Periostracal mineralization in the gastrochaenid bivalve

Spengleria

Antonio G. Checa and Elizabeth M. Harper

1Departamento de Estratigrafía y Paleontología, Universidad de Granada, Avenida Fuentenueva s/n, Granada, 18071, Spain;
2Department of Earth Sciences, University of Cambridge, Downing Street, Cambridge, CB2 3EQ, UK

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Abstract


We investigated the spikes on the outer shell surface of the endolithic gastrochaenid bivalve genus Spengleria with a view to understand the mechanism by which they form and evaluate their homology with spikes in other heterodont and palaeoheterodont bivalves. We discovered that spike formation varied in mechanism between different parts of the valve. In the posterior region, spikes form within the translucent layer of the periostracum but separated from the calcareous part of the shell. By contrast those spikes in the anterior and ventral region, despite also forming within the translucent periostracal layer, become incorporated into the outer shell layer. Spikes in the posterior area of Spengleria mytiloides form only on the outer surface of the periostracum and are therefore, not encased in periostracal material. Despite differences in construction between these gastrochaenid spikes and those of other heterodont and palaeoheterodont bivalves, all involve calcification of the inner translucent periostracal layer which may indicate a deeper homology.

Antonio G. Checa, Departamento de Estratigrafía y Paleontología, Universidad de Granada, Avenida Fuentenueva s/n, 18071, Granada, Spain.
E-mail: acheca@ugr.es

Introduction

The periostracum is the largely proteinaceous layer that is found on the outer surface of shelled molluscs (see Saleuddin and Petit 1983; Salas et al. 2012). It is the first formed part of the shell and plays a number of critical roles in biomineralization of the calcareous portion of the shell. Key of these are the separation of the site of calcification from the contaminating effect of the ambient fluid, formation of the gross template onto which biominerals are secreted and in some taxa protection from corrosion of shell material in environments, such as freshwater or the deep sea, in which waters are unsaturated with respect to calcium carbonate (Taylor and Kennedy 1969; Harper 1997). For many taxa, it has been assumed that the outermost calcified shell layers are deposited directly onto the inner surface of the periostracum sheet and indeed this has been demonstrated within the bivalves for oysters and pterioids (Checa et al. 2005) and the astartid Digitaria digitaria (Salas et al. 2012). However, in other bivalves for example, the external granular prismatic layer of many anomalodesmatans is the product of mineralization of the internal periostracal translucent layer (Harper et al. 2009; Checa and Harper 2010) such that these layers may be regarded as intraperiostracal. In other bivalves, it has long been known that calcification may occur in isolated units also found within the periostracum (Aller 1974; Carter and Aller 1975), although Aller (1974) mistakenly suggested that calcified spikes in Laternula had been prefabricated by the mantle and introduced pre-formed into the periostracum, a supposition corrected by Carter and Aller (1975). In recent years, the number of recorded instances of such intraperiostracal calcification in extant bivalves has increased dramatically, e.g., in anomalodesmatans (Aller 1974; Checa and Harper 2010), some gastrochaenids (Carter and Aller 1975; Carter 1978), certain mytiloids (Carter and Aller 1975; Bottjer and Carter 1980) and lucinids (Bottjer and Carter 1980; Taylor et al. 2004), veneroids (Glover and Taylor 2010) and unionoids (Zieritz et al. 2011). Intraperiostracal spikes have also been reported from fossil forms, for example, in the Modiomorphidae and Permophoridae (Carter 1990; Schneider and Carter 1990).
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Their secretion was initiated by the inner surface of the outer mantle fold and that spikes grew progressively as the free periostracum moved towards the shell margin. The form of these spikes appears very similar to those which occur in many anomalodesmatan bivalves, a fact which led Carter (1978) to discuss the possibility of a close evolutionary relationship between the two taxa. Following detailed work on the morphology, crystallography and genesis of spikes in the anomalodesmatans (Checa and Harper 2010), we have taken the opportunity to undertake a comparative examination of the spikes of *Spengleria*. In particular, we used scanning electron microscopy (SEM) of shell material combined with optical and transmission electron microscopy (TEM) to focus on their relation with the different layers of the periostracum and with the shell, and compared the pattern with what is known from other spiky bivalves.

Huber (2010) records only three species of *Spengleria* but the genus, which ranges across a broad subtropical belt in Americas, Africa and the Indopacific, is in certain need of revision (Paul Valentich Scott, personal communication). In reality only one species, *Spengleria rostrata* has been studied in any detail. The thin and inequilateral valves of *Spengleria* have a marked diagonal radial line which passes from the umbo to a point on the posterior-ventral margin (Fig. 1). This line separates two different styles of surface ornamentation, with the more pronounced ornament being in the posterior sector.

**Material and methods**

**Material**

Our principal study was on material (2 specimens) of *Spengleria rostrata* (Spengler, 1783) collected from Panama City, Florida (USA) from a coral substrate at 60 m (ex Santa Barbara Museum). However, no soft parts were available to complete our study on all aspects of spike formation. As a result
we obtained an individual shell of *Spengleria* sp. (Harvey Reef, North Towartit, Sudan, Red Sea) wherein soft parts were preserved in ethanol, and also obtained the dry shells of an individual of *S. mytiloides* (Lamarck, 1818) (Gulf of Suez). All individuals were at least 15 mm in shell length. Despite the limited number of specimens examined, this combination of material allowed us to make a comparative study of spike formation within members of the genus *Spengleria*.

Microstructural details were determined from fractures of fragments coming from different areas of the shell. Fragments were cleaned for 15 s in an ultrasonic bath and allowed to air dry before SEM observation. Portions of the free periostracum of the two later species were wholly decalcified by immersion in 2% EDTA for several days and sectioned for TEM.

To examine the relationship between the mantle and periostracum, blocks of mantle tissue, along with their attendant periostracum were excised from the posterior and ventral areas of the specimen of *Spengleria* sp. from the Sudan. Unfortunately, the poor histological preservation of the specimen allowed us only to retrieve useable data from one out of six sections attempted.

**Microscopy**

Specimens observed in SEM were previously coated with carbon (Hitachi UHS evaporator). We used the field emission SEM (FESEM) Zeiss LEO Gemini 1530 of the University of Granada. Other samples were gold-coated and observed using a Jeol 820 at the University of Cambridge. Specimens for optical (OM) and TEM were completely decalcified, post-fixed in OsO4 (2%) for 2 h at 4 °C and embedded in epoxy resin Epon 812 (EMS). Semi-thin sections (~0.5 μm) were stained with 1% toluidine blue and observed with an Olympus BX51 microscope. Ultra thin sections (50 nm) were stained with uranyl acetate (1%) followed by lead citrate. They were later carbon-coated and observed with TEM (Zeiss LEO 906E and Zeiss Libra 120 Plus at the University of Granada).

**Results**

**General structure of the shell**

The external appearance and the microstructure of the shell of *Spengleria* differed in the posterior and the ventral-anterior areas of the shell, which were separated by a neat demarcation (Fig. 1). The exterior of the posterior area of the shell was covered by a distinctive light brown to yellowish thick periostracum (Fig. 1), whereas it was transparent over the rest of the shell.

In the posterior area, the periostracum was thick (e.g. 20–25 μm in *S. rostrata*) and it was arranged in wide comarginal folds (Fig. 2A–C) and densely studded with spikes that were arranged in rows either comarginally or slightly obliquely (Fig. 2B); new rows might intercalate from time to time (see Carter 1978; fig. 26). Fractures across the shell showed that in this region, the periostracum was completely detached from the underlying shell. Where the periostracum folded up into ridges there was a marked cavity between the periostracal sheet and the shell, which was filled with crystalline deposits (Fig. 2C) which varied from long slender fibres (Fig. 2D), sometimes associated into bundles, to prisms (Fig. 2E). This was described by Carter (1978) as the acicular prismatic layer. Whatever their morphology might be, these units were characteristically disoriented (Fig. 2D–G). The upper boundary of the crystalline deposits may be flat, due to the periostracum acting as a top surface (Fig. 2E–H). In the intervening areas where the periostracum was down-folded, the crystalline deposits were reduced or absent (Fig. 2C). Either below the fibrous disoriented deposits or directly exposed on the surface, there appeared large fibrous composite prisms with were reclined and elongated parallel to the local growth direction (Fig. 2G,H). These were reported by Carter (1978) as forming a composite prismatic layer extending also to the anterior of the shell (although we were not able to recognize this feature). They had an irregular distribution and might fuse, divide, initiate or disappear in the growth direction (Fig. 2I). These fibrous prismatic deposits were not massive and the units usually appeared partly isolated (Fig. 2J). Prisms frequently interfered with individual fibres (Fig. 2J). In *S. mytiloides*, there were periodic thick organic membranes (~10 μm) (Fig. 2F–I), which were parallel to the shell edge and might eventually be punctured by fibres or prisms of the overlying acicular deposits (Fig. 2G). In plan view these membranes constituted the adoral boundaries of either the thick prismatic or loose fibrous deposits, depending on which one was exposed on the shell surface. Below these deposits, a more regular shell layer of crossed lamellar (CL) layer (Fig. 2K,L) was observed. Although Carter (1978) referred also to a complex CL sublayer below the CL layer, this was not evident in our preparations. However, there was an innermost layer of fine prisms or fibres of aragonite which grew perpendicular to the shell surface. The cross-sectional outlines of these fibres elongated in parallel with the fibres of the underlying CL layer (Fig. 2K,L), which suggested that they grew epitaxially onto the latter. In the examined specimens of both *S. rostrata* and *S. mytiloides*, this finely prismatic layer extended throughout the whole internal shell surface including the very marginal area, which suggested that it might be a final deposit secreted at maturity. If Carter’s (1978) specimens were not fully mature, that might be the reason why this innermost layer remained unnoticed to him.

The anteroventral shell area was also ornamented with thin comarginal ribs with superimposed oblique rows of spikes (Fig. 3A–C). The periostracum was much thinner than in the posterior area (1–2 μm) (Fig. 3A,D) and overlay an external shell layer made of large (some are larger than 50 μm) non-composite prismatic units (Fig. 3D–F). These units tended to be perpendicular to the shell surface, although there was a certain degree of scattering. The prismatic units tended to display...
Fig. 2—A. Posterior shell area of *Spengleria mytiloides*. The thick periostracum has cracked and partly detached in the siphonal area. — B. Detail of the siphonal periostracum of *S. mytiloides*, which is densely studded with spikes. — C. Fracture through the posterior area of *S. rostrata*. The periostracum is completely detached from the underlying shell. Note acicular deposits below the convex-out folds. — D. Detail of acicular deposits below the posterior periostracum of *S. rostrata*. — E. Similar deposits in *S. mytiloides*, composed of prismatic units. — F. General view of the acicular deposits in *S. mytiloides*, which are bounded adorally (top) by an organic membrane. — G. Detail of one such membrane in *S. mytiloides*. It is punctured by adorally and aborally growing prisms. Growth direction is to the top. — H. Surface view of the shell posterior of *S. mytiloides*, with the periostracum mostly removed. Note the periodic distribution of organic membranes. The top surface of acicular deposits is flat due to the contact with the periostracum. — I. Composite prismatic deposits of *S. mytiloides*, which are limited adorally by an organic membrane. — J. Vertical section through composite prisms and associated fibres in *S. rostrata*. — K. Vertical section through the internal crossed-lamellar and fibrous layers of *S. rostrata*. — L. Surface view of an oblique fracture through the crossed-lamellar and fibrous layers. *cl*: crossed lamellar layer, *fl*: fibrous layer; *om*: organic membrane; *pvb*: posterior-ventral band.
an apparently fibrous aspect, which was provided by their mesocrystalline character. The noted scattering in orientation and the fibrous internal aspect might make this microstructure (falsely) reminiscent of either the CL or complex CL structures. Some empty cavities might remain at the junctions of prisms (Fig. 3E). In S. mytiloides, spikes continued as prisms within the shell (Fig. 3F). As noted above, the same innermost fibrous layer described in the posterior portion extended to the rest of the shell (Fig. 3C).

**Morphology, size and distribution of spikes**

Spikes were distributed in rows which were subparallel or slightly oblique to the margin in the posterior area (Fig. 2B) and oblique in the antero-ventral area (Fig. 3A,B). Rows of spikes were aligned with the periostracal crests (Fig. 3A, 4A–C), which were particularly prominent in the posterior area.

The morphology of spikes depended on the area of the shell considered, although there was also some interspecies variation. In general, spikes in the posterior area were slender and conical, with a pointed tip and they protruded well outside the shell surface (Fig. 4A–E). Their outlines were circular to pseudohexagonal. Spikes on ontogenetically older areas of the shell in S. mytiloides were more tooth-like and elongated slightly parallel to the ridge (Fig. 4D,F). In the juvenile posterior area and in the posterior-ventral band of S. mytiloides, spikes were rather highly conical (Fig. 4D,E). Fully grown spikes might protrude up to 70 μm in S. rostrata (Fig. 4A,B) and 30–40 μm in S. mytiloides (Fig. 4C–E). Spikes became blunter and much less protrusive (20 μm) towards the ventral and anterior areas (Fig. 4G,H) and were deeply rooted within the outer shell layer (Figs 3F, 4H). Some spikes might have very low relief at the ventral area of S. mytiloides (Fig. 4I).

Very commonly, spikes displayed growth lines, which were, in general, parallel to the basis of the spike (Fig. 4F), a feature also pointed out by Carter (1978).

**Formation of the posterior periostracum in Spengleria sp**

The mantle of Spengleria sp. in this area consisted of three folds (Fig. 5A), which were not necessarily homologous to the three (outer, middle and inner) mantle folds usually defined for bivalves (Yonge 1957, 1982). The periostracal groove was a small, spirally-shaped structure (Fig. 5B,C) located some 200 μm inwards from the tip of the outer mantle fold (Fig. 5A). It seemed unlikely that this spiral shape was a preservational artifact caused by the admittedly suboptimal material because this appeared to be affecting our
resolution at cellular level and not the spatial arrangement. It was noted that the well-preserved translucent part of the periostracum showed this spiral pattern and a similar topology was observed in Neotrigonia (A. Checa, C. Salas, E. Harper, personal observation). The periostracum initiated at the innermost portion of the periostracal groove as a very thin (a few nanometers thick) dark membrane (Fig. 5C), which was lined on both sides by the microvilli of the mantle cells (Fig. 5D, inset). Despite the poor preservation of the available specimen, the initiation of the dark layer within the incomplete basal cell could be recognized (Fig. 5D). Shortly, secretion of the layered translucent layer began below the dark layer (Fig. 5C). Both the dark and translucent layers increased in thickness within the periostracal groove (Fig. 5C), and this trend continued up to the very tip of the outer mantle fold (Fig. 5E). An adventitious lobe was observed at the internal surface of the outer mantle fold where the periostracum left the periostracal groove (Fig. 5B).
The translucent layer was increased by additional secretions from the external face of the outer mantle fold (Fig. 5E); these were not associated with any groove, thus being purely superficial, and wedge out some distance (~100 μm) backward in the anterior direction.

Unfortunately, spikes were not observed in this histological sample although it was clear (see below) that spikes initiated within the free periostracum.

**Relationship of the spikes with the periostracum**

With the exception of the posterior and posterior-ventral spikes of *S. mytiloides*, spikes were always covered by a thin periostracal layer which wrinkled around the spike (Fig. 4A, B). Optical and TEM revealed that the relationship between spikes and periostracum was more complex than it seemed at first appearance. The posterior periostraca of *S. mytiloides* and *S. rostrata* consisted of a single dark (tanned) layer which was internally sublayered (Fig. 6A). We did not find an evidence in the TEM of a translucent layer that is typical of other bivalves (Bevelander and Nakahara 1967; Saleuddin 1974; Checa and Harper 2010; Salas et al. 2012). The only evidence was provided by SEM observation of the free periostracum of *S. rostrata*, which had split into what might be a dark and a translucent layer, with incipient growing spikes in between (Fig. 6B). In *S. rostrata*, the dark periostracum extended around the spike, at the same time that it tended to gently wedge out towards the tip of the spike (Fig. 6C, D).

In all samples examined of *S. mytiloides*, the posterior spikes showed no sign of any periostracal cover (Fig. 4C, E, F). In juvenile posterior spikes the periostracum might extend for some distance up the spike (Fig. 4D). It was evident in both SEM and TEM that they were directly encased in a more or less shallow excavation in the dark periostracum (Fig. 6E, F) which was particularly evident where the spikes were lost during ultrasonic cleaning. Frequently, these excavations displayed minute bosses of organic material at their bottom, which were ornamented with growth lines (Fig. 6F). The consistency of the pattern observed in all samples excluded the possibility that the periostracum was lost after

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Fig. 6—A. Fracture of the posterior periostracum of *Spengleria mytiloides* showing layering. The outer surface is towards the bottom right of the image. — B. Posterior juvenile periostracum of *S. rostrata*. It is differentiated into an outer dark (dl) and an inner translucent layer (tl). The remains of a forming spike (sp) can also be seen. — C. Thin section through the decalcified posterior periostracum of *S. rostrata*, showing the periostracal envelopes around the spikes. — D. Transmission electron microscopy (TEM) detail of the same sample showing the periostracal envelope. — E. TEM view of the socket left by a detached spike of the posterior area of *S. mytiloides*. No outer periostracal envelope can be discerned. — F. Posterior periostracum of *S. mytiloides*. Many spikes have detached upon ultrasonication revealing that sockets left frequently display bosses. The inset is a detail of one such socket and associated bosses. — G. Posterior shell edge and corresponding free periostracum of *S. rostrata*. — H. Detail of the area labelled H in G, with forming spikes. — I. Detail of the area labelled I in G, in which spikes have not yet begun to grow. — J, K. Free posterior periostracum of *S. mytiloides* seen in plan view (J) and from the shell edge (K). The inset in J shows an initial nucleus, the surface of which is in level with that of the periostracum. — L. Detail of the area labelled L in J. With growth, spikes enlarge and protrude progressively from the periostracum.
spike formation or that its absence was an artifact of preservation.

In both *S. mytiloides* and *S. rostrata*, the dry free periostracum was initially devoid of spikes and thickened from its initiation towards the shell edge. In our specimens of *S. rostrata*, spikes became fully grown within a matter of three to four rows (Fig. 6G–I), whereas in that of *S. mytiloides*, this happened after some seven rows (Fig. 6J–L). Since the initial periostracum was completely smooth, it was evident that the periostracal ridges developed with spike growth.

In *S. mytiloides*, the surface of the initial spikes was exposed (Fig. 6J,L), with no trace of any periostracal cover. These surfaces displayed etching lineations meeting at around 60°, which were similar to those described in biogenic aragonite by Checa *et al.* (2009) and Checa and Harper (2010) (Fig. 6], inset) and which corresponded to traces of the crystallographic a-axes of aragonite. Therefore, initial prisms were \{110\} polycyclically twinned crystals with the c-axis perpendicular to the surface of the periostracum. Initial spikes (those of the first four rows) had their surfaces at the same level as that of the periostracum and began to protrude before reaching their full size (Fig. 6J–L). It was not clear if the high degree of protrusion of fully-grown spikes was the original state or if it was enhanced due to later contraction by drying of the periostracum.

Towards the ventral and anterior shell sides, the periostracum in *S. sp.* and *S. mytiloides* was clearly two layered under TEM, with an outer dark and an inner translucent layer (Fig. 7A–E). It was clear that the dark layer thickened at the expense of the translucent layer (Fig. 7A–C). Observations of the relationships between the periostracal layers and the spikes revealed two types. Close to the posterior-ventral demarcation area (*perv* in Figs 1, 2A), the dark layer completely underlies the spikes (Fig. 7F). In the examined sample of *S. rostrata*, the dark layer also extended onto the spike (Fig. 7F). The same applied to *S. sp.*, but not to *S. mytiloides*, in which there was no periostracal cover. In the ventral and anterior areas, this pattern changed and the spikes were underlain directly by the translucent layer, and in all species, they were covered by a thin dark periostracal layer which thinned towards the spike centre (Fig. 7D,E), in a way similar to that described in anomalodesmatans by Checa and Harper (2010).

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**Fig. 7**—A. Transmission electron microscopy (TEM) detail of the initial free periostracum of *Spengleria* sp., showing the superposition of the dark and translucent layers. — B. General view of the ventral free periostracum of *Spengleria* sp., with growing spikes. The increase in thickness of the dark layer is noteworthy. — C. Detail of the periostracum (frame labeled C in B) of *S. sp.* to show the dark and translucent layers. — D, E. Forming spikes of the ventral areas of *S. sp.* and *Spengleria mytiloides*, respectively. In both cases, the dark layer extends above the spike, but not below, where it is underlain directly by the translucent layer. D is a higher magnification of the area framed in B. — F. Detail of a forming spike of the posterior-ventral area of *Spengleria rostrata*. The dark layer of the periostracum extends below the spike and separates this from the translucent layer. The inset shows a detail of the layered translucent layer. *dl*: dark layer; *tl*: translucent layer.
Discussion

Our observations on the shells and mantle margins of *Spengleria* spp. have allowed us to propose a model for spike formation, summarized in Fig. 8.

Formation of the periostracum and spikes

The periostracum of *Spengleria* initiates at some distance from the tip of the outer mantle fold, within the basal cell and already within the periostracal groove. As in other bivalves, it consists of two layers, an outer dark layer and an inner translucent layer (Fig. 5A–D), with the observed increase in thickness of the dark layer (Figs 5C, 7B) being caused by tanning of the underlying translucent layer (Bevelander and Nakahara 1967; Neff 1972; Bubel 1973; Saleuddin 1974; Checa and Harper 2010; Salas et al. 2012). A feature as yet unique to *Spengleria* is that the translucent layer can be thickened by additional secretions of the external surface of the outer mantle fold (Fig. 5E).

The relationship of the spikes and the periostracal layers is different both between species examined and within different areas of individuals, although in the latter case, the differences are between anterior and posterior sectors of the shell and are consistent within a species. In the antero-ventral sectors of all species, the relation between spikes and the periostracum and their mode of growth is very similar to that reported for the anomalodesmatans by Checa and Harper (2010). At a given
moment, a spike nucleates at the contact between the dark and the translucent layers (Figs 6B, 8A). With time, the spike expands and grows at its base, at the same time that it protrudes progressively from the surface. Growth proceeds around the spike, except at those positions in which it is in contact with the dark layer, i.e. its outer surface. With time, lateral expansion causes the newly secreted parts of the outer surface to come into contact with the overlying dark layer and thus cease to grow. This process explains the formation of the typical cone-like endings of spikes (Figs 4A,B, 7B; see Checa and Harper 2010; fig. 12). Later if the translucent layer mineralizes while spikes are still growing within it, these will finally become incorporated within the outer shell surface (Fig. 8B) as observed in actual specimens (Figs. 3F, 4H). In the specimens examined here, the translucent layer is relatively thin (Fig. 7D,E). It is therefore, hardly conceivable that the large prismatic units of the outer layer, form entirely within the translucent layer, but they may, at least, initiate within it.

In the posterior-dorsal sector of *S. rostrata*, the spikes become separated from the translucent layer by an underlying dark sublayer (Figs 7F, 8C). This prevents their further growth. In the likely, though not observed, event that the translucent layer is later able to mineralize, the spikes would remain isolated within the periostracum, far from contact with the outer shell layer (Fig. 8D). This is probably the case for the posterior-ventral area of the above-mentioned species. In fact, Carter et al. (2012, fig. 143) illustrated a fracture through the postero-ventral shell area of *S. rostrata*; in this specimen, upon desiccation, the periostracum and its encased spikes have detached from the underlying outer shell layer (into which the spikes were shallowly encased); this could not be the case if the spikes were directly attached to the shell and argues for an intermediate periostracal membrane. In the posterior (siphonal) area the periostracum consists of only the dark layer (possibly by transformation of the translucent layer if there was any initially) (Fig. 4B), which will no longer be able to mineralize into an outer prismatic layer. Accordingly, the deposits immediately below would be of the acicular and composite prismatic types (Figs 2C–J, 8E), produced by remote biominalization (Chinzei and Seilacher 1993).

Our observations demand that a totally different explanation has to be provided for the spikes of the posterior and posterior-ventral areas of *S. mytiloides*. Here, the spikes are located within the periostracum but free from any mid and distal periostracal cover and directly exposed on the outer surface (Fig. 4C–F). Since this observation is true even within the free periostracum, it seems most unlikely that there is an initial periostracal cover that has been lost through wear. This fact cannot be attributed to natural or induced bleaching since even the finest surface features are evidently well preserved (e. g. Fig. 6K–L). The only possibility we can entertain is that they are secreted by the mantle epithelium lining the outer periostracal surface. This could either be the outer surface of the middle mantle fold or, most probably, the outer surface of a lobe adventitious to the outer mantle fold (Fig. 8F), similar to the one observed in *Spengleria* sp. (Fig. 5B). Note that the external surface of early spikes is in level with that of the periostracum (Fig. 6J), probably due to the adhering mantle surface preventing them from protruding (Fig. 8F, left). If the adventitious mantle fold does not extend onto the periostracum in this area, the spikes are then free to protrude from the periostracum (Figs 6K,L, 8F, right).

Further development of spikes in the posterior-ventral band of *S. mytiloides* is envisaged as similar to those in *S. rostrata* (Fig. 8D), although the spikes will not finally show any trace of overlying periostracum (Figs 4D,E, 8G). The same is true of the posterior spikes of *S. mytiloides* (Fig. 8H) with regards to the equivalent spikes of the other species (Fig. 8E). This pattern for spike growth in the posterior area of *S. mytiloides* fits with the more columnar aspect of posterior spikes in this species when compared with the conical spikes of *S. rostrata*. In the latter species, spikes basically grow at their bases, where the translucent periostracum is present. In *S. mytiloides*, growth would predominantly be at the tip and at the sides, although some growth at the base has to be implied to explain the increasing depth of rooting of spikes within the periostracum with growth, judging from the depth of the sockets left by detached spikes (Fig. 6F,K,L). Partial embedding of the spikes within the periostracum could also be explained if the mantle epithelium facing the spikes also secreted a translucent layer which later thickened the dark layer on its outer side (Fig. 8F). If spikes were also able to absorb part of the underlying periostracum, the growth lines imprinted on the bosses frequently found at the bottom of cavities (Fig. 6F) could easily be related to the stepped inwards movement towards the periostracum interior. The bosses themselves would be non-absorbable zones of the periostracum, similar to those observed in unionids (personal observation). A combination of these explanations is most likely, since it would account for all the observed features.

Shell formation of *Spengleria* spp

As with spike formation, the mode of shell calcification is also different depending on the shell area. In the siphonal area, the first shell deposits are not in continuity with the thick periostracum (Fig. 2C), which, as noted above, is no longer able to mineralize due to the lack of a translucent layer. Below the periostracum there are composite prisms which either are covered or uncovered by acicular deposits (Fig. 2H,I). These prisms are formed in contact with the mantle but not with the periostracum. This is demonstrated by the fact that they display comarginal growth lines (Fig. 2H) and at least in *S. mytiloides*, are adorally bounded by organic membranes, which are in turn parallel to the shell edge (Fig. 2F–I) (thus presumably replicated by the mantle margin during eventual growth halts). The irregular distribution and morphology of composite prisms is the product of them growing in an open space between the underlying normal shell layers and the periostra-
cum. The cavities left between the composite prismatic deposits and the undulating periostracum are filled with fibrous disorganized deposits (Fig. 2C-J), obviously formed out of the contact with the mantle. They are formed by passive precipitation of crystals within the extrapallial liquid-filled space; they would form at the same time or after the mantle has left their position and is in a more advanced shell growth stage. Their later growth is demonstrated by the fact that some needles and prisms puncture the organic membranes bounding the prismatic deposits in both adoral and aboral directions (Fig. 2G). Also, some fibrous reclined prisms are later able to intercept the aciculae (Fig. 1J). The acicular deposits are thus an instance of remote biomineralization.

For the rest of the shell, the outer coarsely prismatic shell layer is directly in contact with the periostracum (Fig. 3D,F). Here, the spikes are continuous with the outer prismatic layer (Fig. 3F) as in other groups (anomalodesmatans, Neotrigonia). The internal crossed-lamellar and fibrous layers finally extend throughout the whole shell.

**Comparison with other groups of spiky bivalve**

Our observations of the structure of the periostracum and its relationship with the forming spikes and shell have not resolved into a single common pattern for their formation. In the antero-ventral part of the shell, the pattern is very similar to the one described in the anomalodesmatans, in which the spikes are integrated into the shell via common calcification wholly or partly within a translucent periostracal layer (Checa and Harper 2010). Although not studied in detail, these similarities appear also to be shared by the spikes of unionoids (Zieritz et al. 2011) and the bosses of Neotrigonia (personal observations). By contrast, on the posterior side, spikes are encased in the periostracum, which is, in turn, out of the contact with the shell. This pattern, not yet been observed in any other bivalve, appears rather specialized; its functionality is not yet clear.

The similarities between the antero-ventral spikes of Spengleria and spikes of other bivalves are tantalizing perhaps offering support to the suggestion that gastrochaenids might be closely related to the anomalodesmatans as proposed by Carter (1978) and Morris et al. (1991) who noted the similarities in morphology between gastrochaenids and the extinct permorphids. However, molecular evidence has failed to substantiate this model, despite there being no stable position for Gastrochaenidae in the number of molecular analyses that have included Gastrochaena (Spengleria itself has not been included to date), there has been no close relationship with anomalodesmatans (Campbell 2000; Giribet and Wheeler 2002; Giribet and Distel 2003; Taylor et al. 2007), although a combined parsimony analysis of 18S and 28S rRNA by Taylor et al. (2007) did show such a relationship (not produced by either gene alone) but the support was poor.

It seems clear that the intraperiostracal spikes within the heterodont bivalves are not homologous characters. However, basic similarities in the mechanisms by which they form may result from some deeper plesiomorphy. Of particular interest is their association with the inner translucent layer of the periostracum which appears susceptible to calcification. Further research is required to establish whether the translucent layer is homologous between heterodont bivalve taxa.

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**References**


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