

Article

Crystallographic Characteristics of Crossed Mollusc Shell Microstructures with Particular Focus on Scaphopod Shell Crystal Organization

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Abstract

Scaphopod molluscs are marine, infaunal, cosmopolitan animals. They encase their soft tissue with an aragonitic tusk-shaped shell that has at its two ends an orifice, the aperture and the apex. We investigated the shell of the dentaliid species *Fissidentalium metivieri* and *Antalis weinkauffi* and characterized shell crystal organization with electron backscatter diffraction (EBSD) and laser confocal and scanning electron microscopies. Based on crystal size, morphology, organization and growth-line spacing, we distinguish five different crystal arrangement motifs in the investigated shells. We find two slightly different microstructures for the shell proper, two microstructures for the attachment of muscles to the shell and one microstructure for a secondary hard tissue growth product, secreted at the apical orifice. Crystal organization motifs are crossed-lamellar, dendritic and prismatic. Crystal textures are crossed-lamellar, axial-like and crossed-lamellar-like. Crystal organization with a crossed arrangement is utilized for shell formation by representatives of many Ca-carbonate shell-secreting mollusc classes/subclasses: Scaphopoda, Gastropoda, Bivalvia, Polyplacophora (crossed-lamellar), and Patellogastropoda (crossed-foliated). Based on structural–crystallographic attributes, we highlight a basic shell structure that is broadly similar for the species of these mollusc classes/subclasses. However, this basic crystal arrangement motif is significantly modulated by the specific crystal organization that is inherent for a particular mollusc class/subclass.

Keywords: crystal organization of scaphopod shells; crossed-lamellar microstructures and textures; high-resolution EBSD measurements; scaphopod shell crystal microstructure and texture; motifs of crystal arrangements with crossed organizational designs; crystal arrangements of mollusc shells

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1. Introduction

The Scaphopoda form a distinctive class among the Mollusca (e.g., [1–3]). The class is one of the smallest mollusc classes and comprises two orders (the Dentaliida and the Gadilida), 14 families, and, up to now, 586 described species (e.g., [2–12]).

The Scaphopoda are exclusively marine. They are infaunal organisms and are widespread in distribution [13–15]. The animals live fully immersed in soft sediment and dwell within the upper layers (from the sediment surface to about 30 cm depth) of the sedimentary cover. Although scaphopods favor marine habitats down to a few hundred meters water depth [15], they are capable of dealing with considerable bathymetric ranges and can be found in waters as deep as 7000 m [3,14]. Scaphopods tolerate water depths; however, they are not able to tolerate low salinities. Therefore, the Scaphopoda are not observed in waters with salinities below 25‰; thus, they are not present in estuaries and freshwater [7].

Scaphopods are bilaterally symmetrical and elongated animals (e.g., [2,3]). Their organs and other soft tissues are enveloped by a mantle that is open at its two ends (Figure 2 in Reynolds [3]). The shell is secreted by the external surface of the mantle [2,3], which is attached to large (not all) portions of the inner shell surface. At the mantle attachment site, a scar forms, the pallial myostracum or the pallial scar. In a 2D cross-section, the pallial scar is a line of crystals, called the pallial line [3,15].

Characteristic for the Scaphopoda is an external, conical shell, having the form of a curved tube; however, tapered at one of its ends (e.g., [15] and references therein, and Figure 1A of this study). The shell fully covers the soft tissue of the animal [2,3]. It has two orifices: a small one, the apical aperture or the apex, and a large one, the frontal aperture. When the scaphopod organism is alive, the shell is positioned 30° to 40° relative to the sediment surface (Figure 2B in Lamprell and Healy [14], Figure 16.1C in Ponder et al. [15]). The concave side of the shell is oriented upward, and the apex projects about 1 cm out of the sediment surface and into the water (Figure 2B in Lamprell and Healy [14]). The frontal aperture is always within the sediment and is oriented downward (Figure 16.1D in Ponder et al. [15]).

Research on the Scaphopoda started more than two centuries ago, when Keferstein (in Bronn 1862–1866 [16]) recognized that the Scaphopoda form a separate class of the Mollusca. Since then, work concentrated on scaphopod diversity, soft tissue anatomy, scaphopod classification and phylogeny (see references cited in reviews of [2,3,7–9]). Studies dealing with the scaphopod hard tissue dealt with shell morphology, size, surface appearance and ornamentation (e.g., [3,15]) as well as with the determination of the carbonate phase and the chemical composition of the shells [17]. Very little research was conducted on the internal structure of the shells. Nonetheless, based mainly on light microscopy and some secondary electron imaging techniques, it was reported that scaphopod shell crystals are assembled with a crossed-lamellar arrangement pattern (first suggested by Cayeux [18]) and that two main layers form the shell, an outer and an inner shell layer ([3] and references therein).

With the present study, we report crystallographic information on shell crystal organization. The latter was gained with diffraction methods and was complemented with laser confocal imaging. The present study has two objectives. Studies of the last decades have shown that crossed crystal arrangement patterns are utilized for shell formation by species of many mollusc classes and subclasses, such as the Scaphopoda, Bivalvia, Gastropoda and Patellogastropoda (e.g., [15]). It was, however, also reported that there are structural differences in the crossed crystal arrangement patterns for the shells of the species of the above mentioned mollusc classes and subclasses [15]. As scaphopod shell crystallographic data were not available so far, shell crystal organization patterns with a crossed crystal motif could not be juxtaposed and discussed comprehensively for the

above-mentioned mollusc classes and subclasses. This can be done now, as crystallographic data of scaphopod shells are available with the present investigation.

Accordingly, we give, first, an in-depth characterization of the dentaliid shell crystal arrangement pattern (microstructure and texture) and report the latter for the different parts/sections/layers of the dentaliid scaphopod shell. We investigated the shells of *Fissidentalium metivieri* (Scarabino 1995) and *Antalis weinkauffi* (Drunker 1877). While *F. metivieri* forms long (15–18 cm) and relatively thick (1–1.5 mm) shells, *A. weinkauffi* secretes small-sized (10 cm and below), thin (1 mm and below) shells. Previous studies investigated dentaliid shell structure mainly with light and some secondary electron microscopies. Imaging was performed on fracture surfaces, and the samples were taken from somewhere in the shell.

As stated above, our study is based on crystallographic aspects of *A. weinkauffi* and *F. metivieri* shell crystals. Using results of crystal orientation measurements, we discuss shell crystal organization and characterize the mode of crystal arrangement in different shell sections and portions. Crystal organization, morphology and size, thus crystal microstructure and crystal texture, were measured with electron backscattered diffraction (EBSD) and were complemented with laser confocal, SE (secondary electron) and BSE (backscatter electron) imaging. Both EBSD measurements and laser confocal, SE, and BSE imaging were performed on polished shell surfaces. Based on crystal size, morphology, arrangement, growth-line spacing and distribution, we can distinguish, for the investigated shells, three main crystal microstructures and one main crystal texture. Nonetheless, the latter (microstructure, texture) vary for the different sections of the shell. Thus, for the entire scaphopod hard tissue (the shell, the apical tube, the muscle attachment scars), we find, in total, five crystal microstructures and three crystal textures.

Crossed-lamellar (described for aragonitic shells [19]) and crossed-foliated (described for calcitic shells [19]) crystal arrangements are today widely utilized by Ca-carbonate-secreting organisms for shell formation (e.g., [20–24] and references therein). The crossed-lamellar microstructure is reported for scaphopod, gastropod, bivalve and Polyplacophora shells; the crossed-foliated microstructure is known for patellogastropod shells (e.g., [22,25–34]). As with the scaphopod results we have now crystallographic data for all mollusc classes/subclasses with the species constructing their shells with a crossed crystal arrangement pattern, we juxtapose and discuss, as second, shell crystallographic-structural characteristics for the species of the above-mentioned mollusc classes and subclasses. The latter is also feasible, as EBSD measurements render a large body of crystallographic results for shell crystal organization characterization (e.g., [33,35]).

Accordingly, we juxtapose crystallographic-structural differences/similarities of crossed crystal arrangement motifs for the Scaphopoda, Gastropoda, Bivalvia, Polyplacophora, and the Patellogastropoda. (i) We show that for the species of the latter mollusc classes/subclasses there is a broadly comparable crystal arrangement motif, namely, a lamellar shell microstructure. However, this basic crystal organization motif is modulated for each class by variation in crystal arrangement organization. (ii) We, furthermore, demonstrate that the crystal arrangement of gastropod shells and Polyplacophora shell plates are crossed but are not crossed-lamellar. (iii) As last, we discuss that, when crystallographic aspects are regarded, the crossed-foliated crystal microstructure is not the calcitic equivalent of the crossed-lamellar aragonitic microstructure, as postulated previously [19]. The crossed-lamellar and crossed-foliated mollusc shell microstructures are not only different in Ca-carbonate phase, but are also distinct in the mode of crystal organization.

2. Materials and Methods

2.1. Materials

We investigated the shell of the dentaliid species *Fissidentalium metivieri* (Scarabino 1995) and *Antalis weinkauffi* (Drunker 1877). *F. metivieri* was sampled in 1999 at about 750 m water depths in the Tulear region, close to Madagascar. *A. weinkauffi* was sampled in 2010 at about 200 m water depths at Alignay Island in the Philippines. For each species, three shells were investigated. All three shells were scanned with EBSD and imaged with laser confocal and scanning electron (using SE and BSE contrasts) microscopy. The shells were sectioned in two directions: Cut A and Cut B (Figure 1B). Cut A is a section perpendicular to the long axis of the tusk shell; Cut B is parallel to the long axis of the tusk shell.

In addition to the crystallographic characterization of *F. metivieri* and *A. weinkauffi* shells, we discuss in this contribution crystallographic–structural characteristics of crossed crystal arrangements of scaphopod, bivalve, gastropod and Polyplacophora shells. Thus, in addition to the shells of *A. weinkauffi* and *F. metivieri*, we scanned with EBSD the shells of *Fissidentalium metivieri* (Scarabino 1995) and *Antalis weinkauffi* (Röding, 1798) as well. *Laevicardium oblongum* was sampled in littoral waters of Balerna, nearshore Almeria, Mediterranean Sea, Spain. *Glycymeris pilosa* was sampled from subtidal waters of the Paman channel, off Zadar, Adriatic Sea. *Rhyssoplax olivacea* was sampled from the Mediterranean Sea, at Benalmadena coast, littoral of Malaga, Spain. *Acanthopleura vaillantii* was sampled from intertidal waters at Los Negros Escalante, the Philippines. *Helcion concolor* was sampled from intertidal waters at Jeffreys Bay, Indian Ocean, South Africa. *Patella piperata* was collected from intertidal waters in the Canary Islands, Eastern Atlantic Ocean, Spain. *Tanea lineata* was sampled from subtidal waters of the Philippine Archipelago, Western Pacific Ocean.

Subsequent to collection, samples were washed several times with distilled water, air-dried and kept in dry condition until embedding the shells into resin and for further preparation treatments, described below. In the case of *Fissidentalium metivieri* and *Antalis weinkauffi*, we investigated three specimens per species. As *L. oblongum*, *R. olivacea*, *A. vaillantii*, *H. concolor*, *P. piperata* and *T. lineata* are concerned, we investigated two specimens per species. Each species was measured with six to eight EBSD scans.

2.2. Methods

2.2.1. Imaging with Laser Confocal and SEM Microscopies, Electron Backscatter Diffraction (EBSD) Measurements

For laser confocal and SEM microscopy imaging as well as EBSD measurements, the shells were embedded in EPON resin. For obtaining even surfaces, the embedded shells were subjected to eight grinding and polishing steps, using different polishing cloths and polishing agents. The final polishing step consisted of etch polishing in a vibration polisher [36]. The polishing agents were: water and three different suspensions (MetaDi with 0.3 µm large diamond particles, MetaDi with 0.1 µm large diamond particles and the acidic alumina suspension (OP-A)) [36].

To visualize subtle internal structures, samples prepared for EBSD measurements were additionally etched with distilled water for 15 min. These surfaces were imaged with SE, BSE, and laser confocal microscopies.

For laser confocal imaging, the samples remained uncoated. For SE and BSE imaging, the samples were coated with 4 nm of Pt/Pd. For EBSD measurements, the samples were coated with 4 to 6 nm of carbon.

SEM imaging and EBSD measurements were carried out with a Hitachi SU5000 field emission SEM (Hitachi High-Technologies Corporation, Tokyo, 105-8717, Japan)-equipped with a high-resolution Nordlys II Oxford Instruments EBSD and an OXFORD

Instruments X-Max80 EDS detector (Oxford Instruments, High Wycombe, BUCKS, HP12 3SE, UK). During measurements, the SEM was operated at 20 kV; scans were taken with a spot size of 50 to 60 nm, and EBSD measurements were taken with a step size of 200 to 300 nm. Laser confocal imaging was carried out with a Keyence 3D laser scanning confocal microscope (VK-X1000), (Keyence Corporation, Osaka, 533-8555, Japan). Laser confocal imaging was performed in coaxial, reflective mode at 5, 10, 20 and 50 times magnification.

All analyses and data evaluation were performed at the Department of Earth and Environmental Sciences, LMU Munich, Germany.

EBSD crystal orientation data were evaluated with two Oxford Instruments programmes: HKL CHANNEL 5.0 and AZtecCrystal 3.0 (including MapSweeper 3.0). AZtecCrystal and MapSweeper were used for evaluating the scaphopod, bivalve and gastropod measurements. The polyplacophoran and patellogastropod EBSD scans were evaluated with CHANNEL5 and AZtecCrystal. Data evaluation with MapSweeper was performed to improve the angular precision of the scan below 0.1° and to better determine misorientations between adjacent crystals (for further information see references [33,34]).

We performed a statistical evaluation of crystal misorientation data for this contribution. Misorientation data were gained from EBSD measurements. For the statistical evaluation, we used EBSD measurements with very high hit rates ($>70\%$ for aragonite, $>90\%$ for calcite). With the exception of the gastropod shell (*T. lineata*), we evaluated, for each other mollusc class/subclass, misorientations for two species (Scaphopoda: *Fissidentalium metivieri* and *Antalis weinkauffi*, Bivalvia: *Laevicardium oblongum* and *Glycymeris pilosa*, Polyplacophora: *Rhyssoplax olivacea* and *Acanthopleura vaillantii*, Patellogastropoda: *Helcion concolor* and *Patella piperata*). For the class Gastropoda, the shell of only one species (*Tanea lineata*) was investigated. Each shell was scanned with at least five EBSD scans. For each scan, we calculated: (i) the misorientation angle distribution, (ii) the point-to-point misorientation on a trajectory from A to B across four to six adjacent lamellae and (iii) the point-to-point misorientation on a trajectory from C to D in a lamella and following the long axis of the lamella. For each shell and each type of misorientation, we calculated 10 relative frequency–misorientation diagrams.

We refer to differently sized grains. The grain size can be obtained from EBSD measurements. However, as it was not the focus of this study, a statistical grain size analysis is not given in the present study.

2.2.2. Definition of Structural and Crystallographic Terms Used in This Study

Subsequently, we define structural–crystallographic terms that we use in our contribution that are related to crystal microstructure and crystal texture. For further information on EBSD measurements and data evaluation, see reference [33–35].

Microstructure refers to the sizes, morphologies, co- and misorientations, and modes of interlinkage of crystal grains in a material. It is shown with color-coded EBSD maps, where similar colors reflect similar crystal orientations and different colors highlight different crystal orientations. For large parts of the investigated scaphopod shells, we observe the crossed-lamellar microstructure. In a crossed-lamellar mode of crystal organization, the crystals are arranged into bands, also called first-order lamellae. The lamellae are parallel or perpendicular to the shell margin (e.g., [22]). The first-order lamellae comprise smaller units, the second-order sheets. These are substructured by third-order laths [22]. Second- and third-order lamellae of adjacent first-order lamellae dip in opposite directions [22]. The angles between adjacent first-order lamellae vary between 90° and 130° [37].

Texture, or *crystallographic preferred orientation*, relates to the distribution of all crystal orientations within a material. It is illustrated with pole figures, which show either color-coded crystallographic axes orientation data or the contoured version of the density distribution of the crystal orientation poles.

Pole figures are stereographic projections of crystallographic plane-normal or axes orientations and are measured for all pixels of an EBSD map or a selected area (subset). The viewing direction of the pole figures is the same as the viewing direction of the corresponding EBSD maps. All pole figures shown in this contribution display the lower hemisphere. This ensures that the pole figures are displayed in the same spatial orientation as the corresponding EBSD map. With pole figures, we show either individual orientation data points or the density distributions of the orientation data.

We discuss here *axial*, *axial-like*, *crossed-lamellar* and *single-crystal-like* textures.

An *axial texture* is characterized by c-axes clustering in one particular direction and the corresponding a- and b-axes scattering in orientation on a great circle perpendicular to the c-axis orientation (e.g., [31,33]). We describe with this study an *axial-like texture*. We find large scattering for all three crystallographic axes of aragonite; thus, scattering also in c-axes as well as in b- and a-axes. As we do not find clustering of the c-axes, we call the observed texture pattern axial-like. A *crossed-lamellar texture* is characterized, for each first-order lamellar set1 and set2, by two maxima each for the a- and the b-axes orientation distributions and one maximum for the c-axes orientation distributions (e.g., [31–34]). Thus, in the case of a crossed-lamellar texture, when both first-order lamella set1 and set2 are scanned with EBSD, we find in the corresponding pole figure two c-axes, four b-axes, and four a-axes orientation clusters. A *single-crystal-like texture* is present when, in the pole figures, we observe a single orientational maximum for each crystallographic axis of the scanned crystal. Hence, in the case of aragonite, we need to find in the pole figure one cluster each for the c-, b-, and a-axes orientation distributions.

The EBSD *band contrast map* depicts the signal strength of the Kikuchi pattern at each measurement point in the EBSD scan. It is displayed as a gray-scale component in the map; white to light gray colors indicate a high intensity of the Kikuchi signal, corresponding to strong mineralisation; dark gray and black colors point to a weak or absent Kikuchi signal, e.g., when organic matter is scanned.

2.2.3. Scaphopod Shell-Related Terminology Used in This Study

Instead of using the often topologically ill-defined terms of inner and outer shell, we use, for the latter, according to biological convention, the terms distal and proximal shell rims and/or surfaces. The terms distal and proximal are directional terms and describe the relative locations of distal and proximal structures.

The *distal* shell section or surface is the shell portion or surface that is most distant from the median line of the shell arch. Previous work often refers to the distal shell surface as the outer surface.

The *proximal* shell section or surface is the opposite section or surface to the distal shell parts. Previous work often refers to the proximal shell surface as the inner surface.

We follow in this study for the different parts of the scaphopod shell the terminology used by Shimek and Steiner, Palmer and Steiner and Reynolds [2,3,7]. Based on digital tomography imaging, Sigwart et al. [10] visualized in 3D the position of the organ system and orientation of the body axis of the dentaliid scaphopod *Rhabdus rectius*. The authors suggest a different interpretation for the position of the different body parts, relative to the interpretation given in [2,3,7] and suggest a change in the body axis. Accordingly, the nomenclature of the anterior and posterior as well as of the dorsal and ventral shell sections is interchanged (Figure 1E in [10]), relative to the nomenclature used by Shimek and Steiner, and Reynolds [2,3]. Nonetheless, a change in soft tissue organization and orientation does not affect our study. The above-cited studies regard scaphopod organs and other soft tissues, while here we describe crystal organization in the scaphopod shell. The scaphopod shell has two orifices, the frontal aperture and the apical aperture, and two well-

distinguishable shell sides, namely, the convex and the concave side. We use the latter nomenclature for the localization of the observed crystal assembly patterns.

3. Results

We investigated the shell of the scaphopod species *Fissidentalium metivieri* and *Antalis weinkauffi*. We concentrated mainly on the shell of *F. metivieri* (Figures 1–9, S1 and S4–S10); nonetheless, we checked all major findings that we observed for *F. metivieri* for the shell of *A. weinkauffi* as well (Figures 1 and S2–S4). Figure 1A highlights the orientation of a dentaliid scaphopod organism in life position and gives the terms for the shell sections that are used in this contribution. All structures that we show subsequently were obtained from sections through the shells cut in two directions. Cut A runs perpendicular to the long axis of the shell; Cut B runs parallel to the long axis of the shell (Figure 1B).

Previous studies report that scaphopod shells comprise two layers: an outer and an inner shell layer. The observation of these layers was based on light-microscopy imaging. We show in Figures 1C,D and S1 the position and some internal features of these two shell layers. However, the close inspection of the shell structure with laser confocal and SEM imaging reveals for the investigated scaphopod species two further shell layers (Figure 1D–F). We find, in addition to the previously observed outer and inner shell layers, an outermost (red stars in Figure 1D) and an innermost (blue stars in Figure 1E) shell layer. The outermost and the innermost shell layers seam the distal and the proximal surface of the shell (Figures 1E,F and 2C).

F. metivieri and *A. weinkauffi* shells consist of aragonite crystals that are assembled within the shells with a crossed-lamellar arrangement pattern (Figures 2–4, S2 and S3). We see, from distal to proximal, a gradual increase in crossed-lamellar unit size (Figure S4), such that small, submicrometer-sized units, seam the distal shell surface; however, significantly larger crystal entities are towards the proximal surface of the shell (Figure S4). In addition, we observe a strong increase in growth-line presence from distal to proximal shell sections (Figures 1C,D and S1). While at distal and central shell sections the growth lines are widely spaced, the growth lines are very closely spaced at inner/proximal shell portions (Figures 1D and S1). The innermost shell layer (blue stars in Figure 1E,F) does not have a crossed-lamellar crystal organization. It consists of granules and prisms. These become enlarged towards the innermost, proximal, shell surface.

Figures 2–4 and Supplementary Figures S2 and S3 visualize the microstructure and texture of *F. metivieri* and *A. weinkauffi* shell crystals. Crystal orientation is shown color-coded; similar colors indicate similar crystal orientation, and different colors point to different crystal orientations. The outermost shell portion comprises an about 100 µm thick layer of small, submicrometer-sized crystals (Figure 2B,C). These increase in size towards central and proximal shell sections (Figure 2B,D). We find from distal to proximal a gradual transformation in crossed-lamellar unit appearance, from blocky to elongated entities. The large, elongated crystals have lamellar morphology (Figures 2D, 3D, S1 and S4). We furthermore observe a wide spacing of growth lines at distal, relative to their spacing at proximal shell sections (Figures 3 and S1). In general, growth lines do not seem to affect the organization of crystals in the crossed-lamellar units (yellow arrows in Figures 3, 4 and S5). Nonetheless, we find instances as well where growth lines offset crystal organization within the lamellae (Figures 1D and 4A). Crystals at growth lines increase slightly in size and are, rather, prismatic in morphology (Figures 3D and S5).

Pole figures in Figures 2, 4, S2 and S3 visualize the texture patterns of the different shell sections. For the outermost shell layer, the layer that is formed by the submicrometer-sized crossed-lamellar units, we find an axial-like texture pattern (Figure 2E). Nonetheless, the observed texture pattern cannot be termed as clear-cut axial, as we see in the c-axis

pole figure a very broad orientation distribution (Figure 2C). For the central and inner shell portions, we find a clear-cut crossed-lamellar texture (Figures 2F, 4, S2 and S3).

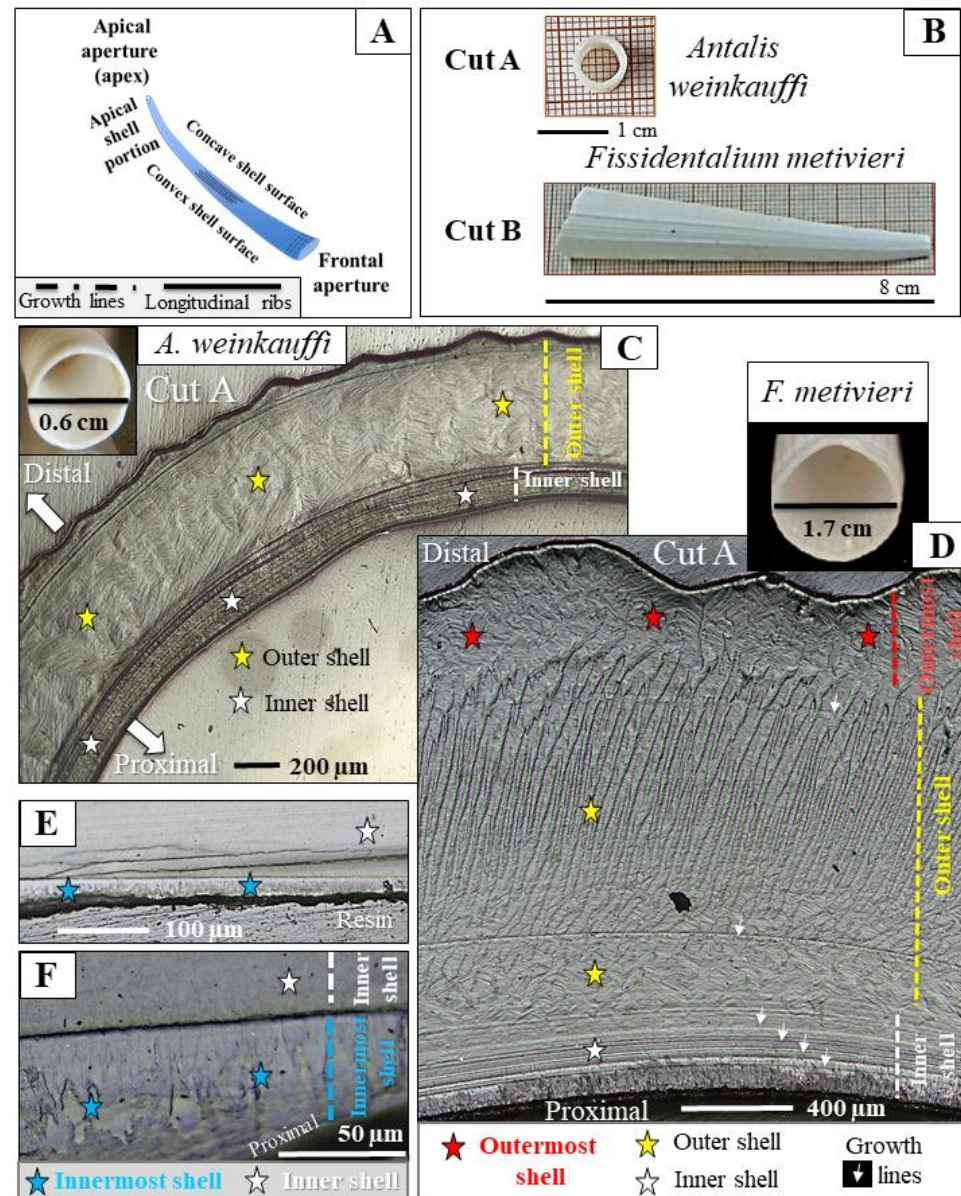


Figure 1. (A) The conchological terms that we use in this contribution. (B) Light microscopy micrographs showing the orientation of the sections that were cut out from the shells (Cut A, Cut B) and were investigated in this study. (C–F) Laser confocal micrographs of the shell of the investigated scaphopod species: *Antalis weinkauffi* and *Fissidentalium metivieri*. Previous studies identified for dentalid shells two mineralized layers: an outer and an inner shell layer [2,3,7,15]. We indicate the position of these layers with yellow and white stars on shell cross-sections, in (C,D). However, we observed additional shell crystal assembly patterns: 1. red stars in (D): the assembly motif of crystals that form the layer at the outermost shell section, 2. blue stars in (E,F): the assembly of crystals that form the layer at the innermost shell section. (C,D) Well observable is the crossed-lamellar arrangement of shell crystals and the differentiation of the latter into an outermost (red stars), an outer (yellow stars) and an inner (white stars) shell section. White arrows in (D) point to the presence of growth lines. The crystals that form the innermost shell section have prismatic morphologies; however, as this study shows, also assembled in the shell with a crossed-lamellar organization pattern. Yellow star in (C): outer shell; white star in (C): inner shell. Red star in (D): outermost shell; yellow

star in (D): outer shell; white star in (D): inner shell. Blue star in (E,F): innermost shell. (B) *A. weinkauffii* and *F. metivieri*, (C) *A. weinkauffii*, (D) *F. metivieri*, (E,F): *F. metivieri*.

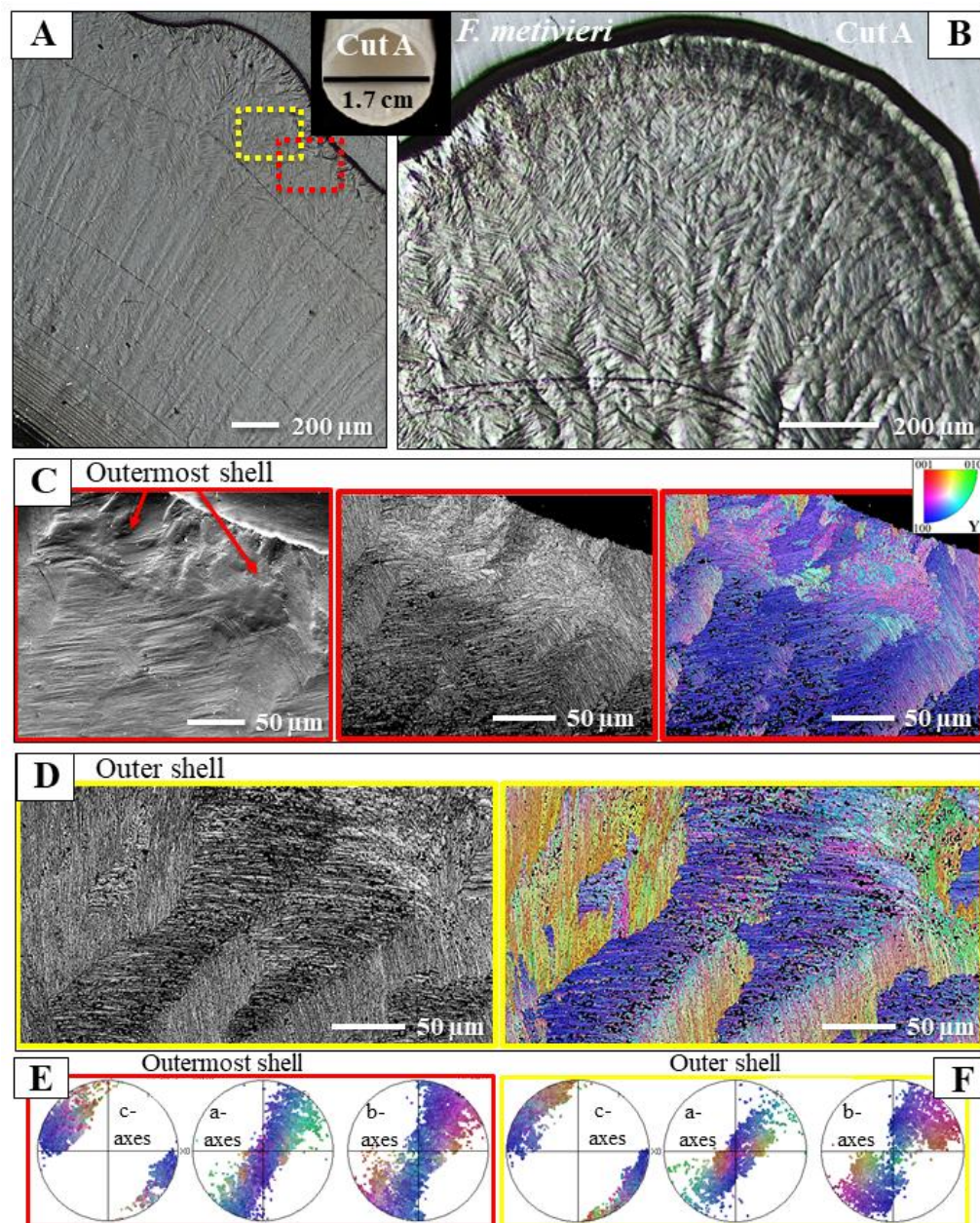


Figure 2. The difference in crystal organization between the outermost and the outer shell sections, respectively (A–F). We observe along the distal surface of the shell a seam of small crystals (C), having a slightly less regular arrangement, relative to the arrangement of the crystals that form the outer shell (EBSD maps in (C,D)). The array of small crystals (of the outermost shell) converges smoothly into the large first-order lamellar units of the outer shell (EBSD map in (D) and image in (A,B)). (B) The increase in crystal unit size from the distal shell surface towards the lamellae of the outer shell is well visible. (E,F) Pole figures highlighting the difference in crystal texture at the outermost (left) and at the outer shell layer (right), respectively. (A,B) Laser confocal microscope image; (C), from left to right: BSE micrograph, EBSD band contrast measurement image (gray-scaled), color-coded crystal orientation map. (D) EBSD band contrast measurement map (gray-scaled) and color-coded crystal orientation map.

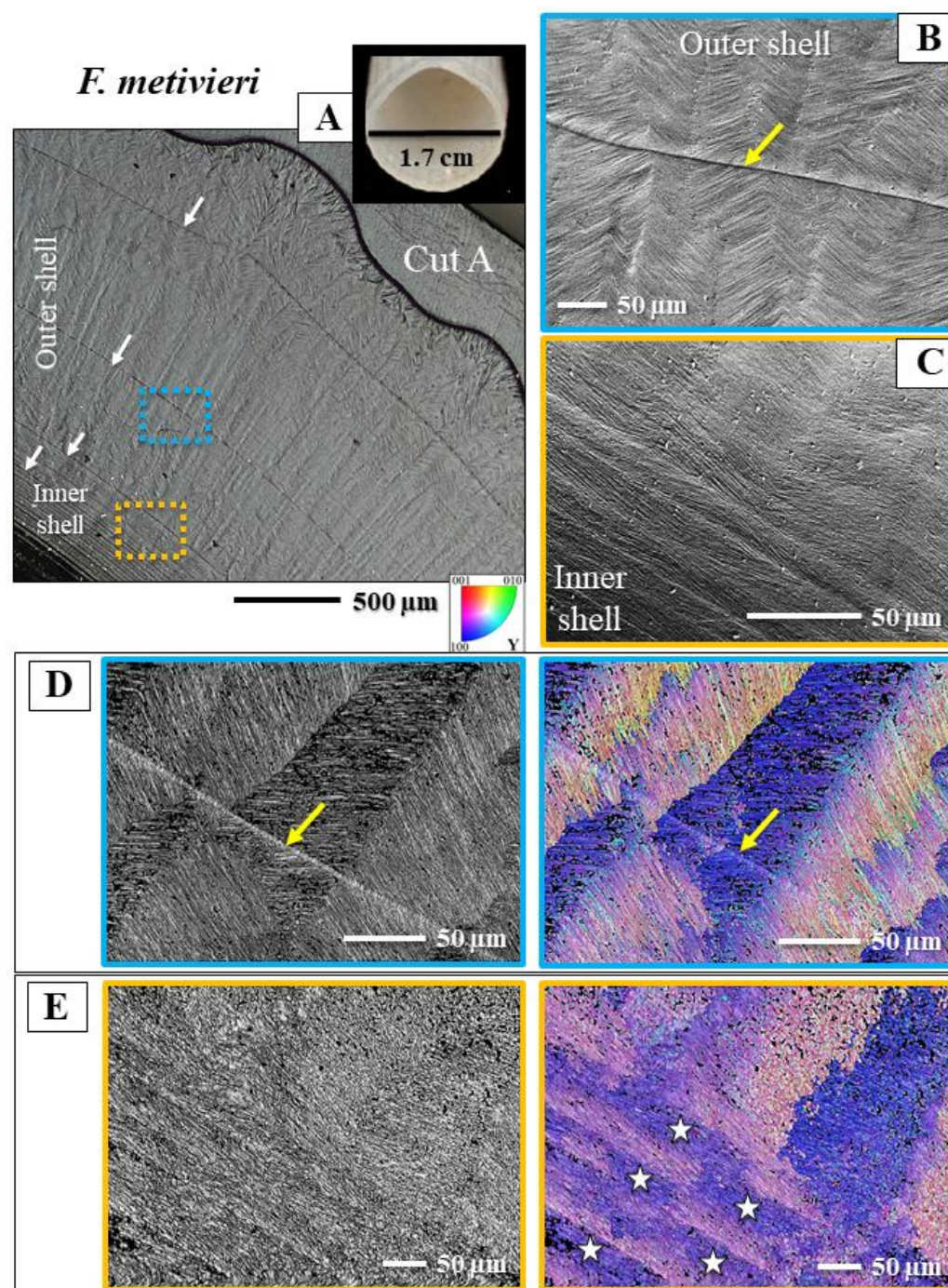


Figure 3. The internal structure and crystal arrangement pattern of the central portion of the outer shell layer and the arrangement of the crystals that form the inner shell layer (A–E). White arrows in (A) and yellow arrows in (B,D) point to growth lines; these become densely packed at inner shell sections (A,C,E). Within the outer shell layer, growth lines do not influence crystal orientation in the crossed-lamellar units (see yellow arrows in (B,D)). (A,B) BSE micrographs. Left in (D,E): EBSD band contrast measurement images, gray-scaled. Right in (D,E): color-coded EBSD crystal orientation maps. See in the color-coded crystal orientation map in (E) the displacement of lamellae across growth increments, the latter indicated with white stars in (D).

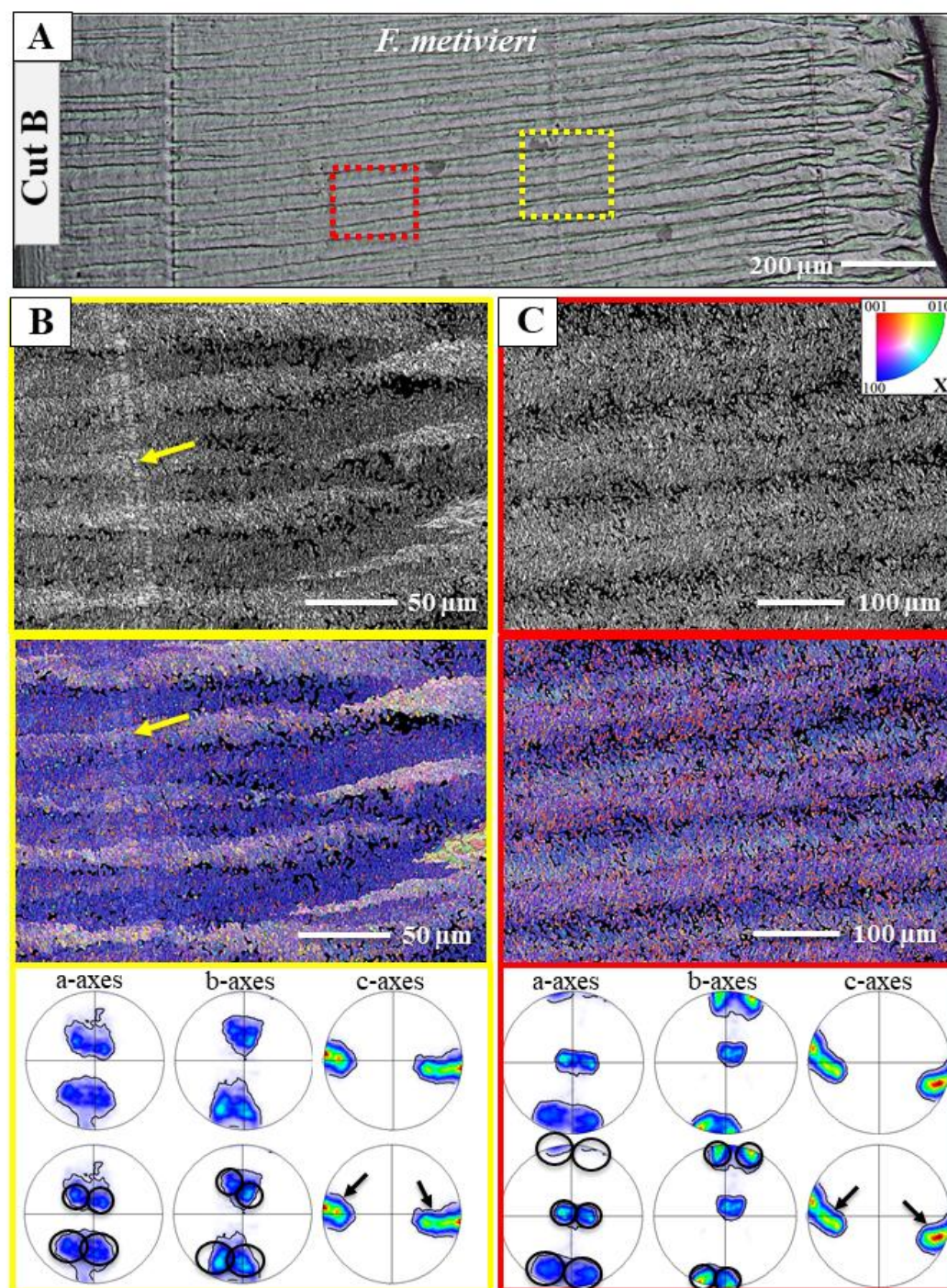


Figure 4. (A–C): Crystal microstructure and crystal texture at the central section of the outer shell. Imaging and EBSD measurements are done on a section cut parallel to the long axis of the shell, Cut B. (A) Laser confocal image; (B,C) EBSD band contrast micrographs (gray-scaled) and crystal orientation maps (in color). Pole figures in (B,C) visualize the corresponding crystal texture. The parallel arrangement of the first-order lamellar sets is well observable. The measurement in (B) is traversed by a growth line (yellow arrow in (B)); we do not observe a marked change in the growth line in the crystal arrangement of the first-order lamella (see color-coded EBSD map in (C)). The pole figures in (B,C) demonstrate the crossed-lamellar texture of the crystals in the EBSD-scanned shell section, as we observe four clusters for the a-axes, four clusters for the b-axes and two clusters for the c-axes. Thus, for a first-order lamellar set1 and set2 crystals, we find, for each set, one cluster for the c-axes and two clusters each for the a- and the b-axes, respectively.

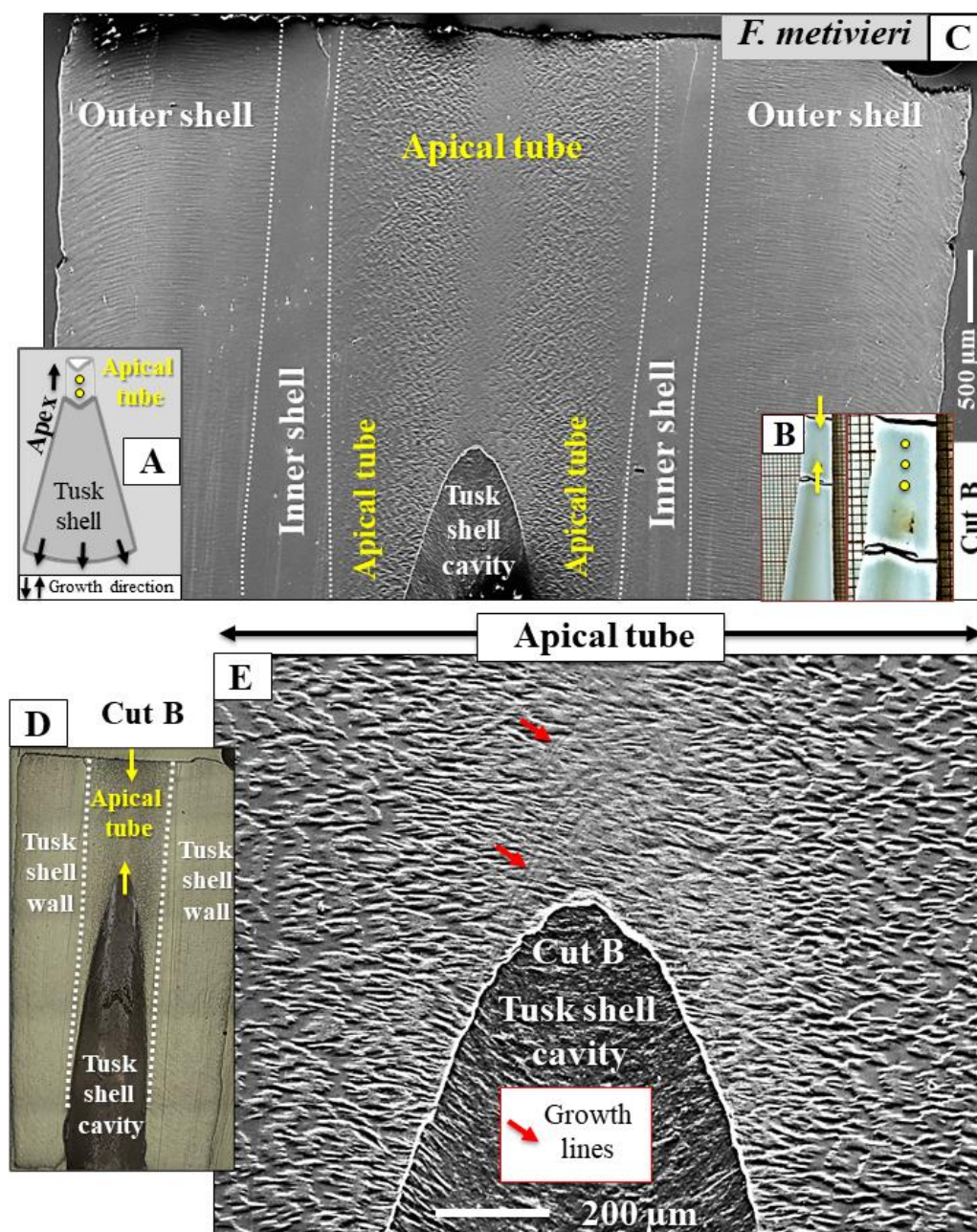


Figure 5. The apical tube of *F. metivieri* at apical shell portions (A–E) and its structural relation to the inner shell layer and the cavity of the tusk shell. The sketch in (A) is modified after [3] and visualizes the position of the apical tube. The latter forms at the apex (yellow arrow and yellow dots in inserts in (B)) and is considered to be the product of secondary mineralization, secreted during shell growth. As the scaphopod organism grows, new shell is added to the frontal aperture and the apex is enlarged. According to Reynolds [3,14], apex orifice enlargement occurs through resorption and decollation. The orifice at the apex needs to be stabilized by hard tissue. A tube (A) forms as the apical tube ((A–E) [14]). The apical tube is strongly mineralized; its crystals are intimately connected to the crystals of the inner shell (C,D). The apical tube consists of a dense aggregation of interconnected aragonite dendrites (E).

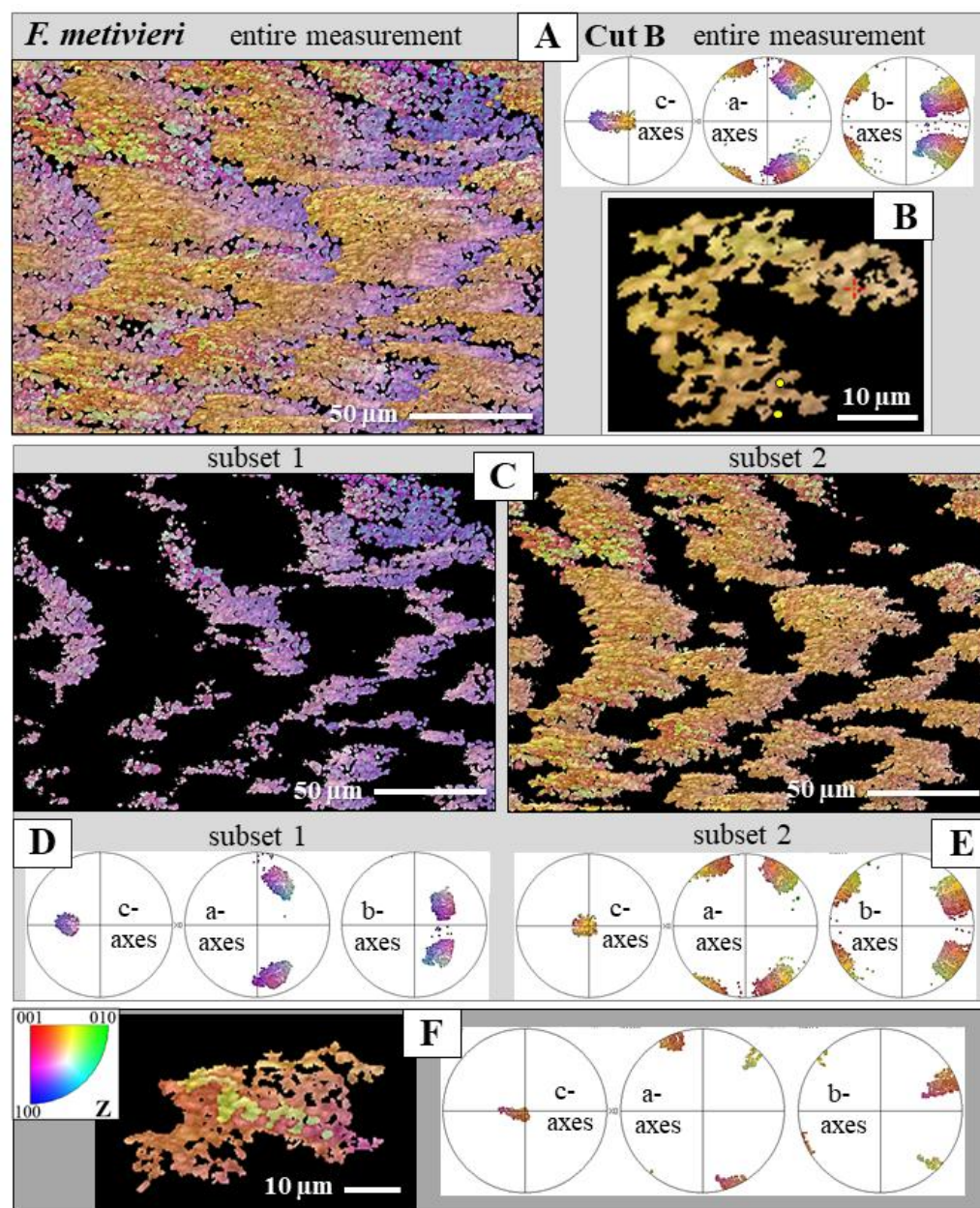


Figure 6. Microstructure and texture of apical tube aragonite (A–F). The apical tube and the tusk shell were sectioned parallel to the long axis of the shell, Cut B. The apical tube crystal units (A–C,F) are dendrites. Individual crystal units (e.g., (B,E)) appear to consist of assemblies of granules. We observe rows of dendritic crystals, alternating slightly in orientation (C). The texture of the apical tube crystal units can be described as crossed-lamellar; see pole figures in (A,B,D–F).

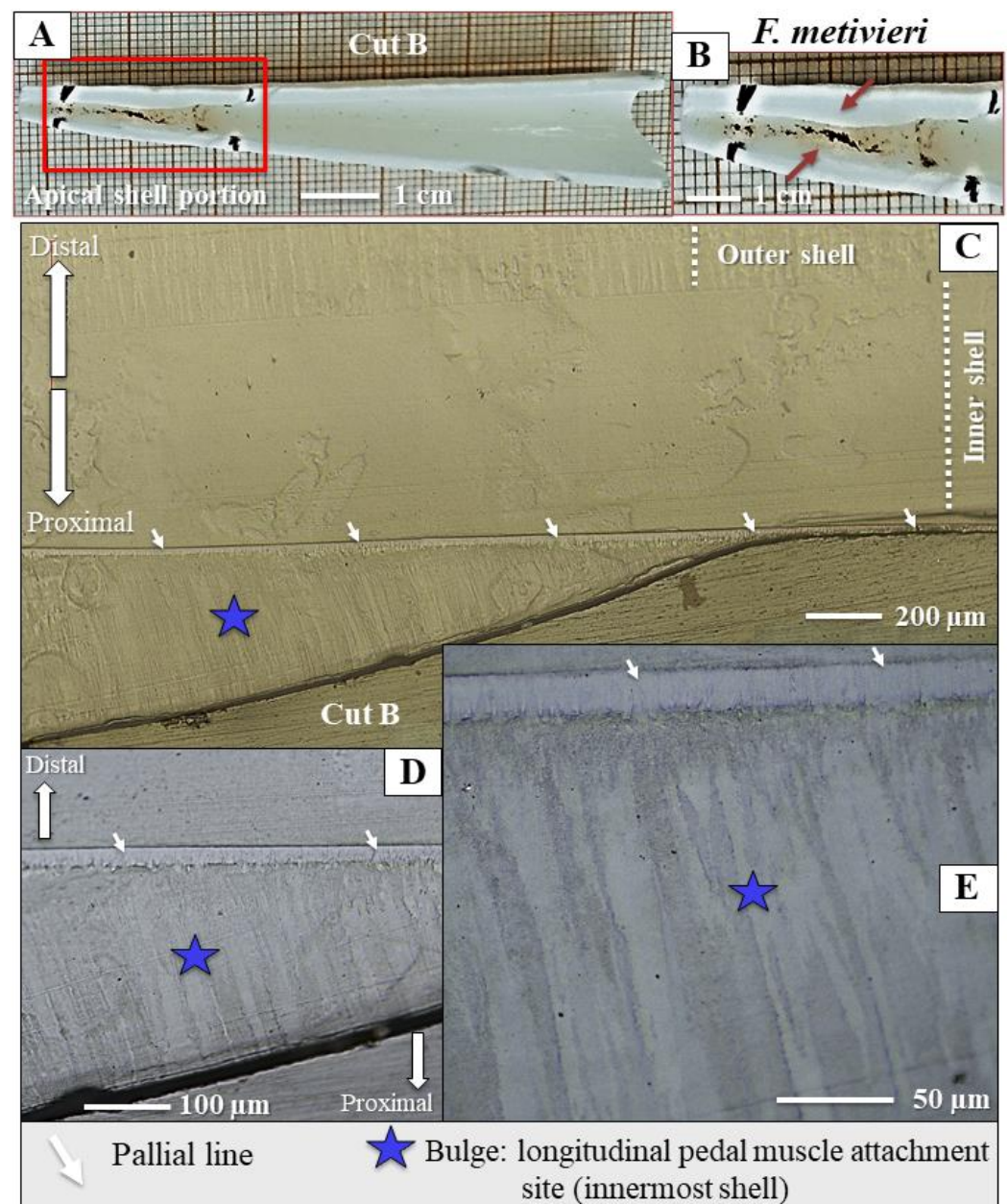


Figure 7. Light microscopy (A,B) and laser confocal microscopy (C–E) images of the mantle muscle attachment and of the dorso-ventral longitudinal pedal muscle attachment scars. The mantle (pallium) secretes the shell. At mantle attachment to the shell, a hard tissue scar or myostracum forms. The pallial scar consists of an assembly of prismatic crystals; in 2D it is a row of prismatic crystals (white arrows in (C–E) and Figure 9B,C). For foot retraction into the shell, Scaphopoda use longitudinal pedal muscles [2,3,7,15]. These attach to the inner surface of the shell (e.g., [2]) and to the pallial myostracum (C–E). The pedal muscle attachment scar is a bulge, an internal shell wall thickening close to the apex (blue stars in (C–E)) that protrudes into the cavity of the tusk shell (red rectangle in (A), red arrows in (B)). The figure in (B) is a zoom-in into the figure in (A). The red rectangle in (A) indicates where the figure shown in (B) was taken. The bulge is circumferential along the inner surface of the shell and is always adjacent and proximal to the pallial line (C,D). Laser confocal images highlight that the bulge also has a prismatic internal structure (D,E), as is the case for the pallial myostracum.

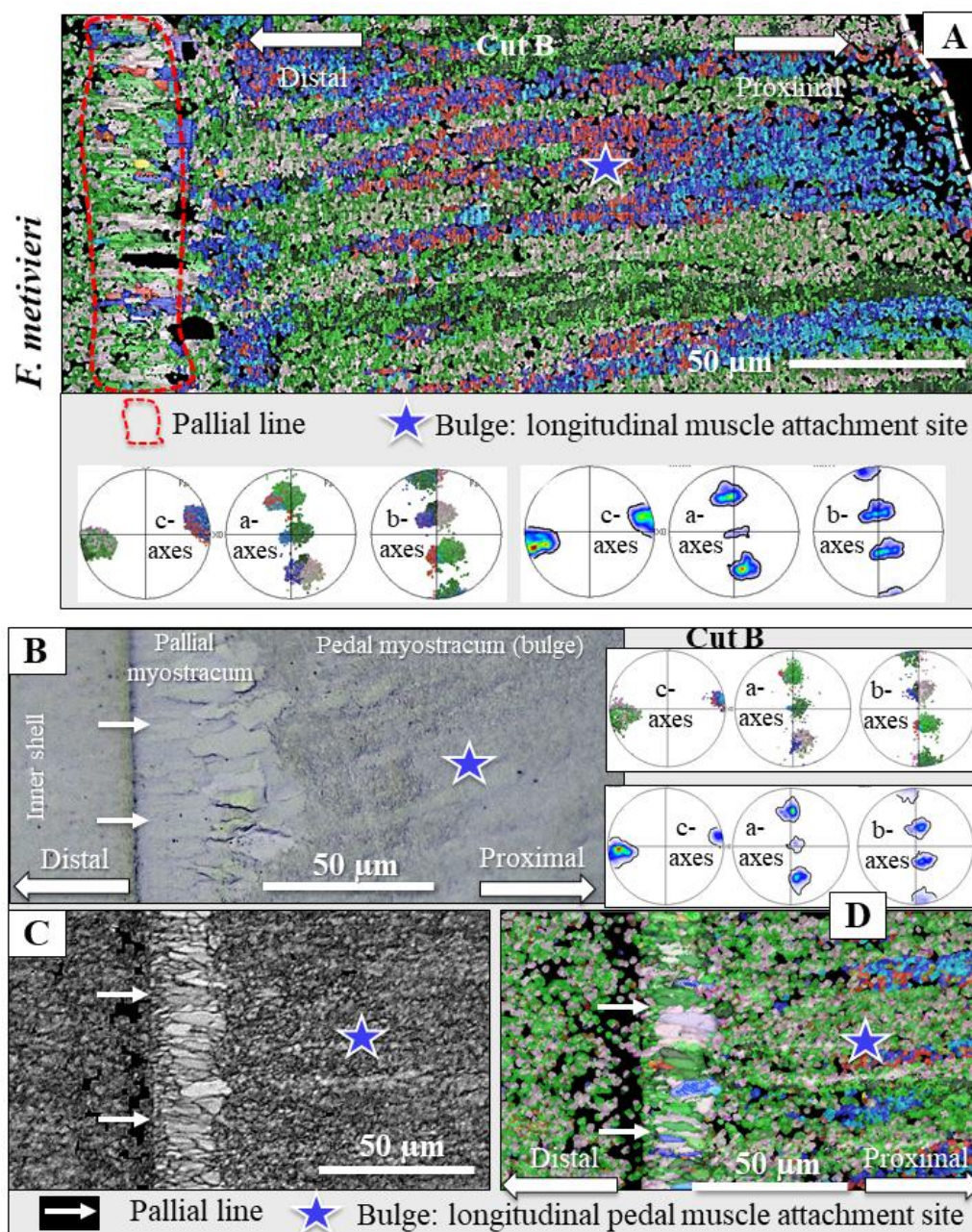


Figure 8. The microstructure and texture of the bulge (blue stars in (A–D)). The shell is sectioned parallel to the long axis of the shell, Cut B. Well observable is the prismatic nature of the pallial line crystals (red rectangle in (A), white arrows in (B,C)) and the transmission of the pattern of crystal orientation from the inner shell to the crystal orientation of the pallial myostracum and further, to the crystals of the bulge. For both EBSD measurements (A,D), we observe a crossed-lamellar texture (see pole figures in (A,D)). (A,D) EBSD measurement, color-coded for orientation. (B) Laser confocal microscopy image. (C) EBSD band contrast measurement image. White dashed line in (A): the proximal surface of the shell.

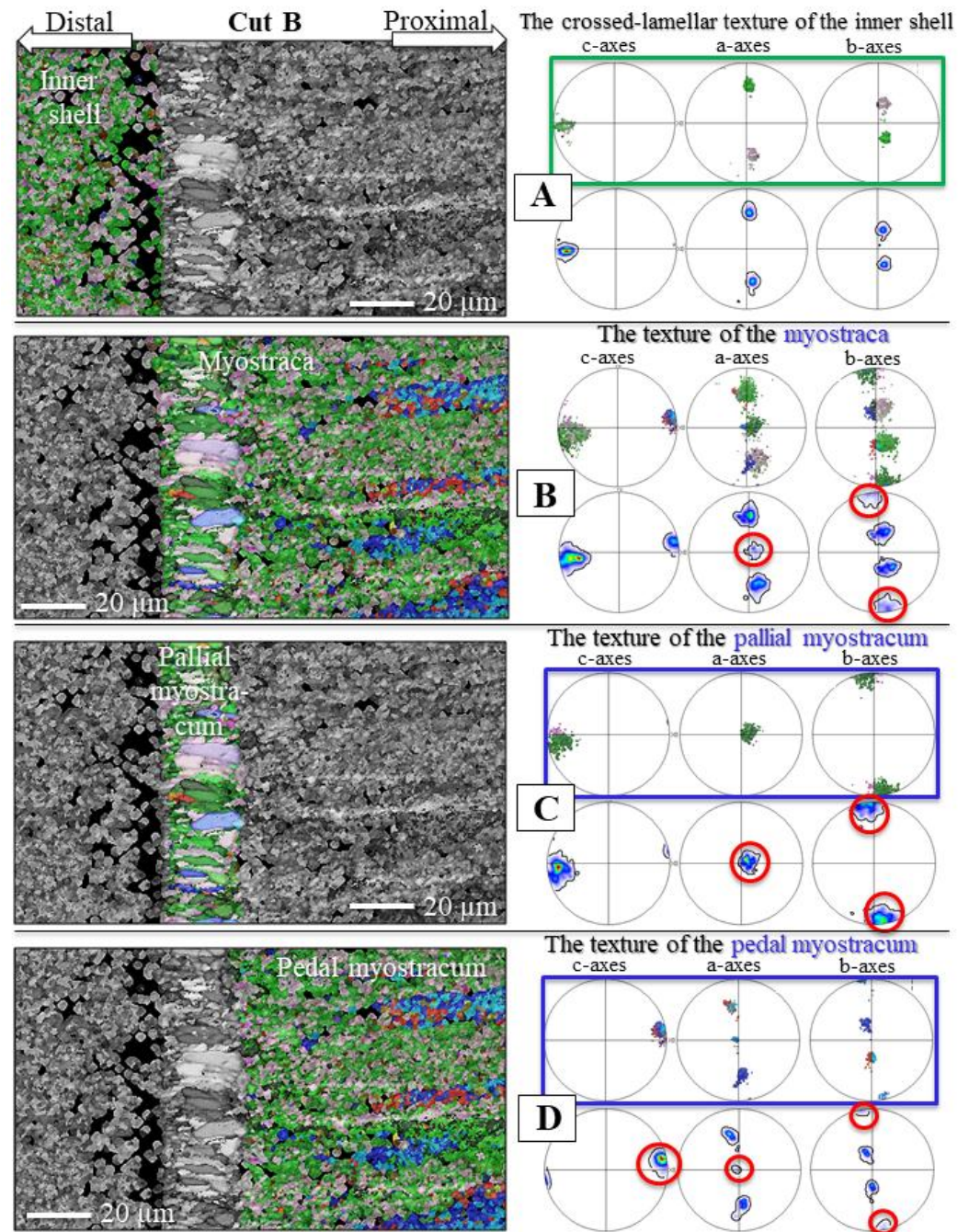


Figure 9. The texture of the inner shell layer (A), of the pallial myostracum (B), and of the longitudinal pedal muscle attachment scar, the bulge (C). The crossed-lamellar texture pattern is transmitted from the inner tusk shell (A) to the pallial (B) and to the longitudinal pedal myostracum (C) (see the c-axes orientations and the a- and b-axes orientations encircled in red). However, at the muscle scars, an additional crystal orientation develops (see the c-axes orientations and the a- and b-axes orientations encircled in red), relative to the orientation of the crystals that comprise the inner shell. (Left) In the figure: EBSD band contrast measurement images (in gray), superimposed, for the relevant subset, with crystal orientation (in color). (Right) In the figure: pole figures showing the texture patterns of the inner shell and of the two myostraca.

The above described structural attributes and crystal organization patterns cover the crystals that comprise the scaphopod shell proper (Figures 1–4, S1–S5). However, we find, at specific shell sections, further microstructures (Figures 5–9 and S6–S10). These are:

- (i) At the apical shell portion, at the very tip of the apex (Figures 5, 6 and S6–S8).

- (ii) Along the proximal surface of the shell, developed as a seam of crystals lining most of the inner shell surface (white arrows in Figures 7, S7 and S8).
- (iii) Along the proximal surface of the shell and proximal to the, above mentioned, seam of crystals.
- (iv) The latter is developed as a mineralized bulge (blue stars in Figures 7, 8, S9 and S10). The bulge is only present at the apical shell section. It is, however, not positioned right at the apex but further towards the frontal aperture (Figures 7A,B, S9D and S10).

As scaphopods grow, a new shell forms at the frontal aperture. This ensures that the shell grows in length and that the orifice at the frontal aperture is enlarged. However, with organismal growth, the orifice at the apex has to be enlarged as well. Enlargement of the apex takes place via shell resorption and decollation [7,37–40], processes that slightly injure the apical orifice. For apical orifice enlargement and restoration, an additional hard tissue is secreted in the form of a pipe or a tube, the apical tube [14]. The pipe/tube might extend outward from the apex ([3] and Figure 5A). We sectioned the apical portion of the shell in two directions and found for *F. metivieri* the apical tube (Figures 5, 6 and S6–S8). Phase detection with EBSD proved that the apical tube consists of aragonite as well. As shown in Figures 5A, S7 and S8, the crystals of the apical tube are intimately fused with the crystals of the inner shell. Furthermore, as shown in Figures S6–S8, the apical tube crystals are always proximal to the crystals of the pallial line, the scar that forms on the inner shell surface when the mantle attaches to the inner surface of the shell (also called the pallial myostracum). Crystal organization and structure of the apical tube are specific (Figure 5). EBSD measurements demonstrate that the microstructure of the apical tube (Figure 6) significantly differs from the microstructure of the rest of the shell (e.g., compare EBSD maps in Figure 3 with the map in Figure 6A). Dendritic units (Figures 6B,F) appear to form rows (subsets 1 and 2 in Figure 6C). The rows have slightly different orientations (see pole figures in Figure 6D,E) and alternate in orientation (Figure 6A). The apical tube dendrites appear to have a granular internal structure (Figure 6). Even though the apical tube microstructure appears to be different from the microstructure of the rest of the shell, the texture pattern of apical tube crystals and the texture pattern of shell wall crystals are similar. With the exception of the seam of the small-size crossed-lamellar units at the distal shell surface, the largest part of shell wall aragonite is assembled with a crossed-lamellar texture (pole figures in Figure 4); the latter is also the texture that we find for the apical tube crystals (pole figures in Figure 6).

We detected a further hard tissue, different in microstructure from that of the shell wall proper and that of the apical tube. At the apical shell section (for the definition of the apical shell section see Figure 1A), we observe a mineralized bulge, consisting of aragonite, running circumferentially along the inner shell surface, and protruding into the cavity of the shell (red arrows in Figures 7A–E, S9 and S10). The crystals of the bulge are intimately connected to the crystals of the rest of the shell. As is the case for the apical tube, the bulge is also always proximal to the pallial myostracum (Figures 7 and S10). The bulge is internally structured and has a prismatic appearance (blue stars in Figures 7C–E and S9B–D). We investigated the extent of the bulge in two *F. metivieri* specimens (Figures 7A,B and S10). We did not find a connection between the bulge and the apical tube. In both specimens, the bulge thins out at its two ends: towards the frontal orifice and towards the apex (Figures S9D,E and S10). We consider the bulge as the attachment site of the longitudinal pedal muscle to the shell, the scar of the pedal myostracum.

Figure 8 shows two EBSD scans on cross-sections covering the inner shell, the pallial myostracum, and the bulge. The difference in microstructure between the inner shell is well observable, on the one hand, and the pallial myostracum and the bulge, on the other. The crystals of the inner shell are fine-grained and granular; the crystals of the pallial my-

ostracum are prismatic (white arrows in Figure 8C); the crystals of the bulge are also prismatic (blue stars in Figures 7 and S9). The textures of the inner shell, that of the pallial myostracum and that of the bulge, are similar (see pole figures in Figure 9). We find for all three shell sections a crossed-lamellar-like texture. However, relative to the mode of crossed-lamellar arrangement of crystals characterizing the inner shell (pole figures in Figure 9A), we find for the pallial myostracum and for the pedal myostracum (bulge) development of an additional crystal orientation for the a- and b-axes (pole figures in Figure 9C,D). Thus, the two myostraca have a prismatic microstructure and a crossed-lamellar texture; however, complemented with an additional mode of crystal organization (Figure 9).

4. Discussion

The combination of EBSD measurements, SE, BSE and laser confocal microscopy imaging discloses many structural characteristics of structural hard tissues. When taking crystal unit size, morphology, growth-line spacing and pattern of crystal organization into account, we are able to identify for the investigated dentaliid scaphopod shells five different crystal arrangement patterns (Table 1): (i) one crystal assembly pattern for the shell proper; (ii) the latter modified at outermost shell section; (iii and iv) two crystal assemblies for the attachment sites of two muscle tissues to the shell; and (v) one crystal assembly pattern for the hard tissue that forms when the apical orifice is enlarged at organismal growth. Despite significant variation in microstructure, we find only three crystal textures: (i) the main crystal texture, (ii) the slight modification of the latter by crystal size, and (iii) the slight modification of the main texture by addition of a further crystal orientation.

Table 1. Summary of the microstructures and textures detected for the investigated dentaliid scaphopod shells.

	Microstructure	Texture
Shell proper (the bulk of the shell)	crossed-lamellar	crossed-lamellar
Shell proper (outermost/distal portion of the shell)	crossed-lamellar	crossed-lamellar-like texture motif influenced by a large spread in crystallite orientation
Mantle attachment scar (pallial myostracum)	Prismatic influenced by a competitive growth crystal growth mechanism	crossed-lamellar-like with a third orientation
Longitudinal pedal muscle attachment scar (pedal myostracum)	prismatic	crossed-lamellar-like with a third orientation
Apical tube	dendritic to prismatic	crossed-lamellar

In essence, the main crystal microstructure and crystal texture of the investigated scaphopod shells is crossed-lamellar; however, modulated in crystal organization at the distal shell portion, at the myostraca and at the apical tube.

4.1. The Dentaliid Scaphopod Shell

Figures 10 and S11 depict shell cross-sections of *Fissidentalium metivieri*, cut perpendicular to the long axis of the shell. The pole figures of Figure 10 show that there is a gradual transformation of the texture from an axial-like texture (red rectangle), measured at the outermost (distal) shell, to a crossed-lamellar texture (orange rectangle) measured

at the inner (proximal) shell. We see well in the pole figures the formation of crystal orientation maxima/clusters, in particular for the a- and b-axes poles, when progressing towards proximal shell sections, relative to the broad a- and b-axes orientation distributions of the axial-like texture at distal shell sections (Figure 10). As is visible from the pole figures in Figure 10, the a-, b-, and c-axes orientation maxima are narrowest for the measurement taken at the proximal shell. Laser confocal microscopy images visualize (Figures 10, S4 and S11):

- (i) The crossed-lamellar nature of the crystals at distal shell sections (Figure S11B) and, most importantly.
- (ii) The increase in crossed-lamellar unit size from distal to proximal shell (Figure S4).

Thus, where the a-, b-, and c-axes orientation maxima are broad and the a- and b-orientations scatter on a great circle, *the first-order lamellar units are small*, while where aragonite crystallographic axes orientation maxima are narrow, *the first-order lamellar units are large*. We conclude from the results shown in Figures 11, S4 and S11 that the microstructure and texture motif of the shell proper become modulated by the crystal unit size. When crystal units are small but are quite irregularly assembled in the shell (Figure 2C,E, Figure 10 red star and Figure S11B,E), even though having a crossed-lamellar appearance, an axial-like texture forms. This texture pattern cannot be termed clear-cut axial, as an axial texture is defined to have one sharp crystallographic axis orientation cluster (in general, the c-axis) and the other crystallographic axes orientations scattering on a great circle, perpendicular to the texture axis [33,35]. For the outermost layer of the investigated scaphopod shells, we do not see a clear-cut c-axis orientation cluster, but a very broad crystal orientation distribution. However, when the crystal units are large and are stringently organized in the shell (Figure S4), for the investigated shells a clear-cut crossed-lamellar texture is developed (pole figures in Figure 10, orange star and in Figure S11F). This crystal size/structure–crystal texture dependency has not been shown yet for another biological Ca-carbonate hard tissue. Our observation demonstrates the high structural flexibility of the crossed-lamellar microstructure, which, today, is the dominant microstructure pattern utilized by molluscs for shell construction [24,29,41,42].

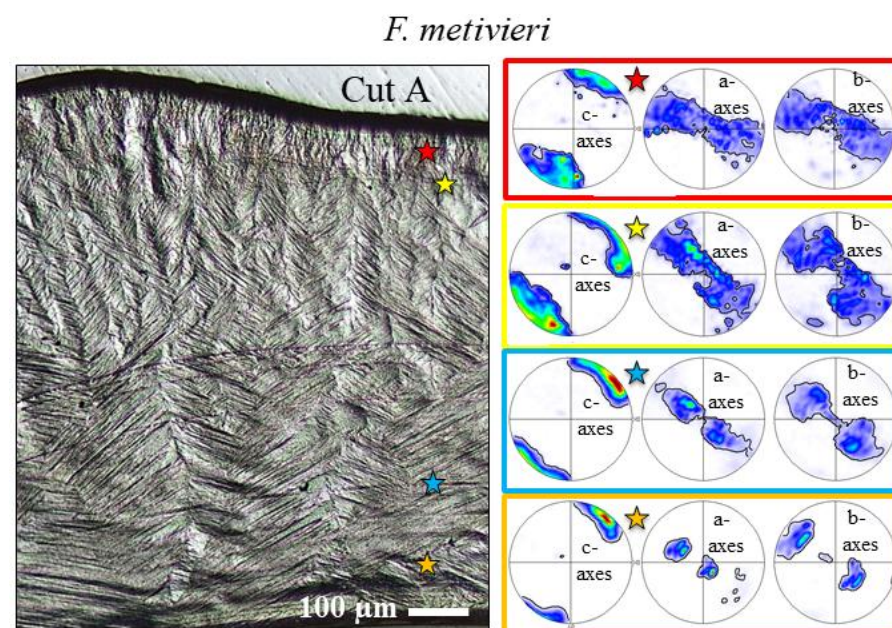


Figure 10. The evolution of aragonite texture on a shell cross-section through the shell extending from the outermost (red star) to inner shell sections (blue and orange stars). The shell was sectioned perpendicular to its long axis, Cut A. **(Left):** laser confocal measurement image with the position of

the EBSD scans indicated by differently colored stars. The corresponding pole figures on the (right) visualize the texture patterns that are present for the different shell sections. In particular, the transformation from an axial-like texture at the outermost, distal, shell section to a crossed-lamellar texture for the rest of the shell, in particular for inner shell sections (regions indicated with orange and blue stars in the laser confocal microscope image). The large scatter in a-, b-, and c-axes orientation along the outermost shell section is well observable, and changes to well-defined maxima for a-, b-, and c-axes orientations at inner shell sections.

The investigated scaphopod shells comprise many growth lines (Figures 1, 3 and S1–S3). Surprisingly, growth-line distribution appears to be structured in the shell, such that at the outermost, outer and central shell sections we observe only a few growth lines, while at inner shell portions, close to the soft tissue of the animal, we find an increased concentration of densely spaced growth lines (Figures 1C,D and S1). The disparate distribution of growth lines led to the suggestion of previous works that the scaphopod shell consists of two layers, an outer and an inner shell layer (Figures 1C and S1). Our results show that growth-line formation does not (or, at least, not markedly) influence the crystal organization of the first-order lamella (yellow arrows in Figures 3 and S5). Nonetheless, we find that at the position of a growth line, crystallite sizes are slightly enlarged and become rather prismatic in shape, in contrast to the laths and sheets that form the first-order lamellae.

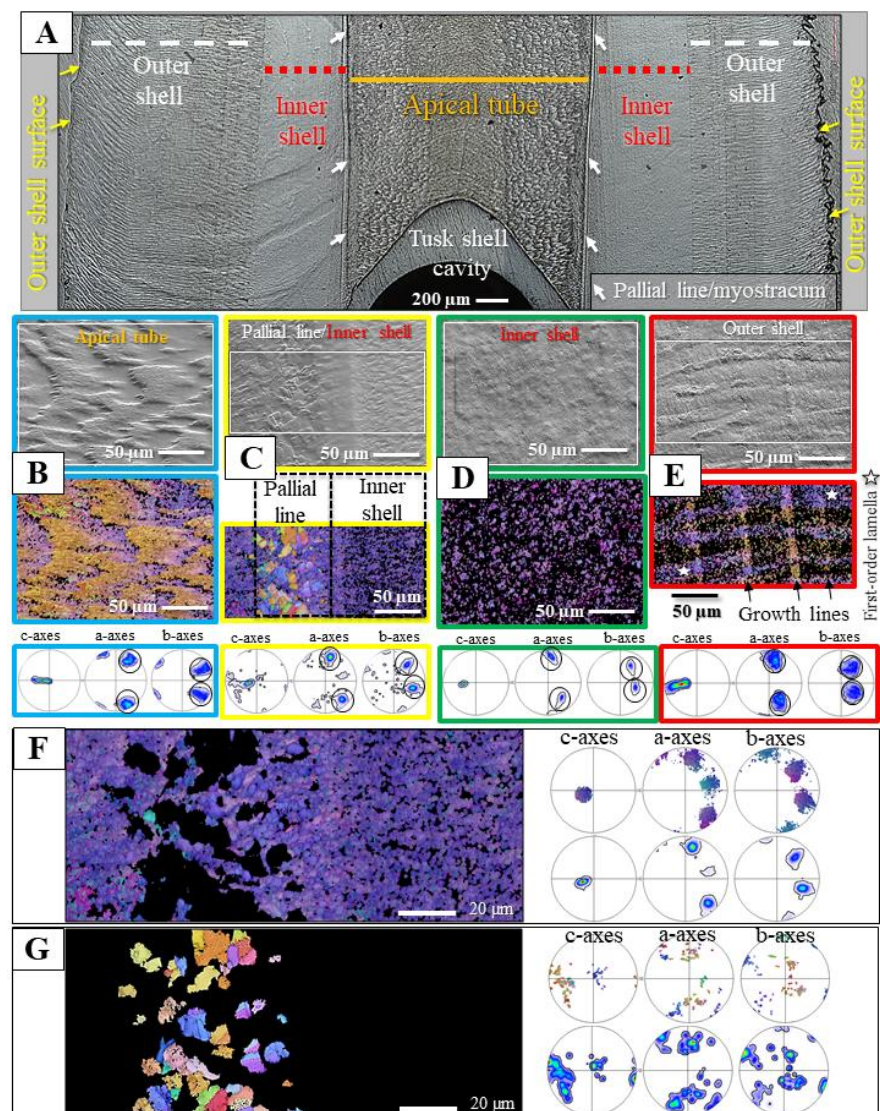


Figure 11. The evolution of aragonite texture on a cross-section through the shell (A) ranging from the apical tube (B), the pallial line (C) via the inner (D) to the outer (E) shell section. We see strong similarity in the texture pattern. The basic texture for all these regions is the crossed-lamellar texture; however, the latter is here modulated by the degree of misorientation between the crystals. We find for the inner shell strongly clustered a-, b-, and c-orientation maxima in the pole figures (C,D), while for the apical tube and the outer shell we see the a-, b-, and c-orientation maxima as well; however, significantly less clustered, relative to the a-, b-, and c-orientation maxima of the inner shell. (C) Note that the crossed-lamellar texture pattern of the inner shell is overlain by the texture of the misoriented crystal assembly of the pallial line. (F) The microstructure and texture of the crossed-lamellar inner shell (right in the EBSD map) and of the apical tube (left in the EBSD map). (G) Microstructure and texture of the pallial line.

4.2. The Apical Tube

The scaphopod mantle and shell are open at their two ends (e.g., [15]). At the orifice of the frontal aperture, the foot and the captacula (the food-catching devices) extend out of the shell. The orifice of the apex facilitates the passage of water through the shell for respiration and for elimination of waste and delivery of gametes [7,14,38,39]. With animal growth, the shell and the orifices have to be enlarged. The frontal aperture becomes steadily enlarged at secretion of the bulk shell. The apex is enlarged through resorption and decollation (Figures 2 and 3 in Lamprell and Healy [14]). However, for apex orifice regeneration, additional hard tissue is secreted (Figure 3 in Lamprell and Healy [14]), often termed secondary secretion. This hard tissue has the form of a tube, termed the apical tube (Figure 2 in Lamprell and Healy [14]). Apical tube material lines the apical shell internally (Figure 3 in Lamprell and Healy [14]) and can extend out from the orifice of the apex (Figure 2 in Lamprell and Healy [14]).

The microstructure and texture of the apical tube are described in this study for the first time. Apical tube microstructure is most interesting (Figures 6 and S8); it is divergent from the microstructure of the shell proper (Figures 2–4) and from the microstructure of the muscle scars (Figures 8 and 9). The apical tube crystals interlink closely with the crystals of the inner shell (Figure 11A). They are not lath- and sheet-shaped, as are the crystals of the crossed-lamellar shell proper, but are rather dendritic (Figure 6B,F). The dendrites appear to consist of granules. Nonetheless, irrespective of this specific microstructure pattern, the texture of the apical tube crystals is crossed-lamellar (see pole figures in Figure 6). Thus, we observe that a crossed-lamellar texture can be formed without a clear-cut crossed-lamellar microstructure and a clear-cut, typical crossed-lamellar crystal/crystallite shape. We measured several times that the shell proper has a crossed-lamellar microstructure and a crossed-lamellar texture. The apical tube forms subsequent to shell formation, subsequent to apex orifice resorption at apex orifice rim regeneration. As shown in Figure 11B–E, the crossed-lamellar microstructure of the shell proper is not transmitted to the crystals of the apical tube, but the crossed-lamellar texture is. As it is well observable in Figure 11B–F (see black circles in the pole figures), we find a similar texture pattern for the outer and inner shell layers, and for the apical tube, only the texture pattern of the pallial line is different (Figure 11F,G). Thus, at the formation of these different shell sections, the crystal texture is kept, and the crystal microstructure is varied.

The attachment of the mantle to the inner shell surface is well observable, due to the specific microstructure of the muscle attachment scar and the pallial line (Figures 9B, S7 and S8). Whenever we imaged the shell proper and the apical tube, we always found the pallial line between these two hard tissue structures, and the apical tube was always running proximal to the pallial line (e.g., Figure S8). This is a clear indication that the apical tube is secreted subsequent to shell proper formation.

4.3. The Muscle Attachment Sites

With this study, we are able to characterize for dentaliid shells the microstructure and texture of two muscle attachment scars. These scars are located intermittently between the shell proper and the muscular system of the foot. Both scars consist of aragonite; one scar forms when the secreting mantle attaches to the shell, the other scar forms when the longitudinal muscle of the foot hydrostat system attaches to the shell [43,44], always to the pallial line (Figures 7 and S9).

All movement of soft and hard tissue requires the action of muscles, and these are part of muscular systems [45–47]. The arrangement and function of muscles in a muscular system are directly related to the skeletal support system of the organism [46]. Vertebrates and invertebrates developed two skeletal support systems and, related to the latter, utilize two different muscular systems [46,47]. One muscular system uses *mineralized tissue as skeletal support*. The associated musculature is arranged in distinct bundles, consisting of striated, oblique or smooth muscles and uses different muscle types for different hard tissue movements [45–47]. Representatives of the latter muscular system are the adductor muscles of bivalved marine organisms ([41,42,48,49] and references therein).

The other muscular system uses a so-called *hydrostatic skeleton* for support [46,47,50,51]; the associated muscular system is termed muscular hydrostat [47]. A muscular hydrostat does not use mineralized tissue as skeletal support; it consists solely of soft tissue, but with a densely packed aggregation of various muscles. A muscular hydrostat system functions as an actuator for movement, but also as skeletal support, even though it does not contain any mineralized tissue. The movements of muscular hydrostat systems rely on the incompressibility of internal fluids for: (i) maintaining the overall volume of the hydrostatic skeleton and (ii) transmitting forces. Hydrostatic skeletons contain cavities filled with body fluid. These skeletons remain constant in volume when pressurized or depressurized by muscle action [46], as the localized contraction of the hydrostatic skeleton results in the movement of the fluid from one portion of the hydrostatic skeleton to the other. The latter enables the transmission of force for initiating soft tissue action, such as elongation, shortening, bending, and torsion. Muscular hydrostats are packed with muscles (Figures 3, 5 and 6 in [2]). These are arranged in 2D as sheets and in 3D as arrays of sheets (Figures 16.2 and 16.3 in [15]), but not as bundles [46]. A prominent example of a muscular hydrostat system is the agile mollusc foot ([52] and references therein). Even though the latter consists only of soft tissue, it performs a variety of actions, such as movement, orientation, anchorage, digging, and many more [52].

In essence, the dentaliid foot and captacula are moved by a muscular hydrostat system [15,52] and (references therein), while the movement of the mantle tissue is performed by a muscular system that acts relative to the hard tissue of the shell. Irrespective of the latter, both muscular systems generate well-observable aragonitic scars at the inner surface of the scaphopod shell proper (Figures 7C,D and S9B,C).

The scaphopod mantle is attached to the shell at its apical region [2,44] and adheres to the inner surface of the shell for about two-thirds of its length (this study). The site of mantle attachment to the shell, the pallial myostracum, is in 2D a line. It is easily observable due to the marked difference in microstructure between the shell and the pallial scar (white arrows in Figures 7, 8B,C, and S7). The thickness of the dentaliid scaphopod pallial myostracum is, for adult animals, about 30 µm and is kept constant for almost the entire length of the scar. Pallial myostracal crystals are granular to prismatic in morphology, increase gradually in size towards the inner shell surface, and are oriented with the aragonite c-axis perpendicular to the inner shell surface (Figure 8). These structural attributes are similar to structural characteristics of the crystals that form the pallial myostraca of other molluscs, e.g., that of the bivalves and of the Polyplacophora [41,42,49,53]. The above-described structural characteristics indicate, for the aragonite of the pallial scar, a

different growth process relative to that of the shell proper, namely, crystal growth through growth competition, as described in detail in [33,34,41,42]. However, despite the marked difference in pallial myostracum and shell proper microstructures, the texture patterns of the pallial myostracum and the shell proper are (almost) similar (Figure 9). We find a crossed-lamellar crystal texture for both the shell and the pallial myostracum (Figure 9A,B). Accordingly, the pallial myostracum adopts the texture pattern of the adjacent shell. This finding has been observed multiple times for a large variety of bivalve shells [33]. However, in addition to the crossed-lamellar texture of the shell, we find that an additional crystal orientation develops for the hard tissue of the pallial myostracum (red circles in the pole figures in Figure 9B,C). This finding is reported here for scaphopod shells for the first time, was, however, observed previously for bivalve shells (Figures 8 and 9 in [31]).

The Scaphopoda move two of their organs in and out of the shell: the foot and the food-catching devices [3,15,44]. The scaphopod foot, a thick, muscular, cylindrically shaped entity of soft tissue, is vital for the organism and is used for many tasks. It can be moved far out of the shell (at maximal extent, more than half of the shell length [44]) and is used for burrowing, locomotion, anchoring and orienting the shell within the sediment [3,15]. Extension and retraction of the foot takes place via the body musculature, via the cooperation of longitudinal, circular, radial, and oblique muscles [54–57]. As stated above, we observed, in addition to the attachment scar of the mantle to the shell, a further muscle scar. However, in contrast to the pallial (mantle) scar, the site of the latter is quite localized. On longitudinal cuts through the shell, it coincides with the presence of a hard tissue bulge (red square, red arrows in Figure 7A,B). The bulge is located at apical shell portions and is circumferential along the inner surface of the shell. The bulge is always proximal to the pallial myostracum (Figures 7 and 8) and has also, as the pallial myostracum, a prismatic internal structure (Figures 7, 8 and S9). We also observe, for the bulge as well, relative to the crossed-lamellar texture of the shell proper, an additional crystal orientation as well (Figure 9D).

Based on the above-described structural characteristics, we consider the bulge to be a muscle scar, namely, the muscle scar of the longitudinal dorso-ventral muscle of the scaphopod foot muscular hydrostat system. The foot is a vital organ for the Scaphopoda [2,3,15]. E.g., scaphopod locomotion through the sediment takes place in such a way that first the foot is extracted from the shell and anchored in the sediment at a slightly different position, relative to the position of the shell. Next, the shell is pulled over the extended foot via contraction of the dorso-ventral longitudinal muscles [44], and this movement initiates a change in the position of the animal. Shimek and Steiner, and Reynolds [2,3] report that the longitudinal muscles attach directly to the shell. Our results show that the bulge is proximal to the pallial myostracum. Thus, the longitudinal muscles do not attach directly to the shell proper, but to the pallial myostracum; the crystals of the bulge are intimately connected to the crystals of the pallial myostracum (Figure 9A,B). Only the pallial muscles attach to the inner surface of the shell proper (Figure 11B). It is observed for modern rhynchonellate brachiopods as well that the adductor muscles that open and close the valves cause the formation of hard tissue bulges for muscle attachment sites to the shell. These adductor muscle scars are always present at inner shell surfaces [49].

4.4. Crossed Crystal Assembly Modes of Mollusc Shells

This study presents the first crystallographic characterization of scaphopod shell crystals, which was determined with EBSD measurements. One main advantage of the EBSD technique is the feasibility of an in-depth evaluation of the measured diffraction data. The microstructure and texture pattern of the hard tissue in question are given with

numerical results. These can be combined with numerical results of other structural attributes of the hard tissue in question, e.g., the misorientation pattern between the crystals. As we now have crystal orientation data on scaphopod shells as well, we can assess similarities and differences in a particular microstructure motif used by species for shell formation of different mollusc classes and subclasses, namely the crossed crystal arrangement motif. Crossed crystal arrangement patterns, such as the crossed-lamellar and the crossed-foliated microstructures, are today the most widely used crystal organization patterns for shell fabrication by the Mollusca (e.g., [24] and references therein). As crossed crystal arrangements are present in the shells of many mollusc classes/subclasses, it was proposed that the crossed mode of Ca-carbonate crystal organization evolved in the geological past independently and several times [20,58]. It was also suggested that the crossed-foliated microstructure is the calcitic equivalent of the aragonitic crossed-lamellar microstructure (Bøggild, p. 251 [19]).

As stated above, with the EBSD measurements and evaluation of the diffraction data, we now have the possibility to check the latter suggestions and will discuss subsequently:

1. Similarities/differences in scaphopod, bivalve, gastropod, and Polyplacophora shell aragonite, described as crossed-lamellar (Figures 12–15 and S12–S14, Table 2).
2. A possible analogy between the crossed-lamellar and crossed-foliated crystal arrangement patterns.

First, for definitional purposes, we detail the nature of the crossed-lamellar crystal organization pattern. We describe the latter for the shell of the aragonitic bivalve *Glycymeris glycymeris* (Linnaeus, 1758). We imaged the crystals that form the crossed-lamellar microstructure on three hierarchical levels (Figure 12A,D,E) and measured their organization with EBSD on two hierarchical levels (Figure 12B–G).

The aragonitic crossed-lamellar crystal arrangement is a hierarchical assemblage of polysynthetically twinned, nanometer-sized laths. The third-order lamellar laths are the basic crystal units of the crossed-lamellar microstructure (red stars in Figure 12F). At a higher hierarchical level, the laths form second-order lamellar sheets (yellow stars in Figure 12D,F). The laths in the sheet have similar c-axis orientations but different a- and b-axes orientations (white and blue arrows in Figure 12D and EBSD measurement in Figure 12E). The difference in a- and b-axes orientation of the laths in a sheet is determined by {110}-aragonite twin formation (see pole figures in Figure 12E). The third-order lamellae, the laths, are internally twinned, such that a single lath can have different orientations of the a- and b-axes in twin relationship. The twins are not between the third-order lamellae, but within the third-order lamellae. Hence, the observation of the {110}-twin misorientation, for aragonite a misorientation at 63°/64°, is fundamental for the identification of a crossed-lamellar crystal arrangement pattern. On a further hierarchical level, the second-order sheets arrange into stacks and generate the first-order lamella (white stars in Figure 12A–C,E).

In color in Figure 12E, we show a first-order lamella; the colors code for crystal orientation. This first-order lamella comprises two subunits, defined by different a- and b-axis orientations (one subunit and orientation is shown in yellow, the other subunit/orientation in red (Figure 12E)). The subunits are related to each other by the {110}-aragonite twin law. Accordingly, for one first-order lamella, we observe in the pole figures a very structured distribution of c-, b and a-axes, namely, a correspondence in c-axis orientation distribution (blue circles in the pole figures in Figure 12E) and a structured variation in b- and a-axes orientations, determined by the misorientation of the polysynthetic {110}-aragonite twins. The crossed-lamellar shell microstructure consists of arrays of first-order lamellae (Figures 12A and S12A), with alternating first-order lamellar sets, set1 and set2 (Figures 12A–C and S12B). Lamella set1 and set2 are defined by different c-, b-, and a-axes

orientations (see pole figures in Figure 12G). Accordingly, for an array of first-order lamella, formed of alternating lamella set1 and lamella set2 of a crossed-lamellar microstructure (EBSD map in Figure 12G), we need to see in the pole figures two c-axis orientation maxima, four b-axes, and four a-axes orientation maxima (EBSD map and corresponding pole figures in Figure 12G).

In conclusion, the aforementioned mode of crystal organization of laths, sheets and lamellae, with the orientation of the crystals being controlled by their mode of assembly on the different hierarchical levels and the {110}-aragonite twin law, is the fingerprint of the (classical) crossed-lamellar microstructure and texture of a structural hard tissue. The mode of crystal organization is reflected in the pole figures with the position and number of the c-, b-, and a-axes orientation maxima (Figure 12B,C,F). To state the crystal organization of a structural hard tissue as crossed-lamellar, the latter described crystal orientation maxima need to be seen in the pole figures.

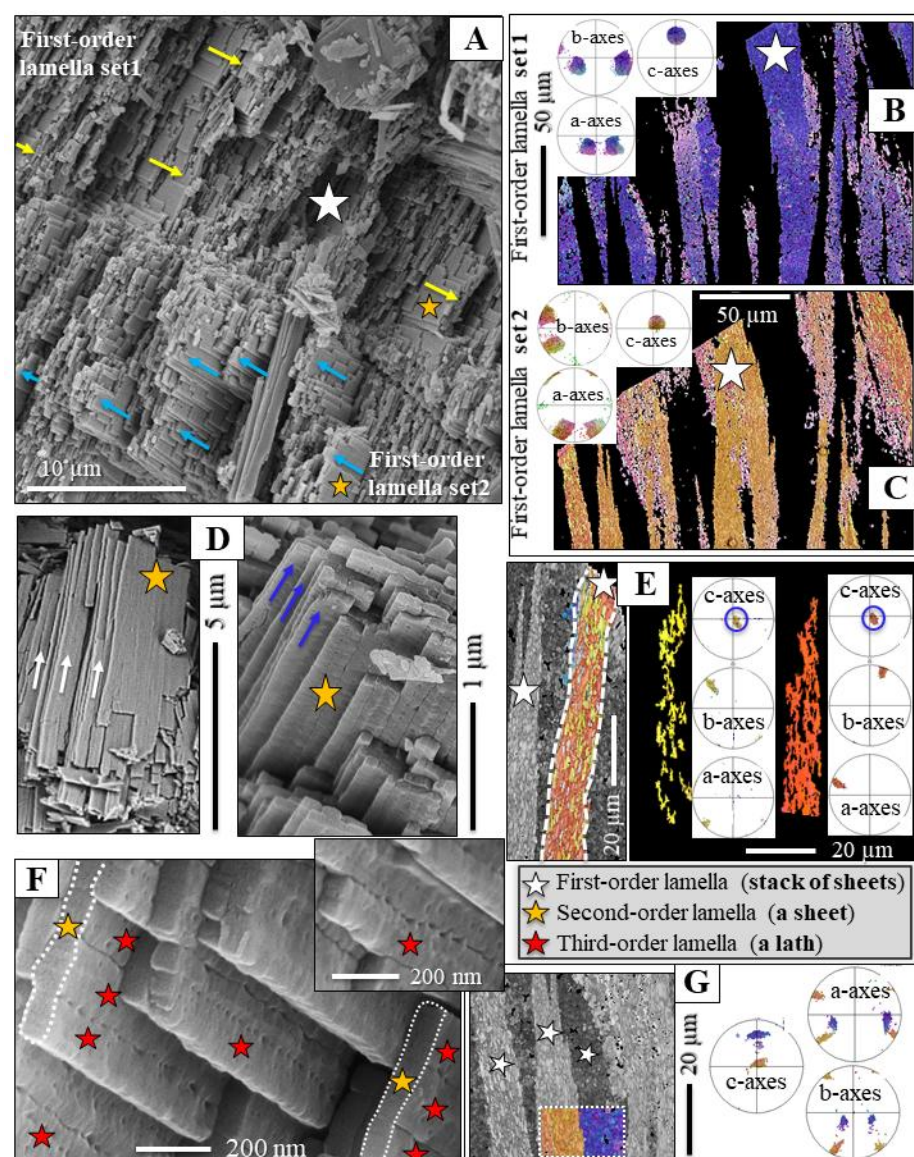


Figure 12. (A–G): Crystal organization in the shell of *Glycymeris glycymeris* (Linnaeus, 1758), the latter formed of crossed-lamellar aragonite. (A,D,F) BSE images of first-order lamella, sets 1 and 2 (A), of the second-order sheets (D), and the third-order laths (F). Indicated by yellow and blue arrows in (A) is the difference in dipping direction of the arrays of second-order sheets in adjacent first-order lamellae. (B,C,E,G) EBSD maps and corresponding pole figures of first-order lamella, set1 and set2 (in blue in (B): first-order lamellar set1; in yellow to red in (C): first-order lamella set2;

the entire measurement is given in Figure S11B). As the color-coded first-order lamella in (E) shows, there is an internal structuring of the latter: we see two main colors; thus, two main crystal orientations. The difference in crystal orientation is defined by a twin relationship between the two crystal assemblies. White and blue arrows in (D) and blue circles in the pole figures in (E) indicate the mode of c-axes orientation of the crystals that form the second-order sheets and the third-order laths. We find for an array of sheets similar c-axes orientations, but different a- and b-orientations (pole figures in (E)). The difference in a- and b-orientations is rooted in the twin relationship within the third-order lamellae. Accordingly, for a crystal assembly with a crossed-lamellar microstructure and texture, formed of adjacent set1 and set2 first-order lamellae (see EBSD crystal orientation map superimposed on the EBSD band contrast map and the pole figures in (G)), we need to find in the pole figures two c-axis orientations, four b-axes, and four a-axis orientations (for each set: one c-axis, two b- and two a-axis orientations). Figure modified after [31,32].

Figures 13, 14, and S13 highlight the lamellar nature of *Fissidentalium metivieri* (Scaphopoda), *Laevicardium oblongum* (Bivalvia), *Glycymeris pilosa* (Bivalvia), *Tanea lineata* (Gastropoda), *Rhysoplax olivacea* (Polyplacophora), *Helcion concolor* (Patellogastropoda) and *Patella piperata* (Patellogastropoda) shells. We show for the shells of these species the microstructures (EBSD maps), the textures (pole figures) (Figures 13 and S13) and the misorientation pattern between the crystals, as measured with the EBSD scan (Figure 14). We give the misorientation (i) within a lamella as well as (ii) between adjacent lamellae.

When entire EBSD scans are regarded, comprising set 1 and set 2 first-order lamellae (left in Figure 13), then we find for *F. metivieri* (Scaphopoda), *L. oblongum*, and *G. pilosa* (Bivalvia) two orientation maxima for the c-axes and four orientation maxima for the b- and the a-axes, respectively. Thus, as required by the classical crossed-lamellar crystal arrangement pattern, for individual first-order lamellae (right in Figure 13), we find one c-axis orientation maximum and two b- and two a-axes maxima and see from the pole figures that the a-, b-, and c-axes orientations between and within the first-order lamellae are determined by the {110}-aragonite twin law (best observable with the pole figures shown for *G. pilosa*, Figure 13C). Thus, for the scaphopod and the bivalve species, we see for shell aragonite a crossed-lamellar crystal organization motif; however, inherent to the species of the different mollusc classes, also some structural modulation of the overall mode of crystal organization.

T. lineata (Gastropoda) and *R. olivacea* (Polyplacophora) are special cases. For entire EBSD scans taken on *T. lineata* shells, we see only one orientation maximum for the a-axes and only two orientation maxima for the b- and c-axes (left in Figures 13D and S13). For individual set 1 and set 2 first-order lamellae of *T. lineata*, we find for the c-, b- and a-axes only one orientation maximum (right in Figure 13D and S13). However, as shown in the misorientation angle diagram of Figure 15D, we observe, for *T. lineata*, the 63°/64°, twin-related, misorientation peak. For entire EBSD scans of *R. olivacea* shells, we find for the c-, b-, and a-axes orientation distributions only one orientation maximum per axis (Figure 13E). The maxima are substructured and comprise submaxima. Well observable is the latter for the c- and a-axes (black arrows in Figure 13E). *R. olivacea* shell aragonite is not twinned; there is no 63°/64° misorientation peak in the relative frequency–misorientation angle diagram (Figure 15E).

Regarding c-axes maxima-overlap for *F. metivieri*, *L. oblongum*, *G. pilosa*, and *T. lineata* (black arrows in the c-axes pole figures of Figure 13 and Table 2), we find significant variation in c-axes maxima-overlap. For example, the two c-axis orientation maxima of *T. lineata* are in very close contact (Figure 13D), while the misorientation between the c-axis orientation maxima of *G. pilosa* is about 60° to 70° (Figure 13C, Table 2).

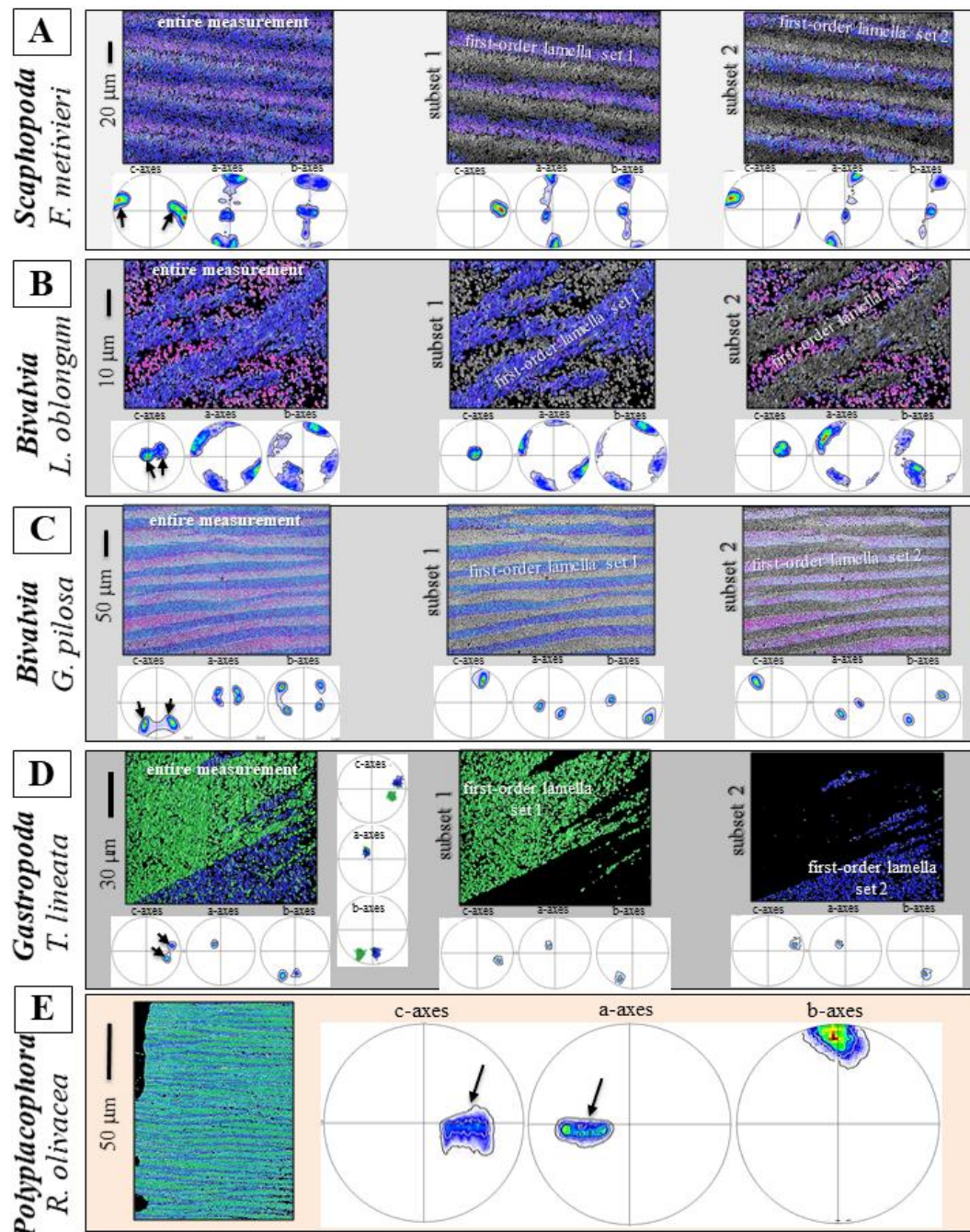


Figure 13. EBSD maps, color-coded for crystal orientation, visualizing the lamellar pattern of scaphopod, bivalve, gastropod and Polyplacophora shells. For the scaphopod and bivalve species, we observe a well-defined crossed-lamellar microstructure and texture, consisting of arrays of first-order lamella sets, set 1 and set 2. We find the related maxima of c-, b-, and a-axis orientation distributions in the corresponding pole figures; for each crossed-lamellar set, one c-axis, two b- and two a-axis orientation maxima. In the case of the gastropod shell, we see for each lamella one c-axis, one b-axis, and one a-axis orientation maximum (this figure and Figure S13). For the polyplacophora shell, we find for the entire measurement one c-axis, one b-axis, and one a-axis orientation maximum; however, we can observe that the c- and a-axes maxima are substructured (black arrows in Figure 13E).

Figure 14A gives the corresponding misorientation pattern for the *F. metivieri*, *L. oblongum*, *G. pilosa*, *T. lineata*, and *R. olivacea* shell microstructures. We show point-to-point misorientations versus distance diagrams across adjacent lamellae (trajectories A to B) and

point-to-point misorientations versus distance diagrams within a first-order lamella, parallel to the long axis of the lamella (trajectories C to D). We see for the scaphopod, bivalve, and gastropod shells:

- (i) Difference in internal structuring within individual first-order lamellae (trajectories A to B).
- (ii) The twinned nature of the aragonite, as the variation in misorientation is between 1° and 65° (trajectories C to D; best observable for *G. pilosa*, *T. lineata*).
- (iii) A slightly increased degree of misorientation for the shells of *F. metivieri* and *L. oblongum*, relative to the degree of misorientation of *G. pilosa* and *T. lineata* shells. Misorientation across lamellae and in a single lamella for *F. metivieri* and *L. oblongum* scatters between 1° and about 90° . Misorientation across lamellae and in a single lamella for *G. pilosa* and *T. lineata* scatters between 1° and about 65° .

Comparing the misorientation diagrams of *F. metivieri*, *L. oblongum*, *G. pilosa* and *T. lineata* to the misorientation diagrams of *R. olivacea*, we find strong differences between the misorientations for *R. olivacea* shell crystals and the misorientations for *F. metivieri*, *L. oblongum*, *G. pilosa*, and *T. lineata* shell crystals (Figure 14). Irrespective of whether the trajectories traverse adjacent lamellae (trajectory A to B) or run within a lamella (trajectories C to D), the degree of misorientation between crystals for *R. olivacea* scatters between 1° and about 20° (Figure 14). In particular, we did not observe a peak in the misorientation angle diagram of *R. olivacea* around 64° . The latter would indicate aragonite {110}-twin-formation, and this is, for *R. olivacea*, never the case.

Figure 14B highlights structural characteristics of the crossed-foliated calcitic shell of the patellogastropods *H. concolor* and *P. piperata*. The shells of these species, as well, have a lamellar microstructure (see EBSD maps in Figure 14B); however, they have a significantly different crystal texture relative to the texture pattern of the shells of the so far discussed aragonitic mollusc species (Figures 13 and S14). We find, for *H. concolor* and *P. piperata*, as well as orientation maxima in the pole figures (Figures 14B and S14B); however, for each lamella, one c-axis orientation maximum and three a*-axes orientation maxima. Regarding the misorientations, we observe a significant difference between the shell of the patellogastropods and the shell of the Polyplacophora, the scaphopod, bivalve and gastropod species. Misorientation between adjacent patellogastropod lamellae is 90° to 95° ; misorientation within a lamella is between 1° and $5^\circ/6^\circ$ (Figure 14B). For the patellogastropod shells, we did not observe a twin-related misorientation. The shells consist of calcite; the calcite twin-related misorientation peak is at 60° , and a peak at 60° misorientation is not observed in the misorientation angle diagrams (Figure 15F,G).

Figure 15 shows relative frequency versus misorientation angle diagrams for *F. metivieri*, *L. oblongum*, *G. pilosa*, *T. lineata*, *R. olivacea*, *H. concolor*, and *P. piperata*. With Figure 15A–G, we give, for each of the above-discussed species, two misorientation angle graphs (shown in red and black) and find, with the exception of *T. lineata* (Figure 15D), a very good correspondence between the two misorientation angle graphs. However, when we compare the misorientation angle distributions of *F. metivieri*, *L. oblongum*, *G. pilosa*, *T. lineata*, *R. olivacea*, *H. concolor*, and *P. piperata*, we see differences in relative frequency of:

- (i) The small-angle misorientations.
- (ii) The $63^\circ/64^\circ$ misorientation.
- (iii) The overall range of the misorientation angle.

Comparing, e.g., (i) the misorientation angle distribution of *R. olivacea* (Figure 15E) to that of the scaphopod, bivalve, and gastropod species (Figure 15A–D) and (ii) to the misorientation angle distribution of *H. concolor* and *P. piperata* (Figure 15F,G), we find immense differences. For *R. olivacea*, *H. concolor* and *P. piperata*, we find only small-angle misorientations; the latter for *R. olivacea* with a very low relative frequency (Figure 15E),

while, for *H. concolor* and *P. piperata*, however, with very high relative frequencies (Figure 15F,G), in contrast to the frequencies of *F. metivieri*, *L. oblongum*, *G. pilosa* and *T. lineata* (Figure 15A–D).

Figure 15H–J summarizes the relative frequencies of the misorientation angles for the discussed species (aragonitic and calcitic). We see:

- (i) A very high peak in small-angle misorientation for the calcitic species (Figure 15I).
- (ii) A very broad distribution of small-angle misorientations (between 5° and 30°) for the aragonitic shell of *R. olivacea* (Figure 15H,I).
- (iii) A well-developed peak at 63°/64° misorientation, indicating the twinned nature of the aragonite of *F. metivieri*, *L. oblongum*, *G. pilosa* and *T. lineata* (Figure 15H,J).

In essence, the main findings of the juxtaposition of structural–crystallographic attributes between *F. metivieri* (Scaphopoda), *L. oblongum* (Bivalvia), *G. pilosa* (Bivalvia), *T. lineata* (Gastropoda), *R. olivacea* (Polyplacophora), *H. concolor* (Patellogastropoda) and *P. piperata* (Patellogastropoda) are (Table 2, Figures 13–15, S13 and S14):

- (i) The aragonite of *R. olivacea* is assembled with a crossed arrangement of crystals. However, the crossed arrangement of *R. olivacea* is not similar to the crossed-lamellar arrangement of crystals that we find for the conchiferan shells (bivalves, gastropods, scaphopods), irrespective of the lamellar microstructure of the hard tissue, the shell being aragonitic, and of previous reports (e.g., Haas [25]).
- (ii) The nature of the crossed-lamellar and the crossed-foliated crystal arrangements is distinct, not only in the Ca-carbonate phase but also in crystal organization, irrespective of the lamellar structure of the hard tissues and of previous reports (e.g., Bøggild [19]).
- (iii) It was suggested that the crossed-lamellar microstructure and texture were invented several times for invertebrate shell formation (e.g., Taylor et al., Wilmot [20,22]). Our study shows that the crossed-lamellar microstructure evolved three times, namely for scaphopod, bivalve and gastropod shell formation. It is now clear that the three groups evolved their crossed-lamellar materials independently. We see the overall crossed-lamellar crystal organization motif modulated and being specific for scaphopod, bivalve and gastropod shells. In addition, their common ancestors (helcioneloids) never had hard tissues with a crossed-lamellar crystal arrangement pattern. Hence, the crossed-lamellar microstructure appeared for hard tissue formation later, when the lineages were already established.

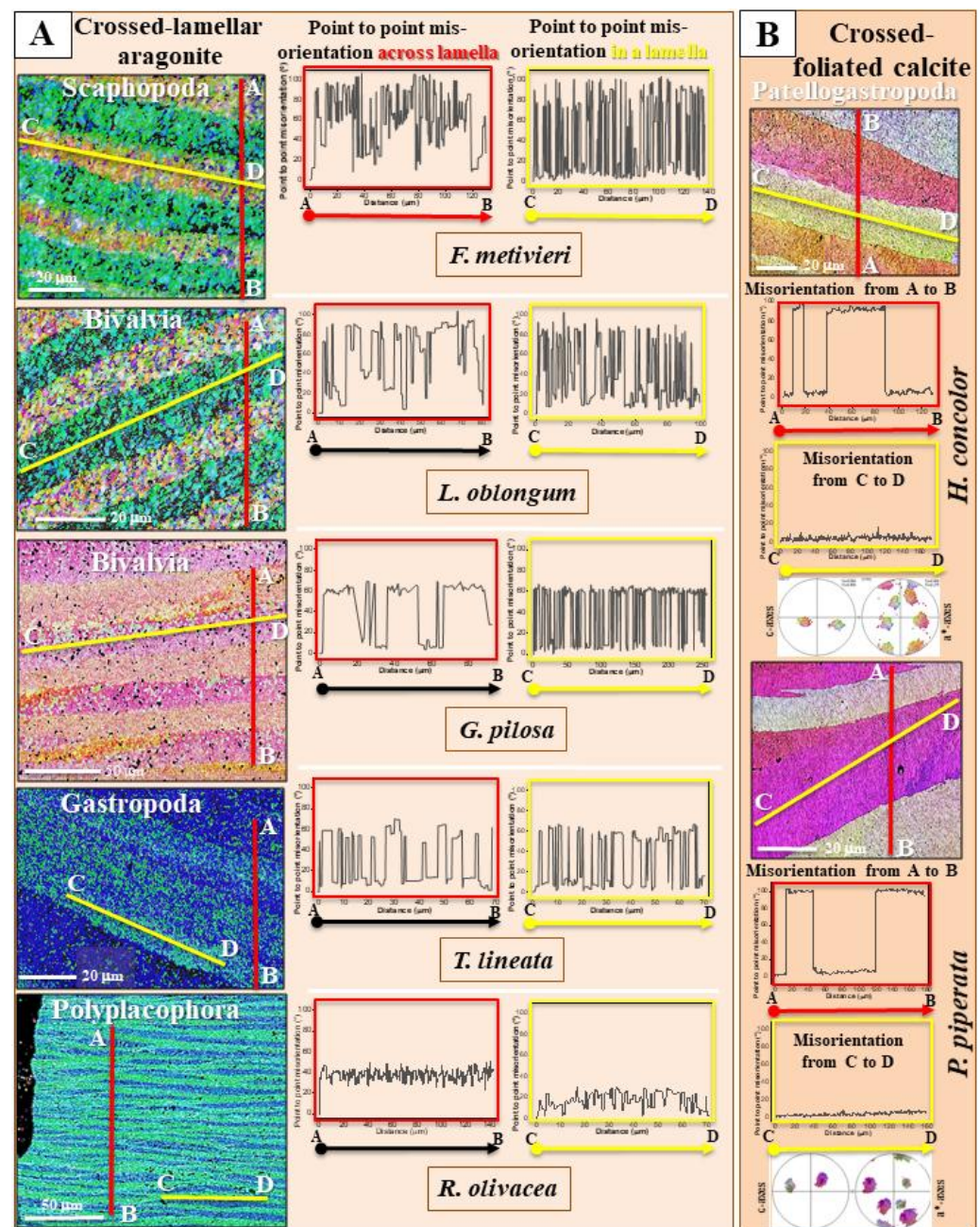


Figure 14. Crystallographic–structural differences/similarities between crystal arrangements of scaphopod, bivalve, gastropod, Polyplacophora, and patellogastropod shells (A,B). Previous work reports a crossed-lamellar mode of crystal orientation for the aragonitic shells and a crossed-foliated mode of crystal orientation for the calcitic shells. It was suggested that the crystal arrangements of the crossed-lamellar and crossed-foliated microstructures are equivalent. We do find laminar microstructures for the shells of the Scaphopoda, Bivalvia, Gastropoda, Polyplacophora, and Patellogastropoda (EBSD maps in (A,B)); however, the textures (Figures 13 and 14B) and the point-to-point misorientations versus distance diagrams (Figure 14) demonstrate that there is significant variation in crystal organization for these laminated microstructures. We find comparability of crystallographic–structural attributes for the shells of *F. metivieri*, *L. oblongum*, *G. pilosa*, and, to some extent, of *T. lineata*. However, we find a big difference in crystallographic–structural attributes of the latter shells and the shells of *R. olivacea*, *P. piperata* and *H. concolor*.

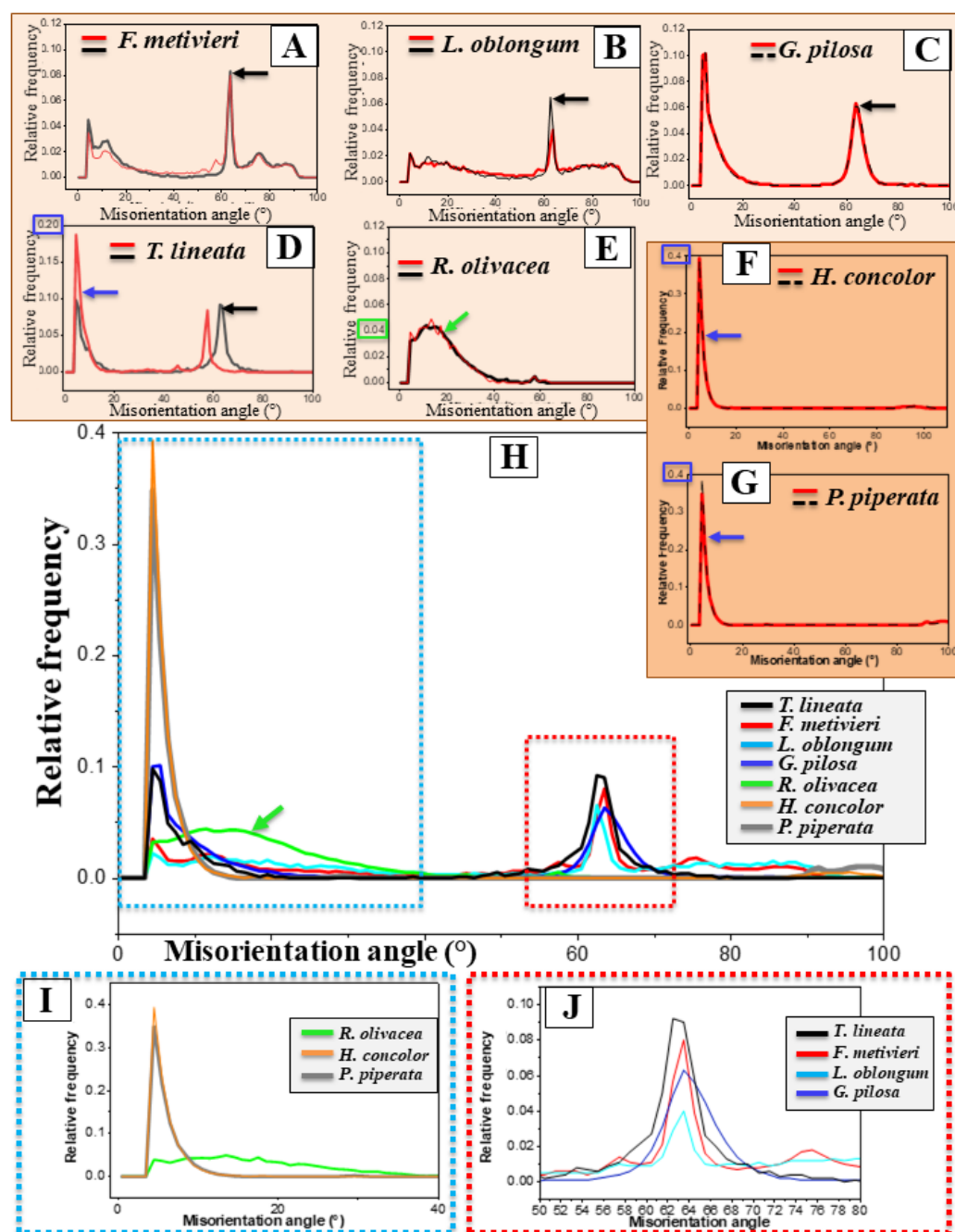


Figure 15. Relative frequency–misorientation angle distributions in Figures 13 and 14 for the discussed mollusc species (A–J). We show differences in small angles ($<10^\circ$) and the $63^\circ/64^\circ$, the aragonite twin-related misorientations. (A–G) Two relative frequency–misorientation diagrams per investigated mollusc species, performed, for each species, on two different shells. Note the very good correspondence of these diagrams per species, with the exception of the diagrams for *T. lineata* (Figure 15D). We observe for *P. piperata* and *H. concolor* (F–H) a huge peak of small-angle misorientation and absence of the twin-related misorientation peak (for calcite, the 60° misorientation). Scaphopod, gastropod and bivalve aragonite is twinned (H,J); polyplacophoran plate aragonite is not twinned (H,I).

Table 2. Summary of Ca-carbonate phase and crystallographic–structural attributes that we obtained for the shells investigated in this study.

	Ca-Carbonate Phase	Laminar Micro-Structure	Clusters in c-Axes Pole Figures	Misorientation Between c-Axes Clusters (°)	Clusters in a-, b- and a*-Axes Pole Figures	Misorientation in a First-Order Lamina (°)	Small Angle Misorientations (°)	Twin-Related Misorientations (°)
Scaphopoda	aragonite	yes	2	40–45	4	90	very low	63–64°
Bivalvia	aragonite	yes	2	60–70	4	60–70	very low to low	63–64°
	aragonite	yes	2	40–45	4	70	very low	63–64°
Gastropoda	aragonite	Yes	2	10–15	4	50–65	low	63–64°
Poly-placophora	aragonite	yes	1	--	1	20–25	very low	absent
Patello-gastropoda	calcite	yes	2	90	6	5–10	very high	absent

5. Conclusions

The Scaphopoda are marine infaunal animals that encase their soft body parts with a continuous, tusk-shaped shell.

We addressed in this contribution the different sections of the *Antalis weinkauffi* and *Fissidentalium metivieri* (dentaliid Scaphopoda) shells and characterized their internal structure, crystal microstructure and crystal texture using EBSD and light and electron microscopes. As crystallographic data are now available for shell crystals of representatives of all mollusc classes and subclasses using crossed crystal arrangements for shell formation, we juxtapose and discuss crystallographic attributes of the different crossed crystal arrangement patterns.

We draw the following conclusions from our study:

1. The scaphopod shell proper, the apical tube, and the muscle attachment scars consist of aragonite. The crystals are arranged in these shell portions with different microstructures.
2. The shell proper has a crossed-lamellar microstructure. From distal to proximal, there is a strong increase in the crossed-lamellar unit size. Distal shell sections are seamed by the smallest crossed-lamellar units. The largest crossed-lamellar units are present at proximal shell sections. The change in crossed-lamellar unit size is smooth.
3. There is an unbalanced distribution of growth lines within the shells. At the outermost, outer, and central shell portions, growth lines are widely spaced. At inner/proximal shell sections, shell portions adjacent to the soft tissue, growth lines are densely spaced.
4. The shell proper has two crystal textures. Most of the shell has a clear-cut crossed-lamellar texture. The seam of small crossed-lamellar crystals at distal shell sections has an axial-like texture. The difference in crystal texture arises, most probably, from the utilization of differently sized crossed-lamellar crystals. We observe an axial-like texture at shell sections with small, less stringently co-oriented crystals, in contrast to shell portions comprising large crossed-lamellar crystals, where the crystal units and crystallites are highly co-oriented.
5. The Dentaliida secrete upon widening the apical orifice a pipe-shaped structure, the apical tube. The crystals of the apical tube consist of aragonite and connect seamlessly with the crystals of the inner shell proper. Apical tube crystals have a dendritic to prismatic microstructure and a crossed-lamellar crystal texture.

6. We find at the inner shell surface the scars of two muscle systems: (i) the scar of the pallial myostracum and (ii) the scar of the pedal myostracum of the muscular hydrostat system of the foot. The pallial myostracum has a prismatic microstructure and a crossed-lamellar-like texture. Pallial myostracum crystals show a gradual increase in prism size towards the inner shell surface and orientation of aragonite c-axes normal to the inner shell surface. These structural characteristics indicate a competitive growth-related process for pallial myostracum crystal formation. The longitudinal muscles of the scaphopod foot attach to an aragonite bulge that is proximal to the pallial myostracum. The bulge is circumferential along the inner surface of the shell and protrudes into the shell cavity. It is not connected to the apical tube. Bulge aragonite has a lamellar to prismatic microstructure and a crossed-lamellar texture. The texture pattern of the inner shell is transmitted to the adjacent muscle scars that form when the muscles attach to the inner surface of the shell. However, we observe, for the scars, formation of a further crystal orientation pattern, in addition to the crossed-lamellar crystal orientation of the inner shell.
7. The crossed (crossed-lamellar and crossed-foliated) mode of crystal organization is today the dominating microstructure and texture pattern of Ca-carbonate mollusc shells. The crossed-lamellar as well as the crossed-foliated crystal arrangements have a lamellar microstructure. Nonetheless, our study shows that there is similarity in crystallographic attributes of the crossed-lamellar microstructure and texture between scaphopod and bivalve shells, and only, in part, between gastropod shells.
8. Even though being lamellar in structure, polyplacophoran shell aragonite and patello-gastropod shell calcite are distinct in microstructure and texture from: (i) the crossed-lamellar crystal arrangement of scaphopods and bivalves and (ii) each other. Hence, even though based on lamellar microstructures, there is no resemblance in crystallographic attributes between the crossed-lamellar shell crystal arrangement of the Polyplacophora and the crossed-foliated shell crystal arrangement of the Patello-gastropoda. The biologically secreted crossed-lamellar crystal organization is not analogous to the biologically secreted crossed-foliated crystal arrangement.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/cryst16080513/s1>. Figure S1: Laser confocal microscope image depicting the microstructure of *F. metivieri* shell cut longitudinally, cut parallel to the long axis of the shell. Note the difference in internal structure between the outermost, outer and inner shell sections, respectively. Figure S2: Microstructure and texture of the outer and inner shell layer of *A. weinkauffi*. The cut is perpendicular to the long axis of the tusk shell, Cut A. Well-observable from the EBSD map and the corresponding pole figures is the crossed-lamellar mode of crystal assembly. A: SE micrograph, B, C: EBSD maps and corresponding pole figures, visualizing patterns of shell crystal organization. The colors in B and C code for crystal orientation. Figure S3: Microstructure and texture outer shell layer of *A. weinkauffi*. The shell was sectioned longitudinally, Cut B. In this mode of sectioning as well, the crossed-lamellar assembly of the shell crystals is also well-visible. Figure S4: Laser confocal microscope images of a shell cross-section of *F. metivieri* and *A. weinkauffi*, Cut A. For both investigated species we observe an increase in crossed-lamellar unit size from distal to proximal shell surfaces. Figure S5: The microstructure pattern that prevails at a growth line. High-resolution EBSD measurements visualize larger-sized crystals at the sites of growth lines, relative to the size of the crystals that form the shell. Figure S6: Laser confocal micrograph depicting a cross-sectional cut through the shell of *F. metivieri*. The cut was taken at apical position. Well observable is the pallial line (white arrows) and the position of the apical tube (white stars). It is, also, well observable that the pallial line and the apical tube are circumferential along the inner surface of the shell. The dashed rectangle indicates the position of micrographs shown in Figure S7. Figure S7: Laser confocal micrographs of a cross-sectional cut through the shell and the apical tube of *F.*

metivieri. The position of the micrographs on the shell cross-section is indicated with a rectangle in Figure S8. Well visible is the assembly of prisms and the typical structure of the pallial myostracum (white arrows) and the difference in internal structure of the growth lines (yellow arrows in both micrographs) and the pallial line (white arrows in both micrographs). Figure S8: Laser confocal micrographs of tusk shell and apical tube cross-sections of *F. metivieri* (A, B). The cut for the images shown in the figure is perpendicular to the long axis of the tusk shell, Cut A. Well-observable is the course of the pallial myostracum (white arrows in A and B). B: microstructure (EBSD map) and texture (pole figures) of the apical tube crystals. While for the tusk shell we find elongated crystals in crossed-lamellar arrangement (A), for the apical tube we find an irregular internal structure, dominated by elongated, granular to vaguely prism-shaped crystals (image of the apical tube in A and EBSD map in B), assembled in the shell without a clear-cut arrangement pattern. Figure S9: The internal structure of the bulge, of the longitudinal pedal muscle attachment scar of *F. metivieri*. A: the internal thickening (red and blue arrows) of the shell close to the apex. B to D: size, shape and organization of aragonite crystals that comprise the bulge (blue stars in A to D). Well-observable is the prismatic shape of the bulge crystals (B, C, D), their difference in shape to the crystals of the shell proper (C), their slight increase in size towards proximal shell surface (B) and their position proximal to the pallial myostracum (B, C, D). Note the yellow arrows in C are supposed to highlight the curved nature of the proximal surface of the bulge; the bulge tapers out (A) towards the apex and towards the frontal aperture (D, E and Figure S10). The bulge is not connected to the apical tube (A and Figure 5B,D). A: light microscopy image, B to E: laser confocal microscopy images. Figure S10: The bulge, consisting of aragonite, protruding into the shell cavity (blue and red arrows in A and B) and being present at the convex as well as concave shell section inner surface close to the apex (A, B). The white, red and blue stars in A to E indicate the extent of the bulge and its tapering/thinning out towards apical as well as frontal aperture directions. A, B: light microscopy images, C to E: laser confocal microscope images. Figure S11: Microstructure and texture of the outermost and outer shell section of *F. metivieri* (A to D) and change of the texture pattern from a rather axial texture, prevailing at outermost shell, to a crossed-lamellar texture, present at the inner shell (C to F). A to C: laser confocal microscopy images. D, E: EBSD map and subsets of the latter with the corresponding texture patterns (E). Figure S12: The crossed-lamellar shell of *Glycymeris glycymeris* (Linnaeus, 1758). A: BSE image of a fracture surface depicting four adjacent first-order lamella set 1 and set 2. White arrows indicate the dipping direction of the arrays of second-order sheets, comprising the first-order lamella. B: EBSD-map, color-coded for crystal orientation, visualizing the, more or less, parallel arrangement and alternation of the first-order lamella set1 and set2. Furthermore, well observable is the internal, crystal orientation-related, structuring of individual first-order lamella (e.g., the lamella marked with a white star in B). For further information see also Crippa et al. 2020a, b [31,32]. Figure S13: EBSD map showing the laminated nature of the *T. lineata* shell. Nonetheless, a classical crossed-lamellar crystal texture, as defined for bivalve shells, is not observable for *T. lineata* from the pole figures, we see for the entire scan two c-axes, but only two b-axes and one a-axes maxima, respectively. For individual first-order lamella we find one c-axes, one b-axes and one c-axes maximum. Figure S14: Overview of different crystal textures derived from crystal organizations forming lamellar microstructures. Lamellar microstructures are utilized today for shell formation by species of five different mollusc classes/subclasses: the Scaphopoda, Bivalvia, Gastropoda, Polyplacophora, Patellogastropoda.

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References

- Palmer, C.P. A supraspecific classification of the scaphopod Mollusca. *Veliger* **1974**, *17*, 115–123.
- Shimek, R.L.; Steiner, G. Scaphopoda. In *Microscopic Anatomy of Invertebrates*; Harrison, F.W., Kohn, A.J., Eds.; Wiley-Liss: New York, NY, USA, 1997; Volume 6B, pp. 719–781.
- Reynolds, P.D. The Scaphopoda. *Adv. Mar. Biol.* **2002**, *42*, 137–236.
- Pojeta, J., Jr. Class Scaphopoda. In *Fossil Invertebrates*; Boardman, R.S., Cheetham, A.H., Rowell, A.J., Eds.; Blackwell: Palo Alto, CA, USA, 1987; pp. 380–386.
- Reynolds, P.D.; Okusu, A. Phylogenetic relationships among families of the Scaphopoda (Mollusca). *Zool. J. Linn. Soc.* **1999**, *126*, 131–154.
- Scarabino, V. Aproximación al número de especies de Scaphopoda actuales. *Com. Soc. Malac. Urug.* **1994**, *7*, 357–361.
- Palmer, C.P.; Steiner, G. Class Scaphopoda. In *Microscopic Anatomy of Invertebrates*; Harrison, F.W., Kohn, A.J., Eds.; Wiley-Liss: New York, NY, USA, 1998; Volume 6.
- Yochelson, E.L. Scaphopoda. In *Functional Morphology of the Invertebrate Skeleton*; Savazzi, E., Ed.; Wiley: Chichester, UK, 1999; pp. 363–367.
- Buckland-Nicks, J.; Gibson, G.; Koss, R. Phylum Mollusca: Polyplacophora, Aplacophora, Scaphopoda. In *Atlas of Marine Invertebrate Larvae*; Young, C.M., Sewell, M.A., Rice, M.E., Eds.; Academic Press: San Diego, CA, USA, 2002; pp. 245–259.
- Sigwart, J.D.; Sumner-Rooney, L.H.; Dickey, J.; Carey, N. The scaphopod foot is ventral: More evidence from the anatomy of *Rhabdus rectius* (Carpenter, 1864) (Dentaliida: Rhabdidae). *Molluscan Res.* **2017**, *37*, 79–87.
- de Souza, L.S.; Caetano, C.H.S. Morphometry of the shell in Scaphopoda (Mollusca): A tool for the discrimination of taxa. *J. Mar. Biol. Assoc. U. K.* **2020**, *100*, 1271–1282.
- Song, H.; Wang, Y.; Shao, H.; Li, Z.; Hu, P.; Yap-Chiongco, M.K.; Shi, P.; Zhang, T.; Li, C.; Wang, Y.; et al. Scaphopoda is the sister taxon to Bivalvia: Evidence of ancient incomplete lineage sorting. *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2302361120.
- Knudsen, J. Scaphopoda and Gastropoda from depths exceeding 6000 meters. In *Galathea Report: Scientific Results of the Danish Deep-Sea Expedition Round the World 1950–52*; Wolff, T., Ed.; Scandinavian Science Press: Copenhagen, Denmark, 1964; Volume 7, pp. 125–136.
- Lamprell, K.L.; Healy, J.M. *A Revision of the Scaphopoda from Australian Waters (Mollusca)*; Supplement 24; Records of the Australian Museum: Sydney, Australia, 1998; pp. 1–189.
- Ponder, W.F.; Lindberg, D.R.; Ponder, J.M. *Biology and Evolution of the Mollusca*; CRC Press: Boca Raton, FL, USA, 2019; Volume 1, pp. 1–214.
- Bronn, H.G. *Die Klassen und Ordnungen der Weichthiere (Malacozoa) Wissenschaftlich Dargestellt in Wort und Bild: Kopffragende Weichthiere (Malacozoa Cephalophora)*; CF Winter: Heidelberg, Germany, 1866; Volume 3.
- Smith, A.M.; Spencer, H.G. Skeletal mineralogy of scaphopods: An unusual uniformity. *J. Molluscan Stud.* **2016**, *82*, 344–348.
- Cayeux, L. Introduction à l'étude pétrographique des roches sédimentaires. In *Mémoires Pour Servir à L'explication de la Carte Géologique Détaillée de la France*; Impr. Nationale: Paris, France, 1916; pp. 469–484.
- Bøggild, O.B. *The Shell Structure of the Mollusks*; Naturvidenskabelig og Mathematisk Afdeling: Kobenhavn, Denmark, 1930; pp. 1–132.
- Taylor, J.D. The structural evolution of the bivalve shell. *Palaeontology* **1973**, *16*, 519–534.
- MacClintock, C. Shell Structure of Patelloid and Bellerophonoid Gastropods (Mollusca). *Peabody Mus. Nat. Hist. Yale Univ. Bull.* **1967**, *22*, 1–140.
- Wilmot, N.V.; Barber, D.J.; Taylor, J.D.; Graham, A.L. Electron microscopy of molluscan crossed-lamellar microstructure. *Philos. Trans. R. Soc. Lond. B* **1992**, *337*, 21–35.

23. Fuchigami, T.; Sasaki, T. The shell structure of the recent Patellogastropoda (Mollusca: Gastropoda). *Paleontol. Res.* **2005**, *9*, 143–168.
24. Checa, A.G.; Griesshaber, E.; Salas, C.; Angiolini, L.; Schmahl, W.W. Mollusc and brachiopod carbonate biomineralization. In *Biomineralization and Bioinspired Materials*; Fratzl, P., Liz Marzán, L.M., Kotov, N.A., Eds.; Wiley/Elsevier/Academic Press: Hoboken, NJ, USA, 2026.
25. Haas, W. Untersuchungen über die Mikro- und Ultrastruktur der Polyplacophorenschale. In *Biomineralisation: Forschungsberichte*; Kommission für Biokristallitforschung, Akademie der Wissenschaften und der Literatur: Stuttgart, Germany, 1972; pp. 3–52.
26. Rodriguez-Navarro, A.B.; Checa, A.; Willinger, M.G.; Bolmaro, R.; Bonarski, J. Crystallographic relationships in the crossed lamellar microstructure of the shell of the gastropod *Conus marmoreus*. *Acta Biomater.* **2012**, *8*, 830–835.
27. Almagro, I.; Drzymala, P.; Berent, K.; Sainz-Díaz, C.I.; Willinger, M.G.; Bonarski, J.; Checa, A.G. New crystallographic relationships in biogenic aragonite: The crossed-lamellar microstructures of mollusks. *Cryst. Growth Des.* **2016**, *16*, 2083–2093.
28. Böhm, C.F.; Demmert, B.; Harris, J.; Fey, T.; Marin, F.; Wolf, S.E. Structural commonalities and deviations in the hierarchical organization of crossed-lamellar shells: A case study on the shell of the bivalve *Glycymeris glycymeris*. *J. Mater. Res.* **2016**, *31*, 536–546.
29. Salinas, C.L.; de Obaldia, E.E.; Jeong, C.; Hernandez, J.; Zavattieri, P.; Kisailus, D. Enhanced toughening of the crossed lamellar structure revealed by nanoindentation. *J. Mech. Behav. Biomed. Mater.* **2017**, *76*, 58–68.
30. Agbaje, O.B.A.; Wirth, R.; Morales, L.F.G.; Shirai, K.; Kosnik, M.; Watanabe, T.; Jacob, D.E. Architecture of crossed-lamellar bivalve shells: The southern giant clam (*Tridacna derasa*, Röding, 1798). *R. Soc. Open Sci.* **2017**, *4*, 170622.
31. Crippa, G.; Griesshaber, E.; Checa, A.G.; Harper, E.M.; Simonet Roda, M.; Schmahl, W.W. Orientation patterns of aragonitic crossed-lamellar, fibrous prismatic and myostracal microstructures of modern *Glycymeris* shells. *J. Struct. Biol.* **2020**, *212*, 107653.
32. Crippa, G.; Griesshaber, E.; Checa, A.G.; Harper, E.M.; Roda, M.S.; Schmahl, W.W. SEM, EBSD, laser confocal microscopy and FE-SEM data from modern *Glycymeris* shell layers. *Data Brief* **2020**, *33*, 106547.
33. Hoerl, S. Calcium Carbonate Biomaterials—Architecture, Design and Nanomechanical Properties of Selected Mollusc, Brachiopod and Echinoderm Skeletal Elements. Ph.D. Thesis, LMU Munich, Munich, Germany, 2025.
34. Hoerl, S.; Micheletti, C.; Amini, S.; Griesshaber, E.; Hess, K.U.; Checa, A.G.; Peharda, M.; Schmahl, W.W. Correlation between nanomechanical properties and microstructural design concepts of bivalve muscle attachment sites. *Mater. Des.* **2025**, *253*, 113845.
35. Schwartz, A.J.; Kumar, M.; Adams, B.L.; Field, D.P. (Eds.) *Electron Backscatter Diffraction in Materials Science*, 2nd ed.; Springer: Boston, MA, USA, 2009; pp. 1–395.
36. Peter, N.J.; Griesshaber, E.; Reisecker, C.; Hild, S.; Oliveira, M.V.G.; Schmahl, W.W.; Schneider, A.S. Biocrystal assembly patterns, biopolymer distribution and material property relationships in *Mytilus galloprovincialis*, *Bivalvia*, and *Haliotis glabra*, Gastropoda, shells. *Materialia* **2023**, *28*, 101749.
37. Bandel, K. Übergänge von einfacheren Strukturtypen zur Kreuzlamellenstruktur bei Gastropodenschalen. *Biomim. Forsch. Ber.* **1979**, *10*, 9–38.
38. Shimek, R.I. Shell Morphometrics and Systematics: A Revision. *Veliger* **1989**, *32*, 233–246.
39. Reynolds, P.D. Mantle-mediated shell decollation increases posterior aperture size in *Dentalium rectius* (Scaphopoda: Dentaliida). *Veliger* **1992**, *35*, 26–35.
40. Steiner, G. Larval and juvenile shells of four North Atlantic scaphopod species. *Am. Malacol. Bull.* **1995**, *11*, 87–98.
41. Hoerl, S.; Griesshaber, E.; Checa, A.G.; Schmahl, W.W. The biological crystals in chomid bivalve shells: Diversity in morphology and crystal arrangement pattern. *Crystals* **2024**, *14*, 649.
42. Hoerl, S.; Le Moine, T.; Peter, N.J.; Amini, S.; Griesshaber, E.; Wang, J.; Harper, E.M.; Salas, C.; Checa, A.G.; Schwaiger, R.; et al. Crystal organisation and material properties of *Chama* and *Glycymeris* myostraca and shells. *Materialia* **2024**, *36*, 102149.
43. Steiner, G. Observations on the anatomy of the scaphopod mantle and the description of a new family, the Fustiariidae. *Am. Malacol. Bull.* **1991**, *9*, 1–20.
44. Steiner, G. The organisation of the pedal musculature and its connection to the dorsoventral musculature in Scaphopoda. *J. Molluscan Stud.* **1992**, *58*, 181–197.
45. Chantler, P.D. Biochemical and structural aspects of molluscan muscle. In *The Mollusca*; Elsevier: Amsterdam, The Netherlands, 1983; Volume 4, pp. 77–154.
46. Kier, W.M. The arrangement and function of molluscan muscle. In *The Mollusca, Form and Function*; Trueman, E.R., Clarke, M.R., Eds.; Academic Press: San Diego, CA, USA, 1988; Volume 11, pp. 211–252.

47. Kier, W.M.; Smith, K.K. Tongues, tentacles and trunks: The biomechanics of movement in muscular-hydrostats. *Zool. J. Linn. Soc.* **1985**, *83*, 307–324.
48. Castro-Claros, J.D.; Checa, A.; Lucena, C.; Pearson, J.R.; Salas, C. Shell-adductor muscle attachment and Ca²⁺ transport in the bivalves *Ostrea stentina* and *Anomia ephippium*. *Acta Biomater.* **2021**, *120*, 249–262.
49. Hoerl, S.; Griesshaber, E.; Weller, D.; Amini, S.; Haussermann, V.; Bitner, M.A.; Achterhold, K.; Pfeiffer, F.; Schmahl, W.W. Crystal organisation of muscle attachment sites of bivalved marine organisms: A juxtaposition between brachiopod and bivalved mollusc shells. *Crystals* **2025**, *15*, 649.
50. Chapman, G. The hydrostatic skeleton in the invertebrates. *Biol. Rev.* **1958**, *33*, 338–371.
51. Clark, R.B. *Dynamics in Metazoan Evolution: The Origin of the Coelom and Segments*; Clarendon Press: Oxford, UK, 1964; pp. 1–313.
52. Wanninger, A. Micromorphology and Gene Expression in Muscle and Shell Development of the Mollusca; Ph.D. Thesis, LMU Munich, Munich, Germany, 2001.
53. Griesshaber, E.; Hoerl, S.; Weller, D.; Salas, C.; Checa, A.G.; Schmahl, W.W. The intricate organization of needle- and rod-shaped crystals of chiton shell plates. *Nat. Sci. Rep.* 2026, Submitted.
54. Trueman, E.R. The burrowing process of *Dentalium* (Scaphopoda). *J. Zool.* **1968**, *154*, 19–27.
55. Kier, W.M. Hydrostatic skeletons and muscular hydrostats. In *Biomechanics: A Practical Approach*; Biewerner, A.A., Eds.; TRL Press at Oxford Univ. Press: New York, NY, USA, 1992.
56. Steiner, G. Phylogeny and classification of Scaphopoda. *J. Molluscan Stud.* **1992**, *58*, 385–400.
57. Wanninger, A.; Haszprunar, G. Chiton myogenesis: Perspectives for the development and evolution of larval and adult muscle systems in molluscs. *J. Morphol.* **2002**, *251*, 103–113.
58. Batten, R.L. The origin of gastropod shell structure. In *Proceedings of the Third North American Paleontological Convention*; Field Museum of Natural History: Chicago, IL, USA, 1982; Volume 1, pp. 35–39.

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