

## En garde! Redefinition of *Nebela militaris* (Arcellinida, Hyalospheniidae) and erection of *Alabasta* gen. nov.

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### Abstract

Molecular data have considerably contributed to building the taxonomy of protists. Recently, the systematics of Hyalospheniidae (Amoebozoa; Tubulinea; Arcellinida) has been widely revised, with implications extending to ecological, biogeographical and evolutionary investigations. Certain taxa, however, still have an uncertain phylogenetic position, including the common and conspicuous species *Nebela militaris*. A phylogenetic reconstruction of the Hyalospheniidae using partial sequences of the mitochondrial Cytochrome Oxidase Subunit 1 (COI) gene shows that *N. militaris* does not belong to genus *Nebela*, but should be placed in its own genus. The morphological singularities (strongly curved pseudostome and a marked notch in lateral view) and phylogenetic placement of our isolates motivated the creation of a new genus: *Alabasta* gen. nov. Based on their morphology, we include in this genus *Nebela kivuense* and *Nebela longicollis*. We discuss the position of genus *Alabasta* within Hyalospheniidae, and the species that could integrate this new genus based on their morphological characteristics.

**Keywords:** Amoebozoa; Biometry; DNA barcoding; Molecular phylogeny; Protist; Taxonomic revision

### Introduction

Testate amoebae are ubiquitous microorganisms in many terrestrial and freshwater to brackish aquatic environments (Meisterfeld 2002; Tsyganov et al. 2016). Their ecological sensitivity, key functional role in microbial foodwebs (Jassey

et al. 2012), and the good preservation of their test in sediments and peat designates them as excellent bioindicators for present and past environmental conditions (Amesbury et al. 2016; Mitchell et al. 2008), ecotoxicology (Amacker et al. 2018), and even forensic science (Seppey et al. 2016). Clear taxonomy is a prerequisite for sound ecological work. However, most original descriptions of testate amoeba species were based only on morphological characters (e.g. test composition, test and pseudostome dimensions) and the validity and phylogenetic position of many taxa are currently unclear. On one hand, several studies have revealed cases of cryptic

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or pseudo-cryptic diversity and proved that fine morphological differences of the shell indeed corresponded to distinct species (Dumack et al. 2016; Heger et al. 2011; Kosakyan et al. 2013; Singer et al. 2015). On the other hand, phenotypic plasticity of the test has also been demonstrated experimentally (Mulot et al. 2017; Wanner 1999; Wanner and Meisterfeld 1994).

Hyalospheniidae (Amoebozoa, Arcellinida) are a widespread family of testate amoebae. They build their test from a proteinaceous matrix (*Hyalosphenia*) (Meisterfeld 2002), which can be reinforced by self-secreted siliceous plates (*Quadrullella*) or by small silica elements taken from preys (e.g. *Nebela*, *Padaungiella*, etc.), a process referred to as kleptosquamy (Lahr et al. 2015). They are mostly found in forest litter (Krashevskaya et al. 2018), fens and *Sphagnum* dominated peatlands (Singer et al. 2018), but also in more extreme environments like cryptogamic crusts in arid environments (Pérez-Juárez et al. 2017). They have been a key element in the debate over protist cosmopolitanism, showing several examples of biogeographic “flagship species”, i.e. organisms with a conspicuous morphology only found in a limited part of the world (Heger et al. 2011; Smith et al. 2008; Smith and Wilkinson 2007). Molecular approaches on Hyalospheniidae opened new horizons in microbial biogeography; indeed, cryptic species showed non-overlapping geographic distribution areas (Heger et al. 2013), disproving the cosmopolitan hypothesis, for testate amoebae at least. Cryptic species also showed diverging ecological preferences for different micro-niches in *Sphagnum* peatlands (Singer et al. 2018), thus further demonstrating the need for improved taxonomy. Consequently, there is currently an active effort to do a systematic revision of testate amoeba taxonomy using combined molecular and morphological approaches (Lahr et al. 2017).

The first molecular surveys of arcellinid testate amoebae were focused on the Small Subunit Ribosomal RNA (18S rRNA) gene and permitted to place the Hyalospheniidae among the Arcellinida (Nikolaev et al. 2005). As the 18S rRNA gene is very conservative (Pawlowski et al. 2012), it is only of limited use for species delineation in testate amoebae. Other markers based on mitochondrial genes (Blandenier et al. 2017; Hebert et al. 2003) were developed to overcome this limitation. The mitochondrial Cytochrome Oxidase Subunit 1 (COI) gene was shown to be useful to explore the cryptic diversity of hyalospheniid species and to resolve phylogenetic relationships within the family (Kosakyan et al. 2013). This resulted in splitting genus *Nebela*, the most species-rich genus of the family, into genera *Padaungiella* (Kosakyan et al. 2012), *Gibbocarina*, *Planocarina*, *Cornutheca*, *Longinebela* and *Nebela* (*sensu stricto*) (Kosakyan et al. 2016).

However, the taxonomic validity and phylogenetic position of many hyalospheniids, including several common taxa, remain unclear. Our focus here is on *Nebela militaris*, an emblematic species in peatlands, where it typically occurs in relatively dry microhabitats (*Sphagnum* hummocks) with

generally low pH (Diaconu et al. 2017; Väiliranta et al. 2012). Its characteristic morphology and good preservation in peat designates it as a valuable bioindicator in ecological and palaeoecological studies of peatlands (Amesbury et al. 2016; Mitchell et al. 2008). Despite its unmistakable morphology, several morphologically similar taxa have been described, but as these descriptions are lacunar (e.g. poor biometry or illustrations, lack of differential diagnosis) their validity is unclear. Although *N. militaris* clearly belongs to the Hyalospheniidae, its morphological characters do not allow a clear assignation to one of the genera as defined by Kosakyan et al. (2016). Its phylogenetic position among the Hyalospheniidae thus remains mysterious. Here, we characterized isolates of *Nebela militaris* from a Swiss peat bog based on detailed morphological observation and a single cell barcoding approach and we clarify its phylogenetic position and propose the new genus *Alabasta*.

## Material and Methods

### Single cell isolation

Cells corresponding to the original description of “*Nebela militaris*” (Penard 1890) were isolated from *Sphagnum* mosses in a wooded peatland (*Pinus mugo uncinata*) in the Swiss Jura Mountains (Le Cachot bog, 47° 00' 15.23" N, 6° 39' 52.83" E). Testate amoeba cells were extracted from ca. 30 g of fresh *Sphagnum* after filtration through a 200 µm mesh filter. Cells were isolated individually under an inverted microscope (Olympus IX81) with a narrow pipette. Light microscopy pictures were taken at 600× magnification (Fig. 1) and morphometric characteristics (length, breadth and width of the aperture) were measured on 20 isolated cells (Fig. 2).

### DNA extraction

We used a specific procedure developed to extract DNA from a single cell of testate amoeba adapted after Chomczynski and Sacchi (1987). We prepared a thiocyanate guanidinium (TG) solution with the following protocol: We first dissolved 60 g of TG in 20 ml of EDTA (0.5 M, pH 8) and 20 ml of H<sub>2</sub>O under agitation at 65 °C then when the solution reached room temperature, we added 5 ml of Sarkosyl 10% (Na *N*-lauroylsarcosinate). Finally the solution was topped up to 100 ml with H<sub>2</sub>O and filtered at 0.2 µm before being stored in the dark.

Each single cell was rinsed several times with distilled water to eliminate contaminants and then transferred into individual PCR tubes containing 50 µl of TG solution. The tubes were heated at 65 °C during 30 min, then 50 µl of isopropanol was added and the tubes then left at –20 °C during 12 h. The cleaning step of the DNA consisted of a first centrifugation at 15,000 rpm during 20 min. The supernatant was

removed before two additional washing steps with 180  $\mu$ l of ethanol (70% and 99% respectively, 15,000 rpm during 5 min). The residual ethanol is evaporated during 2 h under a fume hood.

### PCR amplification and DNA sequencing

The PCR were processed by adding the components and reagents directly into the PCR tubes used for the extractions. Partial sequences of the mitochondrial COI gene were obtained using a nested PCR protocol. The first PCR was conducted using the Arcellinida-specific forward primer ArcelCox (CAA AAT CAT AAA GAT ATT GGD AC) (Kosakyan et al. 2012) and the eukaryote-general reverse primer HCO (TAA ACT TCA GGG TGA CCA AAA AAT CA) (Folmer et al. 1994). The PCR conditions were: Denaturation step at 95 °C for 5 min, then 45 cycles with a denaturation step at 95 °C for 15 s, an hybridation step at 43 °C for 15 s, an elongation step at 72 °C for 1 min, and a final elongation at 72 °C for 10 min. The second PCR was done using hyalospheniid-specific primers HPCOIF (GTT ATT GTT ACT GCT CAT GCC) and HPCOIR (ATA CAA AAT AGG ATC ACC TCC ACC) (Gomaa et al. 2014) with the following conditions: Denaturation step at 95 °C for 5 min, then 40 cycles with a denaturation step at 95 °C for 15 s, an annealing step at 55 °C for 15 s, an elongation step at 72 °C for 1 min, and a final elongation at 72 °C for 10 min. PCR products were purified using a Milipore kit and sequenced with an ABI3730XL DNA sequencer (Applied Biosystems) at Macrogen, Amsterdam NL. Sequences were deposited in GenBank with the following accession numbers: MH616621–MH616624. Light microscopy pictures of the DNA barcoded cells are shown in Fig. 1.

### Phylogenetic analysis

The sequences obtained were 482 nucleotides long and were aligned using BioEdit (Hall 1999) with an exhaustive reference database composed of COI sequences belonging to the Hyalospheniidae family in the GenBank database (Heger et al. 2013; Kosakyan et al. 2012, 2013, 2016; Pérez-Juárez et al. 2017; Singer et al. 2015; Qin et al. 2016). Phylogenetic reconstruction was conducted using the CIPRES Portal (Miller et al. 2010). A maximum likelihood phylogenetic tree was built using the RAxML v.8.2.10 algorithm (Stamatakis, 2014) with the GTR + GAMMA + INVARIANT model and 1000 bootstraps. A Bayesian reconstruction was built with MrBayes (Ronquist and Huelsenbeck 2003) using the GTR + GAMMA + INVARIANT model and ran on two independent chains for 200,000 generations sampled every 100 generations resulting in 4,000 trees, of which 25% were discarded as the burn-in. The trees were rooted using genera *Alocodera* and *Padaungiella* as outgroup based on previous Hyalospheniidae phylogenies (Kosakyan et al. 2016).

## Results and Discussion

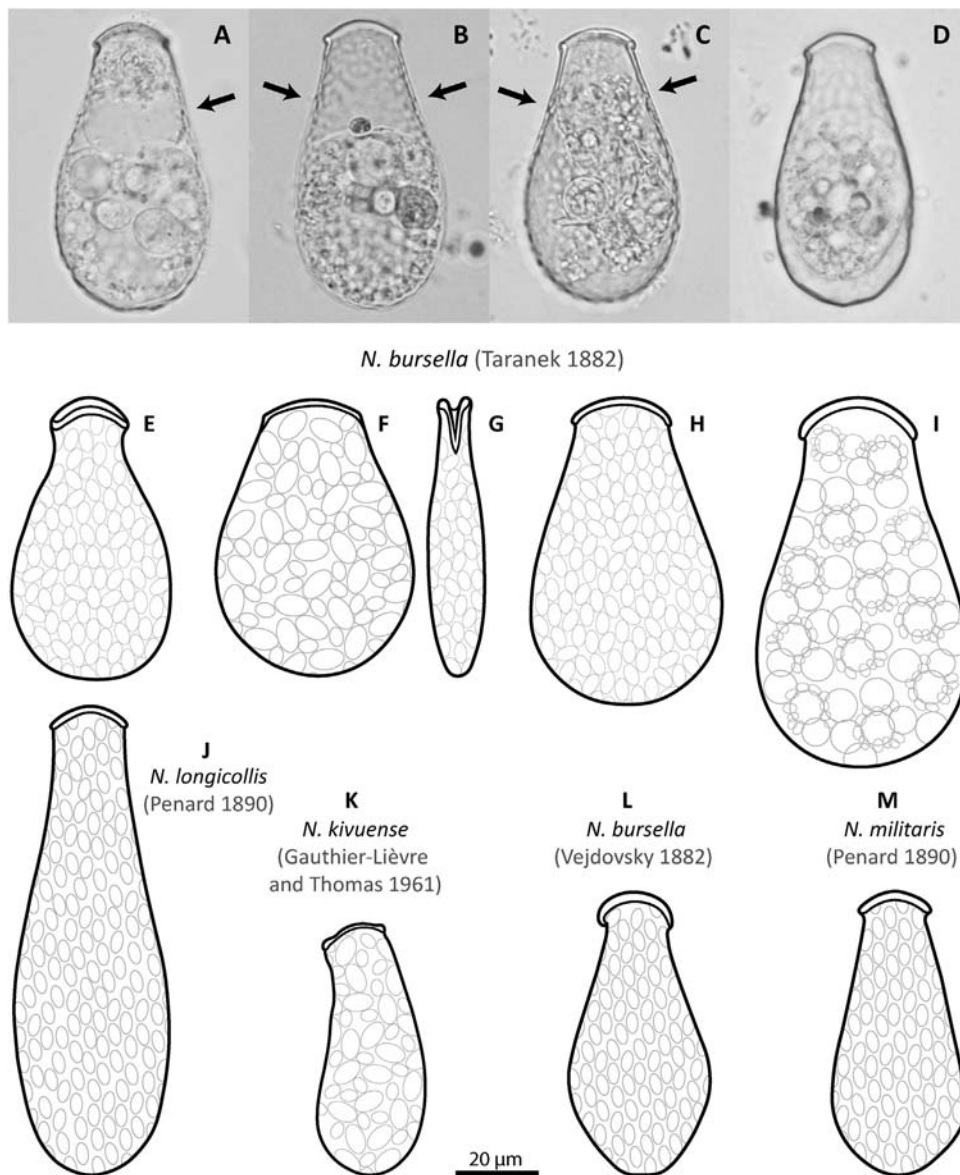
### The morphology of the studied cells is congruent with the original description of “*Nebela militaris*”

The cells described in this study correspond well to the original description of *Nebela militaris* (Penard 1890). They have the same morphology (hyaline, yellowish or colourless and rigid test) and the shape of an ancient cannon (hence the species name given by Penard). The test is proteinaceous and can incorporate silica scales taken from prey. Lateral pores are frequently observed in the first anterior third of the test. The pseudostome is fan-shaped i.e. strongly curved in broad view, with a notch in narrow view, and shows a flare just before the pseudostome (Fig. 1A–D). We recorded the following measurements: Length: 61–77.5  $\mu$ m (mean 67.9  $\mu$ m), width: 31.5–53  $\mu$ m (mean 37.7  $\mu$ m), aperture width (pseudostome long axis): 15.5–20.5  $\mu$ m (mean 18.1  $\mu$ m) (Fig. 2, Table 1). However, our isolates differ slightly from the original description because of their wider test (Fig. 2, Table 1). Also, Penard (1890) originally described the pores on each side of the pseudostome to be infrequent but later stated that they are present in most cases (Penard 1902) which is confirmed by our observations. It is possible that the pores were not visible with the microscope Penard used for the original description as they can be difficult to observe. Nevertheless, a thorough comparison between the original permanent slides made by Penard (pictures available in Wikimedia Commons: [https://commons.wikimedia.org/wiki/Category:Nebela\\_militaris](https://commons.wikimedia.org/wiki/Category:Nebela_militaris)) and the investigated cells confirm that those isolates are the same morphospecies. Moreover, the original types were isolated from the same environment, i.e. *Sphagnum* dominated peatland of the Swiss Jura Mountains (Penard 1890). We thus consider our isolates to belong to the same taxon as “*Nebela militaris*” described by Penard.

### Clarification of the taxonomic status of *Nebela militaris*

The taxonomic history behind *Nebela militaris* is rather complicated as *N. militaris* is morphologically similar to two other taxa: *Nebela bursella* and *N. americana* var. *bryophila*. It is thus unclear whether *N. militaris*, *N. bursella* and *N. americana* var. *bryophila* represent distinct taxa and the confusion encompassing those names comforts the idea that they are merely the same morphospecies.

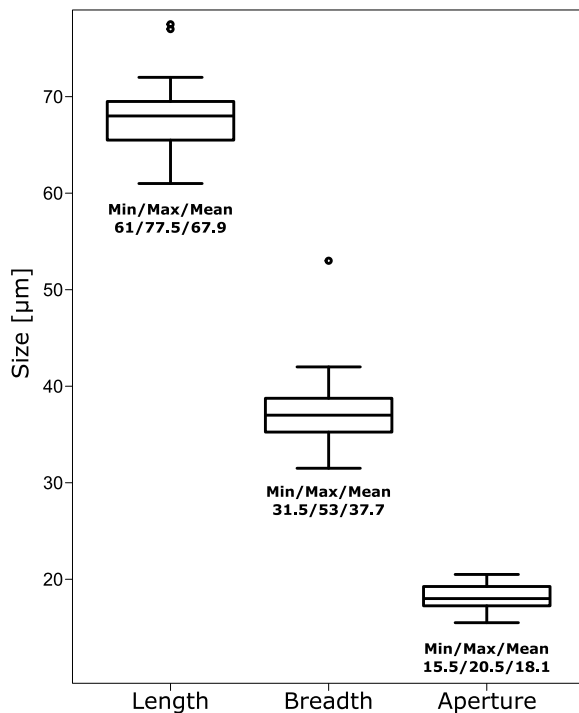
*Nebela bursella* was originally described by Taraneck (1881) as *Nebela bursella* Vejdovský, described again by Vejdovský (1882) and was later reported in Taraneck's monograph (1882). In this monograph, the illustrations that Taraneck includes in the description of *N. bursella* are confusing, since some of them clearly correspond to the two first descriptions of *N. bursella* made by himself and Vejdovský while other depicted specimens are ambigu-



**Fig. 1.** A–D Light microscopy pictures of the barcoded specimens of *Alabasta militaris* (GenBank accession number A: MH616621; B: MH616622; C: MH616623; D: MH616624). The arrows point to the position of the lateral pores. E–I Reproduction of *Nebela bursella* sensu TaraneK as it appears in Plate III Fig. 8, 9, 10, 7 and Plate IV Fig. 16 (Supplementary material), respectively, in TaraneK (1882). J–M Reproduction of *N. longicollis*, *N. kivuense*, *N. bursella*, and *N. militaris*, respectively, as they appear in their original description. The size of E–I and L was approximated as no formal scale was given in the original drawing, but the proportions of E–I were kept. Scale bar = 20 µm.

ous. Indeed, while two illustrations (Plate III Fig. 8 and 12 by TaraneK (1882) (Supplementary material 1), Fig. 8 here reproduced as Fig. 1E) resemble Vejdovský's and TaraneK's original drawings of *N. bursella* with its characteristic curved pseudostome, the other illustrations correspond to specimens that are clearly broader. Based on modern knowledge of Hyalospheniidae systematics, these illustrated specimens should rather be assigned to another species within genus *Nebela* (Plate III Fig. 7 and Plate IV Fig. 16 by TaraneK (1882) (Supplementary material 1), reproduced here as Fig. 1H,I respectively) or, possibly even, given the slit-like aperture, to genus *Heleopera* (Plate III

Fig. 9–11 by TaraneK (1882), reproduced here as Fig. 1F,G), resulting in one description possibly corresponding to three species. This situation led to the misinterpretation that *N. bursella* corresponded to the broader morphotype rather than to the cells illustrated by Vejdovský. This error was not corrected and *N. bursella* was ultimately considered as a synonym to *Nebela tincta* (Awerintzev 1906). In his description of *N. militaris* Penard (1890) states that his new species could be considered by some as mere aberrant specimens of *N. bursella*, thus suggesting that he too was misled. However, unfortunately Penard did not explain what features could be considered as aberrant. He actually based



**Fig. 2.** Length, width and aperture of *Alabasta militaris* (n=20) taken from the same population as the four barcoded specimens (Le Cachot peatland, Swiss Jura Mountains).

his concept of *N. bursella* on Taranek's erroneous lumping and thus confused it with *Nebela tincta* (see Penard 1902). Penard then described *N. militaris*, being certain that it was not only an aberrantly slender form of *N. tincta*, resulting in the redescription of *N. bursella* as *N. militaris*.

*Nebela americana* var. *bryophila* Van Oye (1933) was described as a smaller morphospecies of *Nebela americana* (Taranek 1882). The dimensions and shape of the test are comparable to *N. militaris* but Van Oye (1933) noted the absence of lateral pores on his isolates. As the pores remain difficult to observe, we consider that the original description of *Nebela americana* var. *bryophila* is too lacunar to allow distinguishing it from *N. militaris* and we consider them as synonyms.

For these reasons, we propose to synonymize *N. militaris*, *N. bursella* and *N. americana* var. *bryophila*. Although we are aware that the name *Nebela bursella* precedes the others and thus should prevail, *N. militaris* has been consistently used since Penard's description and is well known to the scientific community working on testate amoebae. Moreover, we performed an extensive literature search and found no report for *N. bursella*, after 1964 (Sudzuki 1964). For these practical reasons, we will invoke article 23.9.3 of the International Code of Zoological Nomenclature to keep *N. militaris* as the valid name even though it is technically a junior synonym.

## Erection of the genus *Alabasta* and its phylogenetic position within the Hyalospheniidae

We obtained four identical partial COI sequences of 482 nucleotides from four different cells. The phylogenetic reconstruction does not place our sequences within the genus *Nebela*, but rather as a sister clade of the genus *Planocarina* (Fig. 3), this position being weakly supported (bs 48, pp 0.97). Moreover, the genetic distance between our sequences and the genetically closest species in genus *Nebela* (i.e. *N. flabellulum*, 18% of dissimilarity) is well above the barcoding gaps calculated for Hyalospheniidae and Amoebozoa in general (Kosakyan et al. 2012; Nassonova et al. 2010; Singer et al. 2018). Our isolates cannot be ascribed to *Planocarina* either due (1) to the genetic distance (ca. 20% of dissimilarity with *P. marginata*), and (2) by the fact that genus *Planocarina* is characterized by the presence of a keel on the posterior part of the test (Kosakyan et al. 2016), whereas it is absent in *N. militaris*. This justifies the creation of a novel genus for *N. militaris* here-after named *Alabasta* that incorporates species with an elongated test and strongly curved pseudostome with a flare and a marked notch in narrow view.

## Species to include in the genus *Alabasta* gen. nov.

Two species fit the morphological description of genus *Alabasta*: *Nebela kivuense* (Gauthier-Lièvre and Thomas 1961) and *N. longicollis* (Penard 1890). We thus formally move those species to genus *Alabasta*.

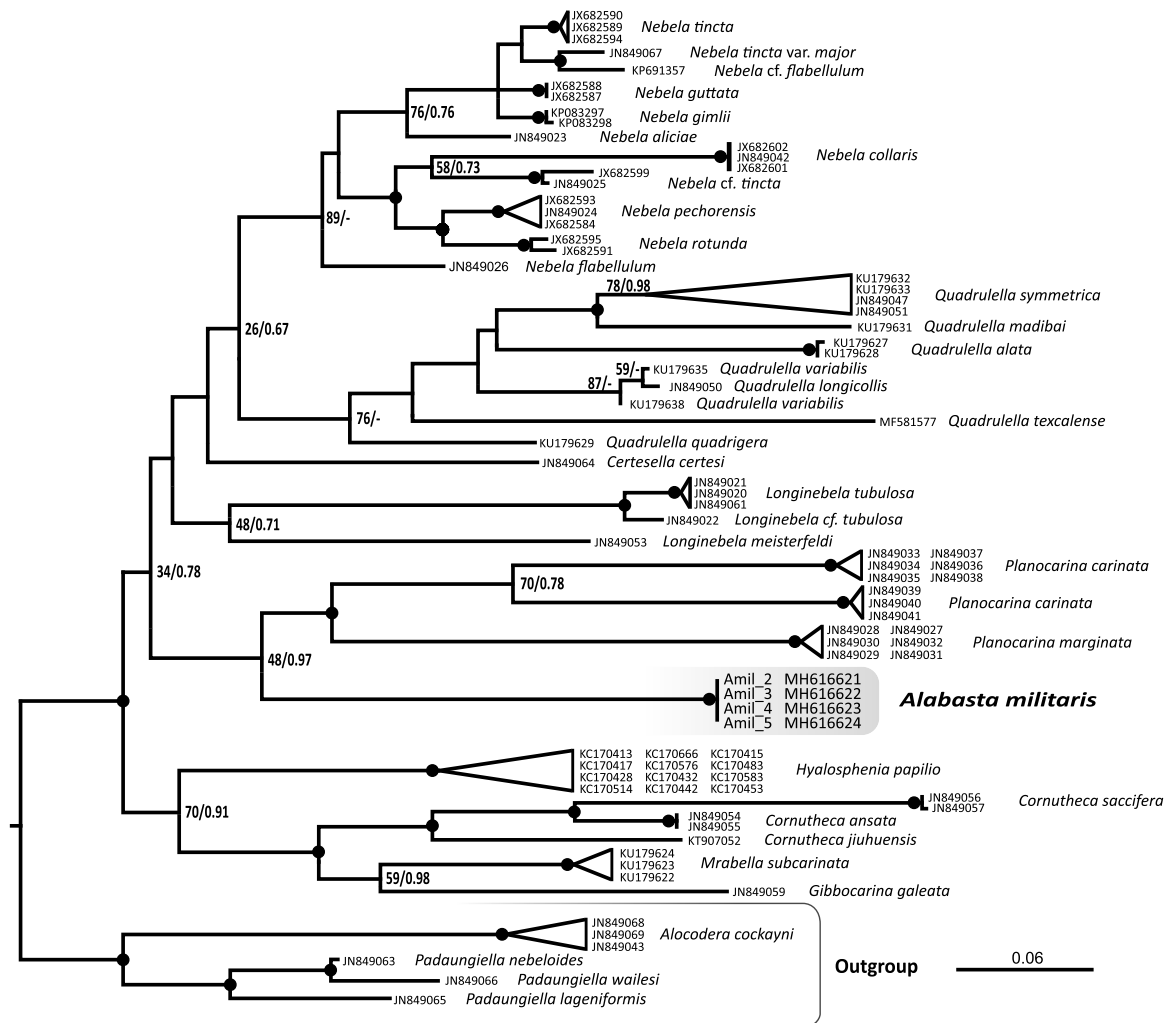
*Alabasta (Nebela) kivuense* (Fig. 1K) was described by Gauthier-Lièvre and Thomas (1961) from an area near Lake Edward in the Democratic Republic of Congo and was recently observed in two wetland-coniferous forest ecosystems in southern Ontario, Canada (Nicholls 2015). *A. kivuense* may potentially be mistaken for *Alabasta (Nebela) militaris* as both species having overlapping length (Table 1), similar narrow piriform tests and deeply curved pseudostome, however the pseudostome of *A. kivuense* is less curved than *Alabasta militaris* (Fig. 1).

*Alabasta (Nebela) longicollis* (Fig. 1V) has been reported only on a few occasions, albeit from several geographical regions worldwide. *Alabasta longicollis* is larger and more elongated than *A. militaris* (almost twice as long and slightly wider, Table 1).

As the original descriptions present clear morphological distinctions between *A. militaris*, *A. kivuense* and *A. longicollis* we keep them as distinct species. However further investigations using molecular data and precise environmental descriptions are needed to investigate the relationships between these three morphologically similar species and clarify the true diversity within this genus.

**Table 1.** Measurements and shape of the different species of *Alabasta* based on the literature.

Species	General shape of the test	Length (L) (μm)	Breadth (B) (μm)	L/B ratio	Pseudostome shape and width (μm)
<i>Alabasta militaris</i>	compressed, narrow piriform	50–82	25–46	1.8–2	deeply curved, 15–22
<i>Alabasta kivuense</i>	compressed, narrow piriform	49–64	18–26	2.4–2.7	very curved, 12–16
<i>Alabasta longicollis</i>	compressed, elongated piriform	120–140	40–45	3–3.1	deeply curved, n.a.

**Fig. 3.** Maximum likelihood phylogenetic tree of all COI gene sequences available on GenBank with the sequences of four single cells of *Alabasta militaris*. Numbers between branches correspond respectively to bootstrap values (bs) and posterior probabilities (p.p.) as calculated with Bayesian inference. The bs and p.p. values are indicated only for supported nodes (bs > 50 and p.p. > 70). Well supported nodes (bs > 80 and p.p. > 90) are marked with a dot.

### Species morphologically related to the genus *Alabasta* gen. nov.

Besides *Alabasta* (*Nebela*) *longicollis* and *A. (Nebela)* *kivuense* there are several species that could be considered as morphologically related to *A. militaris*.

*Hyalosphenia elegans* (Leidy 1879) and *H. insecta* (Harnisch 1938) also share several common morphological

characteristics with *Alabasta*. Furthermore, based on the 18S rRNA gene Lara et al. (2008) demonstrated that *H. elegans* does not branch with *H. papilio* as it is placed as a sister clade to genus *Nebela*. Thus, *H. elegans* and *H. insecta* could potentially also be included in genus *Alabasta*. However, two major differences between these species are 1) the inability of *H. elegans* and *H. insecta* to perform kleptosquamy as commonly observed in *Alabasta militaris*, *A. longicollis* and *A. kivuense* and 2) the presence of circular to oval depressions

at the surface of the shell of *H. elegans* and *H. insecta* that are not observed in *Alabasta* species. Thus, we believe that *H. elegans* and *H. insecta* likely represent yet another clade (likely a new genus), but further molecular data are required to determine this.

## Conclusion

*Alabasta militaris* is a cosmopolitan species that has been reported in the Northern and Southern Hemispheres and in high to low latitudes (e.g. Fernández et al. 2015; Golemansky 1962; Krashevskaya et al. 2017, 2018; Van Oye 1956). However, these observations are based solely on rough morphology-based observation, and a study combining morphometry and single cell barcoding may well reveal an unsuspected diversity of cryptic species (Dumack et al. 2016; Kosakyan et al. 2012, 2013; Lara et al. 2011; Singer et al. 2015). In parallel with findings in other Hyalospheniidae, these closely-related species may have different ecological optima (Singer et al. 2018) and may also have somewhat contrasted functional roles in microbial food webs (Geisen et al. 2018). As *Alabasta militaris* in its present definition has a narrow ecological tolerance, i.e. dry microhabitats in *Sphagnum* (Mazei and Bubnova 2007), the distribution of its lineages is arguably less likely to be structured by geographic distance and barriers to dispersal than larger species such as *Hyalosphenia papilio* (Heger et al. 2013). Finally, single cells transcriptomic studies of amoebae (Kang et al. 2017) may also provide new insight to the evolution of closely related species. The present study of *Alabasta* will pave the way to further studies in this iconic species of protist which may prove a useful model for future studies aiming to understand the general rules that shape ecological, biogeographical and evolutionary process in terrestrial protists.

## Taxonomic actions

### Description of new genus: *Alabasta* gen. nov. Duckert, Blandenier, Kosakyan and Singer

Taxonomic summary:

Arcellinida Kent 1880.

Hyalospheniidae (Schultze 1877) Kosakyan et Lara 2012.

*Alabasta* gen. nov. Duckert, Blandenier, Kosakyan and Singer.

Description: Test rigid, colourless or yellowish, elongated in broad view with a maximal width at about two thirds from the aperture and sides then tapering towards the aperture. Test proteinaceous often with incorporated silica scales taken from euglyphid preys. Pseudostome strongly convex with a flare (i.e. fan shaped) in broad view and a deep notch in profile. Lateral pores usually present at about one third of the distance from the pseudostome to the fundus.

Differential diagnosis: *Alabasta* can be distinguished from similar genera such as *Nebela* and *Longinebela* by its fan-shaped pseudostome (i.e. strongly curved with a flare) in broad view and a deep notch in profile.

Type species: *Alabasta militaris* comb. nov. (Penard 1890) Duckert, Blandenier, Kosakyan and Singer.

Included taxa: *Nebela militaris* Penard 1890; *N. kivuense* Gauthier-Lièvre and Thomas 1961; *N. longicollis* Penard 1890.

Etymology: The name of the genus is derived from the greek word “ἀλάβαστος” (alabaster), in connection with the diaphanous and yellowish aspect of the test of this genus. Also, it appears that *Alabasta* is the name of a fictitious city established in the middle of a desert in the manga *One Piece* (Oda 1999), reminiscent of the ecological preferences of *Alabasta militaris* which is used as a dry indicator in peatlands monitoring.

LSID numbers for the nomenclatural act  
urn:lsid:zoobank.org:act: 7408C1E9-2DD6-4098-97DA-3CF743954B5F and for this publication  
urn:lsid:zoobank.org:pub:72E66220-2093-4765-8D79-413E9B52071D.

## Key to the species

1. Smaller species <100 µm, test narrow piriform in broad view

→ 2

1\*. Larger species: L = 120–140 µm, test elongated piriform in broad view, with an elongated neck.

*A. longicollis*

2. Wider species in broad view (L/B = 1.8–2), pseudostome wide (15–22) µm

*A. militaris*

2\*. Slender species, often laterally curved, in broad view (L/B = 2.4–2.7) pseudostome narrow (12–16 µm)

*A. kivuense*

## Redefinition of *Nebela militaris* Penard 1890

*Alabasta militaris* comb. nov. Duckert, Blandenier, Kosakyan and Singer

1879 *Nebela collaris* (pars) in Leidy, Freshw. Rhiz. N. America, p. 147, pl. 22 Fig. 11, 12, 16 (misidentification).

1881 *Nebela bursella* Vejdovský in Taranek (1881) Sitzber. böhm. Ges. Wiss. p. 230, Fig. 2

1882 *Nebela bursella* Vejdovský in Thierische Organismen der Brunnenwässer von Prag

1882 *Nebela bursella* Vejdovský (pars) in Taranek Abh. böhm. Ges. Wiss. (6) XI (1882), 8, p. 36, pl. III, Figs. 8 and 12.

1890 *Nebela militaris* Penard, Mem. Soc. Phys. Hist. Nat. Genève 31: 164 (creation of *Alabasta* gen. nov. and transfer to the new genus)

**1933 *Nebela americana* var. *bryophila*** Van Oye, Arch. Naturgesch. Abt. B., N.F. 2 (overlapping morphology).

Updated description: Test rigid, colourless or yellowish, compressed in broad view, narrow piriform, reminding the shape of an ancient cannon (hence the species name) with a maximal width at about the first third from the posterior part and the sides of the test tapering towards the aperture. Test proteinaceous often incorporating silica scales taken from euglyphid preys. Lateral pores frequently observed at ca. one third of the distance from the pseudostome to the fundus. Pseudostome fan-shaped (i.e. strongly curved) in broad view, with a notch in profile, and a thick organic lip (Fig. 1.A–D). We recorded the following measurements (Fig. 2): Length: 61–77.5  $\mu\text{m}$  (mean 67.9  $\mu\text{m}$ ), width: 31.5–53  $\mu\text{m}$  (mean 37.7  $\mu\text{m}$ ), pseudostome (aperture) long axis: 15.5–20.5  $\mu\text{m}$  (mean 18.1  $\mu\text{m}$ ). Dimensions based on previous observations: L = 50–82  $\mu\text{m}$ , B = 25–46  $\mu\text{m}$ . Pseudostome 15–22  $\mu\text{m}$  wide.

Differential diagnosis: *A. militaris* may be confused with *A. longicollis* and *A. kivuense*, from which it differs respectively by a shorter test or by a wider test and pseudostome (see Table 1). Also *A. kivuense* is often laterally curved.

Neotype: Due to the absence of a type, we declare the Specimen 516-2 isolated by Penard and mounted on a permanent slide, now deposited at the Natural History Museum of Geneva, Switzerland as the name-bearing type ([https://commons.wikimedia.org/wiki/Category:Nebela\\_militaris#/media/File:Collection\\_Penard\\_MHNG\\_Specimen\\_516-2-1\\_Nebela\\_militaris.tif](https://commons.wikimedia.org/wiki/Category:Nebela_militaris#/media/File:Collection_Penard_MHNG_Specimen_516-2-1_Nebela_militaris.tif)).

Type locality: Jura Mountains, Switzerland

Etymology: The name refers to its general shape which reminds of an ancient military cannon.

Habitat: *Sphagnum* mosses (relatively dry microhabitats such as hummocks), brown mosses, litter, sediments (rare).

Geographical distribution: Apparently cosmopolitan

## Acknowledgments

This work was funded by a SWISSBOL (Swiss Barcoding of Life initiative) project to D.S. D.S., Q.B. and E.L. were also supported by the Swiss NSF (310003A\_143960 and 31003A\_163254). EL is funded by a “Atracción de talentos de la Comunidad de Madrid” project (2017-T1/AMB-5210). We thank the anonymous reviewers for their comments on the manuscript, and the International Commission on Zoological Nomenclature for their help on taxonomic issues.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ejop.2018.08.005>.

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