

Structural Characterization of the Shell of the Land Snail *Megalobulimus* sp.

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The individuals of the family Megalobulimidae (Pulmonata, Gastropoda) are predominantly land snails, and the majority of the species are in extinction process in Brazil because of the predatory collection of the shells. In Gastropods, the shells consist of an outer thin organic periostracum (composed mainly of conchin), and two or three calcareous layers: an outer prismatic portion, a middle lamellate layer, and an inner nacreous layer (hypostracum) [1]. Until the present no detailed study on the shell structure of Megalobulimidae was done. In the present work, the interface periostracum/mineral, and the sublayers of the mineralized shell were studied by SEM.

The shells used in this work were collected in Rio de Janeiro State, and measured approximately 110mm in length and 65mm in width (figure 1). The shell was fragmented and the periostracum was detached from mineral surface mechanically. Further treatments with EDTA 5 % to remove possible mineralized fragments, and with chitinase were performed. The samples were mounted on conductive carbon tape on aluminum stubs and vacuum sputtered with gold. The SEM Hitachi S-4500, operated at 5 kV, and Jeol 5310, operated at 20 kV, were used. In addition, the shell was grounded to powder in order to identify the crystalline phase present, by electron diffraction in a TEM Jeol 1200 EX operated at 80 kV.

The shell of *Megalobulimus* sp. observed by SEM, in cross section (perpendicular to the major axis), is composed by an external periostracum, and at least 9 different distinguishable mineralized layers (figure 2). Each mineralized layer presented crystals oriented differently from those of the neighbor layers (figure 2). The fine powder obtained from the shell showed particles with polygonal shapes (figure 3), and the electron diffraction pattern was compatible with the calcium carbonate aragonite (figure 4). SEM of periostracum treated with chitinase presented no significant digestion, but sometimes it was possible to observe geometrically arranged micro-pores at the external surface (figure 5). Periostracum has two distinct layers: an external one ($\approx 5 \mu\text{m}$ thick), and another one more internal with approximately $2.5\mu\text{m}$ in thickness (figure 6). The internal layer of periostracum presents mammillae-like projections (figure 6) facing to the outer surface of the first layer of the mineralized shell which has depressions (figure 7) with similar dimensions.

The periostracum of *Megalobulimus* sp. probably contains a low proportion of chitin as no significant digestion of the structure was observed. The changes in crystals orientation in the mineral calcareous sublayers should contribute to increase the hardness of the shell and show the complex organization of the related organic matrix. The conchin-mineral interface may work as a key-lock system allowing the adhesion of the two distinct biogenic hard tissues.

References

- [1] R.C. Brusca and G.J. Brusca. Invertebrates. Ed. Sinauer Associates, Inc
- [2] This research was supported by FAPERJ and CNPq Brazilian Agencies.

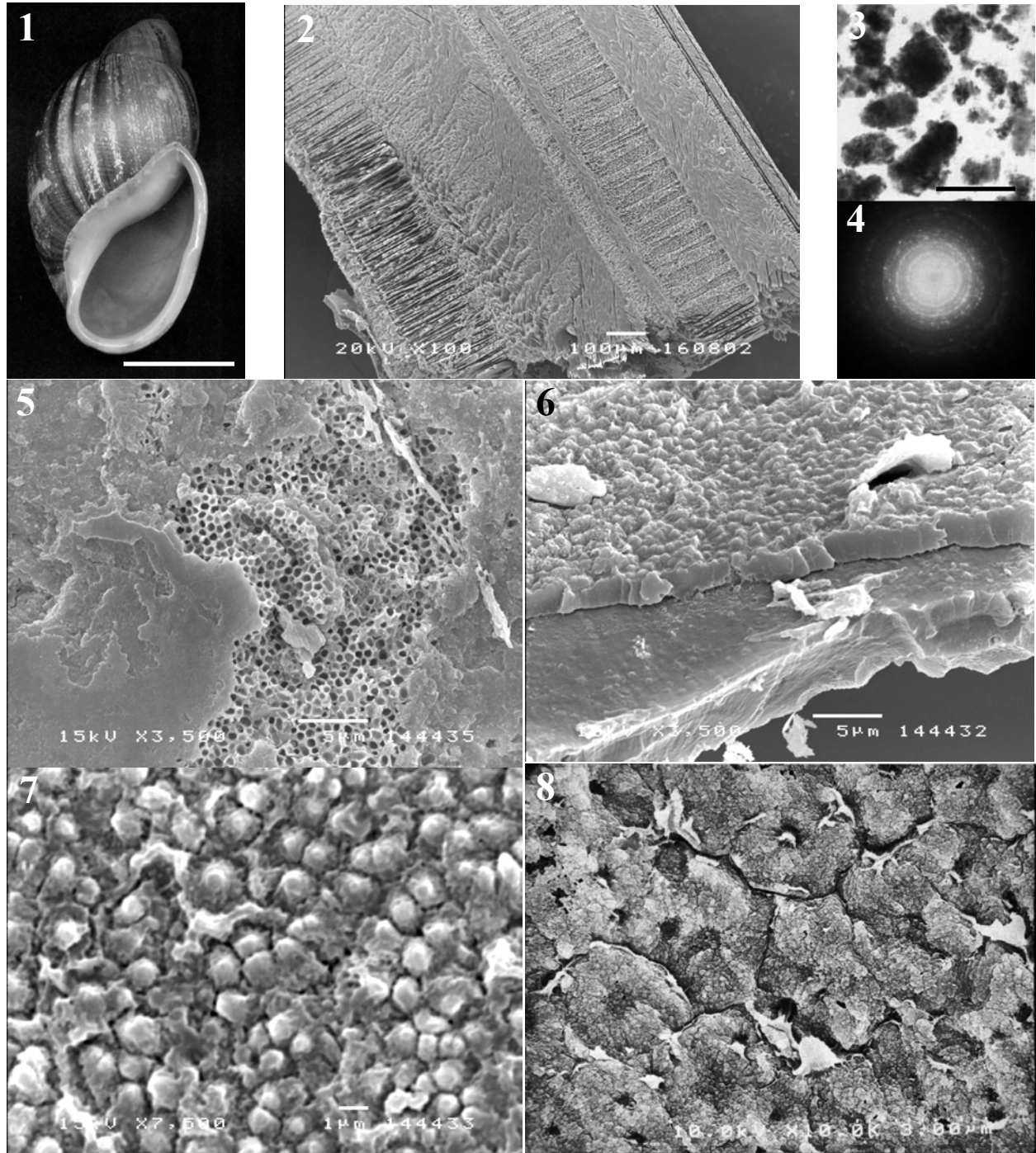


Figure 1: Ventral view of the shell of *Megalobulimus* sp. Bar = 5 cm.

Figure 2: SEM of transversal section showing the different layers. Periostracum - left.

Figure 3: TEM of the shell powder. Bar = 1 μm .

Figure 4: Electron diffraction pattern compatible with the calcium carbonate aragonite.

Figure 5: External part of periostracum treated with chitinase showing micropores.

Figure 6: Periostracum composed by 2 layers, note the mammillae-like structures.

Figure 7: Detail of the internal layer of the periostracum showing the mammillae.

Figure 8: Outer surface of the first layer of the mineral phase showing the depressions.