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Convergent evolution of ventral adaptations for enrolment in trilobites and extant euarthropods

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The ability to enrol for protection is an effective defensive strategy that has convergently evolved multiple times in disparate animal groups ranging from euarthropods to mammals. Enrolment is a staple habit of trilobites, and their biomineralized dorsal exoskeleton offered a versatile substrate for the evolution of interlocking devices. However, it is unknown whether trilobites also featured ventral adaptations for enrolment. Here, we report ventral exoskeletal adaptations that facilitate enrolment in exceptionally preserved trilobites from the Middle Ordovician Walcott–Rust Quarry in New York State, USA. Walcott–Rust trilobites reveal the intricate three-dimensional organization of the non-biomineralized ventral anatomy preserved as calcite casts, including the spatial relationship between the articulated sternites (i.e. ventral exoskeletal plates) and the wedge-shaped protopodites. Enrolment in trilobites is achieved by ventrally dipping the anterior margin of the sternites during trunk flexure, facilitated by the presence of flexible membranes, and with the close coupling of the wedge-shaped protopodites. Comparisons with the ventral morphology of extant glomerid millipedes and terrestrial isopods reveal similar mechanisms used for enrolment. The wedge-shaped protopodites of trilobites closely resemble the gnathobasic coxa/protopodite of extant horseshoe crabs. We propose that the trilobites' wedge-shaped protopodite simultaneously facilitated tight enrolment and gnathobasic feeding with the trunk appendages.

1. Introduction

The ability to enrol the body to form a tight protective ball to deter predatory attacks represents an effective strategy that has evolved multiple times throughout bilaterian evolution, including archetypical examples like xenarthan mammals [1] and several euarthropods such as myriapods [2,3], terrestrial isopods [4,5] and even some insect lineages [6]. Among extinct species, enrolment has been thoroughly documented in trilobites, a diverse group of marine euarthropods typified by the presence of a biomineralized calcitic dorsal exoskeleton (figure 1). Trilobite evolutionary history throughout the Palaeozoic was heavily influenced by their ability to enrol effectively [7–9]. Early Cambrian species show evidence of complete but imperfect (i.e. non-encapsulating) enrolment, leaving open gaps between the thoracic and pygidial spines [10], whereas more derived groups evolved a diverse array of complex interlocking coaptive devices to make this defensive strategy more effective [11–13]. Despite its significance for the long-term evolutionary success of trilobites, enrolment is exclusively known from the perspective of the dorsal exoskeleton due to the paucity of enrolled specimens with exceptionally preserved non-biomineralizing ventral structures (figure 1). Thus, the precise physical mechanisms by which the ventral surface of trilobites could accommodate their numerous biramous appendages and other exoskeletal structures during enrolment remains enigmatic. Attempts to explain how the limbs would be organized relative to each other during enrolment have focused on hypothetical reconstructions of

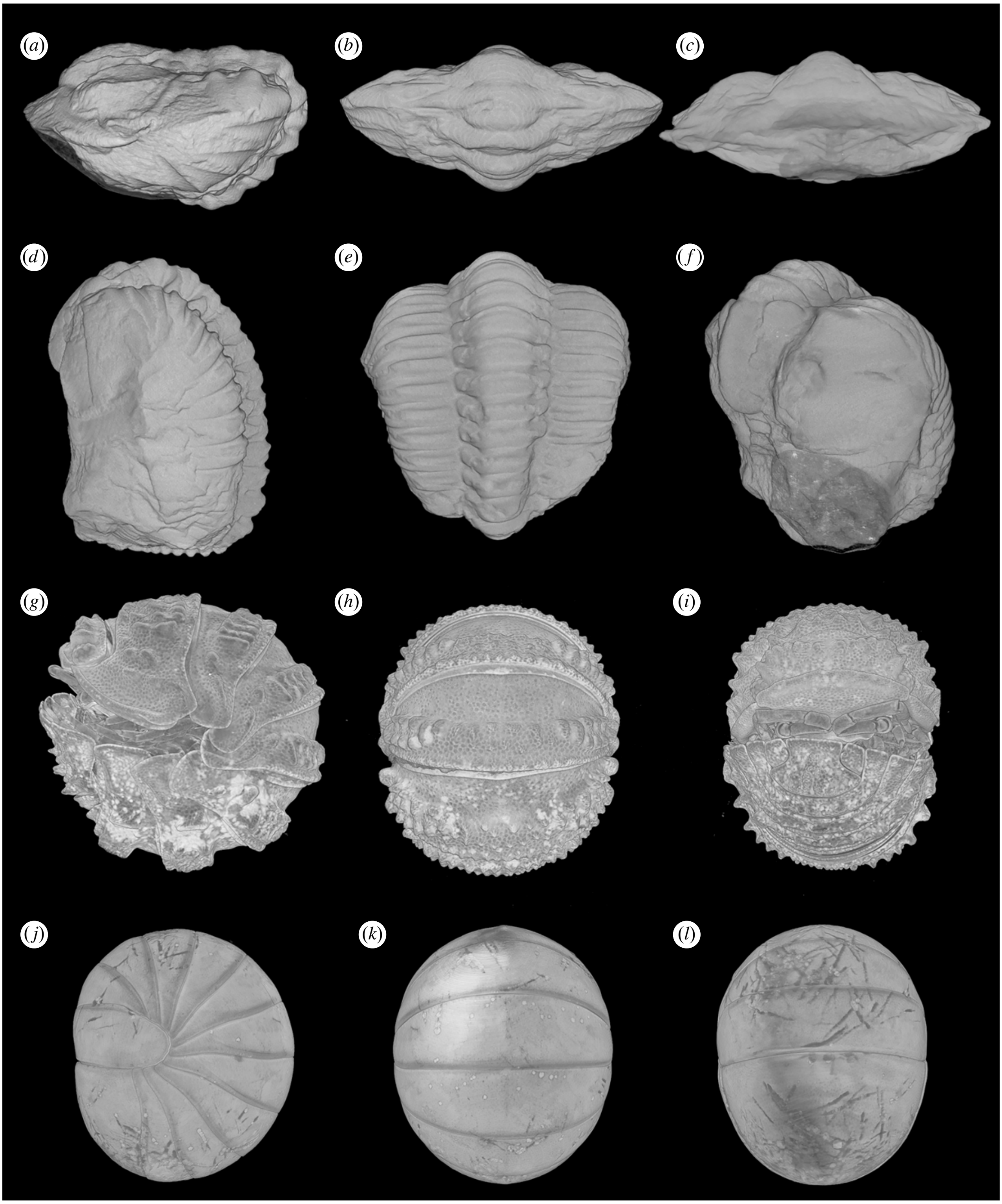

Figure 1. Protective enrolment in trilobites and extant euarthropods. (*a–c*) Tomographic model of fully enrolled *Ceraurus pleurexanthemus* MCZ:IP:186233. (*a*) Lateral view. (*b*) Posterior view. (*c*) Anterior view. (*d–f*) Tomographic model of partially enrolled *Flexicalymene senaria* MCZ:IP:186396. (*d*) Lateral view. (*e*) Posterior view. (*f*) Anteriolateral view. (*g–i*) Tomographic model of partially enrolled terrestrial isopod (MCZ:IZ:90105). (*g*) Lateral view. (*h*) Posterior view. (*i*) Anterior view. (*j–l*) Tomographic model of fully enrolled glomerid millipede (MCZ:IZ:165554-1). (*j*) Lateral view. (*k*) Posterior view. (*l*) Anterior view.

the non-biomineralized structures, like the exceptionally preserved Ordovician trilobite *Placoparia cambriensis* [14]. Although this reconstruction considered the position of the flexible intersegmental tendinous bars based on fossil data, it did not account for the presence of sternites (i.e. ventral exoskeletal plates) that are located in the medial space between each pair of limbs [14]. Moreover, trilobite appendages are infrequently preserved, being only known from *Konservat-Lagerstätten* such as the early Cambrian Chengjiang [15,16], mid-Cambrian Burgess Shale [17,18] and Ordovician Beecher's Bed [19,20]. Trilobite macrofossils from *Konservat-Lagerstätten* are typically highly compressed and their appendages are only found in prone specimens, limiting our understanding of the three-dimensional morphology and organization of the limbs during enrolment. In this context, the precise morphology of trilobite appendages has also not been comprehensively considered in terms of how they would fit in a fully enrolled position. The cross-sectional shape of the trilobite protopodite, for example, has been illustrated as either oval [17,19,21–23], square [20], or authors have omitted them altogether because of the lack of available data [14]. While these differences may seem minor, shape plays a critical role in the function of various body parts [22], and thus this represents a fundamental gap of missing data when reconstructing the early autecology and functional morphology of one of the first successful clades in the evolutionary history of euarthropods.

In this study, we describe the non-biomineralized three-dimensional ventral exoskeletal morphology of trilobites from the Middle Ordovician (Mohawkian) Rust Formation of New York state based on exceptionally preserved fossils with non-biomineralized tissues replicated as calcite casts [24]. Trilobites from the Walcott–Rust Quarry are preserved in various stages of trunk flexure, revealing the intricate coupling of the biramous appendages and sternites to maximize encapsulated enrolment. Comparisons with the three-dimensional exoskeletal and appendicular morphology of extant euarthropods, including a glomerid millipede, a terrestrial isopod and the Atlantic horseshoe crab *Limulus polyphemus*, shows the crucial role of sternite imbrication and protopodite morphology for enrolment. The presence of these functionally similar adaptations in phylogenetically disparate euarthropod lineages demonstrates a profound case of convergent evolution towards a common mechanism for body enrolment separated by over 500 Myr.

2. Materials and methods

All studied specimens are housed at the Museum of Comparative Zoology (MCZ) at Harvard University (Cambridge, MA, USA). Trilobites from the Walcott–Rust Quarry originate from the Middle Ordovician Rust Formation, Trenton Group, in New York state. The exceptionally preserved trilobite fossils are composed of three-dimensional calcite casts of non-biomineralized tissues in a micritic limestone matrix from Layer 3 of the Rust Formation (see [24]). The studied trilobites are mounted as thin sections, produced by Charles D. Walcott in the 1870s [25]. Fossil specimens were imaged at the Digital Imaging Facility (DIF) at the MCZ using a Keyence microscope with transmitted light. Extant euarthropod specimens sampled from the Invertebrate Zoology collections at the MCZ were imaged to analyse their protopodite and sternite morphologies, including *Limulus polyphemus* (MCZ : IZ : 41112), a glomerid millipede (MCZ : IZ : 165554-2) and an oniscidean isopod (MCZ : IZ : 90105). Unfortunately, neither of the two previous specimens can be identified to lower taxonomic levels because they belong to historical collections from the MCZ that were sampled over 100 years ago, and for which species identifications were not available. However, terrestrial oniscidean isopods have morphologically conservative sternites, for example with the porcellioniid *Porcellio scaber* and the armadillidiid *Armadillidium vulgare* from France both displaying two rows of plates with appendages inserting laterally [26]. Glomerid millipede morphology also appears to be stable, with the sternites of the studied specimen bearing striking similarity to the platydesmid *Dolistenus savii* and the siphonophirid *Siphonophora hartii* [27]. All three extant euarthropod specimens were stained with iodine prior to micro-computed tomographic scanning using a Bruker SkyScan 1173 micro-CT scanner at the DIF. Extant specimens were stained in iodine to increase resolution of Micro-CT scanning (see electronic supplementary material information for detailed staining method). Micro-CT imaging was performed at a voltage of 80 kV, wattage of 100 µA, a resolution of 6 µm and with a 0.5 mm thick aluminium filter. Scans were reconstructed as TIFF stacks in NRecon (Bruker Corporation) and visualized and segmented in Dragonfly 2019 4.0 (Object Research Systems, Montreal, Canada). All tomographic data are available through Morphosource (https://www.morphosource.org/) Project 00580889.

One specimen of MCZ:IZ:165554 from the lot of six was dissected and photographed. Dissections were performed using an Ziess Stemi 305 microscope under direct light conditions, and photographs were taken using a Ziess Axiocam 208 colour camera.

3. Results

(a) Sternite morphology and preservation

Specimen MCZ : IP:158251, a thin section of the cheirurid trilobite *Ceraurus pleurexanthemus* in a completely enrolled position (figure 2*a*), reveals the three-dimensional morphology of ventral exoskeletal structures in exceptional detail. The presence of the hypostome and articulating half rings on the same specimen indicates that the section follows the sagittal plane along the midline of the body (figure 2*a*). In addition to showing the pattern of overlap and articulation of the tergites, MCZ : IP:158251 also preserves non-biomineralized ventral exoskeletal structures bound by the body wall as delimited by the presence of sparry and fibrous calcite [24,28,29]. MCZ:IP:158251 features five imbricating and serially arranged ventral structures that dip anteriorly at a 50° angle relative to each other and nearly perpendicular to the dorsal exoskeleton (figure 2*b*). Unlike the dorsal articulating half rings in the same specimen, the ventral structures do not directly overlap each other (figure 2*b*) but are continuous between them based on the distribution of the fibrous calcite. We interpret these ventral features as direct evidence of sternites in *C. pleurexanthemus*, expressed as the thickened exoskeletal plates, connected on the anterior and posterior margins by transverse series of flexible tendinous bars (figure 2*b*). Another specimen of *C. pleurexanthemus* (MCZ: IP:158227) shows a similar sagittal section along the midline of the body, but here the trunk is only partially enrolled, a position that informs the position of the ventral structures under a different configuration (figure 2*c*). MCZ:IP:158227 also preserves five sets of repeating structures, but because of the more abaxial position of the thin section, as indicated by the protopodites seen posteriorly, the sternites are not visible. The anterior ventral structures are underneath the slightly enrolled first five tergites and consist of corrugated bulges with a shorter ventrally concave region between each (figure 2*c*,*d*). The lesser degree of trunk flexure in MCZ:IP:158227 shows that the sternites would be parallel relative to the dorsal exoskeleton in a fully prone position (figure 2*c*,*d*).

Comparisons with three-dimensional datasets of partially and fully enrolled isopods and millipedes supports our interpretation of the ventral morphology of *Ceraurus pleurexanthemus*, as all taxa display the same pattern of anterior imbrication of sternites during enrolment despite their different morphologies (figures 2 and 3). The terrestrial isopod displays a more complex sternite morphology than trilobites, with a row of paired rectangular plates (figure 3*a*–*c*) rather than the single row of hourglass-shaped sternites [14,30]. The anterior edge of the isopod sternite dips ventrally

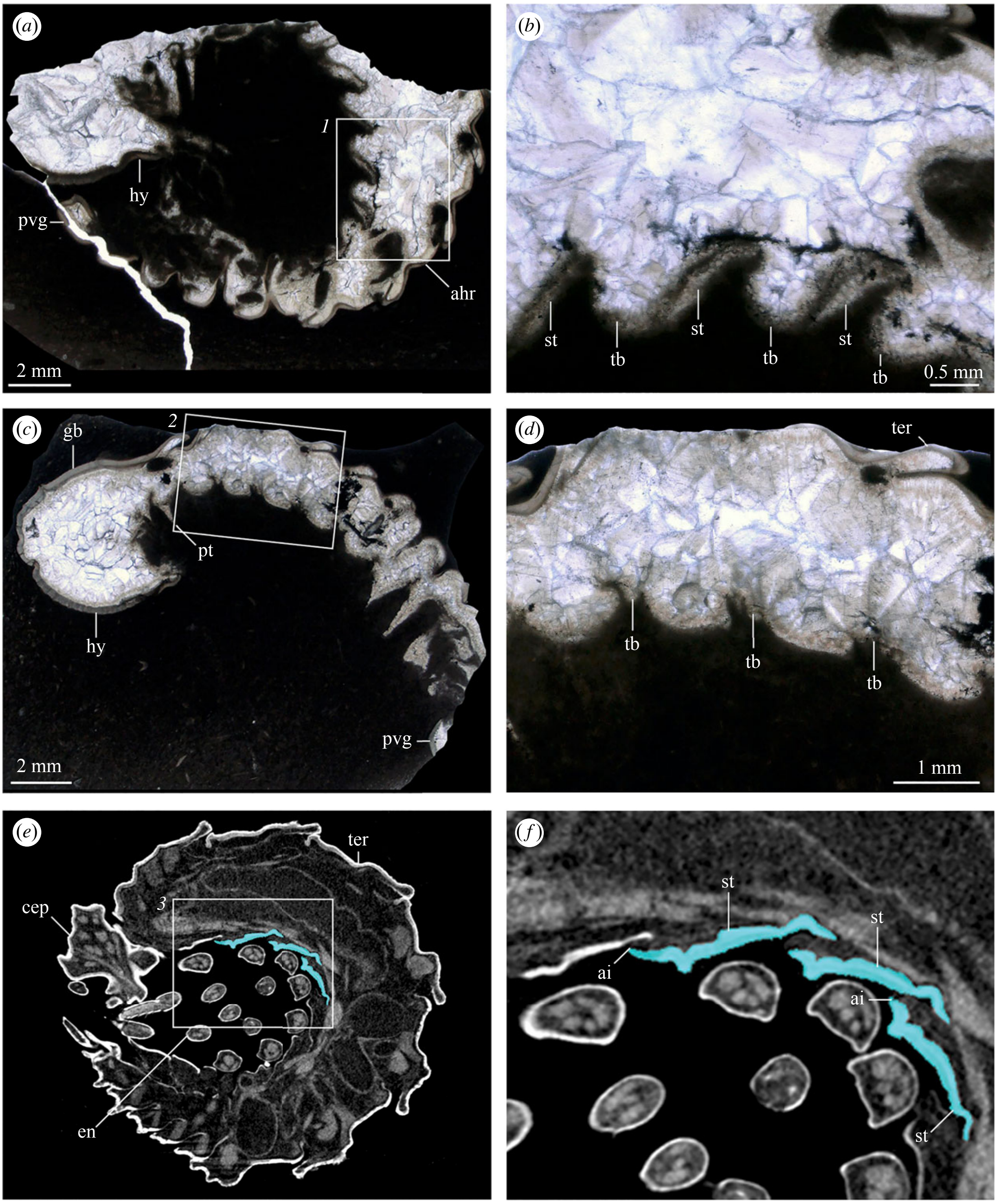

Figure 2. Comparison of sternites and tendinuous bars in *Ceraurus pleurexanthemus* and terrestrial isopod in lateral exsagittal section. (*a*) Photomicrograph of MCZ:IP:158251, a sagittal thin section of a nearly completely enrolled specimen with preserved sternites and tendinous bars. (*b*) Photomicrograph of MCZ:IP:158251 showing magnification of sternites box 1 of (*a*). (*c*) Photomicrograph of MCZ:IP:158227, a sagittal thin section showing tendinous bars in partial enrolment. (*d*) Photomicrograph of MCZ:IP:158227 showing magnification of tendinous bars box 2 of (*c*). (*e*) Tomographic slice of isopod MCZ:IZ:90105 showing corrugation of sternites (blue highlight). (*f*) Magnification of sternites (MCZ:IP:90105). ahr, articulating half ring; cep, cephalon; en, endopodite; gb, glabella; hy, hypostome; pyg, pygdium; st, sternite; tb, tendinuous bar.

during enrolment below the posterior margin of the preceding plate (figures 2*e*,*f* and 3*c*). Glomerid millipedes have wishbone-shaped sternites (figure 3*d*–*f*) with limbs emerging from protopodite/coxa cavities between adjacent ventral plates (figure 3*f*). The elongate lateral portions of each sternite align with the adjacent pleurites (figure 3*e*; electronic supplementary material, figure S1) and imbricate anteriorly during enrolment (figure 3*f*).

(b) Protopodite morphology and preservation

Exsagittal thin sections of Walcott–Rust trilobites such as MCZ:IP:158240 (*Ceraurus pleurexanthemus*; figure 4*a*,*b*) and MCZ:IP:104956 (*Flexicalymene senaria*; figure 4*c*,*d*) show the lateral ventral morphology in three dimensions with exceptional detail. The hypostome and articulating half rings of the tergites are visible in these specimens, similarly to the

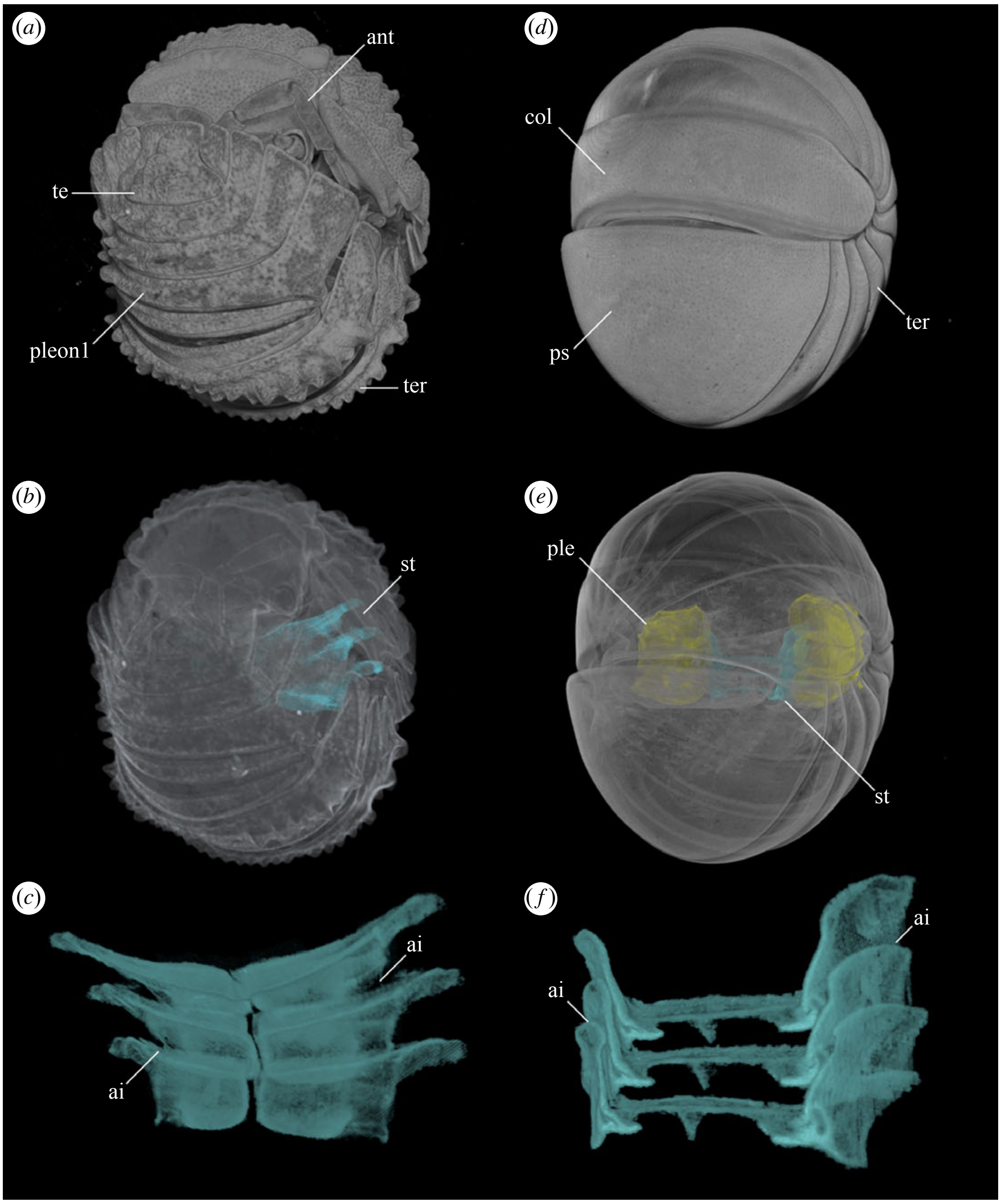

Figure 3. Sternite organization during enrolment in terrestrial isopods and glomerid millipedes. (*a–c*) Micro-CT scan of terrestrial isopod MCZ:IZ:90105. (*a*) Tomographic model of 90% enrolled specimen. (*b*) Micro-CT scan of full specimen with three segmented sternites (blue highlight). (*c*) Micro-CT segmented sternites from three trunk segments. (*d–f*) Micro-CT scan of glomerid millipede MCZ:IZ:165554-2. (*d*) Tomographic model of completely enrolled specimen. (*e*) Reconstruction of full specimen with three segmented sternites (blue highlight) and pleurites (yellow highlight). (*f*) Micro-CT segmented sternites from three trunk segments. ai, anterior imbrication; ant, antenna; col, collum; ple, pleurite; ps, posterior shield; st, sternites; te, telson; ter, tergite.

sagittal thin section in MCZ:IP:158251 (figure 2*a*), but the posterior projections of the hypostome indicate that the more abaxial position near the lateral margin of the axial lobe (figure 4*a*,*c*). In both MCZ:IP:158240 and MCZ: IP:104956, a series of serially repeating wedge-shaped ventral structures are associated with each of the tergites (figures 4*a* and 5*c*,*d*). The presence of fibrous calcite defining these structures indicates that they were originally non-biomineralized [24]. The wedge-shaped ventral structures are widest dorsally and taper ventrally to a point at a 40–50° angle (figure 4*b*,*d*). Specimen MCZ:IP:104956 of *F. senaria* displays a series of 22 wedge-shaped structures, four of which are associated with the cephalon, but none are visible beneath the pygidium (figure 4*c*). MCZ:IP:104956 is 65% enrolled and the series of wedge-shaped structures are angled slightly anteriorly (figure 4*c*). The anterior most wedges have a straight anterior margin that gently curves anteriorly at their mid-section (figure 4*d*). The posterior margin of the wedge is similarly curved, which allows for the succeeding wedges to fit snuggly against one another when in direct contact (figure 4*d*). The terminal tip of the wedge is slightly enlarged and bulbous (figure 4*d*).

Based on their taphonomy and morphology, we interpret the serially repeating wedge-shaped structures observed in both *Ceraurus pleurexanthemus* and *Flexicalymene senaria* as direct evidence of three-dimensionally preserved protopodites, namely the part of the arthropodized biramous appendage that is in direct contact with the body wall [31], as observed in a cross-sectional view from an exsagittal plane. This interpretation is supported by the precise association of a wedge with each tergite (figure 4*c*) and the abaxial position relative to the axial lobe of the dorsal exoskeleton

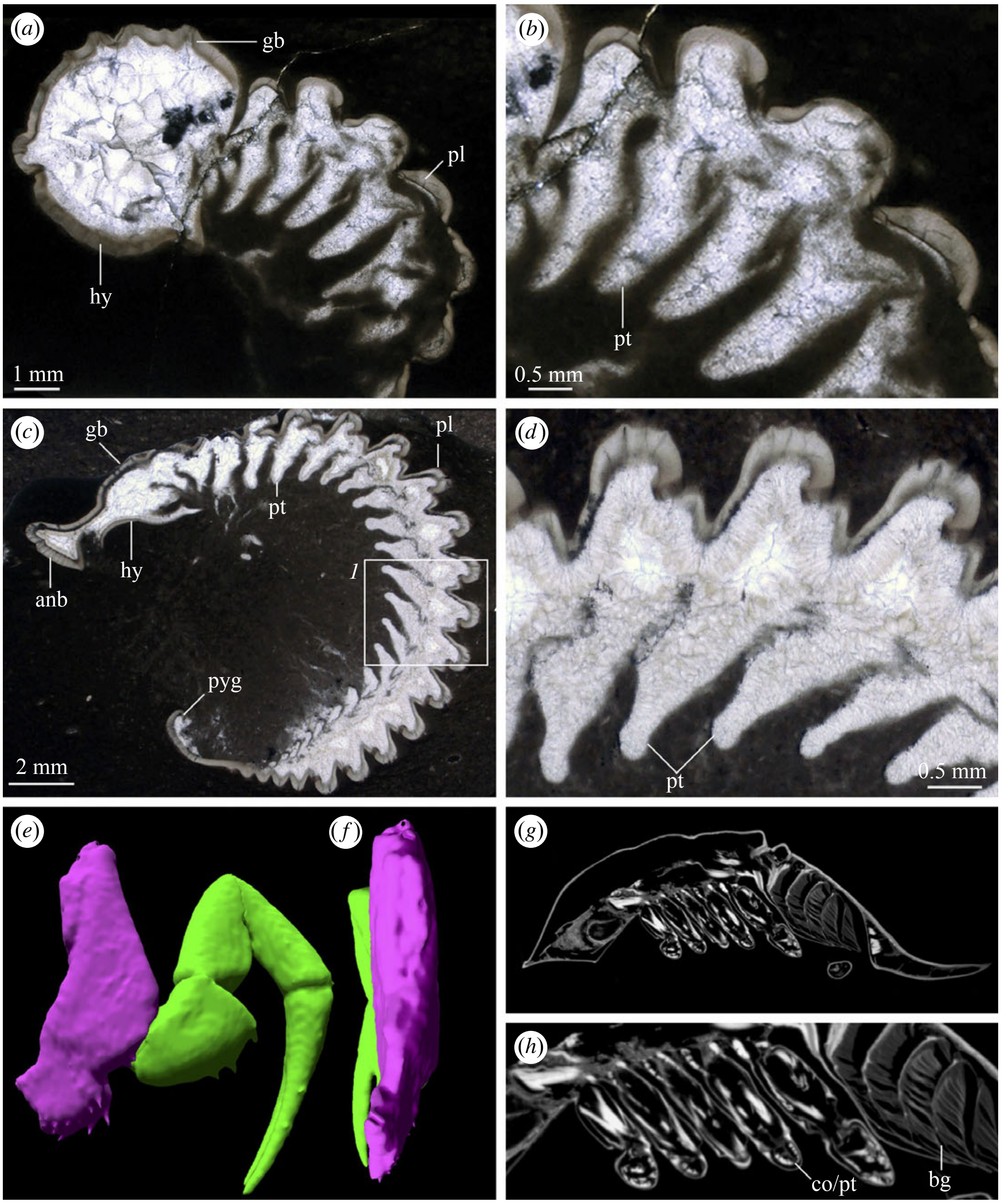

Figure 4. Comparison of coxa/protopodite morphology in Walcott–Rust trilobites and *Limulus polyphemus* in lateral section. (*a*) Photomicrograph of *Ceraurus pleurexanthemus*, an exsagittal thin section of showing protopodites in cross section from a lateral view (MCZ:IP:158240). (*b*) Magnification of protopodites. (*c*) Photomicrograph of *Flexycalymene senaria*, a exsagittal thin section of showing protopodites in lateral view (MCZ:IP:104956), figured in [28] Plate 5, figure 3. (*d*) Magnification of protopodites in box 1 of (*c*). (*e*) Micro-CT segmentation of *Limulus polyphemus* (MCZ:IZ:41112) showing anterior view of walking leg two including coxa/protopodite (purple highlight) and endopodite (green highlight). (*f*) Micro-CT segmentation of *Limulus polyphemus* (MCZ:IZ:41112) showing medial view of walking leg two. (*g*) Tomographic slice of *Limulus polyphemus* (MCZ:IZ:41112) in exsagittal view showing lateral section of protopodite. (*h*) Magnification of coxa/protopodite in lateral section. anb, anterior band of cranidium; co, coxa; gb, glabellae; hy, hypostome; pl, pleural lobe; pt, protopodite; pyg, pygidium.

which can also be seen in a transverse thin section of *C. pleurexanthemus* showing an anterior view of the proximal portion of the appendage (figure 5*a*).

The repeating wedge-shaped structures are not part of the biomineralized dorsal exoskeleton, such as muscle attachment sites or apodemes [32–34] as the original calcite would be clearly visible similar to the tergites (figure 4*b*,*d*). Comparisons with additional thin section specimens of *Ceraurus pleurexanthemus* further strengthen the interpretation of the wedges as protopodites. Specimen MCZ:IP:110933 shows a clear and unobstructed view of one biramous appendage (figure 5*a*), which shows that the laterally splayed protopodites of *C. pleurexanthemus* are subtriangular in anterior view with a nearly vertical medial margin and horizontal ventral margin. The medial margin is studded with gnathobasic spines, and the ventral edge is marked by elongate endites (figure 5*a*). The exopodite is visible dorsally and the endopodite extends from the distal margin of the protopodite (figure 5*a*). The protopodite of MCZ:IP:110933 extends from the lateral edge of the dorsal exoskeleton's axial lobe and partially into the pleural lobe, which closely correlates with the position of the wedge-shaped structures seen in exsagittal thin sections (figure 4*a*,*c*). One specimen (MCZ:IP:110918) of *Flexicalymene senaria* sectioned in coronal (perpendicular to the sagittal and transverse planes) view shows a series of wedge-shaped structures whose apex points towards the midline of the body (figure 5*c*,*d*). The position within the body and the comparison with the two other

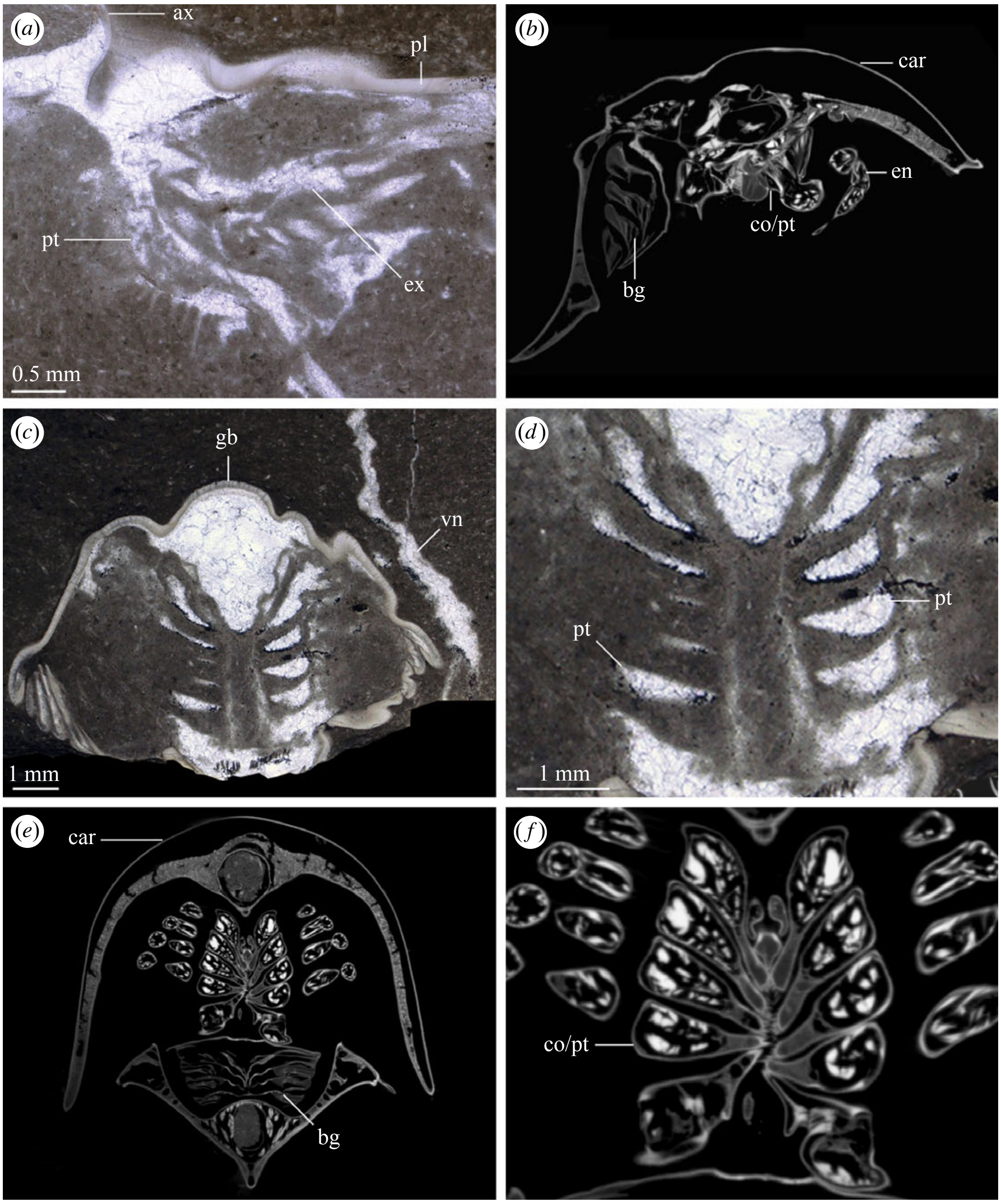

Figure 5. Comparison of coxa/protopodite morphology in Walcott–Rust trilobites and *Limulus polyphemus* in anterior and coronal sections. (*a*) Photomicrograph of *Ceraurus pleurexanthemus*, a transverse thin section of showing an anterior–posterior view of the protopodite (MCZ:IP:110933). (*b*) Tomographic slice of *Limulus polyphemus* (MCZ : IZ : 41112) showing anterior view of coxa/protopodite. (*c*) Photomicrograph of *Flexicalymene senaria*, a transverse thin section showing coronal view of four protopodites (MCZ:IP:110918). (*d*) Magnification of protopodites of MCZ : IP : 110918. (*e*) Tomographic slice of *Limulus polyphemus* (MCZ:IZ: 41112) showing coronal view of coxae/protopodites. (*f*) Magnification of coxae of MCZ:IZ:41112. ax, axial lobe; car, carapace; co/pt, coxa/protopodite; bg, book gill; ex, exopodite; gb, glabella; pl, pleural lobe; pt, protopodite; vn, calcite vein.

views (figure 4*c*,*d*), support the interpretation of these structures representing the protopodites as observed from a dorsal section, which further confirms their wedge-shaped three-dimensional organization.

Comparisons with the three-dimensional morphology of *Limulus polyphemus* support the interpretation of the ventral structures seen in *Ceraurus pleurexanthemus* and *Flexicalymene senaria* as exsagittal and coronal views of protopodites based on the wedge-shaped morphology of the coxa/protopodite (figure 4*e*,*f*). The coxa/protopodite of the walking legs in *L. polyphemus* are dorsoventrally elongate with a large area of attachment to the body wall, the dorsal edge is broad in cross section decreasing in width ventrally (figure 4*e*,*f*). In cross section from an exsagittal view, the coxae/protopodites are broadest dorsally, tapering ventrally (figure 4*g*,*h*). The coxae/protopodites are also wedge-shaped in coronal view, narrowest near the body wall and widening distally (figure 5*e*,*f*), a condition that is also seen in *Flexicalymene senaria* (figure 5*c*,*d*).

4. Discussion

Walcott–Rust trilobites reveal new insights into the ventral morphology of trilobites, with direct implications for understanding their adaptations for enrolment. A single row of hourglass-shaped sternites are known throughout Trilobitomorpha such as *Arthroaspis bergstroemi* [35], *Misszhouia longicaudata* [36,37] and *Sinoburius lunaris* [38]. The pyritized olenid trilobite *Triarthrus eatoni* preserves appendages, sternites and tendinous bars [19], which closely resemble those found in the pliomerid *Placoparia cambriensis* [14] and the possible tendinous bars seen in calymenid *Gravicalymene granulosa* [39]. These examples of preserved sternites are only observable in either ventral or dorsal view due to their preservation in compacted body fossils, therefore not providing information about the three-dimensional morphology, position within the body or movement during enrolment. The Walcott–Rust specimens provide complementary views of the sternites and protopodites that allow to reconstruct their three-dimensional overall morphology.

(a) Sternite position during enrolment

All known cases of sternite preservation in trilobites and non-biomineralized trilobitomorphs point towards the same broad pattern of morphological organization, in which the sternites are successively arranged in an axial row that runs parallel to the dorsal exoskeleton, and which are separated by flexible tendinous bars (figure 2*c*,*d*) [14,35–38]. The sternites are cuticular and have a thinner constitution than the dorsal exoskeleton; however, the ventral side of the body would not be able to physically accommodate the entire sternite series during encapsulated enrolment while maintaining their outstretched disposition without producing excessive tension on either the ventral side, due to over compression, or the dorsal side, due to over extension (figure 5*f*). Instead, the ventral data from Walcott–Rust trilobites demonstrate that the sternites and tendinous bars become corrugated in the transition between prone position to partial and full enrolment (figure 2*a*,*b*), with the anterior edge of the sternite angling ventrally and the flexible tendinous bar bulging (figure 6*a*–*d*). Critically, the same configuration between the sternites and arthrodial membranes are also observed in isopods (figure 3*a*–*c*) and millipedes (figure 3*d*–*f*), with the anterior edge of the sternites dipping ventrally to accommodate tight, encapsulated enrolment. These comparisons indicate that despite the distant phylogenetic relationships between trilobites (extinct stem-group chelicerates), isopods (extant crustaceans) and millipedes (extant myriapods), these euarthropods share fundamentally similar exoskeletal ventral adaptations that facilitate protective enrolment. These findings evidence a striking case of convergent evolution that is heavily influenced by the mechanical requirements and limitations necessary to achieve encapsulation in euarthropods, which have been extensively investigated in terms of their dorsal exoskeletal morphology (e.g. [9,11–13]), but never from the perspective of the ventral anatomy until now. Repeated convergent evolution of the same mechanism in three distantly related euarthropods demonstrates the constraints of achieving complete enrolment with a rigid exoskeleton, and simultaneously the evolutionary advantages that this strategy must confer. Even more notably, sternite morphology varies between the three taxa, from a single row of hourglass-shaped plates in trilobites (figure 2*a*), paired rectangular plates in isopods (figure 3*c*), to wishbone-shaped sternites in glomerids (figure 3*f*), yet all species enrol using the same basic mechanism.

(b) Functional implications for trilobite musculature during enrolment

The new insights into the ventral exoskeletal organization of the trilobite exoskeleton have direct implications for understanding the functional morphology of the trunk musculature during enrolment (figure 6). Three proposed dorsal–ventral muscles would attach from tergites to sternites based on crustacean analogues; the anteriorly descending muscle, the vertically descending muscle and the posteriorly descending muscle [14,40]. Longitudinal ventral muscles have been proposed to be on either side of the body of trilobites, attaching to the tendinous bar [14,41–43]. The reconstruction of an exceptionally preserved specimen of *Placoparia cambriensis* with soft tissues in sagittal cross section [14] bears striking similarities to MCZ:IP:158227 which is not fully enrolled (figure 2*c*). However, the fully enrolled reconstruction illustrates the ventral anatomy exactly the same as in the prone position [14] despite the Walcott–Rust trilobites and MCZ:IP:158251 having been published nearly 100 years before with the corrugated ventral structures highlighted [28]. A proposed mechanism of enrolment and extension for trilobites relied on the contraction of the longitudinal ventral muscle to bring the sternites closer together, enrolling the body versus contraction of the dorsal longitudinal muscle that would extend the body to a prone position [14] (figure 6).

In extant euarthropods that completely enrol their body similarly to trilobites, such as glomeriid millipedes and terrestrial isopods, contraction of the longitudinal ventral muscle flexes the body ventrally [27]. Isopod enrolment, also known as conglobation, requires appropriate and strong musculature to achieve and maintain this posture [44]. However, the sternites are too long (sag.) to allow enrolment without substantial overlap between them (figure 3*c*,*f*). In this context, the sagittal thin section of *C. pleurexanthemus* (MCZ:IP:158251) demonstrates that trilobite enrolment was accomplished by ventrally dipping the anterior margin of the sternites, producing a corrugated outline of sternites and arthrodial membrane (figure 3*c*,*f*). Based on their basic functional morphology, euarthropod enrolment is achieved through contraction of the longitudinal ventral muscle and the vertically descending muscle, bringing sternites closer together and raising the posterior margin dorsally (figure 6*b*,*c*). Returning to the prone position is accomplished through contraction of the longitudinal dorsal muscle and anteriorly descending muscle, which brings the tergites closer together and raises the anterior margin of the sternite to a horizontal position (figure 6*d*).

(c) Functional implications of wedge-shaped protopodites

The overall morphological organization of the trilobite protopodite has been elusive because of its non-biomineralized nature and proximity to the midline, with its three-dimensional structure being especially difficult to reconstruct because of a lack of suitable trilobite fossils that clearly show this structure [17,20,22,34,45,46]. This has left a gap in our understanding of locomotion, enrolment and feeding autecology because of the crucial role of the protopodite [21,22,30,47]. Appendages are most frequently preserved in highly compressed anterior or posterior views such as *Olenoides serratus* from the Burgess Shale [17,20], *Redlichia rex* from Emu Bay Shale [46], or *Hongshiyanaspis yiliangensis* from Hongjingshao Formation [45]. The lack of detailed information regarding the cross section of the trilobite protopodite has resulted in a variety of hypothetical morphological interpretations. For instance, the cross section the trilobite protopodite has been

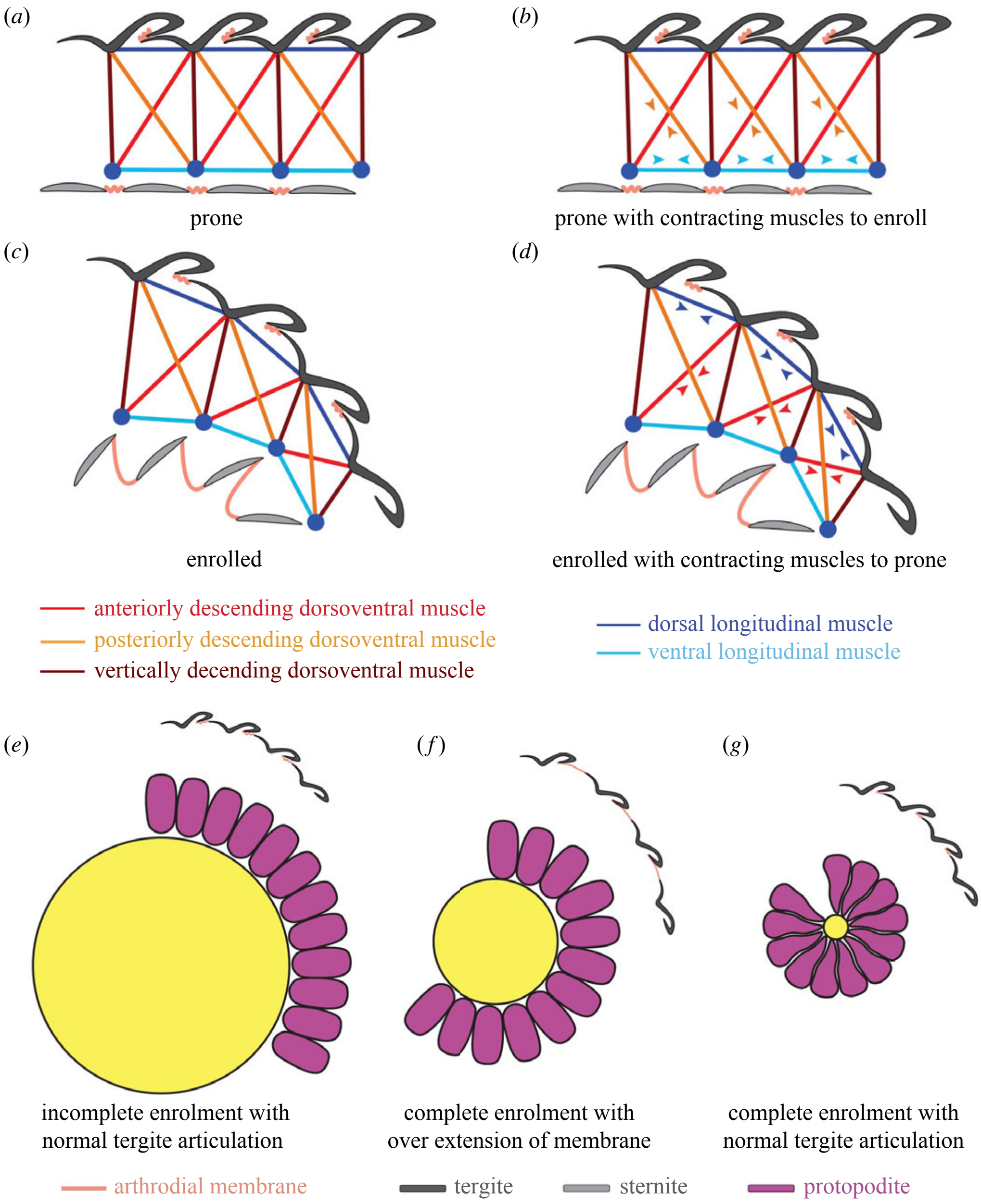

Figure 6. Functional morphology of trilobite enrolment. (*a–d*) Hypothesized muscle attachment in trilobites with contraction indicated by arrow heads. (*a*) Prone position. (*b*) Prone position showing contraction along the ventral longitudinal and posteriorly descending dorsoventral muscles to enrol. (*c*) Enrolled position with corrugation sternites dipping anteriorly and extended tendinuous bars. (*d*) Enrolled position showing contraction of dorsal longitudinal and anteriorly descending dorsoventral muscles to extend the body. (*e–g*) Diagram showing impact of protopodite morphology on complete and tight enrolment. (*e*) Oval protopodites restrict complete enrolment under normal tergite extension. (*f*) Oval protopodites with maximum contraction of the ventral edge cause excessive dorsal extension, exposing the arthrodial membrane. (*g*) Wedge-shaped protopodites based on MCZ:IP:104956 facilitate complete and tight enrolment without over extension of dorsal arthrodial membrane.

reconstructed as having disparate shapes, including oval (e.g. [17,19,21–23,30]), square [20], or this aspect has been omitted them altogether because of the lack of available data [14].

Wedge-shaped protopodites may be common across Trilobita and even more broadly within euarthropods. Specimens of *Isotelus* [48] are preserved with ventral views of three-dimensional protopodites [49,50] which appear as thin transverse to anterolateral bars, a similar condition to that seen in *Triarthrus eatoni* [19,51]. Wedge-shaped protopodites can explain this view, as the *Isotelus* specimens show a coronal cross section similar to *Flexycalymene senaria* MCZ:IP:110918 (figure 4*c*). The thin and elongate appearance of protopodites in *Isotelus* and *T. eatoni* is the result of the coronal view through the ventral edge or middle of the structure resulting in a superficially narrow morphology. The Silurian trilobite *Dalmanites* sp. from the Herefordshire Biota is preserved as three-dimensional calcite casts visualized through serial sectioning [34] similar to the preservation seen in the Walcott–Rust fossils [24]. This allows segmentation of individual appendages which display wedge-shaped protopodites resembling those of *Flexicalymene senaria* and *Ceraurus pleurexanthemus* (figure 4*a–d*). Wedge-shaped protopodites are also found in other trilobitomorphs, such as xandarellids based on a specimen preserved as a three-dimensional external mould in displaying a coronal view of the appendages which plunge into the matrix [52]. The protopodites appear elongate and slender, similar to *Isotelus* and *T. eatoni* discussed above. Given the similar orientation to those specimens, the wedge-shaped protopodite morphology can also account for this specimen with only a two-dimensional view of the structures being visible from the surface. An exception to this appears to be *Agnostus pisiformis* [53] which has an oval coronal cross section [54], but determining protopodite cross section from the published literature is difficult because the required views are rarely illustrated for extant species and infrequently preserved in fossil specimens.

The widely reproduced oval cross section for the protopodite [17,19,21–23,30] would severely hamper the ability of trilobites to enrol effectively. The oval protopodite would make it impossible for trilobites to achieve complete enrolment under the normal observed range of motion of the tergites (figure 6*e*), or alternatively, would require the dorsal side of the body to overextend significantly, leaving open gaps between the articulating tergites and exposing the delicate arthrodial membrane to predators (figure 6*f*). In this context, the distinctively wedge-shaped protopodites of *Flexicalymene senaria* and *Ceraurus pleurexanthemus* (figure 3*a–d*) would play a critical role during enrolment by facilitating a tight body flexure, but without causing dorsal over extension thanks to their narrow ventral margin and form-fitting shape relative to each other (figure 6). Comparisons with the three-dimensional appendage morphology of glomerid millipedes and terrestrial isopods indicate that these extant taxa do not have a wedge-shaped protopodite but differ from trilobites in having medially—(electronic supplementary material, figure S1, S2*a–c*) or laterally—(electronic supplementary material, figure S2*d–f*) attached appendages as opposed to an intermediate condition as in trilobites (figure 4*a*) and other extinct trilobitomorphs. A critical difference between these extant taxa and trilobites, however, is the fact that the former do not use the trunk appendages for food processing, but instead employ the modified mandibles as mouthparts [55], whereas the entire limb series of trilobites has an active role in feeding based on the presence of well-developed gnathobasic spines along the food groove [22,40,56,57]. By contrast, the three-dimensional morphology of the trilobite protopodite is more similar to that of *Limulus polyphemus* both in terms of its transverse and dorsal sections (figures 3*e–h* and 4*f*,*g*), which does engage in aquatic gnathobasic feeding with the entire prosomal limb series [22,47]. Based on these comparisons, we propose that the wedge shape of the trilobite protopodite reflects a unique functional tradeoff between the physical constrains required for tightly accommodating the appendages during enrolment (figure 6*g*), coupled with their pivotal role in food processing during gnathobasic feeding through the ventral groove of the body.

The restudy of the abundant trilobites from the Rust Formation at the MCZ and Smithsonian Institute reveal critical information on the three-dimensional morphology of the biramous appendages in Ordovician taxa, which complements the available compressed macrofossil record from Palaeozoic localities with exceptional preservation. Given the richness of fossil data in these collections, the present study has focused on the proximal organization of the protopodite and mechanisms of enrolment, whereas a more in-depth redescription of the entire appendicular organization of *Ceraurus pleurexanthemus* and *Flexicalymene senaria* is underway. The ability of trilobites to enrol for protection represents an iconic adaptation that heavily influenced the long and successful evolutionary history of these extinct euarthropods. Our new data from the Walcott–Rust Quarry reveal for the first time that, in addition to the coaptative devices on the dorsal calcitic exoskeleton, trilobites also featured ventral morphological adaptations of the non-biomineralized sternites and biramous appendages that played a critical and multifaceted role in their mode of life. We find direct evidence that the fundamental mechanism of sternite corrugation that facilitate complete enrolment in trilobites is also expressed in extant glomerid millipedes and terrestrial isopods, showing a striking case of convergent evolution in phylogenetically distant euarthropod clades separated by hundreds of millions of years.

Data accessibility. All tomographic data are available through Morphosource (https://www.morphosource.org/) Project 00580889.

Supplementary material is available online [58].

Authors' contributions. S.R.L.: conceptualization, investigation, methodology, project administration, visualization, writing—original draft, writing—review and editing; P.A.: methodology, visualization, writing—review and editing; K.N.: conceptualization, methodology, writing—review and editing; J.O.-H.: conceptualization, project administration, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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