Acta Protozool. (2021) 60: 61–75
www.ejournals.eu/Acta-Protozoologica
doi:10.4467/16890027AP.21.007.15381

ACTA
PROTOZOOLOGICA

Certesella larai (Amoebozoa: Arcellinida: Hyalospheniformes) a new soil testate amoeba species from the Dominican Republic and Chile challenges the definition of genera *Certesella* and *Porosia*

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Authors contributions: EM collected samples in the Dominican Republic (together with Enrique Lara). AB, CD and EM performed LM observations, imaging and biometrical measurements. AB performed SEM analyses. EM prepared the tables and figures and wrote the paper with input from AB and CD.

Abstract. Microbial diversity is known to be huge but remains only partly documented. Testate amoebae are a relatively well studied group or free-living protists that build morphologically characteristic shells on which the taxonomy of this group is mainly based. Among testate amoebae, the Hyalospheniformes are especially well documented and are a model group for microbial biogeography. Here we describe a new species of genus *Certesella* from *Sphagnum* mosses and forest litter samples collected in the Dominican Republic and Chile. We name this species *Certesella larai* to honour our esteemed colleague Enrique Lara. This species bears two large pores connected by tube, which are characteristic of the genera *Porosia* and *Certesella*. The new species fits best in the latter genus given the presence of a distinct, sometimes denticulated, neck with a bulge that is absent in genus *Porosia* but occurs in *Certesella*. Its elongated shape (length/breadth ratio 2.03–2.58) only overlaps with *C. certesi*, which is distinguishable by the presence of a distinct longitudinal groove in the neck region that is absent from the new species. Interestingly most specimens lacked the internal teeth which are usually present in members of genus *Certesella*. As such, while the morphology of *C. larai* strongly suggests a belonging to genus *Certesella*, molecular analyses are still required to confirm its phylogenetic position, clarify the relationships among all species in genera *Porosia* and *Certesella* and the definition and validity of these two genera. The presence of this new species in two rather distant regions – Caribbean and central Chile – suggests it is likely widespread, but possibly rare. However, this perception may be due to a sampling bias in favour of *Sphagnum* mosses and wet and organic-rich habitats. This illustrates the need for a more systematic sampling effort to document the full and mostly unknown taxonomic diversity of soil protists.

Key words: Testate amoebae, Hyalospheniidae, genus *Porosia*, genus *Certesella*, forest litter, peat bog

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INTRODUCTION

Testate amoebae are a group of free-living protists commonly found in terrestrial and aquatic habitats. They build characteristic shells (tests) whose morphology and dimensions allow species identification. These tests are well preserved in dry soil, moss samples, lake sediments, peat and permafrost, making them useful proxies in palaeoenvironmental studies (Charman 2001; Ilyashuk et al. 2006; Patterson et al. 2012; Swindles et al. 2019). Testate amoebae are also increasingly used as model organisms for microbial biogeography and several genera of hyalospheniid testate amoebae (e.g., genera *Apodera, Certesella, Alocodera*) are regularly cited as examples of non-cosmopolitan distribution in support for the vicariance hypothesis (Heger et al. 2011b; Smith et al. 2008).

About 2000–3000 testate amoeba taxa have been described (Meisterfeld 2002a, b). However it is currently understood that the vast majority should be treated as complexes of distinct species and that their global diversity is likely much higher. Despite the wealth of chorological data on testate amoebae, many knowledge gaps exist including basic taxonomy and distribution of taxa. This is well illustrated by the few extensive reviews for some regions (Beyens et al. 2016; Beyens et al. 1995; Bobrov et al. 2013; Fernández et al. 2016; Fernández et al. 2015; Qin et al. 2011). Indeed, surveys of testate amoebae in under-studied regions regularly lead to the description of new taxa, which are often highly conspicuous (Bobrov et al. 2015; Féres et al. 2016; McKeown et al. 2021; Reczuga et al. 2015).

In the context of on-going studies on the diversity and geographical distribution of the Circum-Australian group of testate amoebae as well as their morphological and morphometric variability, here we report the finding of a new species in genus *Certesella* from forest litter and peatland samples collected in the Dominican Republic and Chile. This species possesses traits of genera *Certesella* and *Porosia* and call for further studies to clarify the validity of these genera.

MATERIAL AND METHODS

Three samples in which the new species was found were collected in the Dominican Republic and Chile (Table 1). Testate amoebae were extracted from filtered litter or moss samples by shaking the sample in water and then filtering it over a ca. 250 µm mesh. The specimens of interest were isolated with a narrow pipette. The cells were observed and imaged using an Axioplan 2 (Carl Zeiss) light microscope under 200× and 400× magnifications, an inverted DIC microscope Olympus IX81 under 400–1000× magnifications, and a scanning electron microscope Jeol 6060 at a voltage of 20 kV for a detailed study of the structure of the shells.

Nine measurements were taken for shell characters and the length to width ratio calculated (Fig. 1). The average, standard deviation, standard error and coefficient of variation of all measurements were calculated for one population from Chile and one from the Dominican Republic and for the two populations combined. These measurements were compared to the morphometrical measurements reported for known species in genera *Porosia* (Bobrov et al. 2015) and *Certesella*.

RESULTS

Companion species and ecology

The communities of testate amoebae from two different geographical regions in which the new *Certesella* species was found, despite differences in species composition, are characterised by the presence of sphagnobiont and hygro-hydrophilic species from the genera *Difflugia, Pontigulasia, Nebela, Argynnia, Heleopera, Hyalosphenia,* and *Padaungiella* indicating moist to wet conditions (Table 2).

TAXONOMIC DESCRIPTION

Certesella larai sp. nov. (Fig. 1–7, Sup. Fig. 1; Table 3)

Description: Shell elongated pear-shaped (Figs. 1,2,4 & Sup. Fig. 1), compressed with two large, rounded to irregular-elongated elliptical pores clearly visible in broad view, connected by tubes. No visible lateral pore. Punctuations and inner teeth on the neck usually absent but visible in some specimens (Fig. 3). In broad view shell outline oval elongated at the fundus (aboral end of the shell), then narrowing slightly until a bulge near the area of the two large pores where the sides are approximately parallel, and narrowing again at the base of the neck. Neck relatively short with a slight bulge and then again approximately parallel near the aperture (Figs. 1,2 & 4). Shell colourless, covered with oval plates of different sizes and shapes (Fig. 4), most likely recycled idiosomes from euglyphid testate amoebae. Pores surrounded by smaller recycled idiosomes than the main body (Fig. 4). Pseudostome rim smooth and somewhat wavy, lip 2–2.5 µm thick (Figs. 2–4).

Table 1. Description of the sampling locations.

Location	Habitat	Sample type	Coordinates	Elevation [m a.s.l.]	Sample codes
Ebano Verde National Park, Dominican Republic	Secondary vegetation with thick fern cover	Fern litter	19.0385 ; – 70.5233	1293	EM-1384/DR-004
Ebano Verde National Park, Dominican Republic	Broadleaf forest, approx. ¾ of way up from river to the ridge	Broadleaf forest litter	19.0399 ; – 70.5193	1423	EM-1386/DR-006
Parque Nacional Alerce Costero, Los Rios Region Chile	Small *Sphagnum* peatland, with *Fitzroya* trees	*Sphagnum*	–40.171975o –73.491841o	1028	EM-1453/Chi2

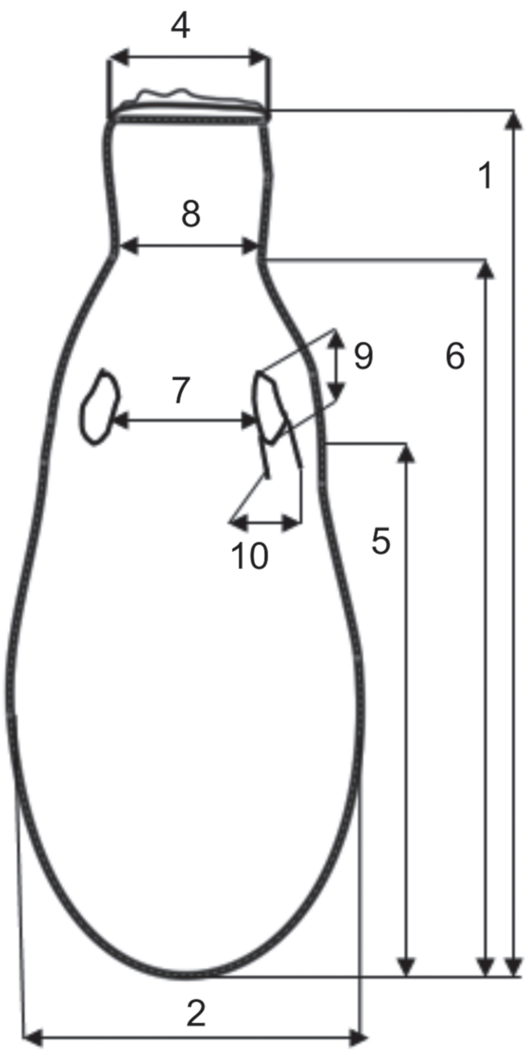

Fig. 1. General shape of *Certesella larai* n.sp. and indication of the morphometrical measurements. 1 – shell length, 2 – shell breadth, 3 – shell length / breadth ratio (not illustrated), 4 – aperture (long axis), 5 – distance from fundus to the pores, 6 – distance from fundus to base of neck, 7 – distance between the pores, 8 – width of the neck at narrowest point, 9 – pore length, 10 – pore width.

Measurements: Shell length: 135.8–153.4 µm, shell breadth: 56.8–68.2 µm, shell depth ca. 50 µm, aperture maximum dimension: 24.0–29.8 µm, distance from the fundus to the pores: 73.0–92.3 µm, distance from the fundus to the base (narrowest point) of the neck: 104.6–125.0, distance between the pores: 22.8–34.1 µm, width of the neck at its narrowest point – 18.5–29.5 µm, pore length – 5.1–12.8 µm, pore width – 3.0–6.4 µm, shell length/breadth ratio 2.03–2.58 (Table 3). The variability of morphological characteristics is generally low (e.g. <6% for characters 1–6 Table 3), and only somewhat higher for the smallest dimensions for which the accuracy of measurements is lower.

Type locality and habitat: Parque Nacional Alerce Costero, Los Ríos Region, Chile, Small *Sphagnum* peatland, with *Fitzroya* trees. In *Sphagnum*. Coordinates: –40.171975°; –73.491841°, elevation 1028 m. a.s.l., EM-1453 / Chi2. Locality of the paratype specimen: Ebano Verde National Park, Dominican Republic. Broadleaf forest litter Forest approximatively three quarters of way up from the river to the ridge. Elevation 1423 m. above sea level. Coordinates: 19.0399°; –70.5193°.

Type specimen: Type: Natural History Museum of Neuchâtel, Rue des Terreaux 14, 2000 Neuchâtel, Switzerland, slide No. 95–2. Paratype: Laboratory of Soil Bioindication, Department of Soil Geography, Faculty of Soil Science, Lomonosov Moscow State University, slide No. 4–2020.

Etymology: The species name was chosen to honour our esteemed colleague Enrique Lara, as a recognition for his major contribution to the molecular taxonomy and phylogeny of testate amoebae. We believe he may be amused by the fact that we name in his honour a species which is not straightforward to classify

Table 2. Occurrence of testate amoeba taxa associated to *Certesella larai* in three samples from Chile and the Dominican Republic.

Taxon	**EM-1453/ Chi2**	**EM-1386/ DR-006**	**EM-1384/ DR-004**	**Chile Only**	**DR Only**	**Chile & DR**	**All 3 samples**
Alabasta militaris		x			x		
Apodera vas f. A (longa)	x	x				x	
Argynnia caudata		x	x		x		
Argynnia columbiana		x			x		
Argynnia dentistoma		x			x		
Argynnia teres		x			x		
Assulina muscorum	x	x				x	
Centropyxis aculeata	x			x			
Centropyxis acuminata	x			x			
Centropyxis constricta	x	x				x	
Centropyxis deflandriana	x		x			x	
Centropyxis elongata	x			x			
Centropyxis latideflandriana		x			x		
Centropyxis lithostoma		x	x		x		
Centropyxis penardi			x		x		
Centropyxis plagiostoma	x			x			
Centropyxis plana v. microstoma	x			x			
Centropyxis stenodeflandriana		x			x		
Centropyxis sylvatica	x			x			
Certesella certesi	x			x			
Certesella martiali	x			x			
Certesella martiali f. A (major)	x			x			
Cryptodifflugia apiculata		x			x		
Cryptodifflugia minuta	x			x			
Cryptodifflugia oviformis		x			x		
Cryptodifflugia oviformis f. fusca.		x			x		
Cyclopyxis eurystoma	x	x	x				x
Cyclopyxis eurystoma v. parvula		x			x		
Cyclopyxis eurystoma v. parvula			x		x		
Difflugia gassowski	x			x			
Difflugia globulus	x			x			
Euglypha ciliata	x			x			
Euglypha ciliata f. glabra		x			x		
Euglypha compressa	x	x	x				x
Euglypha cristata	x	x				x	
Euglypha cristata f. decora		x			x		
Euglypha strigosa	x			x			
Heleopera petricola	x			x			
Heleopera petricola v. amethystea		x			x		
Heleopera petricola v. humicola	x			x			
Heleopera sphagni	x			x			
Heleopera sylvatica			x		x		

Hyalosphenia minuta		x			x		
Hyalosphenia subflava			x		x		
Hyalosphenia subflava f. A (major)		x			x		
Hyalosphenia undans		x			x		
Lesquereusia modesta	x			x			
Nebela barbata	x			x			
Nebela collaris		x			x		
Nebela parvula		x			x		
Padaungiella tubulata	x	x	x			x	
Physochila tenella	x			x			
Plagiopyxis labiata		x			x		
Pontigulasia spectabilis	x			x			
Pseudodifflugia gracilis v. terricola		x			x		
Schoenbornia humicola	x	x	x				x
Sphenoderia fissirostris	x	x	x				x
Trigonopyxis arcula	x	x				x	
Trigonopyxis microstoma	x			x			
Trinema complanatum	x		x			x	
Trinema lineare	x	x	x			x	
Trinema lineare v truncatum	x			x			
Trinema lineare v. minuscula		x			x		
Trinema lineare v. terricola	x			x			
Valkanovia elegans	x			x			
Total number of taxa	38	34	14	25	27	9	4

Fig. 2. Light microscopy images of *Certesella larai* n.sp. from Parque Nacional Alerce Costero, Los Ríos Region, Chile. Left: DIC image of the type specimen deposited at the Natural History Museum of Neuchâtel (slide 95–2). Centre and right: brightfield images of specimens from the same sample. Scale bar 20 µm.

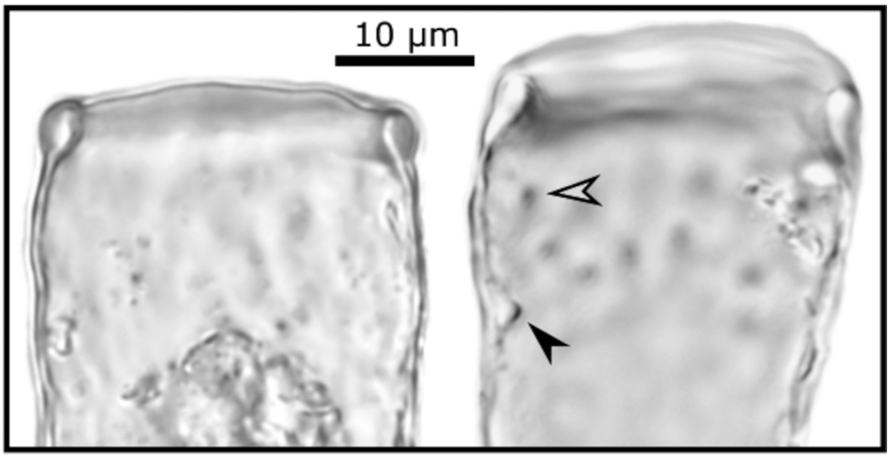

Fig. 3. Light microscopy images of *Certesella larai* n.sp. from Parque Nacional Alerce Costero, Los Ríos Region, Chile, showing the detail of the pseudostome. The right image shows internal teeth fully visible on the flank of the neck (solid arrow and visible only in transparency (empty arrow). Such structures are only visible in ca. 10% of the specimens. Scale bar 10 μm.

based solely on morphological data and clearly calls for molecular phylogenetic analyses … to which he has so greatly contributed.

Comparison between the two populations: The two populations largely overlap in length and width (Fig. 5) and do not differ either in general shape (Sup. Fig. 1) and other characters (not illustrated).

Related species: There is no possible confusion with *C. australis* and *C. murrayi* as their shape is very different (Figs. 6 & 7). The two most similar species are *C. certesi* and *C. martiali*. The shell of the new species is more elongated (length/width ratio: 2.03–2.58) than *Certesella martiali* (1.83-1.90). The length/width ratio overlaps with C. *certesi* (range 1.75–2.92 based on the original description and several subsequent studies (Certes 1889; Deflandre 1936; Heinis 1914; Penard 1911). The contrast is even clearer with the two known species of the genus *Porosia – P. bigibbosa* and *P. paracarinata* (Table 4). Indeed, the dimensions of the new species do not overlap with any known species of genera *Certesella* and *Porosia* in a biplot of length vs. width (Fig. 7). The somewhat irregular, wavy pseudostome lip of *Certesella larai* may be specific to this species; but this should be assessed by a thorough comparative morphological study.

The outline of *Certesella larai* is similar to other species in genus *Certesella* (Figs. 6 & 7). However, most specimens lack the inner teeth on the neck that are considered as one of the characteristic features of this genus. While we consider it unlikely that the lack of internal teeth is an effect of phenotypic plasticity as observed for shell size in *Hyalosphenia papilio* (Mulot et al. 2017), we evaluate that the new species fits best in genus *Certesella*.

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DISCUSSION

Taxonomy

Certesella larai clearly differs in shape and size from all known species of genera *Certesella* and *Porosia* and although we currently lack molecular data, we are therefore confident that this represents a new species. However, the taxonomic position of this new species is unclear from morphology alone and indeed we shifted back and forth between placing this new species into genera *Certesella* or *Porosia*.

The presence of internal teeth on the neck in *Certesella* is considered a major criterion to distinguish it from genus *Porosia* (Tsyganov et al. 2016). Most specimens did not have internal teeth on the neck, which would place this species into genus *Porosia*. However, some individuals (< 10%) did present minute denticulations that, in addition to the general shape and especially the presence of a well-developed neck allowed assigning this species to genus *Certesella* (Fig. 3). Those denticulations, which are often only visible as

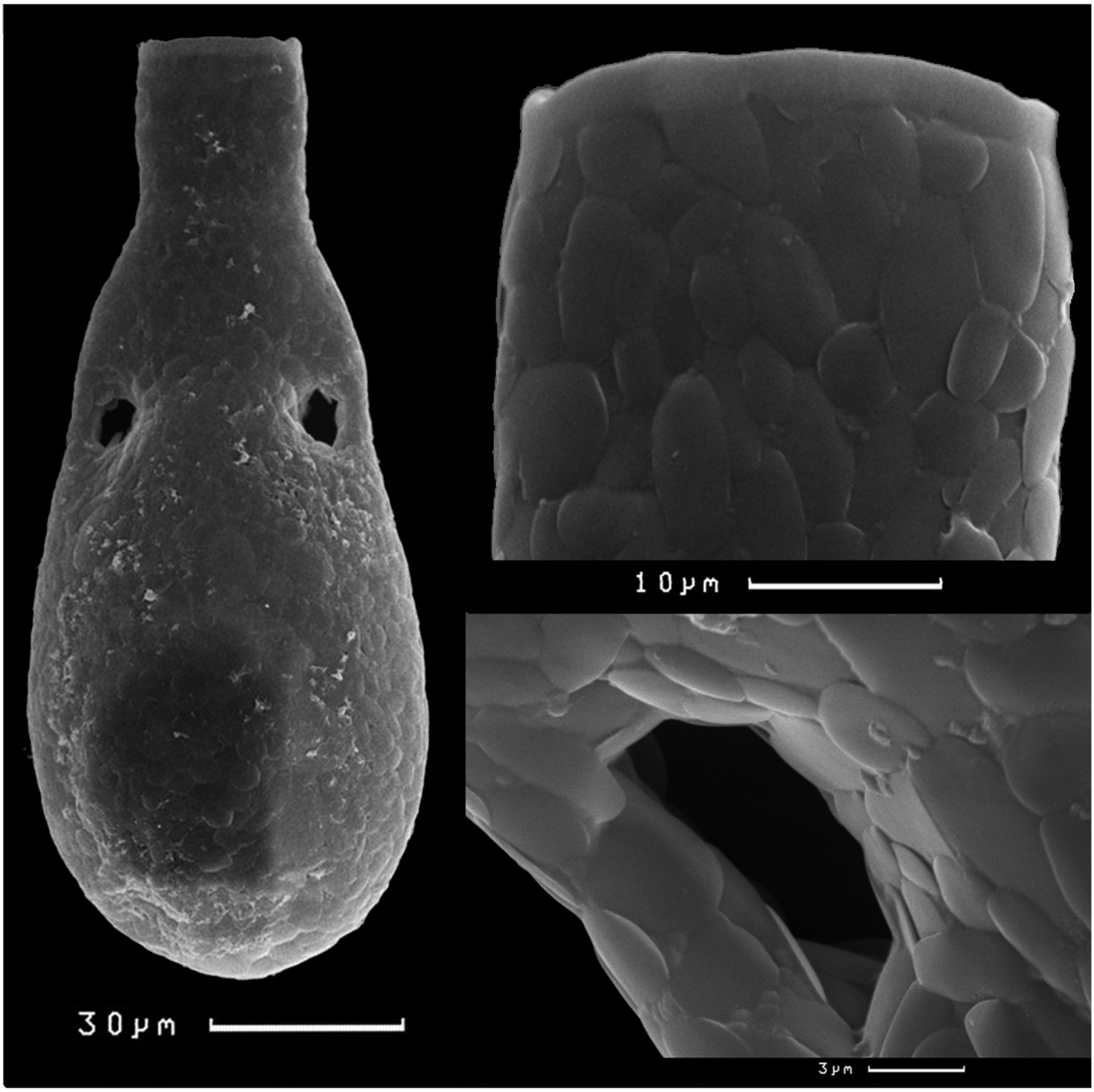


Fig. 4. *Certesella larai* n.sp. scanning electron microscopy images showing the overall shape, the chitinous rim of the pseudostome, the detail of an elongated pore and the plates (presumably recycled from euglyphid testate amoebae) covering the shell.

dark dots, are difficult to observe without high magnification and are clearly absent from most specimens we observed. However, the fact that most specimens of *C. larai* do not present this trait questions the validity of this criterion and blurs the limit between the two genera, as it implies that species closer to *Certesella* than *Porosia* could completely lack internal denticulations.

Among *Certesella*, the overall shape of *C. larai* is most similar to *C. certesi*, and this is an additional reason to place it in the genus *Certesella*. But *C. larai* lacks the characteristic longitudinal groove of *C. certesi*. It can be added that the range of illustrated morphotypes for *Certesella certesi* suggests this taxon corresponds to a species complex unified by this

Table 3. Biometrical measurement (in mm) of *Certesella larai* from two populations from the Domincan Republic and Chile.

	Morphological character	**N**	**Min**	**Max**	**Mean**	**SD**	**Median**	**CV [%]**
Overall								
1	Shell length	51	135.8	153.4	144.6	4.9	144.9	3.4%
2	Shell breadth	50	56.8	68.2	61.9	2.2	62.5	3.6%
3	Shell length / breadth ratio	50	2.03	2.58	2.34	0.11	2.34	4.5%
4	Aperture (long axis)	51	24.0	29.8	27.4	1.3	27.8	4.9%
5	Distance from fundus to the pores	51	73.0	92.3	82.1	4.6	82.4	5.6%
6	Distance from fundus to base of neck	51	104.6	125.0	115.4	4.6	115.2	4.0%
7	Distance between the pores	51	22.8	34.1	29.8	3.2	31.2	10.6%
8	Width of the neck at narrowest point	51	18.5	29.5	25.6	2.4	25.6	9.3%
9	Pore length	50	5.1	12.8	8.9	1.6	8.5	17.6%
10	Pore width	50	3.0	6.4	4.5	0.7	4.3	15.3%
Population 1 – Broadleaf forest litter, Dominican Republic (DR-006; EM-1384)								
1	Shell length	10	139.2	152.6	146.1	4.2	147.0	2.9%
2	Shell breadth	10	56.8	68.2	60.3	2.7	59.5	4.5%
3	Shell length / breadth ratio	10	2.27	2.58	2.43	0.10	2.42	4.1%
4	Aperture (long axis)	10	24.0	28.6	27.4	1.7	28.4	6.1%
5	Distance from fundus to the pores	10	85.2	92.3	85.9	2.2	85.2	2.6%
6	Distance from fundus to base of neck	10	113.6	124.6	118.1	3.2	118.6	2.7%
7	Distance between the pores	10	31.2	32.7	32.3	0.7	32.7	2.2%
8	Width of the neck at narrowest point	10	24.0	29.5	25.5	1.4	25.6	5.5%
9	Pore length	10	8.5	11.4	8.9	1.0	8.5	10.9%
10	Pore width	10	4.3	6.4	4.3	0.0	4.3	0.0%
Population 2 – Sphagnum, Chile (EM-1453)								
1	Shell length	41	135.8	153.4	144.3	5.0	144.8	3.5%
2	Shell breadth	40	56.8	68.2	62.3	2.0	62.5	3.2%
3	Shell length / breadth ratio	40	2.03	2.46	2.32	0.10	2.34	4.2%
4	Aperture (long axis)	41	24.5	29.8	27.4	1.3	27.6	4.6%
5	Distance from fundus to the pores	41	73.0	92.3	81.2	4.5	81.5	5.6%
6	Distance from fundus to base of neck	41	104.6	125.0	114.7	4.7	113.8	4.1%
7	Distance between the pores	41	22.8	34.1	29.2	3.2	29.7	11.1%
8	Width of the neck at narrowest point	41	18.5	29.5	25.6	2.6	25.6	10.1%
9	Pore length	40	5.1	12.8	8.9	1.7	8.5	19.0%
10	Pore width	40	3.0	6.4	4.5	0.8	4.3	16.8%

Table 4. Summary measurements reported for *Certesella* and *Porosia* species. Details of measurements for populations and individual publications are given in Supplementary Table 2.

Species	**Length**	**Breadth**	**L/B**	**Aperture**	**Reference**
Certesella larai sp. nov.	138.2–153.4	56.8–63.9	2.18–2.45	25.6–29.8	Present work
Certesella certesi	80–157	70–90	1.82–2.55		Certes, 1889, Penard, 1911, Heinis, 1914, Deflandre, 1936
Certesella martiali	147–238	77–130	1.55–1.83		Certes, 1889, Penard, 1911, Deflandre, 1936
Certesella australis	199.5–277.5	119–136.5	1.5–1.8		Vucetich, 1973
Certesella murrayi	120–136	95–100			Wailes, 1913
Porosia bigibbosa	128–178	83–123		34–51	Penard, 1890, Wailes & Penard, 1911, Cash et al., 1919, Deflandre, 1936, Ogden & Hedley, 1980, Hoogenraad & de Groot, 1940, Todorov, 2002 and Luketa, 2016
Porosia paracarinata	202–236	142–157		56–67	Bobrov & Kosakyan, 2015

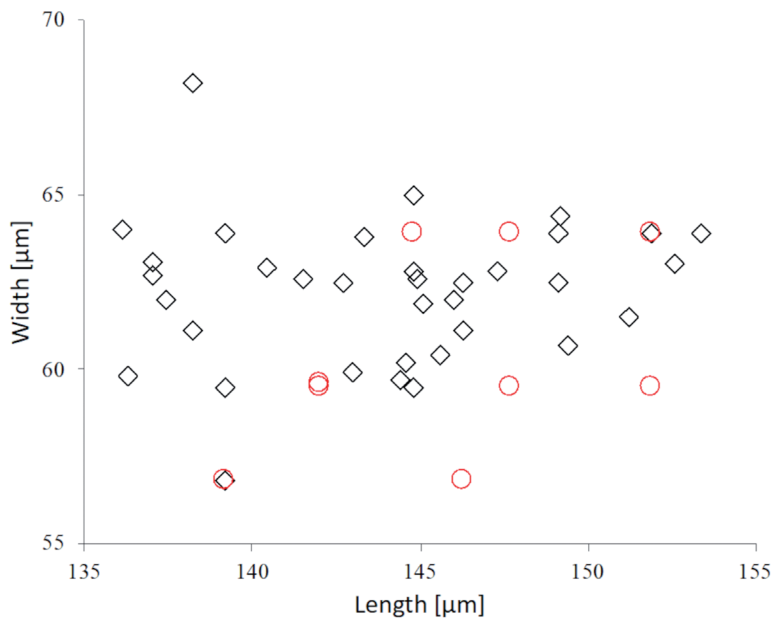


Fig. 5. Biplot of length vs. width of two populations of *Certesella larai* n.sp. from Dominican Republic and Chile.

characteristic longitudinal groove rather than a single species.

The phylogenetic relationship of *Certesella* and *Porosia* remains unclear and currently only *Certesella martiali* has been characterized using DNA barcoding (Kosakyan et al. 2012). Thus, *Certesella* and *Porosia* should be high priority targets for future taxonomic efforts using molecular tools. Furthermore, as *Certesella* has only been found South of the Cancer Tropic while *Porosia* is cosmopolitan, a phylogenetic reconstruction including those two genera would be welcome to clarify the biogeographical history of arcellinid testate amoebae. The outcome may show that *Porosia* is the ancestral type and that *Certesella* is a younger clade, possibly nested within *Porosia* that originated in Gondwana after the breakup of Pangea, which would be consistent with an inferred origin of hyalosphenids at ca. 370 mya (Lahr et al. 2015).

Ecology

Given the fact that *Certesella larai* was rare in the three samples, its specific habitat as well as companion species may not necessarily reflect its ecological optimum. *Certesella certesi*, *C. martiali* and *C. australis* were all described from *Sphagnum* peatlands in South America (Certes 1889; Vucetich 1973; Wailes 1913). In Mexico, Bobrov et al. found *Certesella certesi* only in mountain cloud forests (Bobrov et al. 2013), while in New Zealand Bamforth found it only in lowland podocarp forests (Bamforth 2015). *Certesella martiali* was reported from mosses in Guatemala (Laminger 1973). Heger et al. observed *Certesella* sp. in mosses in several locations across Central America (Heger et al. 2011a). *Porosia bigibbosa* is a rare testate amoeba species in peatlands but is quite common in beech forests in Bulgaria (Todorov 2002) and a new species, *P. paracarinata* was also found in forests in Japan (Bobrov et al. 2015). The discovery of *Certesella larai* suggests that the genus *Certesella* is also not restricted to peatlands. Thus, while hyalospheniids in general are most diverse in acidic and nutrient-poor habitats such as *Sphagnum* peatlands, this is not true for genus *Porosia* and possibly not either for *Certesella*.

As the ecology and diversity of testate amoebae have been much more intensively studied in peatlands than in forests the ecology of some species may not yet be fully understood. The fact that this new species was found in two very distant regions but was rare in the three samples and absent from ca. 100 other neotropical samples may reflect a sampling bias in favour of *Sphagnum* or other habitats which are a priori perceived as more favourable for testate amoebae. This can potentially cause interesting species to be overlooked. Another recent example is the discovery of a new *Quadrulella* species in a semi-desert environment in Mexico (Pérez-Juárez et al. 2017), which contrasts

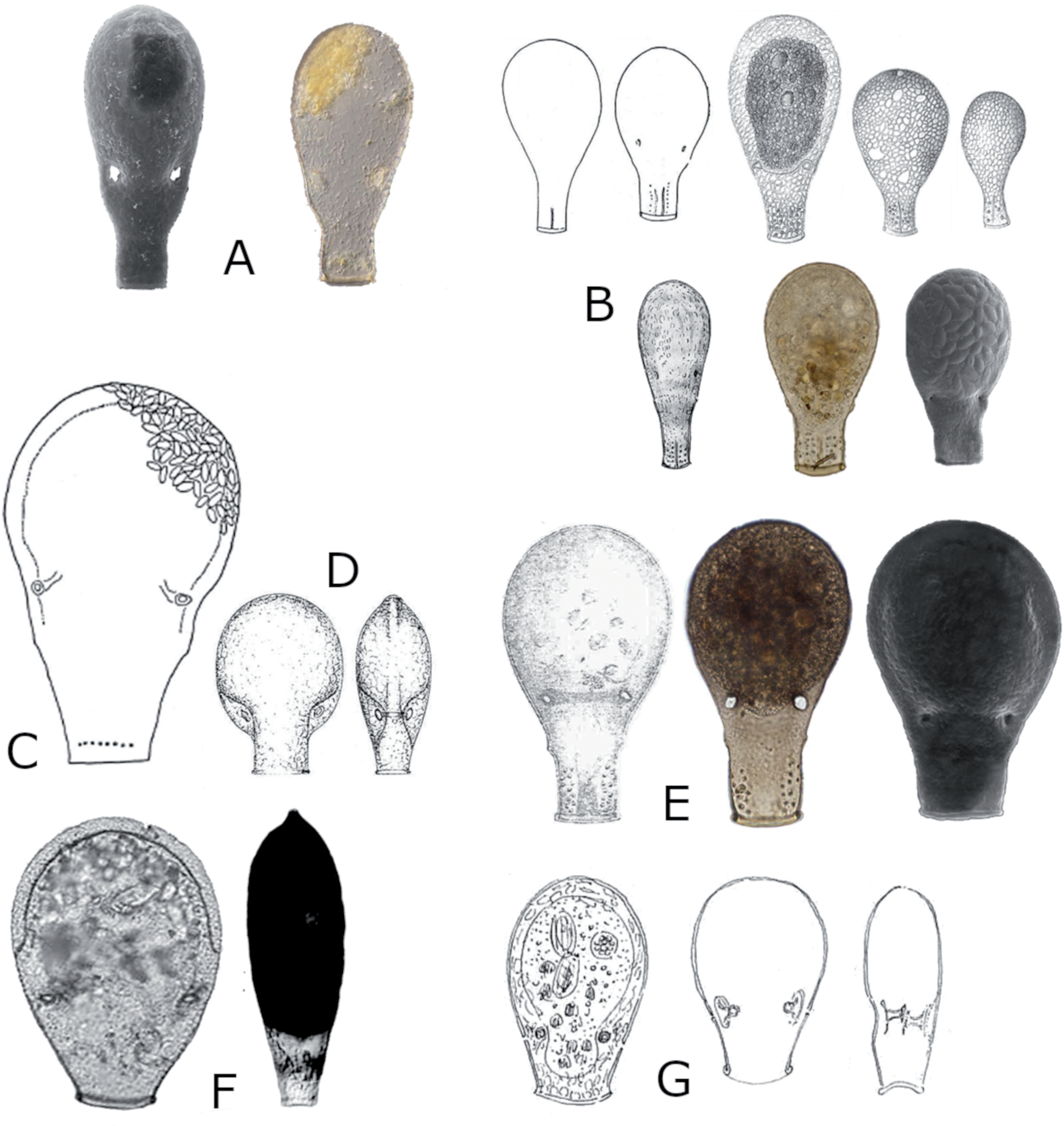


Fig. 6. Comparative overview of the morphology of all known species of genera *Certesella* and *Porosia* species. A: *Certesella larai* n. sp., B: *C. certesi*, C: *C. australis*, D: *C. murrayi*, E: *C. martiali*, F: *Porosia paracarinata*, G*: P. bigibbbosa*. Images are not to scale as some early images lacked a scale.

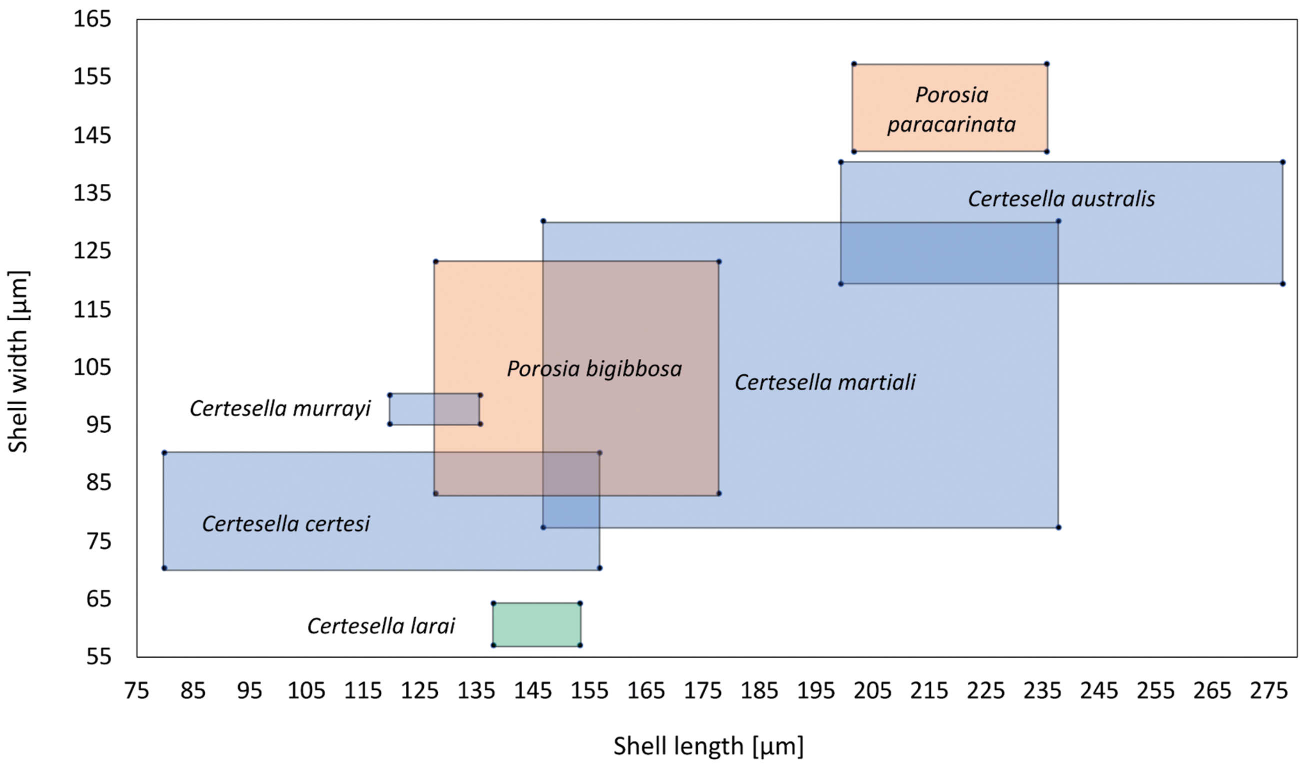


Fig. 7. Maximum range of shell length and width of the three known *Certesella* and *Porosia* species showing a lack of overlap between *C. larai* n.sp. and all other species from the two genera in this two-dimensional space. Each species is illustrated (images not exactly to scale).

with the wetland habitats where species of this genus are usually found. The discovery of these new species illustrates that when under-sampled habitats are studied some surprising discoveries can be made. This should be a motivation for protistologists to continue exploring a broad range of habitats.

Acknowledgements. We thank Dimaris Acosta Mercado for organising the joint sampling trip in the Dominican Republic funded by NSF DEB grant no. 0640052 and under permit no. 00805 Secretaría de Estado y Medio Ambiente y Recursos Naturales of the Dominican Republic. Enrique Lara kindly provided the sample from Chile and helped EM collect samples in the Dominican Republic. This research was supported by the Russian Foundation for Basic Research № 19-05-50093\20. Further funding was provided by Moscow State University and the University of Neuchâtel. We thank two anonymous reviewers who provided useful comments on the manuscript.

REFERENCES

Bamforth S. S. 2015. Composition of Soil Testate Amoebae Communities: Their Structure and Modifications in the Temperate Rain Forests of New Zealand and Tasmania. **62:** 217–226

Beyens L. & Chardez D. 1995. An annotated list of testate amoebae observed in the Arctic between the longitudes 27 degrees E and 168 degrees W. *Arch. Protistenkd.* **146:** 219–233

Beyens L. & Bobrov A. 2016. Evidence supporting the concept of a regionalized distribution of testate amoebae in the arctic. *Acta Protozool.* **55:** 197–209

Bobrov A. & Kosakyan A. 2015. A new species from mountain forest soils in Japan: *Porosia paracarinata* sp nov., and taxonomic concept of the genus *Porosia* Jung, 1942. *Acta Protozool.* **54:** 289–294

Bobrov A., Krasilnikov P. & García-Calderón N. E. 2013. Biogeography of testate amoebae in the soils of Mexico. *Biodiv. Conserv.* **22:** 2837–2855

Certes A. 1889. Protozoaires. *In:* Mission scientifique du Cap Horn. 1882–1883, Tome 6. Zoologie (Ed., Gauthier-Villars et Fils, Imprimeurs-Libraires., Paris

Charman D. J. 2001. Biostratigraphic and palaeoenvironmental applications of testate amoebae. *Quat. Sci. Rev.* **20:** 1753–1764

Deflandre G. 1936. Etude monographique sur le genre Nebela Leidy. *In:* Annales de Protistologie, **5** 201–286

Féres J. C., Porfírio-Sousa A. L., Ribeiro G. M., Rocha G. M., Sterza J. M., Souza M. B. G., Soares C. E. A. & Lahr D. J. G. 2016. Morphological and morphometric description of a novel shelled amoeba *Arcella gandalfi* sp. nov. (Amoebozoa: Arcellinida) from Brazilian continental waters *Acta Protozool.* **55(4):** 221–229

Fernández L. D., Lara E. & Mitchell E. A. D. 2015. Checklist, diversity and distribution of testate amoebae in Chile. *Eur. J. Protistol.* **51:** 409–424

Fernández L. D., Fournier B., Rivera R., Lara E., Mitchell E. A. D. & Hernández C. E. 2016. Water–energy balance, past ecological perturbations and evolutionary constraints shape the latitudinal diversity gradient of soil testate amoebae in south-western South America. *Global Ecol. Biogeogr.* **25:** 1216–1227

Heger T. J., Lara E. & Mitchell E. A. D. 2011a. Arcellinida testate amoebae (Amoebozoa: Arcellinida): Model of organisms for assessing microbial biogeography. *In:* Biogeography of Microscopic Organisms is Everything Small Everywhere? (Ed., Cambridge University Press. 111–129

Heger T. J., Lara E. & Mitchell E. A. D. 2011b. Arcellinida testate amoebae (Arcellinida: Amoebozoa): model of organisms for assessing microbial biogeography. *In:* The importance of being small: does size matter in biogeography? (Ed. Fontaneto D.), Cambridge University Press. 111–129

Heinis F. 1914. Die Moosfauna Columbiens: in: Voyage d'exploration scientifique en Colombie. *Mem. Soc. Neuch. Sci. Nat.* 5: 675–730

Ilyashuk B. P., Andreev A. A., Bobrov A. A., Tumskoy V. E. & Ilyashuk E. A. 2006. Interglacial history of a palaeo-lake and regional environment: A multi-proxy study of a permafrost deposit from Bol'shoy Lyakhovsky Island, Arctic Siberia. *J. Paleolimn.* **35:** 855–872

Kosakyan A., Heger T. J., Leander B. S., Todorov M., Mitchell E. A. D. & Lara E. 2012. COI Barcoding of Nebelid Testate Amoebae (Amoebozoa: Arcellinida): Extensive Cryptic Diversity and Redefinition of the Hyalospheniidae Schultze. **163:** 415–434

Lahr D. J. G., Bosak T., Lara E. & Mitchell E. A. D. 2015. The Phanerozoic diversification of silica-cycling testate amoebae and its possible links to changes in terrestrial ecosystems. 3: 10.7717/peerj.1234

Laminger H. 1973. Die Testaceen (Protozoa, Rhizopoda) einiger Hochgebirgsgewässer von Mexiko, Costa Rica und Guatemala. **58:** 273–305

McKeown M. M., Mitchell E. A. D., Amesbury M. J., Blandenier Q., Charman D., Duckert C., Roland T. P., Swindles G. T., Wood J. R. & Wilmshurst J. M. 2021. The testate amoebae of New Zealand: a checklist, identification key and assessment of biogeographic patterns. *Eur. J. Protistol.*: 125789

Meisterfeld R. 2002a. Order Arcellinida Kent, 1880. *In:* The illustrated guide to the protozoa (Ed. Lee J. J., Leedale G. F. &Bradbury P.), Society of protozoologists, Lawrence, Kansas, USA. **2:** 827–860

Meisterfeld R. 2002b. Testate amoebae with filopodia. *In:* The illustrated guide to the protozoa (Ed. Lee J. J., Leedale G. F. &Bradbury P.), Society of protozoologists, Lawrence, Kansas, USA. **2:** 1054–1084

Mulot M., Marcisz K., Grandgirard L., Lara E., Kosakyan A., Robroek B. J. M., Lamentowicz M., Payne R. J. & Mitchell E. A. D. 2017. Genetic Determinism vs. Phenotypic Plasticity in Protist Morphology. **64:** 729–739

Patterson R. T., Roe H. M. & Swindles G. T. 2012. Development of an Arcellacea (testate lobose amoebae) based transfer function for sedimentary Phosphorus in lakes. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **348:** 32–44

Penard E. 1911. Part VI – Sarcodina – Rhizopodes d'eau douce. *In:* British Antarctic Expedition 1907-09 Under the Command of Sir. E. H. Shackleton, C.V.O.. Reports of the Scientific Investigations. Vol. I – Biology (Ed. Murray J.), William Heinemann, London. 203–263

Pérez-Juárez H., Serrano-Vázquez A., Kosakyan A., Mitchell E. A. D., Rivera Aguilar V. M., Lahr D. J. G., Hernández Moreno M. M., Cuellar H. M., Eguiarte L. E. & Lara E. 2017. *Quadrulella texcalense* sp. nov. from a Mexican desert: An unexpected new environment for hyalospheniid testate amoebae. *Eur. J. Protistol.* **61:** 253–264

Qin Y., Xie S., Smith H. G., Swindles G. T. & Gu Y. 2011. Diversity, distribution and biogeography of testate amoebae in China: Implications for ecological studies in Asia. *EUr. J. Protistol.* **47:** 1–9

Reczuga M. K., Swindles G. T., Grewling L. & Lamentowicz M. 2015. *Arcella peruviana* sp. nov. (Amoebozoa: Arcellinida, Arcellidae), a new species from a tropical peatland in Amazonia. *Eur. J. Protistol.* **51:** 437–449

Smith H. G., Bobrov A. & Lara E. 2008. Diversity and biogeography of testate amoebae. *Biodiv. Conserv.* **17:** 329–343

Swindles G. T., Morris P. J., Mullan D. J., Payne R. J., Roland T. P., Amesbury M. J., Lamentowicz M., Turner T. E., Gallego-Sala A., Sim T., Barr I. D., Blaauw M., Blundell A., Chambers F. M., Charman D. J., Feurdean A., Galloway J. M., Gałka M., Green S. M., Kajukało K., Karofeld E., Korhola A., Lamentowicz Ł., Langdon P., Marcisz K., Mauquoy D., Mazei Y. A., McKeown M. M., Mitchell E. A. D., Novenko E., Plunkett G., Roe H. M., Schoning K., Sillasoo Ü., Tsyganov A. N., van der Linden M., Väliranta M. & Warner B. 2019. Widespread drying of European peatlands in recent centuries. *Nat. Geosci.* **12:** 922–928

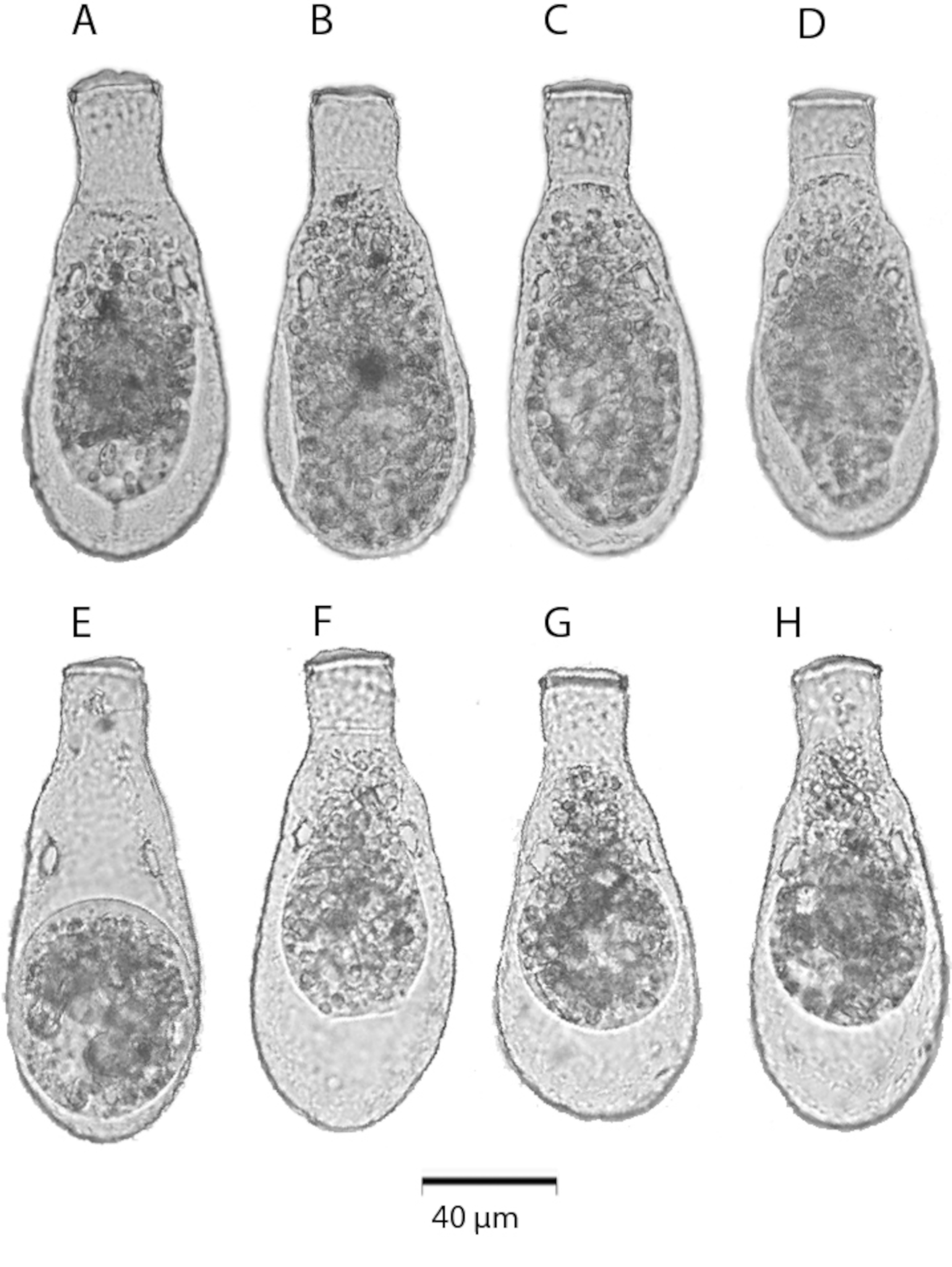
Todorov M. 2002. Morphology, biometry and ecology of *Nebela bigibbosa* Penard, 1890 (Protozoa: Rhizopoda). *Acta Protozool.* **41:** 239–244

Tsyganov A. N., Babeshko K. V. & Mazei Y. A. 2016. A Guide to Testate Amoebae with the Keys to Genera – Monograph, Publishing house of Penza State University, Penza, Russia

Vucetich M. C. 1973. Una nueva especie del genero *Nebela* Leidy. **59:** 79–81

Wailes G. H. 1913. Freshwater Rhizopoda from North and South America. **32:** 201–218

Received 9th April, 2021; revised on 20th August, 2021; accepted on 14th September, 2021



Supplementary Figure 1. *Certesella larai* n.sp. light microscopy images from the Dominican Republic (A–D) and Chile (EH). Scale bar 40 µm. Specimen B is the paratype (deposited in the Laboratory of Soil Bioindication, Department of Soil Geography, Faculty of Soil Science, Lomonosov Moscow State University, slide No. 4–2020).

Table 1. Description of the sampling locations.

Supplementary Table 1. Biometric measurements on two populations of *Porosia elongata* n. sp. from the Dominican Republic and Chile. The morphological character number (see fig. 1)*

Origin and sample codes	1	2	3	4	5	6	7	8	9	10
Chile (EM-1453) – AB_01	149.1	62.5	2.39	29.8	85.2	116.4	32.7	18.5	12.8	4.3
Chile (EM-1453) – AB_02	153.4	63.9	2.40	29.8	85.2	115	32.7	28.4	9.9	4.3
Chile (EM-1453) – AB_03	149.1	63.9	2.33	25.6	85.2	117.9	34.1	28.4	12.8	5.7
Chile (EM-1453) – AB_04	149.1	63.9	2.33	28.4	88	116.4	34.1	24.1	11.4	4.3
Chile (EM-1453) – AB_05	146.3	61.1	2.39	27	85.2	116.4	34.1	18.5	9.9	4.3
Chile (EM-1453) – AB_06	146.3	62.5	2.34	28.4	85.2	117.9	32.7	18.5	9.9	4.3
Chile (EM-1453) – AB_07	139.2	63.9	2.18	28.4	88	110.8	31.2	25.6	11.4	5.7
Chile (EM-1453) – AB_08	139.2	56.8	2.45	28.4	82.4	109.3	31.2	25.6	8.5	4.3
Chile (EM-1453) – AB_09	151.9	63.9	2.38	28.4	89.5	120.7	31.2	28.3	8.5	4.3
Chile (EM-1453) – AB_10	144.8	59.5	2.43	28.4	83.8	116.4	32.7	25.6	8.5	4.3
Chile (EM-1453) – AB_11	146.3	61.1	2.39	28.4	85.2	115	32.7	24.4	11.4	5.7
Chile (EM-1453) – AB_12	146.3	62.5	2.34	28.4	85.2	116.4	32.7	24.4	8.5	4.3
Chile (EM-1453) – AB_13	149.1	62.5	2.39	28.4	92.3	125	32.7	24.4	9.9	4.3
Chile (EM-1453) – AB_14	149.1	63.9	2.33	28.4	79.5	113.6	32.7	25.6	8.5	4.3
Chile (EM-1453) – AB_15	139.2	59.5	2.34	27	79.5	110.8	31.2	25.6	8.5	4.3
Chile (EM-1453) – AB_16	138.2	61.1	2.26	28.4	85.2	109.3	31.2	28.3	11.4	5.7
Chile (EM-1453) – CD_01	144.8	62.8	2.31	28.8	82	119.1	29.7	26.7	10	5.7
Chile (EM-1453) – CD_02	135.8			27.6	74	106.2	28.2	23.8	8.8	4.4
Chile (EM-1453) – CD_03	136.3	59.8	2.28	24.5	73	104.6	25.7	26.1	8.2	4.9
Chile (EM-1453) – CD_04	146	62	2.35	27.4	79.9	112.8	27.2	28.5	11.1	5
Chile (EM-1453) – CD_05	144.9	62.6	2.31	25.1	77.1	110.8	24.5	27.9	5.9	3.5
Chile (EM-1453) – CD_06	145.6	60.4	2.41	25.8	81.4	117.4	25.7	26.1	7.7	5
Chile (EM-1453) – CD_07	149.4	60.7	2.46	27.8	81.6	121.2	32	26.3	8.4	4
Chile (EM-1453) – CD_08	137.4	62	2.22	27.3	75.9	108.7	26.4	26	7.4	4
Chile (EM-1453) – CD_09	137	63.1	2.17	26.2	76.3	113	27.1	24.2	8.2	3.9
Chile (EM-1453) – CD_10	137	62.7	2.19	25.9	76.3	112.6	26.4	24.4	7.6	3.8
Chile (EM-1453) – CD_11	144.56	60.2	2.40	27.2	80.3	113.8	26.3	25.4	8	4.6
Chile (EM-1453) – CD_12	152.6	63	2.42	28.5	82.6	123.3	24.8	24.8	9.8	6.4
Chile (EM-1453) – CD_13	145.1	61.9	2.34	27.8	79.2	118.7	28.5	27.7	7.7	4.4
Chile (EM-1453) – CD_14	141.5	62.6	2.26	28.2	75.2	110.2	28.3	28	7.9	4.6
Chile (EM-1453) – CD_15	151.2	61.5	2.46	26.6	83.3	124.6	30.1	27.7	8.9	4.2
Chile (EM-1453) – CD_16	147.3	62.8	2.35	27.5	81.5	120.3	28.2	26.8	8.9	4.6
Chile (EM-1453) – CD_17	143.3	63.8	2.25	25.2	78.4	115.2	26.9	21.8	6.6	3.8
Chile (EM-1453) – CD_18	144.4	59.7	2.42	27.5	78.4	112.5	22.8	24.4		
Chile (EM-1453) – CD_19	136.1	64	2.13	27	74.1	113.6	26.2	25.6	7.7	3.9
Chile (EM-1453) – CD_20	143	59.9	2.39	28	75	112.3	29.7	27.7	5.1	3.7
Chile (EM-1453) – CD_21	142.7	62.5	2.28	28.6	81.5	113.3	23.8	29.5	9.4	6.2
Chile (EM-1453) – CD_22	140.4	62.9	2.23	27	78.3	112.9	26.5	25.4	8.7	4.6
Chile (EM-1453) – CD_23	138.2	68.2	2.03	25.5	77.7	108	26.5	25.8	7.8	3.3
Chile (EM-1453) – CD_24	144.8	65	2.23	26.5	82.3	117.9	31.4	27.1	8	3
Chile (EM-1453) – CD_25	149.2	64.4	2.32	25.8	78.2	113.7	25.2	26.7	7.2	4.6

Dominican Republic (EM-1384) AB_01	151.9	59.5	2.55	28.4	92.3	123.5	31.2	28.4	9.9	4.3
Dominican Republic (EM-1384) AB_02	151.9	63.9	2.38	28.4	85.2	113.6	32.7	27	8.5	4.3
Dominican Republic (EM-1384) AB_03	147.7	59.5	2.48	28.4	85.2	119.3	32.7	25.6	8.5	4.3
Dominican Republic (EM-1384) AB_04	147.7	63.9	2.31	25.6	85.2	116.4	31.2	24	8.5	4.3
Dominican Republic (EM-1384) AB_05	142	59.6	2.38	24	85.2	117.9	32.7	25.6	8.5	4.3
Dominican Republic (EM-1384) AB_06	146.3	56.8	2.58	28.4	85.2	119.3	32.7	24	8.5	4.3
Dominican Republic (EM-1384) AB_07	144.8	63.9	2.27	25.6	85.2	120.7	31.2	25.6	11.4	4.3
Dominican Republic (EM-1384) AB_08	147.7	59.5	2.48	28.4	85.2	120.7	32.7	24	8.5	4.3
Dominican Republic (EM-1384) AB_09	139.2	56.8	2.45	28.4	85.2	116.4	32.7	25.6	8.5	4.3
Dominican Republic (EM-1384) AB_10	142	59.5	2.39	28.4	85.2	113.6	32.7	25.6	8.5	4.3

*1: Shell length, 2: Shell breadth, 3: Shell length / breadth ratio, 4: Aperture (long axis), 5: Distance from fundus to the pores, 6: Distance from fundus to base of neck, 7: Distance between the pores, 8: Width of the neck at narrowest point, 9: Pore length, 10: Pore width. See also Figure 1.