Ultrastructural analysis of the shells of *Anodontites trapesialis* (Lamarck) and *Anodontites elongatus* (Swaison) (Mollusca, Bivalvia, Etherioidea) from the Mato Grosso Pantanal Region, Brazil [1]

Claudia T. Callil [2] & Maria C. D. Mansur [3]

[1] Programa de Pós-Graduação em Biociências, Pontifícia Universidade Católica do Rio Grande do Sul. Part of doctoral thesis partially supported by CAPES, and SHIFT Program – Pantanal Ecology Project.

[2] Departamento de Biologia e Zoologia, Instituto de Biociências, Universidade Federal de Mato Grosso. Avenida Fernando Corrêa da Costa, 78000-900 Cuiabá, Mato Grosso, Brasil. E-mail: callil@cpd.ufmt.br

[3] Laboratório de Malacologia, Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul. Avenida Ipiranga 6681. 90619-900 Porto Alegre, Rio Grande do Sul, Brasil. E-mail: mcmansur@pucrs.br

---

ABSTRACT. Based on optical and SEM microscopic observations, the projections of the outer surface of the periostracum and inner micro-structures of the shell are described and redefined. The outer surface of the periostracum is practically smooth in both species. Considering a mesoscopic view of the periostracum, *A. trapesialis* (Lamarck, 1819) presents regular corrugations in the form of radial sequences of arches on the disk region, isolated rays or horizontal sequences of rays on the anterior lower region. *A. elongatus* presents corrugations formed by series of oblique arches on the disc and oblique rays on the carina. Under SEM, micro ridges were more evident in *A. elongatus*, but a wide diversity of shapes and patterns of micro fringes were observed in *A. trapesialis*, especially in young individuals. Considering the profile of the shell layers, the periostracum is relatively thin and apparently simple in *A. trapesialis* and thinner in *A. elongatus*. The prismatic layer is thick in both species, consisting of a single series of elongated prisms and wedge-shaped prisms close to the outer surface. The nacreous layer consists of very fine lamellae without pattern or with a slight staircase-like; in *A. elongatus* this layer is divided by a laminar inclusion of conchiolin. The fringes are abundant and diversified in *A. trapesialis*, a species less resistant to desiccation due to the presence of a wide intervalvar gap. The existence of a greater density of micro fringes and spikes in juveniles may be related to the orientation of the animal in order to search for an ideal site for development or for escape from regions subject to seasonal droughts, like Pantanal.

KEY WORDS. Mycetopodidae, periostracum projections, shell layers.

RESUMO. **Análise da ultraestrutura das conchas de *Anodontites trapesialis* (Lamarck) e *Anodontites elongatus* (Swaison) (Mollusca, Bivalvia, Etherioidea) do Pantanal do Mato Grosso, Brasil.** Projeções da superfície externa do perióstraco e micro estruturas da concha foram redefinidas com base na microscopia óptica e eletrônica de varredura. A superfície do perióstraco é praticamente lisa em ambas espécies. Sob vista mesoscópica, *A. trapesialis* (Lamarck, 1819) apresenta corrugações regulares formando seqüências radiais de arcos na região do disco, raios isolados ou seqüências horizontais na região antero inferior. *A. elongatus* (Swainson, 1823) apresenta séries de arcos organizados radialmente no disco e arcos oblíquos na carena. Sob MEV, as micro estrias são mais evidentes em *A. elongatus; A. trapesialis* apresenta micro franjas muito diversas quanto a forma e padrão, principalmente em jovens. Considerando as camadas da concha, o perióstraco é mais fino em *A. elongatus*. A camada prismatica é espessa nas duas espécies, com uma série única de prismas alongados e em forma de cunha próximo à superfície externa. A camada nacarada compõe-se de lamelas muito finas, sem padrão ou do tipo escadaria; em *A. elongatus* esta camada é dividida por uma inclusão laminar de conchiolina. As franjas são abundantes e diversificadas em *A. trapesialis*, espécie menos resistente à dessecação devido à presença de ampla fresta intervalvar. A ocorrência de uma grande densidade de micro franjas e espinhos nos juvenis, estaria relacionada à orientação do animal na busca por local ideal ao seu desenvolvimento ou para escapar das regiões sujeitas às secas sazonais, como o Pantanal.

PALAVRAS CHAVE. Mycetopodidae, projeções perióstraco, camadas concha.

To describe, compare and differentiate the macro- and micro characteristics of the periostracum, as well as the arrangement of the remaining inner layers forming the shells of freshwater bivalves goes beyond a strictly descriptive objective, and represents an important tool to be used in different approaches ranging from Systematics to Ecotoxicology.

Bøggild (1930) characterized the outer surface and the organization of inner structures that compose the shell and described seven morphological types, commenting on the organization of these structures in 50 families of bivalve mollusks. For Unionidae (= Naiadidae sic.), the author described a shell consisting of three layers. The outer layer is the periostracum, a fine organic layer basically consisting of protein, which covers the entire extension of the shell. Below the periostracum is the prismatic layer, consisting of considerably elongated and juxtaposed prisms, and the nacreous layer, consisting of the deposition of horizontal lamellar plates. Using light and scanning electron microscopy, Kobayashi (1969, 1971) detected 11 different basic types of aggregation of the elements that form the calcareous part of the shell of bivalves. A crystallographic characterization of mollusk shells was published by Carter (1990 a, b) who described and illustrated patterns of the organization of the periostracum and of the calcareous structures of various groups.

External periostracal projections recognized by Bottjer & Carter (1980) as of periostracal origin or as adventitious (formed later), were described for some Bivalvia like Arcoida, Mytiloida and Veneroida, and classified according to their morphology and function.

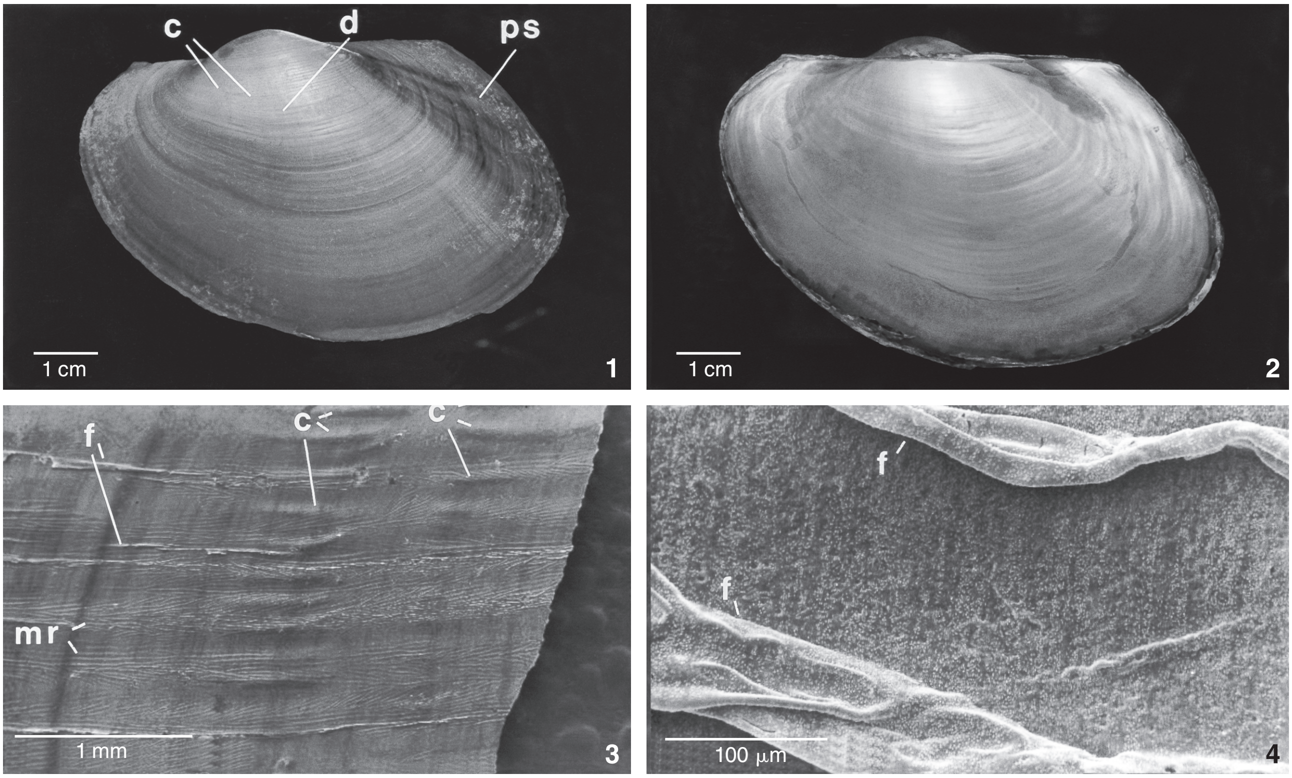
Studies on shell formation and processes of biomineralizatin are relatively few. Saleuddin & Petit (1983) suggested a mechanism for biomineralization in unionid bivalves, summarized as follows: The periostracum is composed by three layers. The outer and middle layers are formed within the periostracal groove and the inner layer by the mantle epithelium. The prismatic shell layer is formed inside the middle layer of the periostracum through a process of vacuolization and antrum formation. By a simmilar process, the nacreous layer is formed from the inner peristracum. More recently, Checa (2000) proposed a different model for the origin of the periostracum and mode of formation of shell layers in Unionidae, that consists: 1) the periostracum is formed by two layers, an outer, thin layer, which is secreted within the periostracal groove, and an inner, thicker layer, which is secreted by the epithelium of the outer mantle fold; 2) calcification initiates within the inner periostracum as fibrous spherulites, which protrude from the periostracum, coalesce and compete mutually, transforming into composite prisms; 3) nacreous tablets begin to nucleate directly on the ends of prism fibers by epitaxy. Transition from composite prisms to nacre is probably induced by merely crystallographic processes.

Little information is available about South American naiads (Hyriidae and Mycetopodidae). Marshall (1925) started the characterization of the periostracum surface for the major Unionoida families and concluded that radial "micro sculptures" seem to be typical of Mutelidae and Mycetopodidae, both belonging to the superfamily Etherioidea, according to Kabat (1997). Bonetto & Ezcurra (1965) defined three basic patterns of striation occurring in the periostracum: folds, macroscopic "sculptures" and microscopic "sculptures". On the basis of this systematization, Bonetto (1966) described and illustrated the pattern of the periostracum folds of Monocondylaeinae and observed that the genus *Fossula* Lea, 1870 had characteristics similar to those of *Anodontites* Bruguière, 1792. According to Bonetto (1967) both genera have rare and sparse periostracum striations and a well-developed prismatic layer. According to a phylogenetic study conducted by Bogan & Hoeh (2000) and Hoeh *et al*. (2001), the genera *Monocondylaea* Orbigny, 1835 and *Anodontites* represent related groups in the family Mycetopodidae.

The aim of the present study is to describe and to compare the form and pattern of outer projections of the periostracum and the arrangement of the inner shell structures of *Anodontites trapesialis* (Lamarck, 1819) and *Anodontites elongatus* (Swainson, 1823), to complement taxomic, biological and ecological studies conducted on both little known Mycetopodidae species, from the Mato Grosso Pantanal (Wetland) region.

MATERIAL AND METHODS

Specimens of *Anodontites trapesialis* (Figs 1 and 2) and *Anodontites elongatus* (Figs 13 and 14) were collected from the lake Baía do Poço (Well Bay) in the municipality of Santo Antônio do Leverger, State of Mato Grosso, Brazil, from May 1998 to April 1999. The shells selected for the study were washed and placed in plastic bags without being submitted to any fixation process. Twenty individuals for each species representing different length classes were analyzed in order to recognize and describe a structural pattern. For scanning electron microscopy, shells from juvenile and adult individuals were washed with distilled water for 24 hours and dried at room temperature. The material was fragmented with a hammer and appropriate portions were selected under a stereoscopic microscope. Fragments from the carina region were used for external analysis of the periostracum, and fragments from the central region of the shell were used for analysis of inner structures. The surface to be fixed was sanded and the fragments were submitted to two consecutive baths under ultrasound for approximately 30 seconds and then fixed on stubs with silver glue or with double-faced carbon adhesive tape. The pieces were sputtered with gold and images were obtained with a scanning electron microscope, model Phillips XL30 and processed in the Microscopy and Microanalysis Center (CEMM) of PUCRS. The used scale for magnifications ranged from 2 to 500 µm. Biometric analysis was performed using the Image Tool software, version 2.0, developed by UTHSCSA (Wilcox *et al*. 1997). The images thus obtained were used for measurements of periostracum, prismatic and nacreous layers thickness, the number and density of folds, ridges and the distances between them as well as the density of



Figures 1-4. *Anodontites trapesialis*: (1) Outer view of the shell; (2) inner view; (3) folds, regular corrugations in form of arches and micro ridges on the periostracum; (4) detail of the folds in the periostracum of a juvenile individual, in the background the surface covered by fringes. (c) Corrugations forming rows of arches, (d) disk, (f) folds, (mr) micro ridges, (ps) posterior slope.

the projections on the periostracum (Tab. II). Mean and standard deviation were calculated for each unit sample and used as a parameter for species comparison.

The projections and structures of the periostracum were recognized following BOTTJER & CARTER (1980) with adaptations for the specific features observed on the periostracum of the studied species. The terminology "macro" and "microsculture" used by MARSHALL (1925) and BONETTO & EZCURRA (1965) for impressions and also for projections on shell surface brought some difficulties concerning periostracum characterization. The terminology sculpture is now suggested only for strongly impressed radial beak sculpture present in most Hyriidae. In view of those divergences observed in the literature a redefinition was necessary and it is summarized in table I.

The calcareous structures were classified according to the criteria proposed by CARTER (1990 a, b). The identification of the species was based mainly on morphological characters following HEBLING (1976) and SIMONE (1994) for *A. trapesialis*, and SIMONE (1997) for *A. elongatus*. The species were considered only at the species level according to the taxonomical revisions of SIMONE (1994, 1997). Voucher specimens were deposited in the mollusks collections of Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, under the numbers MCP 8524, 8525 and 8526, and in the Laboratório de Ecologia Animal –NEPA – from Instituto de Biociências of the Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brazil.

RESULTS

The shells of *Anodontites trapesialis and* A*nodontites elongatus* consist of three different layers: the periostracum, the prismatic layer, and the nacreous layer.

Periostracum – outer surface

Anodontites trapesialis (Figs 1 and 2). With a thickness of 20.1 ± 3.9 µm, the periostracum is olive green in colour, with straw yellow shades in smaller and younger individuals. Larger and older individuals become darker, with a greenish brown colour. The surface presents folds, corrugations, micro ridges and micro fringes (Fig. 3), as described below.

Folds (Macroscopic). The periostracum is practically smooth on the disk region (Figs 3 and 4), with the rare and sparse occurrence of concentric folds (at most 15 per centimetre in radial direction), almost always low and/or worn out. In the

Table I. Definition of folds, corrugations and projections of the periostracal surface of *Anodontites trapesialis* and *A. elongatus* observed respectively by unaided yes, under stereomicroscope and by scanning electron microscopy (SEM).

Periostracum projections	Definitions
Folds	Macroscopically identifiable periostracal commarginal folds. Frequently described as "concentrically" organized in relation to the umbos, but not recommended (Cox *et al.* 1969). They may present an exfoliated aspect as if they were superposed scales, or may simply appear as longitudinal segmentations of the periostracum (but not interrupted).
Corrugations (Fig. 3, 13)	Mesoscopic* observable periostracal corrugations oriented in radial bands perpendicular to the umbos or parallel to the folds. They may appear in the forms of short bands or concentric arches forming stair-like radial rows, or as oblique arches with one end touching a neighbour ray, forming fishbone-like figures. Those regular corrugations reflect a very soft topography of the underlying shell layer (Bottjer & Cartier 1980). They are normally displaced around the disc but little far from the beaks and very different from the macroscopic radial beak sculpture of the Hyriidae that, if present, is strongly impressed on the shell surface.
Micro ridges (Fig. 3, 15,16)	Regular, superficial, periostracal structures that are expressed only on the outer surface of the periostracum (Bottjer & Cartier 1980). Radial, oblique or parallel and very close microstriae mainly present on the carina region. They are visible only at high magnification under the stereoscopic microscope or the SEM. They are usually present at high density, possibly conferring a dull aspect to the surface.
Micro fringes or spikes (Figs. 5-8, 16)	Minute projections observable only by SEM, preferentially on the entire region that covers the carinae. They are organized longitudinally or obliquely in relation to the axis of the micro ridges or may be dispersed throughout the periostracum surface. These micro-ornaments take on varied shapes that may be simple, like aculei, with a triangular or cylindrical base and acuminate or rounded extremities, or compound forming fringes with a digitiform appearance or like small flags.

* Mesoscopic observations were defined as those located at the limit of visualization between the unaided eye and a magnifying lens.

posterior slope the folds are closer (60 per centimetre), higher and overlapping, with a minimum distance of 0.35 mm between them. In juvenile individuals the folds are closer and more conspicuous (Fig. 4), and may be visualized also in the anterior region at an approximate density of 30 folds per centimetre.

Corrugations (Mesoscopic). There are radial sequences of short slightly convex arches (Fig. 3) arranged in rows containing about 70 arches per centimetre. Each individual may present three to seven rows always arranged on the disk region. They are clearly visible not only on the periostracum but also demarcated on the underlying calcareous layer. Bas-relief rays (±10 per mm) occur separately, between the rows of arches or forming sequences perpendicular to the folds, always being more frequent in the anteroventral portion. They are visible only under a magnifying lens and confer a dull aspect to this region of the shell.

Microscopic structures. Ridges with a radial, oblique or horizontal orientation in relation to the folds were occasionally observed (Fig. 3). They do not follow the pattern of the vertical parallel micro ridges observed in most Mycetopodidae, but form more commonly not continuous rows of horizontal ridges or fishbone like structures.

Scanning electron microscopy permitted the observation of very coarse periostracal projections forming rows of fringes (Figs 4 to 8) covering the outer surface of the shell. These minute projections take on different shapes and orientations according to where they are located. They may be simple and cylindrical like fingers (Fig. 5), with the aspect of an aculeus or forming serrated bands or rows of spikes like small triangular flags (Fig. 6), or they form simple and smooth low bands connecting separated spikes like flags with triangular extremities or fissured extremities, taking on a digitiform aspect (Fig. 7). The number of projections ranged from 1700 to 6000 units/mm², with the highest incidence being always associated with the anterior region of the shell of juveniles, which always presented values higher than 5000 projections/mm². In adult individuals, the projections were sparse and worn out or often partially covered by encrustations not secreted by the mollusc. In this case, the distal extremities of the projections are only visible in form of little spikes (Fig. 8).

Anodontites elongatus (Figs 13 and 14). This species presents a very fine periostracum with a thickness of 7.3 ± 1.3 μm. All individuals are dark brown in colour, which can even be black in some larger specimens. Folds, corrugations, micro ridges and projections occur in this species.

Folds (Macroscopic). The periostracum is smooth on the disk region, with only a few folds being observed in the distal portion (26 per centimetre). However, in the region of the posterior slope a high density of high juxtaposed folds (Fig. 15) with an exfoliated aspects was always observed, with values exceeding 50 per centimetre. Lower folds, almost always worn out, occurred in the anterior region, with a density of 40 to 60 folds per centimetre. In juveniles, there was a higher density of

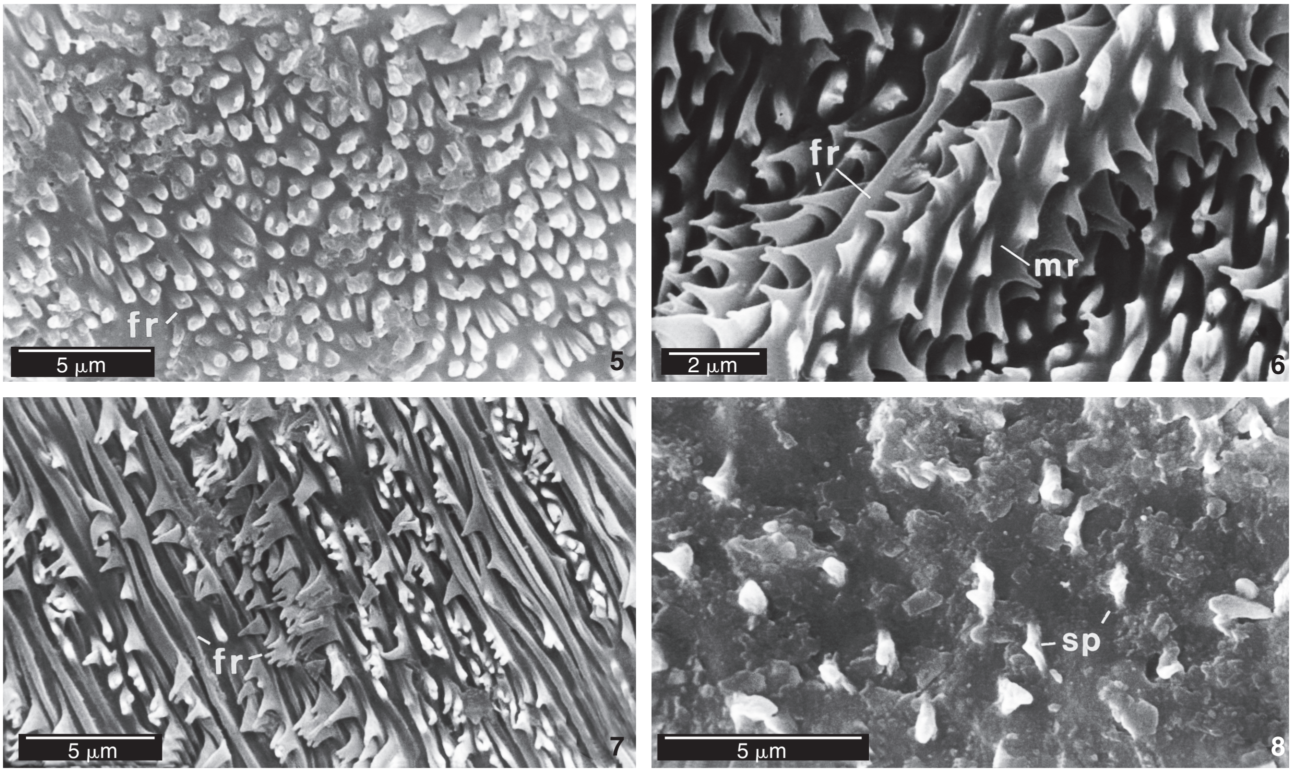


Figure 5-8. (5-7) details of different projections in form of fringes in juvenile individuals and (8) in form of spikes in adult individuals. (fr) Fringes, (mr) micro ridges, (sp) spikes.

folds with a well-preserved exfoliated appearance.

Corrugations (Mesoscopic). Peculiar radial bands occur on the disk region associated with the umbo (Fig. 13). They are formed by two parallel series of oblique arches, forming figures resembling Christmas trees. Each series of oblique arches consist of about 100 corrugations per centimetre. When they occur, the rays are always orientated obliquely to the folds both in the anterior and the posterior regions of the shell. The highest density of these radial bands is associated with the carina, where rays without a defined pattern give an undulated aspect to the periostracum.

Micro ridges are present on the entire shell surface (Fig. 15). Always orientated perpendicular to the folds, they are visible only at high magnification. There are at least 90 microstriae mm^{-1} and the distance between them does not exceed 20 µm.

The projections were only represented by small isolated spikes in this species (Fig. 16), with a constant morphological pattern with a broad base and a tapering extremity and resembling aculei. Their density was always about 2,600 spikes/mm^2 in *A. elongatus*.

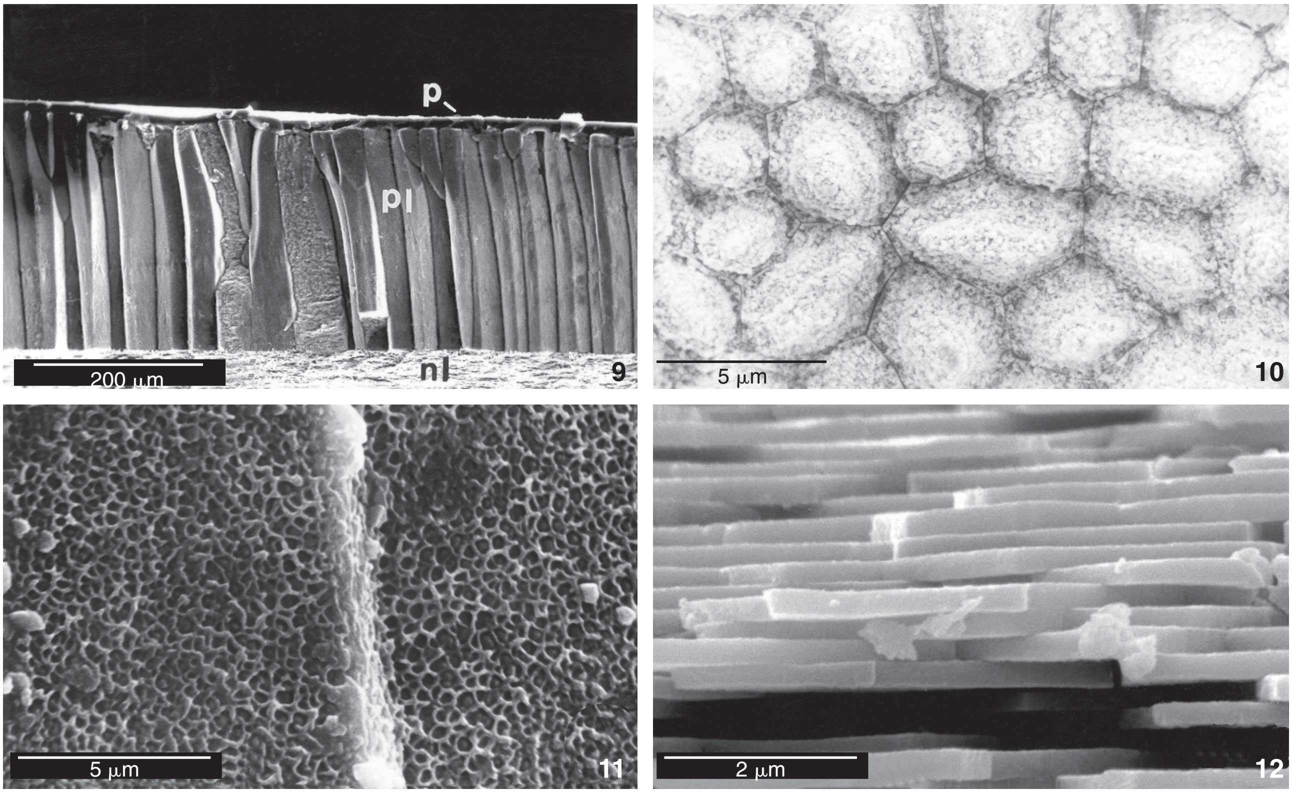
A greater occurrence of spikes, as well as a better integrity of folds and sculptures, was always more evident in juveniles than in adults, as also observed in *A. trapesialis*.

Internal structures. Prismatic and nacreous layers

The prismatic and nacreous layers were distinguishable below the periostracum in the shells of *A. trapesialis* and *A. elongatus*.

It was possible to characterize for both species a uniform prismatic layer consisting of elongated prisms in a parallel arrangement perpendicular to the external surface. The prismatic layer of the two species was classified as regular simple prismatic layer according to the criteria of Carter (1990 a, b) and fibrous aragonitic composite by Checa (2000), with the presence of almost always pentagonal and sometimes hexagonal prisms (Figs 10 and 18). Most of the prismatic units that form this layer are elongated and continuous, extending from the periostracum to the beginning of the nacreous layer. However, smaller wedge-like prisms were also observed, always starting from the outer surface immediately below the periostracum. They are typical competition features according to Ubukata (1994). The surface of the prism is covered with a reticulate lining of a spongy aspect which is visualized only at approximations of more than 2 µm (Figs 11 and 19).

In *A. trapesialis* the prismatic layer is apparently more fragile, formed of smaller prisms measuring around of 450 µm in length and 23 µm in width (Fig. 9). Few wedge-like prisms were observed in the images obtained for these species, and the exist-



Figures 9-12. *Anodontites trapesialis*: (9) shell profile showing the periostracum, the prismatic layer and the nacreous layer; (10) upper view of the prismatic layer; (11) organic film that lines each prismatic unit; (12) nacreous layer. (nl) Nacreous layer, (p) periostracum, (pl) prismatic layer.

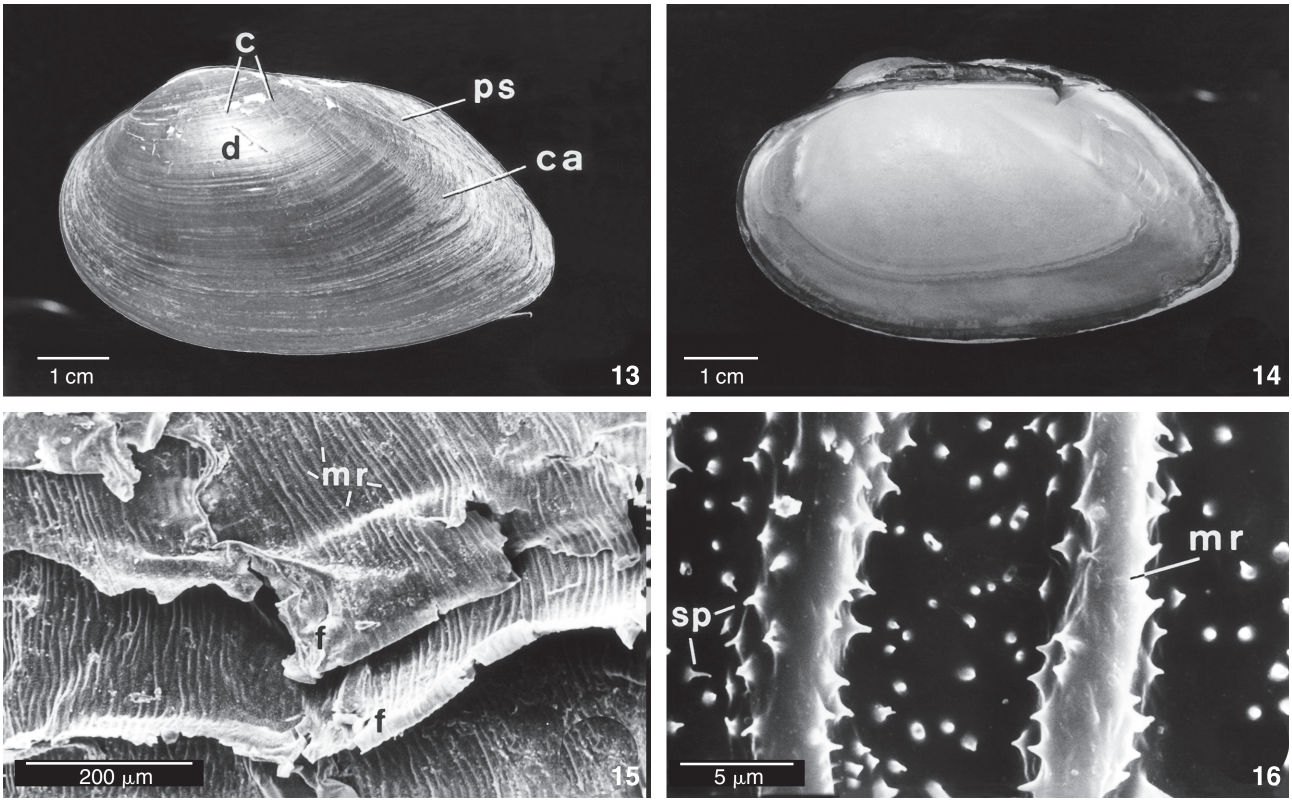
ing ones were always short, not exceeding the upper third of the prismatic layer. With a more robust structure, the shells of *A. elongatus* have a larger prismatic layer (Tab. II), with prisms reaching as much as 580 µm in length and 40 µm in width (Fig. 17). The presence of wedge-like prismatic units was constant (Fig. 18), with prisms of different length reaching as much as two thirds of the length of the main prisms.

The nacreous layer consists of minute superimposed plates (Fig. 12), most of the time without a defined organization. Some shell regions of both species may show a symmetrical oblique arrangement. These characteristics are of the sheet nacreous structure type, among those described by CARTER (1990 a, b), where these lamellae may be organized as the steps of a staircase or may have no defined pattern. In *A. elongatus* the thickness of the nacreous layer was, on average, 650 µm, corresponding to almost three times the thickness of the shells of *A. trapesialis*, whose minimum thickness is about 220 µm (Fig. 21). The size and thickness of each lamellar plate varied between species (Tab. II). An intermediate membrane, the conchiolin layer, very thin, of an irregular pattern, similar to the texture of the periostracum, and running longitudinally along the nacreous layer (Fig. 22), was observed only in *A. elongatus*.

DISCUSSION

During analysis of the studied species, special attention was devoted to the periostracum, since this structure presented more divergences between species. Considered to vary little among unionids, the thickness of the periostracum is 15 to 50 µm in most of the species of this group, classified by TEVESZ & CARTER (1980) as 'intermediate thickness'. Varying around 20.1 µm, the periostracum of *A. trapesialis* perfectly fits this classification. However, the mean thickness of the periostracum of *A. elongatus* was about 7.3 µm, a fact considered to be rare within the superfamily by the cited authors, who believe that an excessively thin periostracum is inefficient in protecting the shell from dissolution.

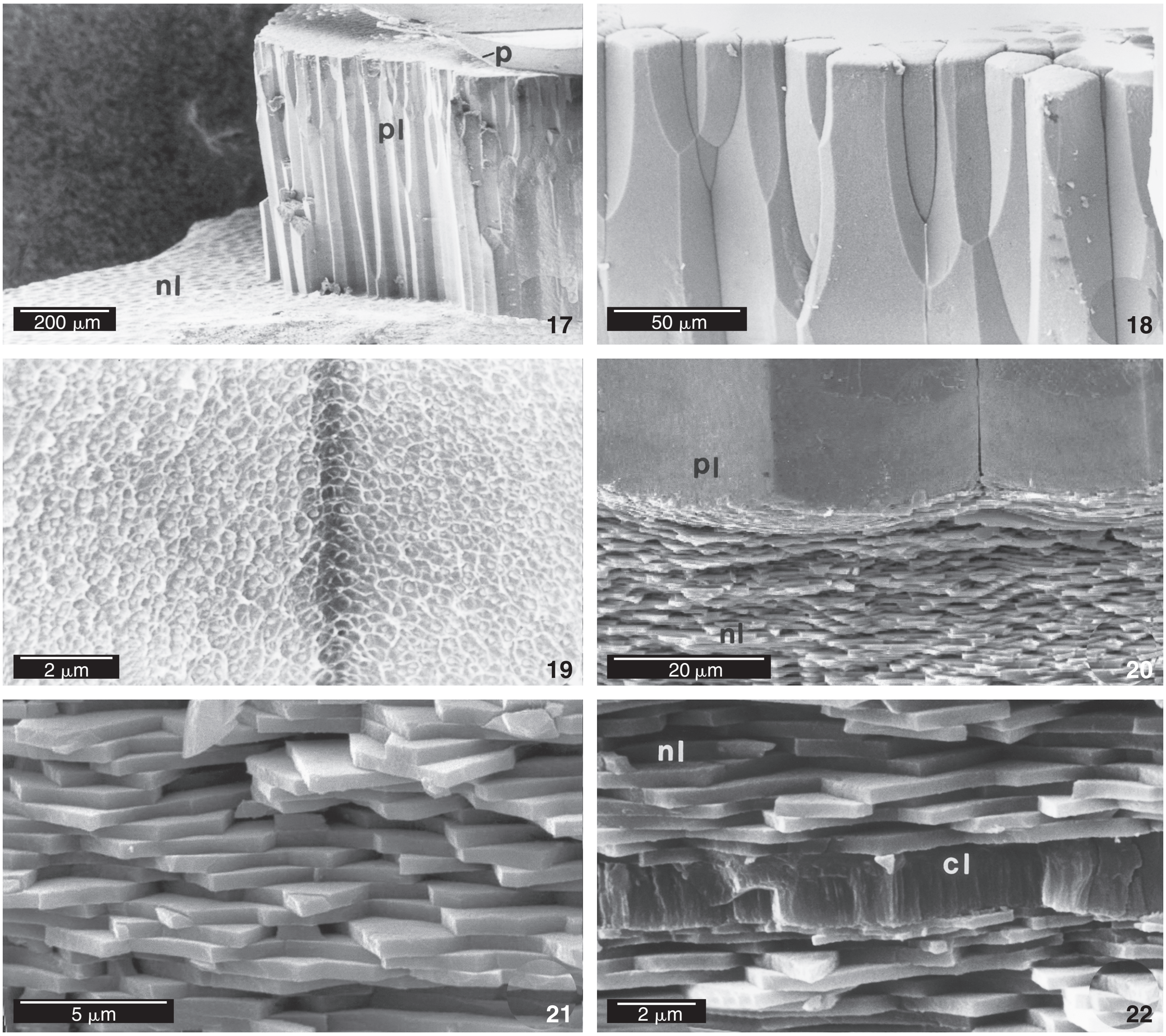
Basically consisting of fibrous proteins and quinones, the periostracum also contains some carbohydrates and lipids, which together form the periostracal units. The biochemical synthesis giving origin to these units is called sclerosis and occurs in the periostracal groove, between the two most external mantle margin folds and located in the outer fold of the mantle. The newly formed periostracum is distally compressed and expelled in the form of a multilamellar entity (SALEUDDIN & PETIT 1983). It is prob-



Figures 13-16. *Anodontites elongatus*: (13) outer view of the shell; (14) inner view; (15) details of the periostracum showing the folds and micro ridges; (16) spikes in the shape of aculei. (c) Corrugations forming rows of arches, (ca) carina, (d) disk, (f) fold, (mr) micro ridges of Mycetopodidae type, (sp) spikes.

Table II. Mean (± standard deviation) values concerning the structures that compose the layers of the shell of *Anodontites trapesialis* and *A. elongatus* from the Baia do Poço (Well Bay), municipality of Santo Antônio do Leverger, Mato Grosso State. Analyses were carried out using scanning electron microscope (SEM).

Structures	*Anodontites trapesialis*	*Anodontites elongatus*
Periostracum		
Thickness	20.11 ± 3.87 µm	7.27 ± 1.29 µm
Distance between folds	170.00 ± 32.50 µm	196.92 ± 40.98 µm
Micro ridges organization	When present, sparse, organized horizontally in oblique or perpendicular rows	Densely grouped, perpendicular to the folds
Micros ridges density	40 per mm	80 per mm
Distance between micro ridges	Not constant	11.61. ± 2.33 µm
Spikes width	1.16 ± 0.20 µm	0.91 ± 0.16 µm
Spikes density	1700 to 6000/mm^2	2600/mm^2
Prismatic layer		
Thickness	482.50 ± 50.46 µm	568.88 ± 12.17 µm
Prism width	23.86 ± 2.13 µm	37.08 ± 11.20 µm
Shape and texture	Hexagonal, spongy	Penta- or hexagonal, spongy
Nacreous layer		
Total thickness	243.75 ± 23.93 µm	650.00 ± 58.30 µm
Lamella thickness	0.22 ± 0.05 µm	0.32 ± 0.06 µm
Conchiolin lamina thickness	Absent	1.71 ± 0.08 µm



Figures 17-22. *Anodontites elongatus*: (17) shell profile showing the periostracum, the prismatic layer and the nacreous layer; (18) detail of the prismatic layer showing the wedge-shaped prisms; (19) the organic film covering each prismatic unit; (20) a detail of the contact between the prismatic layer and the nacreous layer; (21) the laminar plates that compose the nacreous layer; (22) the inclusion of the organic layer (conchiolin layer) in the nacreous layer.(cl) Conchiolin layer, (nl) nacreous layer, (p) periostracum, (pl) prismatic layer.

ably at this time that the sculptures are molded, thus defining the periostracum structure typical of each species.

In both species here studied, the periostracum was practically smooth in a superficial view. The corrugations that could be observed with the unaided eye or under the stereoscopic microscope, were sparse and more frequent in *A. elongatus* than in *A. trapesialis*. This situation was also observed by Bonetto & Ezcurra (1965), who described a periostracum with barely developed "macro sculptures" (actually considered as corrugations) arranged in a linear series of arches. Bonetto (1966) also emphasized the similarities existing in the periostracum of the genera *Anodontites* and some Monocondylaeinae like *Fossula,* which also presents rare and sparse series of corrugations.

The micro ridges of *A. elongatus* were fine, compactly arangend and clearly visible under SEM, always radially organized and perpendicular to the folds, like the definition of "fine radiating threads" given by Marshall (1925) and Bonetto & Ezcurra (1965) to Mycetopodidae micro ridges. Nevertheless those last cited authors stated for *A. elongatus* as quite poor in terms of "micro sculptures", a fact contradicting our observations. Bonetto & Ezcurra (1965) commented on the fact that "micro sculptures" were practically imperceptible in *A. trapesialis*, as also were the folds formed by the periostracum, contradicting also the results of the present researches.

SEM permitted the identification of another structural pattern of minute projections that had not been previously described for this species. Denoted as fringes and or spikes, the minute projections present on the periostracum respectively of *A. trapesialis* and *A. elongatus,* occurred frequently and showed structural diversity, with intra- and interspecific variations.

Anodontites trapesialis is more vulnerable to environmental alterations. This species has a considerable intervalvar gap through which the foot extend and never succeeds in fully closing its valves. This fact prevents this species from isolating itself in the presence of adverse environmental situations. Considering that the specimens here studied originate from the Mato Grosso Pantanal region, an ecosystem subjected to a rigorous cycle of drought and flooding characteristic of wetlands (Junk 2000), the presence of fringes suggests that the function of these structures may be associated with an increased sensitivity to water flow, especially among juveniles. These present a greater density and a distinct morphological pattern of spikes when compared to adult individuals. Thus, the juveniles of *A. trapesialis* may possess a high specialised sensor to water movements. According to Bottjer & Carter (1980) the periostracal projections can be functional for: "achieve stability in the substratum, to minimizing settlement by encrusting epizoans, reducing the hazard of shell penetration by endolithic organisms, camouflaging and protecting the shell margins from predators and extending the range of tactile sensory perceptions away from mantle edge". The authors (op. cit.) considered the latter advantage "as important only in the Mytiloidea, which apparently lack visual perception in the posterior mantle, and which secrete especially long adventitious hairs in certain species". The observed periostracal fringes and spikes of juveniles *Anodontites* are in fact not sensitive structures and not long enough such as the hairs of some mytilids, but rather projections that should promote some kind of vibration do to water current. These signs could be interpreted by the sensitive system of the animal as an order to move away. During sampling in Pantanal Region it was observed that juveniles are rarely found together with adults. The juveniles live mainly on margins and places of low water that are more subjected to the risk of desiccation.

No variation in the structural profile of the periostracum was observed in the studied species, both presenting a simple membrane. However, Waite (1977) demonstrated a difference in the structure of the periostracum, which appears to consist of two different layers: an upper amorphous layer and a weakly fibrous lower layer. In agreement with this, biochemical studies conducted by Saleuddin & Petit (1983) suggest that the newly formed periostracum goes through a remodelling stage through the deposition and polymerization of glycocalcite, processes that are repeated several times.

The importance of the periostracum in the calcification process is shown by the active performance of this membrane (Saleuddin & Petit 1983), which is responsible for: a) nucleation, b) crystal orientation, c) crystal size, and d) crystal type in the formation of the remaining layers forming the shell (Wilbur & Sauleuddin 1983). Checa (2000) recognized only two layers by SEM observations on unionid shells. The external part is composed internally of micron-sized sublayers.

There are several definitions for the different configurations of the prismatic layer among bivalves. Bøggild (1930) categorized them as normal, complex, and compound prismatic structures. Kobayashi (1969, 1971) defined them as prismatic structures and fibrous prismatic structures. In fact and according to the classification of Ubukata (1994) *A. trapesialis* and *A. elongatus* present composite aragonitic prisms that are encapsulated by a surrounding organic matrix (Kobayashi 1969, 1971). Wilbur & Saleuddin (1983) explained that this organic matrix, in addition to the attributes mentioned earlier, also has the function of crystal to crystal adherence, constructing a unified structure.

The structure and arrangement of the prisms are specific for the family and may vary in some details among genera and species (Tolstikova 1974). This fact was not observed in *A. trapesialis* and *A. elongatus*, with the only significant difference concerning prism length, which was approximately on average, 480 µm for *A. trapesialis* and 560 µm for *A. elongatus*. However, the length of these may vary within the same species according to their location on the shell and shell size. In the anterior part the prisms are shorter and in the posterior region close to the margin of the shell, they are longer, as also described by Tolstikova (1974).

Considering the nacreous layers there was a clear difference in the thickness between the two species. *Anodontites elongatus* (650 µm) has a nacreous layer almost three times thicker than *A. trapesialis* (240 µm), explaining the resistance of the shell of *A. elongatus* to external agents, offering better protection to the organism. Bøggild (1930) observed that the lamellar or tabular units that compose the nacreous layer are extremely thin, with values always lower than 0.001 mm. SEM images permitted the exact determination of the height (or thickness) of these units, which measure approximately 0.20-0.30 µm, with more than 2000 superimposed units being necessary to form a 650 µm layer like that of *A. elongatus*. Saleuddin & Petit (1983) described that the formation of this layer is also associated with the periostracum, in the time when products from cell glycocalices adhere to the newly formed organic film, coming from distal cells of the mantle fold. The deposition of this material in randomly arranged plates represents the beginning of the nacreous layer. Checa (2000) describes that nacreous tablets nucleate on previously formed crystals of prismatic layer and the epitaxial growth explain the common orientation of nacreous tablets. Only *A. elongatus* presented segmentation in the nacreous layer by a continuous horizontal layer very similar to the periostracum. KAT (1983) observed within the shell of Margaritiferidae and some Unionidae one or more types of layers named as conchiolin layers, which according to him, closely resembles the periostracum in ultrastructure. These inclusions of organic matter were first described by BØGGILD (1930) so that, due to the intercalation of the organic matrix, the nacreous part is dissolved more slowly by acids. TEVESZ & CARTER (1980) and KAT (1985) described this event and suggested that mollusks with an excessively thin periostracum are inefficient in terms of protection against the dissolution of the calcareous structures of the shell. On the basis of these statements, we may suggest that the presence of these organic sublayers may act by strengthening and protecting the nacreous structure since the periostracum of *A. elongatus* is much thinner (7.0 µm) than that of *A. trapesialis* (20 µm). TEVESZ & CARTER (1980) also stated that the intermediate layers of conchiolin are frequent in Unionidae species, especially those living in environments subjected to acidification, or may be a response of the organisms to exposure to contaminants that penetrate between the shell and the mantle. This question was the starting point for IMLAY (1982) when he suggested the use of freshwater bivalve shells to monitor not only the presence of heavy metals, but also the levels of turbidity, low oxygen concentrations, temperature and pollutants in a general manner.

ACKNOWLEDGEMENTS

We are indebted to CAPES for supporting as fellowship the Doctoral Course to the first author and to Conselho Nacional de Desenvolvimento Científico e Tecnológico for the grant to the co-author (CNPq 302584/2002-1). To Dr. Berenice Anina Dedavid and collaborators from the Microscopy and Microanalysis Center – CEMM – PUCRS, Catholic University, Porto Alegre, Brazil, for the kind assistance and help during SEM sessions. We also thank to the anonymous reviewers for the helpful suggestions and comments on the manuscript.

REFERENCES

BOGAN, A.E. & W.R. HOEH. 2000. On becoming cemented: evolutionary relationships among the genera in the freshwater bivalve family Etheriidae (Bivalvia: Unionoida), p. 159-168. *In*: E.M. HARPER; J.D. TAYLOR & J.A CRAME. **The evolutionary Biology of the Bivalvia.** London, Geological Society, Special Publications, 177p.

BØGGILD, O.B. 1930. The shell structure of the mollusks. **Det Kongelige Danske Videnskabernes Selskabs Skrifter**, **Naturvidenskabelig og Mathematisk Afdeling**, Kopenhagen, **9**, **2** (2): 233-326.

BONETTO, A.A. 1966. Especies de la subfamilia Monocondylaeinae en las aguas del sistema del Rio de La Plata (Moll. Mutelacea). **Archiv fuer Molluskenkunde**, Frankfurt, **95** (1-2): 3-14.

BONETTO, A.A. 1967. El género *Anodontites* Bruguière (Mollusca, Pelecypoda) en el sistema hidrográfico del Plata. **Physis**, Buenos Aires, **26** (73): 459-467.

BONETTO, A.A. & I. EZCURRA. 1965. Notas malacológicas III: 5) La escultura del perióstraco en el género *Anodontites*. 6) El lasidium de *Anodontites trapezeus* (Spix). 7) El lasidium de *Mycetopoda siliquosa* (Spix). **Physis**, Buenos Aires, **25** (69): 197-204.

BOTTJER, D.J. & J.G. CARTER. 1980. Funtional and phylogenetic significance of projecting periostracal structures in the Bivalvia (Mollusca). **Journal of Paleontology**, Iowa, **54** (1): 200-216.

CARTER, J.G. 1990a. **Skeletal biomineralization**: **patterns**, **processes and evolutionary trends I.** New York, Van Nostrand Reinhold, 832p.

CARTER, J.G. 1990b. **Skeletal biomineralization**: **patterns**, **processes and evolutionary trends**, **Atlas and Index**. **II.** New York, Van Nostrand Reinhold, 1-29.

CHECA, A. 2000. A new model for periostracum and shell formation in Unionidae (Bivalvia, Mollusca). **Tissue & Cell**, Amsterdam, **32** (5):405-416.

COX, L.R.; C.P. NUTTALL & E.R. TRUEMAN. 1969. General features of Bivalvia, p. n2-n129. *In*: R. C. MOORE & C. TEICHERT (Eds). **Treatise on invertebrate paleontology.** Lawrence, The Universty of Kansas, vol. 1, XXXVIII+489p.

HEBLING, N.J. 1976. The functional morphology of *Anodontites trapezeus* (Spix) and *Anodontites trapesialis* (Lamarck). (Bivalvia: Mycetopodidae). **Boletim de Zoologia da Universidade de São Paulo**, São Paulo, **1**: 265-298.

HOEH, W.R.; A.E. BOGAN & W.H. HEARD. 2001. A phylogentic perspective on the evolution of morphological and reproductive characteristics in the Unionoida, p. 257-280. *In*: G. BAUER & K. WÄCHTLER (Eds). **Ecology and evolution of freshwater mussels Unionoida. Ecological Studies 145 (14).** Berlin, Springer-Verlag, XXII+394p.

IMLAY, M. J. 1982. Use of shells of freshwater mussels in monitoring heavy metals and environmental stresses: A review. **Malacological Review**, Niwot, **15**: 1-14.

JUNK, W.J. 2000. The Amazon and the Pantanal: A critical comparison and lessons for the future, p.211-224. *In*: F.A. SWARTS (Ed.). **The Pantanal**: **Understanding and Preserving the Worlds largest Wetland.** Minnesota, Paragon House, 301p.

KABAT, A.R. 1997. Correct family names for the freshwater "Muteloid" Bivalves (Unionoida: Etherioidea). **Occasional Papers on Mollusks**, Cambridge, **5** (72): 379-392.

KAT, P.W. 1983. Conchiolyn layers among the Unionidae and Margaritiferidae (Bivalvia): microstructural characteristics and taxonomyc implications. **Malacologia**, Niwot, **24** (1-2): 298-311.

KAT, P.W. 1985. Convergence in Bivalve conchiolin layer microstructure. **Malacological Review**, Niwot, **18**: 97-106.

KOBAYASHI, I. 1969. Internal Microstructure of the Shell of Bivalve Molluscs. **American Zoologist**, Seatle, **9**: 663-672.

Kobayashi, I. 1971. Internal shell microstructure of recent bivalvian molluscs. **Science Reports of Niigata University**, Niigata, **E** (2): 28-73.

Marshall, W.B. 1925. Microscopic sculpture of pearly fresh-water mussel shells. **Proceedings of the U. S. National Museum**, Washington D.C., **67** (2576): 1-16.

Saleuddin, A.S.M. & H.P. Petit. 1983. The mode of formation and the structure of the periostracum, p.199-234. *In*: A.S.M. Saleuddin & K.M. Wilbur (Eds). **The Mollusca**, **4.** New York, Academic Press, Part 1, XX+523p.

Simone, L.R.L. 1994. Anatomical characters and systematics of *Anodontites trapesialis* (Lamarck, 1819) from South America (Mollusca, Bivalvia, Unionoida, Muteoloidea). **Studies on Neotropical Fauna and Environment**, Lisse, **29** (3): 169-185.

Simone, L.R.L. 1997. Anatomy and systematics of *Anodontites elongatus* (Swainson) from Amazon and Paraná Basins, Brasil (Mollusca, Bivalvia, Unionoida, Mycetopodidae). **Revista Brasileira de Zoologia**, Curitiba, **14** (4): 877-888.

Tolstikova, N.V. 1974. Microstructural characteristics of freshwater bivalves (Unionidae). **Paleontological Journal**, Moscow, **8**: 55-60.

Tevesz, M.J.S. & J.G. Carter. 1980. Environmental relationships of shell form and structure of Unionacean bivalves, p. 295-322. *In*: D.C. Rhoads & R.A. Lutz (Eds). **Skeletal growth of aquatic organisms.** New York, Plenum Press, 750p.

Ubukata, T. 1994. Architectural constraints on morphogenesis of prismatic structure in Bivalvia. **Paleontology**, Lawrence, **37**: 241-261.

Waite, J.H. 1977. Evidence for the mode of sclerotization in a molluscan periostracum. **Comparative Biochemistry and Physiology**, Amsterdam, **58**: 157-162.

Wilbur, K.M. & A.S.M. Saleuddin. 1983. Shell formation, p. 235-287. *In*: A.S.M. Saleuddin & K.M. Wilbur (Eds). **The Mollusca 4.** New York, Academic Press, Part 1, XX+529p.

Wilcox, D. C.; S. B. Dove; W. D. McDavid, & D. B. Greer. 1997. Image Tool. Image processing and analysis program for Microsoft Windows 95™ or Windows NT™ Department of Dental Diagnostic Scienc. University of Texas Health Science Center. Disponível em http://macorb.uthscsa.edu/dig/itdesc.html.

Received in 29.X.2004; accepted in 18.VII.2005.