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A new species of testate amoeba, *Arcella prismatica* sp. nov. (Amoebozoa: Arcellinida), from peatlands in Ontario and Quebec, Canada

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Abstract
Arcella is a genus of testate amoebae with a radially symmetrical shell composed of secreted material arranged in hexagonal units. Within the genus, species are distinguished by the morphology and dimensions of the shell. We describe a new species, *Arcella prismatica* sp. nov., discovered in a brown-water lake in the Mer Bleue Bog Conservation Area, a protected wetland in the city of Ottawa, Ontario, Canada. Specimens of the same morphotype have also been found in a subarctic peatland on the James Bay coast, near the village of Chisasibi, Quebec, Canada. The species has a polyhedral shell with a relatively flat dorsal surface and an irregularly crenulated aperture, a combination of characters not found in other members of the genus. The discovery of a novel and evidently widely distributed *Arcella* within the limits of a populous North American city is a reminder that the diversity of microbial eukaryotes is still poorly understood. Further exploration of undersampled peatland habitats can be expected to reveal new organisms and new relationships among known species.

Key words: *Arcella*; Arcellidae; Arcellinida; testate amoebae; Mer Bleue; James Bay peatlands

Introduction

The genus *Arcella* Ehrenberg, 1830 comprises testate amoebae whose shells are radially symmetrical and composed of secreted material, presumably chitinous, arranged in hexagonal units (Meisterfeld 2002). These free-living protists are mainly found in freshwater and moist mosses, but also in soil and dry mosses.

Arcella was erected in 1830 by the pioneering protozoologist C.G. Ehrenberg, for "shelled infusoria" ("*Kapselthierchen*"; Ehrenberg 1830: 74) equipped with a "carapace [that is] shield-shaped" ("*Panzer schildförmig*"; Ehrenberg 1832: 90). Initially, Ehrenberg (1830) described three species, with *Arcella vulgaris* Ehrenberg, 1830 as the type of the genus. In following years, the number of species assigned to the genus increased steadily, and, by the first decade of this century, about 51 nominal species with 71 varieties and subspecies and 27 forms were accepted (R. Meisterfeld unpubl. list, pers. comm. to F.J.S., 24 September 2018). Another 52 species, varieties, and forms had been described, but these were (variously) synonyms, *nomen nudem* ("naked name", i.e., one that does not meet the requirements of the rules of zoological nomenclature), *lapsus calami* ("slip of the pen", i.e., errors), or species belonging to another genus (R. Meisterfeld, unpubl. list, pers. comm. to F.J.S., 24 September 2018).

Recently, the circumscription of *Arcella* has changed, following a major revision of the family based on molecular phylogenetics (González-Miguéns *et al.* 2022). In that work, the new genus *Galeripora* González-Miguéns, Soler-Zamora, Villar-dePablo, Todorov and Lara, 2021 was erected, comprising arcellid species whose shells carry pores around the mouth or aperture. Six new species of *Galeripora* were described and six species previously assigned to *Arcella* were formally transferred to the new genus. Appendix S1 in González-Miguéns *et al.* (2022) also compiled a synonymic list of names in the family, recognizing 63 valid species of *Arcella* and 48 varieties and forms.

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Since that work, three new species of *Arcella* have been described (Useros *et al.* 2023), and five more species historically placed in *Arcella* have been transferred to *Galeripora* (Siemensma 2021). The 61 species that remain in *Arcella* are those that have no pores. It is possible that this genus will be further split, in due course, on the basis of molecular sequence data.

As with other members of the order Arcellinida, which includes about 86 genera (Siemensma 2024), species of *Arcella* are distinguished from one another mainly based on shell morphology (Meisterfeld 2002). Features commonly used to characterize species of *Arcella* include the diameter and height of the test, the form of the aperture or pseudostome (the depth of the invagination, the margin of the opening, which may be smooth, lobed, or crenulate), the shape of the test in profile and ventral view, and notable characters such as depressions or undulations in the dorsal surface and spines or ribs on lateral faces (Nicholls 2005; Reczuga *et al.* 2015; Feres *et al.* 2016; González-Miguéns *et al.* 2022). However, since the application of molecular analysis, it has been demonstrated that the presence of cryptic morphologies makes it impossible to differentiate some closely related species based only on shell morphology (Porfírio-Sousa *et al.* 2017; Soler-Zamora *et al.* 2023).

In this paper, we describe a new *Arcella* species, *Arcella prismatica* sp. nov., based on morphometric and morphological characters. This distinctive and easily identified species was discovered in a drainage arm of the Mer Bleue bog, Ontario, Canada, among floating filamentous algae and aquatic plants, and subsequently found in the Eeyou Istchee region of Quebec, among *Sphagnum* L.

Methods

Mer Bleue

The Mer Bleue bog (45.392376°N, 75.509063°W; Figures 1a,b, 2a) is a large, raised, ombrotrophic peatland within the city limits of Ottawa, Ontario, Canada. It is a remnant wetland, located in a system of channels left by glacial meltwater streams formed during the retreat of the Champlain Sea, during the early Holocene (Mott and Camfield 1969; Fraser *et al.* 2001; Roulet *et al.* 2007; Elliott *et al.* 2012). As post-glacial isostatic uplift pushed the Champlain Sea to the east, the drainage basin now occupied by Mer Bleue filled with fresh water and began a gradual transition from lake and marsh to fen and finally to raised bog. The accumulation of peat began about 8500 years ago, and the transition from fen to bog began about 2000 years later (Bubier *et al.* 2006). As it exists today, Mer Bleue is an isolated pocket of subarctic habitat, supporting an assemblage of plant and animal species usually found much further north in the boreal (taiga) climate zone. Because of its regionally unique ecology, it is designated as a "wetland of international importance" under the Ramsar Convention and classified by the government of Ontario as an "Area of Natural Scientific Interest (ANSI)" (Ramsar n.d.; National Capital Commission n.d.).

On its west side, Mer Bleue has three drainage fingers which discharge slowly into a creek connected with the Ottawa River (Fraser *et al.* 2001).

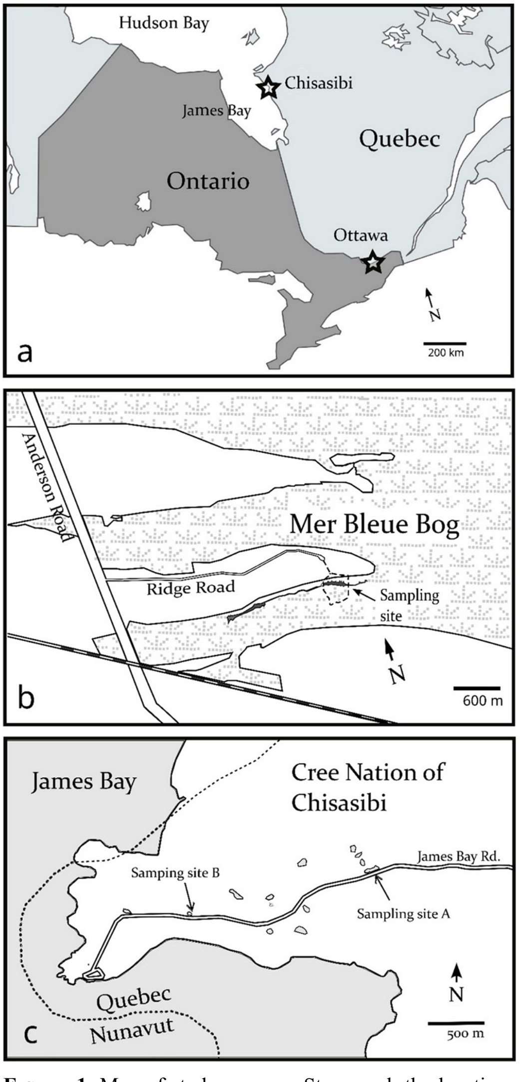

Figure 1. Map of study areas. a. Stars mark the locations of Mer Bleue bog, Ottawa, Ontario, and two sampling sites in Chisasibi, Quebec. b. Close view of Mer Bleue, showing the drainage fingers. The study site (45.392376°N, 75.509063°W) is marked with an arrow. c. Close view of two sampling sites on James Bay Road, outside the town of Chisasibi: site A 53.795167°N, 79.039833°W; site B 53.791556°N, 79.065139°W.

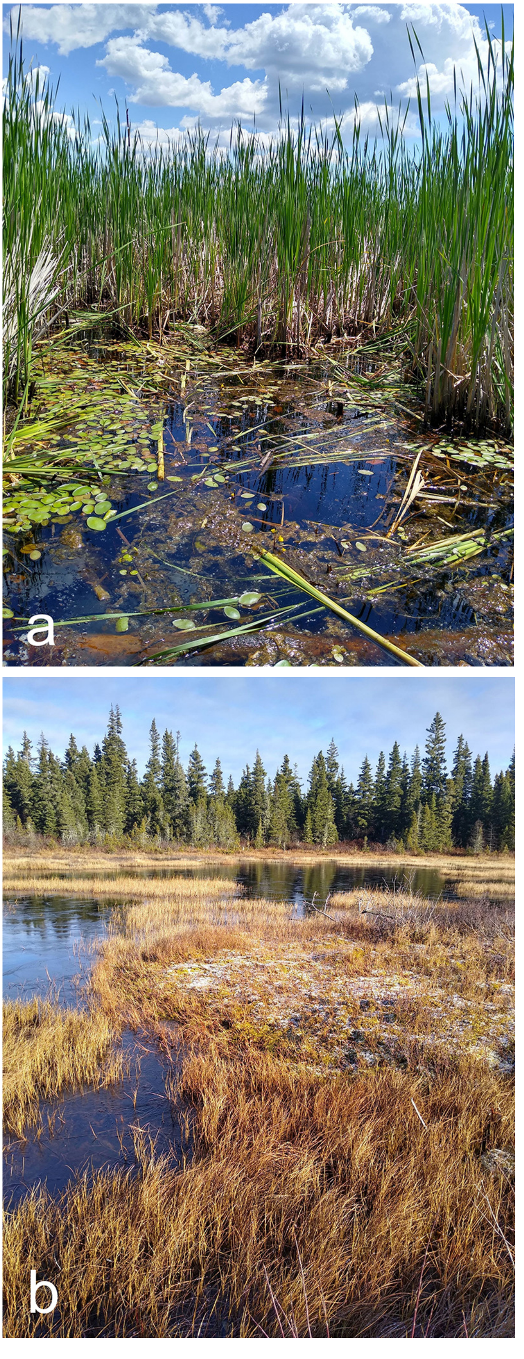

FIGURE 2. Sampling site at a. the type locality in Mer Bleue, Ottawa, Ontario, and b. site A at Chisasibi, Quebec. Photos: Bruce D.S. Taylor.

The southernmost of these channels contains a narrow brown-water bog lake fringed with Broad-leaved Cattail (*Typha latifolia* L.), at the east end of which is a boardwalk installed and maintained by Canada's National Capital Commission. In a shallow marsh accessible from the boardwalk, small mats of filamentous green algae (e.g., *Mougeotia* C. Agardh, *Zygnema* C. Agardh, and *Spirogyra* Link) form near the surface, sometimes among floating stolons of Greater Bladderwort (*Utricularia vulgaris* subsp. *macrorhiza* (Leconte) R.T. Clausen) and other pond species, such as Watershield (*Brasenia schreberi* J.F. Gmelin; Figure 2a). These mats provide a distinctive microhabitat in the low-nutrient water, often dominated by microbial phototrophs and their predators, as well as mixotrophic organisms harbouring algal endosymbionts, such as the ciliates *Stentor pyriformis* Johnson, 1893 and *Dileptus* cf. *viridis* (Ehrenberg, 1833) Foissner, 1987.

Water samples were collected by B.D.S.T. from the same location in 2014 and 2022. On 18 May 2014, samples were drawn from three sites along the Mer Bleue bog interpretive trail, all within 100 m of the location marked in Figure 1b. Three jars, each containing ~100 mL of material, were taken for further study. From each jar, 2–3 mL of material was transferred to a 100-mL polystyrene Petri dish, then examined under a stereo microscope (Acuter 2L, Taoyuan, Taiwan) at 40× magnification. Specimens of the novel arcellid were picked out individually with a glass micropipette, deposited in a watch glass, and rinsed several times in clear water to separate them from other organisms and attached detritus. Specimens were transferred to micro-centrifuge tubes and sent to F.J.S. for study, measurement, and light microscope imagery and to M.C.S.K. for imaging by scanning electron microscopy (SEM). Individual shells were picked from the ethanol-fixed sample, placed on SEM stubs with carbon tabs, arranged with the help of an eyelash brush, and air-dried. Observation and imaging were done in a Hitachi S-570 SEM (Hitachi, Tokyo, Japan) at various magnifications.

On 6 May 2022, the same sampling procedures were repeated, at six sites along the eastern boardwalk at Mer Bleue. Samples from two of these sites contained the novel species *A. prismatica*. Specimens were set aside for imaging and measurement in the light microscope. Observations and measurements were carried out with a Motic 310E compound microscope (Motic, Xiamen, China), equipped with a RisingCam 20 MP USB camera (RisingCam, Hangzhou, China). Digital microphotographs of each shell were recorded at 400× magnification, with RisingView v. 4.11 imaging software (a proprietary version of ToupView, from ToupTek Photonics, Hangzhou, China), and measured with tools incorporated in the same software. Morphometric data (Appendix S1) were gathered on seven traits: number of ribs, diameter of shell, height of shell at the centre, height of shell at the highest side, height of aperture, diameter of aperture, and angle of sides with respect to the ventral plane.

Chisasibi

The Cree Nation of Chisasibi is 944 km north of Mer Bleue in the drainage basin of James Bay, the southernmost part of the Arctic Ocean (Figure 1a). The climate is subarctic but modified by coastal weather effects (Hennigs and Bleau 2017). The biome is northern boreal (taiga), grading into tundra along the coast.

On 4 November 2022, samples were taken from two *Sphagnum* pools (sites A and B in Figure 1c), located ~12 km northwest of the community of Chisasibi. Both sites are small fens of the graminoid type (Sims *et al.* 1982), dominated by grasses and sedges, with shallow expanses of open water broken up by mats of *Sphagnum*. At site A (53.795167°N, 79.039833°W; Figure 2b) 200 mL of water and vegetation were extracted from a partly submerged hummock of Streamside Sphagnum (*Sphagnum riparium* Ångström). At site B (53.791556°N, 79.065139°W), a second sample was collected in a patch of Spiky Bogmoss (*Sphagnum squarrosum* Crome). Both samples were examined under a light microscope and found to contain *A. prismatica*. Sixty-five specimens collected from site A were measured with the same equipment and procedures used for the Mer Bleue samples (Appendix S1). Specimens from site A were imaged in SEM, using a Quanta FEG-250 (Quanta, Eindhoven, The Netherlands), at various magnifications.

Taxonomic Description

Amoebozoa Lühe, 1913, *sensu* Cavalier-Smith, 1998
Class Tubulinea Smirnov *et al.*, 2005
Order Arcellinida Kent, 1880
Infraorder Sphaerothecina Kosakyan *et al.*, 2016
Family Arcellidae Ehrenberg, 1843

Arcella prismatica sp. nov.

Diagnosis

Shell polyhedral with 5–8 ribs. Sides steeply angled and often nearly perpendicular to the ventral plane. Dorsal surface usually flat, concave or slightly domed, rarely with a pronounced dome or peak. Aperture circular, with a crenulated edge. Diameter of the ventral surface 81–115 µm; height 60–87 µm (data from 87 shells collected in Mer Bleue and 65 shells collected in Chisasibi). Pseudopods barely protruding from the aperture and never extending laterally beyond the outer margin of the shell.

Description

The proteinaceous shell is transparent and colourless when newly formed, then gradually darkening to gold, and eventually dark brown. The ventral margin is usually polygonal (Figures 3b,c, 4a), but sometimes nearly circular. The dorsal margin is angular, approximating a polygon (Figure 3h), with five to eight edges, which may be slightly convex or markedly concave. In most individuals, the dorsal region has no folds or creases, but is made up of a continuous surface, usually flat (Figure 3d,e), but also sometimes depressed at the centre (Figures 3i, 4d), or slightly raised (Figure 3a,g). In some cases, the top may be distinctly domed (Figure 4c) or equipped with a peaked ridge or creases (Figures 3f, 4e). The dorsal face may be nearly parallel to the ventral one (Figure 3e), or slightly sloped from side to side (Figure 3d,i). In lateral view some ribs are visible, mostly parallel (Figures 3d,g,i, 5b), but sometimes converging or tapering distally (Figure 3a).

The aperture is about a third as wide as the shell and slightly invaginated. The opening consists of 7–10 relatively large crenulations, often somewhat irregular or ragged in appearance (Figures 3b,c, 4a,f, 5a).

In vivo, the amoeba is usually entangled in filaments of eukaryotic algae or leaves of *Sphagnum*, often in large numbers. Cytoplasm is often greenish from ingested algae. Pseudopods are bluntly lobose, hyaline, and often numerous, but short.

ZooBank registration: urn:lsid:zoobank.org:pub:1767947E-F9D5-44B8-B11A-BA6E4A705344

Type material

The holotype of *A. prismatica* was preserved with Permount SP15-100 mounting medium (Fisher Scientific, Hampton, New Hampshire, USA) on a microscopic slide. Thirteen slides with paratypes were prepared in the same way. The slides were deposited in the Invertebrate Collection at the Canadian Museum of Nature in Ottawa, catalogue number CMNI 2022-0447 (holotype) and CMNI 2022-0448–4460 (paratypes).

Type locality

A cattail marsh at the east end of a brown-water lake in the Mer Bleue Bog Conservation Area, Ottawa, Ontario, Canada, at 45.392376°N, 75.509063°W, among floating filamentous algae and *Utricularia* sp.

Etymology

The species name refers to the typical shape of the shell and derives from the Late Latin *prisma*, meaning a polyhedron with parallel sides and flat parallel faces at each end. Feminine gender.

Morphometrics

See Table 1 and Figure 6.

Differential diagnosis

Arcella prismatica can be easily distinguished

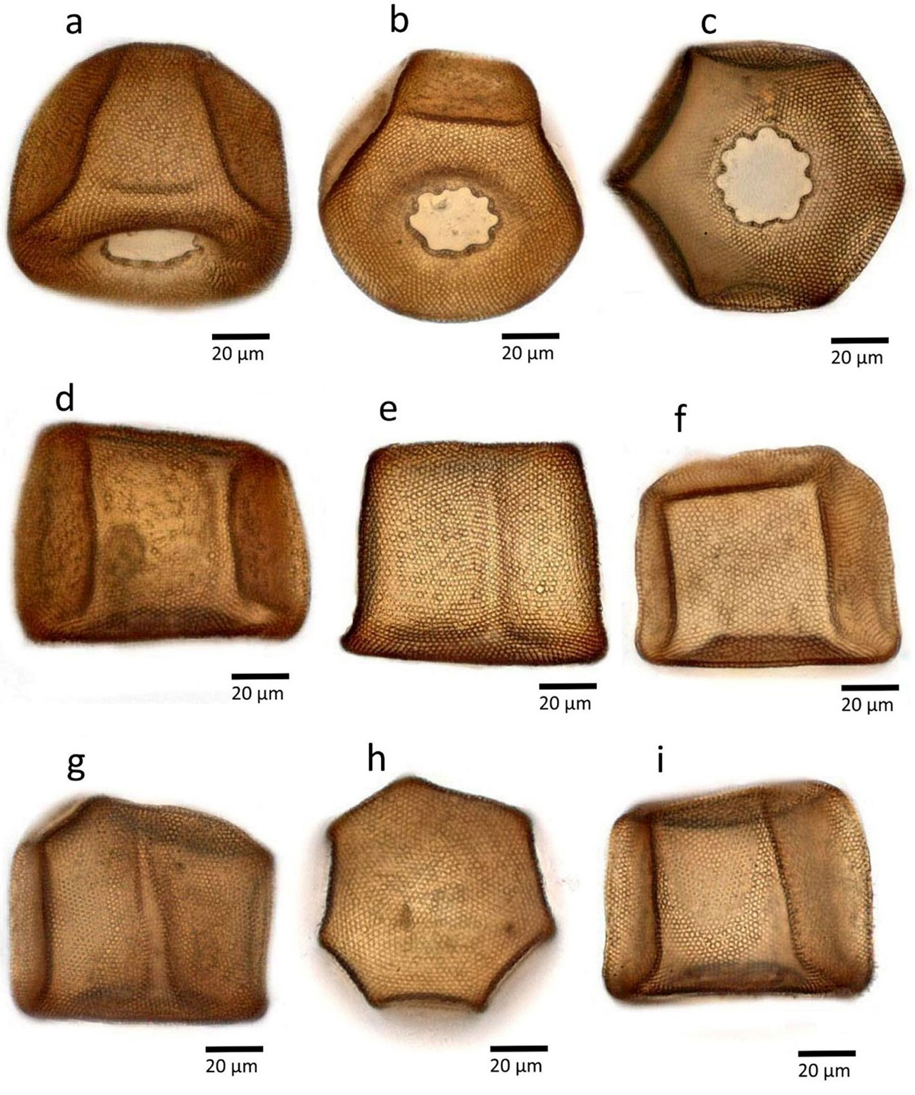

FIGURE 3. *Arcella prismatica* viewed with a light microscope. a. Shell in lateral view. b and c. Ventral area with crenulated aperture. d and e. Shells with flat dorsal surface, lateral view. f and g. Shells with slightly peaked dorsal surface, lateral view. h. Shell with flat dorsal surface, dorsal view. i. Shell with sunken (concave) dorsal surface, lateral view. Photos: Ferry J. Siemensma.

from *Arcella conica* (Playfair, 1918) Deflandre, 1926 and *Arcella costata* Ehrenberg, 1847 and its variety *angulosa* (Perty) Playfair, 1918, all of which have a smooth apertural edge. It can be distinguished from *Arcella spectabilis* Deflandre, 1928, which is more spherical, has many undulations, and never has a flattened dorsal area; and from *Arcella leidyana* Deflandre, 1928, which is almost twice as large, with its smallest diameter at the ventral face and a dorsal surface in the form of a rounded pyramid (Figure 7b from Deflandre 1928: 277)

Discussion

Comparison to similar species

In its type locality—where samples have been collected on numerous occasions between May and October, from 2014 to 2022—specimens of *A. prismatica* are very consistent in size and shape. Morphology and measurements of specimens from James Bay agree with those of specimens from Mer Bleue. The species is easily distinguished from other arcellids found in the same water, such as *Galeripora artocrea*

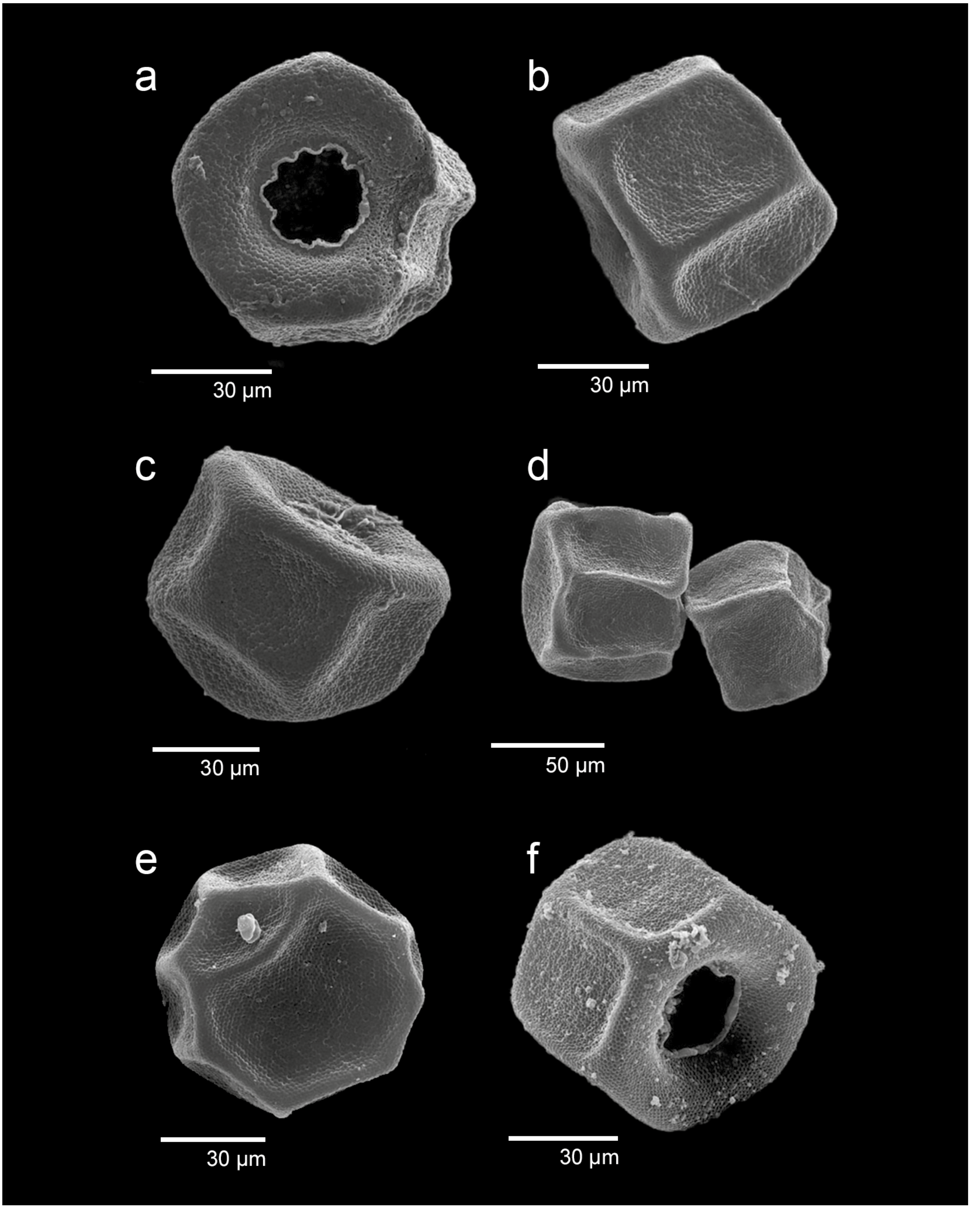

FIGURE 4. Scanning electron micrographs of *Arcella prismatica* specimens from Mer Bleue. a. Ventral view, showing crenulate aperture. b. Shell with flat or concave dorsal surface, lateral view. c. Shell with domed dorsal surface, lateral view. d. Two shells, one with a creased dorsal surface, lateral view. e. Shell with a creased dorsal surface, dorsal view. f. Shell with sides nearly perpendicular to the ventral face. Photos: Michaela C. Strueder-Kypke.

(Leidy, 1876) Gonzalez-Miguens, Soler-Zamora, Villar-Depablo, Todorov & Lara, 2021, *Arcella mitrata* Leidy, 1876*, A. spectabilis*, *Arcella crenulata* Deflandre, 1928, and members of the species complex made up of *Arcella hemisphaerica* Perty, 1852 and *Arcella rotundata* Playfair, 1918. *Arcella conica* (Playfair,

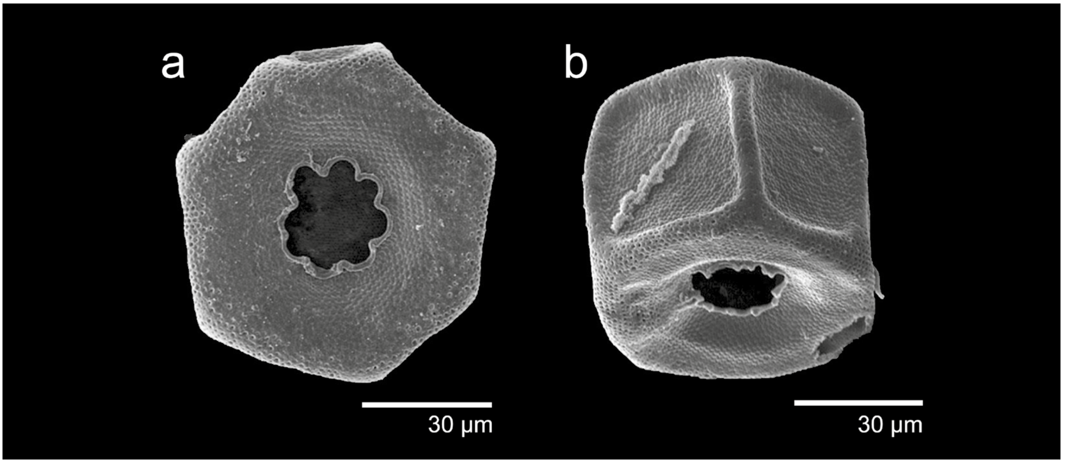

FIGURE 5. Scanning electron micrographs of *Arcella prismatica* specimens from Chisasibi. a. Ventral view, showing crenulate aperture. b. Semi-profile, showing the ribbed sides and flat dorsal surface of the shell. Photos: Michaela C. Strueder-Kypke.

TABLE 1. Morphometrics of *Arcella prismatica* sp. nov. population in Mer Bleue and Chisasibi (see Figure 8, Appendix S1). Measurements in µm.

Character	*n*	Mean	Median	Min.	Max.	SEM	SD	CV
Mer Bleue								
Base diameter	87	94.6	93.8	81.0	114.8	0.6	5.5	5.8
Top diameter	37	78.3	78.2	55.0	97.0	1.5	8.9	11.3
Height at centre	34	74.8	73.9	60.0	87.4	1.1	6.4	8.6
Height at side	34	71.2	72.0	59.7	84.4	1.1	6.2	8.7
Aperture diameter	41	30.5	31.0	26.6	32.9	0.3	1.8	5.9
Aperture height	16	11.1	11.2	8.9	13.2	0.4	1.6	14.8
Angle of sides	36	85.5	85.3	74.0	91.2	0.7	4.2	4.9
Number of ribs	42	6.1	6.0	5.0	8.0	0.1	0.7	12.1
Dorsal flatness*	34	1.0	1.0	0.8	1.1	0.0	1.0	9.4
Chisasibi								
Base diameter	65	97.4	96.8	86.0	114.4	0.8	6.4	6.5
Top diameter	54	84.0	85.2	66.0	95.6	1.0	7.4	8.8
Height at centre	38	71.5	71.3	60.9	86.3	1.3	11.6	7.3
Height at side	38	75.6	73.4	66.3	86.8	0.9	5.5	7.3
Aperture diameter	19	28.2	28.0	26.0	28.3	0.3	1.4	5.0
Aperture height	11	11.4	11.5	9.1	12.8	0.4	1.3	11.1
Angle of sides	35	85.4	85.9	74.1	90.3	0.7	4.0	4.7
Number of ribs	23	6.3	6.0	5.0	8.0	0.2	0.8	13.0
Dorsal flatness*	38	0.9	1.0	0.7	1.1	0.0	0.1	10.6

Note: CV= coefficient of variation; SEM = standard error of the mean.
*Dorsal flatness = height of shell at one side divided by the height at centre.

1918) Deflandre, 1926, which has a similar angular appearance, sometimes appears in samples taken nearby in Mer Bleue, but so far has not been found in the same samples as *A. prismatica*.

Although there are known arcellid morphospecies with which *A. prismatica* shares certain characters, none has the same combination of traits (Table 2).

In 1838, Ehrenberg published an illustration of a polygonal *Arcella* with a flat dorsal surface, which he identified as a variety of *Arcella dentata*. Perty (1852) later assigned this variety to the species *Arcella angulosa* Perty, 1852 and the same morphotype was

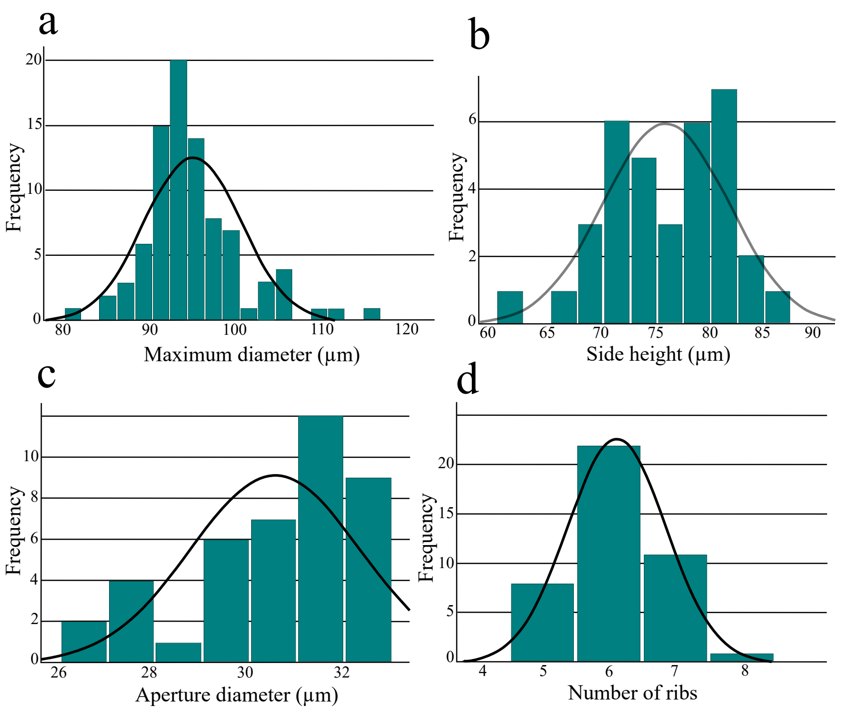

FIGURE 6. Distribution of traits of the Mer Bleue population of *Arcella prismatica*. a. Maximum diameter of the shell (mean = 94.62 µm, SD = 5.52 µm, *n* = 87). b. Height at highest side of the shell (mean = 75.80 µm, SD = 5.84 µm, *n* = 35). c. Diameter of the aperture (mean = 30.54 µm, SD = 1.79 µm, *n* = 41). d. Number of ribs on the sides of the shell (mean = 6.12, SD = 0.739, *n* = 42).

treated by Leidy (1879) as *Arcella vulgaris* var. *angulosa* (Perty, 1852) Leidy, 1879. Later authors regarded the form as belonging to the species *A. costata*, originally found by Ehrenberg in airborne dust (Ehrenberg 1847: 442; Penard 1902; Playfair 1918; Deflandre 1928). With its flat top and polygonal shape, Leidy's *A. vulgaris* var. *angulosa* (=*A. costata* var. *angulosa sensu* Playfair 1918) somewhat resembles *A. prismatica*. However, in that species, the aperture is smoothly circular, without crenulations or lobes, and the angle of the ribbed sides is less steep than that of *A. prismatica* (Figure 7a).

Leidy (1879) also recorded a faceted form of *A. mitrata*, which Deflandre (1928)—evidently without having observed the variant himself—promoted to species level as *A. leidyana* (Figure 7b). Similar to *A. prismatica*, it has nearly parallel sides and a polygonal outline in dorsal view. Deflandre (1928: 277) characterized its aperture as "ondulé plutôt que crénelé" (that is, "wavy rather than crenulate") and specified that the dorsal surface of the shell forms a "pyramidal dome" ("dôme pyramidal"). *Arcella leidyana* is also far larger—roughly twice as wide as the species we describe here, and about 2.35 times as tall—with a ventral face that is narrower than the shell at its maximum diameter (Deflandre 1928). That morphology is not seen in *A. prismatica,* in which the smallest diameter of the shell is never at the ventral face.

Arcella conica has a ribbed, polygonal shell, resembling that of *A. costata*. However, the shell has a peaked or conical dorsal surface, giving it, as Playfair (1918: 640) writes, "the appearance of a marquee-tent" (Figure 7c). The aperture is smoothly circular.

Among his illustrations of *A. mitrata*, Leidy (1879) included another faceted morphotype, which Deflandre (1928) redescribed as *A. mitrata* var. *spectabilis* (Figure 7d). Deflandre's dimensions, derived from Leidy's drawings, indicate that the subspecies is significantly smaller than the "balloon-shaped" forms of *A. mitrata*. Siemensma (2022) found the same morphotype in several Dutch peat bogs, and measurements of his populations (shell height 85–91 µm, diameter 91–98 µm, *n* = 25) agree well with Deflandre's (height 80–90 µm; diameter 86–91 µm). Because of its different shape and size and the fact that distinct populations bloom alongside the larger *A. mitrata*, Siemensma (2022) recognized *A. mitrata* var. *spectabilis* at species level as *A. spectabilis* Deflandre, 1928.

Arcella spectabilis has two characters in common with *A. prismatica*: a roughly polygonal shape

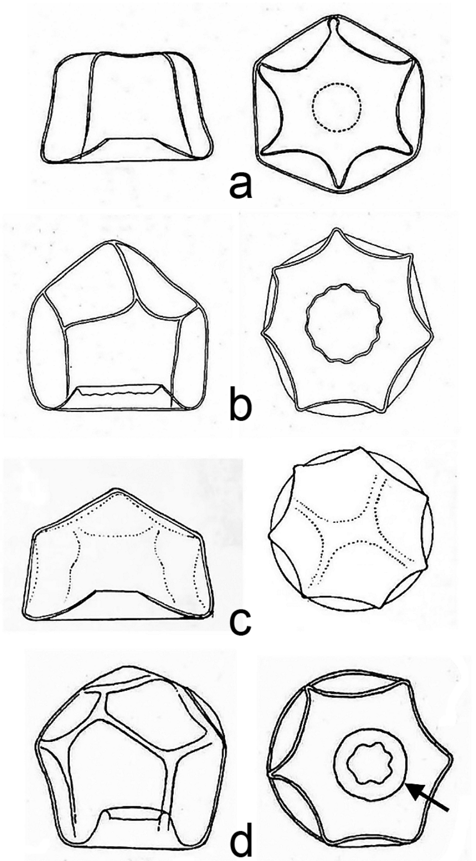

FIGURE 7. Illustrations of similar species, for comparison with *Arcella prismatica*. a. *Arcella costata* (after Ehrenberg, from Deflandre 1928). b. *Arcella leidyana* (after Leidy, from Deflandre 1928). c. *Arcella conica* (from Playfair, 1918). d. *Arcella spectabilis* (after Leidy, from Deflandre 1928). The arrow indicates the "concentric circle" mentioned by Deflandre as a character for *Arcella mitrata* var. *spectabilis*.

in ventral or dorsal view and a crenulate aperture. In Mer Bleue samples taken in 2022 from sites at which both *A. spectabilis* and *A. prismatica* occur, *A. spectabilis* individuals were always significantly ($P < 0.001$) larger than those of *A. prismatica*, with no significant overlap between any of the two species' important dimensions, except for the diameter of their apertures (Figure 9). In all populations of *A. spectabilis*, including those recorded by Leidy (1879) and Siemensma (2022) as well as those found in Mer Bleue, the appearance of the shell in lateral view is very different from that of *A. prismatica*. The dorsal face of *A. spectabilis* is domed and faceted, and never flat, smooth, or concave. Also, in that species the smallest diameter of the shell is at, or near, the ventral plane, whereas the shell of *A. prismatica* (as noted above) is widest at its ventral face. An additional difference concerns the depth of the apertural invagination (or "aperture height" as shown in Figure 8b). *Arcella spectabilis* has a deeply invaginated aperture—roughly twice as deep as that of *A. prismatica*—and a long, everted buccal tube, the top of which appears, in ventral view, as a concentric circle around the aperture, when examined by transmitted light microscopy (see arrow in Figures 7d, 10b). Deflandre (1928: 273) uses the presence of this circle as a diagnostic character for the variety: "bouche à bord crénéle avec cercle concentrique du au tube buccale" (that is, "mouth with crenulated margin with concentric circle due to buccal tube") and as a species character for *A. mitrata* itself. *Arcella prismatica* lacks a buccal tube, and this circular structure is not present in specimens from our study sites (Figure 3b,c) so does not fall within Deflandre's circumscription of var. *spectabilis*, or any variety of *A. mitrata*.

We do not have molecular data for *A. prismatica* or *A. spectabilis*. As there is some resemblance in

TABLE 2. Comparative morphometric data on species similar to *Arcella prismatica*.

Species	Shell		Aperture		Long buccal tube (concentric circle in ventral view)	Aperture crenulations	Dorsal surface
	Diameter, µm	Height, µm	Diameter, µm	Height, µm			
Arcella conica (Playfair, 1918) Deflandre, 1926	68–100	—	20–33	—	Absent	Absent	Peaked
Arcella costata Ehrenberg, 1847	52–100	—	—	—	Absent	Absent	Flat
Arcella leidyana (Leidy, 1879) Deflandre, 1928	184–188	168–176	56–88	22–23	Absent	Absent	Pyramidal
Arcella mitrata Leidy, 1876	100–180	100–162	20	23	Present	Present	Smooth dome
Arcella prismatica n. sp.	81–115	60–87	26–33	9–13	Absent	Present	Usually flat
Arcella spectabilis (Leidy, 1879) Deflandre, 1928*	107–136	109–131	26–36	22–32	Present	Present	Faceted dome

*Mer Bleue population.

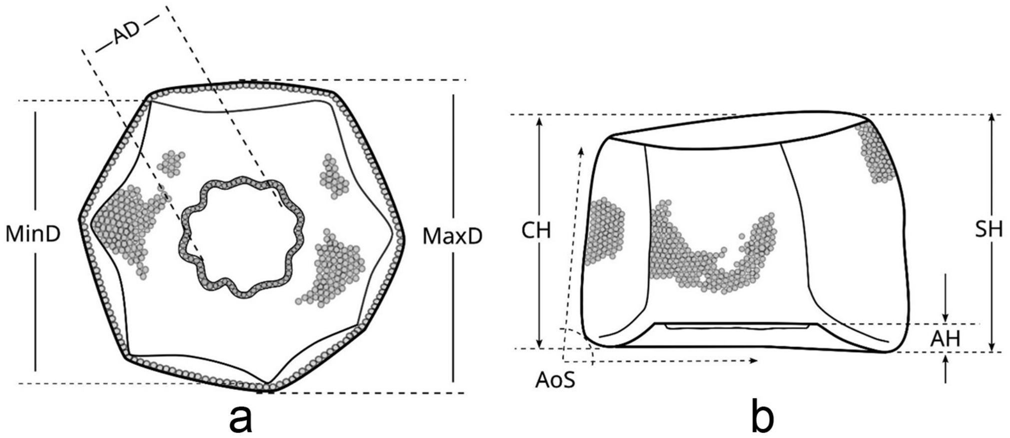

FIGURE 8. Outline of typical *Arcella prismatica*, illustrating measured characteristics. a. Ventral view. MaxD = diameter at base of shell; MinD = diameter at top; AD = aperture diameter. b. Lateral View. CH = height at centre; SH = height at highest side; AH = aperture height; AoS = angle of sides. Drawings: Bruce D.S. Taylor.

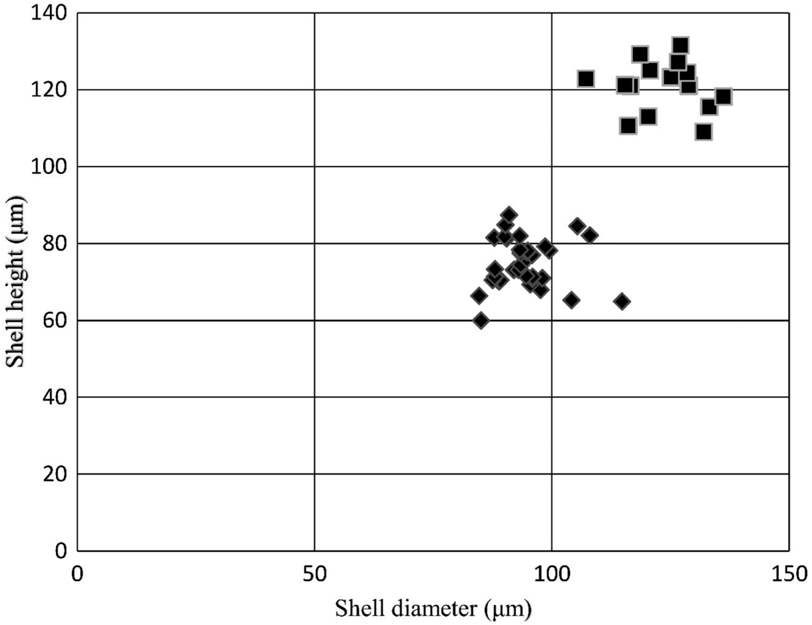

FIGURE 9. Comparison of shell size in populations of *Arcella prismatica* (diamonds) and *Arcella spectabilis* (squares) from Mer Bleue. Both height and diameter were measured (n = 50), and clustered tightly for both species. Shell height in *A. prismatica* (n = 87) differed significantly from *A. spectabilis* (n = 24; t_{106} = 20.09, P < 0.001). Shell diameter in *A. prismatica* (n = 35) differed significantly from *A. spectabilis* (n = 15; t_{48} = 29.58, P < 0.001). Not shown: aperture diameter did not vary significantly between *A. spectabilis* (n = 7) and *A. prismatica* (n = 41; t_{46} = 0.60, P = 0.55). Aperture height varied significantly between *A. spectabilis* (n = 12) and *A. prismatica* (n = 18; t_{28} = 20.71, P < 0.001).

shell shape, it may be asked whether both morphotypes are two different species or just display morphological variation within a single species. Such variation does not occur at random, but is an adaptation to environmental conditions, more influenced by external factors than by genetic inheritance (Mulot *et al.* 2017). Species living in similar environments may have the same overall shape but be distantly related (González-Miguéns *et al.* 2022). Also, intraspecific variability in the morphology of testate amoebae may be an adaptation to the environment, and morphological variability induced by environmental influences

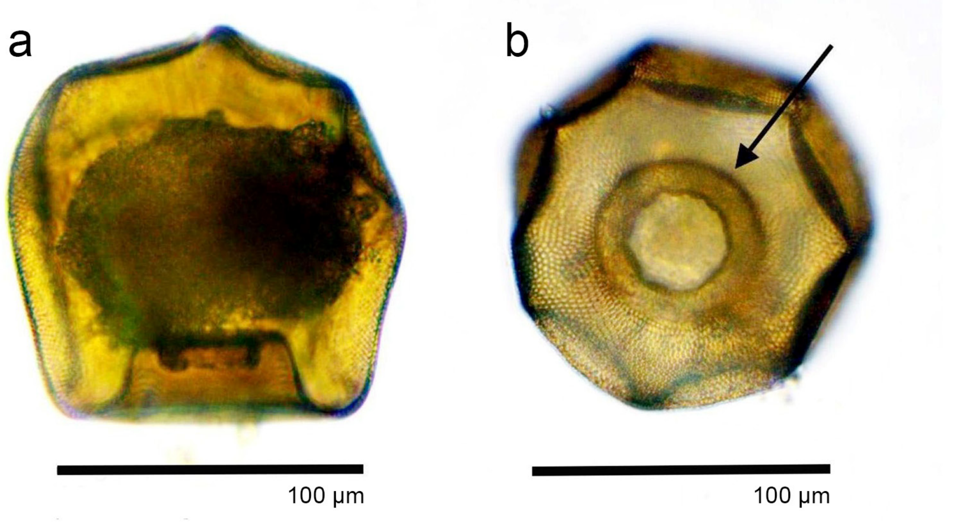

FIGURE 10. Two specimens of *Arcella spectabilis* from Mer Bleue. Arrow (b) indicates the characteristic "concentric circle" surrounding the aperture (Deflandre 1928: 277). The lateral view (a) shows typical features of *A. spectabilis*, with the smallest diameter of the shell at the ventral face, a pronounced buccal tube, and a deeply invaginated aperture. Photos: Bruce D.S. Taylor.

may result in taxonomical complications. Variability in, for example, pore numbers as well as size, is driven by phenotypic plasticity (Bobrov and Mazei 2004; Arrieira *et al.* 2016; Mulot *et al.* 2017). Although the morphotype described here could be a variation of *A. spectabilis,* the environmental conditions in our study were equal for both morphotypes as they are found living close together or intermixed in the same pond. In Mer Bleue populations, *A. spectabilis* blooms alongside *A. prismatica*, in company with other arcellid species (B.D.S.T. pers. obs.). A comparison of the two morphotypes in those populations shows significant differences in size and diameter of the shells, as well as the height (but not the diameter) of the aperture (Figure 9). In samples from Mer Bleue, the two species form distinct populations, with bimodal distribution of sizes and very little overlap in principal dimensions (Tables 1 and 3, Figure 9, Appendix S1 and S2). We consider this an important argument that these taxa are distinct species.

Deflandre's (1928) description of *A. mitrata* var. *spectabilis* is based on the observations and drawings of Leidy (1879). It is obvious that he never saw this species himself, as he mentions only North America in his comments on its geographic distribution. We have found *A. spectabilis* in Europe, at several locations in the Netherlands, always in water bodies with *Sphagnum*. A morphotype like *A. prismatica* has never been observed there. Also, Leidy (1879), who has seen and depicted *A. spectabilis* himself, does not record a morphotype like *A. prismatica* in the text or illustrations of his book. If *A. prismatica* is part of the phenotypic plasticity of *A. spectabilis*, that plasticity should also have been observed by Leidy and in the Dutch and Mer Bleue populations.

There is at least one early record of an arcellid that might be conspecific with *A. prismatica*. In a study of *Arcella* morphology, Cushman and Henderson (1906) included clear photomicrographs of a shell resembling our new species (Figure 11), but do not state where their specimens were collected. They identified it as *A. mitrata*, which Deflandre (1928) recognized as

TABLE 3. Morphometrics of *Arcella spectabilis* population at Mer Bleue (see also Appendix S2). Measurements in µm.

Characters	*n*	Mean	Median	Min.	Max.	SEM	SD	CV
Base (ventral) diameter	21	99.5	98.8	88.6	112.6	1.4	6.6	6.6
Maximum diameter	21	123.3	125.1	107.2	136.2	1.6	7.2	5.9
Height at centre	15	120.8	121.2	109.0	131.5	1.7	6.6	5.4
Aperture diameter	7	31.0	30.9	26.0	35.9	1.1	3.0	9.8
Aperture height	12	26.8	26.8	22.2	31.7	0.8	2.7	10.2

Note: CV = coefficient of variation; SEM = standard error of the mean.

a mistake, although he did not suggest an alternative identification. No view of the aperture was given, so reliable identification is not possible.

Ecology

In Mer Bleue, *A. prismatica* has been found in samples of surface water, in close association with algae and mixotrophic ciliates. It has so far not been retrieved from samples taken in the adjacent *Sphagnum*, the benthos, or in mineralized sediments at the north side of the drainage arm, where the lake is bounded by a forested ridge.

In Chisasibi, the novel amoeba was collected from the margins of two shallow ponds, in grassy fens, among hummocks of *Sphagnum*. These mires support a few small shrubs, but no trees, although they are surrounded on three sides by Black Spruce (*Picea mariana* (Miller) Britton, Sterns & Poggenburgh). *Arcella prismatica* shells—both live and vacant—were most abundant in site A, where they were found entangled in the leaves of *S. riparium*, a transient species associated with wet minerotrophic conditions (Galka *et al.* 2018). The water at both sites is neutral to mildly acidic (pH 6.0–6.2, unpubl. data), and the assemblage of plant species identifies these as "open fens" according to the classification scheme outlined by Sims *et al.* (1982).

Habitat segregation within humic peatland lakes is still poorly understood (Kuczynska-Kippen 2008). It is evident that there are zones in these water bodies that support distinct and undersampled assemblages of microbial organisms and that close investigation of such microhabitats could reveal species rarely found elsewhere (Błedzki and Ellison 2003; Kuczynska-Kippen 2008). The possibility that floating clumps of vegetation in Mer Bleue represent a distinctive microhabitat is supported by the frequent occurrence of unusual ciliate taxa, including undescribed morphotypes of *Frontonia* Ehrenberg, 1838, *Dileptus* Dujardin, 1841, *Leptopharynx* Mermod, 1914, *Metacystis* Cohn, 1866, and *Pelagodileptus* Foissner, Berger & Schaumberg, 1999 (B.D.S.T. pers. obs). However, the appearance of a distinctive microbial life in such floating mats could also be an artifact of spotty sampling. Certainly, the occurrence of *A. prismatica* in less mature peatlands near the coast of James Bay shows that this species is not restricted to the conditions in which it blooms in Mer Bleue.

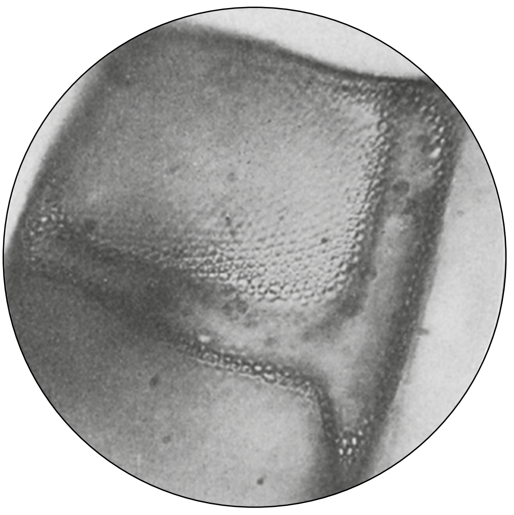

FIGURE 11. *Arcella* sp. ("1200× magnification"), reproduced from Cushman and Henderson (1906), originally identified as *A. mitrata*. Public domain.

In all our sampling sites, the species appears in heavily saturated vegetation in or adjacent to open water, so it is potentially of value for palaeohydrology as a wet indicator. The use of testate amoebae as proxies for palaeoecological conditions is well established (Hendon *et al.* 2001; Booth *et al.* 2008; Mitchell *et al.* 2008; Roe *et al.* 2017) and fossil testate amoebae found in Mer Bleue have already provided a detailed sequential record of environmental changes in the area (Elliott *et al.* 2012; Kopp *et al.* 2013). Although many species of *Arcella* and *Galeripora* have been recorded in analyses of Mer Bleue core samples (Talbot *et al.* 2010; Elliott *et al.* 2012), this novel arcellid has not been mentioned in the palaeoecological literature. To assess the suitability of the species as an indicator further investigation would be necessary.

Implications for arcellinid diversity

Arcella, compared to most groups of testate amoebae, is a well-known and well-characterized genus. The appearance of a novel species of this familiar genus within the limits of a populous North American city is a reminder that there are still large gaps in distributional data on freshwater protists. That this morphotype was quickly found again in samples taken nearly 1000 km away raises the possibility that it might be a common and widespread organism, at least in post-glacial peatlands of eastern Canada. However, genetic data would be needed to determine whether the morphotype, as we have described it, comprises a complex of cryptic species. Because this morphotype has been overlooked in earlier records from North America, its absence from studies conducted outside of the Nearctic biogeographic realm does not need to be interpreted as evidence of endemicity. This, too, could be an artifact of undersampling. That said, it is generally understood that arcellinid amoebae, with their durable and distinctive shells, are potentially useful organisms to test the hypothesis that some microbial species have biogeographically restricted ranges (Heger *et al.* 2012; Reczuga *et al.* 2015; Feres *et al.* 2016). As a species that is readily identified at low magnification and known to bloom prolifically in the locations where it occurs, *A. prismatica* might provide useful data for that discussion.

Author Contributions

Writing – Original Draft: B.D.S.T.; Writing – Review and Editing: B.D.S.T., M.C.S., and F.J.S.; Visualization: B.D.S.T., M.C.S., and F.J.S.; Conceptualization: B.D.S.T. and F.J.S.; Methods: B.D.S.T., M.C.S., and F.J.S.; Investigation: B.D.S.T., M.C.S., and F.J.S.

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SUPPLEMENTARY MATERIALS:

APPENDIX S1. Measurements of *Arcella prismatica* from Mer Bleue, Ontario, and Chisasibi, Eeyou Istchee, Quebec.
APPENDIX S2. Measurements of *Arcella spectabilis* shells from Mer Bleue, Ontario.