**The gnathobasic spine microstructure of Recent and Silurian chelicerates and the Cambrian
arthropodan *Sidneyia*: functional and evolutionary implications**

Russell D.C. Bicknell^{a,*}, John R. Paterson^a, Jean-Bernard Caron^{b,d}, Christian B. Skovsted^e

^a Palaeoscience Research Centre, School of Environmental and Rural Science, University of New
England, Armidale, NSW 2351, Australia; rdcbicknell@gmail.com; jpater20@une.edu.au

^b Department of Natural History, Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario M5S
2C6, Canada; jcaron@rom.on.ca

^c Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario
M5S 2J7, Canada

^d Department of Earth Sciences, University of Toronto, Toronto, Ontario, M5S 3B1, Canada

^e Department of Palaeobiology, Swedish Museum of Natural History, PO Box 50007, SE-104 05,
Stockholm, Sweden; christian.skovsted@nrm.se

*Corresponding author.

E-mail address: rdcbicknell@gmail.com (R. D. C. Bicknell.)

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Abstract: Gnathobasic spines are located on the protopodal segments of the appendages of
various euarthropod taxa, notably chelicerates, and used to crush shells and masticate soft food
items. The gnathobasic spine microstructure of the Recent xiphosuran chelicerate *Limulus
polyphemus* is studied using a sectioned specimen and SEM imagery and compared with the
cuticular structure of similar gnathobases in the Silurian eurypterid *Eurypterus tetragonophthalmus*

from Estonia and the Cambrian artiopodan *Sidneyia inexpectans* from Canada to elucidate the functional morphology of these extinct forms. The thickened fibrous exocuticle in *L. polyphemus* spine tips enables effective prey mastication and shell crushing, while also reducing pressure on nerve endings that fill the spine cavities. The spine cuticle of *E. tetragonophthalmus* has a laminate structure and lacks the fibrous layers seen in *L. polyphemus* spines, suggesting that *E. tetragonophthalmus* may not have been capable of crushing thick shells, but a durophagous habit cannot be precluded. Conversely, the cuticle of *S. inexpectans* spines has a similar fibrous microstructure to *L. polyphemus*, suggesting that *S. inexpectans* was a competent shell crusher. This conclusion is consistent with specimens showing preserved gut contents containing various shelly fragments. The shape and arrangement of the gnathobasic spines is similar for both *L. polyphemus* and *S. inexpectans*, with stouter spines in the posterior cephalothoracic or trunk appendages, respectively. This differentiation indicates that crushing occurs posteriorly, while the gnathobases on anterior appendages continue mastication and push food towards and into the mouth. The results of recent phylogenetic analyses that considered both modern and fossil euarthropod clades show that xiphosurans and eurypterids are united as crown-group euchelicerates, with *S. inexpectans* variably placed within more basal artiopodan clades. These relationships suggest that gnathobases with thickened fibrous exocuticle, if not homoplasious, may be plesiomorphic for chelicerates and deeper relatives. This study shows that the gnathobasic spine microstructure best adapted for durophagy has remained remarkably constant since the Cambrian period.

1. Introduction

The four extant species of Xiphosura, commonly referred to as horseshoe crabs, are taxa familiar to both biologists and palaeontologists (Babcock et al., 2000). Biologists often use xiphosurans as ‘model taxa’ to study aspects of marine chelicerates, whereas palaeontologists study horseshoe crabs largely for their extensive fossil record, spanning *ca.* 480 million years from the Early Ordovician to the Recent (Babcock et al., 2000; Racheboeuf et al., 2002; Heethoff and Norton, 2009; Rudkin and Young, 2009; Van Roy et al., 2010, 2015; Briggs et al., 2012). Furthermore, extant Xiphosura are employed as modern analogues to understand the functional morphology of extinct euarthropod taxa, such as the 507 million-year-old (mid-Cambrian) artiopodan *Sidneyia inexpectans* Walcott, 1911a from the Burgess Shale of British Columbia, Canada (Zacai et al., 2016), the early Cambrian radiodontan *Amplectobelua symbrachiata* Hou, Bergström and Ahlberg, 1995 from the Chengjiang biota of China (Cong et al., 2017), and a variety of large, supposedly predatorial, potentially durophagous eurypterid taxa (Selden, 1981; Babcock et al., 2000; Lamsdell, 2013; Poschmann et al., 2016).

Of the extant xiphosurans, *Limulus polyphemus* (Linnaeus, 1758) is considered here. While the biology of *L. polyphemus* is well documented, the exoskeletal cuticular microstructure is understudied. Two primary layers (in addition to the thin epicuticle) make up the chitinous cuticle of *L. polyphemus*: fibrous endocuticle that underlies a predominantly laminate exocuticle (Hock, 1940; Richards, 1949; Richter, 1969; Dalingwater, 1975; Joffe et al., 1975; Larsen, 1975; Dennell, 1978; Mutvei, 1978; Shultz, 2001; Rubin et al., 2017). No previous cuticle studies have documented the microstructural details of gnathobasic spines on the coxal segments (Fig. 1) of *L. polyphemus* walking legs (i.e., cephalothoracic appendages II to V): the features that allow *L. polyphemus* to masticate soft material and crush thin-shelled bivalved molluscs (Manton, 1964; Wyse, 1971; Wyse and Dwyer, 1973; Botton, 1984; Yamasaki et al., 1988; Botton et al., 2003). While Patten (1894, p. 90) observed that “spines on the mandibles” (presumably referring to the gnathobases) had canals and “a

chitinous tubule”, no one else has sectioned the gnathobasic spines of *L. polyphemus*. The microstructure of the gnathobasic spines is therefore insufficiently documented.

We present new information on the microstructure of gnathobasic spines in *Limulus polyphemus* to determine whether the gnathobases of the Silurian eurypterid *Eurypterus tetragonophthalmus* Fischer, 1839 from Estonia, and the Cambrian artiopodan *Sidneyia inexpectans* from Canada have similar structure and function. *Eurypterus tetragonophthalmus* was chosen because of the three-dimensional preservation of appendage specimens that have been isolated from the carbonate host rock, allowing the spines to be sectioned (Holm, 1898; Seldon, 1981), and the relatively close phylogenetic relationship between eurypterids and xiphosurans (e.g., Legg et al., 2013; Lamsdell, 2013, 2016; Legg, 2014; Garwood and Dunlop, 2014; Selden et al., 2015; Aria and Caron, 2017). While some studies (see Dalingwater, 1975, 1987; Selden, 1981) have detailed some cuticular microstructures of the gnathobasic spines of *E. tetragonophthalmus*, no study has sectioned the spines and imaged the internal features using scanning electron microscopy. *Sidneyia inexpectans* was included as this taxon is one of the oldest gnathobase-bearing artiopodans in the fossil record, and a known durophagous predator/scavenger (Bruton, 1981; Stein, 2013; Zacaï et al., 2016; Bicknell and Paterson, 2017). The exceptionally preserved carbonaceous compression fossils from the Burgess Shale (Gaines et al., 2008) have revealed some of the most intricate morphological details in Cambrian animals, including the internal features of structures such as lobopodian spines and claws (e.g., Caron et al., 2013, fig. 1e–k; Smith and Ortega-Hernández, 2014, fig. 1b–d). These otherwise hidden features are found using scanning electron microscope (SEM) backscatter imaging. This method is used here to show the internal microstructures of the gnathobasic spines of *S. inexpectans*.

2. Material and methods

2.1. *Limulus polyphemus*

One female *Limulus polyphemus* exuvium specimen (370 mm in length, including telson; 220 mm without the telson) housed at the University of New England (UNE; Armidale, New South Wales) was used. The fifth (right-side) cephalothoracic appendage (walking leg 4) was removed, the coxa extracted (Fig. 2A) and embedded in epoxy to make a block. The posterior side of the embedded coxa was ground down to produce a longitudinal section through the gnathobasic spines (Fig. 2B–D). Spines 2–4 were bisected, while spines 5–8 had more material removed to reveal the structural variation through the spines. The sectioned surface of the epoxy block was polished, then photographed under normal (fibre optic) lighting conditions using a Canon EOS 5D digital camera with a Canon MP-E 65 mm 1–5x macro lens. The block was later gold coated and imaged using the JEOL JSM-6010LA Scanning Electron Microscope at UNE. An accelerating voltage of 5 kV was used to acquire the secondary electron (SE) images of spine microstructures (Fig. 3).

2.2. *Eurypterus tetragonophthalmus*

One specimen of a gnathobase-bearing coxa of *Eurypterus tetragonophthalmus* (NRM Ar49987) from the Silurian (late Wenlock; Homerian) Viita Formation in Saaremaa, Estonia, was studied (Fig. 4A); the specimen is housed at the Swedish Museum of Natural History, Stockholm. This specimen was etched from the carbonate matrix using weak acids in the late 19th century and has since been dry stored (Holm, 1898). NRM Ar49987 was also embedded in an epoxy block that was ground down to produce a longitudinal section through the gnathobasic spines (Fig. 4B). Due to the disarticulated nature of the fossil, the anterior and posterior sides of the coxa relative to the body (in life position) are speculative. However, NRM Ar49987 is likely from appendage V (cf. Selden, 1981, fig. 28n). Spines 3, 4, and 6 were bisected and spines 1, 2 and 5 were not sectioned through completely to show microstructural variability (Fig. 4B). The sectioned surface of the block was polished, then imaged using the digital photographic (Fig. 4) and SEM (Fig. 5) techniques and equipment outlined in Section 2.2 above.

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122 2.3. *Sidneyia inexpectans*

123 Three specimens of *Sidneyia inexpectans* gnathobasic appendages from the middle
124 Cambrian (Series 3, Stage 5) Burgess Shale Formation of British Columbia, Canada, were studied.
125 Specimens ROM 63388 (Figs. 6, 7), ROM 64386 (Fig. 8) and ROM 64387 (Fig. 9) were collected from
126 Walcott Quarry and are housed at the Royal Ontario Museum (ROM), Toronto. Two specimens are
127 complete isolated appendages (ROM 63388 and ROM 64387) and one specimen is a fragmentary
128 protopodite showing only the gnathobases (ROM 64386). Specimens were imaged under direct
129 cross-polarized light with a Canon EOS 5D camera. SEM backscatter electron (BSE) images were
130 obtained with the JEOL JSM-6610 at the University of Toronto (Earth Sciences) using an accelerating
131 voltage of 15 kV in high vacuum mode.

132

133 2.4. Terminology

134 The following definitions are provided to clarify the terminology used here:

135 *Endocuticle* – The portion of the cuticle located beneath the exocuticle (Richards, 1951; Dalingwater,
136 1975).

137 *Exocuticle* – The outer layer of cuticle that is hardened and darkened compared to the endocuticle
138 (Richards, 1951; Dalingwater, 1975).

139 *Fibrous cuticle* – Cuticle that has a microstructure similar to thin strands of fibres.

140 *Laminate cuticle* – Cuticle constructed from the deposition of laminae, sometimes found adjacent to,
141 but not extending into the fibrous cuticle. Laminae differ from *lamellae* (not defined here), as lamellae
142 are artifacts of helicoidally-oriented microfibrils (Richards, 1951; Dalingwater, 1975).

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144 3. Results

3.1. *Limulus polyphemus*

The images of the sectioned gnathobasic spines under normal light (Fig. 2B–D) show that the centre of each spine is hollow, with the two major cuticle layers highlighted by different colours. These two layers have been observed in other sections through *Limulus polyphemus* cuticle (Lafon, 1943). The tan-brown outer layer represents the sclerotized exocuticle that extends around the sides and tip of the spine. The white layer beneath the exocuticle is the endocuticle. The boundary between exo- and endocuticle on the sides of the spine is abrupt, whereas the exocuticle at the spine tip (*ca.* 12 times thicker than at the sides) transitions gradually to thin layer of endocuticle (Fig. 2C). The presence of both cuticular layers in the gnathobases of this exuvium shows that the endocuticle was not reabsorbed during ecdysis (Dalingwater, 1975; Mutvei, 1977).

Under the SEM, laminae are observed along the sides of the spines, immediately below the outer surface (Fig. 3D). The total thickness of the laminae in this region of the spine is *ca.* 30 μm , and single lamina are *ca.* 2–5 μm thick. The laminae occur parallel to the relatively solid (featureless) sclerotized cuticle along the spine sides (Fig. 3C, D). This reaffirms that laminate cuticle is part of the exocuticle (Neville et al., 1969; Rubin et al., 2017). The laminae are absent on the spine tip (Fig. 3B). Instead, the thickened exocuticle at the spine tip has fibrous structures that are oriented parallel to the long axis of the spine in the central region and slightly fan out (up to 10° relative to the long axis) on either side (Fig. 3B). The endocuticle on the spine sides and tips has a fibrous microstructure (Fig. 3B, C). The fine fibres are very closely spaced, run parallel to each other, are kinked near the boundary with the exocuticle, and their orientation is oblique to the long axis of the spine (Fig. 3C). These fibrous structures do not extend into the laminate exocuticle on the sides of the spine.

3.2. *Eurypterus tetragonophthalmus*

The sectioned gnathobasic spines of *Eurypterus tetragonophthalmus* under normal light appear to show a ‘cone-in-cone’ structure (Fig. 4B), similar to the internal structure of the gnathobase-like spines in the early Cambrian radiodontan *Amplectobelua symbrachiata* (Cong et al.,

2017, fig. 7G-J). The ‘internal cone’ represents the boundary between the inner surface of solid cuticle and the hollow spine centre (as seen with SEM secondary electron imaging; Fig. 5A, C). Secondary electron images of NRM Ar49987 (Fig. 5) show that the entire spine cuticle has a laminate construction, reaffirming observations by Dalingwater (1975, pl. 3, figs. 5, 6). The laminae are parallel to the curvature of the inner and outer margins of the spine (Fig. 5C, D). In rare cases, the laminae appear to be slightly sinuous (Fig. 5B), but this could be a taphonomic artefact. The laminae are *ca.* 1–3 μm thick, which is comparable to cuticle in other eurypterid taxa, but thinner than those observed in *Limulus polyphemus* and trilobites (Dalingwater, 1973a, b, 1975).

3.3. *Sidneyia inexpectans*

Although it has been noted that the gnathobases of *Sidneyia inexpectans* are strongly sclerotized (Zacai et al., 2016), previous studies of this taxon have not documented any internal spine structure (Bruton, 1981; Stein, 2013; Zacai et al., 2016). These studies have also noted the differentiation in gnathobasic spine shape from the anterior to posterior appendages. Bruton (1981, p. 637) observed that the two anteriormost appendage pairs (his “coxae 1 and 2”)—considered by Stein (2013, p. 173) as belonging to the cephalon—have long, thin gnathobasic spines (e.g., Fig. 7A), whereas the spines on the more posterior appendages are stout (e.g., Figs. 6B, 9A) – a view supported by Stein (2013). Conversely, Zacai et al. (2016) proposed that the anterior appendages possess the more stout (teeth-like) gnathobasic spines and the posterior appendages have the longer spines, although no new figured material or additional discussion supported this reinterpretation. For comparison, the anterior walking legs of *Limulus polyphemus* have a combination of fine and stout spines and the pushing legs (cephalothoracic appendage pair VI) have the most stout spines, which we interpret (following Bruton, 1981) to be the case in *S. inexpectans* (*contra* Zacai et al., 2016).

Due to the compacted, carbonaceous preservation of the specimens on matrix, the spines of *Sidneyia inexpectans* could not be sectioned to uncover internal details. However, distinctive internal features of the gnathobases were found using SEM-BSE imaging at a high accelerating voltage, as electrons under such conditions can penetrate deeper beneath the surface (Orr and Kearns, 2011). The BSE images show two layers of sclerotized cuticle in some well preserved *S. inexpectans* spines (Figs. 7B, 9B, C): a thin outer layer that is underlain by much thicker cuticle. The outer layer of cuticle is up to 10 μm thick (Fig. 9C). The cuticle that underlies the outer layer has a fibrous texture (e.g., Figs. 6D, 7C, 8D). The fibres are oriented approximately parallel to the long axis of the spine in the central region and fan out on either side. This is similar to *Limulus polyphemus* spine tips (Fig. 3B). However, unlike *L. polyphemus* and *Eurypterus tetragonophthalmus*, the gnathobasic spine cuticle of *S. inexpectans* lacks laminae. This apparent absence of laminate structure in the thin outer layer (where it might be expected) may be because of taphonomic alteration of microstructures, the preservational fidelity of carbonaceous compression fossils, or the resolution of BSE imaging and detecting variable carbon concentrations across the spines (Orr and Kearns, 2011).

Some appendage specimens of *Sidneyia inexpectans* (e.g., ROM 64386) suggest that the gnathobases are hollow towards the base of the spine (e.g., Fig. 8B–D). The boundary between fibrous cuticle and the central cavity is demarcated by a dark line near the spine base (Fig. 8C, D). In very well preserved spines (e.g., Fig. 8D), the extent of the fibrous cuticle shows that only the bottom third of the spine was hollow. This demonstrates that the gnathobases of *S. inexpectans* have relatively thicker cuticle at the spine tips compared to the other taxa studied here.

4. Discussion

4.1. *Limulus polyphemus*

The hollow centres of *Limulus polyphemus* gnathobasic spines are filled with tissue and nerves endings and function as sensory structures that respond to chemical, mechanical and thermal stimuli (Hayes and Barber, 1967; Wyse, 1971), as well as mastication tools. The spines must therefore be constructed to maximize mastication ability and minimize pressure on tissue and nerves. The thick, sclerotized spine tips allow for food manipulation (including shell-crushing) and protection of sensitive tissues. The gradual transition from exocuticle to endocuticle along the axis of the spine tip likely minimizes various stresses that result from repetitive plastic deformation during mastication. This would maintain cuticle strength and potentially create a cushioning effect. The fibres aligned parallel to the long axis of the spine tip also reinforce the spine in the direction of maximum force. This confirms that cuticular fibres are often oriented parallel to the direction of greatest stress acting on any feature (Dalingwater, 1985; Wilmot, 1990).

Limulus polyphemus gnathobases seem to lack laminae at the spine tips (at least in the specimen considered here). As cuticle, and specifically laminae, are the extracellular components of *L. polyphemus* integument secreted from epidermal cells, laminae should occur along the entire margin of the spine (Richards, 1951; Neville, 1975; Dalingwater and Miller, 1977; Dalingwater, 1985). One possible explanation for this absence may be that laminae are not structurally robust when subjected to excessive force, thus requiring more solid (homogeneous) cuticle at the spine tip. Another plausible scenario is that the laminae on the spine tip were worn off due to regular food manipulation, especially when crushing shells. During subsequent moult stages, the laminae would therefore be redeposited. As the specimen studied here represents an exuvium, other specimens (such as newly moulted individuals) should be examined in future to test these ideas.

4.2. *Eurypterus tetragonophthalmus*

The cuticular microstructure of the gnathobasic spines in *Eurypterus tetragonophthalmus* differs markedly to *Limulus polyphemus* and *Sidneyia inexpectans* (Fig. 10). In *E. tetragonophthalmus*, the spines are constructed entirely of laminate cuticle, whereas those of *L.*

polyphemus and *S. inexpectans* are largely composed of fibrous cuticle. Furthermore, individual laminae of *E. tetragonophthalmus* are thinner than those observed in *L. polyphemus*. These differences suggest that the gnathobasic spines of *E. tetragonophthalmus* were perhaps not equipped to deal with excessive forces. By extension, *E. tetragonophthalmus* was possibly limited to using its gnathobases (at least on prosomal appendages I–V) to masticate soft prey or perhaps thin-shelled items. However, it is important to note that *E. tetragonophthalmus* has large, stout gnathobases on the posteriormost prosomal appendages ('appendage VI'; Selden, 1981, figs. 8, 31r, u–w, z, aa). Selden (1981, p. 20) noted that some gnathobases on this appendage show "extreme abrasion", indicating that these spines may have masticated harder prey items. Thus, it is likely that *E. tetragonophthalmus* had durophagous tendencies, as suggested for other eurypterids (Poschmann et al., 2016). This pattern of having more robust crushing spines on the posterior appendages is also observed in *Limulus polyphemus* and *S. inexpectans* (see Sections 3.3 and 4.3). It is possible that the appendage VI gnathobases of *E. tetragonophthalmus* have a similar fibrous microstructure to the spines of *L. polyphemus* and *S. inexpectans*, but this can only be confirmed when more specimens become available for sectioning.

After examining sections of the gnathobasic spines of *Limulus polyphemus*, hollow centres in *Eurypterus tetragonophthalmus* spines were somewhat expected. The presence of these cavities—if once filled with sensitive tissue—suggests that *E. tetragonophthalmus* also used the spines as sensory tools, in addition to food mastication (Hayes and Barber, 1967; Wyse, 1971). This is supported by the existence of fine pore canals seen in other specimens of *E. tetragonophthalmus* gnathobases (e.g., Dalingwater, 1975, pl. 3, fig. 5; Selden, 1981, fig. 23n, o), which may have housed sensory structures. Unfortunately, these pore canals, as well as the 'setal sockets' observed by Dalingwater (1975, pl. 3, fig. 6), were not found in the spines illustrated here (Fig. 5). This is best explained by the different imaging methods used, as these fine structures were illustrated by Dalingwater (1975) and Selden (1981) using transmitted light microscopy.

4.3. *Sidneyia inexpectans*

The opportunity to investigate and extend the understanding of gnathobasic microstructures back to the Cambrian is made possible by exceptionally preserved fossils, such as those from the Burgess Shale. A variety of animals from this deposit have been studied using SEM-BSE imaging, which show well preserved microstructures within non-biomineralized cuticular features, such as claws, spines, radulae, and other sclerites. Examples include the lobopodian *Hallucigenia sparsa* (Walcott, 1911b) (Caron et al., 2013; Smith and Ortega-Hernández, 2014), the priapulid *Ottoia tricuspidata* Smith et al., 2015, and the stem-group molluscs *Odontogriphus omalus* Conway Morris, 1976 and *Wiwaxia corrugata* (Matthew, 1899) (Caron et al., 2006; Smith, 2012, 2014). However, this imaging technique has not been used on the gnathobases of Cambrian euarthropods until now.

The SEM-BSE imaging of *Sidneyia inexpectans* gnathobases shows that this taxon has a similar microstructure to the gnathobasic spines of *Limulus polyphemus*, especially the thickened fibrous cuticle in the spine tips of both species (Fig. 10). As discussed in Section 4.1, this fibrous microstructure likely allows *L. polyphemus* to apply considerable force during mastication of prey items (including shells), while also providing a level of protection of sensitive tissue housed within the central spine cavity. This is probably also the case for *S. inexpectans*. Also noteworthy is the relative thickness of the cuticle in the spine tips of *L. polyphemus* and *S. inexpectans*, which appears much thicker in the latter species. This difference may relate to feeding habits. *Limulus polyphemus* has a varied diet that occasionally includes bivalved molluscs (Botton, 1984). Rare *S. inexpectans* specimens preserving shelly cololites (containing fragmentary brachiopods, trilobites and other biomineralised taxa) suggest that it was a durophage, but probably also consumed soft prey (Bruton, 1981; Zacaï et al., 2016; Bicknell and Paterson, 2017). Given the robust gnathobasic spine tips (Figs. 6–9) and variable shelly diet (Zacaï et al., 2016; Bicknell and Paterson, 2017), it is possible that a durophagous habit for *S. inexpectans* was normal, rather than the exception. It is important to note, however, that the relatively thicker spine tips of *S. inexpectans* do not necessarily indicate that it was a more effective durophage compared to *L. polyphemus*. While the gnathobasic microstructures are

broadly similar and well suited for shell-crushing, the mechanical strength of the two taxa would be markedly different given the overall size discrepancy of these two species, particularly with respect to appendage musculature. Thus, *L. polyphemus* is capable of consuming larger (and possibly thicker-shelled) prey compared to *S. inexpectans* (Zacai et al., 2016).

The gnathobasic spines of *Sidneyia inexpectans* commonly display an irregular saw-toothed pattern, with large spines adjacent to at least one smaller spine (Figs. 6B, C, 7A, B, 8A, B, 9A, B). Given the two-dimensional nature of the fossils, this arrangement may represent a single row of spines, or the superimposition of at least two rows resulting from compression (cf. Bruton, 1981, p. 637). Sets of gnathobases of various euarthropods—including *Limulus polyphemus* and *Eurypterus tetragonophthalmus*—are typically arranged into two or more rows of spines (of different shapes and sizes) (e.g., Fig. 11; Manton, 1964, figs 14–16; Selden, 1981, fig. 3). The spine rows are roughly parallel and usually offset (Fig. 11). These gnathobasic spine rows would likely show a similar (irregular) saw-toothed pattern to that seen in *S. inexpectans* if they were compressed into a single plane. Moreover, some appendage specimens of *S. inexpectans* appear to show slight overlap of the gnathobasic spines (e.g., Fig. 7A; Bruton, 1981, pl. 7, figs. 46, 49, 50, pl. 10, figs. 71, 77, pl. 11, figs. 81–83; Stein, 2013, fig. 6C, D, G; Zacai et al., 2016, fig. 3F). This evidence suggests that there is more than one row of spines with variable sizes. A similar saw-toothed pattern is seen in the preserved gnathobases of the durophagous chelicerate *Wisangocaris barbarahardyae* Jago et al., 2016 (their fig. 4A–K) from the lower Cambrian (Series 2, Stage 4) Emu Bay Shale of South Australia.

Sidneyia inexpectans has more robust and stout gnathobases on the posterior appendages, comparable to *Limulus polyphemus* (see Section 3.3) (Bruton, 1981; Stein, 2013). This pattern suggests that *S. inexpectans* likely used the posterior appendages for crushing harder food items (including biomineralized taxa) before passing them anteriorly towards the mouth (Bruton, 1981; Zacai et al., 2016). Like *L. polyphemus*, the more elongate gnathobases on the anterior appendages were probably used to masticate soft prey, as well as push any food items into the mouth opening (Manton, 1964; Botton, 1984).

4.4. Evolutionary implications

Microstructural features are often used as characters in phylogenetic analyses of the Arthropoda. It is therefore important to discuss these new observations in the context of established relationships between modern and fossil euarthropods. A comprehensive phylogenetic analysis of extinct and extant arthropods (Legg et al., 2013), including all three species studied here, demonstrated that xiphosurans and eurypterids represent separate, derived clades within the Euchelicerata. This same study placed *Sidneyia inexpectans* within the Vicissicaudata, a clade positioned outside crown-group chelicerates, but united with xiphosurans and eurypterids in the Artiopoda (Legg et al., 2013, figs. 2, 4). A subsequent study by Legg (2014, fig. 5) produced a similar topology using implied character weighting, but equal character weighting placed *S. inexpectans* as a stem-group euchelicerate. Other phylogenetic analyses have also demonstrated a close relationship between xiphosurans and eurypterids, but typically show *S. inexpectans* to be in a more basal position outside of the chelicerates (e.g., Lamsdell, 2013; Selden et al., 2015; Aria and Caron, 2017). Although these analyses differ in terms of taxon sampling, character selection, coding, and methodology, the relatively consistent phylogenetic positions of these three taxa suggests that perhaps gnathobasic spines with thickened fibrous cuticle (as seen in *Limulus polyphemus* and *S. inexpectans*) is the plesiomorphic condition for chelicerates and more basal sister taxa. This would indicate that the laminate cuticle in the gnathobases of *Eurypterus tetragonophthalmus* and possibly other eurypterids (Dalingwater, 1973a, 1975) is a derived condition. However, this can only be confirmed by determining the gnathobasic microstructure for a broader range of chelicerates and other artiopodans. An alternative hypothesis is that *L. polyphemus* and *S. inexpectans* converged upon a similar gnathobasic spine morphology for the purpose of crushing shells and other hard food items.

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Fig. 1. Line drawing of a transverse section (in anterior view) through the prosoma of *Limulus polyphemus*, showing the walking legs with gnathobasic spines. The gnathobases are located on the coxa and point towards each other. Drawing modified from Selden (1981, fig. 9).

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Fig. 2. The gnathobasic spines of *Limulus polyphemus* (photographed under normal light). (A) Posterior side of the coxa on the fifth (right-side) cephalothoracic appendage (walking leg 4), displaying gnathobasic spines prior to sectioning. (B) The sectioned spines shown in A. (C) Close-up of sectioned spines 2 and 3, showing the tan-brown exocuticle, white endocuticle, and central cavity. (D) Close-up of sectioned spines 5 and 6, showing both exocuticle and endocuticle. Numbered spines correspond to those described in the text and labelled in Fig. 3. Abbreviations – Gn: gnathobasic spine; End: endocuticle; Exo: exocuticle.

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Fig. 3. SEM-SE images of the sectioned gnathobasic spines of *Limulus polyphemus*. (A) Transect through all sectioned gnathobasic spines; boxes outline the three areas illustrated in B–D. (B) The tip of spine 3 showing the thick fibrous exocuticle (grey arrow); note the fibres oriented parallel to the long axis of the spine. (C) Close-up of the side of spine 3 showing the fibrous endocuticle and solid exocuticle. (D) Close-up of the edge of spine 4 showing the laminate exocuticle (grey arrow). The outermost layer around the spine edge is the epoxy resin (as labelled in C) and is not a part of the cuticle. Numbered spines correspond to those described in the text and labelled in Fig. 2.

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Fig. 4. The gnathobasic spines of *Eurypterus tetragonophthalmus* (NRM Ar49887) (photographed under normal light). (A) The set of gnathobasic spines prior to sectioning. (B) The sectioned spines shown in A. White arrows pointing to the boundary between the solid cuticle and the hollow centre of the spine. Numbered spines correspond to those described in the text and labelled in Fig. 5.

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Fig. 5. SEM-SE images of the sectioned *Eurypterus tetragonophthalmus* gnathobasic spines. (A) Transect through all sectioned gnathobasic spines; boxes outline the three areas shown in B–D. Spine 4 is located below the sectioned plane, so was not visible when using the SEM. (B) Close-up of the axial region of spine 6 showing the slightly sinuous laminae. (C) Close-up of the axial region of spine 3 showing the curved laminae that contour the inner margin of the cuticle. (D) Close-up of the side of spine 2 showing the laminae that run parallel to the outer margin of the cuticle. Numbered spines correspond to those described in the text and labelled in Fig. 4.

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Fig. 6. The gnathobasic spines of *Sidneyia inexpectans* (ROM 63388A). (A) Partial set of appendages showing the two types of gnathobases (boxed areas), detailed in B and Fig. 7. (B) Close-up of stout spines, as shown in A; box outlines the area shown in C. (C) SEM-BSE image of the spines boxed in B; box outlines the area shown in D. (D) SEM-BSE image of spine boxed in C, showing the cuticular fibres oriented parallel to the long axis of the spine in the central region and fanning out on either side.

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Fig. 7. The gnathobasic spines of *Sidneyia inexpectans* (ROM 63388A). (A) Close-up of the second type of longer, thinner gnathobasic spines, as shown in Fig. 6A; box outlines the area shown in B. (B) SEM-BSE image of the spines boxed in A; box outlines the area shown in C. The white arrows indicate the thin outer layer of cuticle. (C) SEM-BSE image of spine boxed in B, showing the thick, fibrous cuticle; note the fibres oriented parallel to the long axis of the spine in the central region.

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Fig. 8. The gnathobasic spines of *Sidneyia inexpectans* (ROM 64386). (A) Well preserved row of gnathobasic spines; box outlines the area shown in B. (B) SEM-BSE image of spines shown in boxed area of A; boxes outline the areas shown in C and D. (C) SEM-BSE image of spines showing boundary between cuticle and central basal cavity (white arrows). (D) SEM-BSE image of a spine showing well preserved fibrous cuticle and the boundary between cuticle and the central basal cavity (white arrow).

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Fig. 9. The gnathobasic spines of *Sidneyia inexpectans* (ROM 64387). (A) Complete appendage with well preserved gnathobasic spines; box outlines the area shown in B. (B) SEM-BSE image of spines shown in boxed area of A, with white arrows indicating the thin outer layer of cuticle; box outlines the areas shown in C. (C) SEM-BSE image of a spine in B, with white arrows indicating the thin outer layer of cuticle.

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Fig. 10. Simplified reconstructions of the cuticular microstructure of the gnathobasic spines in *Limulus polyphemus*, *Eurypterus tetragonophthalmus*, and *Sidneyia inexpectans*. For *L. polyphemus*, only the relative thickness of the exocuticle and endocuticle is shown, as well as the orientation of fibres within each of these cuticular layers. The spine of *E. tetragonophthalmus* consists entirely of laminate cuticle, and the spine of *S. inexpectans* consists of a thick fibrous cuticle covered by a very thin outer cuticle. The grey area of each spine represents the central cavity that houses the soft tissues (including nerves).

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Fig. 11. The gnathobasic spines of *Limulus polyphemus* on the fifth cephalothoracic appendage (walking leg 4); the same specimen illustrated in Fig. 2. (A) Anterior view of coxa. (B) Posterior view of coxa. (C) View from the sagittal plane showing offset spine rows.

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*Limulus
polyphemus*

*Eurypterus
tetragonophthalmus*

*Sidneyia
inexpectans*