

A new species of *Sphaerium* Scopoli, 1777, from southern Brazil (Bivalvia: Sphaeriidae)

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ABSTRACT

Sphaerium cambaraense new species is described based on samples collected in the beginning of the summer near the headwaters of Taquari River (Jacui River Basin) on the basaltic plateau of southern Brazil. This is the first record for the genus *Sphaerium* in Brazil and in South America outside the Andes. *Sphaerium cambaraense* is characterized by a relatively large and solid shell, a high triangular shell outline and a solid hinge plate. Compared with *Sphaerium forbesi* (Philippi, 1869), *S. cambaraense* has a more strongly triangular shell outline and beaks not prominent. *Sphaerium lauricochae* (Philippi, 1869), another similar species from Bolivia, Chile, and Peru, has a more rounded shell outline.

Additional Keywords: Freshwater, Rio Grande do Sul, South America

INTRODUCTION

According to Dreher-Mansur and Meier-Brook (2000), the family Sphaeriidae is represented by two subfamilies: Euperinae, including the genera *Eupera* Bourguignat, 1854, and *Byssanodonta* d'Orbigny, 1846; and Sphaeriinae with three genera, *Sphaerium* Scopoli, 1777, *Musculium* Link, 1807, and *Pisidium* Pfeiffer, 1821.

Four species of the genus *Sphaerium* are known from South America: *Sphaerium aequatoriale* Clessin, 1879, from Ecuador (Kuiper and Hinz, 1984); *S. forbesi* (Philippi, 1869) recorded from Peru and Bolivia (Haas, 1949), from Bolivia (Haas, 1955), from Colombia, Peru, Bolivian Andes (Kuiper and Hinz, 1984), and Chile (Ituarte, 1995); *S. lauricochae* (Philippi, 1869), from Peru, Bolivia and Chile (Kuiper and Hinz, 1984), and Chile (Ituarte, 1995); *S. titicacense* (Pilsbry, 1924), from Peru and Bolivia (Kuiper and Hinz, 1984). According to these authors, they are concentrated in the Central Andes from Ecuador to North Chile including high-altitude Bolivia, at 2000 to 4700 m altitude; varying in size between 3 and 12.5 mm. Haas (1949) reports

Sphaerium (*S.*) *boliviense* (Sturany, 1900), from the highlands of Bolivia and from a lake in Junin, Peru. He examined also material of this species collected by Sioli in the regions of the rivers Maué-Açú and Tapajós, tributaries of the Amazon River, which constitutes the first record of *Sphaerium* for Brazil. According to Kuiper and Hinz (1984), *S. boliviense* is a junior synonym of *S. forbesi*.

The record of *Sphaerium observationis* by Mansur et al. (1991) for Mirim Lagoon in southern Brazil is a misidentification, as that species is not a *Sphaerium*. According to the revision by Ituarte (1995), *Pisidium observationis* Pilsbry, 1911, only occurs in the southern Argentina, not in Brazil.

Sphaerium cambaraense new species is the fifth *Sphaerium* species from South America and the first species of the genus described from southern Brazil, in the highlands near the Atlantic Ocean, a location geographically very distant from the Andes and Amazon River.

MATERIALS AND METHODS

Specimens were collected with a plastic sieve with mesh size of about 0.8 mm. Specimens were sorted from the sediment with fine feather tweezers; anesthetized in small vials containing water with menthol crystals, fixed in a 5% formalin solution for 24 hours, rinsed for 24 hours in tap water and preserved in 70% ethanol. Soft parts of specimens for scanning electron microscopy (SEM) were removed with tweezers, shells cleaned with a soft and fine brush and rinsed several times in distilled water. Dried shells were glued on stubs with light-silver glue (Porolon Equipment, Herts) or metallic adhesive tape (TO66 Silver tape 9 mm, Hert-Scotch), coated with gold, and observed either in a Cambridge Stereoscan 250 Mk2 or Philips scanning electron microscopes. Shell microstructure was studied by fracturing shells at the middle of the height, parallel to commarginal ridges. Terminology for shell microstructure follows Dydych-Falniowska (1983) and Dreher-Mansur and Meier-Brook (2000).

Stomach nomenclature follows Purchon (1958, 1960). The shape indices, height index [$I = H/L$] and convexity index [$Ci = W/H$], were calculated according to the criteria followed by Ituarte (1996).

Abbreviations use in the text are: MACN, Museo Argentino de Ciencias Naturales, Buenos Aires; MCN, Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre; MCP, Museu de Ciências e Tecnologia da Pontifícia Universidade Católica, Rio Grande do Sul, Porto Alegre, Brazil.

Genus *Sphaerium* Scopoli, 1777

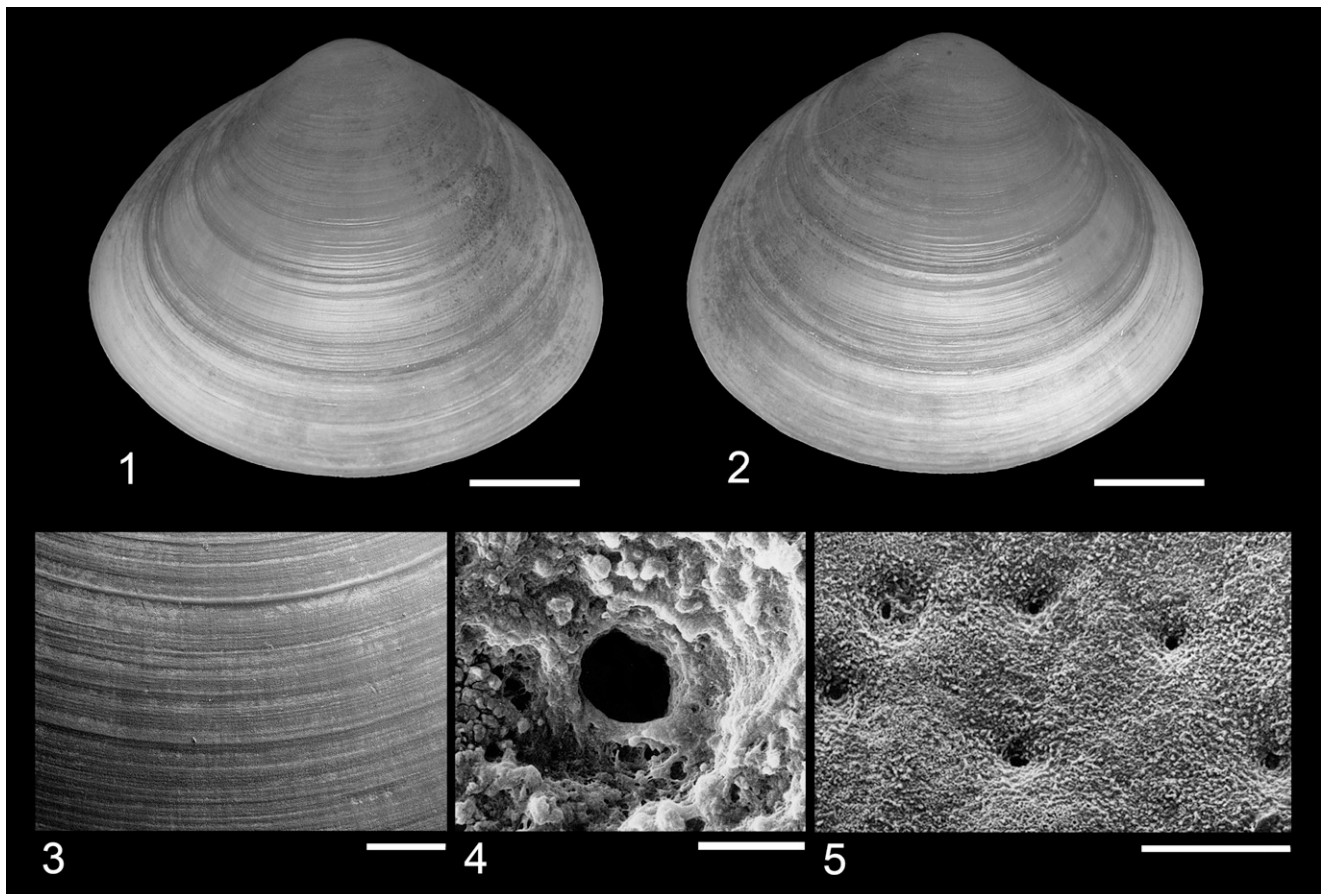
Sphaerium cambaraense new species
(Figures 1–20)

Diagnosis: Distinguished by the relatively large and solid shell with trapezoidal tending to triangular shell outline, low and wide beaks, subcentrally located, without marked nepionic cap, and broad and solid hinge plate.

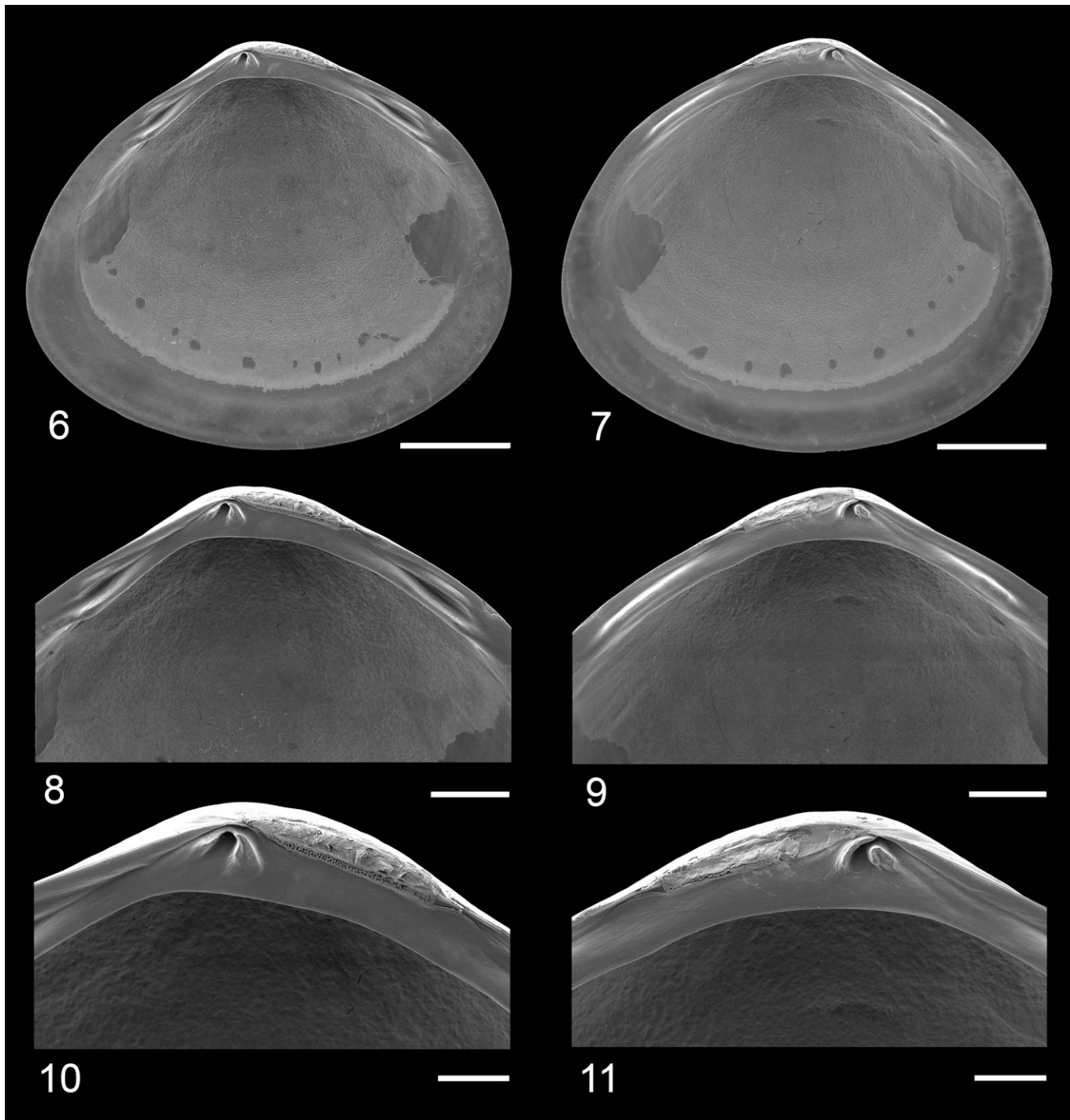
Description: SHELL: Solid, relatively large (maximum observed L: 11.22 mm), slightly convex ($Ci = 53 \pm 5$). Shell outline high ($I = 85 \pm 2$), trapezoidal tending to triangular. Dorsal margin has pronounced curve. Anterior and posterior margins gradually descending and

gently curved below middle of total height, without marked angle. Posterior end slightly truncated, oblique (Figures 1, 2). Ventral margin long and evenly curved. Shell surface silky, glossy, with very fine irregularly distributed radial lines, weaker on beaks and irregular commarginal, sometimes coarse, striae (12 or more per 0.5 mm in the middle of the shell (Figure 3). Outer shell surface light yellowish brown; pale brown at beaks, more ventrally grayish brown with complete or incomplete yellow concentric bands running from anterior to posterior margins; a large yellow band near ventral margin. Beaks without marked embryonic cap. Inner shell surface white, grayish at muscle scars. Beaks slightly prosogyrous, low and wide, slightly raised above dorsal margin, subcentrally located (Figures 1, 2, 6, 7).

Hinge plate strikingly curved, solid, broad, reaching 0.5 mm width in middle region in specimens of 11 mm length, slightly narrower at level of cardinal teeth. Hinge line arched, particularly below cardinal teeth (Figures 6–9). Cardinal teeth strong, close to dorsal margin. Right cardinal teeth strong, short, strongly curved, posterior end enlarged in a grooved cup (Figure 10). Left cardinal teeth: outer cardinal tooth, C4, thin, strikingly oblique, located immediately behind C2, anterior end slightly overlapping



Figures 1–5. *Sphaerium cambaraense* new species. **1, 2.** Holotype MCP Mol. Outer view of left and right valves. **3.** Detail of outer shell surface. **4, 5.** Inner shell surface, detail of pores. Scale bars: 1, 2 = 4 mm; 3 = 400 μ m; 4 = 4 μ m; 5 = 40 μ m.



Figures 6–11. *Sphaerium cambaraense* new species. Paratype (MACN-In 37063). **6.** Inner view of right valve. **7.** Inner view of right valve. **8.** Hinge of right valve. **9.** Hinge of left valve. **10.** Detail of right cardinal tooth (C3) and ligament. **11.** Detail of left cardinal teeth (C2 and C4) and ligament. Scale bars: 6, 7 = 2 mm; 8, 9 = 1 mm; 10, 11 = 500 μ m.

C2; inner cardinal tooth, C2, short, high, columnar, deeply arched into a V-shape (Figure 11). Right lateral teeth somewhat short, strong, with distal cusps (Figure 8); left lateral teeth relatively long, strong, and high (Figure 9). Ligament internal but exteriorly visible, slightly protruded in larger specimens (Figures 10, 11).

SHELL MICROSTRUCTURE: Inner shell surface perforated by numerous pores ($55/400 \mu\text{m}^2$ to $13/160 \mu\text{m}^2$) (Figures 4, 5) representing the opening of tubuli that cross entire calcareous part of shell (shown in part in Figure 12). Openings of pores on inner surface surrounded by a funnel-shaped depression and in mouth circled by a rim

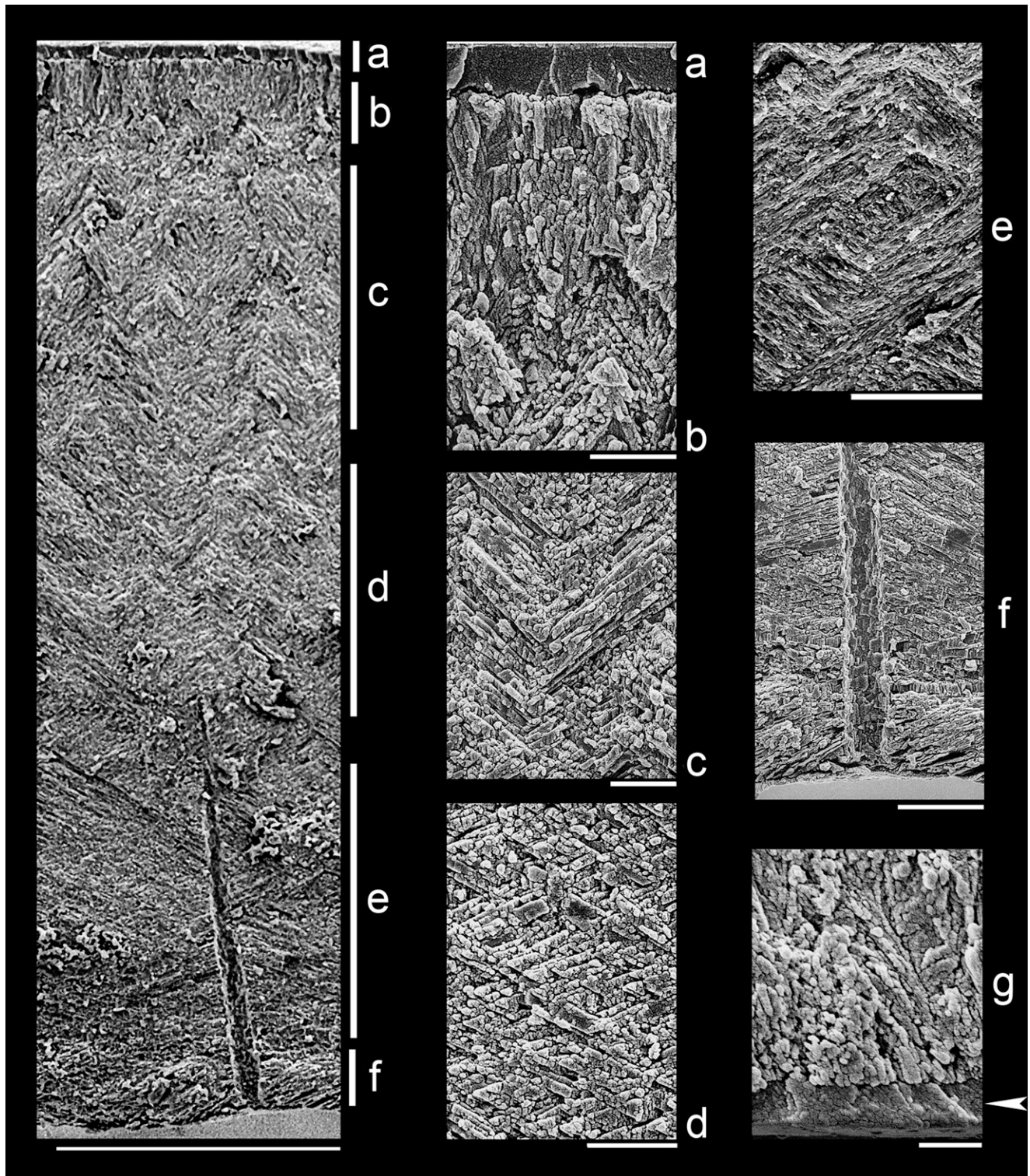
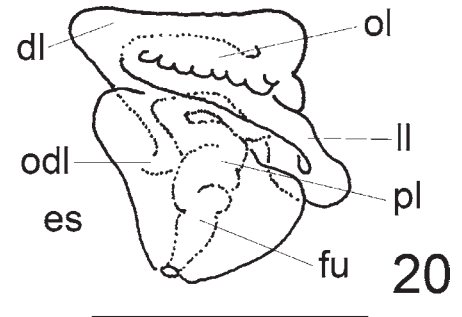
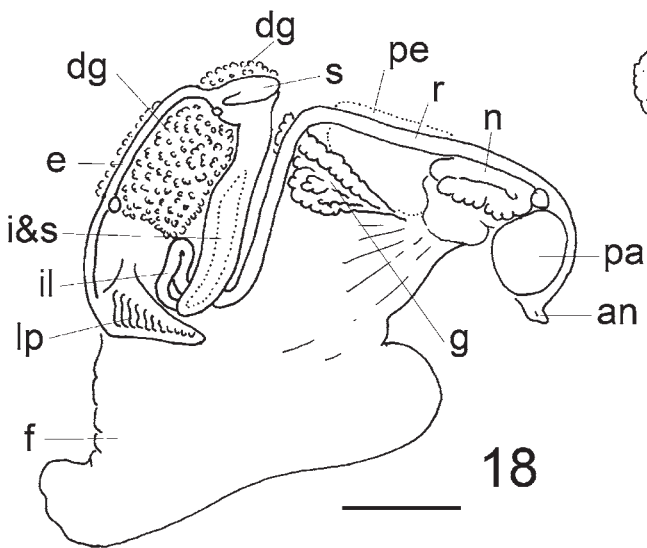
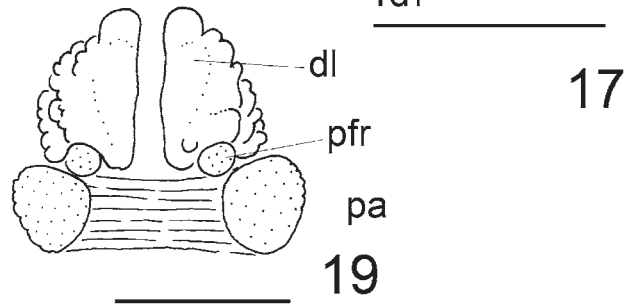
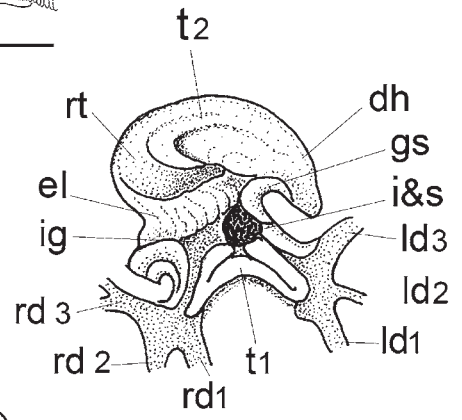
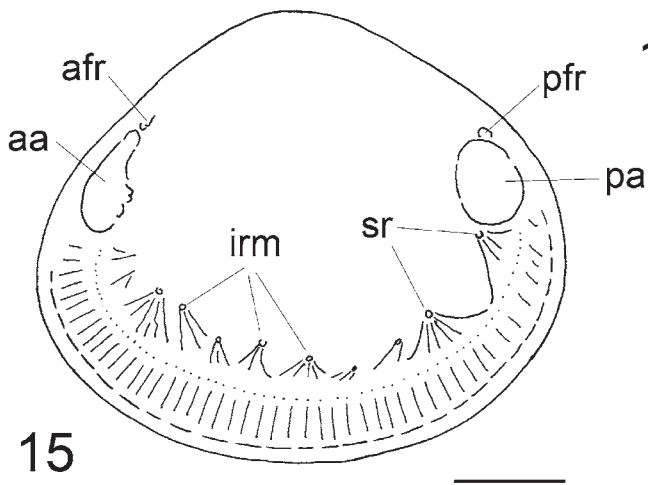
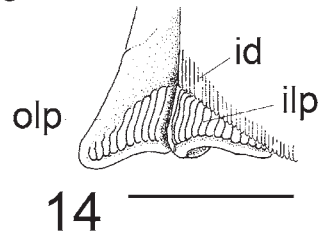
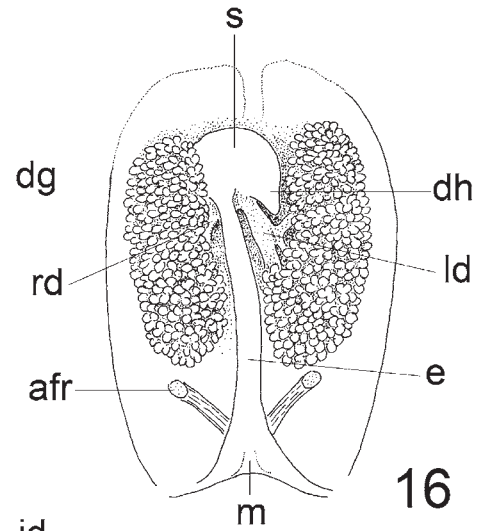
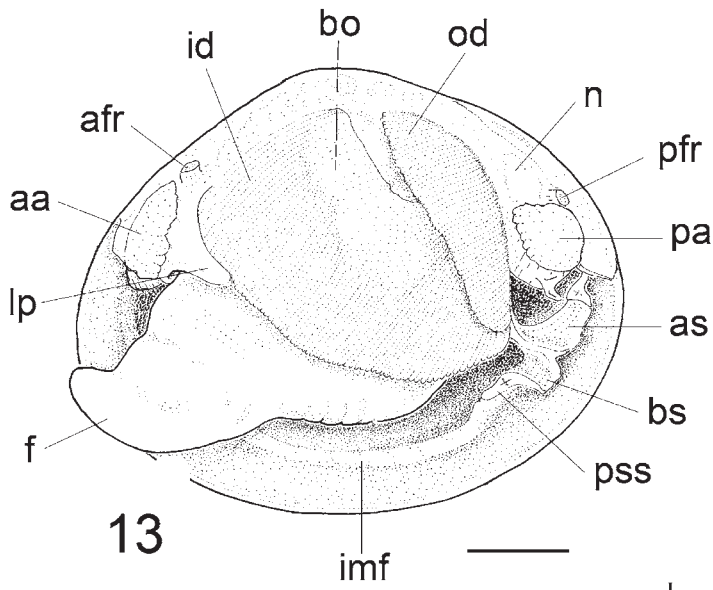


Figure 12. *Sphaerium cambaraense* new species. Shell microstructure from **a**: periostracum (top) to **f**: the endostracum (bottom). Inserts **a–g** are details of: **a**, periostracum; **b**, granular layer; **c**, diagonal layer forming a composite prismatic structure; **d**, diagonal layer, crossed structure; **e**, diagonal layer forming a pseudo crossed lamellar structure; **f**, palisade structure; **g**, diagonal layer, and internal surface of the endostracum (arrow). Scale bars: 12 = 50 μm ; inserts: a–d = 4 μm ; e = 20 μm ; f = 10 μm ; g = 2 μm .



(Figure 4). Shell relatively thick reaching 200 μm in central area (Figure 12). Shell structure consists of five layers: periostracum, granular layer, diagonal layer, palisade, diagonal layer, and endostracum. Below the 2 μm thick periostracum (Figure 12, **a**), a granular 10 μm thick layer appears (Figure 12, **b**); grains concentrated in some points irregularly and sparsely distributed, looking like cone-shaped bars arranged perpendicularly to shell surface. These change into irregularly and compactly arranged grains of different sizes. More internally, shell structure changes gradually into a diagonal layer (Figure 12, **c**, **d**, **e**), where three different patterns may be distinguished: first (about 50 μm thick), composite prismatic structure, showing vertical feather-like pattern (Figure 12, **c**); second (about 40 μm thick), occupies the central part of shell, gradually changing into a cross-lamellar structure (Figure 12, **d**); the third (approximately 60 μm), resembles a pseudo crossed-lamellar structure (Figure 12, **e**). Close to the inner shell surface there are one or two palisade layers, narrow (each about 1 μm thick) (Figure 12, **f**). The palisade structure is followed by a diagonal layer with lamellae oriented in one direction (Figure 12, **g**); below, a very narrow layer, the endostracum (1.0 to 1.5 μm thick) (Figure 12, arrow).

ANATOMY (FIGURES 13–20): Inner and outer demibranchs well-developed, outer smaller demibranch reaching half of height of inner demibranch (Figure 13). Brood sacs occupy anterodorsal part of inner demibranchs, embryos contained in sacs showed different developmental stages, denoting sequential brooding. Up to six developing embryos found in largest examined specimen (≥ 11 mm length). Largest, tertiary, brood sac contained two embryos (≥ 1 mm length) (Figure 13); secondary sac located under largest one, contained three or four small embryos. Primary sac attached lower on inner demibranch. (Specimens for this study were collected at the beginning of the Southern Hemisphere summer (January, 1994) and many of the specimens larger than 9 mm showed brood sacs.) Anal and branchial openings extended in diverging short siphons, nearly equal in size. Anal siphon wider at base and more stretched out (Figure 13). Two labial palps (Figure 13, 14) on each side of mouth, triangular-shaped in lateral view; opposite contacting walls with 12 small folds, tapering toward distal ends. Mantle musculature (Figure 15) with relatively short siphonal retractors; inner radial mantle muscles arranged in eight to nine bundles (Figure 15). Fan-shaped stomach (Figures 17, 18) bent to right side, laterally covered by digestive gland. Dorsal hood relatively short and left duct well developed. Stomach internally (Figure 17) shows very simplified structures

on dorsal hood and right side such as short gastric shield under dorsal hood; minor typhlosole and rejecting tract beginning at right side after descending from dorsal hood; and an elevation slightly wrinkled between intestinal groove and rejection tract; anterior fold absent. Intestine opening associated to style sac in center of floor; major typhlosole arched in front with two expansions that end respectively at left and right duct openings, not penetrating in ducts, which allocates the stomach to Type IV; left and right duct openings well developed and ramified in three secondary ducts.

Intestine short and simple (Figure 18), anterior part associated to style sac, broad, descending straight to floor of visceral mass; mid-intestine strikingly stretched forming single loop; hindgut straight, ascending and bending toward rectum that ends into an anal papilla. Nephridia of closed type; dorsal and outer lobes fused, impossible to distinguish in dorsal view (Figures 19, 20); external wall of outer lobe ornamented with many small, rounded extrusions; nephridia in young specimens usually of open type and with separated lobes. Funnel relatively long and wide; proximal loop with smooth walls forming three ascending rings then run backward up to posterior adductor muscle, turning laterally in direction to lateral loop. Lateral loop straight, elongated, running along outer side and partially covered laterally by outer lobe. Excretory sac sub-triangular, rounded in front and relatively small. No valve at the entering of the distal loop into excretory sac.

Type Locality: Lajeado da Margarida (50°15.75' W, 29°0.87' S; 870–880 m altitude) on Camisa River, Antas River Basin, considered to be the headwaters of Taquari River, the main tributary of Jacuí River, South Atlantic Brazilian Basin.

Type Material: Holotype MCN 38821; Paratypes: MCN 33919 (33 specimens), MCP 9109 (6 specimens), MACN-In 37063 (two specimens), 12 Jan. 1994.

Etymology: The specific epithet refers to the City and Municipality of Cambará, close to the type locality.

Distribution and Habitat: Known only from the type locality. The Municipality of Cambará is situated in northeastern Rio Grande do Sul State, Brazil, in the highest part named Planalto Riograndense. From the physiographic point of view, this region is characterized by a basaltic shield covered by low grass steppes and *Araucaria* Forest mixed with the southeastern limits of the Atlantic Forest. The altitude varies from 850 to 1050 m, and in the winter, temperatures fall below freezing and there is the occasional snow. The rivers that cross the region, flanked by a low gallery forest, have

Figures 13–20. *Sphaerium cambaraense* new species. Schematic drawings of soft anatomy. **13.** Gross anatomy (left mantle lobe removed). **14.** Folded surfaces of inner and outer left labial palps. **15.** Outer view of left mantle lobe showing the inner radial mantle muscles. **16.** Dorsal view of the stomach and digestive gland. **17.** Floor of the stomach after removing the roof. **18.** Inner view of the organs in the visceral mass. **19.** Dorsal view of nephridia, posterior adductor muscle and posterior foot retractors. **20.** Lateral view of left nephridium. Scale bars: 13, 15, 16, 18 = 2 cm; 14, 17, 19, 20 = 2 mm.

hard bottoms formed by flattened basaltic stones, and currents are strong. The collecting sites were small ponds along the river course, where currents were lower, allowing the accumulation of decayed leaves and very soft, dark, and fine sand deposits, not deeper than 1 m, where specimens settle. Together with the *Sphaerium* samples, many specimens of one species of *Pisidium* sp. (MCN 33918), and one of *Diplodon* sp. (MCN 33920) not yet identified, were found.

DISCUSSION

Sphaerium cambaraense is similar to *Sphaerium forbesi* (Philippi, 1869) (from Bolivia, Chile, and Peru). However, *S. cambaraense* has a more decidedly triangular shell outline, beaks not full with not marked nepionic shell; in addition, *S. cambaraense* is larger than *S. forbesi*. *Sphaerium lauricochae* Philippi, 1869, also reported from Bolivia, Chile, and Peru, differs from *S. cambaraense* by its more rounded shell outline. In relation to shell thickness and microstructure, *S. cambaraense* is similar to the European species *Sphaerium rivicola* (Lamarck, 1818) and *Sphaerium corneum* (Linnaeus, 1758); Dyduch-Falniowska (1983) reported for these species (as well as for *Musculium lacustre* (Müller, 1774) and several *Pisidium* species) six different layers, (1) periostracum, (2) homogeneous-granular layer, (3) granular layer; (4) diagonal layer (composite prismatic structure), (5) palisade structure, and (6) endostracum. Only *S. corneum* and *S. rivicola* showed a different structure for the diagonal layer, referred to as "crossed-lamellar structure" (Dyduch-Falniowska, 1983). This structure was also found in *S. cambaraense*, however, the diagonal pattern of the plates has a different arrangement: in *S. rivicola* the oblique plates show a horizontal herringbone pattern and in *S. cambaraense* some rows of plates are oblique not forming a horizontal pattern. These latter resemble in part the pseudo crossed-lamellar structure found in the Corbiculidae. The periostracum layer in *S. cambaraense* is thinner than in *S. corneum* and the homogeneous granular layer is lacking. The simplified condition of posterior and right side of stomach is quite similar to that found in the Euperinae (Dreher-Mansur and Meier-Brook, 2000).

The nephridium is relatively similar compared to *S. corneum* (Dreher-Mansur and Meier-Brook, 2000), but the excretory sac and proximal loop are shorter and the valve at the insertion of the distal loop into excretory sac is lacking in *S. cambaraensis*; the multilobed surface of lateral lobe is observed for the first time in *Sphaerium*.

Based on the similar morphology of the nephridium, Kornushin (1998) proposed that South American species traditionally assigned to *Sphaerium* actually belong to *Musculium*. According to Park and Ó Foighil (2000), the usefulness of the fine anatomy of nephridium in the family Sphaeriidae is relative, due to its high morphological plasticity. Cooley and Ó Foighil (2000), based on mitochondrial 16S rDNA gene sequences observed that the *Sphaerium/Musculium* clade exhibit moderate to low levels of genetic divergences and

the same asynchronous or sequential brooding pattern (i.e., brooding sacs contain more than one developing generations of embryos). Nevertheless, Cooley and Ó Foighil (2000) recognized *Musculium* as a monophyletic group. We allocate the new species in *Sphaerium* until more evidence is available to help with this question. More recently, a phylogenetic analysis of the Sphaeriinae (Lee and Ó Foighil, 2003) based on a molecular study of nuclear (ITS-1) and mitochondrial (16S) gene sequences of 15 species from North and South America, Europe, Asia, and Australia, recovered a strongly supported monophyletic group of sequential brooders (*Musculium* and *Sphaerium*). However, the analyses indicate that *Musculium* and *Sphaerium* sensu lato are not natural groups, proposing a new classification system comprising five subgenera within *Sphaerium*: *Sphaerium* sensu stricto Scopoli, 1777; *Musculium* Link, 1807; *Amesoda* Rafinesque, 1820; *Sphaerinova* Iredale, 1943, and *Herringtonium* Clarke, 1973. As these subgeneric groupings, however well supported by molecular data, are not defined from the morphological point of view, it is not possible at this point to place *S. cambaraense* within the new scheme.

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LITERATURE CITED

- Cooley, L. R. and D. Ó Foighil. 2000. Phylogenetic analysis of the Sphaeriidae (Mollusca: Bivalvia) based on partial mitochondrial 16S rDNA gene sequences. *Invertebrate Biology* 119: 299–308.
- Dyduch-Falniowska, A. 1983. Shell Microstructure and Systematics of Sphaeriidae (Bivalvia, Eulamellibranchiata). *Acta Zoologica Cracoviensia* 16: 251–296.
- Haas, F. 1949. Land und Süßwassermollusken aus dem Amazonas Gebiete. *Archiv für Molluskenkunde* 78(4/6): 149–156.
- Haas, F. 1955. On some small collections of inland shells from South America. *Fieldiana, Zoology* 34(35): 361–387.
- Ituarte, C. 1995. Nuevos registros de *Pisidium* Pfeiffer, 1821 y *Sphaerium* Scopoli, 1777 (Bivalvia: Sphaeriidae) en Chile, Bolivia y Noroeste argentino. *Neotropica* 41(105–106): 31–41.
- Ituarte, C. F. 1996. Argentine species of *Pisidium* Pfeiffer, 1821, and *Musculium* Link, 1807 (Bivalvia: Sphaeriidae). *The Veliger* 39 189–203.
- Kornushin, A. 1998a. Notes on the anatomy of some species of *Sphaerium* s. (Mollusca, Bivalvia) from the tropical region with revision of their taxonomic status. *Vestnik zoologii* 32(3): 3–12.

- Korniushin, A. 1998b. A comparative investigation on nephridia in fingernail and pill clams. *Malacological Review*, Supplement 7: 53–63.
- Kuiper, J. G. J. 1962. Notes sur le systématique des pisides. *Journal de Conchyliologie* 102: 53–57.
- Kuiper, J. G. J. and W. Hinz. 1984. Zur Fauna der Kleinmuscheln in den Anden (Bivalvia: Sphaeriidae). *Archiv für Molluskenkunde* 114(4/6): 137–156.
- Lee, T. and D. Ó Foighil. 2003. Phylogenetic structure of the Sphaeriinae, a global clade of freshwater bivalve molluscs, inferred from nuclear (ITS-1) and mitochondrial (16S) ribosomal gene sequences. *Zoological Journal of the Linnean Society* 137: 245–260.
- Mansur, M. C. D. and C. Meier-Brook. 2000. Morphology of *Eupera Bourguignat* 1854, and *Byssanodonta Orbigny* 1846 with contributions to the Phylogenetic Systematics of Sphaeriidae and Corbiculidae (Bivalvia: Veneroidea). *Archiv für Molluskenkunde* 128(1/2): 1–59.
- Mansur, M. C. D., C. Schulz, M. G. O. Silva, and N. M. R. Campos-Velho. 1991. Moluscos bivalves límnicos da Estação Ecológica do Taim e áreas adjacentes, Rio Grande do Sul, Brasil. *Iheringia, Série Zoologia*, 71: 43–58.
- Park, J. K. and D. Ó Foighil. 2000. Sphaeriid and corbiculid clams represent separate heterodont bivalve radiations into freshwater environments. *Molecular Phylogenetics and Evolution* 14: 75–88.
- Purchon, R. D. 1958. The stomach in the Eulamellibranchia; Stomach type IV. *Proceedings of the Zoological Society of London* 131: 487–525.
- Purchon, R. D. 1960. The stomach in the Eulamellibranchia; stomach types IV and V. *Proceedings of the Zoological Society of London* 135: 431–489.