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Microstructure of the Embryonic Shell of *Nautilus belauensis* (Cephalopoda): Evidence from Oxygen Plasma Etching

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ABSTRACT

We used oxygen-plasma etching to study the microstructure of the embryonic shell of *Nautilus belauensis.* Nacreous tablets of the first septum are composed of a varying number of crystalline sectors that consist in turn of numerous parallel vertical lamellae 0.1 μm in thickness. The central portion of each tablet is occupied by an organic accumulation containing numerous mineral granules. The prismatic layer of the first septum on its adoral side is composed of thin prismatic units 0.1 to 0.2 μm in diameter. These units are themselves composed of consecutively stacked crystalline discs, 0.1 to 0.2 μm in thickness, which are separated by thin, horizontal, organic sheets.

INTRODUCTION

The embryonic shell of *Nautilus belauensis* Saunders, like the shell of other molluscs, is composed of calcium carbonate and organic compounds (glycoproteins). Therefore, study of the microstructure of this shell has broad implications for molluscs in general. In order to study the microstructure, we must etch either the mineral or organic components that form the shell. Several techniques for etching calicum carbonate have been described in previous studies (e.g., Grégoire, 1962; Crenshaw and Ristedt, 1975; Mutvei, 1979). For

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example, Mutvei (1979, 1980) etched shells using solutions of (1) glutaraldehyde (GL), (2) glutaraldehyde-acetic acid-Alcian blue (GLAA), or (3) chromium sulfate (CS). In this paper, we introduce a new technique called oxygen-plasma etching for the study of molluscan shell microstructure. In this technique, the organic component of the shell is removed by oxidation.

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MATERIAL AND METHODS

The embryonic shell of *N. belauensis* used in this study was obtained as part of the captive-breeding program of *Nautilus* species at the Waikiki Aquarium. Details of the collection, handling, and breeding of these animals are given in Carlson (1987). Their embryonic development is described in Arnold (1987, 1988) and Arnold et al. (1987). The shell studied is typical of the score of shells obtained as part of this program. This shell is at the two-chambered stage of development and its overall morphology is described in Landman et al. (1989: figs. 4–8).

The shell was removed from a recently dead embryo, washed thoroughly in filtered sea water, dehydrated through a graded series of ethanol, and then embedded in Bueler's Epo-Kwick resin. The resultant block and shell were ground and later polished with aluminum oxide to produce a parasagittal cross section. This section cuts across shell layers at an oblique angle, broadly exposing their surfaces.

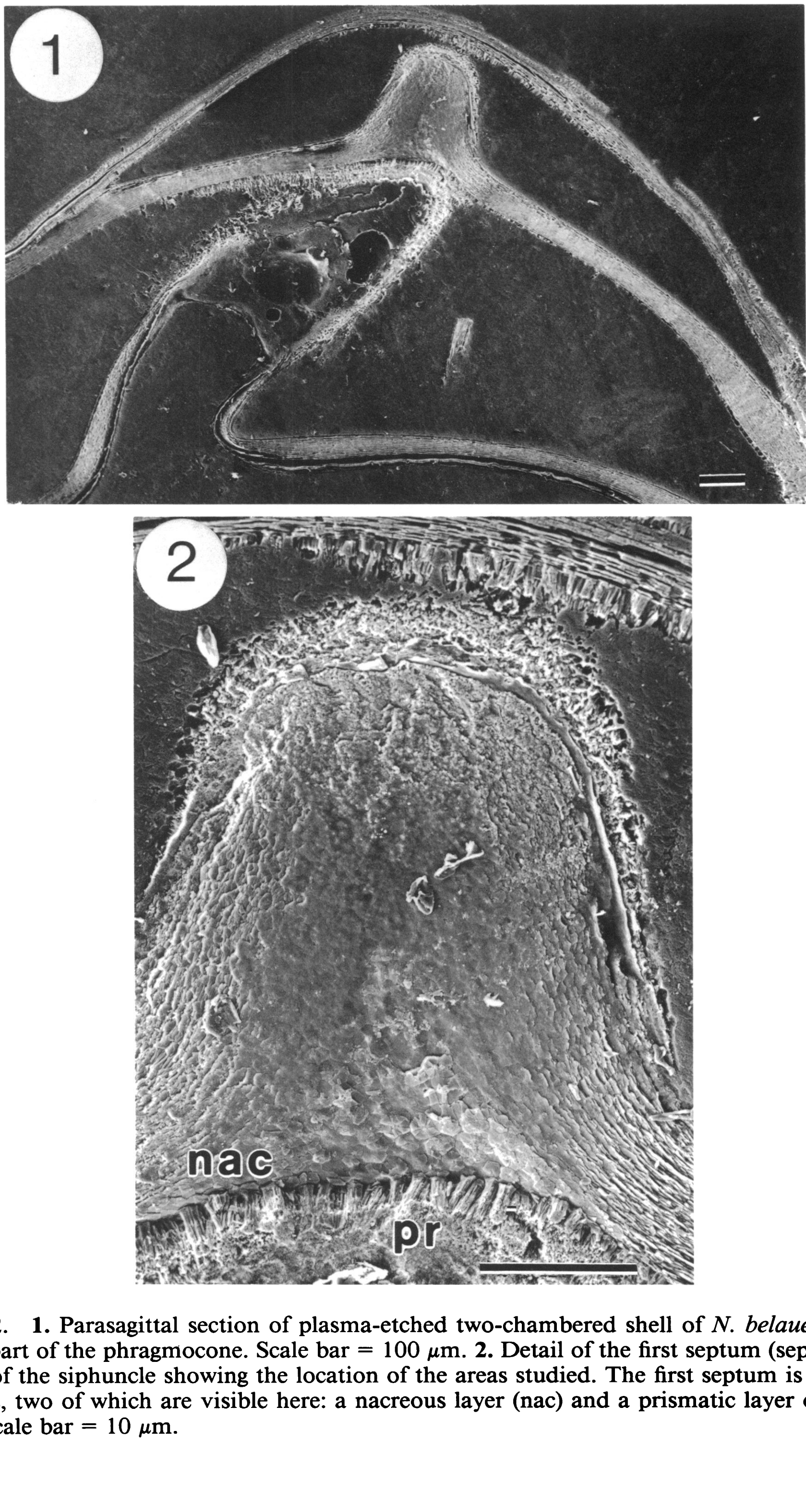
A "peel" of the shell was made with an acetate sheet to remove any loose surface material. This peel can be directly observed but its usefulness is limited to mapping because of its relatively poor resolution. The whole block face was then etched with oxygen plasma in a SPI Plasma Prep. (Structure Probe Inc., Westchester, PA) for two four-minute periods with an interval of cooling between the etchings. The block containing the shell was placed directly over the vacuum vent and the maximum power level was used with the radio frequency tuned to give maximum-glow discharge (70–75 watts). See Kuzirian and Leighton (1983) for details of the apparatus used in this study.

The entire block face was then gold coated on a rotating stage of a shadow caster. The embedded and etched shell was examined with a JOEL 840 scanning electron microscope at the Marine Biological Laboratory at Woods Hole, Massachusetts and useful magnifications of up to 50,000× were obtained.

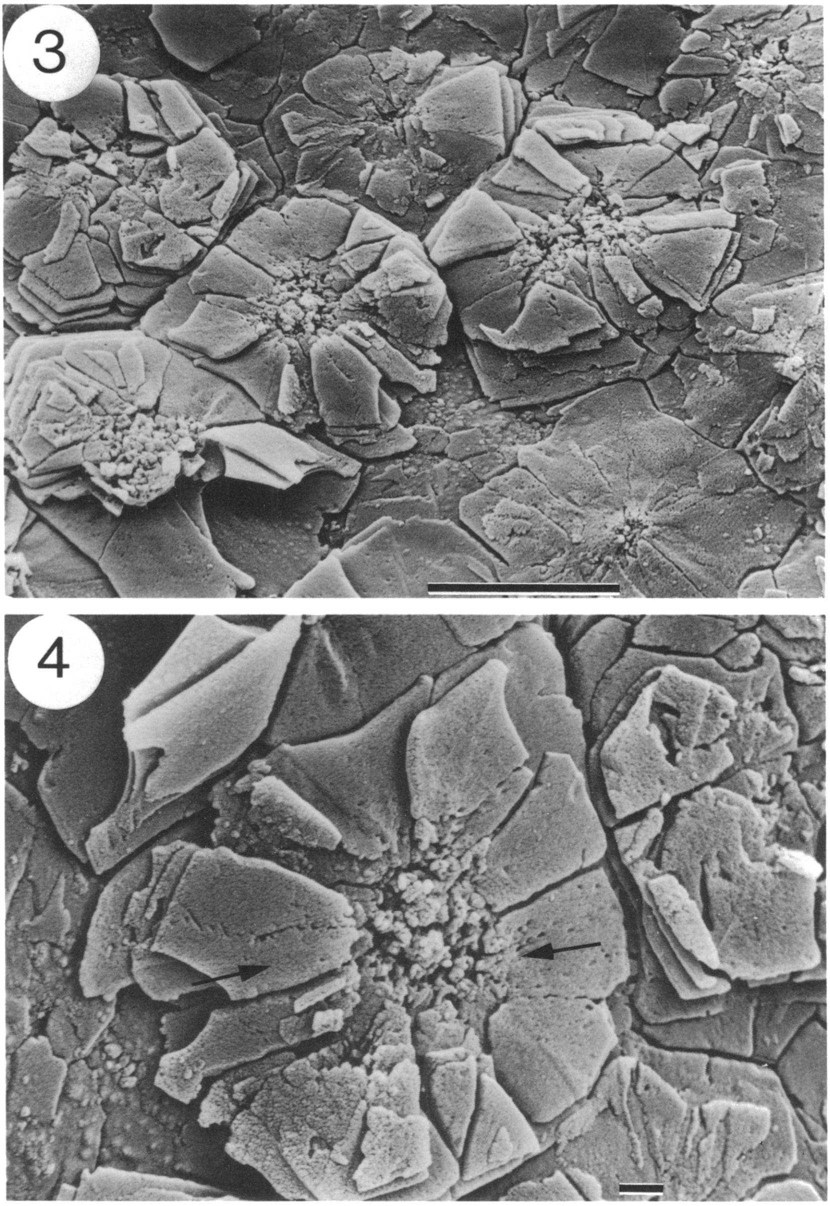
RESULTS

The plasma-etched embryonic shell is shown in figures 1 and 2. The septa are composed of three layers: (1) a spherulitic prismatic layer on the adapical side, (2) a thick nacreous layer (nac), and (3) a prismatic layer on the adoral side (pr). The latter two layers are well exposed at the transition of the first septum and its septal neck.

The nacreous layer of the first septum is composed of polygonal tablets 10 to 15 μm in diameter, which are arranged in vertical stacks. The surface of the nacreous layer is visible at the transition between the first septum and its septal neck because part of the overlying prismatic layer was ground off during section preparation (fig. 2). Some areas of the nacreous surface appear to have been more extensively etched than others, due perhaps to the fact that the nacreous surface was slightly curved and, therefore, was exposed



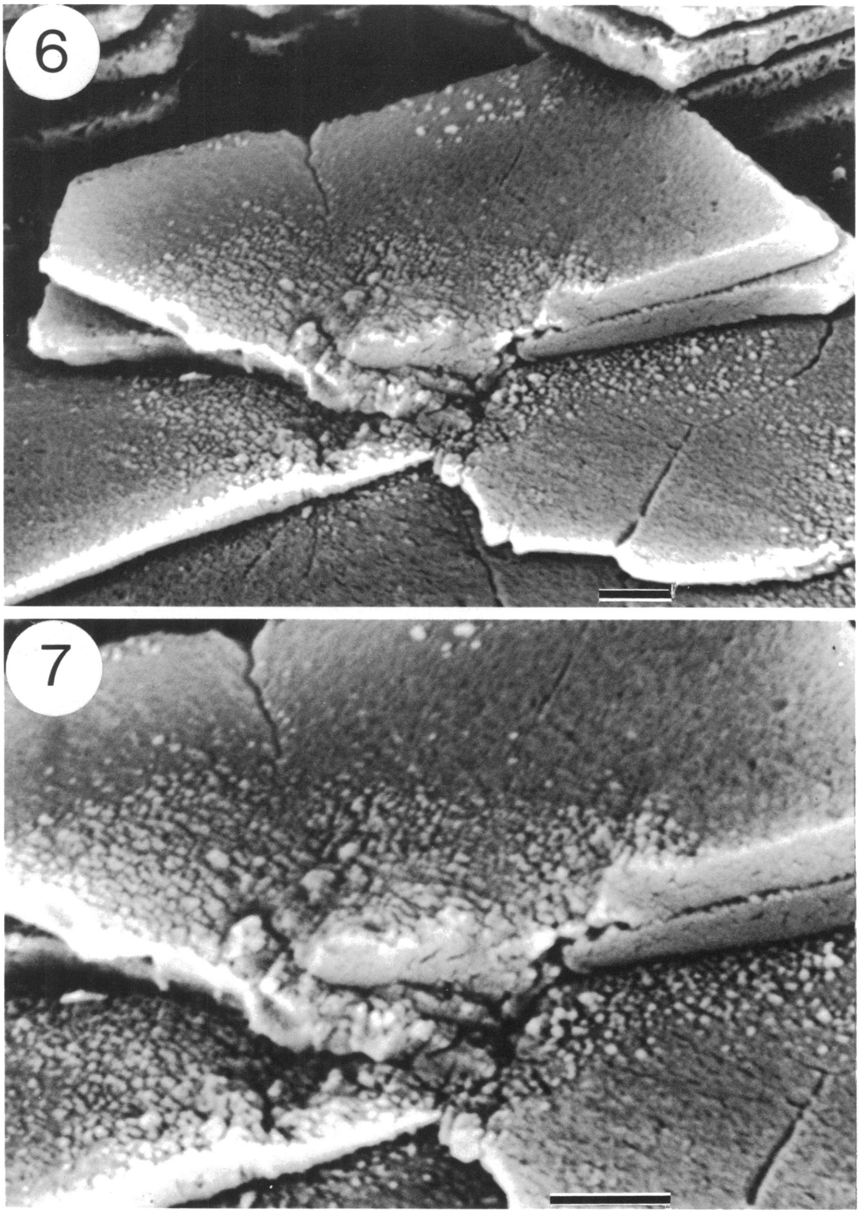
Figs. 1, 2. **1.** Parasagittal section of plasma-etched two-chambered shell of *N. belauensis* showing the apical part of the phragmocone. Scale bar = 100 μm. **2.** Detail of the first septum (septal neck) and distal end of the siphuncle showing the location of the areas studied. The first septum is composed of three layers, two of which are visible here: a nacreous layer (nac) and a prismatic layer on the adoral side (pr). Scale bar = 10 μm.



Figs. 3, 4. **3.** Close-up plasma-etched nacreous tablets showing the presence of a variable number of crystalline sectors and a central organic accumulation. Scale bar = 10 μm. **4.** Detail of a nacreous tablet; arrows indicate the direction of parallel thin vertical lamellae composing the crystalline sectors. The central organic accumulation contains numerous small crystalline granules. Scale bar = 1 μm.

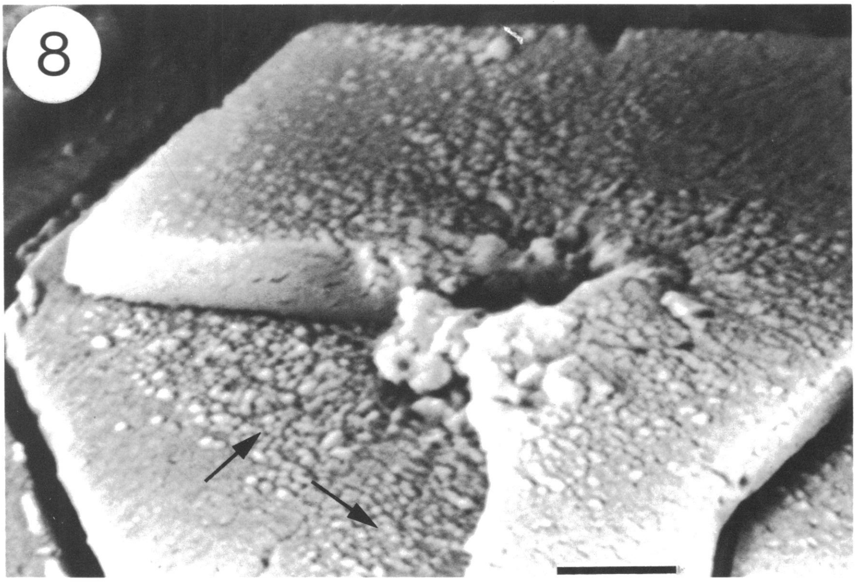
to the plasma at different angles. In areas that were more extensively etched, each nacreous tablet consists of 4 to 10 radially arranged mineral sectors (figs. 3–5). The presence of these sectors suggests that the tablets are twinned, similar to the twinning in nonbiogenic aragonite (Gossner, 1930; Mutvei, 1978, 1980: fig. 1e–h). However, conclusive crystallographic evidence for twinning has not yet been presented. The mineral sectors are sep-

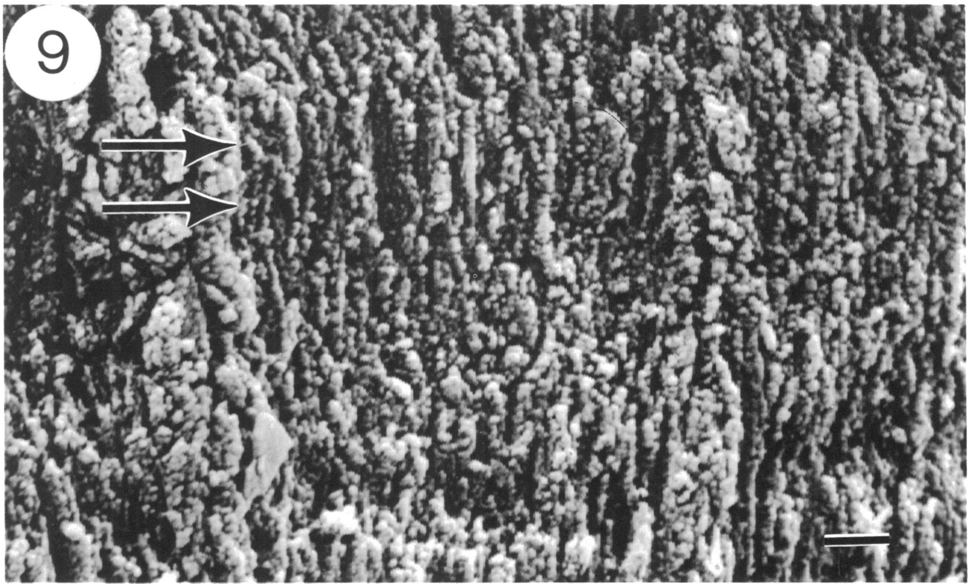
Fig. 5. Detail of the boundary between the plasma-etched prismatic and nacreous layers. The prisms are composed of consecutively stacked mineral discs (indicated by arrows). Scale bar = 1 μm.



Figs. 6, 7. Surfaces of consecutive, plasma-etched nacreous tablets showing the presence of parallel rows of small tubercles on the central portion of each surface. The orientation of these tubercles coincides in consecutive tablets (best seen in the left-hand side of the figures). Scale bars = 1 μm.

arated by narrow interspaces that originally contained thin, vertical, organic sheets, now removed by the etching (compare with Mutvei, 1980: pl. 1C, fig. 1c, d). In several tablets, the individual sectors are apparently composed of numerous parallel, indistinct vertical lamellae that are approximately 0.1 μm in thickness (fig. 4, arrows). By analogy with nonbiogenic aragonite, this structure is probably the result of polysynthetic twinning (Gossner, 1930). The orientation of the lamellae in adjacent sectors is difficult to dis-





Figs. 8, 9. **8.** Surfaces of two consecutive, plasma-etched nacreous tablets showing the difference in the orientation of the parallel rows of tubercles in adjacent crystalline sectors (indicated by arrows). **9.** Plasma-etched section of the prismatic layer forming the supraseptal ridge at the junction of the second septum and outer shell wall. The prisms are composed of consecutively stacked mineral discs separated by narrow interspaces (arrows). The alignment of these discs in adjacent prisms produces thin, horizontal, mineral lamellae. Scale bars = 1 μm.

tinguish, and therefore, we cannot determine how many of the sectors are interpenetrant twins and how many are cyclic twins (Mutvei, 1980).

The large central portion of each tablet is distinctly porous and consists of numerous, irregularly spaced granules of mineral composition (figs. 3–5). The interspaces between the granules were originally filled by an organic matrix, now removed by the etching. This central area represents the central organic accumulation previously described in nacreous tablets etched in a solution of GLAA (Mutvei, 1980).

In the nacreous tablets that were less extensively etched, the interspaces between the crystalline sectors were only partially distinguishable, and the central organic accumulations were less porous (figs. 6–8). The adoral surfaces of these tablets show numerous small tubercles, approximately 0.1 μm in diameter. These tubercles are absent or only weakly developed on the periphery of the tablets. The tubercles are arranged in parallel rows and the orientation of these rows appears to differ in adjacent crystalline sectors (fig. 8, arrows). However, the orientation is similar in overlying sectors. These tubercles may represent the end portions of aragonitic crystallites that form the vertical lamellae described above (compare with Mutvei, 1980: fig. 1).

The prismatic layer of the first septum originates on the adoral surface of the nacreous layer where four to six polygonal tablets of decreasing diameter grade into columnar prisms (fig. 5). These prisms are solid in the basal region but distally subdivide into numerous, thin, elongate prismatic units approximately 0.1 to 0.2 μm in diameter (fig. 5, upper part; see also Bandel, 1977). Horizontal mineral lamellae separated by narow interspaces are visible across the prisms (fig. 5, arrows). Each lamella is 0.1 to 0.2 μm thick and represents the alignment of individual minute discs within adjacent prisms.

These discs are better exposed in the prisms that form the supraseptal ridge at the junction between the second septum and outer shell wall (fig. 9; "septal cement," Grégoire, 1962). Each prism is 0.1 to 0.2 μm in diameter and is composed of consecutive mineral discs 0.1 to 0.2 μm thick. These discs are separated by narrow interspaces originally occupied by thin, horizontal, organic sheets, now removed by the etching. The position of the discs in each thin prism corresponds to that in adjacent prisms so that the discs form thin, horizontal, mineral lamellae (fig. 9, arrows) similar in thickness to those of the septal prismatic layer (compare with fig. 5).

DISCUSSION

Although additional experiments need to be performed, it appears that oxygen-plasma etching successfully removed the organic components of the embryonic shell without severely damaging the mineral components. Even the extremely thin and delicate organic sheets that encase the minute, crystalline discs composing the aragonitic prisms (the "intracrystalline, organic matrix") were removed. This resulted in an improvement in the resolution of the mineral components.

Many structural features, previously recognized by GL, GLAA, and CS etching (Mutvei, 1979, 1980) were observed in greater detail in the plasma-etched shell. For example, the nacreous tablets are composed of a varying number of crystalline sectors, suggesting that the tablets are aragonitic twins. Adjacent sectors are apparently separated by thin, vertical, organic sheets. The center of each tablet is occupied by an organic accumulation composed of mineral granules embedded in an organic matrix. The crystalline sectors are composed of numerous, parallel, vertical, crystalline lamellae, each 0.1 μm thick, which probably formed as a result of polysynthetic twinning. The lamellae consist, in turn, of rows of acicular crystallites. The end-portions of these crystallites may appear on the adoral surfaces of the nacreous tablets as tubercles approximately 0.1 μm in diameter.

In addition to corroborating earlier observations about nacreous tablets, new data was obtained about the prismatic microstructure of the shell. For example, each prism has a diameter of 0.1 to 0.2 μm. It is composed of consecutively stacked crystalline discs, about 0.1 to 0.2 μm in thickness, probably separated from each other by very thin, horizontal, organic sheets, removed in our preparation by plasma etching. The position of the crystalline discs in adjacent prisms coincides, and as a result, horizontally continuous crystalline lamellae appear. Thin, horizontal, organic sheets of about similar spacing have also been reported in the prisms and spherulites of the archaeogastropod *Haliotis* (Mutvei et al. 1985: pl. 1, figs. 4, 5).

Thus, it appears that oxygen-plasma etching may provide new insights into the microstructure of the embryonic shell of *Nautilus belauensis*. By removing obscuring organic material, it was possible to increase resolution and demonstrate that there is a common particle size of approximately 0.1 to 0.2 μm that appears to characterize both nacreous and prismatic microstructure. In addition, these small particles appear to be aligned and arranged by the organic materials removed during the oxygen-plasma etching process.

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