L2-127

Anales del Instituto de Biología Universidad Nacional Autónoma de México,
Serie Zoología 72(1): 1-8. 2001

A new species of *Difflugia* (Rhizopoda: Testacea) from Argentina

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Resumen. Una especie nueva de *Difflugia* Leclerc, se encontró sobre vegetación flotante de un cuerpo de agua somero de la provincia de Chaco, Argentina. *D. rafaeli* sp.nov. se caracteriza por presentar una teca esférica (relación longitud/diámetro ≅ 1) con un cuello recto, corto pero conspicuo. El pseudostoma es desde arriñonado hasta triangular con bordes redondeados. Dimensiones de la teca: longitud: 124.2-147.5 μm; diámetro: 130.8-147.5 μm; altura del cuello: 11.0-17.6 μm. La nueva especie también se caracteriza biométricamente. Se concluye que existen diferentes *phena* (con protuberancias o sin ellas) dentro de la población.

Palabras clave: amibas testáceas, especie nueva, cuerpo de agua somero, Chaco, Argentina.

Abstract. In the floating vegetation of a swamp in the province of Chaco, Argentina, a new species, *Difflugia* Leclerc was found. *D. rafaeli* sp. nov. is characterized by a spherical shell (ratio length/diameter ≅ 1) with a straight, short but conspicuous neck. Pseudostome kidney- shaped to triangular with round angles. Shell dimensions: length: 124.2-147.5 pm; diameter: 130.8-147.5 pm; height of neck: 11.0-17.6 pm. The new species is also biometrically characterized. It is concluded that there are different *phena* (with and without protuberances) within the population.

Key words: testate amoebae, new species, swamp, Chaco, Argentina.

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Introduction

As part of the research that we are conducting on the subtropical fauna of fresh water testate amoebae, we recorded the following species in a swamp in the province of Chaco, Argentina: *Centropyxis aculeata* f. *bacillaris* Lena & Zaidenwerg, *C. discoides* (Penard) Deflandre, *Cucurbitella crateriformis* Gauthier-Lièvre & Thomas, *C. obturata* var. *micropyla* Dioni, *Difflugia acuminata* Ehrenberg, *D. capreolata* Penard, *D. corona* Wallich, *D. corona* f. *tuberculata* Vucetich, *D. lobostoma* Leidy, *D. pleustonica* Dioni, *D. pyriformis* Perty, *D. stellastoma* Vucetich, *D. urceolata* Carter, *Difflugia* sp., *Euglypha tuberculata* Dujardin, *Euglypha* sp., *Lesquereusia modesta* Rhumbler, *L. modesta* var. *caudata* (Playfair) Thomas & Gauthier-Lièvre, *L. spiralis* (Ehrenberg) Bütschli, *Lesquereusia* sp., *Netzelia tuberculata* (Wallich) Netzel, and *N. wailesi* (Ogden) Meisterfeld. In the floating vegetation of the same swamp a new species of *Difflugia* Leclerc was found, which is described and illustrated in this work, as well as biometrically characterized.

Material and methods

Specimens were obtained from the submerged roots of the water cabbage *Pistia stratiotes* at La Cava swamp, Department of San Fernando, province of Chaco, Argentina. It is a water body situated on the westside of the Paraná River valley (27° 27' S, 58° 57' W), about two kilometers from the main course of the river. It is an environment with abundant organic sediments, anoxic almost to the surface during the months of greater temperature (January and February). It is densely covered by macrophytes -which prevent wind stirring and reduces drastically the incident light in the first centimeters beneath vegetation-, with an acid water trend, and practically isolated from the Paraná river's hydrodynamics. The water in the dense, shaded regions of this swamp has a low level of dissolved oxygen (measurements taken at the surface have rarely shown more than 5% saturation and often nil), as it is characteristic of tropical swamps of South America. More details about the environmental characteristics of this Chaco swamp can be found in Bruquetas de Zozaya & Neiff (1991) and Bruquetas de Zozaya & Poi de Neiff (1993). At the sampling moment (October), air temperature was 24°C, water temperature 26°C, dissolved oxygen 0.7 mg/l and pH 6.8.

The tests, previously fixed in formaldehyde 4%, were measured by means of a light microscope at a magnification of 400x (objective 40x, ocular 10x). For the study of shell morphology, shells were coated with gold-palladium and examined with a JEOL T100 scanning electron microscope (SEM).

Biometric characterization was made following Schönborn & Peschke (1988). Ideal individuals were constructed following Schönborn *et al.* (1983) and Schönborn & Peschke (1988) (Table 1).

Difflugia rafaeli sp. nov.

Diagnosis. Shell almost spherical ($\bar{x}$ total length= 138.0 μm; $\bar{x}$ 7 maximum diameter = 138.2 μm), with a straight and short neck. Pseudostome small, variable, from kidney-shaped to triangular with rounded angles; its diameter ($\bar{x}$= 36.5 μm) corresponds, approximately, to a quarter of the shell diameter.

Description. Shell spheroidal, with a short, straight, and conspicuous neck (Figs. 1-3, 7). Coating formed by irregular, siliceous particles, from small (3-4 μm) to medium (12-17 μm) size, that do not project distinctly off the contour of the test. Some of the specimens (33%, n= 30) have rounded protuberances on their tests (Fig. 2) which are also easily recognizable in the light microscope. The pseudostome, proportionally small in comparison with the diameter of the test, varies from kidney-shaped to triangular with rounded angles (Figs. 4, 9). Its edge is constituted by small size particles arranged in quite a regular way which, as seen by the light microscope, appears to have a thin rim. The neck, formed by small elements of homogeneous size, has thicker particles on its base, arranged in a row in a necklace manner (Figs. 2-3). The organic cement network that joins the coating elements has rings with a central pore. Some of them are well defined (Fig. 5, arrow), about 2 μm in external diameter and 0.56 μm in internal diameter; the others are less noticeable, with pores range between 0.66 μm and 0.75 μm. An even smaller mesh is infrequently seen beneath the pores (Fig. 6, arrow). The empty tests (63%, n = 30) are greyish, whereas those shells with protoplasm are opaque and dark brown-coloured.

Type-locality. La Cava swamp (27° 27' S, 58° 57'W), department of San Fernando, province of Chaco, Argentina.

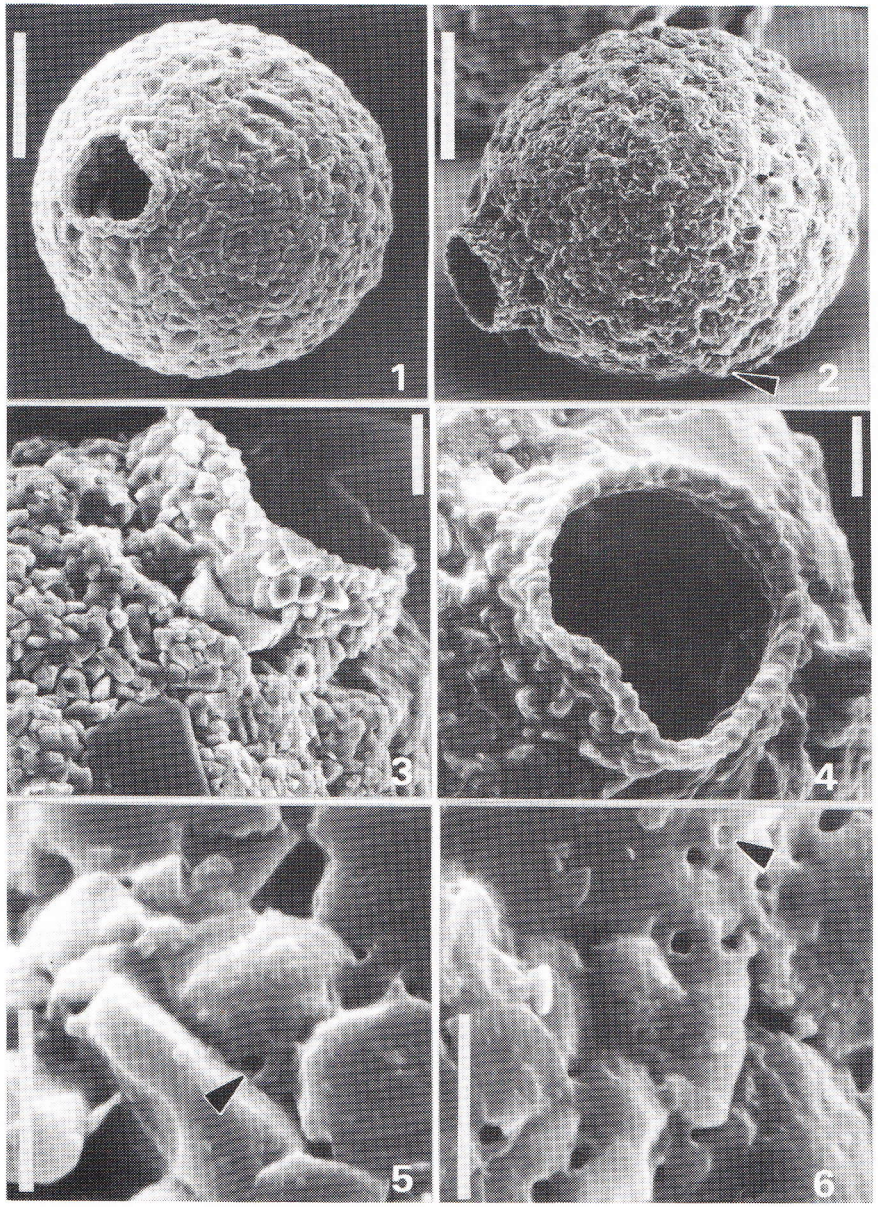
Holotype. Permanent slide in Canada balsam deposited at the Museo de La Plata, Departamento Científico Zoología de Invertebrados (Protistas), La Plata, Argentina (MLP), No. 002.

Paratypes. Several specimens deposited in the same collection, No. 003.

Etymology. The specific name is dedicated to our friend and colleague, Lic. Rafael Urréjola, of the Scanning Electron Microscopy Service of the Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Argentina.

Discussion

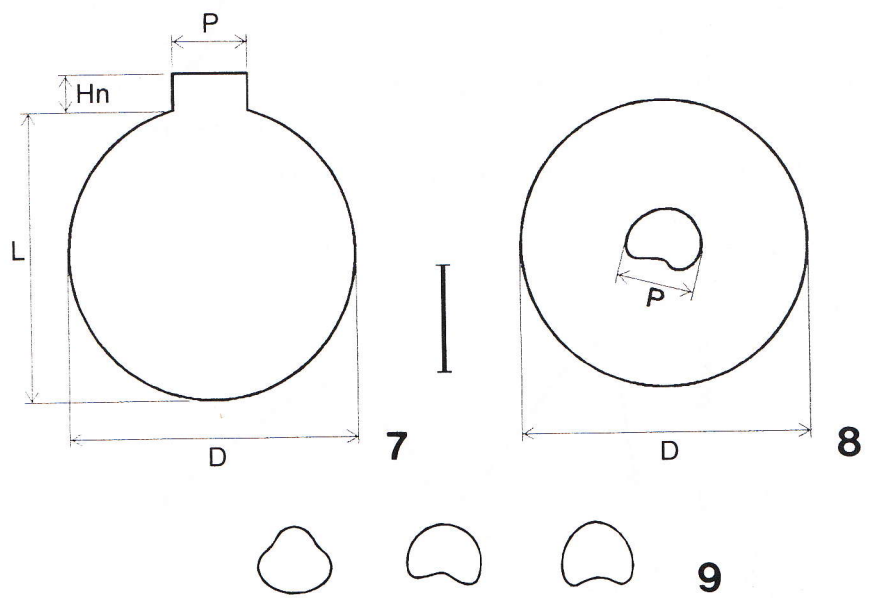
The genus *Difflugia* Leclerc 1815 is characterized by a shell composed of agglutinated mineral particles and diatom-frustules, whereas the genus *Netzelia*



Figs. 1-6. *Difflugia rafaeli* sp. nov. 1.Ventro-lateral view (scale bar = 50 μm. 2. Specimen with protuberances (arrow) (scale bar = 50 μm). 3. Detail of the neck (scale bar = 10 μm). 4. Detail of the pseudostome (scale bar = 10 μm). 5—6. Detail of the organic network (5. Arrow indicates a ring and pore. 6. Arrow indicates organic mesh) (scale bar = 5 μm).

Ogden 1979, aside from the usual mineral particles and diatom-frustules, is able to construct the shell of autogenous siliceous components (idiosomes). We include *D. rafaeli* sp. nov. in the genus *Difflugia* Leclerc due to the fact that the study of the shell morphology does not give evidence of idiosomes.

We compared the new species to those *Difflugia* species, whose morphology coincides with the globose, ovoid-globose, collared, and lobed groups, according to the sections created by Gauthier-Lièvre & Thomas (1958). From the comparison with the species belonging to the globose and ovoid-globose groups, it results that none of them has a neck whereas the new species here described does have it. From the comparison with the species belonging to the collared group, we concluded that the greater affinity of *D. rafaeli* is with *D. brevicolla* Cash and *D. kabylica* Gauthier-Lièvre & Thomas. Both of them have a well defined neck but shorter than that of *D. rafaeli*, and their pseudostomes are circular. On the other hand, the ratio diameter/pseudostome is proportionally smaller in our specimens than in those of *D. brevicolla* and *D. kabylica*. The new species also differs strongly in the ratio total length/maximum diameter which, unlike *D. brevicolla* and *D. kabylica*, has an almost circular figure. In the case of the *Difflugia* belonging to the lobed group, we have compared the new species only to those which, besides



Figs. 7-9. *Difflugia rafaeli* sp. nov. 7-8. Ideal individual, lateral (7) and ventral (8) view, constructed using the median values as axes (D: maximum diameter; Hn: height of neck; L: total length; P: diameter of pseudostome). 9. Three different forms of the pseudostome. Scale bar = 50 μm.

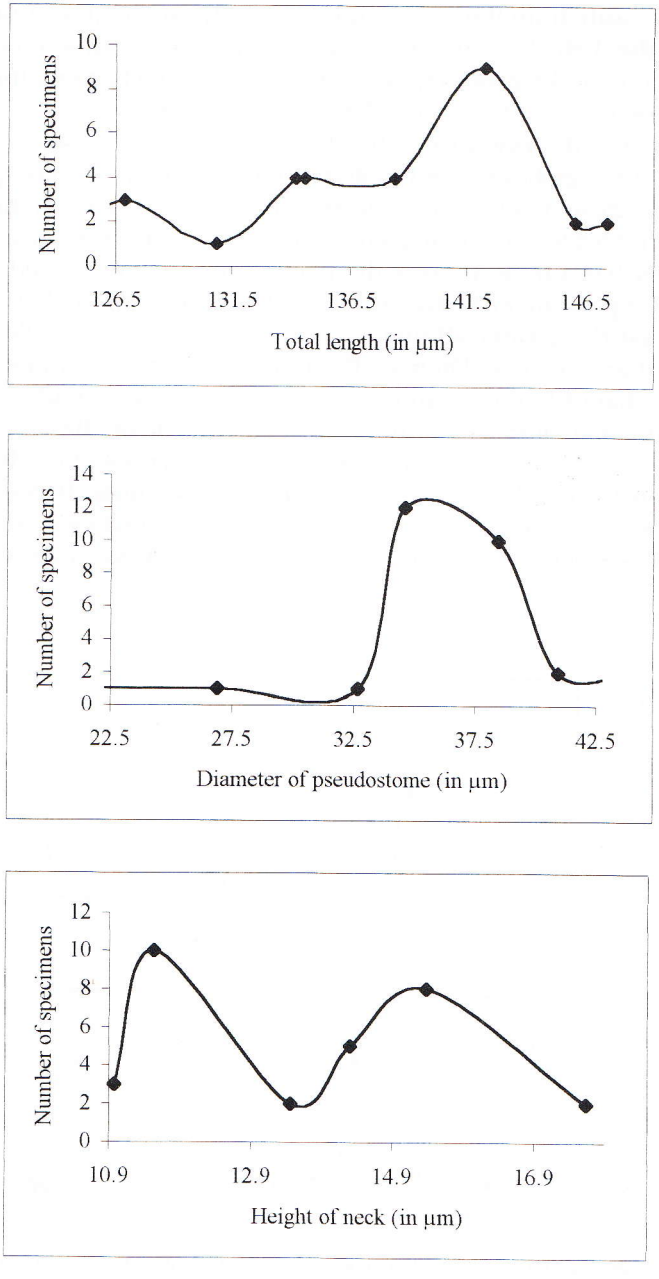


Fig. 10. Frequency analysis of shell length (upper);diameter of pseudostome (middle); and height of neck (lower) of *Difflugia rafaeli* sp. nov.

being lobed, have protuberances on their shell (as 33% of our specimens). Among these, the most similar species is *Difflugia muriformis* Gauthier-Lièvre & Thomas 1958, since it presents some similar dimensions as, for example, total length and maximum diameter. However, according to the original description of Gauthier-Lièvre & Thomas and our own observations, *D. muriformis* should be included in *Netzelia*, just as it happened with *N. tuberculata* (Wallich) Netzel, which was initially included in *Difflugia*.

We conclude that we are in the presence of a size monomorphic population because, in agreement with the biometrical treatment carried out (Fig. 10), it was not possible to separate the sample into independent populations.

In a previous survey of the testate amoebae of Argentina (Vucetich & Lopretto 1995), 71 species of *Difflugia* were listed. Among them, *D. corona* f. *tuberculata* Vucetich 1973, *D. muriformis* Gauthier-Lièvre & Thomas 1958, and *D. pleustonica* Dioni 1970, are characterized by having a regular arrangement of protuberances on the surface of the shell. As the typical form of *D. corona* and *D. corona* f. *tuberculata* are often found together in the same samples (Vucetich 1973, 1978), further biometrical studies in progress will help to reveal whether they are separate entities.

Furthermore, we suggest there are different *phena* (i.e., different forms or phenotypes occurring within a single population) in *Difflugia rafaeli*, as it has been described by Schönborn (1962) for *Difflugia limnetica*. For this reason, we agree on the use of Mayr's (1963) term "polyphenism" (in the sense of a "nongenetic" change of phenotype) to describe the existence of such variants in our new species.

Table 1. Biometric characters from *Difflugia rafaeli* sp. nov. (number of specimens= 30; all measurements in μm from light microscopy)

Character	min	max	$\bar{x}$	M	s	v	L_1	L_2
total length (L)	124.2	147.5	138.0	138.5	6.1	4.4	125.7	150.2
maximum diameter (D)	130.8	147.5	138.2	138.0	4.4	3.2	129.4	147.0
diameter of pseudostome (P)	21.0	47.6	36.5	35.6	4.9	13.5	26.6	46.3
height of neck (Hn)	11.0	17.6	13.5	13.9	2.1	15.3	9.4	17.6
L /D	0.92	1.09	1.00	1.00	0.05	4.58	0.91	1.09
D/P	2.96	6.72	3.87	3.84	0.66	17.05	2.55	5.19

min = minimum; max = maximum; $\bar{x}$ = arithmetical mean; M = median; s = standard deviation; v = coefficient of variation (%); L_1 = 95% confidence interval, lower limit; L_2 = 95% confidence interval, upper limit.

Acknowledgements. We thank Marcela A. Blanco for her assistance in biometrics analysis. Support from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina, to which one of us (ECL) belongs, is gratefully acknowledged.

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Recibido: 14.VIII.2000
Aceptado: 6.XII.2000

Fab. - FCNyM
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