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# Cuticular reticulation replicates the pattern of epidermal cells in lowermost Cambrian scalidophoran worms

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The cuticle of ecdysozoans (Panarthropoda, Scalidophora, Nematoida) is secreted by underlying epidermal cells and renewed via ecdysis. We explore here the relationship between epidermis and external cuticular ornament in stem-group scalidophorans from the early Cambrian of China (Kuanchuanpu Formation; ca 535 Ma) that had two types of microscopic polygonal cuticular networks with either straight or microfolded boundaries. Detailed comparisons with modern scalidophorans (priapulids) indicate that these networks faithfully replicate the cell boundaries of the epidermis. This suggests that the cuticle of early scalidophorans formed through the fusion between patches of extracellular material secreted by epidermal cells, as observed in various groups of present-day ecdysozoans, including arthropods. Key genetic, biochemical and mechanical processes associated with ecdysis and cuticle formation seem to have appeared very early (at least not later than 535 Ma) in the evolution of ecdysozoans. Microfolded reticulation is likely to be a mechanical response to absorbing contraction exerted by underlying muscles. The polygonal reticulation in early and extant ecdysozoans is clearly a by-product of the epidermal cell pavement and interacted with the sedimentary environment.

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## 1. Introduction

The epidermis is a fundamental cellular tissue of Metazoa [1] which acts as an interface between the external medium and the body interior [2]. It includes epithelial cells and extracellular material. Epithelial cells form a tissue with complex junction systems and secrete two types of extracellular substrates: basally, the extracellular matrix (ECM), and apically, the glycocalyx (layers of proteins and sugar) and the cuticle [2]. Although absent in placozoans and ctenophores, the glycocalyx plays a crucial role as a matrix on which other extracellular molecules (e.g. chitin, collagen) can anchor and eventually form a cuticular structure. A cuticle occurs in all present-day ecdysozoans (Nematoida, Scalidophora, Panarthropoda), with a consistent three-layered structure [2,3]. Ecdysozoans display a great variety of cuticular structures often associated with sensory organs (e.g. setae). Cuticular moulting is common to all extant representatives of Ecdysozoa and is a key diagnostic feature of the group. Exuviae found in early Cambrian scalidophoran worms from China (ca 535 Ma [4]) suggest that ecdysis has a very ancient origin and evolves through a suite of genetic innovations in the three ecdysozoans clades (Scalidophora, Nematoida, Panarthropoda [4,5]). Cambrian scalidophorans have very diverse cuticular features that are best preserved in Burgess Shale-type Lagerstätten [6,7] and small carbonaceous fossil (SCF) [8] and small shelly fossil (SSF) [9,10] assemblages in which (SSF) scalids, platelets and pharyngeal teeth are preserved in three dimensions as exemplified by stem-group scalidophorans such as Eopriapulites, Eokinorhynchus [9,10] and palaeoscoleccids [11–14]. These microscopic cuticular elements have been studied in detail in various groups of early Cambrian scalidophorans and provide diagnostic characters useful to their classification. Recently, Wang et al. [4] found a new type of micro-ornament in scalidophoran worms from the early Cambrian Kuanchuanpu Formation (ca 535 Ma). It consists of very fine reticulation on both the external and internal surface of exuviae. The relationships between the external cuticular features and

the underlying epidermal tissues of ancient animals have been largely overlooked. In extant arthropods such as centipedes, the polygonal surface pattern of the cuticle faithfully reproduces the geometry of the underlying epidermal cells [15]. A comparable epidermis-to-cuticular correspondence has been established in crustaceans such as ostracods [16,17], isopods [18] and decapods [19], but has never been observed in other ecdysozoans groups such as the Scalidophora and the Nematoida. Here, we describe two types of micro-reticulate cuticular patterns in lowermost Cambrian scalidophoran worms from the Kuanchuanpu Formation, which have direct counterparts in extant priapulidworms such as *Priapulus caudatus* and *Halicryptus spinulosus*, suggesting that the reticulation observed on the external surface of the Cambrian worms is the direct expression of the epidermis cellular pavement. Our results provide key information on the cuticle formation of basal ecdysozoans that complement recent studies on their moulting process [4].

## 2. Material and methods

### (a) Fossil material

The studied specimens were collected from Bed 2 of the early Cambrian Kuanchuanpu Formation (Fortunian Stage, Terreneuvian Series) at the Zhangjiagou Section near Dahe town, Xixiang County, Shaanxi Province [20]. The Xixiang area was palaeogeographically located on the northwestern margin of the Yangtze Platform during the Ediacaran and Cambrian periods. The fossilbearing horizon correlates to the *Anabarites trisulcatus*–*Protohertzina anabarica* assemblage biozone [21], which indicates the Meishucunian Stage (equivalent to the Fortunian Stage). U–Pb dating of zircons from tuff layers (Meishucun Section, Yunnan Province) gives an estimated age of  $536.5 \pm 2.5$  Ma [22]. Several horizons of the Kuanchuanpu Formation have yielded abundant secondarily phosphatized SSFs that are extracted from calcareous rocks through digestion in 8–10% acetic acid [23] and picked out from residues under a binocular microscope. These SSF assemblages contain abundant fossils with cnidarian affinities [24,25], possible deuterostomes [26], diverse scalidophoran worms [9,10,27,28], cyanobacteria [29] and problematic forms [30]. Our study is based on 22 specimens in which cuticular ornament is particularly well preserved. All specimens are deposited in the collections of the Early Life Institute of Northwest University, Xi'an, China (see electronic supplementary material, table S1). These specimens were mounted on stubs, coated with gold and observed under a FEI Quanta 400 FEG Scanning Electron Microscope (SEM; high vacuum and 20 kV) at the State Key Laboratory of Continental Dynamics (SKLCD), Northwest University, China. X-ray microtomography (MicroCT) was performed at SKLCD in order to reconstruct the three-dimensional morphology of the reticulate microstructures of two specimens (ELIXX93-335 and ELIXX95-85). MicroCT images were processed with 'Dragonfly 4.0' software.

### (b) Biological material

*Priapulus caudatus* was collected from the Gullmarsfjord ( $58^{\circ}15'30''$ – $58^{\circ}16'39''$  N;  $11^{\circ}25'36''$ – $11^{\circ}26'34''$  E; depth approx. 40 m; salinity approx. 33 PSU (practical salinity unit)), near the Kristineberg Marine Research Station (KMRS; Göteborg University), Sweden, and *H. spinulosus* from the brackish waters of the Baltic Sea (at  $58^{\circ}50'53''$ – $58^{\circ}50'48''$  N;  $17^{\circ}32'22''$ – $17^{\circ}32'32''$  E; depth approx. 16 m; salinity approx. 7 PSU), near the Askö Laboratory, Baltic Sea Centre (Stockholm University). *Priapulus caudatus* was dredged from muddy, poorly oxygenated sediments and kept in tanks at low temperature before being fixed (glutaraldehyde) and desiccated (Leica Critical Point Dryer) for microscopic observations. *Halicryptus spinulosus* was prepared via comparable

standard methods. *Priapulus caudatus* and *H. spinulosus* secrete mucus which sticks sedimentary particles and detritus to the external surface of the trunk [31], thus preventing clear observation of the cuticular microstructures. Fine cuticular structures were best observed in freshly moulted specimens and exuviae. Images of dissected and whole specimens were taken with a Zeiss Merlin compact scanning electron microscope (SEM) at the Centre Technologique des Microstructures (CT $\mu$ , University Claude Bernard Lyon 1). Some specimens were embedded in resin (EPON), sliced (Ultramicrotome Reichert Ultracut S) and stained with Toluidine blue for observation of cellular and cuticular features using optical microscopy.

### 3. Results

#### (a) Cuticular reticulation in early scalidophorans

Scalidophoran worms are frequent elements of SSF assemblages from the early Cambrian Kuanchuanpu Formation, and are represented by at least five different species and more than nine unnamed forms [9,10,28,32]. They consist of relatively rare complete specimens and more frequent fragmentary tubular remains, all secondarily preserved in calcium phosphate and showing very fine external details of cuticular features (e.g. sclerites, scalids and pharyngeal teeth). Some of these tubular elements are exuviae which display either a positive or a negative relief (figure 1d,m), indicating that these worms were able to turn their flexible cuticle inside out during ecdysis in a manner similar to that of extant priapulids [4]. SEM observations have revealed very fine reticulate ornament over the entire external surface of the cuticle, characterized by a polygonal network with interconnected narrow ridges or furrows (figure 1c,f). This micro-ornament may be locally less well defined due to factors of preservation (figure 1b,g). A second type of reticulate pattern, characterized by wavy ridges, occurs on the inner surface of exuviae (figure 1n,o).

##### (i) Reticulation on sclerites

Reticulation is well defined in the two types of exuviae, especially around the basal part of sclerites. In exuviae with a positive relief, it consists of five- to seven-sided polygons delimited by straight interconnected ridges (SIR), which form a rather regular network. In some cases, elongate polygons or polygons with a more irregular shape are evident. SIR relief decreases towards the apex of the sclerites. Measurements of more than 200 polygons were made in 10 different specimens (electronic supplementary material, table S1). The diameter of the polygons ranges from 4 to 12  $\mu\text{m}$ , their perimeter length is from 14 to 31  $\mu\text{m}$  and SIR width varies from 1.3 to 2.0  $\mu\text{m}$  (figure 1b,c; electronic supplementary material, table S1). The exact counterpart of this polygonal network was observed in exuviae with a negative relief (i.e. sclerites pointing inwards instead of outwards) in which SIR faithfully correspond to interconnected furrows (SIF). SIF encircle slightly convex polygonal areas. Measurements of about 200 of these polygons in 5 specimens give values similar to those obtained from sclerites with a positive relief (polygon diameter and perimeter, 4–15 and 20–43  $\mu\text{m}$ , respectively; electronic supplementary material, table S1; figure 1e,f). Virtual tomographic sections show that SIR are the exact counterparts of SIF (figure 1h).

##### (ii) Reticulation on flexible parts of the cuticle

A different type of reticulate pattern is evident in areas where

the cuticle is devoid of sclerites and supposedly more flexible. It is characterized by strongly microfolded interconnected ridges (MIR) (figure 1k,l) which contrast with the straight outline of SIF (figure 1c). These ridges bear tiny V-shape, evenly distributed microfolds (figure 1l). The diameter of these polygons ( $n = 130$  in 7 different specimens) ranges from 5 to 19  $\mu\text{m}$  with a wall thickness of 0.4–2.0  $\mu\text{m}$ . Their perimeter varies from 19 to 47  $\mu\text{m}$ . Individual polygons have 9 to 26 microfolds (microfold length between 3.0 and 4.6  $\mu\text{m}$ ; see electronic supplementary material, table S1). Exuviae with a negative relief show a reticulate pattern characterized by punctuated interconnected furrows (PIF) around polygonal areas (figure 1n,o). As for SIR and SIF (observed on sclerites), PIF are the inverted counterparts of MIR. The smooth microfolded walls of MIR match exactly with the punctuated outline of PIF as confirmed by measurements ( $n = 100$ ; polygon diameter: 5–15  $\mu\text{m}$ ; number of pits: 10–22) and tomographic cross-sections (figure 1p,q). Some polygonal areas seem to be stretched in one direction (figure 1o).

## (b) Reticulation and cuticle structure in extant priapulid Worms

In *P. caudatus* (figure 2a,b), reticulation occurs along both the trunk (figure 2c–e) and the proboscis (figure 2f,g), with polygonal diameters of 9–14 and 10–21  $\mu\text{m}$ , respectively (electronic supplementary material, table S1). The three to five-sided polygons observed on the trunk are delimited by straight weakly elevated interconnected ridges and form a rather regular network (figure 2c,d). Microfolded polygons (resembling the MIR-type in fossils) preferentially occur in the strongly contracted areas of the trunk (figure 2e). Their diameter and thickness are 7–11  $\mu\text{m}$  and 1.2–1.6  $\mu\text{m}$ , respectively. Microfolds are evident both on the walls and inner areas of the polygons (figure 2e).

Transverse sections observed under the SEM show that the cuticle of *P. caudatus* sits above the epidermal cells and is made of numerous, densely packed laminated layers (figure 2b,c). The cuticle thickness varies from 4.7 to 10  $\mu\text{m}$  (see also [33]). However, clear subdivisions (e.g. exocuticle, endocuticle) could not be observed in SEM. Reticulation seems to affect the most superficial part of the cuticle, presumably the epicuticle (figure 2c). Sections through embedded specimens reveal the configuration of epidermal cells and also the relation between the epidermis and the cuticle. Transverse sections show that the epidermis has a single layer of subrectangular cells (figure 2b,o,q). However, the height (10–20  $\mu\text{m}$ ) and width (approx. 10  $\mu\text{m}$ ) of these cells depend on the location and orientation of the microtome sections (figure 2m,o–q) and therefore show some variations. The boundaries between epidermal cells correspond to the external polygonal ridges observed on the external surface of the cuticle. Longitudinal sections through the epidermal layer show that the layer of epidermal cells forms a welldefined pentagonal and hexagonal network (cell size between 8 and 25  $\mu\text{m}$  in diameter; figure 2r–t).

A comparable reticulation was observed (SEM) in freshly moulted specimens of *H. spinulosus* (figure 2h–j), the diameter of polygons (7–16  $\mu\text{m}$ ) being virtually the same as in *P. caudatus*. The strongly wrinkled appearance of the cuticle is probably due to its extreme thinness and disappears after

a few days following ecdysis (figure 2i). In *H. spinulosus*, reticulation also occurs along the inner surface of fresh exuviae (figure 2k), with five- to seven-sided polygons of 5–16  $\mu\text{m}$  and 0.8–1.3  $\mu\text{m}$  in size and wall thickness,

respectively (electronic supplementary material, table S1).

## 4. Discussion

### (a) Polygons replicate epidermal network

SIR/SIF and MIR/PIF clearly represent the positive/negative expressions of two types of cuticular micro-ornament. The consistent size range of polygons (4–19  $\mu\text{m}$ ; see electronic supplementary material, table S1) overlaps with that of epidermal cells in *Priapulid* (8–25  $\mu\text{m}$ ). Moreover, as seen in *Priapulid*, epidermal cells form a regular polygonal network in which cell boundaries match with the walls of the reticulation (figure 2o–q; electronic supplementary material, table S1). These comparative studies with modern priapulids suggest that the reticulation evident on the cuticle of Cambrian scalidophorans similarly replicates the pavement of underlying epidermal cells from which the cuticle was secreted. In present-day ecdysozoans, epidermal cells secrete a cuticle characterized by three distinct layers: a basal layer with chitin, a middle layer of homogeneous structure and an apical layer having three very thin lamellae [2,3,34], establishing a faithful correspondence in size and shape between the network of epidermal cells and that of the cuticular reticulation. A typical example is in the case of centipedes which display a regular hexagonal network over the external surface of their cuticle. In these myriapods, reticulation appears on the epicuticle and clearly replicates the epidermal cell pavement evident at the beginning of moulting [15]. A comparable geometrical replication occurs in crustacean ostracods and other extant arthropod groups [16,19]. Prior to moulting, ecdysozoans secrete specific enzymes that loosen contact between the basal part of the old cuticle and the epidermal cells [33,35] (figure 3a,e). The growth of epidermis via mitosis (e.g. [36,37]) results in new epidermal cells relatively smaller than in their pre-moult state (figure 3b,c,f). Their apical surface characterized by a wavy appearance [38] bears microvilli and starts to secrete individual cuticular patches [33,36] (figure 3b) which fuse together to eventually form a new cuticular layer as the epidermis stretches horizontally (figure 3c,d,g). The accumulation of material secreted by microvilli at the boundary between adjacent epidermal cells [39,40] most probably induces the formation of cuticular ridges in this particular boundary area (figure 3d–i). This process leads to an exact correspondence between the epidermal and the cuticular reticulate networks (figure 3d,h). Although our fossil material does not show details of the cuticle ultrastructure (figure 1j) and underlying epidermal cells (figure 1h,j), we hypothesize that the cuticle of early Cambrian scalidophoran worms was formed through a comparable process. Thus, fundamental aspects of ecdysis [4] and cuticle formation (this study) seem to have been established early in the evolution of ecdysozoans (at least not later than 535 Ma), possibly before the rise and major diversification of arthropods.

### (b) Microfolds in reticulation

We suggest that the microfolds associated with the reticulation of the cuticle of Cambrian worms may be original features. The cuticle of extant scalidophorans such as *P. caudatus* and *H. spinulosus* is very thin (approx. 4.7–10  $\mu\text{m}$ ) and responds in a flexible manner to the contraction and relaxation of circular and longitudinal muscles [33] by creating transverse microfolds, parallel to annuli. The reaction of worms to fixing

chemicals (e.g. glutaraldehyde, formaldehyde) often results in extreme body contraction and cuticular folding which, when exerted on reticulate structures, generate microfolds along the walls of the polygons (figure 2e). The microfolded features observed on the cuticle of Cambrian worms, exemplified by MIR/PIF, may be similarly explained by muscular contractions, possibly induced by environmental stress that caused the animal's death. However, these microfolds are not seen in the inner areas (floors) of the polygons. In addition, the V-shaped microfolds observed along the polygonal ridges have a consistent amplitude and a virtually constant number (figure 11). By contrast, the microfolds seen in extant priapulids are distributed more irregularly and seem to affect the whole cuticular structure (figure 2e). We suggest that the microfolds associated with the cuticle reticulation evident in Cambrian worms may be original features. They may characterize freshly moulted worms in which the cuticle is extremely thin and wrinkled as seen in extant *H. spinulosus* (figure 2k), or result from unknown external or internal factors. Interestingly, MIR/PIF are mostly found in areas where the cuticle is supposed to be thinner and flexible, whereas SIR/SIF preferentially occur around thick sclerites. Microfolds may be seen as a physical solution to absorb plastic deformation due to muscular contractions, especially where the cuticle is thin. That would explain why non-deformable sclerites lack such wavy appearance. Similarly, *Eopriapulites sphinx*, another scalidophoran worm from the Kuanchuanpu Formation, shows a complex wrinkled cuticular pattern with closely spaced annuli and short longitudinal folds running across each annulus [27]. This pattern may represent another solution to absorb contraction exerted by both circular and longitudinal muscles. An alternative third hypothesis is that the microfolded walls of polygons might be the external replica of interdigitated junctions between epidermal cells. Such junctions might have strengthened contacts between cells in body areas subjected to frequent contractions [41–43].

In summary, both reticulation types (SIR/SIF and MIR/PIF) support the hypothesis that early Cambrian scalidophorans secreted their cuticle in a similar way to that of modern ecdysozoans, that is: (i) that individual epidermis cells served as a matrix for the deposition of molecules such as chitin, which resulted in the formation of the cuticular layer; and (ii) that the reticulate pattern of the cuticle replicates the boundaries between epidermal cells. SIR/SIF seem to be mainly associated with relatively thick cuticular structures, typically trunk sclerites, whereas MIR/PIF preferentially occur in areas where the cuticle is thinner and more flexible. MIR/PIF may result from the absorption of body contraction exerted by underlying muscles, cuticular shrinkage during the early step of cuticle formation or (more improbably) from a special type epidermal cell junctions. Reticulation also appears in other ecdysozoan groups (e.g. palaeoscolecids, panarthropods; figure 4; electronic supplementary material, table S1).

### (c) Evolutionary significance

The oldest known body fossil record of ecdysozoans is at ca 535 Ma, as exemplified by the diverse stem-group scalidophoran worms from the Kuanchuanpu Formation. No other ecdysozoan group (e.g. panarthropods) is known from this early Cambrian (Terreneuvian) biota. This suggests that scalidophorans diverged early in the evolutionary history of ecdysozoans and that the last common ancestor of ecdysozoans has yet to be found in the Precambrian. Various phylogenies based on genomic and morphological data have been proposed over the recent years and reveal important

uncertainties and controversies [52] in the divergence pattern and relationships of major ecdysozoan lineages. The simplified phylogenetic tree used here (figure 4) to plot the occurrence of reticulate patterns of the cuticle through geological times is that of Nielsen [34], in which Panarthropoda (Arthropoda, Onychophora, Tardigrade) and Cycloneuralia (Scalidophora, Nematoda, Nematomorpha) are sister groups. The stemgroup Scalidophora which accommodates various worm categories (e.g. palaeoscolideans and taxa from the Kuanchunapu biota) and the stem-group Onychophora (lobopodian panarthropods) were added to the tree. It is evident that various types of cuticular patterns developed during the Cambrian period within both the Scalidophora and the Panarthropoda (e.g. arthropods and lobopodian stem-group onychophorans; figure 4; electronic supplementary material, table S3). The oldest known types of reticulation occur in scalidophorans from the Kuanchuanpu Formation and consist of simple hexagons and other polygons with either straight (SIR/SIF) or microfolded (MIR/PIF) walls. Although the nature of the last common ancestor of ecdysozoans remains to be defined, we suggest that this animal had a cuticle, grew through successive moulting stages (ecdysis [4]) and replicated its epidermal cellular pavement to form a regular cuticular network. Cuticular reticulation in Ecdysozoa appears to be nothing than an initial by-product of cell division during cuticle growth. However, this basic process evolved to produce many different variants through time. An ancient differentiation may have occurred when cuticles became more complex and thicker (e.g. strengthening features) as exemplified by the SIR/SIF and MIR/PIF types (Kuanchuanpu scalidophorans) found in the rigid and flexible cuticular areas, respectively. During the course of evolution, the increased complexity of cuticular features (e.g. sclerotization, mineralization) associated with possible epidermal differentiation [53–55] gave rise independently in almost all groups (except nematodes) to a great variety of patterns of reticulation. In contrast with arthropods, the cuticular ornament of scalidophoran worms has remained virtually unchanged and poorly diversified since the early Cambrian.

**Data accessibility.** All fossil specimens from the Kuanchuanpu Formation are deposited in the collections of the Shaanxi Key Laboratory of Early Life and Environments, Northwest University, China. Extant *P. caudatus* and *H. spinulosus* were collected from Kristineberg Marine Research Station and Baltic Sea, respectively. Some figures, tables and text have been uploaded as electronic supplementary material.

**Authors' contributions.** J.H. conceived the project. D.W., J.H. and J.V. interpreted the fossil material and wrote the paper with input from the other authors. J.S. and Y.-f.S. reconstructed the microtomographic data. All authors read and approved the final manuscript.

**Competing interests.** We declare we have no competing interests.

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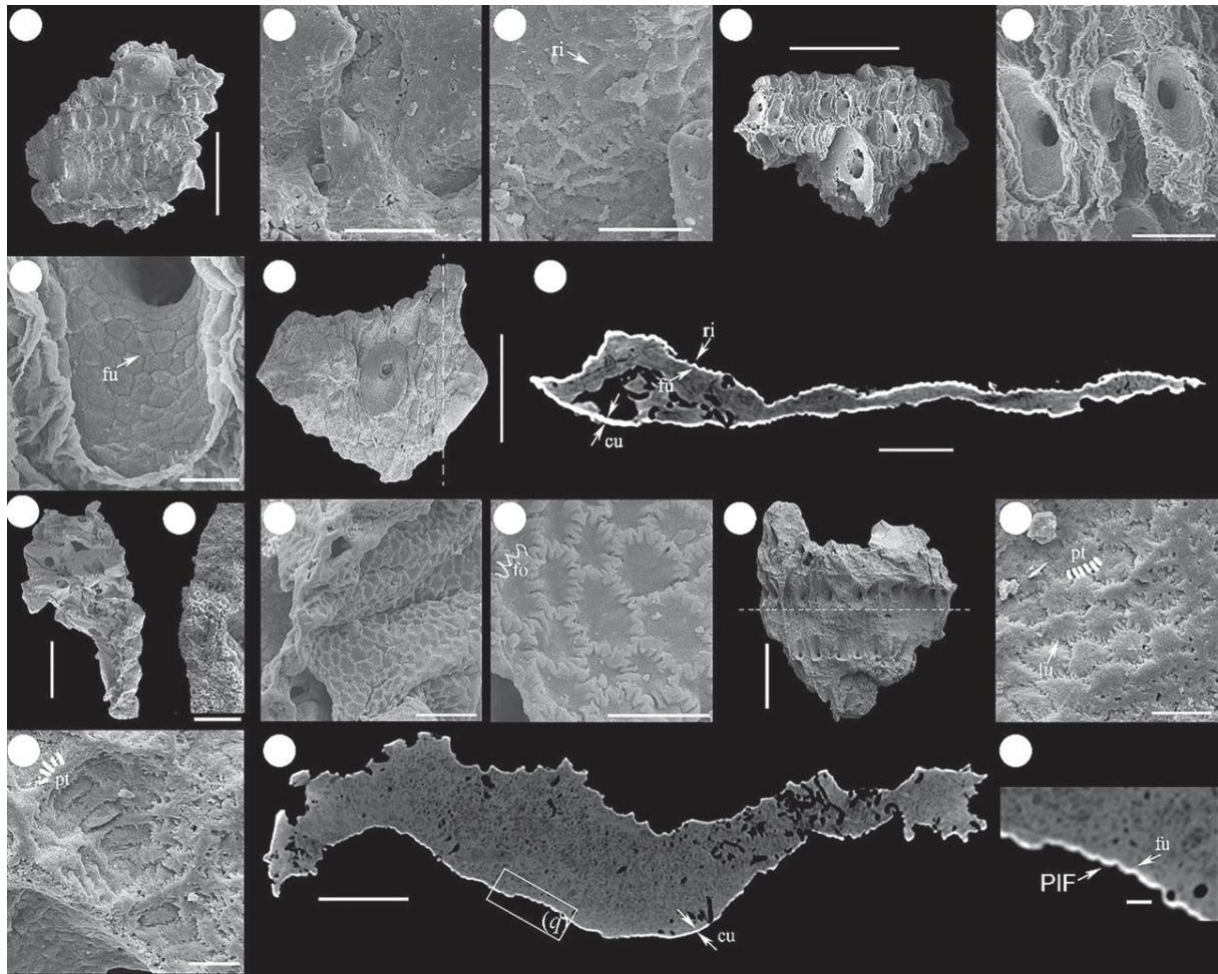
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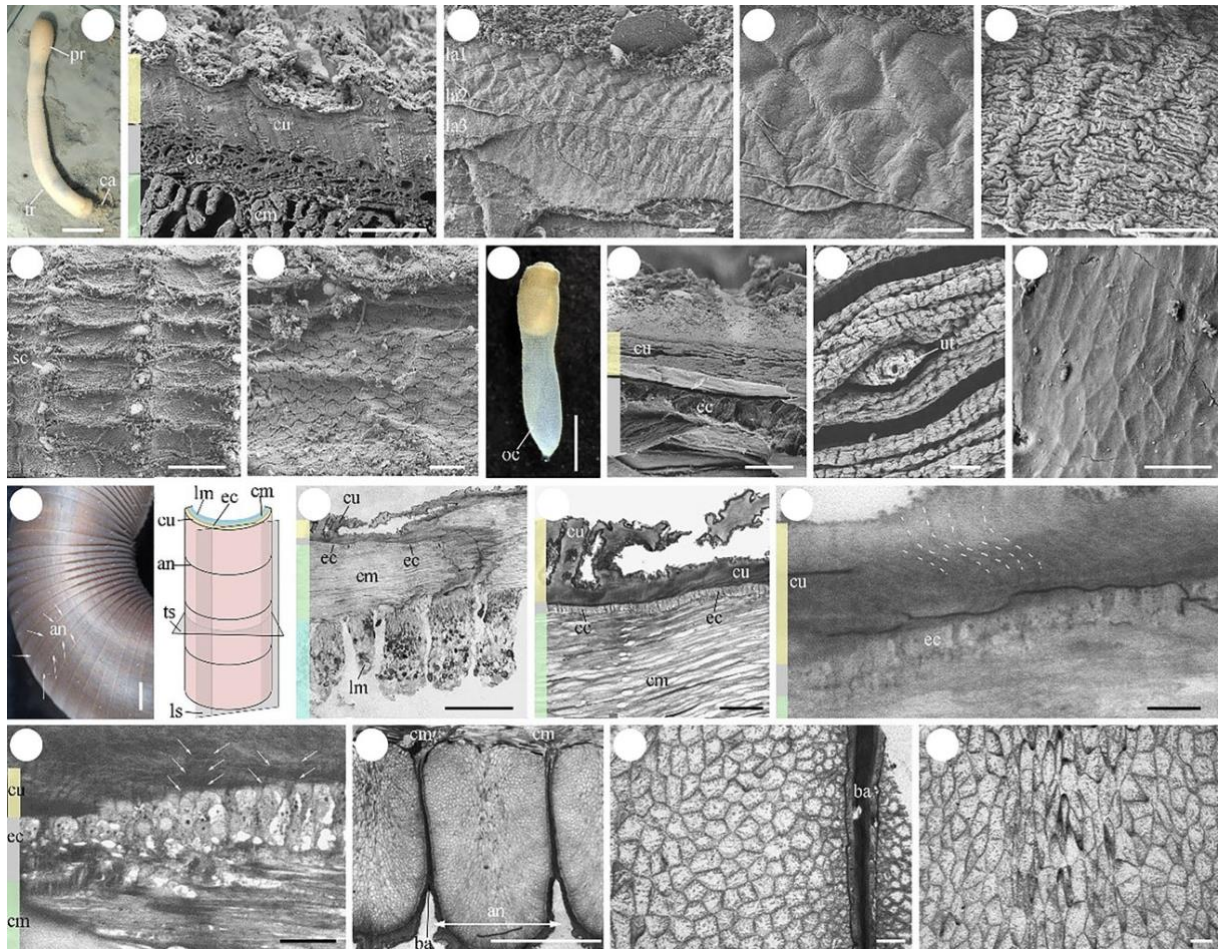
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## References

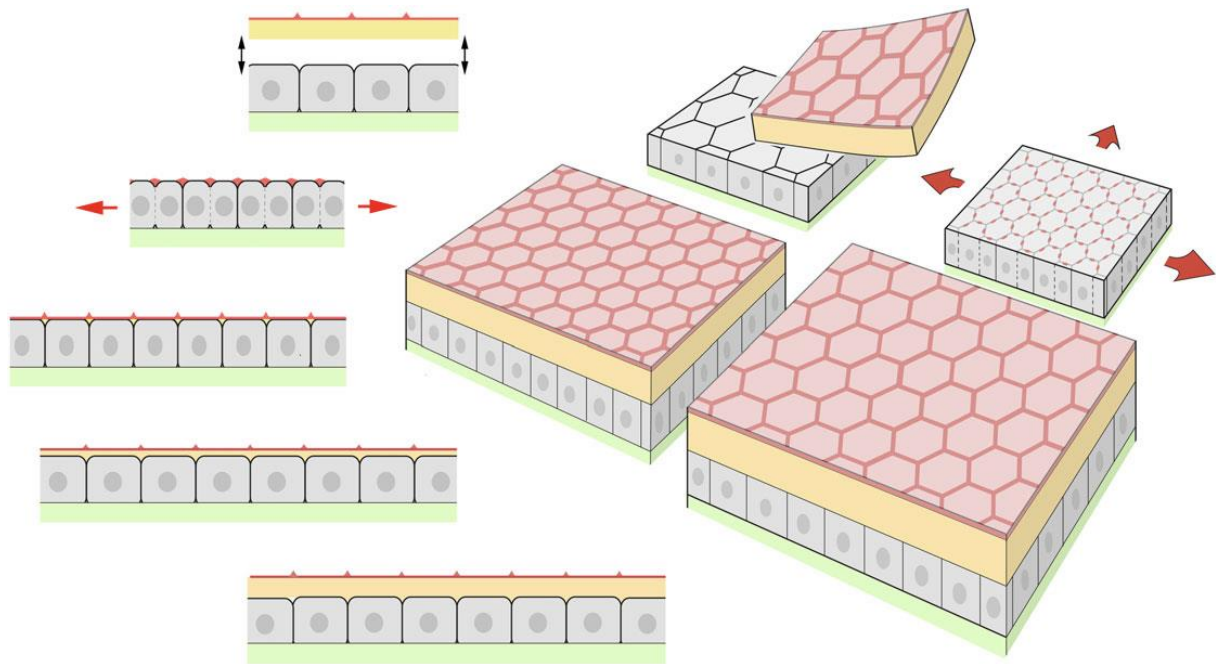
- Gibson WT, Gibson MC. 2009 Cell topology, geometry, and morphogenesis in proliferating epithelia. *Curr. Top. Dev. Biol.* **89**, 87–114. (doi:10.1016/j.cotd.2009.09.004-2)
- Schmidt-Rhaesa A. 2007 *The evolution of organ systems*, pp. 54–73. Oxford, UK: Oxford University Press.
- Brusca RC, Moore W, Shuster SM. 2016 *Invertebrates*, pp. 639–910, 3rd edn. Sunderland, MA: Sinauer Associates.
- Wang D, Vannier J, Schumann L, Wang X, Yang XG, Komiya T, Uesugi K, Sun J, Han J. 2019 Origin of ecdysis: fossil evidence from 535-million-year-old scaldiphoran worms. *Proc. R. Soc. B* **286**, 20190791. (doi:10.1098/rspb.2019.0791)
- Schumann I, Kenny N, Hui J, Hering L, Mayer G. 2018 Halloween genes in panarthropods and the evolution of the early moulting pathway in Ecdysozoa. *R. Soc. Open Sci.* **5**, 180888. (doi:10.1098/rsos.180888)
- Smith MR, Harvey THP, Butterfield NJ, Kouchinsky A. 2015 The macro- and microfossil record of the Cambrian priapulid *Ortoia*. *Palaeontology* **58**, 705–721. (doi:10.1111/pala.12168)
- Conway Morris S. 1977 Fossil priapulid worms. *Spec. Pap. Palaeontol.* **20**, 1–95.
- Harvey THP, Butterfield NJ. 2017 Exceptionally preserved Cambrian loriferans and the early animal invasion of the meiobenthos. *Nat. Ecol. Evol.* **1**, 22. (doi:10.1038/s41559-016-0022)
- Liu YH, Xiao SH, Shao TQ, Broce J, Zhang HQ. 2014 The oldest known priapulid-like scaldiphoran animal and its implications for the early evolution of cycloneuralians and ecdysozoans. *Evol. Dev.* **16**, 155–165. (doi:10.1111/ede.12076)
- Zhang HQ, Xiao SH, Liu YH, Yuan XL, Wan B, Muscente AD, Shao TQ, Gong H, Cao GH. 2015 Armored kinorhynch-like scaldiphoran animals from the early Cambrian. *Sci. Rep.* **5**, 16521. (doi:10.1038/srep16521)
- Müller KJ, Hinz-Schallreuter L. 1993 Palaeoscolecid worms from the middle Cambrian of Australia. *Palaeontology* **36**, 549–592.
- Han J, Zhang XL, Zhang ZF, Shu DG. 2003 A new platy-armored worm from the early Cambrian Chengjiang Lagerstätte, South China. *Acta Geol. Sin.* **77**, 1–6. (doi:10.1111/j.1755-6724.2003.tb00103.x)
- Han J, Liu JN, Zhang ZF, Zhang XL, Shu DG. 2007 Trunk ornament on the palaeoscolecid worms *Cricocosmia* and *Tabelliscolex* from the Early Cambrian Chengjiang deposits of China. *Acta Palaeontol. Pol.* **52**, 423–431.
- Conway Morris S. 1997 The cuticular structure of the 495-Myr-old type species of the fossil worm *Palaeoscolex*, *P. piscatorum* (? Priapulida). *Zool. J. Linn. Soc.* **119**, 69–82. (doi:10.1111/j.1096-3642.1997.tb00136.x)
- Fusco G, Brenca C, Minelli A. 2000 Cellular processes in the growth of lithobiomorph centipedes (Chilopoda: Lithobiomorpha). A cuticular view. *Zool. Anz.* **91**, 91–102.
- Okada Y. 1982 Structure and cuticle formation of the reticulated carapace of the ostracode *Bicoronuothere bisanensis*. *Lethaia* **15**, 85–101. (doi:10.1111/j.1502-3931.1982.tb01124.x)
- Okada Y. 1981 Development of cell arrangement in ostracod carapaces. *Paleobiology* **7**, 276–280. (doi:10.1017/S00948373000405X)
- Schmalfuss H. 1978 Morphology and function of cuticular micro-scales and corresponding structures in terrestrial isopods (Crust., Isop., Oniscidea). *Zoomorphologie* **91**, 263–274. (doi:10.1007/BF00999815)
- Giraud-Guille M-M. 1984 Calcification initiation sites in the crab cuticle: the intraprismatic septa—an ultrastructural cytochemical study. *Cell Tissue Res.* **236**, 413–420. (doi:10.1007/bf00214245)
- Li ZP. 1984 The discovery and its significance of early Cambrian small shell fossils in Hexi area Xixiang Shaanxi. *Geol. Shaanxi* **2**, 73–78.
- Steiner M, Li GX, Qian Y, Zhu MY. 2004 Lower Cambrian small shelly fossils of northern Sichuan and southern Shaanxi (China), and their biostratigraphic importance. *Geobios* **37**, 259–275. (doi:10.1016/j.geobios.2003.08.001)
- Sawaki Y et al. 2008 Internal structures and U–Pb ages of zircons from a tuff layer in the Meshuocunian formation, Yunnan Province, South China. *Gondwana Res.* **14**, 148–158. (doi:10.1016/j.gr.2007.12.003)
- Müller KJ. 1962 Aus der Praxis—Ein einfacher behelf für die listungstechnik. *Palaeontol. Z.* **36**, 265–267. (doi:10.1007/BF02986978)
- Wang X, Han J, Vannier J, Ou Q, Yang XG, Uesugi K, Sasaki O, Komiya T, Sevastopulo G. 2017 Anatomy and affinities of a new 535-million-year-old medusozoan from the Kuanchuanpu Formation, South China. *Palaeontology* **60**, 853–867. (doi:10.1111/pala.12320)
- Shao TQ, Tang HH, Liu YH, Waloszek D, Maas A, Zhang HQ. 2018 Diversity of cnidarians and cycloneuralians in the Fortunian (early Cambrian) Kuanchuanpu Formation at Zhangjiagou, South China. *J. Paleontol.* **92**, 115–129. (doi:10.1017/jpa.2017.94)
- Han J, Conway Morris S, Ou Q, Shu DG, Huang H. 2017 Meiofaunal deuterostomes from the basal Cambrian of Shaanxi (China). *Nature* **542**, 228–231. (doi:10.1038/nature21072)
- Shao TQ, Liu YH, Wang Q, Zhang HQ, Tang HH, Li Y. 2016 New material of the oldest known scaldiphoran animal *Eopriapulites sphinx*. *Palaeoworld* **25**, 1–11. (doi:10.1016/j.palwor.2015.07.003)
- Shao TQ et al. In press. New macrobenthic cycloneuralians from the Fortunian (lowermost Cambrian) of South China. *Precambrian Res.* 105413. (doi:10.1016/j.precamres.2019.105413)
- Yang XG, Han J, Wang X, Schiffbauer JD, Uesugi K, Sasaki O, Komiya T. 2017 Euendoliths versus ambient inclusion trails from Early Cambrian Kuanchuanpu Formation, South China. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **476**, 147–157. (doi:10.1016/j.palaeo.2017.03.028)
- Shao TQ et al. 2015 New small shelly fossils (*Acanthocassis* and *Xinlispina* gen. nov.) from the Fortunian stage (early Cambrian) in southern China. *Acta Geol. Sin. (English Ed.)* **89**, 1470–1481. (doi:10.1111/1755-6724.12558)
- Oeschger R, Janssen HH. 1991 Histological studies on *Halicryptus spinulosus* (Priapulida) with regard to environmental hydrogen sulfide resistance. *Hydrobiologia* **222**, 1–12. (doi:10.1007/BF00017494)
- Liu YH et al. 2018 New armoured scaldiphorans (Ecdysozoa, Cycloneuralia) from the Cambrian Fortunian Zhangjiagou Lagerstätte, South China. *Pap. Palaeontol.* **5**, 241–260. (doi:10.1002/spp2.1239)
- Lemburg C. 1998 Electron microscopical localization of chitin in the cuticle of *Halicryptus spinulosus* and *Priapulites caudatus* (Priapulida) using gold-labelled wheat germ agglutinin: phylogenetic implications for the evolution of the cuticle within the Nemathelminthes. *Zoomorphologie* **118**, 137–158. (doi:10.1007/s004350050064)
- Nielsen C. 2012 *Animal evolution: interrelationships of the living phyla*, pp. 238–310, 3rd edn. Oxford, UK: Oxford University Press.
- Chapman RF. 2013 *The insects—structure and function*, pp. 463–498, 5th edn. Cambridge, UK: Cambridge University Press.
- Wigglesworth VB. 1973 The role of the epidermal cells in moulding the surface pattern of the cuticle in *Rhodnius* (Hemiptera). *J. Cell Sci.* **12**, 683–705. (doi:10.1007/BF02986978)
- Bennet-Clark HC. 1971 The cuticle as a template for growth in *Rhodnius prolixus*. *J. Insect Physiol.* **17**, 2421–2434. (doi:10.1016/0022-1910(71)90089-8)
- Gibson MC, Patel AB, Nagpal R, Perrimon N. 2006 The emergence of geometric order in proliferating metazoan epithelia. *Nature* **442**, 1038–1041. (doi:10.1038/nature05014)
- Locke M. 1966 The structure and formation of the cuticular layer in the epicuticle of an insect, *Calpodes ethlius* (Lepidoptera, Hesperidae). *J. Morphol.* **118**, 461–494. (doi:10.1002/jmor.1051180403)
- Botting JP, Muir LA, Van Roy P, Bates D, Upton C. 2012 Diverse middle Ordovician palaeoscolecidan worms from the Builth-Llandrindod Inlier of central Wales. *Palaeontology* **55**, 501–528. (doi:10.1111/j.1475-4983.2012.01135.x)
- Moritz K. 1972 Zur Feinstruktur integumentaler Bildungen bei Priapuliden (*Halicryptus spinulosus* und *Priapulites caudatus*). *Z. Morphol. Tiere* **72**, 203–230. (doi:10.1007/BF00391552)
- Higgins RP, Storch V. 1989 Ultrastructural observations of the larva of *Tubiluchus cordilicola* (Priapulida). *Helgoländer Mar. Res.* **43**, 1–11. (doi:10.1007/BF02365545)
- Higgins RP, Storch V, Shirley TC. 1993 Scanning and transmission electron microscopical observations on the larvae of *Priapulites caudatus* (Priapulida). *Acta Zool.* **7**, 301–319. (doi:10.1111/j.1463-6395.1993.tb01245.x)
- Borner J, Rehm P, Schill RO, Ebersberger I, Burmester T. 2014 A transcriptome approach to ecdysozoan phylogeny. *Mol. Phylogenet. Evol.* **80**, 79–87. (doi:10.1016/j.ympev.2014.08.001)
- Vannier J, Calandra I, Gaillard C, Zylinska A. 2010 Priapulid worms: pioneer horizontal burrowers at the Precambrian-Cambrian boundary. *Geology* **38**, 711–714. (doi:10.1130/g30829.1)
- Kesidis G, Slater BJ, Jensen S, Budd GE. 2019 Caught in the act: priapulid burrowers in early Cambrian substrates. *Proc. R. Soc. B* **286**, 20182505. (doi:10.1098/rspb.2018.2505)
- Thompson I, Jones DS. 1980 A possible onychophoran from the middle Pennsylvanian Mazzone Creek beds of northern Illinois. *J. Paleontol.* **54**, 588–596.
- Smith MR, Ortega-Hernández J. 2014 *Hallucigenia*'s onychophoran-like claws and the case for Tactopoda. *Nature* **514**, 363–366. (doi:10.1038/nature13576)
- Yang J, Ortega-Hernández J, Gerber S, Butterfield NJ, Hou JB, Lan T, Zhang XG. 2015 A superarmored lobopodian from the Cambrian of China and early disparity in the evolution of Onychophora. *Proc. Natl Acad. Sci. USA* **112**, 8678–8683. (doi:10.1073/pnas.1505596112)
- Murdock DJ, Gabbott SE, Purnell MA. 2016 The impact of taphonomic data on phylogenetic resolution: *Helenodora inopinata* (Carboniferous, Mazon Creek Lagerstätte) and the onychophoran stem lineage. *BMC Evol. Biol.* **16**, 1–14. (doi:10.1186/s12862-016-0582-7)
- Garwood RJ, Edgecombe GD, Charbonnier S, Chabard D, Sotby D, Giribet G. 2016 Carboniferous Onychophora from Montceau-les-Mines, France, and onychophoran terrestrialization. *Invertebr. Biol.* **135**, 179–190. (doi:10.1111/ivb.12130)
- Giribet G, Edgecombe GD. 2017 Current understanding of Ecdysozoa and its internal phylogenetic relationships. *Integr. Comp. Biol.* **57**, 455–466. (doi:10.1093/icb/ixc072)
- Waku Y. 1978 Fine structure and metamorphosis of the wax gland cells in a psyllid insect, *Anomoneura mori* Schwartz (Homoptera). *J. Morphol.* **158**, 243–274. (doi:10.1002/jmor.1051580302)
- Neil JV. 2000 Factors influencing intraspecific variation and polymorphism in marine podocopid Ostracoda, with particular reference to tertiary species from southeastern Australia. *Hydrobiologia* **419**, 161–180. (doi:10.1023/A:1003983918339)
- Guillot C, Lecuit T. 2013 Mechanics of epithelial tissue homeostasis and morphogenesis. *Science* **340**, 1185–1189. (doi:10.1126/science.1235249)



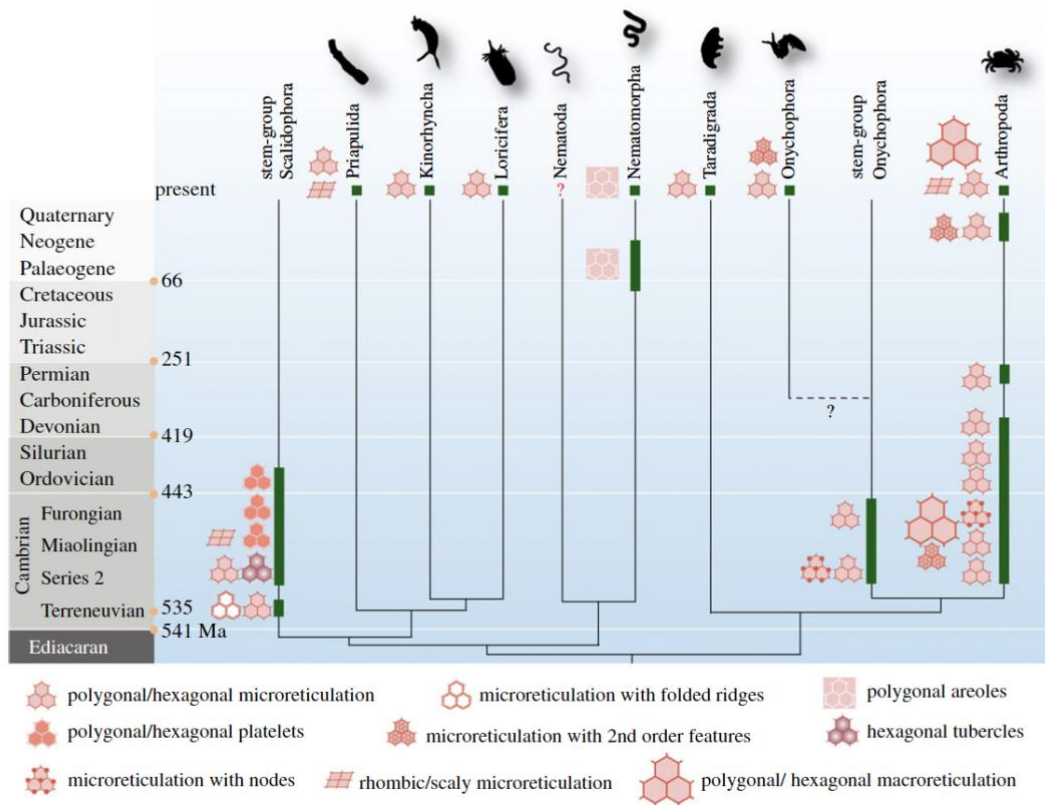
**Figure 1.** Reticulation in scalidophoran worms from the early Cambrian Kuanchuanpu Formation, China. (a–c) Reticulation on the external surface of sclerites; ELIXX106-520, general view and close-ups showing polygons with SIR. (d–f) Reticulation on the internal surface of sclerites; ELIXX78-205, general view and close-ups showing polygons with SIF. (g,h) ELIXX93-335, general view and microCT image of a virtual section through the specimen (dotted line); each ridge on the outer surface corresponds to a furrow on the inner surface. (i–l) ELIXX73-212, reticulation on the external surface of flexible cuticle; (i) general view and close-ups showing reticulation with MIR (9–26 V-shaped microfolds); (j) a broken part of phosphatized cuticle with no visible microstructures. (m–q) ELIXX95-85, general view and close-ups showing reticulation with PIF (10–22 pits) and elongated PIF between sclerites (external surface of an inverted exuvia; see Wang et al. [4]); (p,q) microCT images of a virtual section through the specimen (dotted line in e). cu, cuticle; fo, fold; fu, furrow; PIF, punctuated interconnected furrows; pt, pit; ri, ridge. Scale bars represent: 500  $\mu\text{m}$  (a,e,g,i,m), 200  $\mu\text{m}$  (p), 100  $\mu\text{m}$  (e,h), 50  $\mu\text{m}$  (b,k), 20  $\mu\text{m}$  (c,f,l,n,o,q), 4  $\mu\text{m}$  (j).



**Figure 2.** Reticulation and epidermal cells in *Priapulus caudatus* and *H. spinulosus* from Sweden. (a–g) *Priapulus caudatus*. (a) General view of a live specimen on sediment. (b) Transverse section through the cuticle (details of epicuticle surface obscured by sediment particles and debris). (c,d) Oblique section showing the layered structure of the cuticle (each layer with reticulate network); general view and close-up. (e) Reticulate surface affected by microfolds (on both ridges and the floor of the polygons). (f) Scaly reticulation on the proboscis. (g) Close-up of f. (h–k) *H. spinulosus*. (h) General view of a moulting specimen with old cuticle partly detached from the body. (i) Transverse section through trunk showing cuticle epidermal cells and circular muscles. (j) Newly moulted cuticle with reticulation and unsclerotized tubuli. (k) Reticulation along the internal surface of an exuvia. (l–t) *Priapulus caudatus* from Sweden; microtome sections of specimens embedded in resin (toluidine blue staining). (l) Trunk annuli of *Priapulus caudatus*. (m) Simplified diagram of trunk to show location of transverse and longitudinal sections. (n) Transverse section through trunk cuticle (cuticle, epidermal cells, circular muscles, longitudinal muscles in light grey, red green and white, respectively). (o) Transverse section showing epidermis (single layer) between cuticle and circular muscle. (p,q) Sections showing correspondence between the boundaries of epidermal cells and the walls of cuticular reticulation. (r–t) Intermediate section showing boundaries between annuli (dark blue lines) and reticulation within annuli; general view and details showing subhexagonal network near the boundaries and more elongated one in central part of annulus. an, annulus; ba, boundaries of annuli; ca, caudal appendage; cm, circular muscle; cu, cuticle; ec, epidermal cells; la, layer; lm, longitudinal muscles; ls, longitudinal section; oc, old cuticle; pr, proboscis; tr, trunk; ts, transverse section; ut, unsclerotized tubuli. Light grey, purple and light green colours represent the cuticle, epidermal cells and circular muscles, respectively. Scale bars represent: 2 cm (a), 500  $\mu$ m (n,r), 100  $\mu$ m (o), 20  $\mu$ m (c,g,i,j,k,p,q,s,t), 10  $\mu$ m (b,d,e) and 5  $\mu$ m (h). (Online version in colour.)



**Figure 3.** Idealized diagram to explain cuticular reticulation in Cambrian scalidophoran worms, based on analogues in extant priapulids (*Priapululus*, *Halicryptus*; see text for details). (a–e) Successive steps in cuticle formation: (a) old cuticle splitting from the epidermis layer (ecdysis); (b) mitosis within epidermis (dividing cells) and formation of small cuticle patches at the boundary between cells; (c) epicuticle layer is formed and bears external reticulation; underlying cuticular layer starts growing; (d) intermediate step; (e) whole cuticle is formed. (f–i) Block diagrams successively showing ecdysis, cell division and initial step of cuticle secretion (as in figure 2) and expansion (red arrows) of epidermal layer and cuticle, leading to final reticulate pattern (polygons have the same size in f and i). Epicuticle is depicted in red/pink; undifferentiated exo- and endocuticle in yellow; epidermal cells in grey; and circular muscles in light green. (Online version in colour.)



**Figure 4.** Occurrences of cuticular patterns of reticulation plotted on a phylogenetic tree of Ecdysozoa based on Nielsen’s phylogeny (supported by morphological characters, see [34]) showing two sister groups (Cycloneuralia and Panarthropoda). For alternative phylogenies based on molecular data, see [44]. The stem-group Scalidophora to which the present fossil material belongs and the stem-group Onychophora (lobopodian panarthropods) are added to the tree. The scalidophoran fossil record goes back to the lowermost Cambrian (e.g. body fossils from the Kuanchuanpu Formation, Terreneuvian) but may extend to the Precambrian–Cambrian boundary as exemplified by trace fossils probably made by stem-group scalidophoran worms (e.g. *Treptichnus* [45,46]). Black question mark and dotted lines represent the first fossil record crown Onychophora [47]. Some lobopodian panarthropods (e.g. *Hallucigenia*) are likely to belong to the stem-group Onychophora [48,49]. Whether onychophorans from the Carboniferous Mazon Creek and Montceau-les-Mines Lagerstätten belong to the crown-group Onychophora or not is currently debated [47,50,51]. The chronology of divergence between groups below the basal Cambrian is hypothetical. Reticulation of the cuticle is found in all ecdysozoan clades except Nematoda (red question mark) and may be present in the last common ancestor of ecdysozoans (see text). Green bars represent the appearance of reticulation. Microreticulation corresponds to polygon size of 10–60  $\mu\text{m}$  that replicates epidermal cell boundaries; macroreticulation to polygons of larger size that may result from more complex cellular processes. (Online version in colour.)