Acta Protozool. (2015) 54: 283–288
www.ejournals.eu/Acta-Protozoologica
doi:10.4467/16890027AP.15.023.3537

ACTA
PROTOZOOLOGICA

Nebela kivuense Gauthier-Lièvre et Thomas, 1961 (Amoebozoa, Arcellinida), Missing for a Half-century; Found 11,500 km from "home"

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Abstract. In 1961, the testate amoeba *Nebela kivuense* Gauthier-Lièvre et Thomas, 1961 was described for the first and only time from an area near Lake Edward in the Democratic Republic of the Congo (at 0.002° N Latitude). The lack of recent reports of this species, despite exhaustive surveys of the testate amoebae fauna of the major continents of the world, suggested that *N. kivuense* was a rare species perhaps endemic to a small, local equatorial region of the African continent. This paper reports its rediscovery from two wetland-conifer forest ecosystems in southern Ontario, Canada (at 44° N Latitude), thus changing dramatically our previous perception of its very restricted global distribution. This has implications for the idea held by many students of biogeography that there is a special category of microscopic protists that contains truly rare species and their rarity, perhaps together with specific habitat requirements and tolerances, limits opportunities for dispersal around the world. The *N. kivuense* story is a clear example of the dangers of inferring endemism from rarity.

Key words: *Nebela*, testate amoeba, Hyalospheniidae, protist biogeography.

INTRODUCTION

The testate amoeboid genus *Nebela* is one of several moss and soil-dwelling genera recently assigned to the Hyalospheniidae (Amoebozoa, Arcellinida) by Kosakyan *et al*. (2012, 2013). *Nebela* species are characterized by a hyaline organic test to which are cemented various siliceous scales of other protists, notably those of the genera *Euglypha*, *Trinema*, *Corythion*, and others. These are scavenged from the environment from dead cells, or may be actively ingested during predatory feeding on living prey, the scales of which are then secreted to the exterior of the *Nebela* test (Meisterfeld 2002). *Nebela* tests are usually flattened disc-shaped or elongated bulb-like structures possessing an elliptical pseudostome with a smooth thickened rim.

Among the very smallest *Nebela* species is *N. kivuense*, the only verified record of which is from a single location (the type locality) in East-Central Africa (Gauthier-Lièvre and Thomas 1961). This taxon also appeared among a listing of 22 *Nebela* species from Romania (Godeanu 1972). That report remains unsubstantiated, however, owing to the lack of any accompanying descriptive material (measurements, drawings, images, etc.) that would support its identification. The purpose of this note is to report the occurrence of *N. ki-*

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vuense from Ontario, Canada, because it has important implications for the theories of biogeography of small rare protists.

MATERIALS AND METHODS

Samples of moss from shaded, wet fen habitats in south-central Ontario were collected in April and May of 2000 and July of 2001 by picking several whole plants, including the older brown ground-level portions, and rinsing vigorously with distilled water in 500 mL plastic bottles to dislodge testate amoebae attached to stems and leaves. Several such samples were collected within plots of approximately 100 m in diameter at two locations in separate watersheds. These were located at: 1) 44°08′30″N, 79°06″W (Uxbridge Brook of the Pefferlaw River watershed), and 2) 44°10′40″N, 79°07′30″W (Beaver River watershed). At both locations, the arboreal overstory was dominated by White Spruce (*Picea glauca*), Black Spruce (*Picea mariana*), Balsam Fir (*Abies balsamea*) and Eastern White Cedar (*Thuja occidentalis*). Samples containing *Nebela kivuense* were associated with the mosses *Hylocomium splendens* (Hedw.) B.S.G., *Sphagnum wulfianum* Girgensohn, and two other unidentified *Sphagnum* species.

Laboratory methods have been described elsewhere (e.g., Nicholls, 2007). Measures of central tendencies of test morphology were summarized using the descriptive statistical methods described in Costat (Cohort Software 1995). Owing to some similarity in the shape of the tests of *Nebela kivuense* and *Nebela militaris*, which co-occurred in some of the same samples, measurements of specimens of both taxa were made and compared statistically.

RESULTS AND DISCUSSION

The sizes and shapes of test of *N. kivuense* in the Ontario collections generally agreed well with those described and illustrated (Fig. 1) by Gauthier-Lièvre and Thomas (1961). Ontario specimens were more variable in the degree to which they were slightly curved in plan view (Figs 2–6, 12a, 13a, 14), and in the degree of taper from the widest region near the aboral end to the anterior in both plan (Figs 2–6, 12a, 13a, 14) and lateral (Figs 7–11, 12b, 13b) views. In all tests examined, the pseudostomal aperture was outlined by a thickened rim of organic cement (Fig. 15). A cyst plate was found in some specimens constructed about 15–20 µm from the anterior pseudostomal aperture and spanning the entire width of the interior of the test (Fig. 16); cysts were large, spherical and thick-walled (Fig. 17).

Test dimensions from the 39 specimens of *N. kivuense* measured in the Ontario samples (Table 1) agree well with those given by Gauthier-Lièvre and Thomas (1961); their values being, lengths = 58–63 µm, widths = 18–22 µm, and pseudostomal apertures = 13–15 µm. It might be argued that the Ontario specimens were larger than those in the type material, but this difference is not likely significant if the number of specimens from the type material was much lower (these data were not given by Gauthier-Lièvre and Thomas).

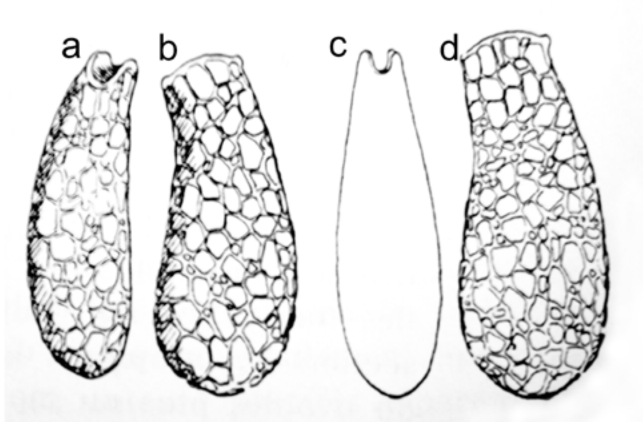


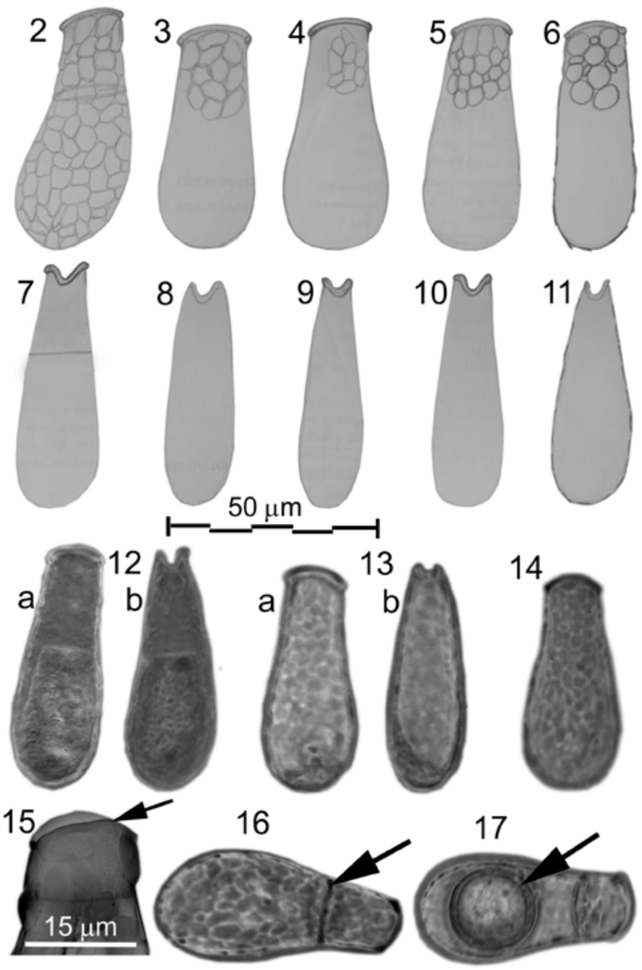
Fig. 1. A reproduction of Figs 1e and 1f from Gauthier-Lièvre and Thomas (1961) illustrating the shape of the test of *Nebela kivuense*; a and c – lateral (edge) views; b and d – plan views.

The other difference between the two populations relates to the relative curvature of the tests. Most of the tests in the African samples were apparently slightly curved, while only a few of the Ontario specimens were curved. These differences are also considered to be within the range of normal intra-specific variation, owing to the great similarity in general size and shape of the tests of the two populations. Most notable also, is the agreement in the apparent selection of both large and small scales of "foreign" testate amoebae used to construct the *N. kivuense* tests in both the African and Canadian populations. *N. kivuense* appears to have often preferred the larger elliptical or circular scales of *Puytoracia*, *Sphenoderia* and large *Trinema* species (Figs 18–21). This is in marked contrast to the scales appearing in the tests of many other much larger *Nebela* species which tend to select the smaller scales of other testate amoebae species (see, for example, the SEM images of several *Nebela* species in Kosakyan *et al*. 2012).

In both the African and Canadian populations, there was a copious application of organic cement securing the testacean scales to the integument of the *N. kivuense* tests. This was applied often along straight lines without following the curved edge of the scales such that the shape of the scales observed with lower magnification

Table 1. Descriptive statistics (measures of central tendency) for test lengths, widths and test aperture width in *Nebela kivuense* and *Nebela militaris* (Ap. Width = width of the pseudostomal aperture; CO-VAR = coefficient of variation). All measurements in µm.

N. kivuense (n = 39)				*N. militaris* (n = 54)		
Test Length	Test Width	Ap. Width		Test Length	Test Width	Ap. Width
55.1	23.0	13.8	MEAN	72.0	39.7	19.6
55	23	14	MEDIAN	72	40	20
49	20	12	MINIMUM	64	34	17
64	26	16	MAXIMUM	82	46	22
6.1	6.2	7.2	% CO-VAR	5.7	7.3	7.1



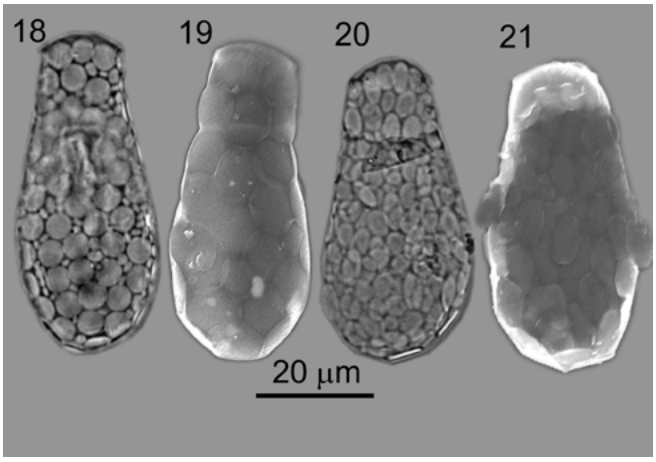
Figs 2–17. Diagramatic representations and light and electron microscope images illustrating test morphology of *Nebela kivuense*. Figs 2–6, 12a, 13a and 14 – variations in test shape as seen in plan views together with the lateral view of the same tests (Figs 7–11, 12b, 13b). Fig. 15 – scanning electron microscope image showing the flattened anterior region of a test with its thickened rim of organic cement around the pseudostomal aperture (arrow). Fig. 16 – well developed internal cyst plate (arrow). Fig. 17 – thick-walled spherical cyst (arrow).

light microscopy was often perceived to be polygonal rather than truly circular or elliptical. Gauthier-Lièvre and Thomas (1961) erroneously described the scales included in the tests of the African specimens as being "polygonales irrégulières", and this feature was reproduced in their drawings (Fig. 1). What appeared to be very thin polygonal plates in some of the Canadian material, were revealed to be circular or elliptical scales, after dissolution of the organic cement in concentrated HNO_3 (or after mounting in the high refractive index media, Styrax or Canada Balsam; Figs 18–21); in no cases were any polygonal non-scale agglutinated materials found in tests of this species.

Because errors in identification can lead to false conclusions about geographic range of testate amoebae and other small protists (Mitchell and Meisterfeld 2005), it is important to ask the question: "is it possible that *N. kivuense* is more widespread globally than the title of this paper suggests, but is 'hiding' behind the name of another similar species?". It is necessary, therefore, to draw attention to the features that distinguish *N. kivuense* from other *Nebela* species. *N. kivuense* is among the smallest of the known *Nebela* species, so there are only a limited number of options for its misidentification. Because there was overlap in the lengths of tests of *N. kivuense* and *N. militaris* reported in the *Nebela* literature, and because *N. miliaris* and *N. kivuense* co-occurred in some of the Canadian samples, I compared the tests dimensions in these two taxa (Table 1; Fig. 22). Despite the minor overlap in test lengths, these are clearly two separate species on the basis of pseudostomal aperture and test widths that were much greater in *N. militaris*. Furthermore, it cannot be argued that *N. kivuense* might be an "ecological variant" of *N. militaris* because populations of both taxa co-occurred in the same ecosystems.

A similar but less rigorous comparison was made of *N. kivuense* with five other *Nebela* taxa based on literature descriptions (Table 2). It is apparent that *N. kivuense* is a unique species that has a low probability of being confused with other *Nebela* species in the published literature.

The discovery of *Nebela kivuense* in temperate North America has implications for biogeography. An



Figs 18–21. Dried tests with circular or elliptical scales "scavenged" from other species of testate amoebae. Figs 18, 20 – light microscope images of tests mounted in Canada Balsam. Figs 19, 21 – scanning electron microscope images of two different tests.

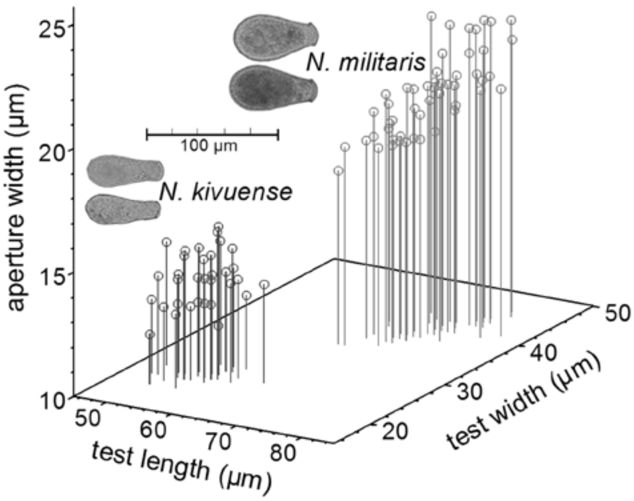


Fig. 22. Comparison of test length, width and pseudostomal aperture width in *Nebela kivuense* and *N. militaris*, co-occurring in the Ontario (Canada) samples.

Table 2. Comparisons of test size and shape in *Nebela kivuense* with other small *Nebela* species.

Taxon	Test Length (L) and Width (W) (µm)	Notes
Small unnamed form[1] of *Nebela minor* Penard, 1883 [described by Penard (1902)]	L = 55–70 W = ?	The degree of taper of the neck region of the test is far greater than that found in *N. kivuense* (i.e., the ratio of test width to pseudostome width is much greater in the Leidy/Penard taxon).
N. gimlii Singer et Lara, 2015: Singer *et al.* (2015)	L = 68–78 W = 50–62	Its much wider test and its lack of the elongated tapered neck region of the test set this apart from of *N. kivuense*.
Nebela cylindrica Bonnet, 1979: Bonnet (1979)	L = 48–50 W = 17–22	It cannot be confused with *N. kivuense* because its test is only slightly flattened (hence the specific epithet, "cylindrica") with a thin poorly developed collar surrounding the pseudostomal aperture.
Nebela tubulata Brown, 1911; (assigned to *Padaungiella*, Lara et Todorov [in Kosakyan *et al*. 2012]); (Mitchell, 2003; http://istar.wikidot.com/id-keys/Nebela)	L = 55–74 W = 28–48	This species has a prominent anterior portion with nearly parallel sides and a markedly bulbous and much wider posterior; there is no possibility of taxonomic overlap with *N. kivuense*.
Nebela militaris Penard, 1890; see also Penard (1902) and Deflandre (1936)	L = 50–72 W = 25–38	The test shape is noticeably more "bulbous" in plan view in *N. militaris* (i.e., has a much lower length-to-width ratio than in *N. kivuense*; see also Fig. 22).
Nebela militaris var. *curvata* Golemansky, 1962	L = 90 W = 40	This taxon was described (Golemansky, 1962) as a large *N. militaris* with a markedly curved test. Its much larger size readily distinguishes it from *N. kivuense*.[2]

[1] Penard (1902) said that this taxon was likely the same as the form illustrated in Leidy's (1876) Plate XXII, Figs 11, 12 and 16, which Leidy had inappropriately lumped in with *N. collaris* Ehrenberg. The modern concept of *N. collaris*, however, includes only forms with test lengths in the range of 95–115 µm (Kosakyan *et al*. 2013). This leaves out the small "form" described by both Leidy and Penard.
[2] Also, there have been no previous reports of *N. militaris* var. *militaris* with slightly curved tests in plan view or tests with false-polygonal plate structures (as described above for *N. kivuense*) in either *N. militaris* var. *militaris* or *N. militaris* var. *curvata*.

argument has been made that there are few barriers to the global distribution of small, single-celled protists (Fenchel and Findlay 2004). Owing to the relative ease by which small organisms and their cysts are blown by wind or transported long distances in the plumage of water birds, there would appear to be a size threshold above which these transport vectors are much less effective. A prevailing hypothesis suggesting that small protists are more likely to be "everywhere", while protists generally above a size of 100–150 μm tend to exhibit more regional tendencies in their world distributions (Wilkinson 2001, Yang *et al*. 2010). It has also been argued that there are truly rare species whose distribution is controlled by special environmental tolerances and requirements, irrespective of cell size (Foissner 2007); such species will include endemic species that are particularly adapted to polar, high alpine, or other "harsh" or unique environments.

The type locality for *Nebela kivuense* as described by Gauthier-Lièvre and Thomas (1961) was an area of wet moss between Lubèro and Butembo in the Democratic Republic of the Congo (DRC). This is about 50 km E of Lake Edward, about 150 km N of Lake Kivu on the border between the DRC and Rwanda. Lake Edward lies on the Equator at an elevation of 920 m, and experiences typical African equatorial climate with a short rainy season in October and a longer rainy season in April and May. Although the original collection site was not described in any detail, the type locality for *N. kivuense* was most probably along the main road north of Lubèro, that skirts the western boundary of Virunga National Park and passes through the southwestern foothills of the Rwenzori Mountains ("Mountains of the Moon"). The equatorial climate at the elevation of Lake Edward would be moderated towards a slightly more sub-tropical climate on the lower slopes of these mountains.

In marked contrast, the climate of the area near the Canadian habitats of *N. kivuense*, at 44° North Latitude, was characterized by a winter period from December to March with an average temperature of –4.7°C (1981–2010) when the ground is frozen and covered with snow. Total annual average precipitation for this region of Ontario (Udora weather station) was 885 mm (82% rain, 18% snow, as water equivalent). The daily average temperature for the warmest month (July) was 20°C and –7°C for the coldest month (January).

Until now, it could be assumed from the limited data on *N. kivuense*, that it might be characterized as a very rare tropical species. More likely, based on the recent discovery in Canada, it would appear to have a wide climate tolerance and could be expected to grow in many moist, moss-dominated habitats in both temperate and equatorial locations. The lack of reports of this species do, however, suggest that it is a very rare species; and the Canadian discovery further suggests that very rare species masquerading as endemics can achieve global distributions and remain regionally rare.

Acknowledgements. I thank Ranganathan Santhanam, University of Ontario, Institute of Technology for assistance with electron microscopy.

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Received on 11th February, 2015; revised on 27th February, 2015; accepted on 13rd March, 2015