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ORIGINAL ARTICLE

Nebela jiuhuensis nov. sp. (Amoebozoa; Arcellinida; Hyalospheniidae): A New Member of the *Nebela saccifera - equicalceus - ansata* Group Described from *Sphagnum* Peatlands in South-Central China

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ABSTRACT

Hyalospheniids are among the most common and conspicuous testate amoebae in high-latitude peatlands and forest humus. These testate amoebae were widely studied as bioindicators and are increasingly used as models in microbial biogeography. However, data on their diversity and ecology are still very unevenly distributed geographically: notably, data are lacking for low-latitude peatlands. We describe here a new species, *Nebela jiuhuensis*, from peatlands near the Middle Yangtze River reach of south-central China with characteristic morphology. The test (shell) has hollow horn-like lateral extensions also found in *N. saccifera, N. equicalceus (=N. hippocrepis),* and *N. ansata*, three large species restricted mostly to *Sphagnum* peatlands of Eastern North America. Mitochondrial cytochrome oxidase (COI) data confirm that *N. jiuhuensis* is closely related to the morphologically very similar North American species *N. saccifera* and more distantly to *N. ansata* within the *N. penardiana* group. These species are all found in wet mosses growing in poor fens. Earlier reports of morphologically similar specimens found in South Korea peatlands suggest that *N. jiuhuensis* may be distributed in comparable peatlands in Eastern Asia (China and Korea). The discovery of such a conspicuous new species in Chinese peatlands suggests that many new testate amoebae species are yet to be discovered, including potential regional endemics. Furthermore, human activities (e.g., drainage, agriculture, and pollution) have reduced the known habitat of *N. jiuhuensis*, which can thus be considered as locally endangered. We, therefore, suggest that this very conspicuous micro-organism with a probably limited geographical distribution and specific habitat requirement should be considered as a flagship species for microbial biogeography as well as local environmental conservation and management.

THE systematic use of high-resolution morphological and molecular methods has increased considerably our estimations on protist diversity, by revealing the existence of numerous new lineages (Epstein and Lopez-Garcia 2008), providing better estimates of the true diversity within known lineages (Pawlowski et al. 2012), and demonstrating the existence of limited geographic distributions (Darling et al. 2007; Heger et al. 2013; Merkado et al. 2013; Watts et al. 2011). Thus, molecular data have brought support to views based on morphological studies that suggested the existence of regional endemism in free-living protists (Foissner 2006; Smith and Wilkinson 2007) and narrow ecological specialization (Foissner et al. 2003) and also raising the question of the possible existence of endangered protist species (Cotterill et al. 2008). However, global biodiversity assessments of soil protists suffer from a strong geographical bias in sampling (Bonnet 1977; Foissner 2006).

Our focus here is on testate amoebae, a diverse group of unicellular protists that build a test (shell), in which a single amoeboid cell is enclosed (Meisterfeld 2002a,b). They occur worldwide in a range of terrestrial, wetland,

freshwater, and marginally marine habitats. They play important roles in microbial food webs and biogeochemical cycles (Puppe et al. 2014; Vohnik et al. 2011; Wilkinson and Mitchell 2010) and especially so in oligotrophic environments such as peatlands (Jassey et al. 2013). They are also commonly used as indicators in biomonitoring and paleoecological studies (Charman 2001; Payne 2013).

The vast majority of testate amoebae were described based on morphological characteristics of the test, while molecular data are only available for a limited number of the ca. 2500 described taxa. Molecular studies typically revealed the existence of an unexpected specific richness within single morphospecies, as shown in the *Nebela tincta–collaris* complex (Kosakyan et al. 2013; Singer et al. 2015). Studies performed over broad biogeographic regions also showed that some of these cryptic taxa had restricted distributions that could partly be explained by environmental characteristics (Heger et al. 2013). Thus, molecular data add to the already documented evidence for more or less restricted geographical distribution of several taxa, especially among arcellinid testate amoebae (Heger et al. 2011b).

So far over 20 species of genus *Nebela* have been reported from China (Qin et al. 2011; Shen 1983), all of which were supposed to have a cosmopolitan distribution. Most *Nebela* species reported from China were found in freshwater lakes and in soils (Qin et al. 2009, 2013a; Shen 1983), while data from peatlands remain very limited (Li et al. 2010; Qin et al. 2012, 2013b; Song et al. 2014). We were, therefore, expecting to find new species in our prospections of Chinese low-latitude peatlands. Indeed, these environments have a restricted distribution in this region, which is located several thousands of kilometers south of the vast expanses of higher latitude peatlands in Asia. Furthermore, as these peatlands are under threat due to increasing direct and indirect human impact (Qin et al. 2013b), it is especially urgent to document this unknown and potentially threatened diversity.

We present here a new species with a characteristic morphology encountered in a *Sphagnum* peatland located in the Shennongjia Mountains near the middle Yangtze River region. We describe it as *Nebela jiuhuensis* spec. nov. (Arcellinida, Hyalospheniidae) based on morphometry and molecular (COI) data. We discuss its phylogenetic position, ecology, geographical distribution, and possible conservation status.

MATERIALS AND METHODS

Sampling, isolation, and morphometry

Dajiuhu peatland (31°24′–31°33′N, 109°56′–110°11′E, Figure S1) is located in the Shennongjia Mountains of Southern Central China, near the middle Yangtze River region. The study site is located at 1700–1800 m a.s.l. Mean annual temperature is 7.4 °C, and estimated annual precipitation is 1528.4 mm (Xie et al. 2013; Zhao et al. 2007).

The depth to the water table was measured in 54 sampling sites at the end of July and early August in 2012, after allowing the water level to stabilize for 24 h. Water pH and conductivity were measured in the field for each site using a Hach pH probe (model no 54650-10). *Sphagnum* samples were kept in plastic bags, brought back to the laboratory, and processed immediately. The mosses were shaken in water and filtered between 20- and 250-μm mesh filters. The obtained size fraction was observed at 400× magnification. *Nebela jiuhuensis* was found only in one of these sites. We returned to the same peatland and collected five more samples at the same site in April 2014 to obtain enough material for detailed morphological and molecular analyses.

Selected specimens were placed on an aluminum-stub and coated with a thin layer of gold for scanning electron microscopy imaging using a Quanta 200 microscope operating at 20 kv. A total number of 31 individuals were found and investigated for morphometry. Six measurements were taken on each test (Fig. 1): (1) length, (2) width, (3) width of the aboral part of the test, (4) aperture width, (5) distance from the aperture to the base of the flat keel (i.e. the extension of the test present between the horn-like extensions and the main part of the test), and (6) length of the flat keel (i.e. distance from the base of the flat extension of the test present between the horn-like extensions and the main part of the test to the place where the test margin becomes hollow again).

Molecular analyses

A total of 18 active *N. jiuhuensis* individuals were isolated for molecular analyses. In each tube, a single cell was isolated and its DNA extracted using a guanidine thiocyanate protocol (Chomczynski and Sacchi 1987) as described in Kosakyan et al. (2013). We applied a semi-nested PCR protocol to amplify a fragment of the mitochondrial cytochrome oxidase gene subunit I (COI). We first amplified a ca. 700 bp fragment with the broad-spectrum COI primers LCO1490 and HCO2198 (Folmer et al. 1994), and this amplicon was used subsequently as a template for a second reaction using the general primer HCO2198 and the hyalospheniid-specific primer Arcelcox1F (CAAAATCA TAAAGATATTGGDAC) described in Kosakyan et al. (2012), the second amplicon had a length of about 620 bp. Reaction mixtures contained DNA, 13 μl Premix taq (EX Taq Version 2.0) (TaKaRa, Dalian, China), and 1 μl of each forward (F) and reverse (R) primer (20 p mol/μl) were complemented by RNA-free water (TaKaRa) in a total volume of 25 μl. The amplification profile was the following: initial denaturing at 95 °C for 3 min followed by 40 cycles (denaturation at 95 °C for 15 s, annealing at 40 °C for 15 s, and extension at 72 °C for 1 min) and a final extension at 72 °C for 8 min. Sequencing was performed on an ABI-3730 DNA analyzer, and the resulting sequences were deposited in GenBank under accession number KT907052.

Phylogenetic analysis

The obtained sequence was aligned manually using BioEdit software (Hall 1999). The alignment is available

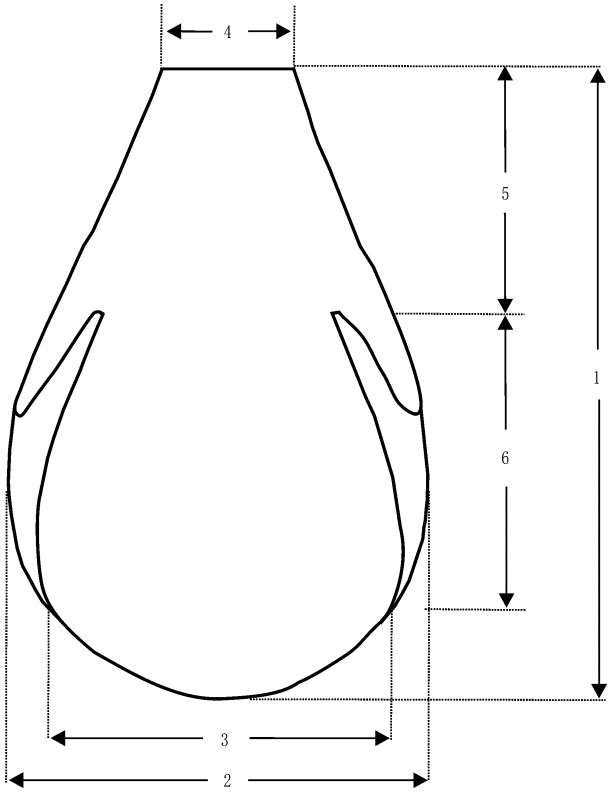

Figure 1 Test outline showing the main characters of *Nebela jiuhuensis* n. sp. 1-test length, 2-test width, 3-width of the aboral part of the test, 4-aperture width, 5-distance from aperture to the base of the keel, and 6-length of the keel.

from the authors upon request. Maximum likelihood trees were reconstructed using the RAxML v 7.2.8 algorithm (Stamatakis et al. 2008) as proposed on the Black Box portal (http://phylobench.vital-it.ch/raxml-bb/). The GTR+Γ+I model was considered during the analysis. Model parameters were estimated in RAxML over the duration of the tree search. The tree was rooted with *Padaungiella* (*P. lageniformis*-JN849065, *P. wailesi*-JN849066, *P. nebeloides*-JN849063) and *Alocodera* (*A. cockayni* JN849043, JN849069, JN849068) species (Kosakyan et al. 2012). The tree was viewed using Fig Tree (program distributed as part of the BEAST package http://tree.bio.ed.ac.uk/software/figtree/). Divergences between sequences were calculated using BioEdit. Missing data were not considered in the calculation.

RESULTS AND DISCUSSION

Taxonomic clarification of the *Nebela equicalceus-hippocrepis-saccifera* group

Before presenting and discussing our results, we need to clarify the somewhat confusing taxonomy of the *Nebela equicalceus-hippocrepis-saccifera* group.

- Leidy described *Nebela equicalceus* in 1874 (Leidy 1874) and mentioned the existence of another form.
- Leidy later (Leidy 1879) re-named *Nebela equicalceus* as *N. hippocrepis*. In fact in his 1879 monograph, he used the name *N. hippocrepis* and mentioned the two synonyms (*Difflugia* (*Nebela*) *equicalceus*. Leidy: Proc. Ac. Nat. Sc. 1874, 156 and *Nebela equicalceus*. Leidy: Proc. Ac. Nat. Sc. 1876, 118, Fig. 12), but he did not specifically justify the change of name. Regardless, this action is nonvalid ("unjustified emendation" ICZNarticle § 33.2.3). The correct name is, therefore, *N. equicalceus* (Leidy 1874).
- In his 1879 monograph, Leidy (Leidy 1879) included illustrations of the two species as *N. hippocrepis*. The illustration shown on plate XXIV (Fig. 13) corresponds to *N. saccifera*.
- In 1913, Wailes described this second form of *N. equicalceus/hippocrepis* as *Nebela saccifera* (Wailes 1913).
- The study by Kosakyan et al. (2012) included *N. hippocrepis* (which should have been referred to as *N. equicalceus*). The identification of this species was matter of internal debate among authors, but we now consider that the illustration (Fig. 3 C & D in Kosakyan et al. 2012) clearly shows that the test has a hollow keel and not a flat keel. The light and scanning electron images show that the fundus of the test is compressed in side view, but it is not a flat keel as observed in *N. carinata* or *N. equicalceus*. We have, therefore, corrected the name of this species in GenBank and hereafter refer to the two corresponding sequences (JN849056 and JN849057) as *N. saccifera*.

Morphology and morphometry

In the *Sphagnum* samples from Dajiuhu peatland, we observed a large *Nebela* species with distinctive morphology (Fig. 1, 2). The test is laterally compressed, pyriform in broad lateral view and tapering from the middle of the test to the aperture. The aperture is oval in front view with an organic lip. In broad lateral view, two short hollow horn-like extensions are visible, beginning from the middle and pointing towards the bottom of the test (Fig. 2, 3). A flat extension of the test (i.e. "keel") is present between the horn-like extensions and the main part of the test giving the test a smooth general outline in broad lateral view. In narrow lateral view, the test is narrowly pyriform with the aboral end somewhat pointed. The test is colorless, covered by more or less oval transparent plates, interpreted as recycled siliceous scales taken from euglyphid prey. The test is relative large, with mean length of 199 μm ± 16.3 μm (± SD, $n = 31$) and mean width of 118 ± 7.9 μm (± SD, $n = 31$). The aperture is 37 ± 3.8 μm wide. Coefficients of variation of most measured characters are around or < 10% (Table 1).

Phylogenetic analysis and comparison with similar species

The relative large size and its hollow horn-like lateral extensions suggest that *Nebela jiuhuensis* is related to

N. saccifera Wailes (1913), *N. equicalceus* Leidy (1876) (syn.: *N. hippocrepis*), and *N. ansata* Leidy (hereafter referred to as the *Nebela saccifera* group).

COI sequencing and subsequent phylogenetic analyses confirmed this relationship, placing *N. jiuhuensis* in a basal position with respect to *N. ansata* and *N. saccifera* with a strong support. *N. jiuhuensis* can be readily distinguished from *N. ansata* based on morphology. *Nebela ansata* also possesses two horn-like projections but lacks the flat extension of the test between these projections. Morphologically, the species most similar to *N. jiuhuensis* is *N. saccifera*. It is on average slightly larger, but the two overlap (*N. saccifera*: 203–240 μm; *N. jiuhuensis*: 176–226 μm, average = 199 μm) has a similar keel, but the lateral extensions are shorter and more slender (Fig. 2–5). However, the two species only have 84% similarity in their COI sequence.

Other species of *Nebela* such as *N. carinata* Leidy (1879) and *N. marginata* Penard (1902) possess a more or less developed keel. However, unlike in the *N. saccifera* group, their keel is flat (rather than hollow) and they lack the horn-like lateral extensions (Table 2). These species branch together elsewhere in the phylogenetic tree (Kosakyan et al. 2012; Fig. 6). *Nebela galeata* Penard and *N. penardiana* Deflandre possess a hollow keel (barely visible in *N. penardiana*) but lack the horn-like lateral extensions and the flat extension of the test. These species are closely related to the *N. saccifera* group (Kosakyan et al. 2012; Fig. 6).

Biogeographical considerations

Microorganisms with an unmistakable morphology have been suggested as good models for testing biogeographic hypotheses even before the use of molecular methods (Foissner 2006; Heger et al. 2011a; Smith and Wilkinson 2007; Smith et al. 2008). The whole *N. saccifera* group with their large size and characteristic morphology clearly belongs to this category. It is indeed unlikely for these taxa to have been missed by researchers. Species from this group have limited distributions: *N. saccifera*, *N. equicalceus,* and *N. ansata* are only found in peatlands in Eastern North America (Table 2, Fig. 7; Heger et al. 2011b; Leidy 1879).

One possible exception is a record of *N. equicalceus* from Nepal (Bonnet 1977). Bonnet surprisingly did not discuss this new record despite his strong interest for biogeography. However, unfortunately, no illustration was given in the original publication, and we did not find this species in the permanent slide collection deposited by Louis Bonnet at the Natural History Museum of Geneva. We find this record unusual for two reasons: (1) the study

Figure 2 Light micrograph of *Nebela jiuhuensis* n. sp. from the Dajiuhu peatland of Shennongjia Mountains, central China. Scale bar = 40 μm.

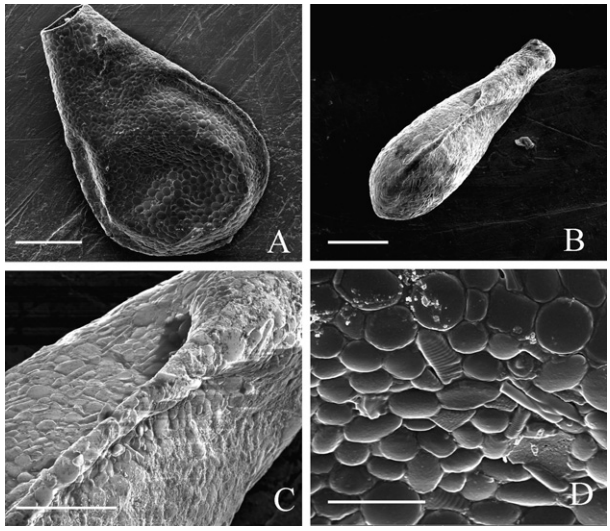

Figure 3 Scanning electron microscopy photographs of *Nebela jiuhuensis* n. sp. (**A**) Front view, (**B**) lateral view, (**C**) a little "horn" at the beginning of the keel, (**D**) detail showing the test including recycled silica scales possibly from *Trinema* with some diatom frustules. Scale bars: A and B = 50 μm, C = 20 μm, D = 10 μm.

Table 1. Morphometric characteristics of *Nebela jiuhuensis* nov. spec

Character	Min	Max	Mean	SD	SE	CV (%)
1-Test length	176	226	199	16.3	2.9	8.2
2-Test width	101	130	118	7.9	1.4	6.7
3-Width of the aboral part of the test	90	119	105	6.7	1.2	6.4
4-Aperture width	30	46	37	3.8	0.7	10.4
5-Distance from aperture to the base of the flat keel	76	106	91	7.5	1.4	8.3
6-Length of the flat keel	45	96	74	11.7	2.1	15.9

Measurements in μm, $n = 31$ individuals. SE, standard error of the mean; SD, standard deviation; CV, coefficient of variation in %; Min, minimum; Max, maximum; *n*, number of individuals investigated. See Fig. 2.

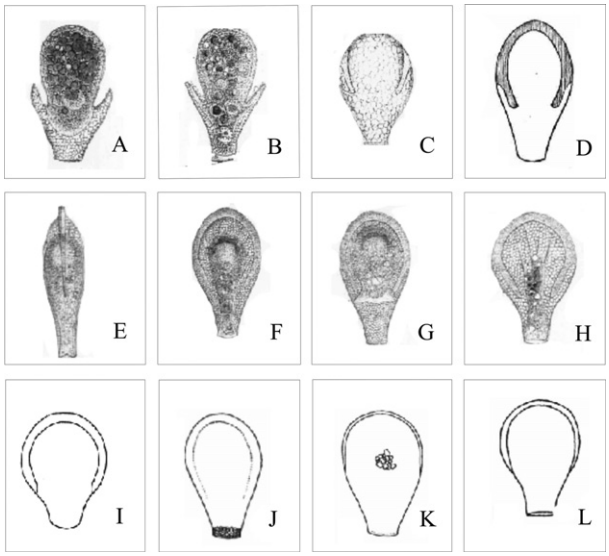

Figure 4 Drawings from the original descriptions of some species of testate amoebae from genus *Nebela* possessing a keel. (**A, B**) *N. ansata* (from Leidy 1879); (**C**) *N. saccifera* Wailes 1913; (**D**) *N. equicalceus* (syn.: *N. hippocrepis*) Leidy 1876; (**E–H**) *N. hippocrepis* Leidy 1879; (**I**) *N. carinata* Leidy 1879; (**J**) *N. galeata* Penard 1902; (**K**) *N. marginata* Penard 1902; (**L**) *N. spumosa* Awerintzew 1906.

site (in central Nepal) is very distant from the East Coast of North America and, at that time, constituted the first finding for this group in Eurasia, and (2) the habitat where it was found was markedly different from the places where members of this group had been found until and since then. Indeed, Bonnet found this species in "xerophilic mosses growing on shallow soil and rocks at high elevation on a ridge above the treeline at 3900 m." For these reasons, we consider highly unlikely that the observed organism belonged to *N. equicalceus*. However, the observed cells had "short horns" and were found at the top of a mountain pass (in a place called Taunja) and thus very exposed to distant eolian contamination (Bonnet L., personal communication). Thus, we may indeed hypothesize that (1) this record does not correspond to *N. equicalceus* but to another, closely related species and (2) that the ecological characteristic of the sample may not necessarily reflect the true ecological optimum of this species. Therefore, (1) we still consider *N. equicalceus* as endemic to Eastern North America, and (2) there is most likely another new species to describe in this group from Nepal.

Nebela jiuhuensis has been found exclusively in Eastern Asia. To date, it has been reported only from Dajiuhu peatland and from another peatland in Hunan Province, also near the Middle Yangtze reach. In a survey of testate amoeba diversity in a *Sphagnum* peatland in Korea, Chung et al. (1992) reported a species from the *N. saccifera* group designated as *N. equicalceus*. However, its morphology differs *N. equicalceus* by its smaller size (181–197 μm), which does not fit the range given in the original description but is in agreement with the range observed

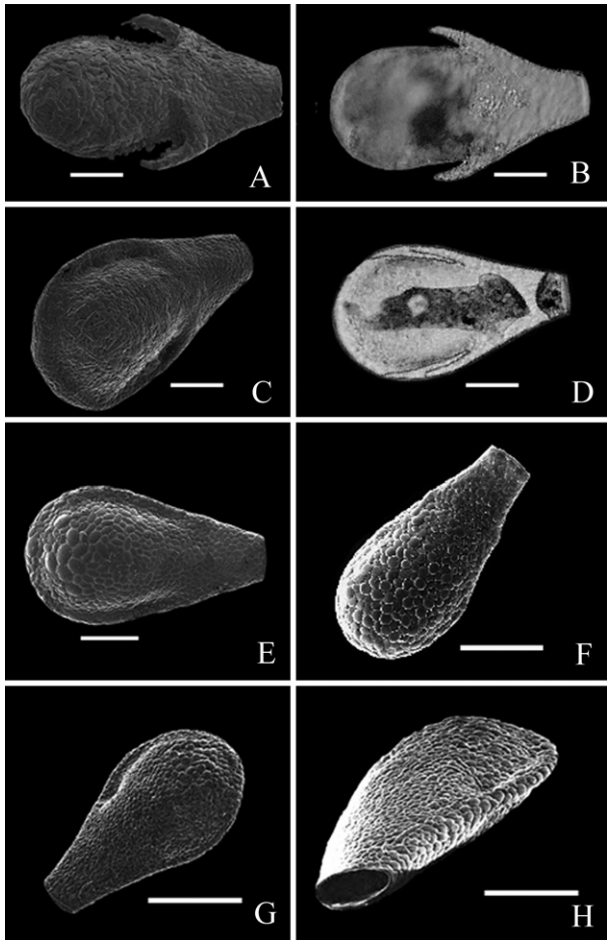

Figure 5 Scanning electron and light micrographs of taxa in *Nebela saccifera* group: (**A, B**) *N. ansata*; (**C, D**) *N. equicalceus*; (**E**) *N. galeata*; (**F**) *N. penardiana* (A–F) from Heger et al. 2011a; Kosakyan et al. 2012; (**G, H**) *N. jiuhuensis* (from Chung et al. 1992, assigned to *N. equicalceus*). Scale bars: A–F = 50 μm, G = 75 μm, H = 43 μm.

for *N. jiuhuensis*. Based on this and the geographical distribution of species in this group, we interpret this taxon as belonging to *N. jiuhuensis* (Table 2, Fig. 5G). In addition, the habitat where it has been found matches well the *terra typica* of *N. jiuhuensis*. The distribution of *N. jiuhuensis* thus encompasses Eastern Asia including Korea and the subtropical part of China.

Ecology and conservation

Members of the *N. saccifera* clade are found in wet habitats, such as *Sphagnum*-dominated peatlands, like other large hyalospheniids possessing a keel such as *N. carinata, N. galeata, N. marginata*, and *N. spumosa* (Heger et al. 2011b; Hoogenraad and de Groot 1952; Kosakyan et al. 2012; Leidy 1874, 1876, 1879; Wailes 1913). *Nebela jiuhuensis* has been found in a *Sphagnum* moss carpet floating over a water surface in association with the arcellinid testate amoeba species *Difflugia oblonga* and

Table 2. Morphological characteristics of *Nebela jiuhuensis* and other species of the *Nebela saccifera* group. Data from original descriptions. Measurements in μm

Taxon	Length	Width	Aperture width	Keel	Habitat	Location	Citation
N. jiuhuensis	176–226	101–130	30–46	Only between the lateral arms (horns)	*Sphagnum* peatland	Shennongjia, South-central China	Present work
*N. jiuhuensis**	181–197	103–133	29–35	Only between the lateral arms (horns)	*Sphagnum* swamp	Taekwanryŏng, South Korea	Chung et al. 1992
N. saccifera	203–240	126–145	38–45	Only between the lateral arms (horns)	*Sphagnum* peatland	Lakehurst, Absecom, Good Ground, Long Island, U.S.	Leidy 1879; Wailes 1913
N. equicalceus	252–260	140–160	28–40	Around the posterior end of the test	Wet *Sphagnum* or other mosses peatland	New Jersey, U.S.; Nepal; Nova Scotia, Canada	Leidy 1879; Bonnet 1977; Kosakyan et al. 2012*
N. ansata	216–260	132–164	28–52	A thin keel around the test	*Sphagnum* peatland	Absecon, New Jersey, U.S.; Nova Scotia, Canada	Leidy 1879; Heger et al. 2011a,b; Kosakyan et al. 2012

*Listed as *N. hippocrepis*.

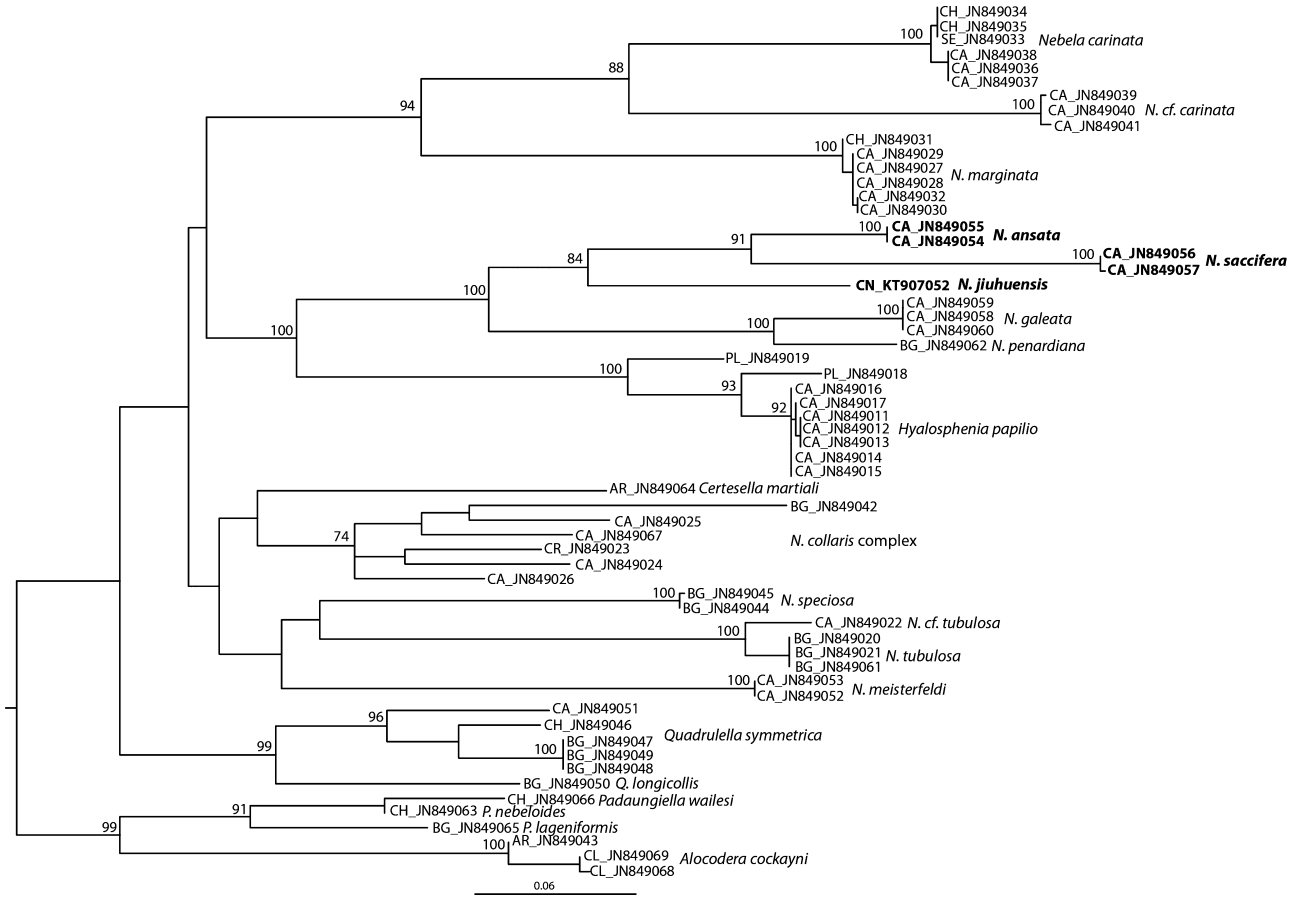

Figure 6 Maximum likelihood bootstrap consensus tree of species in the *Nebela saccifera* group based on COI data.

D. bacillifera. Under these conditions, the surface is under the influence of both groundwater and rainwater. The site is, therefore, minerotrophic. The average water table depth was 1.5 cm, pH 4.9, and water conductivity 13.41 μS/cm^2. These ecological conditions correspond to the optima of other members of the *N. saccifera* clade

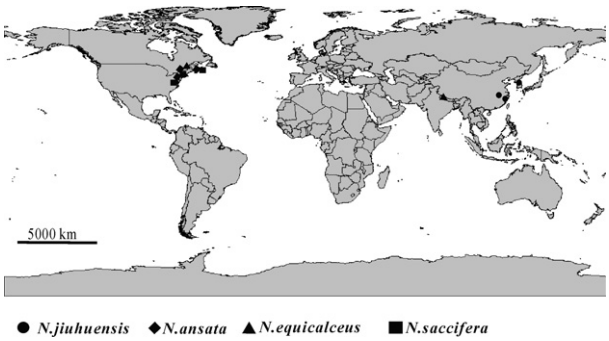

Figure 7 World map showing the collection sites for all known species of the *Nebela saccifera* group. The problematic record of *N. equicalceus* from Nepal most likely represents another new species of the group (see text for further discussion).

(Chung et al. 1992; Heger et al. 2011b). For example, *N. ansata* was found in a site with a water table depth of 3 cm and pH of 4.14 in eastern North America (Heger et al. 2011b). These environmental conditions can be encountered only rarely in some peatlands or portions thereof, where human impact is minimal. In Dajiuhu peatland, this represents only ca. 2% of the total surface, corresponding to the area untouched by human activities. The fact that *N. jiuhuensis* was only found in this habitat suggests that it has a narrow tolerance and can be found only in this restricted area. It is indeed unlikely for such a large and conspicuous species to be abundant and widespread in this site as we did not find it in the analysis of tens of samples from different microhabitats in previous studies (Qin et al. 2012, 2013b; Qin Y., Gu Y., Lamentowicz M., Wang H., Zhang Q., Zhang G., Mitchell E. A. D., unpubl. data).

Dajiuhu peatland is currently under threat from direct human impact, such as drainage for agriculture, pollution, *Sphagnum* harvesting, and peat extraction (Qin et al. 2012). As a consequence, the potential habitat of *N. jiuhuensis* is likely to decline, and this species can thus currently be considered as locally threatened. This also holds true for several other equally rare testate amoeba species occurring at the site: *Argynnia caudata*, *Nebela barbata*, and c.f. *Hoogenraadia humicola*. This clear threat for local (if they occur elsewhere) or possibly global (if they only occur at this site) extinction should be interpreted as a warning sign of ecosystem deterioration, and should lead to concrete action for its preservation. Dajiuhu peatland belongs to the middle Yangtze reach region, which is one of the few (17) regions recognized both as a Global Biodiversity Hotspot and a Global 2000 Priority Ecoregion in China (Mittermeier and Mittermeier 1997; Olson and Dinerstein 1998). In addition, the Shennongjia Mountains are considered to be one of the most important hotspots for biodiversity in China.

The narrow tolerance of *N. jiuhuensis*, combined to its large size and unmistakable morphology, allows an easy visual detection even at low magnification (e.g. 100×). If, as we believe, its presence testifies for good local environmental conditions, it could be used as a "biomonitoring flagship species" to assess environmental degradation in Dajiuhu peatland or in other similar environments in Eastern Asia. Using protists as biomonitoring flagship species for the conservation of a particular ecosystem may seem a provocative idea, as the very existence of these organisms is unknown to the general public and to policy makers, as has been pointed out for nonvertebrate animals (Cardoso et al. 2011). It is nevertheless clearly time to bring protists to the front of the (biology) scene, as the scientific community is becoming increasingly aware of their global importance for ecosystem function. Similar steps have already been taken elsewhere: a study revealed an immense ciliate diversity in a particular kind of ephemeral water bodies near Salzburg (Austria), the Krauthügel ponds, which led to their protection (Cotterill et al. 2013). We defend this viewpoint as protistologists but also as natural history researchers (Cotterill and Foissner 2010). Far from being an unlikely tool for biomonitoring and environmental conservation, protists indeed have all the characteristics to make them essential tools for environmental assessment (Payne 2013), and *N. jiuhuensis* may be a first instance where testate amoebae can be used directly for environmental conservation.

The next step toward this goal will be to document precisely the distribution and ecology of this species both within the sites where it has already been found and once its ecological requirements are better documented to search for it in potential suitable habitats in a broader region. Based on these data, it will then be able to clarify the true conservation status of this species and to what extent it can indeed be used as a biomonitoring flagship species.

CLASSIFICATION SUMMARY

Amoebozoa
Tubulinea
Arcellinida Kent 1880
Hyalospheniidae (Schulze 1877) Kosakyan and Lara 2012
Nebela Leidy 1874
Nebela jiuhuensis Qin, Mitchell & Lara

Type specimen. The holotype specimen for morphological analysis has been placed on a glass slide covered with Canada balsam in the state Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan, China, under catalog number D2010-54.
Type locality. Dajiuhu peatland (Figure S1) in the Shennongjia Mountains, south-central China.
Type ecosystem. Floating *Sphagnum* moss carpetin wet fen; average water table depth was 1.5 cm, pH 4.9, and water conductivity 13.41 μS/cm^2.
Geographical distribution. Known from south-central China (Shennongjia Mountains, 31°24′–31°33′N, 109°56′–110°11′E) and South Korea (indicated as "Taekwanryöng" but without any geographical coordinates or other more

detailed information, probably in the Taebaek Mountains, Gangwon Province, South Korea).

Etymology. The Latin word "jiuhuensis" has been named in reference to the peatland "Dajiuhu" where the species were firstly observed. "Jiuhu" means nine lakes in Chinese.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Landscape of the Dajiuhu peatland in the Shennongjia Mountains of Southern Central China.