

## QTA of prismatic calcite layers of some bivalves, a link to trichite ancestrals

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**Abstract.** Eight calcite layers of bivalves are examined in the view of QTA. We use the QTA information to compare calcite prisms of a fossil species, *Trichite*, with other outer prismatic calcite layers. The results indicate that the fossil species exhibit textures close to the ones of Pteriomorphia, but considerably different from the ones of mytilids, as expected from molecular analysis. Texture analysis results point out that inside the Pterioids, the Pectinoidea family is probably more distant than Pterioidea and Pinnoidea ones, coherently with other studies, and perhaps closer to Mytiloidea. However, texture analysis seems to favour a closer relationship of Trichites with Pinnoids than with Pteriids.

### Introduction

Modern bivalved molluscs make their calcareous shells out of layers of two or more of the following microstructures (aragonitic prisms, nacre, crossed-lamellar, complex crossed-lamellar and homogeneous, and, or calcitic prisms and foliae). There is good evidence that the earliest bivalves, recorded from the Middle Cambrian, which were wholly aragonitic, comprising a thin outer layer of prisms and a substantially thicker layer of nacre [1,2], and that the diversity of microstructures arose relatively rapidly, most by the Ordovician [3]. Quite why this diversity of structures evolved is unclear but raises extremely interesting questions as to the potential benefits of the more derived microstructures. What is well established is that the microstructural arrangements shown by different bivalve taxa is of considerable phylogenetic value [4,5,6].

Of particular interest is the evolution of calcitic microstructures from the originally wholly aragonitic primitive shell. Calcitic microstructures are found in only two of the subclasses of the Bivalvia: the Pteriomorphia and the Heteroconchia. In each of these subclasses calcite prisms are found in two apparently separate clades and it is thus entirely reasonable to suggest this mineralogy has evolved on at least four separate occasions [7,8]. Within the Heteroconchia, outer layers of calcite prisms characterise the rudists, large, bizarre extinct bivalves which dominated shallow marine carbonate platforms for much of the Mesozoic, and also occur in some members of the Chamidae, an extant family of tropical bivalves [8]. Within the Pteriomorphia, a large clade of calcite secreters, the pterioids, includes major groups such as the oysters, scallops, pinnoids and pteriids, many of which have expanded their calcitic layers such that they may account for most of the shell thickness. This expansion of the calcitic shell layers is most extreme in the oysters where the only aragonite present is restricted to the muscle attachment sites and ligament [9]. The other pteriomorphian group to have some members to secrete calcite is the mytiloids (mussels). In both the chamids and mytiloids, however, there is clear evidence that not all members secrete calcite, implying that even within these taxa the ability to secrete calcite has evolved more than once.

The study of crystallographic textures in gastropods has revealed that this may be a method of revealing phylogenetic relationships [10]. It may be instructive to study bivalve microstructures in the same way. This paper focuses on a QTA study of the calcite prisms of a number of modern pteriomorph bivalves. Specifically, we examined and compared calcite prisms from two mytilids,

which are only very distantly related and almost certainly represent totally separate innovations of calcite prisms, and a number of pteriods. Our study also included a sample of the prismatic calcite shell of the enigmatic Mesozoic bivalve *Trichites sp.*

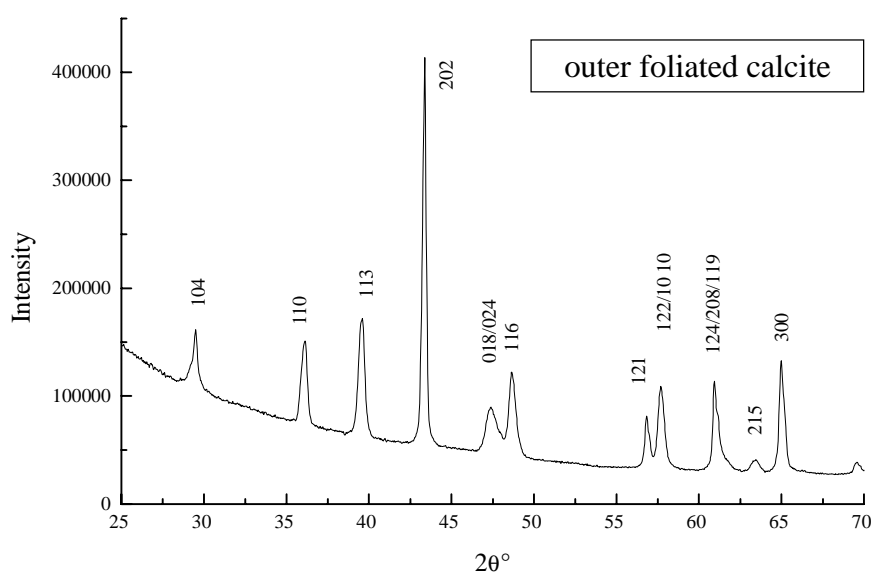
Traditionally, *Trichites* is placed within the Pinnoidea, a pteriomorph group which normally has regular ham-shaped shells and live partially embedded in soft sediments, anchored by byssal threads. Pinnoids do have flexible shells comprised of thin layers of calcite prisms, lined internally by nacre. *Trichites* appears to adopt a rather different life habit, lying on one side on top of the sediment and is frequently highly distorted and display torsion, therefore implying that if it is indeed a pinnoid it is a rather aberrant form. We try in this work to show how QTA may on the contrary indicate that *Trichites* should not be placed in the Pinnoidea.

## Experimental details

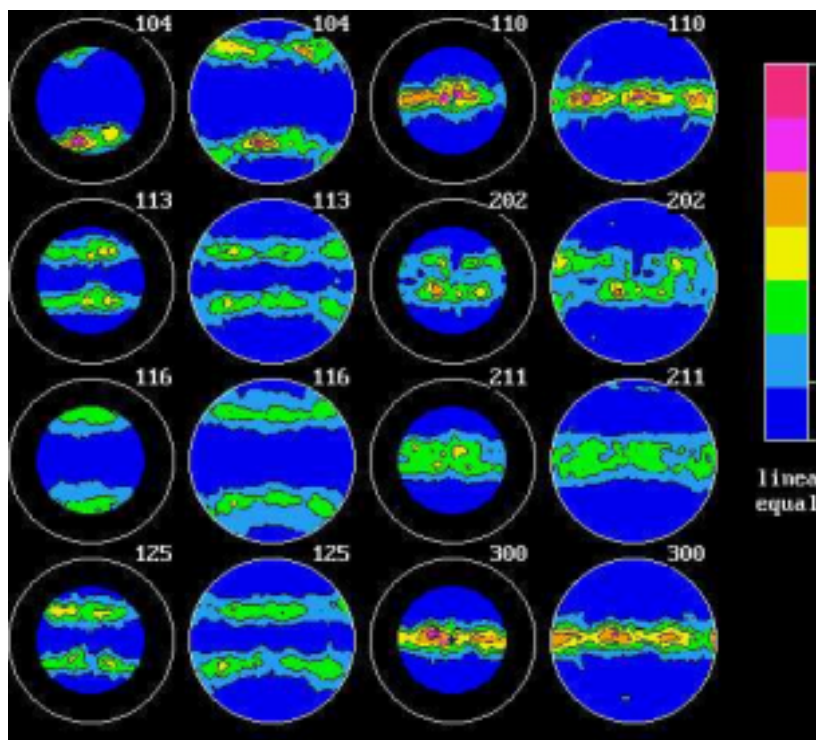
We studied the texture of 8 calcite layers, divided in 5 outer prismatic layers: the Pteriods fan shell *Pinna nobilis*, *Pteria penguin* and scallop *Amusium parpiraceum*, the Mytilids deep ocean mussel *Bathymodiolus thermophilus* and common mussel *Mytilus edulis*. The prismatic layer of the fossil *Trichite sp.* and the inner foliated layer of the oyster *Crassostrea gigas* were also investigated. The orientation of the main axes of the shells was defined by the normal **N** to the shell (centre of pole figures), the growth direction **G** of the shell (vertical axis of the pole figures) and the growth lines direction **M** [10].

X-ray texture measurements were carried out with a Huber four-circle goniometer mounted on an X-ray generator equipped with a curved position sensitive detector (INEL CPS-120), as detailed elsewhere [11]. The QTA analyses were operated using the WIMV algorithm as implemented in the Beartex package [12], considering a triclinic sample symmetry. Nine pole figures or multipole figures ( $\{012\}$ ;  $\{104/006\}$ ;  $\{110\}$ ;  $\{113\}$ ;  $\{202\}$ ;  $\{024/018/116\}$ ;  $\{211/122/1010\}$ ;  $\{125\}$ ;  $\{300/0012\}$ ) were used in the OD refinement. During this stage, the overlaps were resolved manually in order to get the best reproducibility of the experimental pole figures.

A typical summed x-ray diffraction diagram obtained on the outer prismatic layer of the common mussel *Mytilus edulis* is presented in Fig. 1. It shows the experimental resolution achieved, with the reflections used for the OD refinements.



**Fig. 1:** Summed diagram of the outer prismatic layer of *Mytilus edulis*



**Fig. 2:** Experimental and recalculated pole figures of *Bathymodiolus thermophilus*. Equal area projections. Logarithmic density scale. Max: 6.3 mrd; Min: 0 mrd.

Fig. 2 illustrates the quality of the OD refinement on the deep ocean mussel *Bathymodiolus thermophilus*. One can see the good reproducibility obtained, with RP factors of  $RP_0 = 25\%$  and  $RP_1 = 17\%$  for this sample. As already observed for shells textures [13], the texture reaches high degrees (Tab. 1).

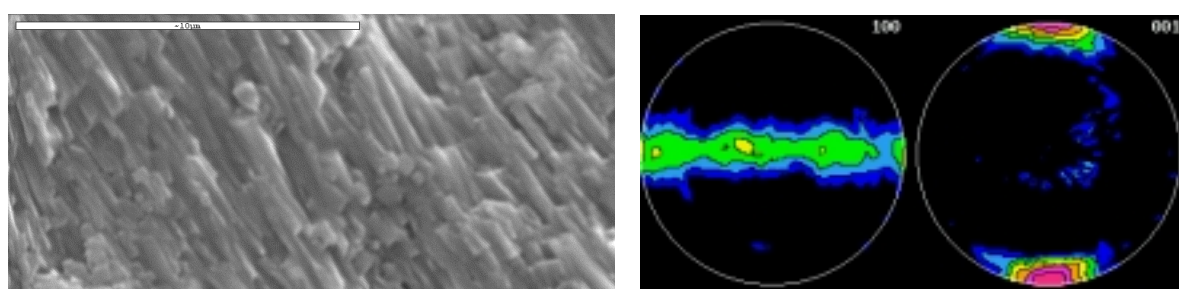
### Texture analysis results

Table 1 relates the results obtained on the 8 samples. The textures are refined with relatively low RPs in general. Looking at the c and a-axis distributions, we observe regular trends among the analysed species. All the textures show high strengths, as in aragonite layers [10], with ODF minima of 0 for all examined layers.

The inner foliated calcite of the oyster *Crassostrea gigas* has a much higher texture strength than all the measured prismatic layers. The two *Pinna nobilis* and *Pteria penguin* prismatic calcite layers exhibit close textures, with c-axes oriented parallel to **N** and a-axes at random around c, only different by their dispersions (differences in texture strengths). *Trichites* show inclined c-axes, though only  $15^\circ$  of deviation from **N**, and a much higher texture strength than *P. nobilis* and *P. penguin*. Both examined mussels, *Mytilus edulis* and *Bathymodiolus thermophilus*, show very inclined c-axes, with a single-crystal-like distribution of the a-axes. This is also the texture characteristic of *Amussium parpiraceum*. These three species have texture strengths lower than *P. nobilis*, *P. penguin*, *Trichites* and foliated calcite, and each of them a distinct textural signal, considering their c- and a-axis distributions. Also, the crystal prisms as seen using SEM look elongated along an axis which does not correspond to any of the a, b or c axes of calcite, as illustrated in Fig. 3 for *B. thermophilus*. This is an example of the non-trivial relationship existing between SEM and texture investigations, already demonstrated for aragonite [10,14], and proving the non-redundancy of the two approaches that may be used in phylogenetic discussions.

|                                   | Layer type | ODF Max (mrd) | ODF min (mrd) | RP0 (%) | RP1 (%) | c-axis     | a-axis     | {001} Max (mrd) | F <sup>2</sup> (mrd <sup>2</sup> ) | - S |
|-----------------------------------|------------|---------------|---------------|---------|---------|------------|------------|-----------------|------------------------------------|-----|
| <i>Pinna nobilis</i>              | OP         | 303           | 0             | 50      | 29      | // N       | random     | 68              | 29                                 | 2.3 |
| <i>Pteria penguin</i>             | OP         | 84            | 0             | 29      | 15      | // N       | random     | 31              | 13                                 | 1.9 |
| <i>Amusium parpiraceum</i>        | OP         | 330           | 0             | 53      | 33      | // G       | <110> // M | 20              | 31                                 | 2.6 |
| <i>Bathymodiolus thermophilus</i> | OP         | 63            | 0             | 25      | 18      | // G       | // M       | 27              | 13                                 | 1.9 |
| <i>Mytilus edulis</i>             | OP         | 207           | 0             | 41      | 25      | 75° from N | <110> // M | 23              | 21                                 | 2.2 |
| <i>Trichites</i>                  | P          | 390           | 0             | 52      | 28      | 15° from N | random     | 56              | 41                                 | 2.2 |
| <i>Crassostrea gigas</i>          | IF         | 908           | 0             | 45      | 31      | 35° from N | // M       | >100            | 329                                | 5.1 |

**Tab. 1:** Results for the 8 samples analysed.



**Fig. 3:** SEM microstructure of *B. thermophilus* (left) and corresponding {100} and {001} recalculated pole figures. G is vertical on pole figures, horizontal on the SEM picture. Scale bar: 10 µm

## Discussion

There is good evidence to suggest that the earliest bivalves were all entirely aragonitic and of rather simple construction from two microstructural types. However, later bivalves evolved a further 5 microstructural types (2 of them calcitic) and utilised these in a variety of different arrangements. The result is that the bivalve microstructure and mineralogy is extremely diverse across the class and is, therefore, thought to be a valuable character for phylogenetic analysis. Two major types of calcite microstructure are identified: prisms, which usually occur on the outside of the valve, and foliae which tends to occupy the bulk of a shell (e.g. oysters: *C. gigas*). Of these calcite prisms are perhaps the most interesting because there is good evidence that calcitic prisms have evolved at least four times independently within the Bivalvia, in the mussels, pteriods, chamids and the extinct rudists.

The data presented here is in majority from the calcitic prismatic layer of bivalves from members of the sub-class Pteriomorphia. This broad group encompasses two of the calcite secreting clades, the mussels and the pteriods. It is therefore of great value to compare the texture of the calcite crystals within members of these taxa.

The textures observed revealed important differences between the mussels and the pteriod specimens studied, as well as important differences within these groups. The mussels *B. thermophilus* and *M. edulis* both have calcitic outer shell layers. It is clear from texture analysis (see Tab. 1) that the two are separate innovations of the calcitic shell layer, with different alignment schemes of their a-axes. However both species support their separation within the mussels, implying at least that calcite secretion within even the mussels is diphyletic. More widespread analysis of the crystalline textures of different calcite-secreting mytilids, combined with molecular

data should resolve the matter if exactly how many times calcitic layers have evolved within the group.

Within the pterioids, the calcite prisms of *A. papiraceum* show very inclined c-axis, and in this respect share more with those of the mussels than the other pterioids. This is interesting because in the phylogenetic scheme, based on a study of 18S rDNA of a wide range of pteriomorphians [15] clearly demonstrated that the Pinnoidea and Pterioidea are sister-taxa, and that the Pectinoidea are more distantly related. If this is the case, it raises the possibility that the ability to secrete calcite is not monophyletic within the pterioids, and had in fact evolved several times. This appears to be supported by our data.

The pterioids are represented by *P. nobilis*, *P. penguin*, *Trichites sp.*, and *A. papiraceum*. Although these ostensibly all belong to the same clade of calcite secreters it is important to establish the degree of similarity or difference between the various taxa. In particular, recent molecular schemes have placed the pteriids (i.e. *P. penguin*) as a sister-group to the pinnoids (*Pinna* and possibly *Trichites*). All three species exhibit similar textures, but *Trichites*, if we except its slightly inclined c-axis distribution, shows an ODF maximum closer to the pinnoids than to the pteriids.

*A. papiraceum* belongs to the superfamily Pectinoidea which, according to some phylogenetic schemes at least, is further removed from the pteriids and pinnoids, than are the mussels. If this is so, then calcite secretion in the pteriooid group may be polyphyletic and thus we might expect the *Amussium* to display different textures to both the *Pinna* + *Pteria* and the mussels. This is actually what we have measured as textures in *Amussium*, which presents a clearly not random alignment of its a-axes (i.e. is different of *Trichites*, Pterids and Pinnoids), a c-axis distribution in the plane of the shell (not like *M. edulis*) and  $\langle 110 \rangle$  directions aligned with **M** (where *B. thermophilus* has its a-axes).

## Conclusion

The calcite layers of the taxa analysed here show different texture patterns that allow us to put some light on their place in the phylogeny. Only one species from each sub-family has been studied and of course more studies would be needed, but general trends are confirmed by recent molecular investigations. For instance the Pectinoidea superfamily looks from texture analyses closer to the mitiliids than to the pterioids where it was placed up to now. Also, the studied *Trichite sp.* seems to be more closely related to Pinnoidea than to Pterioidea. The six analysed taxa show six different texture patterns of prismatic calcite layers, which are also distinguishable from the one of the foliated calcite. These texture patterns present common characteristics, as in the case of aragonitic layers of molluscs. The foliated calcite layer appears to be much strongly textured than all the prismatics. No apparent crystalline epitaxial-like relationship is detected between the aragonitic inner nacre and the calcitic outer layers of *B. thermophilus*.

As for the aragonitic layers observed in previous works, the SEM-microstructure and texture characterisations provide non-redundant information, because they are not everytimes linked in a simple manner, also in calcite layers.

Our results have demonstrated that studies of crystalline texture has the potential of providing valuable phylogenetic information that may be used, in conjunction with molecular studies to supplement and modify the previous phylogenetic trees built from gross morphological characters alone. In particular we have shown that it is possible to use these techniques to unravel the evolution of different microstructures by highlighting differences between structures which appear similar

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## References

- [1] J.D. Taylor: *Palaeontology* **16** (1973) 519.
- [2] B. Runnegar: *Alcheringa*, **9** (1985) 245.
- [3] J.C.W. Cope: *Palaeontology* **40** (1997) 713.
- [4] J.D. Taylor, Y.W.J. Kennedy, A.D. Hall: *Bulletin of the British Museum (Natural History)*, Zoology Series, Supplement, **3** (1969) 1.
- [5] J.D. Taylor, Y.W.J. Kennedy, A.D. Hall: *Bulletin of the British Museum (Natural History)*, Zoology Series, **22** (1973) 253.
- [6] J.G. Carter: *Skeletal biomineralization: patterns, processes and evolutionary trends*. New York, Van Nostrand & Reinhold (1990).
- [7] J.G. Carter: In *Skeletal growth of aquatic organisms*, L. D.C. Rhoads & R.A (Eds.) (1980) pp69-113. New York and London: Plenum Press
- [8] E.M. Harper: *J. of Molluscan Studies* **64** (1998) 391.
- [9] H.B. Stenzel: *Science* **142** (1963) 232.
- [10] D. Chateigner, C. Hedegaard, H.-R. Wenk: *J. Structural Geology* **22** (2000) 1723.
- [11] J. Ricote, D. Chateigner: *Boletín de la Sociedad Española de Cerámica y Vidrio*, **38**[6] (1999) 587.
- [12] H.R. Wenk, S. Matthies, J. Donovan, D. Chateigner: *J. Applied Crystallography* **31** (1998) 262.
- [13] D. Chateigner, C. Hedegaard, H.-R. Wenk: "Textures of Materials" (Ed. J.A. Szpunar), NRC Research Press, vol. 2, pp 1495-1500 (1999)
- [14] C. Hedegaard, D. Chateigner, H.-R. Wenk: Submitted *J. of the American Malacological Society*.
- [15] G. Steiner, S. Hammer: In "The Evolutionary Biology of the Bivalvia". E. M. Harper, J. D. Taylor, & J. A. Crame (Eds.), Geological Society of London, Special Publications, 177 (2000) pp. 11-29.