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A New Species from Mountain Forest Soils in Japan: *Porosia paracarinata* sp. nov., and Taxonomic Concept of the Genus *Porosia* Jung, 1942

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Abstract. A new species, *Porosia paracarinata*, is described from mountain forest litter, Bijodaira, Japan. This is the second species in the genus *Porosia*; until now, the genus was monospecific with the type species *Porosia bigibbosa*. *P. paracarinata* sp. nov. is distinguished from *P. bigibbosa* by the presence of a wide lateral keel. Test ultrastructure of *P. paracarinata* sp. nov. was documented using light and scanning electron microscopy. Morphometric analyses showed that this species is only slightly variable. The main morphological variability is due to the size of the lateral keel, which can vary from very wide (13.13 µm) to very narrow (3.75 µm). Ecological notes and morphological comparisons between *P. paracarinata* and other closely related species are discussed. The taxonomic concept of previously monospecific genus *Porosia* is expended.

Key words: *Porosia*, Japan, morphometry, *Porosia paracarinata*, testate amoebae.

INTRODUCTION

Hyalospheniid testate amoebae were described in the 19th century (Leidy 1874). Originally, the family included only a few genera (Leidy 1874, Schulze 1877). Most hyalospheniid species were grouped into the genus *Nebela* (Leidy 1879). The genus *Porosia* was proposed to include a single species, *N. bigibbosa* Penard, 1890, for differing significantly from other *Nebela*-s by two distinct lateral depressions with two large invaginated pores situated on each side, connected by internal tubes (Jung 1942).

In the Japanese mountains we found a species clearly sharing the morphological peculiarities of the genus *Porosia*, the details of which are quantitatively presented here. Thus, the taxonomic concept of the genus *Porosia* needs to be expanded, which is also proposed in this paper.

MATERIALS AND METHODS

Testate amoebae were isolated from litter and soil (taking the uppermost 5 cm). Samples were taken in Bijodaira, Mount Tateyama, Japan (Fig. 1). Cells were studied using Motic BA300 (China) light microscope. Scanning micrographs were made according to the technique described in Ogden (1980) using Jeol 6060A (Japan) scanning electron microscope.

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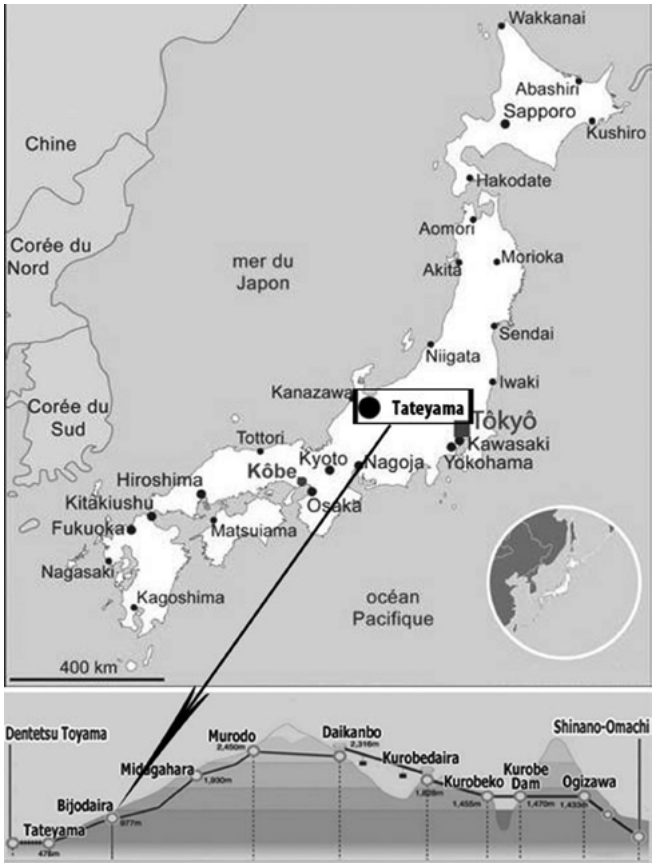

Fig. 1. Map of Japan, with indication of Mount Tateyama (in the first figure) and with the detailed locality called Bijodaira (in the second figure) where *Porosia paracarinata* was found (figure modified from http://klettertechnik-bauservice.de/images/pulau-honshu).

The following measurements were taking for the 30 cells: 1) test length; 2) test breadth; 3) aperture diameter; 4) distance from the posterior end of the test to the keel starting point; 5) distance from aperture to large pores; 6) breadth of test in the area of the large pores; and 7) keel breadth (Fig. 2). The following descriptive statistics were calculated: mean (x); median (M); standard deviation (SD); extreme values (Min and Max). Statistical analysis was performed using PAST 3.04.

RESULTS

Species description

Arcellinida Kent 1880

Hyalospheniidae (Schulze 1877) sensu Kosakyan and Lara 2012

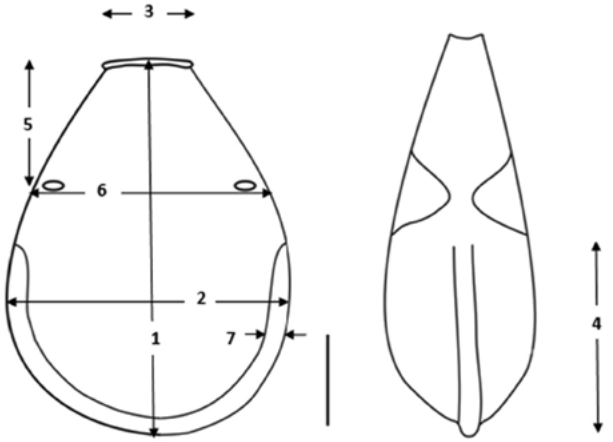

Fig. 2. Outline of *Porosia paracarinata*: ventral (left) and lateral (right) views. 1–7 – measured characters of the test (see Table 1). Scale bar: 50 μm.

***Porosia paracarinata* Bobrov et Kosakyan (Figs 2, 3, 4)**

Diagnosis: Test large, pyriform, laterally compressed, with rounded posterior part, narrow elliptical in profile. At approximately 1/3 of test length starting from aperture, two distinct lateral depressions with two large invaginated pores are situated on each side, which are connected by internal tubes as in *Porosia bigibbosa*. In profile, small lateral pores can be observed, just anterior to the large pores. At approximately after 1/2 of the length of the test counted from aperture, a lateral keel emerges such as in *Nebela carinata*, the width of which can vary between individuals (see Table 1, Figs 3, 4). Test is colorless, composed of circular or elliptic or irregular plates, often covered by the thin layer of organic cement. Length = 202–236 μm, Breadth = 142–157 μm. Aperture is oval, 56–67 μm wide, curved, surrounded with a thick organic lip.

Morphometry and morphological variability: Morphometric analyses of *Porosia paracarinata* show that species is characterized by low variability (Table 1). The largest standard deviation (SD) was observed in test length. The most stable morphological character is the thickness of the organic lip surrounding the aperture. The width of the lateral keel can also be variable among the individuals starting from very narrow (3.75 μm, see Fig. 4) to very wide (13.13 μm, see Fig. 3).

Ecology: *Porosia paracarinata* inhabits wet leaves (forest litter layer A0 or L-layer) in the mixed forest of *Fagus* sp. and *Cider japonica*.

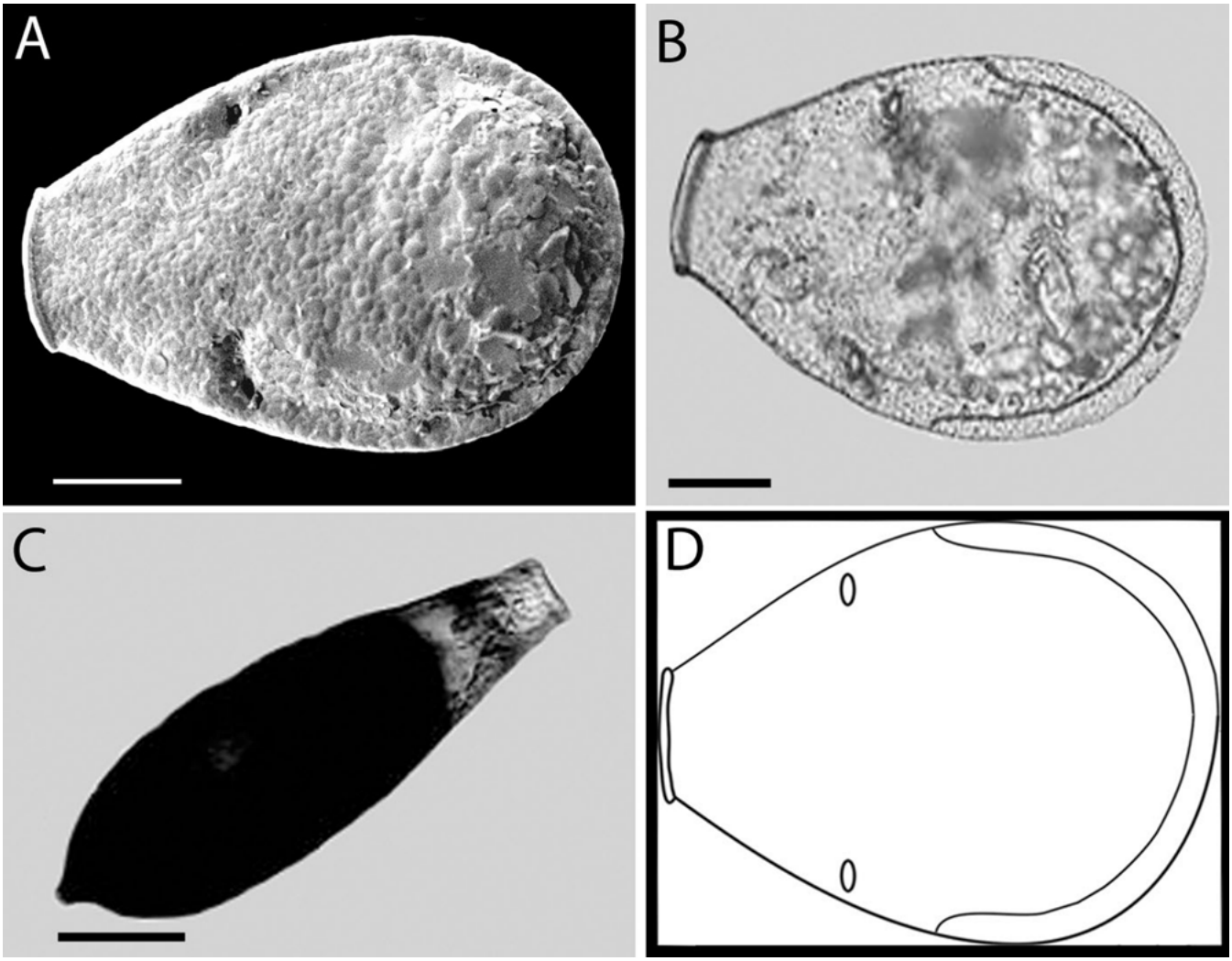

Fig. 3. *Porosia paracarinata* sp. nov. **A –** SEM image of *P. paracarinata* with wider keel; **B –** LM image of *P. paracarinata* with wider keel; **C** – *P. paracarinata* in profile view; **D –** schematic draw of *P. paracarinata*. Scale bars: 50 μm.

Table 1. Biometric characterization of *Porosia paracarinata* sp. nov. based on 30 measured specimens. SD – standard deviation; Min – minimum; Max – maximum. Measurements of characters (in μm) shown on Fig. 2: 1– test length; 2 – test breadth; 3 – aperture diameter; 4 – distance from posterior end of the test to the keel starting point; 5 – distance from aperture to large pores; 6 – breadth of the test in the area of the large pores; 7 – keel breadth.

N = 30	1	2	3	4	5	6	7
Mean	223,15	151,13	62,42	94,13	85,50	98,98	8,25
Median	225,00	150,00	62,69	91,88	86,25	97,50	7,88
SD	9,27	5,60	3,55	8,19	5,81	5,60	2,94
Min	202,50	142,50	56,25	82,50	75,00	93,75	3,75
Max	236,50	157,50	67,60	112,50	93,75	112,25	13,13

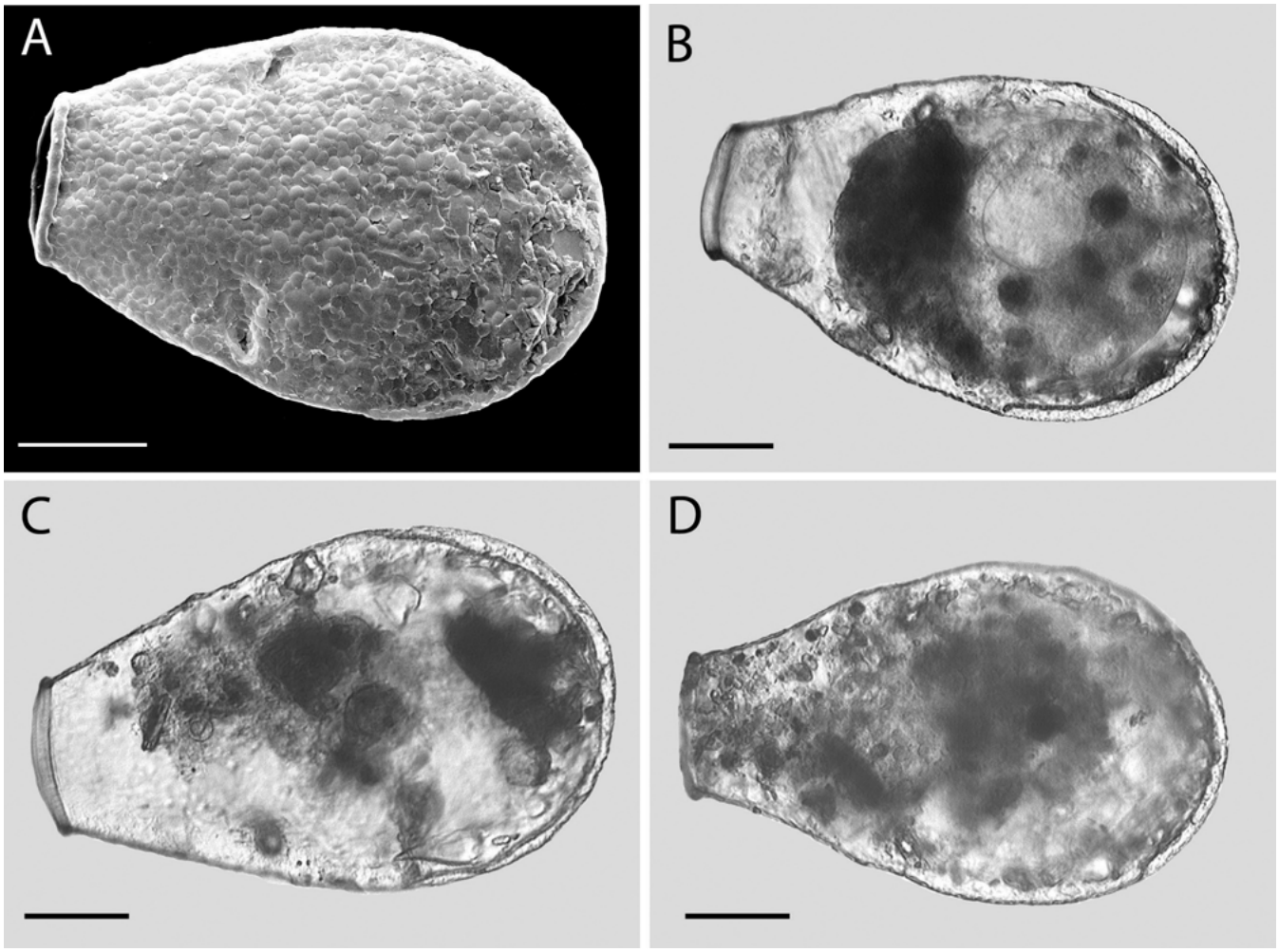

Fig. 4. Keel variation in *Porosia paracarinata* sp. nov. **A –** SEM image of *P. paracarinata* with narrow keel; **B** – LM images of *P. paracarinata* with narrow; **C** – very narrow and **D** – almost disappearing keel. Scale bars: 50 μm.

Type locality: Japan, Honshu island, mountains of Tateyama, Bijodaira, 36 34′58.93N, 137 27′39.49E, alt 997 m.

Etymology: The species name is emphasizing a morphological distinct character such as lateral keel which can be comparable with *Nebela carinata*.

Haplotype: The type specimen is located in Dr. A. Bobrov's collection (Preparation B-1/2015), Department of Soil Sciences, Moscow State University named after M. V. Lomonosov.

List of the associated testate amoebae: *Porosia paracarinata* was found in associations with the following species: *Arcella arenaria compressa, A. arctocrea aplanata, A. rotundata aplanata, Centropyxis aculeata, C. elongata, C. spinosa, C. sylvatica, Cyclopyxis eurystoma, C. eurystoma parvula, Plagiopyxis bathystoma, Heleopera lata, H. petricola, H. petricola amethystea, Hyalosphenia subflava, Padaungiella lageniformis, P. marginata, P. wailesi, Argynnia dentistoma, A. caudata, Physochilla griseola, Schoenbornia humicola, Phryganella hemisphaerica, Assulina muscorum, Quadrulella symmetrica, Tracheleuglypha acolla, Euglypha cristata decora, Sphenoderia fissirostris, Trinema complanatum, T. enchelys, T. lineare, T. lineare terricola, Cryptodifflugia oviformis fusca*.

Related species – similarities and differences: *Porosia paracarinata* shares interesting morphological features with other hyalospheniid genera such as *Ne-*

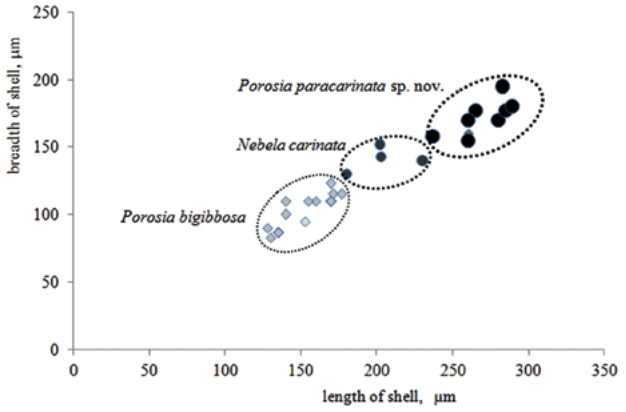

Fig. 5. Schematic demonstration of relationship of *Porosia paracarinata* sp. nov. and two closely related species, such as *Nebela carinata* and *Porosia bigibbosa*, based on morphological characters (average morphometric characteristics of species based on literature data and personal observations*).

* For average morphometric characteristics of species the following literature data are used – *Porosia bigibbosa* (Penard 1890, Cash *et al.* 1919, Wailes and Penard 1911, Deflandre 1936, Hoogenraad 1940, Ogden and Hedley 1980, Todorov 2002, Tsukii 2005); *Nebela carinata* (Cash *et al*. 1909, Ogden and Hedley 1980).

bela, particularly *Nebela carinata*, *N. marginata* because of the distinct lateral keel; *Certesella* because of two distinct lateral depressions with two large invaginated pores, situated on each side. *Porosia paracarinata* can be easily distinguished from the *Nebela* species, which do not have lateral depressions with large invaginated pores. However, there can be some confusion between certain *Certesella* species for which these kind of pores are typical. *Certesella australis* can be especially tricky because, like *Porosia paracarinata*, it also has a lateral keel. However, it is a much more elongated species with a distinct neck. With close and detailed observation, it is possible to see little pores situated in a parallel line to the aperture, which are the result of internal indentations typical for *Certesella* species. It could be possible that *Porosia paracarinata* is an intermediate species between those of *Nebela*, *Certesella* and *Porosia*. This new species differs from *Porosia bigibbosa* by larger dimensions and the presence of the lateral keel. Fig. 5 schematically shows the relationship of *P. paracarinata* with two possible closely related species such as *P. bigibbosa* and *Nebela carinata*.

DISCUSSION

According to literature data, *Porosia bigibbosa* found by different authors showed a quite variable morphology. None of the authors mentioned the presence of a lateral keel (Leidy 1879, Wailes and Penard 1911, Cash *et al*. 1919, Deflandre 1936, Hoogenraad and Groot 1940, Ogden and Hedley 1980, Török 1995, Todorov 2002). However, the examples stemming from literature can be confusing, especially when the findings published under the name *P. bigibbosa* can have possible affinities to *P. paracarinata*. The first example is unpublished, but it is quite obvious: In 2005, Tsukii published several photos of the *Porosia bigibbosa* from Japan (locality called Oze-shokubutu-kenkyu-mihonen, Oze-ga-hara (Oze marsh), Katashina village, Gunma Pref.) in the internet (http://protist.i.hosei.ac.jp/PDB/Images/Sarcodina/Porosia/sp_02.html), where the cells have an obvious lateral keel similar to *P. paracarinata* with a narrow keel. Katashina is located about 145 km north-east of Bijodaira (the place where *P. paracarinata* was discovered).

Another interesting fact is that it is possible that the genus of *Porosia* is much more diverse than we first expected: i.e. *Nebela bohemica* illustrated in Chattopadhyay and Das 2003, Figs 154–155) can be quite confusing. In the SEM micrograph (see Chattopadhyay and Das 2003, Fig. 154) the two large pores and the absence of any internal dentations in the neck show that this is obviously *Porosia*. It could have some affinity with *P. paracarinata* since in the LM image (see Chattopadhyay and Das 2003, Fig. 155) the test has a keel, but in the individual encysted it is hard to see the invaginated pores. On the other hand, in the SEM image (Fig. 154) it is also hard to see if the test has a keel or not because of the upside down position of the test. Another interesting example is the individual sample illustrated as *P. bigibbosa* in Lara *et al*. 2008 (see Fig. 1g), which is much smaller than the typical species and has a distinct lateral keel, similar to *P. paracarinata*, with the exception that the keel is starting near the lateral pores and not above lateral pores as in *P. paracarinata* (Fig. 2). Beside this, the smaller size (110 µm) of the individual sample illustrated in Lara *et al*. (2008), is indicating that this could well be another species within the genus. We also observed variation of a lateral keel in the individual samples of our studied population (see Table 1). Molecular data is needed to clarify if these variations belong to the distinct genotypes or are sim-

ply the result of phenotypic plasticity. Currently, only one *Porosia* (named as *P. bigibbosa*, but morphologically distinct from typical *P. bigibbosa*) rSSU sequence is available (Lara *et al.* 2008), and new molecular data based on other genes is necessary to understand the position of individual taxa within the genus.

Taxonomic concept of genus *Porosia* Jung 1942

Since the genus *Porosia* is not monospecific anymore, an expansion of the concept of this genus is required.

Original description of the genus according to Jung 1942 is the following:

Ahnlich wie Penardiella mit Porendurchbohrungen am Halsansatz. Doch fehlen die Porenlochgruppen in der Nahe des Pseudostoms. Rein boreal mit nur einer Art: P. bigibbosa (Penard, 1890), die sehr selten in Europe, dagegen in Spitzbergen haufiger ist. Vielleicht kaltstenotherm? Schalenende breitgerundet. l = 135–170 u, br = 87–110 u, m = 34–35 u.

Translation: Similar to *Penardiella* [currently *Certesella*] with pore holes on the base of the neck. However the pore punctuations on the base of the neck near aperture is lacking. Purely boreal with only one species *P. bigibbosa* (Penard 1890), in Europe very rare, but more prevalent in Spitzbergen [currently Svalbard, Norway]. Maybe cold-stenothermic? Posterior end of the test is broadly rounded, length 135–170 µm, breadth 87–110 µm, aperture 34–35 µm wide.

Expansion of the taxonomic concept for the genus *Porosia* (Jung 1942) sensu Bobrov et Kosakyan:

Test is pyriform, with rounded posterior end, laterally compressed. In front view two distinct lateral depressions with two large invaginated pores are situated on each side, which are connected by internal tubes as in genus *Certesella*. In profile, small lateral pores can be observed, just anterior to the large pores. The lateral keel can (or can not) be present surrounding 1/3 of posterior lateral margin (keel is important distinctive character between *Porosia* species: *Porosia bigibbosa* – lacking of lateral keel, *Porosia paracarinata* – presence of lateral keel). Test composed of euglyphid shell plates embedded in unstructured cement. Aperture is curved, surrounded with organic lip.

Habitat: sphagnum mosses, litter, soil, rare genus.

This genus is closely related to *Certesella* but lacks the punctuated neck.

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