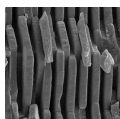# Crystallographic texture of Late Triassic gastropod nacre: evidence of long-term stability of the mechanism controlling its formation

JIŘÍ FRÝDA, KLAUS BANDEL & BARBORA FRÝDOVÁ

An analysis of the crystallographic texture of nacre (mother-of-pearl) in the Late Triassic gastropod *Wortheniella coralliophila* is presented. The primary aim of this study was to test the time stability of the crystallographic texture pattern of gastropod nacre over an extremely long time span. Our results show that the crystallographic axes of individual aragonite platelets in the Late Triassic *Wortheniella coralliophila* have an identical arrangement to those in living vetigastropods. The same microstructure as well as the crystallographic texture of nacre in Recent and Late Triassic gastropods implies the same biological mechanisms for its formation. Our study suggests that the proteins controlling the shape and orientation of individual nacreous platelets in living gastropods have not changed since the Late Triassic. The molecular mechanisms driving the origin and the development of gastropod nacre are thus extremely old and have remained unchanged for at least 220 million years. • Key words: Gastropoda, nacre, crystallographic texture, time stability.

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Several months ago, a team of Japanese scientists (Suzuki *et al*. 2009) reported the discovery of two novel proteins, named Pif80 and Pif97, which seem to be the key players in controlling the crystal structure of nacre in pearl-forming oysters. The mechanisms determining the shape and orientation of individual calcium carbonate crystals in molluscan shells are very complex and poorly understood even though they had been intensively studied during past decades (see Checa & Rodrigues-Navarro 2005, Marin *et al*. 2008, and Suzuki *et al*. 2009, for details and references). The application of electron microscopy has enabled us to analyze the detailed distribution of aragonite and calcite in individual shell layers as well as their microstructural patterns. This research has revealed the extremely high microstructural disparity of molluscan shells (*e.g.*, Carter 1990). However, this work is far from complete because the shell microstructure of many, highly diversified groups of molluscs has not yet been studied. For example, the shell microstructure of living members of the Neritimorpha (a highly diversified order of marine, freshwater and terrestrial gastropods) has only been characterized in a few species (Sasaki 2001). Similarly, data on the shell microstructure for the vast majority of 605 family rank taxa of gastropods (Bouchet *et al*. 2005) are still not available.

The research aimed at understanding the mechanisms of shell formation on a molecular basis started at the same time as studies of microstructural patterns of molluscan shells. It has been shown that molluscan shells represent a complex composite material containing not only calcium carbonate crystals but also various organic macromolecules. These macromolecules form a matrix – a framework in which mineral crystals grow. The major components of the organic matrix are polysaccharide b-chitin, a relatively hydrophobic silk protein, and a complex assemblage of hydrophilic proteins, many of which are unusually rich in aspartic acid (see Addadi *et al*. 2006, and Marin *et al*. 2008, for details and references). Biochemical studies of molluscan shell formation have produced evidence that macromolecules present in the shell control the polymorphism of calcium carbonate. Aragonite is a phase which dominates in molluscan shells, but both aragonite and calcite shell layers are present in many species. Falini *et al*.
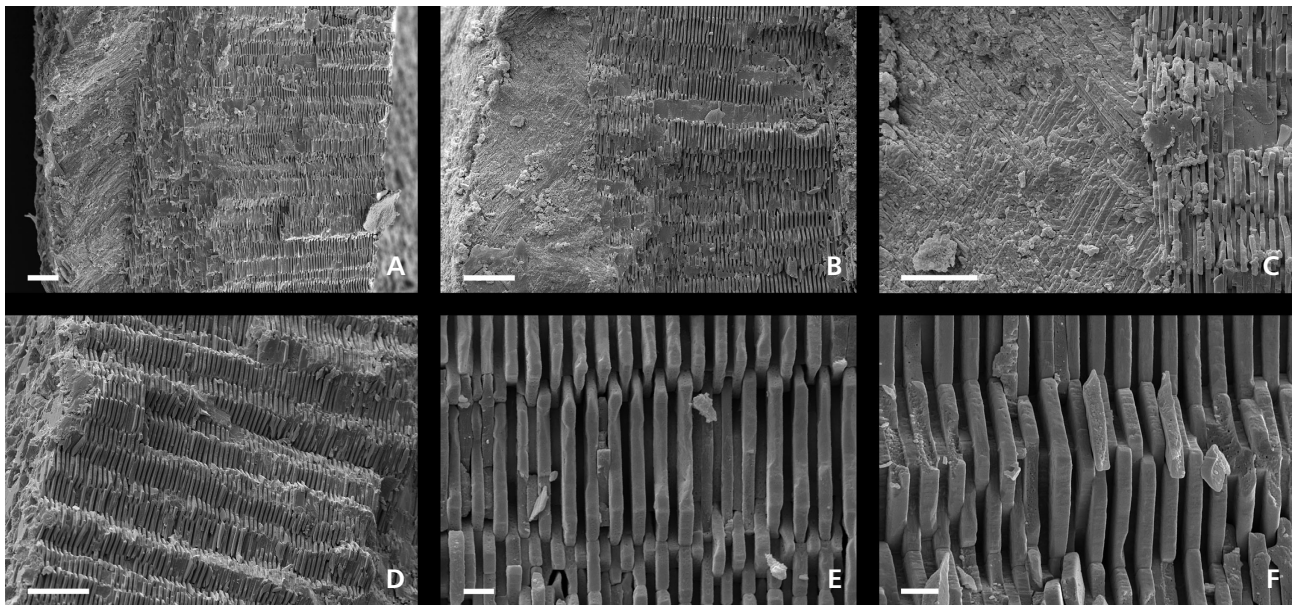
Figure 1. Secondary electron images of shell fragments of the Late Triassic *Wortheniella coralliophila* Kittl, 1891, from the St. Cassian Formation. • A, B – overall views of broken shell showing its outer layer (left) composed of spherulite sectors and inner nacreous layer (right). • C – detailed view of the boundary between spherulitic and nacreous layers. • D – view of the inner layer showing the columnar arrangement of nacreous platelets. • E, F – detailed views of nacreous platelets. Localities: A, D and F – Campo, B, C and E – Misurina; both localities near the Cortina d'Ampezzo, Dolomites. Scale bars: A, B, D – 20 μm; C – 10 μm; E, F – 2 μm.

(1996) showed that macromolecules extracted from the aragonite shell layer induced the growth of aragonite *in vitro* on a substrate of polysaccharide b-chitin and hydrophobic silk protein. Under the same conditions, the macromolecules extracted from the calcite shell layer induce the growth of calcite. Falini *et al*. (1996) suggested that these macromolecules are responsible for the precipitation of either aragonite or calcite also *in vivo*. This discovery began a period of intensive research to identify the particular macromolecules responsible for the biomineralization processes as well as to understand shell formation at the molecular level (see details in Marin & Luquet 2004, Suzuki *et al*. 2009). These studies have revealed many important insights into the mechanisms of shell formation on the molecular level (see Marin *et al*. 2008 for summary). On the other hand, during the last decade, studies of the inorganic part of molluscan shells (biocrystals of aragonite or calcite) have become less frequent and have been focused mainly on analyses of the mechanical properties of particular shell layers (*e.g.*, Bertoldi *et al*. 2008) or on models explaining the growth of biocrystals (*e.g.*, Checa *et al*. 2006; Checa & Rodrigues-Navarro 2001, 2005). However, studies of the shell microstructure can also provide important data for understanding molluscan phylogeny. It has been known since the beginning of the last century (Bøggild 1930) that particular types of shell microstructures are limited to certain phylogenetic lineages of living mollusks (*e.g.*, nacre is lacking in all living gastropods except for some members of the Vetigastropoda). In addition, it has been shown that

microstructural data may characterize "high-level" taxa and therefore are an important source of information for phylogenetic analyses of major molluscan groups (*e.g.*, MacClintock 1968, Bandel 1979, Carter 1990, Ponder & Lindberg 1997).

The Patellogastropoda may represent one of the few good examples of the usage of microstructure data for family rank taxonomy. Shells of these exclusively marine gastropods are very simple (limpet), but their internal shell structure is the most complex among all living gastropods. In contrast to their shell microstructure, morphological features of their shells thus provide only a limited phylogenetic signal. The patellogastropod shells are formed of several different aragonite and/or calcite shell layers. Many non-redundant characteristics diagnostic of particular patellogastropod clades may be inferred from such complex shell structures (MacClintock 1968, Bandel & Geldmacher 1996, Fuchigami & Sasaki 2005). Also, the oldest known member of the Patellogastropoda was identified only by its shell microstructure (Hedegaard *et al*. 1997). However, the usage of shell microstructure data for the determination of phylogenetic position of fossil molluscs is exceptional rather than common.

Molluscan shells are formed by thousands of minute crystals of aragonite and/or calcite arranged in different shell layers. Therefore, this very complex composite material can provide three basic types of information on its inorganic component (*i.e.*, information on the shell microstructure, phase composition, and crystallographic
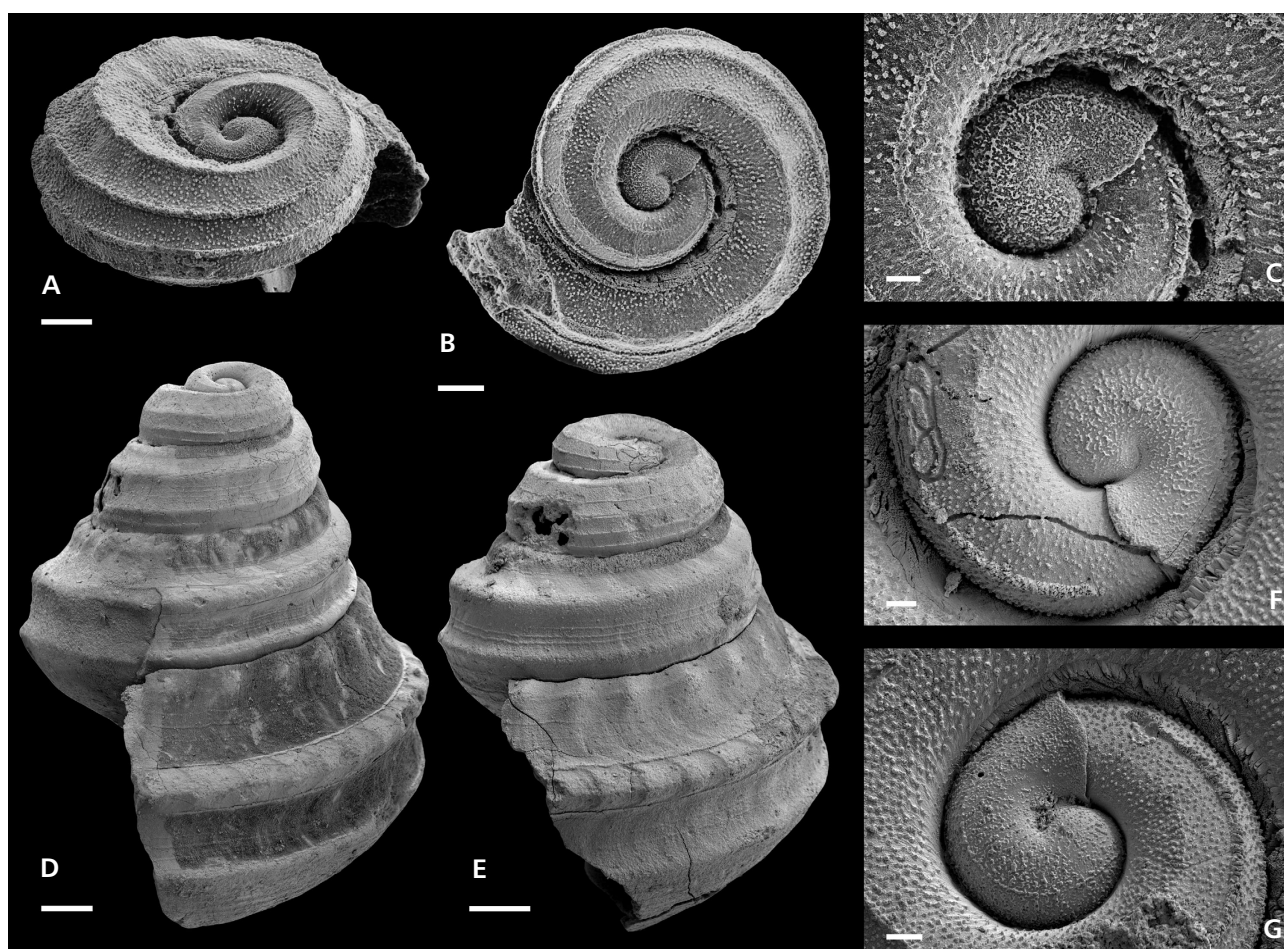
Figure 2. Late Triassic *Wortheniella coralliophila* Kittl, 1891, and *Wortheniella subpunctata* (Laube, 1868) from the St. Cassian Formation. • A, B – oblique and apical views of juvenile teleoconch of *Wortheniella coralliophila* showing almost planispirally coiled first whorl and protoconch in depression. • C – detailed view of protoconch in specimen figured in A and B. • D, E – oblique lateral views of teleoconch in *Wortheniella coralliophila* showing its selenizone and flat apex. • F – detail of the protoconch of specimen figured in E. • F – detail of protoconch of *Wortheniella subpunctata* (Laube, 1868). Localities: A, B, C, D and G – Misurina; E and F – Alpe di Specie; both localities near the Cortina d'Ampezzo, Dolomites. Scale bars: A, B – 100 μm; C – 30 μm; D, E – 200 μm; F, G – 20 μm.

texture). The microstructure characterizing the shape of biocrystals has been the most studied property of molluscan shells. Data on phase composition (*i.e.*, on the type of mineral forming a particular shell layer) are very rare and for many major molluscan groups are missing (they have been frequently inferred indirectly from shell microstructural type of a particular shell layer). The crystallographic texture, describing the arrangement of crystal axes of individual biocrystals, also provides an important set of characters for definition of the nature of individual shell layers. It has to be noted that shell layers having the same microstructure may have different crystallographic textures and *vice versa*. Thus, an analysis of crystallographic texture and microstructure of molluscan shells provides non-redundant characteristics. However, in contract to the microstructures, there are almost no data on crystallographic textures of molluscan shells. So far, only a few

shell layers in several tens of the many tens of thousands of living molluscan species have been studied (Hedegaard 1997, Hedegaard & Wenk 1998, Chateigner *et al*. 2000). Data on the crystallographic texture of shells in extinct molluscan species practically do not exist.

In this paper, we present results of crystallographic texture analysis of nacre in the fossil gastropod, *Wortheniella coralliophila* Kittl, 1891, from the Late Triassic St. Cassian Formation. The present study is part of an on-going project focused on the evolution of nacre in different groups of living and extinct molluscs. The main goal of this short paper is to test whether the crystallographic texture of gastropod nacre has changed during the past 200 Ma. The crystallographic texture is under biological control as is the shell microstructure, and therefore its study can reveal how stable are the biological mechanisms controlling biomineralization processes during an extremely long time interval.

Material and methods

Gastropod nacre (mother-of-pearl) was selected for the test of time stability of the crystallographic texture of molluscan nacre over an extremely long period of time for several reasons. First, nacre is considered to be one of simplest and also one of the oldest of all molluscan microstructures. In addition, gastropod nacre occurs only in one of several major gastropod clades which makes phylogenetic as well as taxonomic placement of studied gastropods easier (see Discussion). Nacre is formed of regularly arranged aragonite platelets (Fig. 1). In fossil molluscs, aragonite in the shell usually became unstable and transformed into the more stable calcite during diagenesis. Thus, the presence of an aragonitic shell layer is a good indicator of the preservation of the original microstructural as well as textural features in fossil shells. Findings of aragonitic shells in fossil molluscs are generally less frequent with increasing geological age. Aragonitic fossils are relatively common in the Cenozoic, rare in Mesozoic, and extremely rare in Late Paleozoic strata. No aragonitic molluscan shells have been found in Lower Paleozoic beds.

For our test, we decided to use a very old gastropod taxon with known phylogenetic position. For this reason the Late Triassic *Wortheniella coralliophila* Kittl, 1891, from the St. Cassian Formation was selected (Fig. 2). The phylogenetic position of this species was recently revised (Bandel 2009). Development of a typical protoconch (Fig. 2C, F) as well as presence of nacre (Fig. 1) in the Late Triassic *Wortheniella coralliophila* Kittl, 1891, places it in the archaeogastropod clade (Bandel 1982, Schwardt 1992, Sasaki 1998; see Discussion). The genus *Wortheniella* Schwardt, 1992, is present as several common species in the Late Triassic St. Cassian Formation, from which the studied specimens of *Wortheniella coralliophila* originated. The St. Cassian Formation has provided the most important and most diversified Triassic gastropod fauna known to date (list of papers dealing with the St. Cassian gastropods and locality data can be found in Bandel 1991, 1993a, 2007, 2009). The age of the Late Triassic *Wortheniella coralliophila* may be estimated to be about 220 Ma. The crystallographic texture of *Wortheniella coralliophila* was compared with data on living vetigastropods. Living vetigastropods have a uniform crystallographic texture (see Discussion) and therefore we present here data for only two species (Eastern Pacific *Tegula funebralis* (Adams, 1855) from the Oregon coast and *Haliotis asinina* Linnaeus, 1758, from the Queensland coast; lgt. J. Frýda), belonging to two phylogenetically distant clades of vetigastropods. The first species is a member of the family Turbinidae Rafinesque, 1815 (Turbinoidea Rafinesque, 1815), and the second of the family Haliotidae Rafinesque, 1815 (Haliotoidea Rafinesque, 1815; see Bouchet *et al*. 2005).

Gastropod shells selected for crystallographic texture analysis were gently broken to preserve information on the biological orientation of each shell fragment which may be valuable in future research. Several shell fragments of each species were studied under SEM (Fig. 1). Other fragments selected for crystallographic texture analysis were separately embedded in epoxy resin, and the biological orientation of each shell fragment was marked. Subsequently, each shell fragment was polished using a progressively finer polishing medium (down to 5 μm grit SiC followed by several minutes with 3 μm diamond, 1 μm diamond, 0.25 μm diamond, and 0.05 μm colloidal silica). Samples were cleaned in an ultrasonic bath, dried at room temperature, and then carbon coated. Electron Backscatter Diffraction (EBSD) analysis was carried out in the Department of Geochemistry at the Czech Geological Survey using a Nordlys detector connected to a CamScan scanning electron microscope. The diffracted backscatter electrons producing a series of Kikuchi bands were collected by the Nordlys detector, and the crystallographic orientation of each measured biocrystal was evaluated by the EBSD acquisition software (HKL CHANNEL 5). 1000 to 5000 measurements were carried out on each nacre sample on a regular grid with a spacing of 15 μm. The maximum size of nacreous platelets is about 15 μm (Fig. 1) and so the number of measurements roughly corresponds to the number of measured nacreous platelets in each shell sample. The orientation of each measured nacreous platelet was calculated and expressed as three Euler angles by the software package HKL CHANNEL 5. These values were subsequently recalculated as nine direct cosines for three basic crystallographic directions of aragonite (*i.e.*, [100], [010], and [001]) and crystallographic direction [101] by the software package (Škeble). These crystallographic directions were plotted in a stereographic projection (Fig. 3) using the commercial software Spheristat (Pangaea Scientific). For the main crystallographic directions, three eigenvectors were calculated (their directions and magnitudes). Eigenvalues were then used for the calculation of two Woodcock's ratios (C and K) that, in turn, served for numerical evaluation of a textural type for each group of calculated vectors (Table 1). The C value is defined as $\ln(E3/E1)$ and the K value as $\ln(E3/E2)/\ln(E2/E1)$, where E1, E2, and E3 are eigenvalues. The values of Woodcock's ratios were plotted on a modified Flinn diagram (Fig. 4).

Results

The SEM results show a well-preserved nacreous layer in all studied gastropod species including the Late Triassic *Wortheniella coralliophila* (Fig. 1). Results of texture analysis clearly indicate that the crystallographic direction [001] (*c* axis of aragonite) of all nacreous platelets in the
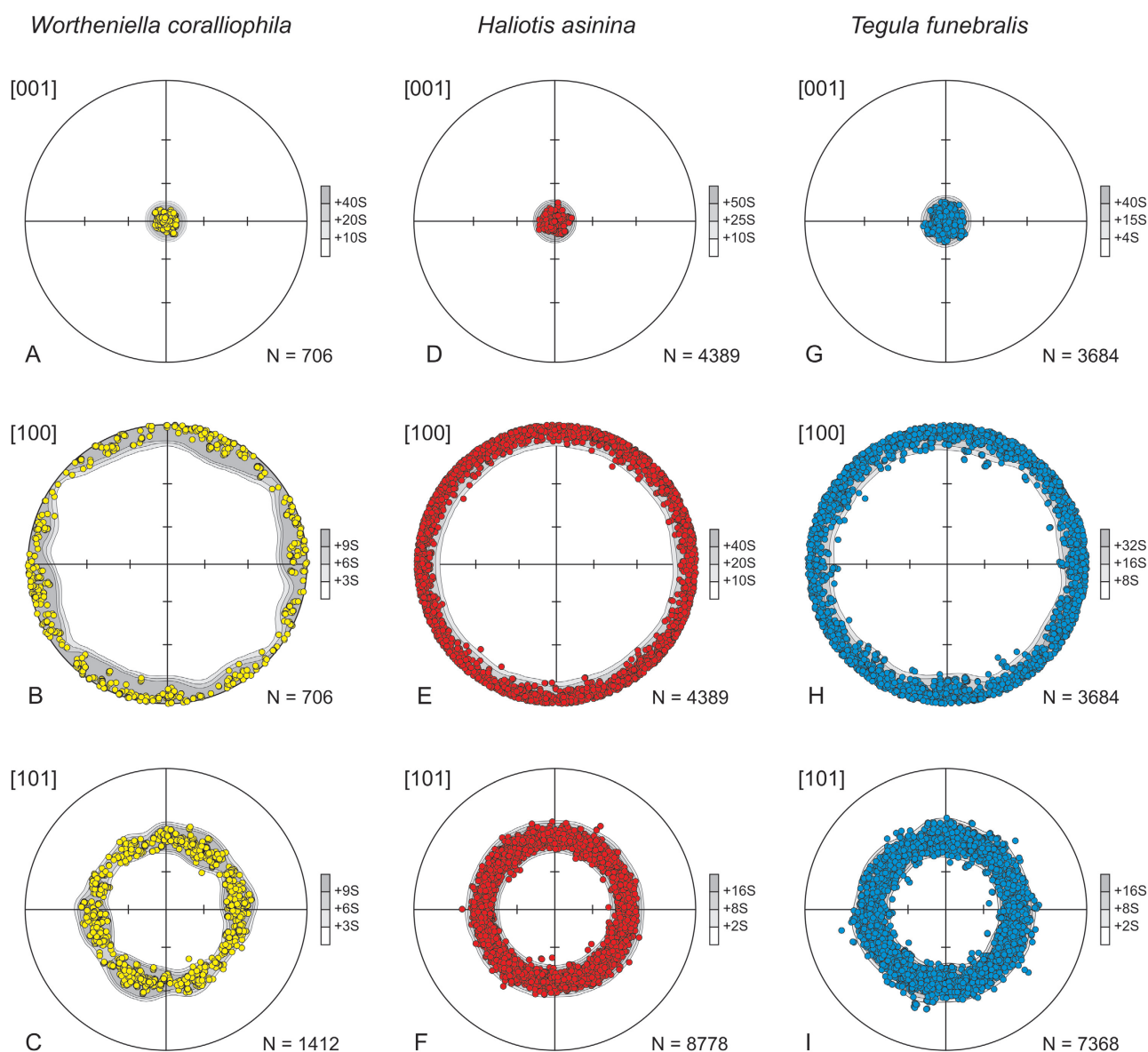
Wortheniella coralliophila   *Haliotis asinina*   *Tegula funebralis*

Figure 3. Results of crystallographic texture analysis of nacre in the Late Triassic *Wortheniella coralliophila* Kittl, 1891, and Recent *Haliotis asinina* Linné, 1758, and *Tegula funebralis* (Adams, 1855) figured in stereographic projection. • A–C – *Wortheniella coralliophila*. • D–F – *Haliotis asinina*. • G–I – *Tegula funebralis*. For all projections the direction perpendicular to the page is indentical with the direction perpendicular to the shell surface, direction from left to right is identical with growth direction of the shell (*i.e.*, adapertural direction). A, D, G – crystallographic direction [001], B, E, H – crystallographic direction [100], C, F, I – crystallographic directions [101] and [$\bar{1}$01]; see text for detailed description.

Late Triassic *Wortheniella coralliophila* is perpendicular to the shell surface (Fig. 3A). Woodcock's C value for this crystallographic direction equals 5.506(5), and is much higher than the critical value C_{95} (= 0.19) for a uniform distribution (significance level 0.05, Table 1). In other words, there is statistically significant evidence that the crystallographic *c* axes of all nacreous platelets are not uniformly distributed. Woodcock's K value for the same crystallographic direction equals 8.8(1). Both Woodcock's ratios plotted on a modified Flinn diagram clearly show that

the crystallographic axes *c* for all nacreous platelets in *Wortheniella coralliophila* form a strongly developed cluster (Fig. 4). The same results were observed for the crystallographic *c* axes of nacreous platelets in the living turbinid, *Tegula funebralis,* and the haliotid, *Haliotis asinina* (Table 1 and Fig. 3D, G).

On the other hand, the ordering of two perpendicular crystallographic directions [100] and [010] (*i.e.* the crystallographic *a* and *b* axes of aragonite) is different from that of [001]. The *a* and *b* directions have the same textural pattern
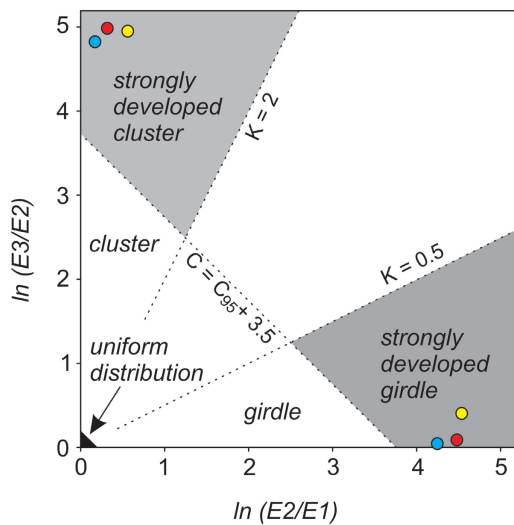
Figure 4. Calculated values of Woodcock's ratios plotted on a modified Flinn diagram. *Wortheniella coralliophila* (yellow), *Haliotis asinina* (red) and *Tegula funebralis* (blue); see text for details.

Table 1. Calculated Woodcock's ratios (C and K) for crystallographic directions [100] and [001] (*i.e.*, crystallographic *a* and *c* axes of aragonite), and the critical value C_{95} for a uniform distribution at significance level 0.05 for all studied species.

	Woodcock's Ratio					
	Wortheniella coralliophila		*Haliotis asinina*		*Tegula funebralis*	
Direction	[100]	[001]	[100]	[001]	[100]	[001]
C_{95}	0.19	0.19	0.15	0.15	0.16	0.16
C	4.941	5.506	4.561	5.298	4.282	4.986
stD	0.106	0.005	0.015	0.002	0.014	0.001
K	0.089	8.848	0.019	16.162	0.009	29.148
stD	0.002	0.134	0.000	0.125	0.000	0.205

and so only the direction [100] (*i.e.*, crystallographic *a* axis of aragonite) is discussed and plotted (Fig. 3B). Woodcock's C value for the crystallographic direction [100] in *Wortheniella coralliophila* equals 4.9(1), and is much higher than the critical value C_{95} (= 0.19) for a random distribution (Table 1). However, Woodcock's K value for the same crystallographic direction equals only 0.089(2). Therefore, both values plotted on the modified Flinn diagram show that the crystallographic *a* axes of all nacreous platelets form a strongly developed girdle (Fig. 4). The same results were observed for the living turbinid, *Tegula funebralis,* and the haliotid, *Haliotis asinina* (Table 1 and Fig. 3E, H). In other words, the crystallographic texture of nacreous platelets in all studied gastropod species is strongly uniaxial. This means that one axis (*c* axis of aragonite) is in a strongly preferred orientation and is perpendicular to the shell surface, whereas the other two perpendicular axes (*a* and *b* axes of aragonite) are randomly oriented. This textural type can be shown by a stereographic projection of crystallographic directions [001], [101] and [$\bar{1}$01] because all intersections of the latter vectors with a projection sphere appear in the same hemisphere (Fig. 3C), unlike intersections of [100] and [010] vectors. Note that the total number of plotted points for [101] and [$\bar{1}$01] vectors represents twice the number of measurements because these two crystallographic vectors could not be distinguished.

In total, the results of the textural analysis clearly demonstrate that the crystallographic texture of the nacreous shell layer in the Late Triassic *Wortheniella coralliophila* is identical with the crystallographic textures of nacre in the living turbinid, *Tegula funebralis,* and the haliotid, *Haliotis asinina* (see Figs 3 and 4).

Discussion

Nacreous microstructure is secreted only in molluscs and occurs in four of the eight living molluscan classes, in Bivalvia, Tryblidia (Monoplacophora), Cephalopoda and Gastropoda. There are two microstructural patterns of nacre, sheet and columnar, which differ in the stacking mode of nacreous platelets. The nacreous platelets form sheets in Bivalvia and Tryblidia, and columns in Gastropoda (Fig. 1) and Cephalopoda. It has been shown that molluscan classes differ in the growth mechanisms of their nacre, causing its different microstructural and textural features (Wise 1970; Wada 1972; Mutvei 1978, 1980; Checa & Rodríguez-Navarro 2001, 2005; Checa *et al.* 2008). Thus, each of the living molluscan classes has its own type of nacre (Chateigner *et al.* 2000).

The gastropod nacre is known only in one of five major living clades, in the majority of members of the Vetigastropoda. In vetigastropods, nacre is absent in some trochoideans (Phasianellidae, Halistylinae and Skeneidae) and in all species of Lepetodrilidae, Fissurellidae and Scissurellidae (Carter 1990, Ponder & Lindberg 1997, Geiger *et al.* 2008). Hedegaard (1997) suggested that gastropod nacre was derived within the Vetigastropoda. On the other hand Frýda *et al.* (2004, and in press) showed that vetigastropod nacre is homologous with the nacre in some fossil cephalopods and pointed out that nacre is plesiomorphic in vetigastropods.

Recently published data on the internal relationships among major gastropod clades [*e.g.*, Ponder and Lindberg 1997 – (Patellogastropoda (Neritimorpha (Vetigastropoda (Heterobranchia + Caenogastropoda)))) and McArthur & Harasewych 2003 – (Patellogastropoda (Vetigastropoda (Neritimorpha (Heterobranchia + Caenogastropoda))))] appear to support a derived nature of gastropod nacre, because the Vetigastropoda are interpreted as not being the basal gastropod clade. Patellogastropoda lack nacre and

are considered to be the sister taxon of the rest of the gastropods. The latter interpretation was based on analyses of morphological and anatomical data but is in conflict with the results of some molecular analyses (see references in Attipis *et al*. 2008). In addition, the interpretation of the patellogastropod fossil record seems to be also in conflict with their basal position (see Frýda *et al*. 2009 for discussion and references). The Patellogastropoda, as the first gastropod offshoot (Ponder & Lindberg 1997), had to have split off from the rest of the gastropods (*i.e*. orthogastropods) before the Early Ordovician (Frýda & Rohr 2004, Frýda *et al.* 2008a). The oldest fossil record for patellogastropods is, however, of a Late Triassic age (Hedegaard *et al*. 1997). Thus, there is no certain fossil record for the first half of the presumed patellogastropod evolution as an independent gastropod clade (*i.e*., missing data for about 250 Ma). Regardless as to whether gastropod nacre represents a plesiomorphic or derived character, it had to be lost independently in several vetigastropod lineages if the most recent model of vetigastropod phylogeny (Geiger *et al*. 2008, fig. 12.7) is to be accepted.

A direct study of the evolution of gastropod nacre is difficult because the oldest known nacreous gastropods come from the Carboniferous (*e.g.*, Bandel *et al*. 2002) and there is only a low number of Late Paleozoic gastropods with a well-documented nacreous layer. Classification of the Paleozoic archaeogastropods at the family rank is still very unstable (compare Knight *et al*. 1960, Wagner 1999, and Bouchet *et al*. 2005). Nevertheless, Geiger *et al.* (2008) mentioned Euomphaloidea as the only vetigastropod group lacking nacre in their list of "major vetigastropod groups" originating in the Paleozoic. Thus all living vetigastropods lacking nacre originated after the Paleozoic time. The phylogenetic position of the extinct Euomphaloidea is uncertain (Bandel & Frýda 1998; Nützel 2002; Frýda *et al*. 2006, 2008a). There is no reason to place them, together with all other extinct Paleozoic archaeogastropod families, within the Vetigastropoda because the latter clade is diagnosed by anatomic features that cannot be examined in extinct groups. Paleozoic Euomphaloidea as well as Macluritoidea may represent basal or sister taxa to the Archaeogastropoda (also including Vetigastropoda) as shown by their protoconch morphology (Frýda & Rohr 2006). The members of the first group lack nacre (Bandel & Frýda 1998), but there are no shell microstructure data for macluritoidean gastropods.

On the other hand, nacre was documented in species of the superfamily Porcellioidea Koken, 1895 *in* Zittel (1895), including some extinct archaeogastropods (Kiel & Frýda 2004). Bandel (1993b) was the first to recognize the connection between the Paleozoic Porcelliidae and Mesozoic Cirridae and united them in one group. The oldest porcellioidean gastropods come from Silurian strata (Frýda 1997) and they became extinct during the Cretaceous

(Bandel 1993b). Morphological disparity within the Paleozoic porcellioideans is high, uniting sinistrally coiled shells with an apertural slit (Bandel 1993b) or tremata (Frýda *et al*. 2008b) or lacking the slit (Frýda & Blodgett 1998, 2004; Frýda & Farrell 2005). Some porcellioideans have bilaterally symmetrical adult shells, which were developed secondarily from sinistrally coiled shell (Frýda & Blodgett 1998). In addition, openly coiled juvenile teleoconchs were documented in two Carboniferous genera (Bandel & Frýda 2004, Frýda 2004). The Porcellioidea have been separated from the rest of the archaeogastropods (and therefore from vetigastropods if this group already existed) since the Silurian. Thus indirectly suggesting that archaeogastropods already had nacre in the Silurian if we reject the hypothesis that nacre originated independently in two archaeogastropod groups. This interpretation would suggest that nacre was present in the oldest gastropod groups (at least since the Silurian) and may represent a plesiomorphic feature not only in vetigastropods but also in all gastropods.

The studied species, the Late Triassic *Wortheniella coralliophila,* belongs to genus *Wortheniella* Schwardt, 1992, which was recently united by Bandel (2009) with the genera *Bandelium* Schwardt, 1992 and *Rinaldoella* Bandel, 2009 and placed into the family Wortheniellidae Bandel, 2009, of the Eotomarioidea Wenz, 1938. The latter superfamily was separated from the rest of the archaeogastropods during the Ordovician (type genus *Eotomaria* Ulrich & Scofield, 1897, is Ordovician). If this model is correct, then it suggests the presence of nacre in the Ordovician (otherwise gastropod nacre had to be developed independently at least twice – in the eotomarioidean clade and in the ancestral groups of living vetigastropods).

Conclusions

New data on the Late Triassic gastropod *Wortheniella coralliophila* provides the first evidence for extremely long-term stability of a textural pattern in gastropod nacre. Extraordinary long-term stability of the morphological features of gastropod nacre has already been observed (Batten 1972, Bandel 1991, Bandel *et al*. 2002). The latter authors showed that the nacre of fossil gastropods is composed of many small platelets arranged in columns, thus having the same microstructure as the nacre of living gastropods. However our study is the first to show that not only the external shape and arrangement of nacreous platelets in living and extinct gastropods is identical, but also their crystallographic texture. The crystallographic axes of individual aragonite platelets in the Late Triassic *Wortheniella coralliophila* that lived about 220 million years ago have an identical arrangement with those in living vetigastropods. If the phylogenetic position of the genus *Wortheniella* is correct (*i.e*. a member of the superfamily Eotomarioidea

Wenz, 1938, which has been separated from the rest of the archaeogastropods since the Ordovician – see Bandel 2009 and Knight *et al*. 1960), then gastropod nacre may have remained unchanged even longer – from the Ordovician to Recent.

Extremely long-term stability of microstructural features and crystallographic texture of gastropod nacre also implies that the biological mechanisms driving nacre origin and development were extremely stable in time. Suzuki *et al*. (2009) recently discovered two novel proteins (Pif80 and Pif97) that control the crystal structure and texture of nacre in living pearl-forming oysters. Our results thus suggest that the same or similar proteins controlling the shape and orientation of individual nacreous platelets in gastropods have not changed since at least Late Triassic, thus for 220 Ma. The present study indirectly suggests that the molecular mechanisms driving the origin and development of gastropod nacre are extremely old and have remained unchanged for several hundred million years.

Acknowledgements

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