisting mainly of fallen needles (coniferous semi-decomposed litter – organic surface soils). Mountain gravelly seasonally freezing soils with high moisture content formed on volcanic rocks.

Type specimen: Laboratory of Soil Bioindication, Department of Soil Geography, Faculty of Soil Science, Lomonosov Moscow State University, slide No. 2-2010.

Etymology: The new species was named because of the discoid shape of the shell.

Table 3. Measurements (in µm) of different species of the genus *Assulina*.

Species and reference	Length of the shell (L)	Breadth of the shell (B)	Depth of the shell	Breadth of the aperture	L/B
<i>A. collaris</i> Schönborn and Peschke 1988	41–57	22–34	15–19	6–10	1.7–1.9
<i>A. muscorum</i> Cash <i>et al.</i> 1915	28–58	19–50	16–22	6–16	1.2–1.8
<i>A. muscorum</i> Ogden and Hedley 1980	45–53	32–48	18–22	12–18	1.1–1.4
<i>A. seminulum</i> Cash <i>et al.</i> 1915	60–90	50–75		16–15	1.2
<i>A. seminulum</i> Ogden and Hedley 1980	72–82	62–74	25–35	21–23	1.1–1.2
<i>A. scandinavica</i> Cash <i>et al.</i> 1915	80–120	70–110	39–42	15–30	1.1
<i>A. scandinavica</i> Ogden and Hedley 1980	107–114	102–103	39–42	26–30	1.0
<i>A. discoides</i> sp. nov. present studies	84–88	84–88	26–30	23–27	1.0

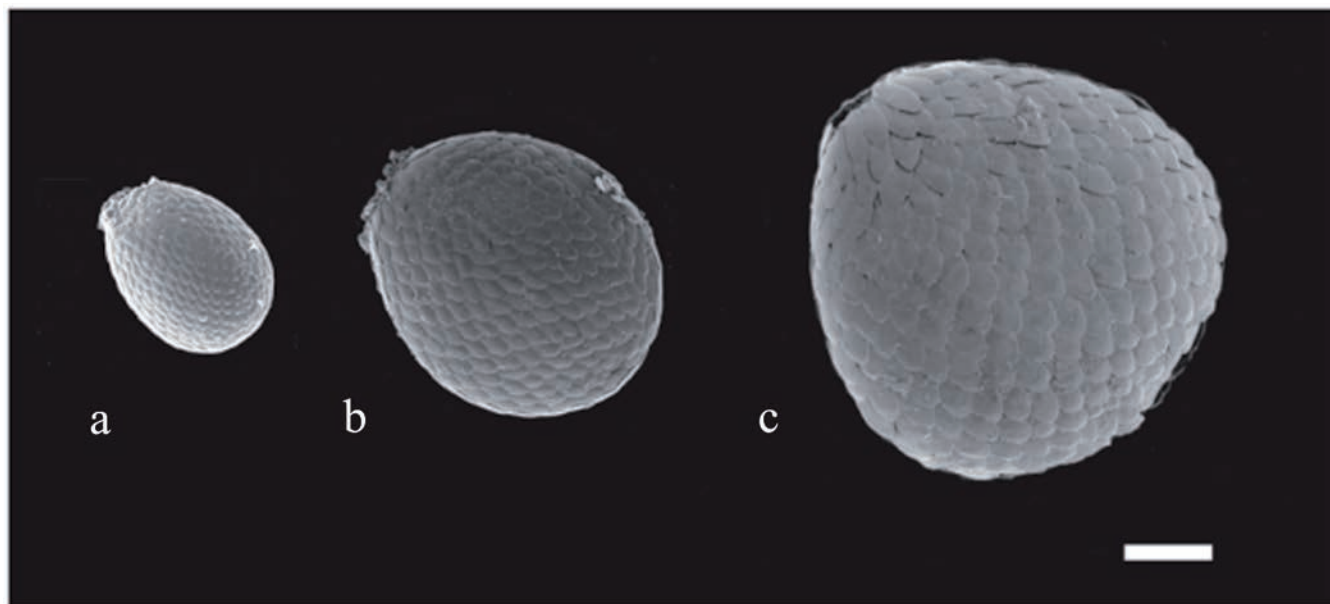


Fig. 17. Scanning electron microscope images of *Assulina muscorum* (a), *A. seminulum* (b) and *A. scandinavica* (c). The three cells are represented at the same scale. Scale bar: 20 μm (after Lara *et al.* 2011).

Related species – similarities and differences: *Assulina discoides* is easily distinguished from all other species of the genus *Assulina* (Fig. 17) by its disk-shaped shell. In contrast, *Assulina scandinavica* is almost triangular and has a sharp tapering of the shell from the broad aboral region (Ogden and Hedley 1980). The new species differs from the brown *Assulina muscorum* by being colorless, larger in size, and smaller length/width ratio (Table 3). An important feature is also a monomorphism of the new taxon that allows it to be easily identified in samples with co-occurring congeners, such as *Assulina muscorum*.

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