How the mollusc got its scales: convergent evolution of the molluscan scleritome

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Abstract

Uniquely among gastropods, the scaly-foot gastropod carries numerous dermal sclerites of unknown origin. The vestigial operculum, previously reported to be absent in this species, is reduced and straightened, but present in all individuals. Comparison with sclerites of the chitons (Polyplacophora: Chitonoidea) revealed stark differences in secretion mechanism and composition of the scales, despite superficial similarity. The scaly-foot sclerites are secreted in layers covering outpockets of epithelium and are proteinaceous, while chiton sclerites are secreted to fill an invaginated cuticular chamber and are calcareous. Marked differences in underlying epithelium of the scaly-foot gastropod's sclerites and operculum suggest that the sclerites do not originate from multiplication of the operculum. This convergence highlights the ability of molluscs to adapt mineralised dermal structures, which is supported by an extensive early fossil record of molluscs with complex scleritomes. Sclerites of Cambrian taxa with molluscan affinities are also morphologically variable; this may cast doubt on the affinity of specific taxa to chitons, or the larger clade Aculifera. Comparisons with independently-derived similar structures in living molluscs is informative for inferring homology among fossils and their position with respect to the enigmatic evolution of molluscan shell forms in deep time.

Keywords

Scaly-foot gastropod, Polyplacophora, Aculifera, *Wiwaxia*, *Halkieria*

Introduction

The scaly-foot gastropod is the only known extant or extinct gastropod with dermal sclerites, which cover the foot, imbricating like roof-tiles (Warén *et al*., 2003; Fig. 1A). It was first recovered from hydrothermal vents of the Kairei vent field, Central Indian Ridge, Indian Ocean (Van Dover *et al*., 2001), and has since been found at two more Indian Ocean vent fields (Nakamura *et al*., 2012; Tao *et al*., 2012; Tao *et al*, 2014). The sclerites attracted great interest as they were coated in a layer of iron sulfide (pyrite FeS_2 and greigite Fe_3S_4) and were assumed to have a defensive function against predators (Warén *et al*., 2003). Suzuki *et al*. (2006) proposed that the mineralisation of the sclerites are likely to be mediated by the gastropod through controlling concentration of sulfur compounds. Iron biomineralisation is however not always observed, as one population does not have an iron sulfide layer (Nakamura *et al*., 2012). Although the species is thus far not formally described, and the true function of the sclerites is unclear, both genetic and morphological investigations reliably place scaly-foot gastropods within Peltospiridae (Warén *et al*., 2003), a family in the clade Neomphalina (previously included in Vetigastropoda but now considered as a separate clade).

Mollusca is a very diverse phylum and the extant classes exhibit a great disparity in body armour and shell forms. For example, Polyplacophora (chitons) have eight shell plates compared with two shells in Bivalvia and one in most gastropods and some cephalopods (which has been reduced or lost in some groups; Furuhashi *et al*., 2009). Scale-like structures are uncommon among the extant mollusca and were thought to be a distinctive characteristic uniting a proposed clade 'Aculifera' (Caudofoveata + Solenogastres + Polyplacophora; Sigwart & Sutton, 2007). The scaly-foot sclerites are superficially very similar to those of other molluscs, especially sclerites on chiton girdles (Fig. 1). Given the distant phylogenetic position of the two and that no other gastropods possess sclerites, these are unlikely to be directly homologous structures.

In addition, the similarity of scaly-foot gastropod sclerites to those of Cambrian metazoans has also been suggested (Warén *et al*., 2003; Suzuki *et al*., 2006). Many groups of Cambrian metazoans are known for having dermal sclerites (e.g., halkieriids, tommotiids, sphogonuchitids, etc.; Bengtson, 1992), and one group, the halwaxiids, has been proposed by some to represent stem-group aculiferans (Vinther & Nielsen, 2005; Vinther, 2009). Halwaxiida is a clade of metazoans characterised by a body covered by sclerites and many have shell plates. The most notable members are *Halkieria*, with two shells, one on the anterior end and one on the posterior end; *Wiwaxia*, with no shell (Fig. 1E); and *Orthrozanclus*, with only one anterior shell. The systematic position of halwaxiids has been extensively debated, and has been argued to be related to annelids, brachiopods, or molluscs. One important argument of halwaxiids' molluscan affinity has been the similarity of sclerites to those of the Mollusca, especially chitons. Vinther (2009) described a complex canal system in *Sinosachites delicatus* (Jell, 1981) and noted its resemblance to chiton aesthetes. Recently the idea of halwaxiids as early molluscs has been reinforced by similarities of *Wiwaxia* mouthparts to modern molluscan radulae (Smith, 2012), yet sclerites continue to be a central piece of evidence tying halwaxiids to molluscs (Smith, 2014). From evidences of merged sclerites in halkierrids, it has also been proposed that shell plates of molluscs may have arisen from sclerites fusing together (Missarzhevsky, 1989; Bengtson, 1992).

The discovery of the scaly-foot gastropod implied that dermal sclerites may be a rapidly evolving, derived structure, and brings the homology of halwaxiid and molluscan sclerites to question, as mentioned briefly by Warén *et al*. (2003) and Smith (2014). Although it has been suggested in the past that the scaly-foot sclerites are homologous to an operculum and represent an operculum duplication event (Warén *et al*., 2003), the discovery of a true operculum in scaly-foot gastropods (Nakamura *et al*., 2012), casts doubt on this hypothesis. In this study, the anatomy of scaly-foot gastropods' sclerites is investigated to shed light on its origin and how it compares with its operculum, as well as similar structures in aculiferans.

Methods

Over 300 specimens of the scaly-foot gastropod, ranging from juveniles of 5-10 mm shell length to adults of up to 45 mm shell length, from all three known localities were examined: Longqi vent field (Tao *et al*., 2014), Southwest Indian Ridge, 37°47.03'S 49°38.97'E ('Tiamat Chimney'), depth 2785 m, on-board RRS *James Cook* expedition JC67 with ROV *Kiel 6000* fixed and stored in 4% formalin (around 200 specimens); Solitaire vent field, Central Indian Ridge, 19°33.41'S, 65°50.89'E, depth 2606 m (about 100 specimens); Kairei vent field, Central Indian Ridge, 25°19.24'S, 70°02.43'E, depth 2415 m to 2460 m (20 specimens); Central Indian Ridge specimens are from the collections of Japan Agency for Marine-Earth Science and Technology, fixed and stored either in 4% formalin or 99% ethanol.

For scaly-foot gastropod specimens, specimens from the three known localities were used to examine the ontogeny of vestigial operculum. For detailed morphological examination, the operculum and adjoining tissue, including sclerites were dissected from the foot of adult and juvenile specimens from the Longqi vent field. For chiton specimens, a section of the girdle was removed from the right side of the body adjacent to valve II. The dissected tissues were decalcified in 2% EDTA for 48 hours and prepared for sectioning following the methods of Ruthensteiner (2008): implementing dehydration in an acetone series, embedding in Epon expoxy resin following the manufacturer's instructions (SIGMA 45359 Epoxy Embedding Kit), and serial sectioning at 1.5 µm using an automated rotary microtome (Leica RM2255) fitted with a diamond knife (DiaTome HistoJumbo 8 mm). Resulting sections were stained with the high contrast monochromatic Richardson's stain and examined with a compound microscope (Olympus BX41) with digital images captured via a SLR camera attached to the microscope trinocular (Olympus E-600).

Results and Discussion

The examination showed that the scaly-foot gastropod sclerites (Fig. 1A,B) are clearly constructed in very different manner to chiton scales (Fig. 1C,D), and are made of different materials. The superficial similarity of these scleritomes is limited to a functional convergence, and not indicative of any apparent anatomical homology.

Polyplacophoran scales are highly variable among chiton species, but are predominantly formed of aragonite (Eernisse & Reynolds, 1994). For example, *Enoplochiton niger* has prominent large aragonitic calcareous scales embedded within a cuticular layer covering the girdle muscle block (Fig. 1C). These scales do not form on any kind of stalk but sit on a compressed secretory epithelium, which sits at the bottom of a pocket within the cuticle, and the scales appear to grow through secreting calcareous material to fill the cavity (Blumrich, 1891; Fig. 1D). These large scales are typical of the superfamily Chitonoidea; other species have various structures but all grow through the same approach of an infilled pocket of epithelium underlying the girdle cuticle (Blumrich, 1891; Leise & Cloney, 1982).

Through the cross-section of a decalcified scaly-foot gastropod sclerite (Fig. 1B), clear growth lines are visible. The sclerites appears to not contain calcareous material, but instead are made almost entirely of conchiolin as previously reported (Warén *et al.*, 2003).

The sclerite contains a projection of pedal tissue from the base to approximately 1/3 of the length to the tip. The tissue is mainly pedal musculature penetrated by nerve fibres, on the contact surface with the sclerite a single layer of tall columnar epithelial cells is present. The sclerite appears to be secreted in layers covering the epithelial layer, and it thus grows by a new layer pushing older layers outwards.

Clear differences in the underlying epithelium suggest that the sclerites do not represent multiplication of the operculum (Fig. 2B). The scaly-foot gastropod operculum is attached to the foot in a layer of epithelial cells, which stops abruptly at the end of attachment. The operculum is attached to the foot by a small pad, and the anterior edge rests in a pocket surrounded by tall columnar epithelial cells, which are approximately double the height of those at the operculum attachment.

In adults, the operculum is effectively buried in layers of sclerites (Fig. 2A). Initial investigations did not distinguish the operculum from the surrounding sclerites (Warén *et al*., 2003), and a more recent report suggested that only about half of the adults possessed an operculum (Nakamura *et al*. 2012). Careful examination of adults from all three populations revealed that in fact all have an operculum. The adult operculum has a very relaxed coiling and elongate shape, but in juveniles it is multispiral and circular (as reported in Nakamura *et al*., 2012 for a population from Solitaire Field; Fig. 2C); this is confirmed to be the case for all three populations examined here.

A major question remains as to the anatomical origin of the scales; if the secretory epithelium and structure of the sclerites does not correlate to the structure of the operculum, the scales apparently represent an independent mineralisation of other aspects across the surface of the foot. Peltospirids, and the closely related gastropod clade Vetigastropoda (Aktipis & Giribet, 2012), are notable for repeated emergence of tentacles around the operculum and the on the posterior part of the foot (McLean, 1989; Warén & Bouchet, 1989). The tentacles are highly elastic and typically considered to be primarily sensory in function (Fretter, 1989), yet they also represent plastic multiplied outpockets of epithelium widespread across the foot.

The presence of sclerites has been a central theme in arguments about the placement of certain enigmatic Cambrian fossils within the Mollusca (Smith, 2014). Although the molluscan affinities of *Halkieria* and *Wiwaxia* among others is based on multiple lines of evidence (Vinther, 2009; Smith, 2012), the complex dorsal scleritomes on these taxa (Fig.

1F) have been used to compare them directly with living representatives of Polyplacophora and spiculose aplacophorans (Vinther, 2009). The scales of the scaly-foot gastropod have also been compared to these Cambrian fossils and do share obvious similarities (Warén *et al*., 2003; Figure 1).

These fossil taxa, broadly grouped as Halwaxiida (Conway Morris & Caron, 2007), represent a broad morphological diversity of individual sclerites within and among taxa, similar to the structural diversity observed within other classes of molluscs. *Halkieria* and sachtid sclerites have a central spine that had a tissue-filled canal (Vinther, 2009), which are potentially more analagous to the scales of the scaly-foot gastropod than cuticular elements in chitons or aplacophorans.

The distinctive armour of the scaly-foot gastropod has inspired extensive research (Suzuki *et al*., 2006; Yao *et al*., 2010) and has been a subject of interest to the wider public (e.g., Barley, 2010). These ongoing studies will provide a sound basis to understand the anatomical origin of mineralised structures in the scaly-foot gastropod and other molluscs, and the interpretation of such structures in the deep molluscan fossil record. The presence of sclerite elements has multiple independent evolutionary origins, possibly driven by myriad adaptations on the notably plastic molluscan bauplan.

Molluscs *sensu lato* are capable of creating diverse mineralised dermal structures, including two classes of aplacophoran molluscs (Solenogastres, Caudofoveata), chitons, and other gastropods such as the spiculose Acochlidea (Rankin, 1979; Jörger *et al*., 2010). The relationships among the living classes of molluscs remain contentious (e.g. Smith *et al.*, 2011; Stöger *et al*., 2013; Sigwart & Lindberg, 2014). Fossils are an essential line of evidence to establish the topology of molluscan phylogeny, which remains one of the major questions in metazoan evolution (Telford & Budd, 2011; Telford, 2013; Sigwart & Lindberg, in press). The affinity of halwaxiids to any specific class within Mollusca requires more than a 'just so' story, and a detailed analysis of morphological homology.

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Figures

Figure 1. (A) Apex view of a scaly-foot gastropod showing the shell and numerous sclerites, (B) Semithin central section through a fully grown scaly-foot gastropod sclerite showing dermal tissue canal penetrating the scale and layering of the scale conchiolin, (C) *Enoplochiton niger* showing sclerites on the girdle (ZSM Mol-20034094, La Heradura, Chile, coll. 17 Nov. 2003), (D) Semithin section of the same specimen (*E. niger*) girdle sclerite, (E) *Wiwaxia corrugata* Walcott, 1911 (ROM 61510), (F) Cross section of *W. corrugata* sclerites (ROM 62270). Scale bars: 10 mm (A, C), 5 mm (E), 1 mm (B, F), 0.5 mm (D). Parts (E) and (F) reproduced from Smith (2014) with permission from the Palaeontologial Association.

Figure 2. (A) Operculum of an adult scaly-foot gastropod seen *in situ*, with layers of sclerites above the operculum removed, (B) Semithin section through the scaly-foot gastropod operculum and scales with arrowhead indicating proximal end of operculum attachment, (C) Ontogeny of scaly-foot gastropod operculums: from top juvenile, sub-adult, adult specimens. Scale bars: 5 mm (A), 1 mm (B), 2 mm (C).

Figure 1

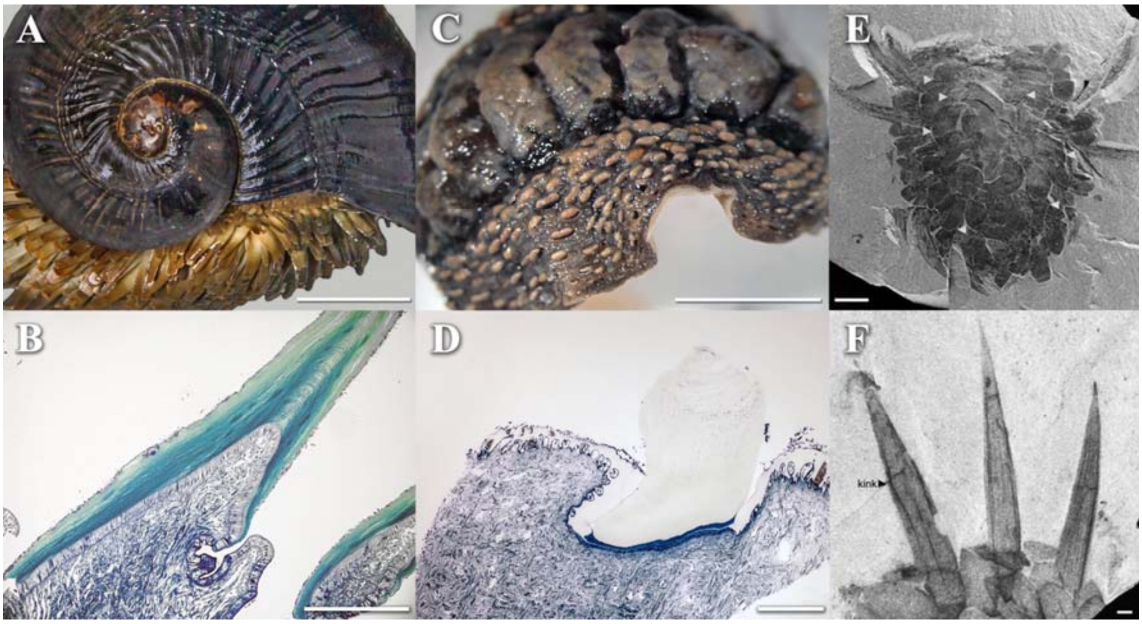

Figure 2