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Biogenic Crystallographically Continuous Aragonite Helices. The Microstructure of the Planktonic Gastropod *Cuvierina*

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The pteropod *Cuvierina* constructs very lightweight, thin, flexible and resistant shells, with the most unusual microstructure: densely packed, continuous crystalline aragonite fibers which coil helically around axes perpendicular to the shell surface. The high degree of fiber intergrowth results in a particular interlocking structure. The shell is constructed by guided self-assembly, outside the animal's soft body. A prerequisite to understand its formation is to resolve the underlying crystallographic building principle. This is basic in order to use this hierarchically structured and highly functional biomaterial as inspiration for the production of new materials. It teaches us about the optimization of structures over millions of years of evolution under strict consideration of energetic costs and efficient use of available resources and materials. By using a combination of spatially resolved diffraction and imaging techniques, which complement at different levels of resolution, we were able to describe how helical coiling proceeds. Despite their curling, the fibers are continuously crystalline and show a preferred crystallographic growth direction. When the latter can no more be maintained due to the imposed curving, abrupt changes across twins permit to continue growth in the desired direction. This is a nice example of how crystallographically continuous fibers can grow helically.

1. Introduction

Mollusks are unrivaled masters in calcification among metazoans. Partly thanks to this ability they have become the second most diverse group of invertebrates within the marine realm.[1] The microstructures making up their shells are particularly interesting as hierarchically-ordered functional materials due to their excellent properties, such as toughness, elasticity, lightweight and softness. Although the mechanical properties of

the constituting materials (calcium carbonate crystals, organic matter) are relatively weak, their combination and optimization of synergistic effects give rise to outstanding properties of the hybrid material. Unravelling the growth mechanism and deciphering the biological strategies that lead to such sophisticated, hierarchically structured biocomposites is not only of interest for the development of new functional materials, but might help to find energy- and resource-efficient synthetic routes built on principles of self-assembly.

Among the different microstructures that have been described in the literature,[2,3] there is renewed interest in the so-called aragonitic helical fibrous microstructure (AHFM).[2,3] It is the most unusual of all molluscan microstructures, since it is made of very thin aragonite fibers which coil helically for several turns along an axis perpendicular to the shell's surface. The AHFM has been reported in species of the eight genera belonging to the Cavolinioidea (Table S1, Supporting Information), coiling being invariably dextral or right-handed.[4,5] Therefore, the AHFM is a trait characteristic to this superfamily.[6]

This microstructure has only recently attracted the attention of materials scientists because of its unusual organization, crystallography and biomechanical performance. However, the only three studies concerned with the crystallographic structure of the material [7-9] agree solely on the fact that the *c*-axes of fibers are parallel to the coiling axis, whereas there is big disparity between results with respect to the rest of the crystallographic directions. There is uncertainty, first, about the exact relationship between the crystal axes and the fiber morphological axes, and, second, regarding crystallographic continuity of fibers. In particular, Li et al. [9] concluded that the fibers consist of two families of curved fibrous blocks stacked together along the shell

thickness and which complete the helical assembly. This is in contrast to the continuity of individual fibers observed with scanning electron microscopy (SEM).

Part of the disparity of the results can be attributed to the methods employed, which provide a too wide (X-ray diffraction, XRD) or too local (transmission electron microscopy, TEM) view of the aggregate. We have carried out an in depth study of the crystallography of the fibers making up the AHFM of two species of the genus *Cuvierina* using two high resolution techniques, electron back-scatter diffraction (EBSD) coupled to SEM, and TEM, which cover the micro- and the nanoscale, respectively. By integrating data coming from the two techniques, a coherent crystallographic model is aimed to be arrived at. In particular, our aim is to answer questions such as: do fibers have a preferred growth direction? are they crystallographically continuous? and, if so, do their crystal axes twist during coiling? otherwise, how do they manage to twist?

2. Results

2.1. Morphology of Fibers

The shells of *Cuvierina* are small (<1.2 mm), vase-shaped, and thin (**Fig. 1**a). Inspection of extensive longitudinal (**Figure 1**b) and transverse fractures reveals that, as in all the specimens of cavolinioideans examined, the fibers invariably coil dextrally and complete up to three and a half turns (**Figure 1**c). The axes of the spirals are invariably perpendicular to the shell surface and the morphological (not crystallographic) orientation of fibers is exactly the same at a particular depth within the shell[2,8] (**Figure 1**c, d). The latter is specifically evident from an inspection of the internal shell surfaces, where the exposed fibers have exactly the same orientation (**Figure 1**e). When the fibers

can be seen curving in horizontal fractures (**Figure 1**f), it is because the fracture in fact has gone through increasingly deeper planes within the shell.

The helix lead (or pitch) and angle increase towards the shell interior (**Figure 1**c). Our estimations range from 12° for the outermost turn to almost 30° for the most internal turns (**Figure 1**c), the latter figure being well above previous values.[2] The helix radius (~10-12 μm in the specimen of **Figure 1**c) is similar for the different helices and remains more or less constant throughout the shell thickness

Given the extensive vertical dimensions of their cross sections (**Figure 1**g to i), fibers at a distance <2 radii should intersect twice per turn, which is particularly evident in horizontal fracture (**Figure 1**f). After crossing, both fibers reappear and continue to grow (**Figure 1**g, h). This process accounts for the complex angular cross-sectional outlines of fibers (**Figure 1**i) [see also [2,8,9]] and, at the same time, implies that the outlines are permanently changing during growth. No interruption of fibers has been observed.

2.2. EBSD Data

All the EBSD maps obtained on inclined sections (**Figure 2**a) are characterized by broad horizontal bands composed of laterally imbricated green arches, scattered with small blue patches which correspond to areas where the fibers have been cut in parallel (b1 bands in **Figure 2**a, b; Figure S2, Supporting Information). The arches reflect the orientation of the sectioned fibers. Between the green bands there are areas with oblique series of blue dots, which are a continuation of the green arches and which converge towards the center of the intermediate bands between the green ones (b2 bands in **Figure 2**a). These correspond to fibers cut at an angle close to the leading angle (b2 in **Figure 2**b).

The 001 pole maximum (**Figure 2**b) shows an extremely reduced spread (<10°), which is placed at 30° to the center of the diagram (i.e., the inclination of the cutting plane). This implies that all fibers have a common *c*-axis, which coincides with the coiling axis of the spirals, and which is exactly perpendicular to the shell surface. The 100 and 010 pole figures show a clear arch-like distribution (**Figure 2**b), which would give the false impression of a continuous (i.e., non-preferred) distribution for both the *a*- and *b*-axes. According to the 010 pole figure, the *b*-axes of the fibers sectioned in parallel (b1 fibers in **Figure 2**a, b) are at an inclination of ~30° to the cutting plane, whereas the *a*-axes are perpendicular to the fiber axes. The (mainly light) blue areas scattered within the green bands correspond to crystals which are in a different orientation (up to some 60° to the fiber axes). According to their position in the pole figures, the distribution of crystallographic axes for the dark blue fibers (b2 in **Figure 2**b) is identical to those for the fibers in green (b2 in **Figure 2**b). Fibers cut at very high angles (cutting plane inclination + lead = ~60^0; Figure S1, Supporting Information; b3 in **Figure 2**b) are very rarely recorded in the map because the cross sections of their constituent crystallites (see below) are too small to provide indexable patterns. The distribution of crystal axes is consistent with those of the previous cases (b3 in **Figure 2**b).

In the maps on sections at a low angle to the surface the fibers are exposed to a large extent, which permits an easy recognition of their orientation (**Figure 2**c). The spread of the 001 maxima is similar to those on inclined sections. The distributions of the 100 and 010 pole figures are again arch-like. By comparing the orientation color maps with the corresponding color pole figures, a consistent correspondence between the elongation of fibers and the orientation of the *b*-axes is evident. Occasionally some grains display two

or even three orientations (indicated in **Figure 2**c), with a common *c*-axis and with the *b*-axes at ~60°, which is strongly suggestive of internal {110} twinning.

2.3. TEM data

In TEM sections fibers appear elongated when cut close to their long axis. One of the cut fibers was oriented along a zone axis and is thus highlighted by a strong diffraction contrast (**Figure 3**a). The lattice fringes reveal that the [001] axis points towards the shell surface, in agreement with previous descriptions,[8,9] while the [010] is in plane and offset by approximately 15° with respect to the long axis of the fiber (i.e., the helical lead angle) (**Figure 3**b, c). Images taken further down along the helical axis from the same sample display fibers that were cut transversally (**Figure 3**d), thus demonstrating the interlocked fiber cross-sections.[2,8,9] The inset in **Figure 3**d shows three neighboring fibers oriented with their respective [100], [110] and [010] orientations parallel to the viewing direction. While the [100] and [110] can be related by twinning, the [010]-oriented fiber is rotated along the *c*-axis by 31.9° with respect to the [110] oriented fiber. The varying contrast (**Figure 3**a, d-f) demonstrates that even similarly aligned fibers can show different crystallographic orientations. This confirms the EBSD observations that morphologically aligned fibers can grow along different crystallographic orientations. Different contrast between neighboring fibers is also evident from the plane view images (**Figure 3**g, h). The annular dark field STEM image shown in **Figure 3**g reveals identically oriented fibers in similar contrast and fibers with slightly different orientation and different contrast between them.

The question that remains to be answered is how continuous crystalline aragonite fibers can grow helically. Evidence for a variation in growth direction along individual fibers is provided by TEM images of fibers that were cut close to their long axis. In the

example shown in **Figure 3**e and f, the respective bright- and dark-field images contain contrast variations that are caused by twinning, but also due to misalignment of small crystallographic domains (several tens of nm in diameter). Due to the twinning, the projected growth direction in this fiber switches between <010> and <110>. Twinning therefore seems to play a role in enabling the curved growth of crystalline helical fibers.

More insight on this aspect is obtained by looking at the fibers in top-view. The respective TEM and STEM views reveal a high density of twinning planes at various directions, frequently along or close to the fiber axis (see **Figure 4**a-d). **Figure 4**e shows a crystallographically continuous, curved section of a fiber. Lattice fringe analysis reveals that it extends along the <110> direction at the top right side and along the <020> direction at the bottom left side. It is therefore evident that the change in growth direction is related to twinning. Indeed, not only polysynthetic twinning, but also polycyclic twinning is observed within individual fibers, such as in **Figure 4**f. Finally, fiber intergrowth (**Figure 4**a, b, e), as described in the SEM part, is regularly observed in the TEM plane views, as well as some more rare instances of splitting (**Figure 4**g).

3. Discussion

3.1. Crystallography of Helical Fibers

The AHFM of the shell of *Cuvierina* is composed of thin fibers which show an astounding degree of regularity in orientation, in which all fibers in a same depth plane within the shell are oriented exactly in parallel. This morphological degree of order is to some point a reflection of the crystallographic order that characterizes this material. According to EBSD data, the *c*-axes of fibers are highly co-oriented in parallel to the axes of the helices and strictly perpendicular to the shell surface, something which was

previously recognized.[9] With regards to the *a*- and *b*-axes, the latter are in the projection of the fiber axis onto the plane parallel to the shell surface; that is, fibers tend to grow along the projected *b*-axis (b_P), whereas the *a*-axis is perpendicular to the fiber in the same plane (**Figure 5**). Nevertheless, for these axes, there is a certain degree of scattering, as the horizontal projections of some fibers are deviated from the preferred *b*-axis orientation for up to ~60^0 (e.g., **Figure 2**c, bottom map). EBSD data also imply that deviated fibers commonly consist of crystals twinned on {110} (**Figure 2**). TEM sectioned fibers commonly orient with the *b*-axis in the growth direction and show a high incidence of twins, either of the polycyclic or polysynthetic kind. This causes frequent changes in orientation of the *b*-axes, but, in general, these remain at a relatively low angle to the growth direction (ca. <30^0). Generally, TEM data match EBSD data, although the former qualitatively show a higher incidence of fibers aligned along <110>. The cases for fibers growing along the b_P-axis and in the direction of the {110} plane are sketched in **Figure 5**.

Taking into account the constancy in the orientations of crystal axes and the increase observed in the lead angle towards the shell interior (**Figure 1**c), the planar upper and lower faces of the fibers cannot correspond to particular crystallographic planes.

3.2. Fiber Growth

On the basis of the combined SEM, TEM and EBSD observations, the following model for the formation of crystalline twisted fibers is derived. Due to the spiral trajectories of fibers and assuming invariant crystal orientations, growth along the *b*-axis projected on the fiber axis (b_P; **Figure 5**, left) can only be maintained for reduced angular distances until the growth direction becomes markedly deviated from the preferred crystal axes. Then, new directions for b_P, which are more conveniently oriented with respect to the

spiral path, have to be selected. Imagine a fiber whose b_P -axis is oriented exactly in the growth direction (1 in **Figure 6**a); after 30^0 of angular movement along the spiral path, the different orientation of its b_P-axis becomes ~30^0 (2 in **Figure 6**a); from here on, a crystal (obtained by a single twin operation) whose b_P-axis is at ~60^0 to the b_P-axis of the previous crystal, becomes more conveniently oriented with respect to the helical path. After another 30°, the new crystal will have its b_P-axis parallel to the growth direction (3 in **Figure 6**a). In this way, along a complete turn, the orientation of the b_P-axis in or close to the fiber axis can be maintained by shifting the orientation of the b_P-axis every ~60^0 due to twinning. This process can easily result from the observed high frequency of twins of different kinds (**Figure 4**). Along the spiral path, intervals in which growth is mainly along b_P will alternate with others in which fibers are mainly oriented along {110} twinning planes (**Figure 6**b) or intermediate directions. Twinning allows the formation of helically twisted fibers with preferential growth along the b_P-axis (as shown by EBSD), while at the same time retaining morphological (as observed by SEM; **Figure 1**) and crystallographic continuity (as observed by TEM; **Figures 3** and **4**). This is in contrast to the recent claim that fibers must be discontinuous since they have two different growth directions (010 and 110) in different sectors of the helices.[9] Note that these observations can be fully explained by our model of growth by periodic shifting from a <010> to a <110> direction (**Figure 6**b).

4. Conclusions

The AHFM microstructure of cavolinioideans is unique in being composed of helical fibers which run from the external to the internal shell surface at the same time that they coil helically. This implies that in these gastropods calcification of the shell necessarily proceeds from the external to the internal shell surface, where the mantle is adhered.

This is clearly unlike in trochospiral gastropods in which calcification is in the adoral growth direction.

Our study provides a consistent picture of the crystallography of this sophisticated material for the genus *Cuvierina*, but which we believe is general for the Cavolinioidea, given the homogeneity of the microstructure across the group.

Preferential growth direction along b_P in the AHFM is unique. Other types of aragonitic microstructures show preferred growth directions along either the *c*-axis (fibrous prismatic),[10] the *a*-axis (foliated aragonite)[11] or the projected <110> directions (crossed-lamellar).[12] Except for the first case, which is also the common case in inorganic fibrous aragonite (e.g. [13]), preferential growth results from the action of particular organic molecules, which are able to promote/prevent growth by adhering to particular crystal faces.

The above described growth strategy harnesses the peculiar crystallographic characteristics, in terms of preferred growth directions promoted by particular proteins and twinning development, of aragonite. Although this is hypothetical, it is doubtful that a microstructure similar to the AHFM could be constructed with e.g. calcite. Whatever the process is, it is clear that it follows a non-classical crystallization (as it is built from small units forming a meso-crystal [14,15] (Figure S2; Supporting Information).

Olson et al. [16] showed that in biogenic aragonite (nacre) tilting of the *c*-axis occurs across superposed tablets. This feature is much more intense in biogenic prismatic calcite.[17,18] The small spread of the 001 pole figure maxima in the AHFM of *Cuvierina* (**Figure 2**) suggests that no appreciable tilting of crystal axes occurs in this material.

Although helical coiling can be related to screw dislocation-driven in the case of helical nanowires,[19] the dislocation core is absent in the fibers composing the AHFM. A comparable mechanism is known in nacre [20] and in biogenic calcite (semi-nacre[21]

and foliated calcite [22]), although, in these cases, the step size of the dislocation is the thickness of one tablet or lath, and not one atomic plane, as in nanowires. As with crystal lattice tilting (see above) no evident influence of such mechanisms have been found.

Although overall, the *b*, and, secondarily, the <110> directions, are dominating as growth directions, it is evident that spiral growth is not guided by crystallographic growth directions, which conversely have to adapt to the changes in curvature imposed by helical growth. Hence, it can be assumed that the helical growth has to be achieved by some form of unveiled templated growth. The only aspect left to solve is how the templating of the structure works, for which we envisage two alternative possibilities: (1) helical growth is under strict biological control, i.e. growth is guided by an underlying genetic programming mediated by cellular secretion; (2) calcification happens around a self-organized biological scaffold, i.e. the twisted plywood chitin scaffold found in arthropod exoskeletons [23,24], this usually being interpreted as a liquid crystalline cholesteric phase.[25,26] Whatever the case, nature demonstrates how to grow continuous helically twisted crystalline fibers, which is something challenging for material science.

4. Experimental section

Material: Dead shells of *Cuvierina columnella* (Banc Atlantis, N-O. "le Suroit" Seamount 2, Atlantic Ocean, 34º 22,4'N, 30º27,8'W, dredged from 1340 m),acquired on loan from the Muséum National d'Histoire Naturelle, Paris, were well preserved enough for crystallographic studies. Specimens of *Cuvierina urceolaris* from Olango Island (dredged from 150 m), Balicasag Island (180 m) and Mactan Island (200 m), the Philippines, were purchased from Conchology Inc.

Scanning Electron Microscopy (SEM): Five specimens (three of *C. columnella* and two of *C. urceolaris*) were fractured, ultrasonicated and coated with carbon (Hitachi UHS evaporator) for SEM observation (Zeiss Leo Gemini 1530 and Zeiss Auriga Cross-Beam Station) at the Centro de Instrumentación Científica (CIC) of the Universidad de Granada (Spain). Specimens of other cavoliniodeans (*Cavolinia inflexa*, *Cavolinia longirostris*, *Diacria trispinosa*, *Diacria quadridentata* and *Clio pyramidata*) were also examined for comparison.

Electron Backscatter Diffraction (EBSD): This is a SEM-based method for local measurements of crystal orientations. Initially developed for the study of metals, it is increasingly applied to biominerales. The EBSD technique analyzes the diffraction pattern produced when backscattered electrons are diffracted by the most superficial lattice planes of a crystalline material. The diffraction patterns are captured and transferred from the charge coupled device (CCD) camera of the detector to the computer. Once indexed, the patterns provide information about the the space group of the crystal structure and the orientation of the crystal lattice. Data can be obtained in the form of orientation maps or pole figures. The colors in the EBSD maps correspond to the color coded orientations in the inverse pole figure map. This technique provides resolution on a sub-µm scale (see below) and is therefore a good complement to the broader scale, X-ray-based techniques.

One complete specimen of *Cuvierina columnella* was fractured and cleaned from organic matter (particularly the external cuticle) with commercial bleach (4% active Cl) for 3 min; the concave shell surface was analyzed without further polishing, although this procedure proved unsuccessful. In addition, two shells of *Cuvierina columnella* and one of *C. urceolaris* were sectioned and polished along three directions: parallel to the shell surface, perpendicular to the shell axis, and at ~ 30^0 to that axis. A longitudinal

section of the fibers along a certain angular distance was exposed to the electron beam by sectioning the shell at the latter angle. In particular, along a single turn, each fiber was cut at a variable angle from the lead angle minus the sectioning angle to the lead angle + the sectioning angle (Figure S1, Supporting Information). Accordingly, the fibers were cut in parallel when the lead angle-sectioning angle = 0 (once per turn). The 30° angle was chosen because it agrees with highest estimated lead angle of the internal turns (25-30°; see below). Polishing was carried out on horizontal diamond-impregnated discs (Struers Planopol 2 polishing machine) with grit sizes 3, 1 and 0.25 μm. A final polishing with colloidal silica was conducted. We used two equipments. First, we used an Inca Crystal (Oxford Instruments) detector coupled to a Gemini-1530 (Carl Zeiss) Field Emission SEM (FESEM) from the Center for Scientific Instrumentation (Universidad de Granada), operated at 20 kV and with a beam diameter ~1 μm. To avoid excessive charging, samples were coated with 2 nm of carbon in a Baltec MED 020 electron beam evaporator. The second equipment was a Hikari EDAX detector coupled to a FEI FESEM Quanta 3D at the Institute of Metallurgy and Materials Science of the Polish Academy of Sciences (IMIM, Krakow, Poland). Operation in low vacuum mode (0.45 Torr) made coating unnecessary. A special X-ray cone was attached to the SEM pole piece to minimize the so-called "skirt effect" of the primary electron beam by reducing the gas-path length. The microscope operated at 15 kV with a beam current of 5.7 nA and the beam diameter was 25 nm. Analysis softwares HKL CHANNEL5 (Oxford Instruments) and TSL OIMTM (version 5.3) were used to post-process the EBSD measurements. By far, the most informative and complete maps (i.e., with a higher percent of indexable analyses) were those in which the shells were sectioned either at 30°or parallel to the shell axis, which were performed at the IMIM with a with a very small beam size. In all cases, the percent of indexable patterns was

relatively low due to the small crystallite size. The width of fibers is only 200-300 nm and due to the abundant twinning (see TEM results), the actual single crystalline domains are much smaller than that.

Transmission electron microscopy: Cross section and plane view samples for TEM analysis were prepared by cutting embedded shell pieces of *Cuvierina columnella* perpendicular and parallel to the shell surface, respectively. Thin cuts were first mechanically polished and subsequently thinned down to electron transparency with a GATAN precision ion polishing system (PIPS) at the Fritz-Haber Institute of the Max Planck Society in Berlin. TEM analysis was carried out using an image Cs corrected FEI Titan microscope that was operated at 300 kV.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the authors.

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Figure captions

Figure 1. Optical (a) and SEM (b-i) observations of *Cuvierina* shells. (a) View of a complete specimen of *C. columnella*, with anatomical directions indicated. (b) Fragment showing a longitudinal fracture through the shell of *C. columnella*. The boxes show the positions equivalent to those of other panels in this figure. (c). View of a longitudinal fracture through the shell of *C. urceolaris* showing perfectly arranged helices coiling for about 3.5 whorls (indicated). The pitch increases from the outer to the inner whorls. (d) Longitudinal fracture through the shell of *C. columnella*, showing the external helices. The orientations of some fibers is indicated with arrows. (e) View of the internal shell surface of C. *columnella*, showing the even orientation of the fibers. The sample has been slightly bleached. (f) Surface view of a fracture through the shell of *C. columnella*. The fracture runs roughly parallel to the outer surface of the shell and exposes fibers at different depths. It shows the high frequency of interpenetration between fibers. (g, h) Two views of the twisting fibers of *C. columnella*, showing the high degree of interpenetration. This is particularly evident where the fibers are seen in lateral view (arrows). (i) Transversely fractured fibers of *C. columnella*, showing the complex outlines obtained by interpenetration.

Figure 2. EBSD Inverse pole figure maps. (a) Inverse pole figure map of a section through the shell of *Cuvierina columnella*, inclined at 30° to the coiling axis (see Figure S1, Supporting Information). The inset is the color key (valid also for (c)). The labels to the right of the figure refer to the different orientations of the fibers with respect to the sectioning plane shown in (b). (b) Pole figures for the same map. The position on the

pole figures and the inferred distribution of axes for three differently oriented and/or inclined types of fibers (b1, b2, b3) are indicated below. (c) Image quality (gray scale) and inverse pole figure maps (color), and corresponding pole figures for the whole maps, performed on sections at a low angle to the shell surface. The orientation of crystals at particular positions are indicated with parallelepipeds on the quality maps. The longest dimensions of their grey faces ({001} faces) are the *b*-axes and the shortest dimensions, the *a*-axes.

Figure 3. TEM views of fibers in vertical and horizontal sections. (a) Longitudinally cut fiber with a strong diffraction contrast. (b, c) Progressively higher resolution images and deduced crystallographic orientation. The Fast Fourier Transform of the lattice fringes is shown as an inset in (b). The *c*-axis points in the direction of the helical axis and perpendicular to the shell surface. The *b*-axis is at an angle of ca. 15° with respect to the long axis of the fiber. (d) Fibers were cut transversally, exposing their interlocked cross-sections. The inset shows the respective orientations of the fiber sections (false colors correspond to the indicated orientations). (e, f) Bright- (e) and dark-field (f) images of longitudinally cut fibers. Twinning planes can be identified by thin streaks along the *c*-axis direction in (e) (some are indicated with arrows). The dark field image highlights differently oriented crystalline domains along the fiber section. (g, h) Diffraction contrast in annular dark field STEM (g) and TEM (h) images recorded from plane view samples. The red line in (h) indicates a possible plane and viewing direction for a TEM image recorded from a cross section sample, such as the one shown in (d).

Figure 4. TEM views of fibers in plane sections. (a-d) TEM images, demonstrating that the {110} twinning planes can be oriented at different angles with respect to the long axis of the fiber section. The insets in (d) show the Fast Fourier Transform (FFT) of the lattice fringe for the two fibers; the right fiber consists of two polycyclically twinned crystals (upper right inset). (e) Progression of the growth direction from <110> to [020] within a continuous fiber. The inset correspond to the FFT of the small box. (f) Presence of polycyclic twinning in a single fiber; f1 shows the orientations (in different colors) for the two areas framed in (f); f2 is the FFT of the lattice fringes of the small box. (g) Example of fiber splitting (arrows) and intergrowth.

Figure 5. Crystallographic model for the fibers of *Cuvierina*. The *c*-axis is always strictly parallel to the coiling axis. In the preferred growth direction (left sketch) the *b*-axis is at the lead angle to the fiber local axis, and the *a*-axis is perpendicular to the fiber axis. In some instances, fibers have been observed growing along a <110>direction, contained within a {110} twinning plane (right sketch). b_P is the projection of the *b*-axis onto the fiber axis.

Figure 6. Model for the crystallographic changes during rotation of fibers. (a) Detail of the change in orientation with the angle of rotation. At position 1, the *b*-axis of the orange crystal is parallel to the growth direction, but deviates progressively with the angle of rotation; at position 2, the angle of deviation of the *b*-axis of the orange crystal is exactly the same as that of a new crystal obtained by a {110} twin (green crystal); from here on, the deviation of the *b*-axis of the green crystal becomes progressively

reduced until becoming 0° at position 3 (at 60° from position 1). (b) Theoretical changes expected during an incomplete turn; growth direction shifts from being along the *b*-axis to being along <110> and back every 60°. Colors indicate similar orientations of crystallites. The actual relationship between spiral radius (~21 μm) and fiber width (~300 nm) has been observed.