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Evaluating the Taxonomic Identity in Four Species of the Lobose Testate Amoebae Genus *Arcella* Ehrenberg, 1832

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Summary. The taxonomic identity in microbial eukaryotes remains an impediment to discussing ecology, biogeography and phylogeny, mainly due to a lack of standards in organism descriptions and few comparative works. The lobose testate amoebae (Arcellinida) present an ideal study system, as progress is severely hindered due to taxonomic confusion. In the present survey, we have examined the morphology, biometry and ecology of 2400 individuals in the genus *Arcella* Ehrenberg, 1832, collected from the Tiete River in Sao Paulo, Brazil. We then contrasted these new data with 26 previously described species, varieties and forms, looking for consistencies and trying to establish distinct entities. Using a combination of morphology and multivariate statistics we were able to determine 4 distinct taxa (*Arcella hemisphaerica*, *Arcella discoides*, *Arcella gibbosa* and *Arcella brasiliensis*), each of them encompassing a number of other non-distinct nominal taxa. We describe in detail each of the 4 taxa with notes on ecology and biogeography, and list the indistinguishable names in an effort to make identification and taxonomy in the testate amoebae a more objective and precise exercise by clarifying the taxonomic identity.

Key words: Taxonomic identity, Arcellinida, *Arcella*, Morphology, Biometry.

INTRODUCTION

A biological name refers to a set of individuals found in nature. It is a human construct that is linked to natural entities by type specimens and/or descriptions, this link defined as the taxonomic identity (Patterson and Larsen 1992), also referred to as taxonomic concept in certain contexts (Kennedy *et al.* 2006).

The name should explicitly and objectively refer to a natural entity, with the purpose of enabling discussion and future changes with sufficient stability (see ICZN (Ride *et al.* 1999), ICBN (Mcneill *et al.* 2006) and ICNB (Lapage *et al.* 1992) for objectives of biological nomenclature). This objectivity should be present regardless of species concepts and methods used in the description of an organism, lineage or population (de Queiroz, 1998). Taxa (i e., names) are the currency with which we build hypothesis in many biological disciplines; including phylogenetics, ecology and biogeography. Hence confusion or inaccuracy in this foundation is a severe impediment.

In the lobose testate amoebae (Arcellinida), the problem of taxonomic identity is beginning to surface as studies reveal non-monophyly of morphologically established genera, such as *Nebela* and *Hyalosphaenia*

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(Lara *et al.* 2008). The strongly supported phylogenetic reconstructions indicate that in each case, there are in nature more entities that were not possible to be separated on the basis of morphology alone. Yet the historical taxonomic confusion, a result of the lack of preserved specimens, and the lack of standardized approach to descriptions, makes it difficult to rename organisms in an objective way that refers back to the organisms originally identified and described (Lahr *et al.* 2008). Furthermore, this is a serious impediment for areas dependant on taxonomy and identification such as ecological studies, and biogeographic surveys (Smith *et al.* 2008).

In order to achieve meaningful discussion regarding the monophyly of genera or historical relationships among them, first it is necessary to flesh out the identity of less inclusive taxa established by traditional methods, as was done with *Centropyxis* (Lahr *et al.* 2008), by determining which are the entities with enough evidence to be called a distinct taxon using methods comparable to those of the original descriptions. Such a framework will help resolve the problem confounding research in other disciplines such as phylogenetics, ecology and biogeography of microbial eukaryotes.

The present study focuses on establishing the identity of four nominal species in the genus *Arcella* Ehrenberg, 1832, by using a combination of morphometry, multivariate statistics and morphological data in order to thoroughly compare natural populations to previously described taxa.

The genus *Arcella* comprises the lobose testate amoebae whose tests are made up with secreted material, presumably chitinous, arranged in hexagonal units; there are more than fifty decribed species, and many more varieties and forms (Meisterfeld 2002). Many of the descriptions are incomplete and based on few specimens, which is a common problem in lobose testate amoebae (Lahr and Lopes 2006) and other groups of microbial eukaryotes (Patterson and Larsen 1992).

We analyzed a large number of individuals (2400) belonging to the genus *Arcella* from a natural population in the Tiete River, Sao Paulo, Brazil, which could be ambiguously assigned to 7 nominal species and 18 more infra-subspecific taxa. We performed a comparative morphometric analysis of the collected specimens and previously reported variation on the nominal species to test the validity of those taxa.

MATERIAL AND METHODS

Samples were taken from two localities at Ecological Park of the Tietê River, São Paulo – Brazil in February and August 2004. The first locality was at the river itself (23º29′374″S, 46º31′500″WO), a flowing water environment, and the second at a marginal lake (23º29′055″S, 46º30′939″WO) 100 m away from the river. In each locality, samples were taken separately from the sediment and from roots of floating aquatic plants. Sampling methods, biometry, morphological analysis and scanning electron microscope (SEM) preparations follow (Lahr and Lopes 2006). Specimens preserved in 70% ethanol and stubs used for ultrastructural analyses are deposited at Laboratorio de Malacologia, at Instituto de Biologia, Universidade de Sao Paulo (IBUSP).

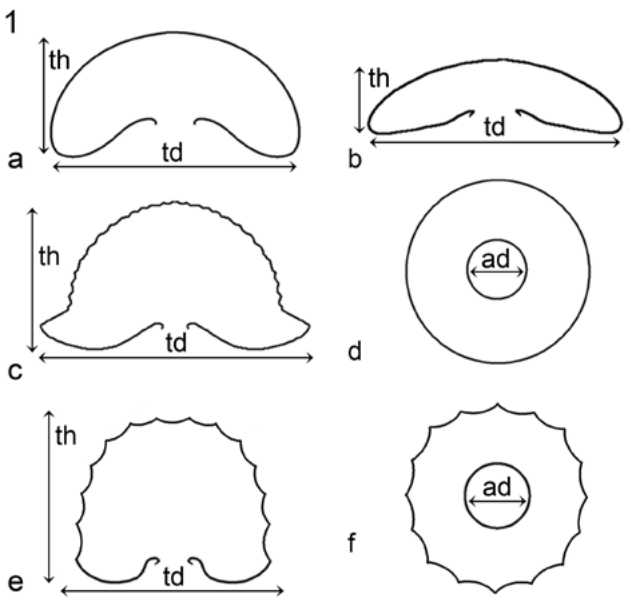
We extracted 2400 individuals that were examined, identified, and measured using a light microscope under magnification of 200––400 ×, depending on the specimen. We decided to restrict measures to a 5 μm resolution, given the tri-dimensional characteristics of the test. Several individuals, representing a wide range of variability for each the nominal taxa, were chosen for examination with the SEM.

Three morphometric characters were measured in order to allow comparisons with previous literature: test diameter (td), test height (th) and aperture diameter (ad) (Fig. 1). Statistical analyses were performed using the software STATA 9.1 (StataCorp 2005).

Specimens were classified to putative taxa using qualitative and morphometric characters (Fig. 1) from original and subsequent descriptions (references in Table 1). Using these data, the numbers of specimens unambiguously assigned to each taxon as well as the number assignable to multiple taxa were tallied (Table 1), to a total of 26 nominal taxa. Four complexes are readily distinct by qualitative disjunctive morphological features: 1 – *A. hemiphaerica* + *A. rotundata*; 2 – *A. discoides* + *A. megastoma* + *A. polypora*; 3 – *A. gibbosa* and 4 – *A. brasiliensis*. These four groups were further analyzed separately. Terminology used in morphological descriptions follows (Foissner and Korganova 2000, Lahr and Lopes 2006, Lahr and Lopes 2007) and (Luftenegger *et al.* 1988).

The diagnostic utility of the morphometric characters was evaluated by using box plots and multivariate statistics for complexes 1, 2 and 3. *A. brasiliensis* is a clearly distinct group by qualitative morphological characters and does not need further statistical analysis.

Distributions of characters for each of the nominal species, inside each of the three groups, were compared using box plots. Principal component analysis (PCA) was used to determine how specimens classified to the nominal species related in multivariate space (Pimentel 1979). PCA analysis was run using a correlation matrix, standardized loadings on principal components (not shown) were examined to determine the relative contributions of each morphometric character to each component, and factor scores were plotted to examine how species partitioned in morphospace. For each of the complexes *A. hemisphaerica* + *A. rotundata* and *A. discoides* + *A. megastoma* + *A polypora*, we ran two PCAs, one using only unambiguously identified taxa where we can plot dots referring to nominal species (746 and 765 individuals, respectively), and one using the full dataset where we can't designate names due to ambiguity (1125 and 1160 individuals, respectively). In both cases the results are essentially identical. For *A. gibbosa*, we have only plotted a non-named full (106 individuals) dataset looking for any sharp distinctions, since all infra-subspecific taxa were ambiguous.



Figs 1a–f. Schematic outlines of tests of *Arcella* species, showing position of measured axis. **a** – lateral view of *A. hemisphaerica*; **b** – lateral view of *A. discoides*; **c** – lateral view of *A. brasiliensis*; **d** – apertural view for *A. hemisphaerica*, *A. discoides* and *A. brasiliensis*; **e** – lateral view of *A. gibbosa*; **f** – apertural view of *A. gibbosa*. Abbreviations: th – test height; td – test diameter; ad – aperture diameter.

RESULTS

The morphometric variables analyzed for each of the three complexes (*A. hemisphaerica* + *A. rotundata*, *A. discoides* + *A. polypora* + *A. megastoma* and *A. gibbosa*) coupled with the analysis of morphospace by multivariate statistics shows no distinction among taxa embedded within each group. *A. brasiliensis* is a distinct rare taxon that can be unambiguously identified by morphology alone. Here, we examine, on a case-by-case basis, the evidence for non-distinction and provide a single description for each of the four complexes, while preserving the names and authorities of nominal taxa under a list of previously described non-distinct entities. The name adopted for the whole complex is the oldest one available according to the Principle of Priority.

A. hemisphaerica + *A. rotundata* complex

We identified 1120 individuals as either of *A. hemisphaerica* or *A. rotundata* and 450 of those individuals ambiguously fit descriptions of 8 more infra-subspecific taxa based on previous descriptions (see Table 1). The three morphometric characters analyzed shows complete overlap for the two species and infra-subspecific taxa (Figs 2a–d). The result of PCA analysis shows that the two species are indistinguishable (Figs 2e, f), the plot using unambiguously identified specimens reveals that the distinction is arbitrary (Fig. 2e). We attribute the "striped" pattern in the principal components plot to the limitation of measures taken in the light microscope: we have determined that measures would be rounded to increments of 5 µm, to account for limited resolution of the microscope and tridimensional characteristics of the test. The only qualitative character that could further divide this group is the presence of undulations or depressions in the abapertural surface, but this is a natural variation of tests in *A. hemiphaerica*, as observed in laboratory clonal cultures (Lahr D., pers. obs.). Therefore, we consider these nominal taxa referring to a single entity with 9 non-distinguishable nominal taxa within.

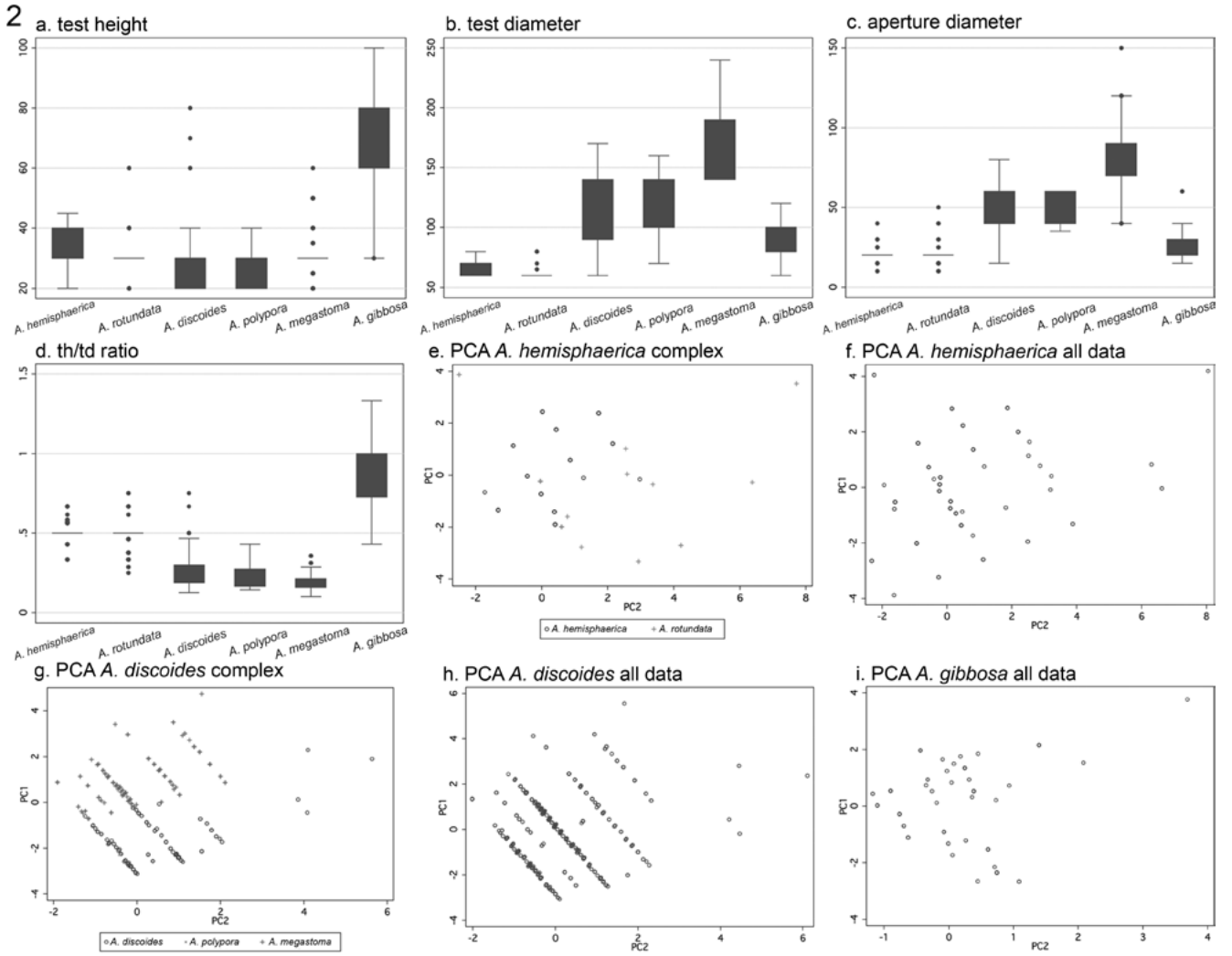
Arcella hemisphaerica Perty, 1852
Previously described non-distinct taxa
Arcella hemisphaerica Perty, 1852, Pl. 9, Fig. 5
Arcella hemisphaerica fma *undulata* Deflandre, 1928: 214, Figs 122–124
Arcella hemisphaerica var. *depressa* Playfair, 1918, Pl. 34, Fig. 7
Arcella hemisphaerica var. *tuberculata* Stepanek, 1963: 56, Figs 7, 54
Arcella rotundata Playfair, 1918, Pl 34, Fig. 1
Arcella rotundata var. *stenostoma* Deflandre, 1928: 233, Figs 226–232
Arcella rotundata var. *stenostoma* fma *undulata* Deflandre, 1928: 235, Figs 233–234
Arcella rotundata var. *aplanata* Deflandre, 1928: 235, Figs 235–239
Arcella rotundata var. *alta* Playfair, 1918, Pl 34, Fig. 2
Arcella rotundata var. *alta* fma *undulata* Stepanek, 1963: 58, Figs 38, 40
Examined material: 1120 individuals under LM, 20 individuals under SEM

Morphology: Test circular in apertural view (Fig. 3), hemispheric in lateral view (Fig. 4). Abapertural region may be provided with depressions giving the test an undulate appearance (Figs 5, 6). Whole test made of chitinous material built from box-like, hollow, hexagonal units (Figs 7, 8). A small pore (1–2 µm) is present at each vertex of each hexagon (Fig. 9). Hexagons appear

Table 1. List of diagnosis and morphometric amplitude for the nominal taxa in the genus *Arcella* studied from Tiete River, Sao Paulo. Amplitudes are based on the original description and expanded by subsequent researches. Measures in µm. Characters as designated in Fig. 1. NT – number of specimens assigned to this taxon alone, NU – number of specimens that could be assigned to this and other taxa, ? – not stated in any taxonomic work, ST – same as parent taxon.

Nominal taxa	Diagnosis	Morphometric range						References
		td	th	ad	th/td	NT	NU	
A. hemisphaerica Perty, 1852	test hemispheric in lateral view	33–69	23–45	11–20	0.48–0.75	597	447	Perty 1852, Penard 1890, Playfair 1918, Wailes 1919, Deflandre 1926, Deflandre 1928, Decloitre 1954, Green 1975, Vucetich 1973, Ogden and Hedley 1980, Van Oye 1956
A. hemisphaerica fma *undulata* Deflandre, 1928	depressions on abapertural surface	42–51	33–40	11–16	?	0	114	Deflandre 1928, Decloitre 1954, Decloitre 1965, Vucetich 1973, Van Oye 1956, Hardoim and Heckman 1996
A. hemisphaerica var. *depressa* Playfair, 1918	abapertural surface less depressed (low th/td ratio)	34–57	21–36	10–15	?	0	391	Playfair 1918, Deflandre 1928
A. hemisphaerica var. *tuberculata* Stepanek, 1963	tubercules on abapertural surface	55	41	20	?	0	53	Decloitre 1976, Stepanek 1963
A. rotundata Playfair, 1918	subspheric test, low th/td ratio	47–62	25–30	12–17	0.53–0.55	44	354	Deflandre 1928, Green 1975, Van Oye 1956
A. rotundata var. *stenostoma* Deflandre, 1928	relatively small aperture (low ad/td)	39–60	22–30	9.3–16	0.47–0.54	0	51	Deflandre 1928, Vucetich 1973, Dioni 1970
A. rotundata var. *stenostoma* fma. *undulata* Deflandre, 1928	relatively small aperture, depressions on abapertural surface	40–48	20–25	12–15	?	0	10	Deflandre 1928, Decloitre 1948, Vucetich 1973, Dioni 1970
A. rotundata var. *aplanata* Deflandre, 1928	more flattened than type form (lower th/td)	64–86	24–33	9.5–30	0.35–0.40	26	9	Deflandre 1928, Van Oye 1956
A. rotundata var. *alta* Playfair, 1918	higher th/td	36–53	22–32	10–15	0.6–0.62	3	16	Playfair 1918, Deflandre 1928
A. rotundata fma *undulata* Stepanek, 1963	depressions on abapertural surface	ST	ST	ST	ST	0	43	Stepanek 1963
A. discoides Ehrenberg, 1871	low th/td, circular in apical view	90–157	25–57.3	21–52	0.2–0.25	100	294	Deflandre 1928, Decloitre 1948, Green 1975, Vucetich 1973, Dioni 1970, Hardoim and Heckman 1996, Velho 1996

A. discoides var. *difficilis* Deflandre, 1928	marginal rim	108–128	25–36	37–48	0.25	0	37	Deflandre 1928
A. discoides var. *scutelliformis* Playfair, 1918	higher than type, higher th/td	44–95	25–37	14–34	0.29–0.34	62	104	Deflandre 1928, Van Oye 1956
A. discoides var. *foveosa* Playfair, 1918	depressions on abapertural surface	53–78	25–38	18–28	0.3–0.35	0	12	Playfair 1918, Deflandre 1928
A. discoides var. *pseudovulgaris* Deflandre, 1928	presence of marginal rim	76–128	25–39	20–48	0.27–0.29	9	43	Deflandre 1928
A. discoides var. *pseudovulgaris* fma. *undulata* Deflandre, 1928	marginal rim, depressions on abapertural surface	118–122	25–40	42–50	?	0	16	Deflandre 1928
A. discoides var. *pseudovulgaris* fma. *arcuata* Deflandre, 1928	marginal rim, arched test in lateral view	103–168	25–41	39–75	?	15	173	Deflandre 1928, Vucetich 1973
A. polypora Penard, 1890	ring of pores surrounds aperture	75–200	25–42	37–43	0.2–0.29	5	132	Penard 1980, Deflandre 1928
A. polypora var. *curvata* Deflandre, 1928	arched test in lateral view	120–135	25–43	48–57	?	0	168	Deflandre 1928
A. megastoma Penard, 1902	subspheric test, low th/td ratio, high number of nuclei	140–402	25–45	40–216	0.22	400	300	Deflandre 1928; Green 1975; Vucetich 1972/1973; Hardoim and Heckman 1996; Velho 1996
A. megastoma fma *arcuata* Deflandre, 1928	arched test in lateral view	198–215	25–45	83–110	?	0	124	Deflandre 1928
A. gibbosa Penard, 1890	circular domed test, hemispherical in side view, regular depressions on the abapertural surface	70–140	45–80	18–32	0.53–0.69	7	64	Deflandre 1928, Decloitre 1948, Decloitre 1954, Green 1975, Vucetich 1973, Velho 1996, Tsyganov 2006
A. gibbosa var. *levis* Deflandre, 1928	smooth abapertural surface	90–95	60	30	?	0	2	Deflandre 1928, Dioni 1970
A. gibbosa var. *mitriformis* Deflandre, 1928	higher abapertural dome, high th/td	55–95	46–90	14–28	0.63–0.94	22	77	Deflandre 1928, Decloitre 1948, Decloitre 1954, Vucetich 1973
A. gibbosa var. *aplanata* Van Oye, 1956	flattened abapertural dome, low th/td	55	?	15	0.27	0	13	Van Oye 1956, Decloitre 1976



Figs 2a–i. a–d – box plots of morphometrical measures for the seven nominal species recognized within the genus *Arcella*. Boxes contain the center 50% of values, box edges are the first and third quartiles, whiskers cointain 1.5 midranges, outliers represented by dots; **e–i** – plots of principal component 1 (PC1) versus principal component 2 (PC2) for the various complexes encountered within the genus *Arcella*; **e** – principal component analysis plot of the individuals in the *Arcella hemisphaerica* complex that could be unambiguously assigned to either *A. hemisphaerica* or *A. rotundata*; **f** – PCA plot of all individuals assigned to the *A. hemisphaerica* complex; **g** – PCA plot of the individuals in the *A. discoides* complex that could be unambiguously assigned to either *A. discoides*, *A. polypora* or *A. megastoma*; **h** – PCA plot of all individuals assigned to the *A. discoides* complex; **i** – PCA plot of all the individuals assigned to the *A. gibbosa* complex.

inflated at both sides of test (apertural and abapertural). Aperture central (Fig. 10), invaginated, surrounded by a small, curled lip (Fig. 11). Test light yellow to deep brown. Provided with two nuclei, as observed in the specimens that had cytoplasm.

Biometry: All measures are highly variable (CV between 10.24–15.99, Table 2) but within the limits usually expected for populations of testate amoebae (Bobrov and Mazei 2004). Aperture diameter presents the lowest standard error of the mean (0.1). Size frequency analysis shows that *Arcella hemispherica* complex has a main size class and a wide size range for all measured characters. For test diameter, 77.7% of measures are within 55–65 µm. For test height, 96.1% of measures are within 25–35 µm and for aperture diameter, 91.7% of measures are within 15–25 µm.

Table 2. Biometric characterization of the investigated *Arcella* species from Tiete River, Sao Paulo. Characters are as designated in Fig 1. Measurements in µm. x – arithmetic mean, M – median, Min – minimum, Max – maximum, SD – standard deviation, SE – standard error of the mean, CV – coefficient of variation in %, n – number of investigated specimens.

	x	Min	Max	SE	SD	CV	n
Arcella hemisphaerica							
Test height	34.61	20.0	60.0	0.15	5.15	14.88	1126
Test diameter	68.48	60.0	80.0	0.22	7.52	10.98	1126
Aperture diameter	20.54	10.0	50.0	0.10	3.28	15.99	1125
th/td ratio	0.51	0.25	0.75	0.00	0.05	10.24	1126
Arcella discoides							
Test height	29.59	20.0	80.0	0.23	7.83	26.46	1163
Test diameter	140.35	60.0	240.0	1.20	40.99	29.20	1163
Aperture diameter	65.06	15.0	150.0	0.69	23.47	36.08	1160
th/td ratio	0.23	0.10	0.75	0.00	0.08	36.06	1163
Arcella gibbosa							
Test height	71.65	30.0	100.0	1.78	18.29	25.53	106
Test diameter	84.67	60.0	120.0	1.46	15.00	17.71	106
Aperture diameter	24.43	15.0	60.0	0.65	6.74	27.57	106
th/td ratio	0.85	0.43	1.33	0.02	0.20	23.72	106
Arcella brasiliensis							
Test height	84.00	80.0	100.0	4.00	8.94	10.60	5
Test diameter	144.00	110.0	160.0	9.27	20.74	14.40	5
Aperture diameter	32.00	20.0	40.0	3.74	8.37	26.10	5
th/td ratio	0.59	0.50	0.73	0.04	0.09	15.06	5

Ecology and geographic distribution: This taxon is known from all continents (Ogden and Hedley 1980). In Brazil, the species has been registered in plankton samples for Mato Grosso (Green 1975), Minas Gerais (Dabés 1995, Bonecker *et al.* 1996); has also been registered from samples of aquatic macrophytes from Mato Grosso (Hardoim and Heckman 1996). The present survey encountered most individuals (81.4%) associated to the roots of aquatic plants in a running water environment (the river itself). To a lesser degree, the individuals were sampled for all other micro-habitats surveyed, except the sediment of the river (Table 3).

Remarks: The individuals observed here are concordant to *Arcella hemisphaerica* recent re-description in (Tsyganov and Mazei 2006/2007), and we have not found individuals that would fit their new combination *Arcella intermedia* (Deflandre, 1928), this probably corroborates the status of that entity as a distinct species.

A. discoides + *A. polypora* + *A. megastoma* complex

We identified 1163 individuals to one of the three species, and 572 of those individuals ambiguously fit descriptions of 9 more infra-subspecific taxa, as portrayed in Table 1. The three morphometric characters

Table 3. Ocurrence according to habitat of the *Arcella* species studied from Tietê River. A river and a lake were sampled, and within each, two different microhabitats: the sediment and the roots of floating aqatic plants. Numbers in %, n – number of specimens.

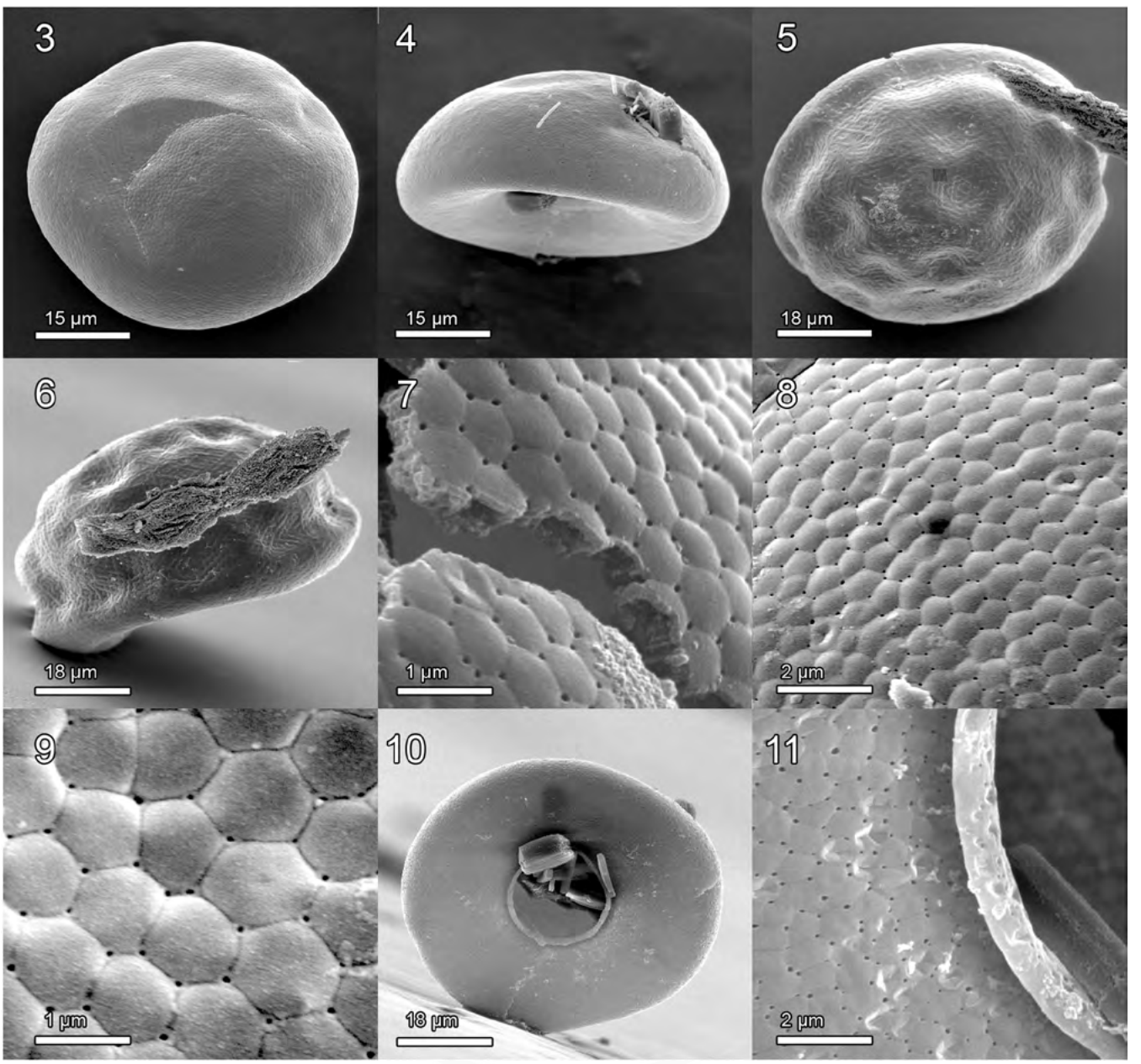
Habitat	River		Lake	
	Roots	Sediment	Roots	Sediment
Arcella hemisphaerica (n = 1125)	81.4	0.0	13.3	5.3
Arcella gibbosa (n = 106)	12.8	0.0	68.1	19.1
Arcella discoides (n = 1195)	57.7	4.8	33.0	4.5
Arcella brasiliensis (n = 5)	20.0	0.0	20.0	60.0

analyzed shows overlap between the specific taxa (Figs 2a–d), and the PCA shows an arbitrary division between *A. discoides* and *A. megastoma* (Figs 2g, h). *A. polypora* is within the range of *A. discoides* in morphometric space (Fig. 2g). Here again we attribute the "striped" pattern in the principal component plot to limited resolution of the light microscope. A few individuals of *A. discoides* located to the right of the PC2 axis might constitute a separate entity, but we were not able to identify a diagnostic feature for those specimens. Further sub-cellular characterization and molecular work are needed to be able to differentiate those particular organisms. The variation in number of nuclei might represent a character worth looking into. Therefore, we consider these nominal taxa referring to a single entity, with 11 nominal taxa within.

Arcella discoides Ehrenberg, 1871
Previously described non-distinct taxa
Arcella discoides – Ehrenberg, 1871: 259, PL 3, Fig. 1
Arcella discoides var. *difficilis* – Deflandre, 1929: 257, Figs 327,328
Arcella discoides var. *scutelliformis* – Playfair, 1918, Pl 34, Fig. 8
Arcella discoides var. *foveosa* – Playfair, 1918, Pl 34, Fig. 9
Arcella discoides var. *pseudovulgaris* – Deflandre, 1928: 261, Figs 340–344
Arcella discoides var. *pseudovulgaris* fma. *undulata* – Deflandre, 1928: 261, Fig. 345
Arcella discoides var. *pseudovulgaris* fma. *arcuata* – Deflandre, 1928: 261, Figs 346–348
Arcella polypora – Penard, 1890: 156 Pl 8, Fig. 2, 9
Arcella polypora var. *curvata* – Deflandre, 1928: 265 Figs 357–362
Arcella megastoma – Penard, 1902: 409
Arcella megastoma fma. *arcuata* – Deflandre, 1928: 268, Figs 373–374
Examined material: 1198 individuals under LM, 19 individuals under SEM

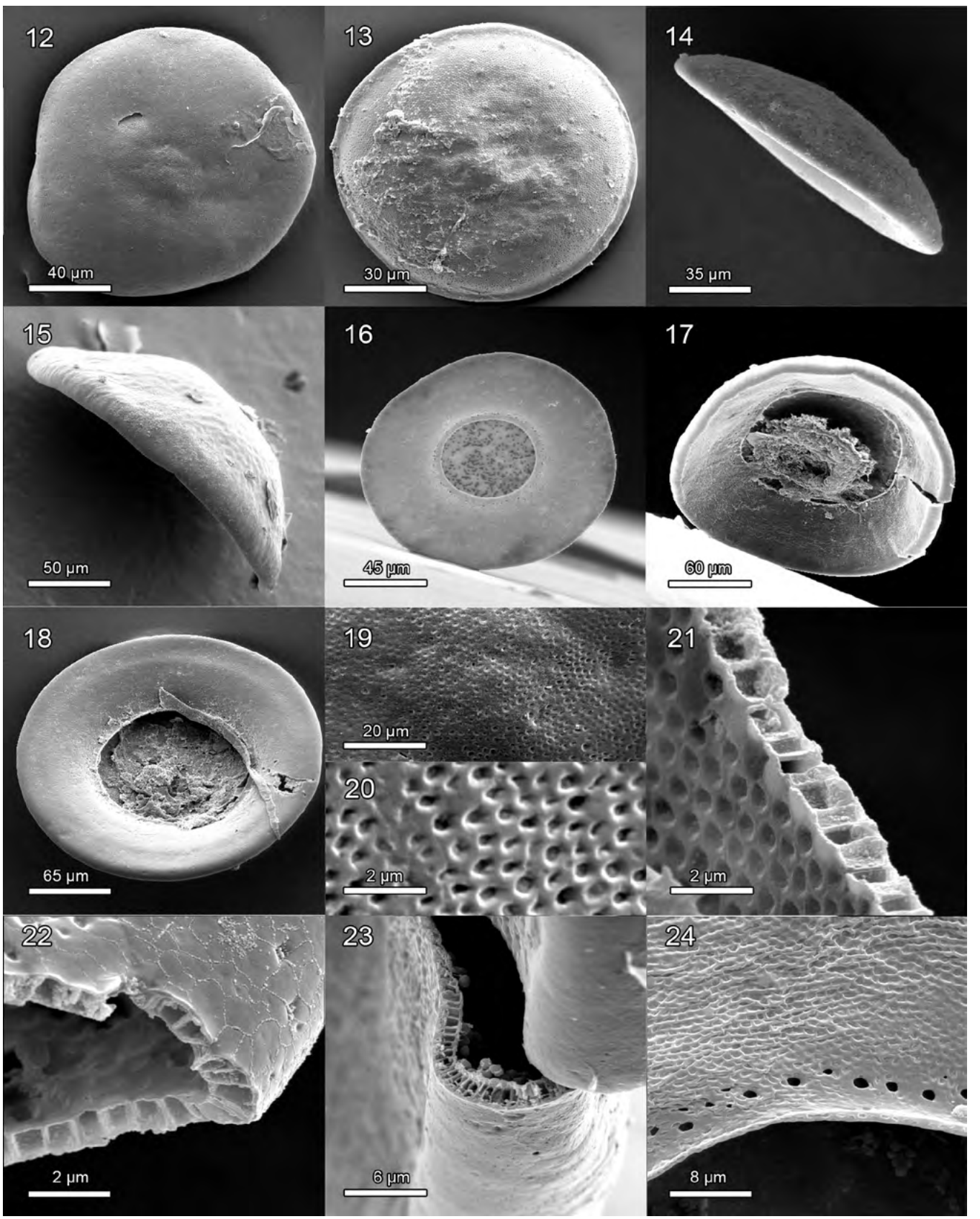
Morphology: Test circular to elliptic in apertural view (Figs 12, 13), flattened to a disc shape in lateral view (Fig. 14), sometimes arched (Fig. 15), arched tests look elliptical in apertural view. Aperture central (Figs 16, 17, 18), circular in most cases, elliptical in those who present an arched test (Fig. 17, long axis of aperture is perpendicular to long axis of test), delimited by a small curled lip. Whole test made of chitinous material built from box-like, hollow, roughly hexagonal units (Figs 19, 20, 21). The conspicuous pattern created by the building units is not visible at the abapertural region due to an additional covering layer (Figs 22, 23), which may cover the whole abapertural region evenly. At the apertural region, there is no covering layer, therefore, the pattern is visible, and the building units appear collapsed (Fig. 24). A ring of pores, usually 3 μm in diameter each, located outside the aperture is often, but not always present (Fig. 24). Test light yellow to deep brown. Variable number of nuclei (two or more).

Biometry: All measures are highly variable (CV between 26.46–36.08, Table 2). Test height presents the lowest standard error of the mean (0.23). Size frequency analysis shows that *Arcella discoides* complex has a main size class and a wide size range for all measured characters. For test diameter, 72.78% of measures are within 100–150 μm. For test height, 69.86% of measures are within 20–30 μm and for aperture diameter, 65.18% of measures are within 50–100 μm.



Figs 3–**11.** Morphology of the *Arcella hemisphaerica* complex. **3** – Superior view of an *A. hemisphaerica* individual with a smooth test; **4** – lateral view of an individual with a smooth test; **5** – superior view of an individual with a test provided with undulations; **6** – lateral view of an individual with a test provided with undulations; **7** – high magnification view of the abapertural region of the test, showing the hexagonal, hollow, box-like building units; **8** – lower magnification showing variation in the shape of the building units; **9** – close up of the building units, showing the single pore at the edge of each vertex; **10** – apertural view of an individual, showing centrally located aperture; **11** – high-magnification of the edge of the aperture, showing the delimiting curled lip.

Ecology and geographic distribution: This taxon is recorded for the Americas, Europe, Africa, Asia and Australia (Ogden and Hedley 1980). In Brazil, records are from mosses in Rio de Janeiro (Cunha 1913), (Wailes 1913), plankton samples for Ceara (Cunha 1913), Mato Grosso (Green 1975, Hardoim and Heckman 1996), Goiás (Lansac-Tôha *et al.* 2000), Sao Paulo (Durigan *et al.* 1992, Oliveira *et al.* 1992, Sipaúba-Tavares *et*



al. 1995), Minas Gerais (Dabés 1995), Mato Grosso do Sul (Daday 1905, Velho and Lansac-Tôha 1996, Lansac-Tôha *et al.* 1997, Bonecker *et al.* 1998, Velho *et al.* 1999) and Paraná (Lopes 1993, Nunes *et al.* 1996, Velho and Lansac-Tôha 1996, Lansac-Tôha *et al.* 1997, Velho *et al.* 1999). The present survey sampled most individuals associated to the roots of aquatic plants both in the river (57.7%) and in the lake (33%). This is the only taxon that was sampled from the sediment of the river (Table 3).

Remarks: The main feature that would separate some individuals of *A. megastoma* is the large number of nuclei, but this is a variable character and apparently increases with the size of test, it could also be separated by a large apertural diameter, even if there is some overlap with other nominal taxa. Despite the observation that *A. polypora* has a mean number of nuclei around 10, the variation around that mean is evidence that in nature the variation is much higher (Hegner 1920). *A. polypora* would nevertheless be distinct by the ring of pores surrounding the aperture, but this ring is seen in other nominal taxa as well, proving to be an ambiguous character.

A. gibbosa complex

We identified 106 individuals as *A. gibbosa*, and within these, 77 ambiguously fit 3 infra-subspecific taxa. The morphological ranges shows no discrete differentiation and the PCA does not reveal any grouping of specimens (Fig. 2i). We therefore consider all four names referring to a single entity.

Arcella gibbosa Penard, 1890
Previously described non-distinct taxa
Arcella gibbosa – Penard, 1890: 155, Pl 5 Figs 96–98, Pl 6, Fig. 1
Arcella gibbosa var. *levis* – Deflandre, 1928: 229, Fig. 207
Arcella gibbosa var. *mitriformis* – Deflandre, 1928: 230, Figs 208–218
Arcella gibbosa var. *aplanata* – Van Oye, 1956: 334, Fig. 4
Examined material: 106 individuals under LM, 15 individuals under SEM

Morphology: Test circular in apertural view, with a high domed profile in lateral view (Figs 25, 26, 27). Abapertural region provided with well-marked depressions, variable in depth. Whole test made of chitinous material built from box-like, hollow, hexagonal units (Figs 28, 29). Usually, more than one small pore (1–2 μm) is present at each vertex of each hexagon at the abapertural region (Fig. 30), while at the apertural region most vertices are provided with only one pore (Fig. 31). Hexagons appear inflated at the abapertural region (Figs 29, 30) and collapsed at the aperture region (Figs 31, 32). Aperture central (Fig. 27), invaginated surrounded by a curled lip (Fig. 33). Test light yellow to deep brown. Provided with two nuclei.

Biometry: All measures are highly variable (CV between 17.7–27.6, Table 2). Aperture diameter presents the lowest standard error of the mean (0.654). Size frequency analysis shows that *Arcella gibbosa* complex has a main size class and a wide size range for all measured characters. For test diameter, 75.47% of measures are within 70–90 μm. For test height, 63.2% of measures are within 70–80 μm and for aperture diameter, 94.3% of measures are within 10–20 μm.

Ecology and geographic distribution: This species has been registered for Europe, South and North America and Africa (Ogden and Hedley 1980). In Brazil, registers are for plankton samples in Mato Grosso (Green 1975), Minas Gerais (Bonecker *et al.* 1996, Bonecker *et al.* 1997), Goias (Lansac-Tôha *et al.* 1999, Lansac-Tôha *et al.* 2000), Mato Grosso do Sul and Parana (Velho and Lansac-Tôha 1996, Lansac-Tôha *et al.* 1997, Velho *et al.* 1999), from mosses in Rio de Janeiro (Wailes 1913) and from macrophyte samples in Rio Grande do Sul (Torres and Jebram 1994, Torres 1998). The present survey recorded most individuals (68.1%) from this

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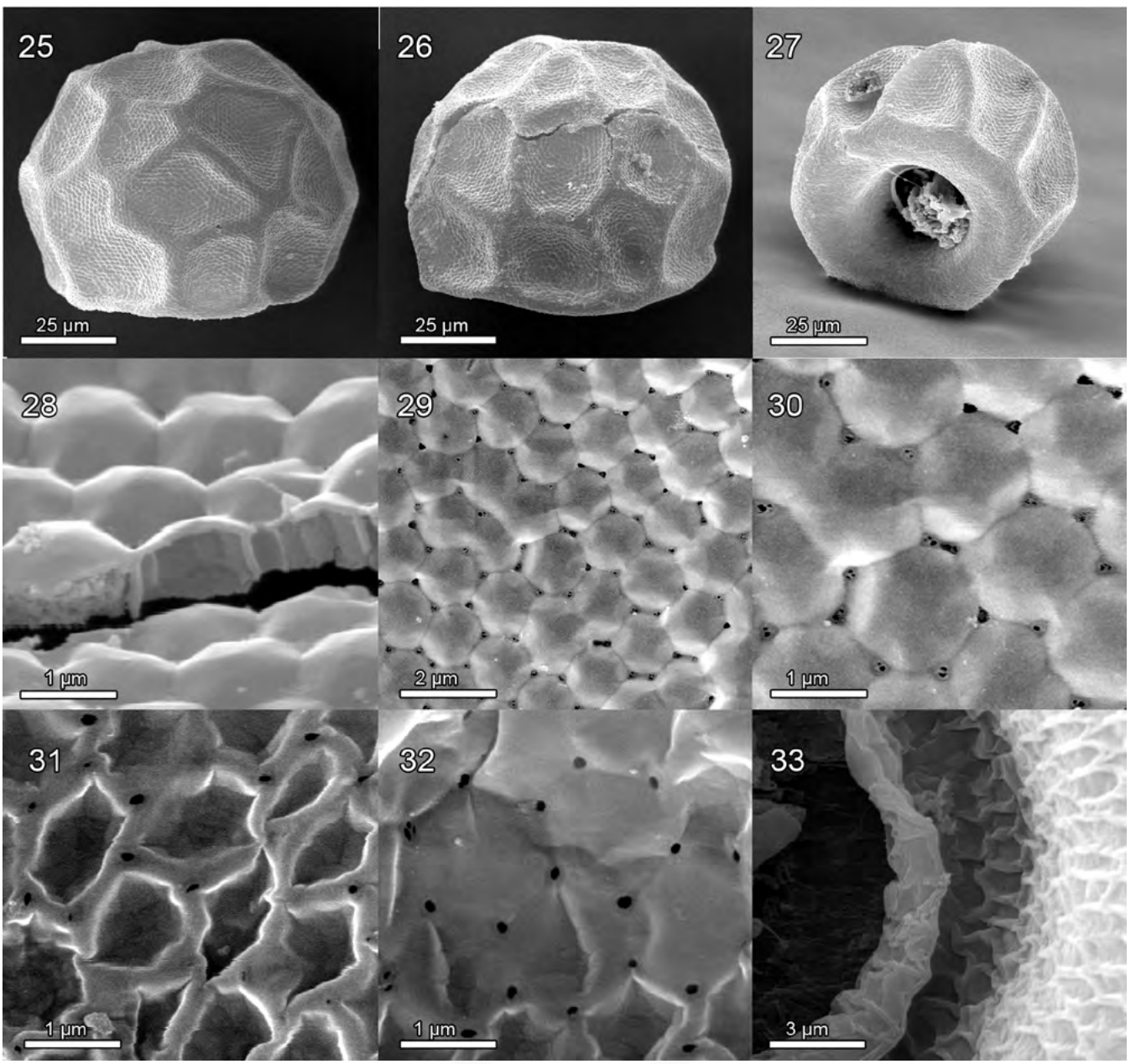
Figs 12–24. Morphology of the *Arcella discoides* complex. **12** – superior view of a smooth test individual, with no marginal rim; **13** – superior view of an individual provided with a thin marginal rim; **14** – lateral view of a non-arched individual; **15** – lateral view of an arched test individual; **16** – apertural view of a non-arched individual; **17** – apertural view of an arched individual; **18** – apertural view of an individual provided with a thin marginal rim; **19** – appearance of the test at the abapertural region; **20** – high magnification of the test surface on the abapertural region; **21** – view of the test wall showing the inside part and hollow box-like building units; **22** – view of the cracked test wall on the edge of test, showing additional covering layer on the abapertural region; **23** – view of the cracked test wall showing lack of covering layer on the apertural region; **24** – close up on the edge of the central aperture, showing delimiting curled lip.



Figs 25–**33.** Morphology of the *Arcella gibbosa* complex. **25** – lateral view of an individual showing large depressions and ridges on the abapertural region of the test; **26** – lateral view of another individual showing variation in the depressions and ridges; **27** – apertural view showing smooth apertural region and centrally located aperture; **28** – high magnification of the abapertural region of the test, showing a crack where the hollow building units can be seen; **29** – general aspect of the test wall on the abapertural region; **30** – close up of the abapertural region of the test showing more than one pore at each vertex of each building unit; **31** – close up of the apertural region test wall showing collapsed building units with only one pore at each vertex; **32** – another close up of the apertural region test wall showing more or less inflated building units; **33** – edge of aperture showing the delimiting curled lip.

species associated to the roots of aquatic plants in the marginal lake, a lenthic habitat. Individuals were also recorded for the other sampled micro-habitats, except the sediment of the river (Table 3).

Remarks: Our results are largely concordant with (Tsyganov and Mazei 2006) recent re-description of *Arcella gibbosa*, to which we only add non-distinctiveness of other nominal taxa. The distinction between *A.*

gibbosa and *A. hemisphaerica* is corroborated in our analysis.

Arcella brasiliensis

We have identified 5 individuals belonging to the species *A. brasiliensis*. This is a rare amoeba that is clearly defined by morphological features. We include here the first account for its test ultra-structure and provide a thorough description.

Arcella brasiliensis Cunha, 1913
Examined material: 5 specimens under LM, 1 under SEM.

Morphology: Test circular in apertural view (Fig. 34), hemispheric in side view (Fig. 35), with a distinct marginal ring. Abapertural surface provided with depressions, promoting an undulate aspect (Fig. 35). Test built with hexagonal, hollow, box-like building units (Fig. 36), a single pore is sometimes present on the vertex of an hexagon. Apertural region smooth (Fig. 37). Aperture circular, central, invaginated, delimited by a curled lip (Fig. 38). Test light yellow to brown.

Biometry: Due to limited number of individuals, we only report the morphometric ranges in Table 2.

Ecology and geographic distribution: This species is recorded only for Brazil, from Rio de Janeiro (Cunha 1913), Minas Gerais (Dabés 1995), Goias (Lansac-Tôha *et al.* 2000), Mato Grosso do Sul and Parana (Velho and Lansac-Tôha 1996, Lansac-Tôha *et al.* 1997), and Rio Grande do Sul (Torres 1998). The present survey sampled only five individuals, so this is a rare species in the Tiete River (Table 3).

Remarks: Previous descriptions (Cunha 1913, Deflandre 1928, Velho and Lansac-Tôha 1996) portray this species with a smooth abapertural dome, but all individuals analyzed had small depressions, forming undulations. The original drawing shows the marginal rim with an alternate pattern of undulations, but (Deflandre 1928) attributes the pattern to an optical illusion. Our SEM micrographs show the absence of such an alternate pattern. The individuals measured in our study are also compatible to *Arcella marginata* Daday, 1905, and this might be a non-distinct taxon, but we refrain from doing any further speculations given the small number of individuals measured.

DISCUSSION

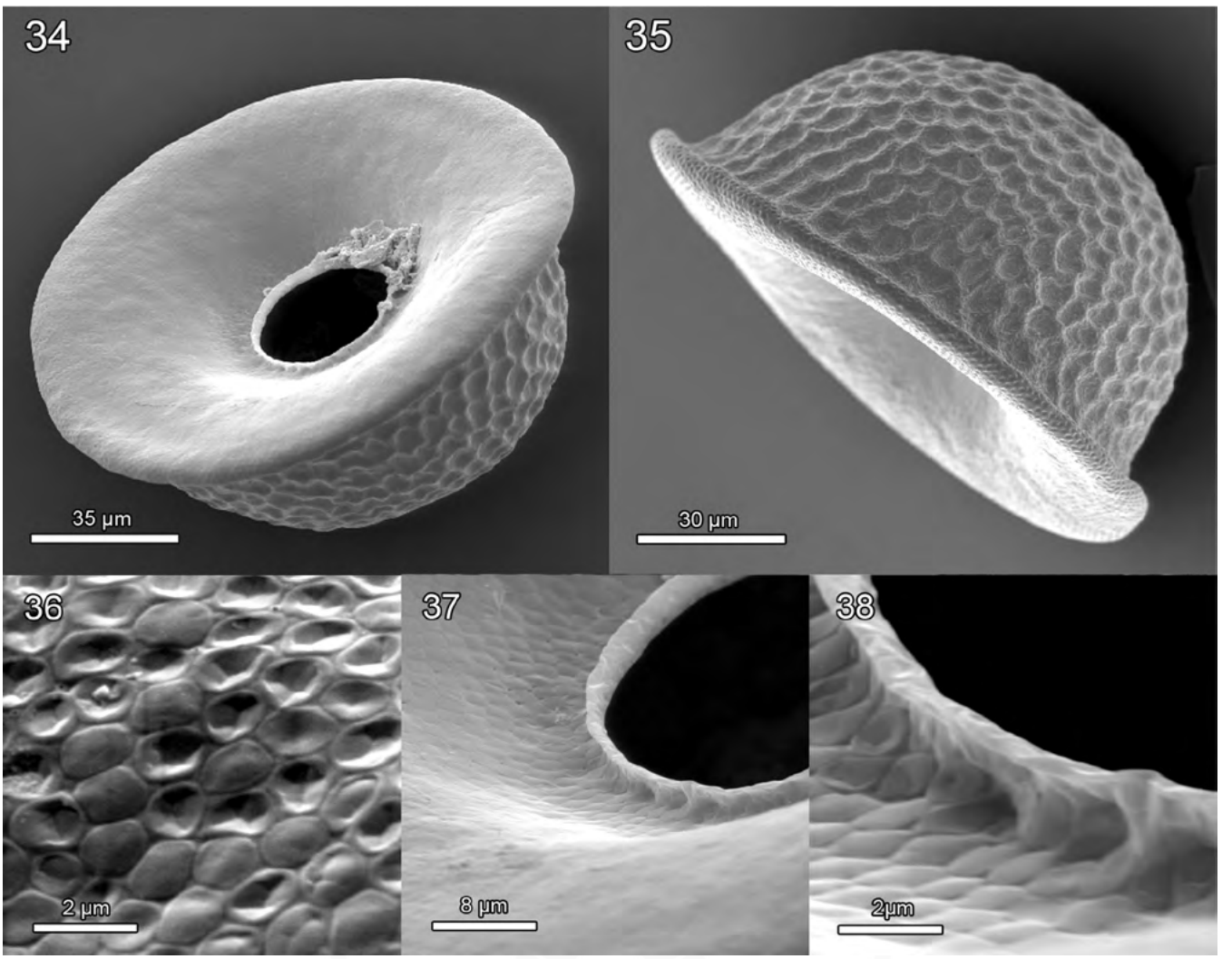
Taxonomy and morphology

We identified four distinct morphological entities, three of them being a complex of associated indistinct nominal taxa. Each complex has its unique features, and can also be identified by ratios between test height and test diameter. For three of the species complexes (*A. hemisphaerica*, *A. gibbosa* and *A. discoides*) the biometric data if analyzed together would effectively determine that no distinction can be made between them.

However, pronounced disjunctive qualitative morphological features may separate them unambiguously: *A. hemisphaerica* has a distinct semi-circle contour in lateral view, despite some *A. discoides* being able to reach the same th/td ratio, the test contour remains distinctive as a disc shape. *A. gibbosa* has large depressions and ridges along the sides of the test, despite some *A. hemisphaerica* possessing a certain amount of depressions, it is never quite as pronounced as in *A. gibbosa*. *A. discoides* has a very flattened lateral profile , while some individuals may reach a test almost as high as *A. hemisphaerica*. *A. brasiliensis* has a much more pronounced marginal rim than what is present in some of the other species. Since we have not measured rims in the other species, we do not rule out that these might as well be the extreme end of the distribution for marginal rims.

The ratios between morphometric measures also bring novel and potentially useful distinctive characters, especially test height/test diameter ratio. Early on (Deflandre 1928) had divided the genus into four groups: the "tall tests" with a th/td ratio close to 1; the "hemisphaeric tests" th/td = 0.5; the "short tests" th/td = 0.3 and the "keeled tests," with presence of the marginal rim (keel). Curiously, our survey shows that each species analyzed here will fit into one of those categories, however, the present survey uses an unprecedented number of biometrical data (Table 1), hence Deflandre's hypothesis might be well supported.

There are two other possible distinctive characters, only observable by SEM. The first are collapsed hexagons at the apertural region of *A. discoides* and *A. gibbosa*, which might be an artifact of sample preparation and therefore misleading as a diagnostic character. The second is the number of pores at the vertex of each hexagon in the abapertural region: *A. gibbosa* usually has two, *A. hemisphaerica* usually has one and *A. discoides* usually shows an additional covering layer at the



Figs 34–38. Morphology of *Arcella brasiliensis*. **34** – apertural view, showing centrally located aperture; **35** – lateral view showing conspicuous marginal rim and undulations in the abapertural region; **36** – close up of the abapertural region test wall, showing building units with sometimes a pore present at the vertex; **37** – smooth apertural region; **38** – aperture edge with delimiting curled lip.

abapertural region. This has the potential to be indeed a defining character, as observed in small ultrastructural differences in the genus *Cyphoderia* (Todorov *et al.* 2009). If so, it correlates to distinctions made by test height/test diameter ratio. This would be a useful character in ecological surveys for confirming an initial biometric identification by observing a few specimens under the SEM.

Nomenclatural remarks

We corroborate the need for thorough comparative studies in the less inclusive testate amoebae groups (genera and species). The number of non-distinct nominal taxa found is evidence that few comparative surveys have been carried in the genus *Arcella.* Maintaining information on previously described entities allows more in-depth studies to flesh out distinct entities, as has been done in (Tsyganov and Mazei 2006/2007), where *A. intermedia* is separated from *A. hemisphaerica*.

If the considerations present in ICZN (Ride *et al.* 1999) are to be followed, especially the determinations for the species group (Article 45) and the Principle of Coordination, a more immediate problem arises: infra-subspecific taxa are sometimes described using the same epithet, for

instance *Arcella megastoma* fma. *arcuata* and *Arcella discoides* fma. *arcuata*, both described by (Deflandre 1928), are deemed by the rules of nomenclature to be *Arcella arcuata* Deflandre, 1928. Still, they originally referred to different organisms; therefore the taxonomic identity of the two initial names gets confused.

The case of *Arcella hemisphaerica* and *Arcella rotundata* also brings up an interesting point. These species are distinguishable morphologically, but indistinguishable by measurements. Given the knowledge of variation in clonal cultures of testate amoebae, an argument can be made that if an observer sets out to find a particular morphotype, he or she will be overlooking the fact that the morphotype in question might be a variant of another morphotype. The case of spines in *Centropyxis aculeata* (Lahr *et al.* 2008) illustrates this fact well and should be extended to other morphological characteristics such as keels and number of pores.

Only a comprehensive review with all existing names would point out matters like this. However, without studying actual specimens, such a revision would be of limited use, for example in this study we have determined that *A. megastoma* and *A. discoides* are non-distinct entities as described, therefore the nomenclatural problem is dismissed.

CONCLUSION

In sum, we propose the solution – when a complete revision is not granted – to treat all names that are non-distinguishable under one operationally distinct entity, and list all non-distinct names under the oldest one. This way, taxonomic information is not lost, the appearance of old names with new authorities is avoided, and furthermore, the taxonomic concept used by the current researcher is objective and explicit, and allows challenging by subsequent studies. Once the original taxonomic identity is clarified, further studies taking into account sub-cellular features and molecular sequences will be able to properly identify cryptic entities, and report them in an intelligible manner.

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