Supplementary Materials: The Caudofoveata (Mollusca) Spicule as a Biomineralization Model: Unique Features Revealed by Combined Microscopy Methods

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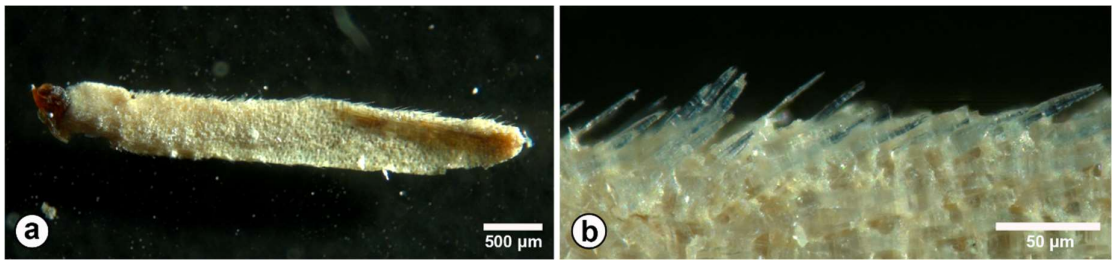

Figure S1. Image of Falcidens sp (Caudofoveata) mollusk obtained with a stereoscope. (**a**) Whole organism presenting a tegumentum covered by small and thin spicules; (**b**) Detail of the previous image magnified 18×. From this figure, we concluded that the spicules were transparent and had lengths of 60 µm and thicknesses of about 2 µm (middle region of the organism).

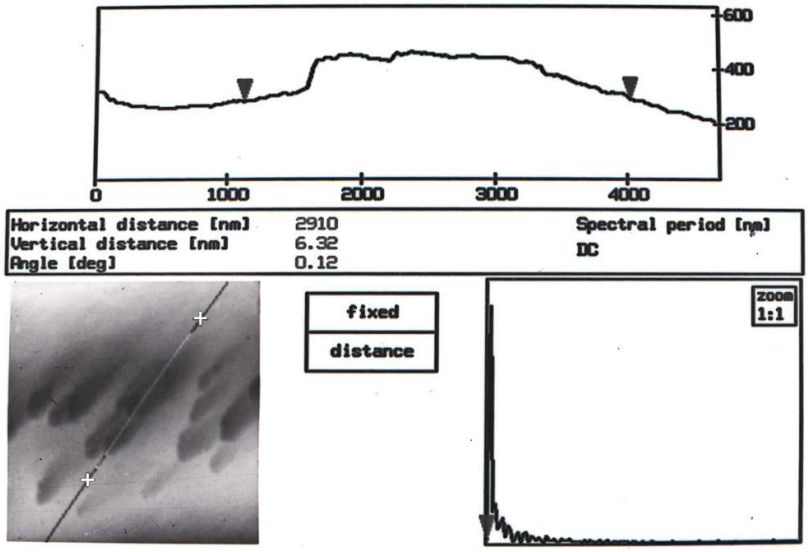

Figure S2. Topographic analysis of the dorsal face of Falcidens sp. spicule by AFM. Top figure: Line profile obtained with the AFM from the image at the bottom left. Bottom left: AFM image obtained in "height mode" (constant force). The white crosses correspond to the arrowheads of the line profile. Note that the upper lateral faces of the crystallite region are about 200 nm above the reference level (plane of the arrowheads in the line profile. In this image, the crystallite major axis is parallel to the spicule major axis.

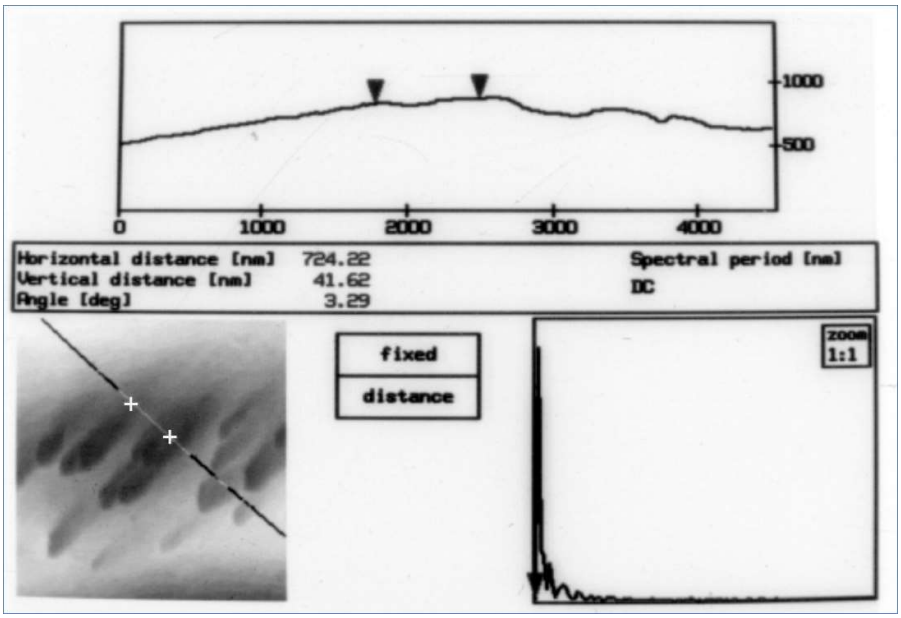

Figure S3. Topographic analysis of the dorsal face of Falcidens sp spicule by AFM in a section plane perpendicular to the one in Figure S2. Top figure: Line profile obtained with the AFM from the spicules shown at the bottom left. Bottom left: AFM image obtained in "height mode" (constant force). The white crosses correspond to the arrowheads of the line profile. Note that the line profile between the two crosses was almost flat, which means that laterally, the upper surface of the crystallites was at the same height as the intercrystallite region.

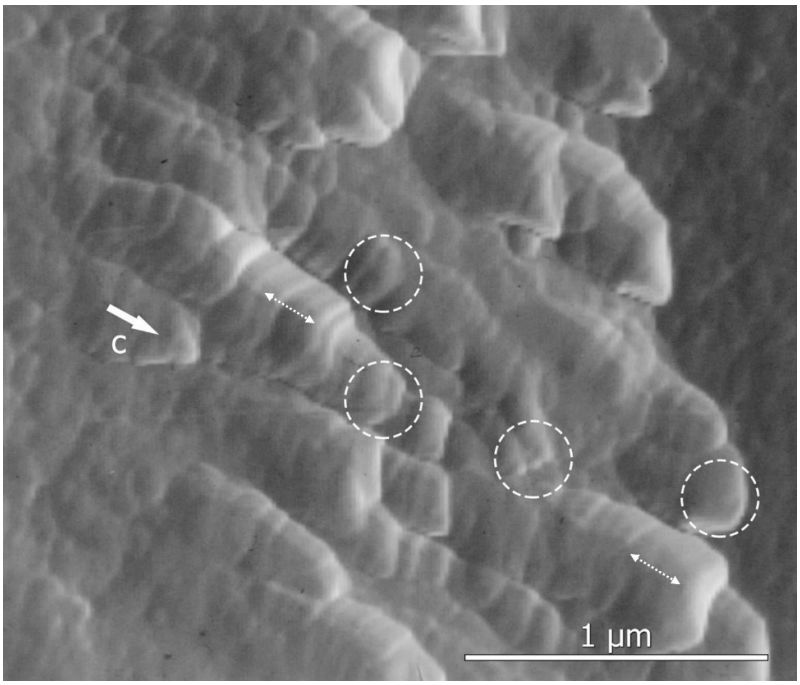

Figure S4. AFM image ("force image" obtained at "constant height") of the dorsal surface of a *Falcidens sp.* spicule. Many crystallites are seen oriented in parallel. Circles indicate oblique faces that present relative angles compatible with aragonite crystal faces. The optical axis (*c*-axis) is parallel to the image plane (white arrow) and also parallel to the *c*-axis of the whole spicule (see Figure 8 of the main manuscript text). The double arrow suggests possible pseudohexagonal twins.

Table S1. Angles between oblique faces, measured directly from the AFM image in Figure S3. The two first lines in the table correspond to regions of the kind indicated by circles in Figure S3.

Faces	Angles
0 1 1/0 0 1	35.76 − 180 = 144.24°
0 1 1/0 −1 1	71.53 − 180 = 108.5°
0 2 1/0 −2 1	110.4 − 180 = 69.6°
0 2 1/0 0 1	55.23 − 180 = 124.77°

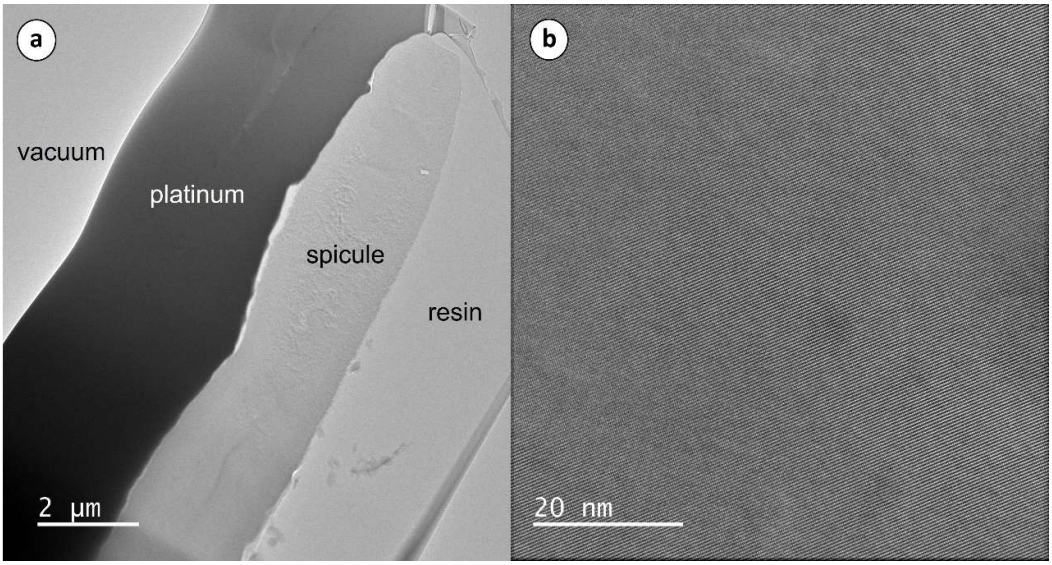

Figure S5. Electron microscopy analysis of a lamella obtained by FIB-SEM from an epoxy resin embedded spicule. (**a**) Lamella observed in HRTEM. The different regions of the image field are indicated; (**b**) HRTEM image of a small region of the spicule showing a structure typical of a single crystal.

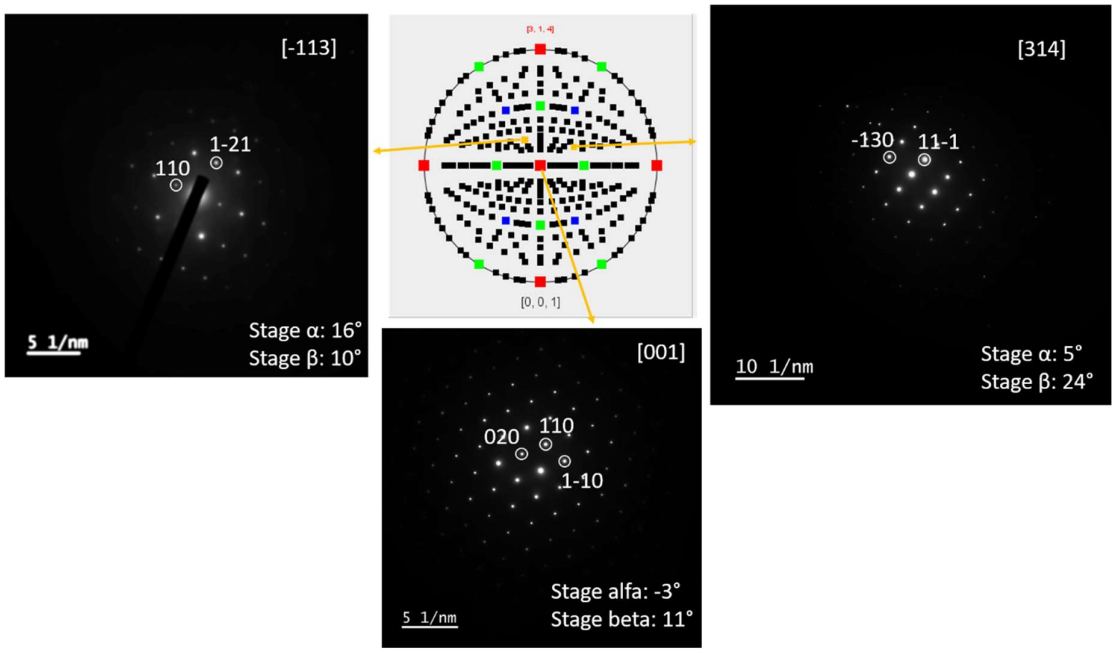

Figure S6. Diffraction patterns obtained from a lamella in different orientations/zone axes, determined by the use of the goniometer. The patterns showed unequivocally that the regions analyzed were single crystals of the calcium carbonate aragonite. Specifically, the pattern corresponding to

the [001] zone axis reinforces the one presented in Figure 8 of the original text, which was obtained by sectioning the spicule perpendicular to its major axis.