

ACTA PROTOZOLOGICA

On a New Species of Euglyphid Testate Amoeba, *Scutiglypha cabrolae*, from the Licancabur Caldera Lake, Central Andes

Willem H. DE SMET¹ and John A. E. GIBSON²

¹ University of Antwerp, Department of Biology, Campus Drie Eiken, Belgium; ² University of Tasmania, Marine Research Laboratories, Tasmanian Aquaculture and Fisheries Institute, Hobart, Australia

Summary. A new testate amoeba *Scutiglypha cabrolae* sp. n. is described from a small lake located in the caldera of Licancabur volcano, central Andes, at 5916 m above sea level. Its morphology was investigated using light and scanning electron microscopy. The main characteristics of the new species are: thick-set ovate test, length/width ratio 1.4; relatively small size, mean length \times width: $69 \times 48 \mu\text{m}$; 3–4 rows of apertural plates, apertural plates of first row lozenge-shaped, oral margin with median tooth and (5)–6 lateral toothlets; body-plates scutiform, broad, with weakly crenulated oral and aboral margin.

Key words: Testate amoebae, Euglyphida, *Scutiglypha* sp. n., Licancabur, Andes.

INTRODUCTION

The numerous water bodies of the High-Andes, many of them saline due to the low precipitation rates and high evaporation rates, experience extreme environmental conditions of, for example, low temperatures, low-oxygen content, low atmospheric pressure and high UV radiation. In this respect they are particularly interesting for the study of the invertebrate biodiversity at the species and community level, as well as for the study of the physiological adaptations of these organisms to extreme conditions. As a cold habitat at tropical latitudes extending from Colombia in the north to Chile

in the south, the Andes, moreover, is probably very relevant biogeographically as a migration corridor for cold-stenothermal organisms (see, for example, Löffler 1968, 1984). The extreme conditions of this region have also attracted the attention of the NASA High Lake Project, designed to obtain critical astrobiological information about the limits of life in these unique environments, and scientific clues about potential planetary analogs such as Martian paleolakes (see Cabrol *et al.* 2003, submitted). Among the lakes studied by the High Lake Project is one of the highest lakes in the world, located in the caldera of Licancabur volcano (6017 m) on the border of Chile and Bolivia ($22^{\circ}50'07''\text{S}$, $67^{\circ}53'07''\text{W}$). The study of one-off zooplankton samples of the lake revealed a community composed of a single species of rotifer, chironomid and testate amoeba, respectively, two copepods, three cladocerans and two ostracods, of which the two ostracods, two of the cladocerans and the testate amoeba are new to science.

Address for correspondence: Willem H. De Smet, University of Antwerp, Dept. of Biology, Lab. of Polar Biology, Limnology and Palaeobiology, Universiteitsplein, 1, B-2610 Wilrijk, Belgium, E-mail: willem.desmet@ua.ac.be

This paper describes the new testate amoeba, which belongs to the genus *Scutiglypha* Foissner et Schiller, 2001, which is characterized by filose pseudopodia and a test composed of scutiform siliceous plates biosynthesized by the resident amoeba.

MATERIALS AND METHODS

Samples were collected from a small lake (100 × 40 m, maximum depth 5.2 m) located in the caldera of Licancabur volcano at 5916 m a.s.l., in the central Andes. The samples of littoral plankton were taken on 4 February 2007 using a 20 µm mesh plankton net, and were immediately fixed in 95% ethanol. For permanent slide preparations and scanning electron microscopy (SEM), specimens were washed with distilled water, then transferred to round cover glasses diameter 10 mm, and dried at room temperature. Permanent slide preparations were obtained by mounting the air-dried tests in Naphrax®. For SEM the cover glasses containing the tests were glued to a SEM stub and sputter coated with gold. Preparation of individual test plates was done by dissolving the organic cement using dilute NaOCl solution following the protocol by De Smet (1998). SEM was performed with a Philips SEM-515 microscope operated at 20 kV. Measurements of test characteristics were done on SEM-microphotographs.

RESULTS

Systematics

- Rhizaria Cavalier-Smith, 2002
Cercozoa Cavalier-Smith, 1998 emend. Adl *et al.*, 2005
Silicofilosea Adl *et al.*, 2005
Euglyphida Copeland, 1956 emend. Cavalier-Smith, 1997
Euglyphidae Wallich, 1864 emend. Lara *et al.*, 2006
Scutiglypha Foissner et Schiller, 2001

Scutiglypha cabrolae sp. n., Figs 1–3

Diagnosis: Test ovate, thick-set, length/width ratio 1.4; cross-section and aperture circular; size relatively small, mean length × width: 69 × 48 µm; 3–4 rows of apertural plates, apertural plates of first row ± lozenge-shaped, oral margin with median tooth and (5)–6 lateral toothlets; body-plates scutiform, broad, with weakly crenulated oral and aboral margin; two spines, appendix of body plates.

Type locality: Small lake in caldera of Licancabur volcano (22°50'07"S, 67°53'07"W) at 5916 m a.s.l., central Andes. Littoral plankton.

Type specimen: A holotype and paratype mounted in Naphrax® on permanent glass slide preparations deposited in Muséum National d'Histoire Naturelle, Paris, catalogue number MNHN: ZS 51/boîte PROTO 1-39 (holotype) and MNHN: ZS 52/boîte PROTO 1-40 (paratype).

Etymology: This new species is dedicated to Dr Nathalie Cabrol (NASA Ames Research Center, Space Science Division), in recognition of her will and interest in taking samples in these inhospitable high places.

Description: The test is thick-set ovate with a length/width ratio of 1.4, circular in cross-sectional view, and composed of about 100–120 siliceous plates. The plates are arranged imbricately in alternate longitudinal rows. The apertural and body-plates appear identical within each transversal row, but differ in shape and/or dimensions in the successive rows; no distinct transversal rows can be observed with the fundus-plates, the dimensions of which also vary largely in order to fit the hemispherical shape of the fundus. The aperture is terminal and circular, bordered by 3–4 rows of plates, with 8 plates in the first row and 10 in the rows following. All apertural plates (Figs 3a–f) are more or less lozenge-shaped with rounded aboral margin and denticulate oral margin. The denticulation is most pronounced in the first row with each plate bearing one large median

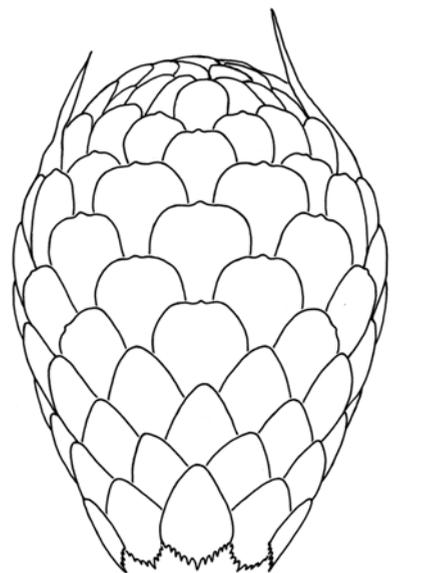


Fig. 1. *Scutiglypha cabrolae* sp. n., drawing of test, lateral view. Scale bar: 50 µm.

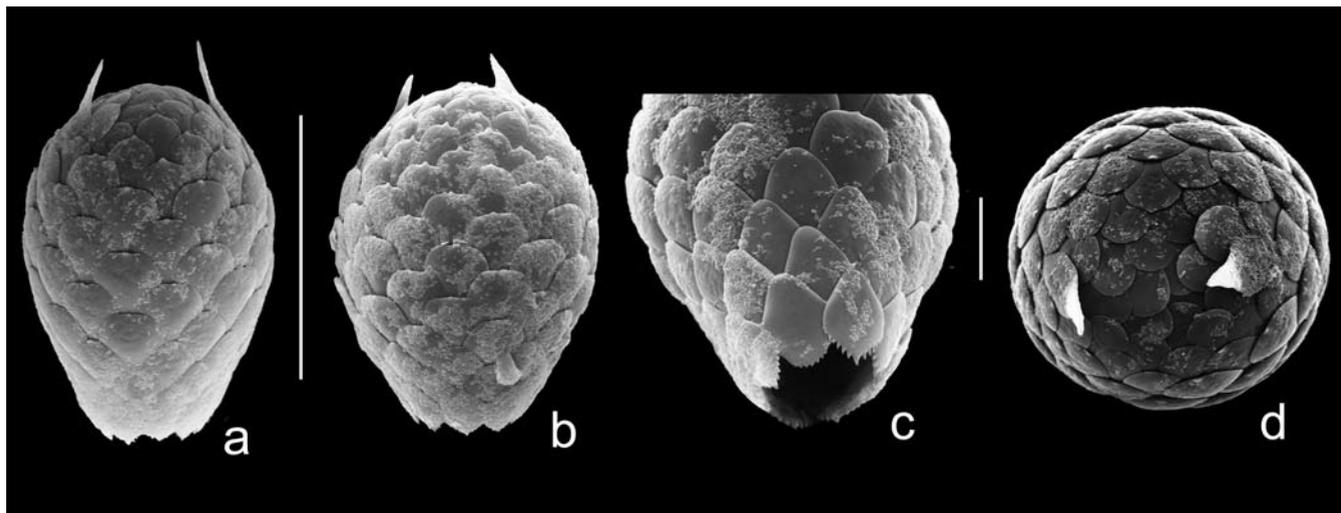


Fig. 2. *Scutiglypha cabrolae* sp. n., SEM photographs of test. **a, b** – lateral view; **c** – aperture, oblique frontal view; **d** – fundus, distal view. Scale bars: 50 μm (a, b), 10 μm (c, d).

tooth and (5)–6 smaller teeth on both sides (Figs 3a–c). The length and number of the denticles is decreasing progressively in the successive distal rows of apertural plates, whereas the length of these plates is increasing (Figs 3d–f). The transition of aperture-plates to body-plates (Figs 3g–i) is abrupt, without intermediate forms. About 5–7 rows of body plates are present, of which the third or fourth are usually the widest. The body-plates are broadly scutiform with both the oral and aboral margin weakly crenulated (oral margin of the first row of body plates occasionally straight cut with 3–5 small denticles instead of crenulated); their greatest width is situated near the aboral third of the plate. The median projection at the aboral margin of these plates is usually weakly developed (Fig. 2a); in specimens with a more pronounced median projection (Fig. 2b) its length shows an increasing tendency towards the fundus. The body-plates gradually change into smaller fundus-plates, distally becoming more and more ellipsoid without crenulation (Figs 3j–n); a single rounded fundus-plate can be present (Fig. 3o). All specimens studied display a fundus bearing two sub-terminal spine-plates (Figs 3p, q), one of them with often very short spine, arising at equal distance from the apex. The spine-plates show a weakly crenulated oral margin and a short robust spine at the aboral margin; short spines continuous with plate (Fig. 3q), longer spines weakly offset (Fig. 3p). A few small internal ellipsoid plates (Figs 3s, t: i), situated under the fundus-plates, can be present.

Measurements: Measurements of morphometric variables of the test are given in Table 1. With a mean test length, width and apertural diameter of 68.5, 48.4 and 16.4 μm , respectively, *S. cabrolae* sp. n. ranks among the medium sized Euglyphidae. Variability of test length and width was low, with coefficients of variation < 10%; a higher variability was noted for the diameter of the aperture (18%). Measurements of the different plates are shown in Table 2. The size of the body-plates (length \times width) varies from 10.0 \times 8.0 μm to 15.7 \times 13.2 μm depending on the row of plates. In view of these size differences only data for the row with the largest body-plates are presented in Table 2. The length of the spine of the spine-scales varies from 3–13 μm (mean 8 μm).

The size of the globular nucleus is 20.2–23.2 \times 18.6–18.8 μm (mean, $N = 5$: 21.4 \times 18.7 μm).

Ecology: The environmental conditions within the crater (Cabrol *et al.* submitted) are characterized by aridity, strong evaporation, intense solar UV irradiance, and a thin 480 millibar atmosphere. Temperature extremes range from -40°C to $+9^{\circ}\text{C}$, with daily averages of -12°C in winter and -5°C in summer. Ice cover forms in April and thawing typically occurs in September. The lake water is subsaline with concentrations of dissolved solids totalling circa 1.2 g L^{-1} , with Ca^{2+} and SO_4^{2-} as dominant ions. The concentrations of nitrates, nitrites and reactive phosphate were below the detection limits, and the content of dissolved organic carbon was

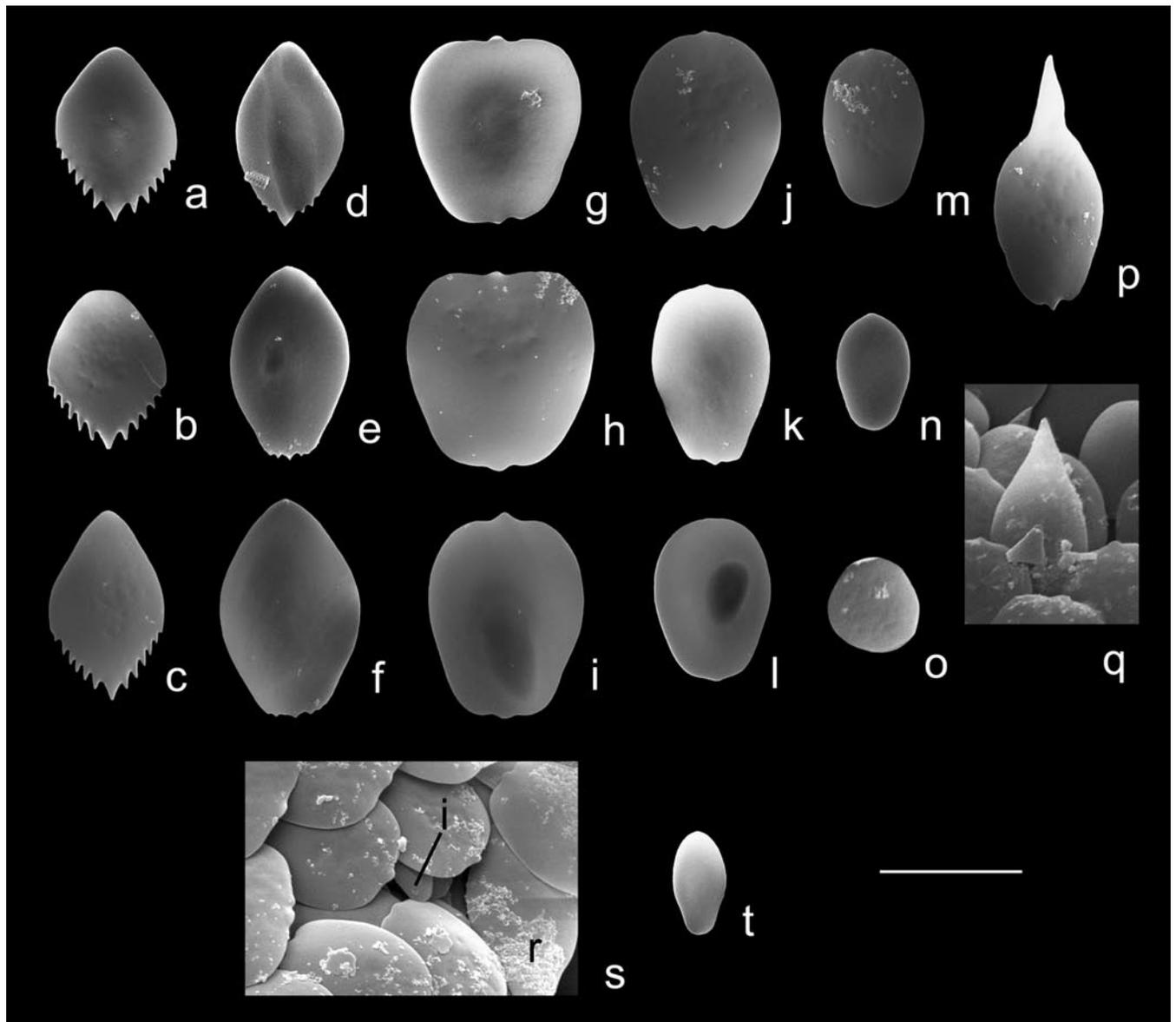


Fig. 3. *Scutiglypha cabrolae* sp. n., SEM photographs of siliceous plates composing test. **a–f** – apertural plates; **a–c** – of 1st row; **d** – of 2nd row; **e** – of 3rd row; **f** – of 4th row; **g–i** – body-plates; **g** – of 2nd row; **h** – of 5th row; **i** – of 7th row; **j–o** – fundus-plates; **p, q** – spine-plates; **s, t** – internal plates. **a–p** and **v** of same specimen, **i** – internal plate, **r** – spine plate. Scale bar: 10 μ m.

measured to be 2.44 mg L⁻¹. Levels of UV radiation are consistently high at summer solstice (UV-A + UV-B = 116 W m⁻², or 160% that at sea level), and shorter wavelength radiation (UV-C) was also detected at the lake shore, with a peak radiation at solar noon of 9.6–14.6 mW m⁻².

DISCUSSION

Foissner and Schiller (2001) established the genus *Scutiglypha* to accommodate those species of the genus *Euglypha* Dujardin, 1841 the tests of which are built of scutiform body-plates. There is some query about the validity of the former genus and intermediates have

Table 1. Morphometric characteristics of *S. cabrolae* sp. n., measurements in μm . SE – standard error of the mean, SD – standard deviation, CV – coefficient of variation in %, Min – minimum, Max – maximum, N – number of individuals investigated.

Character	Mean	Median	SE	SD	CV	Min	Max	N
Test length	68.5	68.5	1.0	4.5	6.6	55	75	20
Test width	48.4	48.5	1.0	4.6	9.5	36	56	20
Aperture diameter	16.4	16.5	0.6	2.9	17.6	11	21	20
Length/width ratio	1.42	1.40	0.02	0.1	6.3	1.3	1.6	20

Table 2. Dimensions of test plates of *S. cabrolae* sp. n. in μm . SE – standard error of the mean, SD – standard deviation, CV – coefficient of variation in %, Min – minimum, Max – maximum, N – number of plates measured.

Plate type	Aperture-plate 1 st row	Aperture-plate 2 nd row	Aperture-plate 3 rd row	Aperture-plate 4 th row	Body-plate	Fundus-plate	Spine-plate + spine
<i>Length</i>							
Mean	12.6	13.2	13.3	15.3	14.9	–	–
Median	12.6	13.1	13.3	15.5	14.7	–	–
SE	0.6	0.04	0.2	0.1	0.1	–	–
SD	1.7	0.1	0.5	0.2	0.5	–	–
CV	13.3	1.1	4.0	1.6	3.6	–	–
Min	10.0	13.0	12.4	15.0	14.3	6.7	15.4
Max	15.1	13.4	13.9	15.6	15.7	14.3	25.1
N	9	5	5	5	12	20	5
<i>Width</i>							
Mean	8.9	7.9	8.4	9.8	11.5	–	–
Median	8.6	7.6	8.3	9.9	11.6	–	–
SE	0.3	0.2	0.2	0.1	0.3	–	–
SD	1.0	0.4	0.5	0.3	0.9	–	–
CV	11.0	5.4	5.4	2.3	7.8	–	–
Min	7.6	7.6	7.7	9.3	10.0	5.2	7.1
Max	10.9	8.7	9.1	10.0	13.2	10.5	7.6
N	9	5	5	5	12	20	5

been reported (see for example Foissner and Schiller 2001, Wylezich *et al.* 2002), but the specific shape of the body-plates and the test pattern complexity, viz. the array of differently shaped types of plates of different dimensions, present in *Scutiglypha* seems a valid feature for the distinction of the two genera. The genus

Euglypha, as originally defined, is a large, common genus containing more than 40 morphospecies and twice as many sub- and infra-subspecific taxa, of which only a few have been studied by modern methods (Meisterfeld 2002). The major problems with the genus are on the one hand excessive splitting of taxa, and on the other

hand the tendency, as was pointed out already by Wailes and Penard (1911), to record all Euglyphae under a few well-known species or to uncritically synonymize (e.g., Decloitre 1962) different taxa. Of the many sub- and infra-subspecific taxa described on a morphological basis it is actually impossible to tell whether they are merely examples of phenotypic plasticity or genetically different lineages. On the other hand it is also evident from the ssu rDNA sequence comparisons by Wylezich *et al.* (2002) and Lara *et al.* (2007) that molecular divergences may reflect characteristic combinations of sometimes subtle different morphological features that can only be observed with scanning electron microscopy. They also showed that in euglyphid testate amoebae molecular data support that shape and size of the plates, plate arrangement, and test dimensions are decisive characters for the determination at the species level.

To date five species displaying scutiform body-plates have been described: *S. acanthophora* (Ehrenberg, 1841), *S. aspera* (Penard, 1891), *S. australica* (Playfair, 1917), *S. crenulata* (Wailes, 1912) and *S. scutigera* (Penard, 1911). Contrary to Foissner and Schiller (2001), who tentatively treated *S. australica* and *S. crenulata* as synonyms, we consider these valid species in view of the obvious differences in plate morphology and test dimensions.

In *S. aspera* and *S. scutigera* the oral and aboral margins of the body-plates are simply rounded, provided or not with a rounded median process, whereas in the other species, as well as in *S. cabrolae* sp. n. they are crenulated.

There is some controversy regarding the taxonomic status of *S. acanthophora*. This controversy basically centres on the fact that as originally described *Euglypha acanthophora* body-plates are elliptical (see, e.g., Wailes 1915, Playfair 1917). Despite this, the euglyphid pictured sub *E. alveolata* Dujardin, 1841 in Leidy (1879), showing a small projection at the aboral margin of the body-plates, was synonymized with *E. acanthophora* by Wailes (1915), and a *Euglypha* with distinctly crenulated body-plates illustrated by scanning electron microscopy was identified as *E. acanthophora* by Ogden (1981). Meisterfeld (in Foissner and Schiller 2001) and Wylezich *et al.* (2002) found intermediate forms, with only slightly crenulated body-plates and smaller dimensions, between typical *E. acanthophora* and *S. crenulata*, respectively. Foissner and Schiller (2001) argued that the taxa and transitions of Ogden and Meisterfeld are varieties of *E. crenulata*, or that *E.*

acanthophora and *E. crenulata* are members of a sister species complex. Whether these are natural variations within the species, or we are actually dealing with different species does not matter for the distinction of *S. cabrolae* sp. n. The new species is distinguished from the '*acanthophora* type' with crenulated body-plates by for example its more pronounced ovate shape with more strongly arched sides, the smaller length/width ratio (1.4 vs. 2.0–2.1) and the larger body-plates (on average $14.9 \times 11.5 \mu\text{m}$ vs. $8.7\text{--}11.2 \times 7.2\text{--}9.0 \mu\text{m}$).

Scutiglypha crenulata and its var. *minor* Wailes, 1912 are characterized by the elongate-ovate test with the sides less arched as in the new species, and the greater dimensions (Wailes 1912): length \times width $115\text{--}140 \times 67\text{--}77 \mu\text{m}$ and $80\text{--}100 \times 45\text{--}60 \mu\text{m}$ instead of $55\text{--}75 \times 36\text{--}56 \mu\text{m}$. The aperture of *S. crenulata* is bordered by 2–3 rows of apertural plates, 12–14 in each row, whereas 3–4 rows each with 8–10 plates are present in *S. cabrolae* sp. n. The aperture-plates of *S. crenulata* have their aboral margin crenulated or provided with a blunt process (aboral margin simply rounded in *S. cabrolae* sp. n.), and the oral margin bears 3–5 denticles (5–6 in *S. cabrolae* sp. n.) laterally from the median tooth. The oral and aboral margins of the body-plates of *S. crenulata* are conspicuously crenulated showing three distinct processes, contrary to the weak crenulation with minute and shallow median process in *S. cabrolae* sp. n.

Dioni (1970) described and pictured a species sub *Euglypha crenulata* Wailes from the Paraná (Argentina) with almost identical dimensions to that of *S. cabrolae* sp. n., but distinguished from the latter by the scutiform body-plates showing broadly rounded oral and aboral margins, the latter with blunt process medially (oral and aboral margin of scutiform body-plates crenate in *S. cabrolae* sp. n.), and the more and longer flexuous spines occurring from the posterior third of the test on, contrary to the two sub-terminal and robust spines in *S. cabrolae* sp. n. (however, spines and especially their number and length are a relatively weak character given that they are sometimes shorter or lacking in cultures (Meisterfeld, pers. comm.).

Based on the shape of the body-plates *S. cabrolae* sp. n. is apparently most closely related to *S. australica*. The new species is distinguished from the latter by its more thick-set habitus, resulting in a smaller length/width ratio of 1.4 vs. 1.9 in *S. australica*. The size of *S. cabrolae* sp. n. is on average smaller than the size of *S. australica* and its var. *elegans* (Playfair 1917) (length \times width of nominal species and variety: $64\text{--}137 \times 32\text{--}72$

μm , aperture 19–36 μm). Only two rows of aperture-plates are reported in *S. australica*, contrary to the 3–4 rows in the new species. The shape of the body-plates is rather similar in both species but dimensions are apparently smaller in *S. australica* (10–12 \times 7–10.5 μm vs. 10.0–14.9 \times 8.0–11.4 μm in *S. cabrolae* sp. n.). In the description of the body-scales of *S. australica* Playfair (1917: 661) also states that ‘only the two minute spikes (our median processes at the oral and aboral margin) overlap, leaving a minute foramen on either side.’ However, this must be considered as an incorrect observation, and the illustration of the body-plate (loc. cit. Pl. 40, Fig. 2), suggesting that the oral margin is broader than the aboral margin, must be considered upside down.

Examining Miocene fossilized specimens of *S. crenulata* by scanning electron microscopy Foissner and Schiller (2001) found that in the most fully decayed specimens the plates dissolve from the margin to the centre, becoming smaller and ellipsoidal. The small elliptical plates, illustrated in their Fig. 7 (loc. cit.) were considered to represent final stages of the disintegration process, although they did not exclude that it could be fundus-plates as well. In our preparations of *S. cabrolae* sp. n. we observed similar small plates (Fig. 3t) which are located in the fundus region at the inner side of the body plates (Figs 3s: i), proving that they are not disintegration artefacts.

The occurrence of *S. cabrolae* sp. n. in plankton samples is rather unusual for Euglyphidae, which are primarily inhabitants of aerophytic mosses, sphagnum and organic soils, or show a benthic-periphytic way of life. Most probably this must be ascribed to everyday temperature induced circulations of the lake water, wind mixing and/or stirring up of benthos/periphyton from the shallow littoral when the samples were taken, as is also suggested by the presence of ostracods and chironomid larvae. However, studies (see for example Velho *et al.* 1999) show that testate amoebae, e.g., *Euglypha acanthophora*, can be the dominant zooplankters in the littoral zone of lentic and lotic environments as well. Their occurrence in the planktonic environment appears not determined only by washing out from the bottom and from the shoreline vegetation, but may represent stages necessary to guarantee the success of the populations as well.

Most Euglyphidae apparently have a world-wide distribution (see for example Decloitre 1962, Stout 1969). However, analyses of sequences of ssu rRNA

coding regions reveal that widely distributed morpho-species can split into different clades (Wylezich *et al.* 2002, Meisterfeld 2006). This illustrates that the apparent cosmopolitanism of certain species is artificial and caused by the lack of morphological characters allowing for taxonomic resolution. The morphologically well-defined *S. cabrolae* sp. n. is to date only known from its type locality, and its most closely related congener, *S. australica*, has only been reported from Australia, suggesting that both species may represent Gondwanan elements that evolved after Australia’s last Gondwanan links were severed during the Early Tertiary. Considering the specificity of the extreme habitat of the Licancabur caldera lake, *S. cabrolae* sp. n. also could be a local endemic. The volcanic cone of Licancabur was constructed largely in the late Pleistocene (De Silva and Francis 1991) and it would seem, therefore, that colonization (and speciation as well in case *S. cabrolae* sp. n. proves to be a local endemic), must have occurred at the earliest during the Pleistocene/Holocene transition.

Owing to the excellent preservation of siliceous test plates in lake sediments (Douglas and Smol 1987, Foissner and Schiller 2001) and the species-specific shape of the plates, it should be possible using sedimentary profiles, to reconstruct the colonization history of lake Licancabur and to determine the ancestor of recent *S. cabrolae* sp. n.

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