

**Euarthropods on a roll:
Comparative anatomy of the trunk musculature
of volvatory isopods and millipedes.**

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Abstract

Defensive enrollment, or volvation, is a widely successful antipredatory strategy known from several animal groups, ranging from xenarthran mammals to select euarthropods. In the latter, oniscomorph millipedes and armadillidiid isopods have independently evolved the ability to enroll into near-perfect spheres, representing a striking instance of convergent evolution whose biomechanical basis remains largely unexplored. In this study, we used natural history museum specimens and X-ray micro computed tomography to generate high-fidelity 3D models of the internal and external trunk anatomies of Oniscomorpha and Armadillidiidae, with particular focus on the musculature responsible for enrollment. The segmented specimens showed good overall muscle preservation, validating the use of decades-old and chemically fixed museum samples for micro-CT-based investigation of euarthropod muscles. Tomographic models reveal complex trunk musculatures including dorsal, ventral, and dorsoventral elements in both taxa. Large tergal apodemes provide attachment sites for the trunk musculature, which anchors ventrally to the armadillidiid sternites and oniscomorph pleurites, respectively. However, the muscle groups responsible for enrollment, including abduction and adduction, differ between taxa, with higher complexity in armadillidiids. Volume estimates indicate that armadillidiids feature higher overall investment in trunk muscles than oniscomorphs, and higher relative investment in enrollment. We hypothesize that the complexity and large muscle masses displayed by armadillidiid isopods reflect different adaptive strategies, constraints imposed by their distinct exoskeletal architecture, or both.

I. Introduction

Volution, the defensive enrollment of the body, is a common strategy employed by a large diversity of animals to resist predation (Cazzolla Gatti et al., 2020; Eisner & Davis, 1967; Tuf & Ďurajková, 2022). Conglobating into a tight ball presents the combined benefits of protecting the organism's often delicate underside while exposing a hard, subspherical surface that potential predators can't easily process (**Figure 1**). This adaptive breakthrough can be traced back to the Cambrian Period, when trilobites enrolled to shield unarmored or otherwise vulnerable ventral structures (Losso et al., 2023). Volvatory behaviour has since convergently evolved in a number of more derived bilaterian taxa, from select euarthropods (crustaceans, myriapods, insects) to cingulatan (armadillos), pholidatomorph (pangolins) and erinaceous (hedgehogs) mammals (Bellini et al., 2019; Moller-Krull et al., 2007; Superina & Loughry, 2012; Tang et al., 2019). In euarthropods, complete enrollment is best exemplified by armadillidiid isopods (Smigel & Gibbs, 2008) and oniscomorph diplopods (Hadrián & Šipoš, 2016), while some hybosorid beetles (Ballerio & Grebennikov, 2016; Lu et al., 2022), cuckoo wasps and other insects (Perry & Nalepa, 2003) perform an incomplete form of conglobation, relying on the sole articulation of intertagmal joints with little intersegmental movement.

The efficacy of euarthropod volvation is easily demonstrated by its evolutionary success, yet it presents several biomechanical challenges. First, the external morphological characteristics of the exoskeleton must enable its enrollment. Dorsal exoskeletal structures need to coaptively interlock to minimize potential weaknesses, while the spatial arrangement of the ventral pieces must accommodate associated appendages in a central cavity. Similarly, the shape of their protopodites (the proximalmost portion of the leg (Boxshall, 2004)) may physically restrict the tight body flexure required for successful volvation (Losso et al., 2023). Second, enrolling and maintaining tonic immobility requires high investment in relevant musculature. Muscles

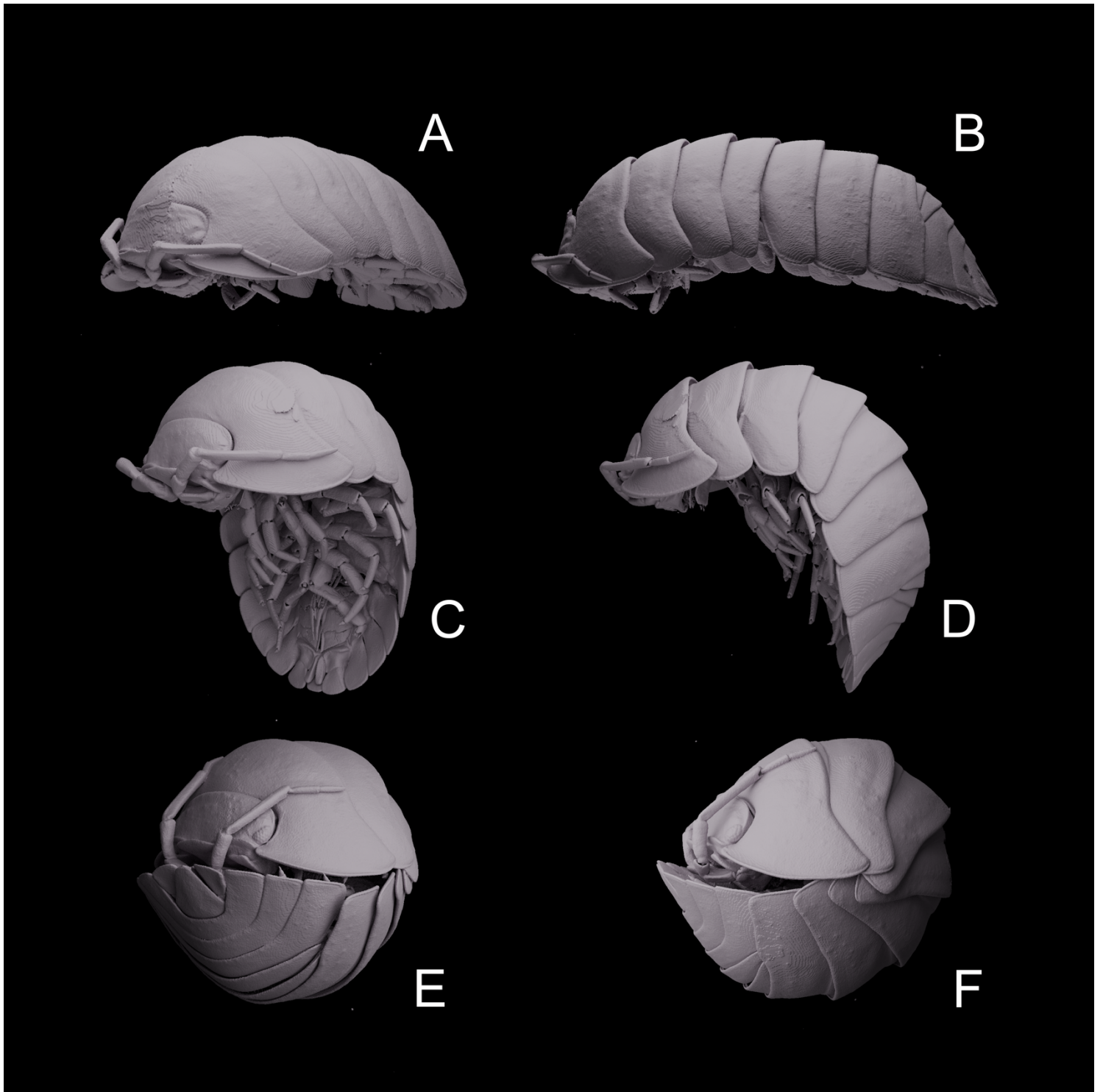


Figure 1 | Isopod enrollment sequence (MCZ:IZ:CRU-7087A – C) in oblique (left) and lateral (right) views.

inside the body cavity must enable rapid transitions from a prone state to a rolled-up state, and vice versa, with the strength necessary to withstand predation attempts. Third, this

musculature must work together with internal exoskeletal features to ensure enrollment is mechanically efficient.

The great diversity of volvatory euarthropods display an equally varied set of external exoskeletal features involved in their defensive behaviour. In conglobating Coleoptera, a series of grooves and keels locks exoskeletal pieces into place while enrolled (Ballerio & Grebennikov, 2016). This striking adaptation likely limits the energetic cost of tonic immobility while increasing resistance to forced unenrollment. In armadillidiid isopods, the dorsal exoskeletal plates imbricate in a repeating pattern while prone, only leaving the posterior tergal margins exposed. The tergites retain significant overlap once enrolled, representing potential points of purchase for predators. Perhaps as a direct response to this shortcoming, isopod tergites can occasionally bear protruding spikes, variable in number, shape and size, for further protection. Extant oniscomorph (“pill”) millipedes, on the other hand, never bear tergal spikes. They also differ from armadillidiid isopods by their extremely smooth conglobated shape, forming near-perfect spheres with minimal tergal overlap (Hadrián & Šipoš, 2016). The anterior tergal margins bear transverse grooves that have been hypothesized to help support preceding tergites when the animal is enrolled (Hadrián & Šipoš, 2016; Hannibal & Feldmann, 1981). Previous research on colobognathan diplopods has highlighted the presence of a sclerotized, intucked fold at the posterior margins of the tergite, protecting the arthrodial membrane (Manton, 1961). Whether this structure is present in Oniscomorpha and how it might interact with the anterior tergal margin remains unclear.

The internal adaptations that underpin euarthropod volvation remain largely unexplored. This is particularly true of the highly specialized armadillidiid isopods and oniscomorph millipedes, whose muscular architectures have received little attention. Previous research on Oniscidea has focused on the cephalic and appendicular musculature (Schmitz, 1986), but the trunk muscles of these organisms have yet to be studied. In diplopods, Manton (1961) provided a comprehensive description of the trunk musculature of Colobognatha based on dissected specimens. This work suggested that the partial, spiral-like enrollment of these animals is mediated by ventral, dorsal, and dorsoventral muscles, which together with large prothragmal apodemes impose

rotational forces on exoskeletal pieces (Manton, 1961). While they inform a basic template for high euarthropod trunk flexure, these findings are unlikely to fully reflect the enrollment musculature of the distantly related Oniscomorpha, and extrapolating them to armadillidid isopods would be methodologically inappropriate. Another challenge may be presented by specimen availability and preservation, since long-term storage in ethanol has been shown to cause muscle damage in both vertebrates (Leonard et al., 2022) and aquatic euarthropods (Begić et al., 2022), though this remains unstudied in the specific cases of Armadillidae or Oniscomorpha. An assessment of the oniscomorph and armadillidid trunk musculatures integrating potential preservation biases is therefore needed.

In this study, we used natural history museum specimens to characterize the trunk musculatures of armadillidid isopods and oniscomorph diplopods through a combination of micro-computed tomography (micro-CT), 3D segmentation and volume rendering. The high-fidelity, three-dimensional models obtained from this workflow reveal good overall preservation of muscular structures in ethanol over long time scales (> 100 years) and confirm that enrollment behaviour depends on the synergistic action of multiple trunk muscles. Armadillidid isopods display higher structural complexity and larger muscular volumes in comparison to Oniscomorpha, indicating higher overall investment in volutary behaviour. Only part of these structures are conserved across taxa, highlighting the role of divergent adaptations in enabling a remarkable case of functional convergence.

II. Materials and methods

II.1 | Specimens

All specimens used for this study are housed at the Museum of Comparative Zoology (MCZ, Harvard University, Cambridge, Massachusetts, USA). Three armadillidid isopods belonging to the same lot (*Armadillidium depressum*, MCZ:IZ:CRU-7087, donated circa 1908, hereafter referred to as MCZ:IZ:CRU-7087A-C) were chosen for direct comparison over a full enrollment sequence. To compare muscular structures

across isopod and oniscomorph taxa, an unidentified armadillidiid isopod (part of lot MCZ:IZ:90105, enrolled) and an unidentified glomerid pill millipede (part of lot MCZ:IZ:165554, enrolled) were further added to the dataset. Also collected over 100 years ago, these specimens were micro-CT-scanned and the sternite morphology studied by Losso et al. (2023). Finally, two sphaerotheriid pill millipedes (*Zoosphaerium* sp., MCZ:IZ:81272, prone & MCZ:IZ:83746, enrolled) were chosen to increase our taxonomic sampling of Oniscomorpha. These specimens were picked for their relative recency (MCZ:IZ:83746: 1990; MCZ:IZ:81272: 2008) and to assess the effect of storage time on muscle degradation.

II.2 | Specimen preparation and imaging

All seven specimens underwent micro-computed tomographic scanning following chemical staining (**Figure 2**). To improve scanning resolution, specimens MCZ:IZ:90105, MCZ:IZ:165554 and MCZ:IZ:CRU-7087A-C were stained for 5 days using a 1.25% Lugol's iodine solution (1:2 mixture of iodine and potassium iodide). Due to their significantly larger size, staining of both *Zoosphaerium* specimens (MCZ:IZ:83746; MCZ:IZ:81272) was performed in the same solution over 21 and 23 days respectively. Once stained, specimens were rinsed, wrapped in poly-fil and placed in liquid-tight containers before submersion in 70% ethanol. This imaging setup ensured the chemical stability of the specimens while holding them firmly in place, eliminating potential vibrations during scanning. Imaging was performed on a Bruker Skyscan 1173 micro-CT scanner at the Digital Imaging Facility, Harvard University. Scanner settings for all specimens are presented in **Table 1**.

Table 1 | micro-CT parameters for all specimens used in this study.

Specimen	Voltage (kV)	Current (μ A)	Resolution (μ m)	Filter
MCZ:IZ:165554	80	100	6	Al 0.5mm
MCZ:IZ:90105	80	100	6	Al 0.5mm
MCZ:IZ:CRU-7087A	80	100	13.5	Al 0.5mm
MCZ:IZ:CRU-7087B	80	100	9.3	Al 0.5mm
MCZ:IZ:CRU-7087C	70	214	11.9	N/A
MCZ:IZ:83746	80	187	27.3	N/A
MCZ:IZ:81272	80	100	15	Al 0.5mm

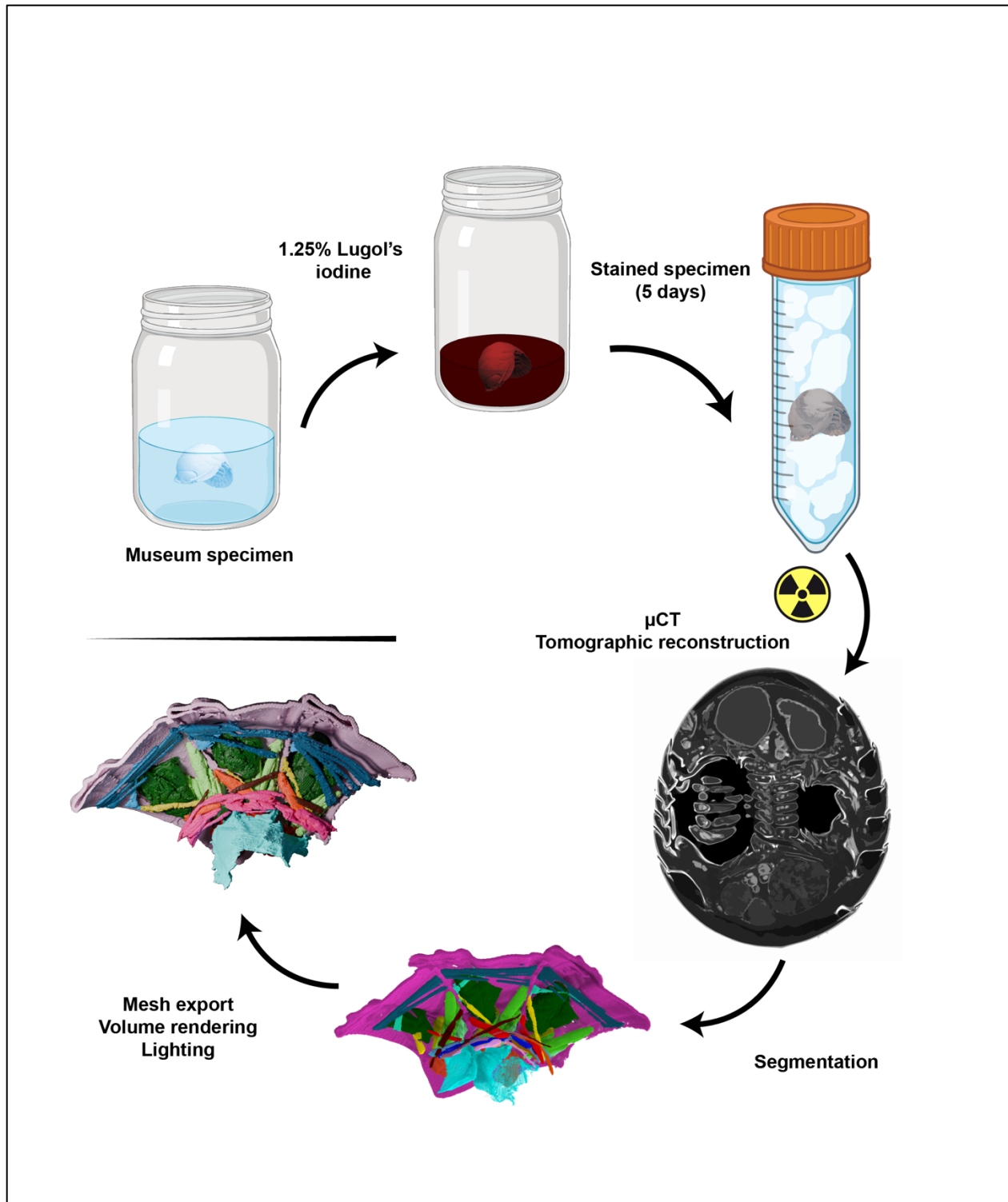


Figure 2 | General workflow developed for this study. Museum specimens preserved in 70% ethanol were stained in a Lugol's iodine solution for 5-23 days before micro-CT scanning. After reconstruction, regions of interest were segmented, visualized, and exported as mesh files in Dragonfly before volume rendering in Blender.

II.3 | Processing of regions of interest, volume estimates and 3D rendering

Tomographic scans were reconstructed as TIFF stacks in NRecon (Bruker Corporation) and processed in Dragonfly 2019 V4.0 (Object Research Systems, Montreal, Canada). Specimen segmentation was performed on segments 3-5 (Armadillidiidae, part of the pereon) and diplosegments 7-9 (Oniscomorpha). Regions of interest were selected on two-dimensional tomographic slices using a combination of thresholding and painting (3D brush) before semi-automated cleanup using the *Process Islands* menu. Total trunk volumes and muscle volumes were acquired from the *Statistical properties* panel in Dragonfly as displayed in the *Volume (labeled voxels)* field.

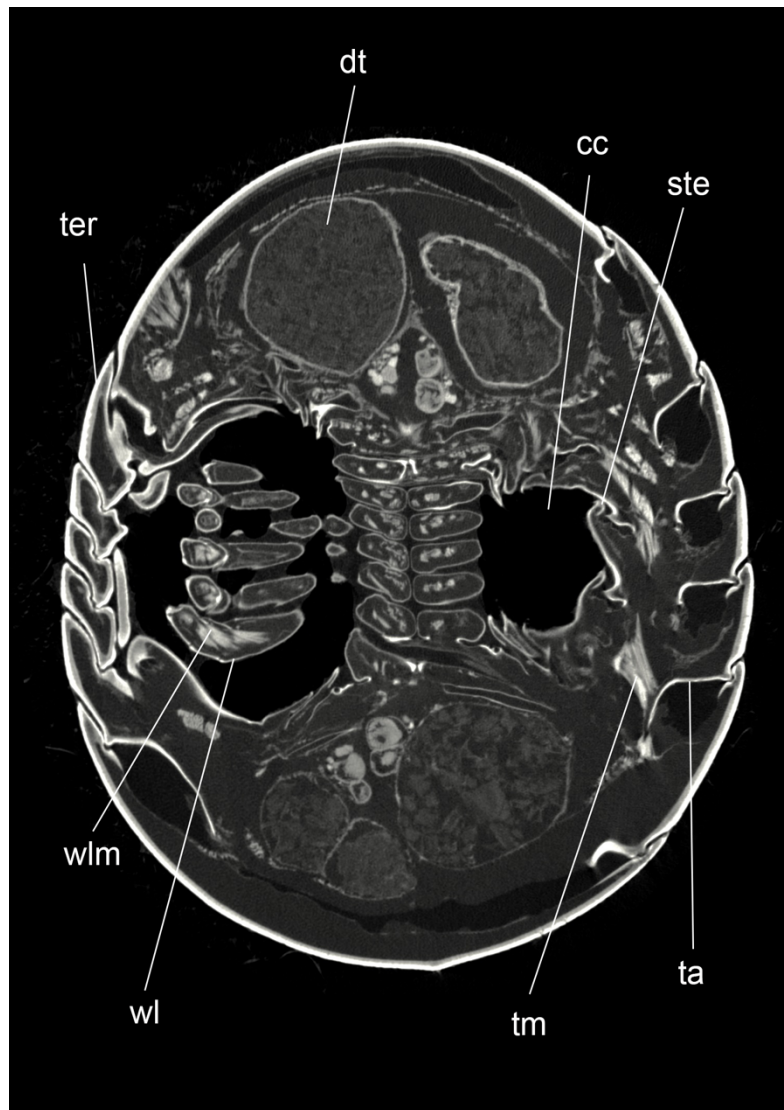
Individual regions of interest were exported as mesh files (.stl, *Threshold* = 30 for exoskeletal pieces and 50 for muscles) and imported to Blender V4.1.1 (Blender Foundation) for 3D rendering. Scenes were lit by a single *Area* light source placed posteriorly and rendered using the *Cycles* engine with *Transparent* film. Object transparency effects were achieved by lowering the material alpha and setting *Material properties* > *Viewport Display* > *Settings* > *Blend Mode* to *Alpha Blend*. Whenever transparent objects were present in the scene, harsh edges were eliminated by setting *Render* > *Light Paths* > *Max Bounces* > *Transparent* to 1000. Renders were exported as .png images and assembled in Adobe Photoshop 2024.

III. Results

III.1 | Specimen imaging

All isopod specimens were successfully stained, their musculature showing increased x-ray attenuation compared to surrounding, non-muscular tissue. Similarly, tomographic slices obtained from specimen MCZ:IZ:165554 (Oniscomorpha) showed high contrast between the exoskeletal pieces, muscles and other organs (**Figure 3**). This was not the case for the *Zoosphaerium* specimens (MCZ:IZ:83746; MCZ:IZ:81272; Oniscomorpha), however, for which chemical staining failed. Tomographic slices obtained from these individuals showed virtually no muscle detail, and their segmentation was not attempted.

Figure 3 | Two-dimensional frontal slice obtained after tomographic reconstruction of MCZ:IZ:165554 (Oniscomorpha), showing exoskeletal pieces, muscles, and internal organs. Abbreviations: ter, tergite; ste, sternite; tp, tergal prothorax; wl, walking leg; cc, central cavity; dt, digestive tract; wlm, walking leg muscle; tm, trunk muscle.



III.2 | Effects of long-term ethanol storage on muscle preservation.

Long-term (> 100 years) storage of specimens in 70% ethanol led to good overall preservation, though it generated diverse patterns of muscle alteration (**Figure 4**). Isopod specimens MCZ:IZ:90105 and MCZ:IZ:CRU-7087C showed an excellent degree of preservation, retaining the full three-dimensional structure of their trunk musculatures with no apparent porosity (**Figure 4 A-B**). In comparison, the muscles of all remaining specimens showed weak to moderate damage, characterized by alterations to their spatial arrangement, texture, structural integrity, or a combination of these properties. The trunk musculature of MCZ:IZ:CRU-7087B (Armadillidiidae) displayed the worst level of preservation of all specimens, its spongy texture suggesting significant decay. This was particularly true of the least voluminous structures, which appeared to be partially destroyed (**Figure 4 C-D**). The third armadillidiid specimen belonging to this lot, MCZ:IZ:CRU-7087A, showed an intermediate level of preservation. While its musculature remained structurally intact with no significant porosity, it showed some degree of shrinkage and displacement compared to the better preserved MCZ:IZ:CRU-7087C. Finally, the trunk muscles of MCZ:IZ:165554 (Oniscomorpha) were well preserved overall, though some either separated from their attachment points or broke in half (**Figure 4 E**). The dorsal muscles show the worst level of preservation for this specimen, retaining little three-dimensional information.

III.3 | Internal anatomy of the armadillidiid and oniscomorph trunks

The trunk musculatures of armadillidiid isopods and oniscomorph diplopods share a similar overall arrangement, comprising dorsal, dorsoventral and ventral muscle groups that attach between various exoskeletal features (**Figure 5-6**). This is consistent with Manton (1961)'s account of colobognathan millipedes, which described their trunk musculature and exoskeletal characteristics on the basis of dissected specimens. We will

refrain from using the detailed terminology introduced in this study, however, as structure homology cannot be robustly assessed from tomographic data.

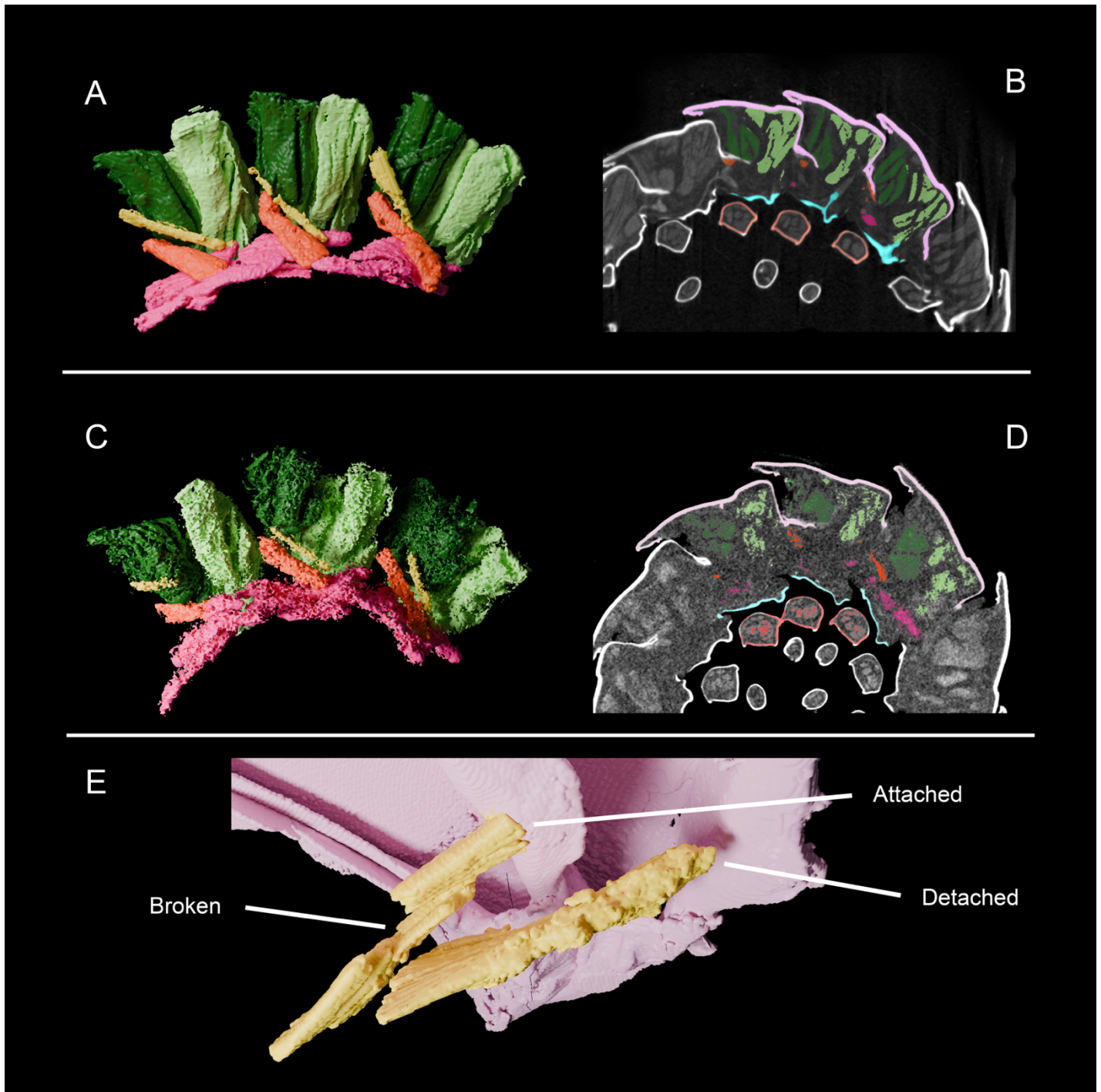


Figure 4 | Variability in muscle preservation following long-term museum storage. A-D: variability between specimens from the same lot (Armadillidiidae). A: MCZ:IZ:CRU-7087C, 3D medial view. B: MCZ:IZ:CRU-7087C, 2D medial view. C: MCZ:IZ:CRU-7087B, 3D medial view. D: MCZ:IZ:CRU-7087B, 2D medial view. E: variability between the long dorsoventral muscles of MCZ:IZ:165554 (Oniscomorpha), which show deterioration as either breaks or separation from their attachment points.

III.3.1 | Biomineralized structures

In both armadillidiids and oniscomorpha, the tergites consist of tapered exoskeletal plates and bear a pronounced internal process on their anterior margins, the prophragma (**Figure 6 A-D**). The prophragma differs in its shape between the taxa, however, forming a continuous recurved hook in MCZ:IZ:165554 (Oniscomorpha, **Figure 5 A,C**) and a ventrally extending structure in isopods (MCZ:IZ:90105, MCZ:IZ:CRU-7087, **Figure 5**

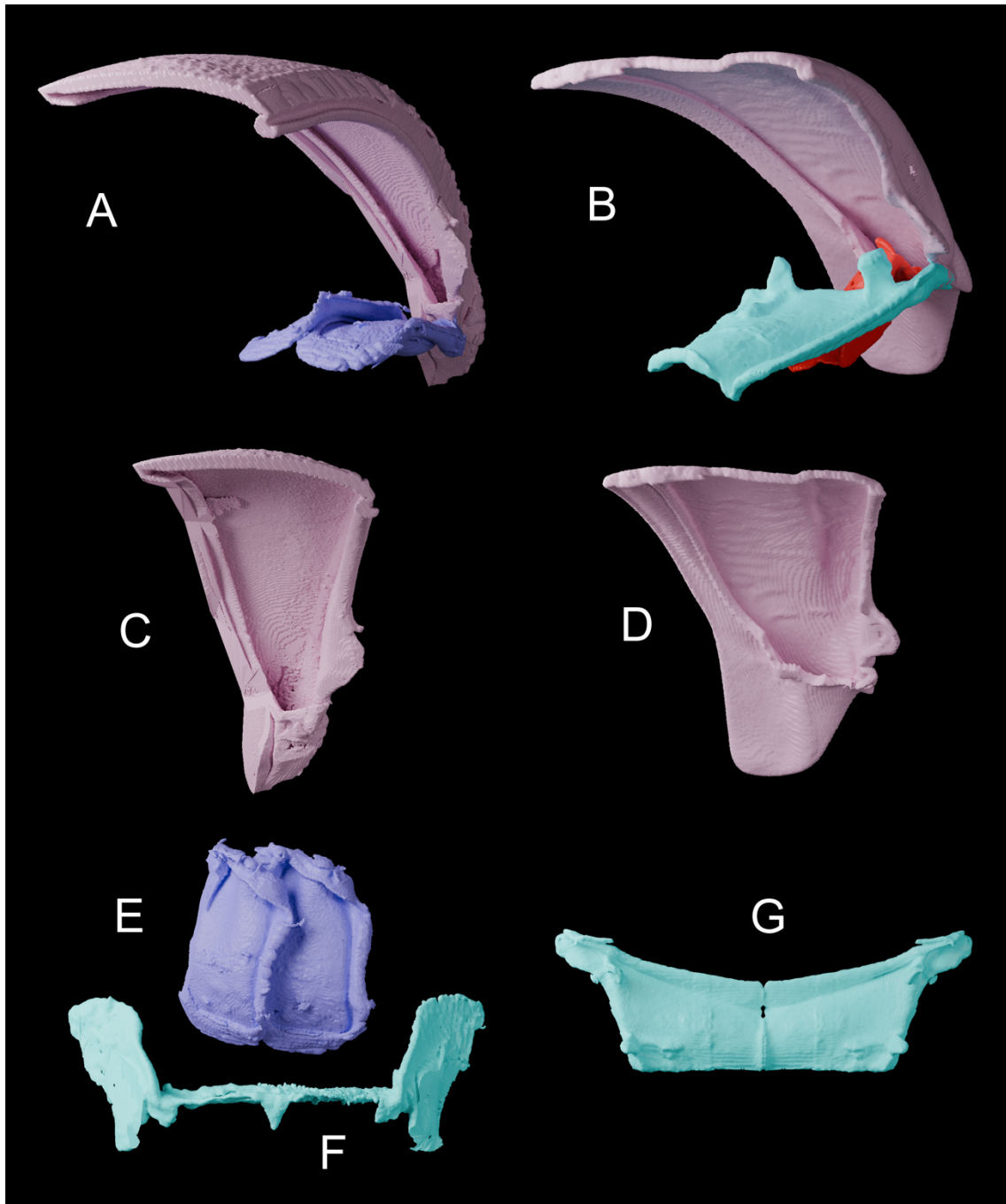


Figure 5 | Oniscomorph (left) and armadillidiid (right) exoskeletal plates. A-B: articulation of the tergite with ventral exoskeletal plates (pleurites and sternites, 15 respectively). **C-D:** tergal face and apodeme. **E:** pleurites (Oniscomorpha, dorsal view). **F-G:** sternites (ventral view).

B,D). The dorsal aspect of the oniscomorph prophragma thus forms the transverse tergal groove reported by Hannibal & Feldmann (1981) and Hadrián & Šipoš (2016) (**Figure 5 A**). Centrally positioned along the dorsoventral extension of the prophragma sits a pronounced, tooth-shaped and posteriorly directed apodeme. This structure is present in oniscomorphs and armadillidiids, its angularity more pronounced in the latter. It provides a structurally robust point of attachment for several trunk muscles (see below). Finally, the posterior tergal margins of both taxa form large intucked folds, similar to the structures reported by Manton (1961) in colobognathan diplopods. In Oniscomorpha, the posterior tergal margins of fully enrolled specimens rest on the anterior grooves borne by their following neighbours, thereby minimizing tergal overlap and contributing to the spherical conformation of the animal.

The sternites of the oniscomorph diplopod MCZ:IZ:165554, which were segmented but masked from medial views to enhance image legibility, do not provide support for the trunk musculature. They articulate laterally with the pleurites and consist of flattened, parallel blades, each supporting a median spinoid process (**Figure 5 F**). At the points of articulation, the sternites flatten in the shape of spatulas, which cover the dorsomedial edges of the pleurites. In isopods, the sternal plates are subrectangular in shape, tapering laterally to narrow apices aligned with the anterior margins (**Figure 5 G**). The lateral apices (“handles”) of the sternites articulate with the tergites, resting below the apodemes. Finally, the oniscomorph pleurites are saddle shaped (**Figure 5 E**). These plates sit in a partial overlap, the posterior margin of each covered by its following neighbor. Wider than long, they each support a robust anterior process that provides points of attachment for dorsoventral muscles.

III.3.2 | Trunk musculature

In Armadillidiidae, ventral longitudinal muscles extend between the posterior margins of the sternites (**Figure 6A**). These muscles are composed of several parallel perimysia that extend paraxially in a cylinder (MCZ:IZ:90105) or dorsoventrally flattened sheet (MCZ:IZ:CRU-7087, all specimens). Similarly, dorsal longitudinal muscles comprising 5 - 6 perimysia extend paraxially between the tergal prophragma, close to the

sagittal plane of the body. These fibers are also arranged in a dorsoventrally flattened sheet, but transition from a parallel arrangement in the posterior segments (e.g., segment

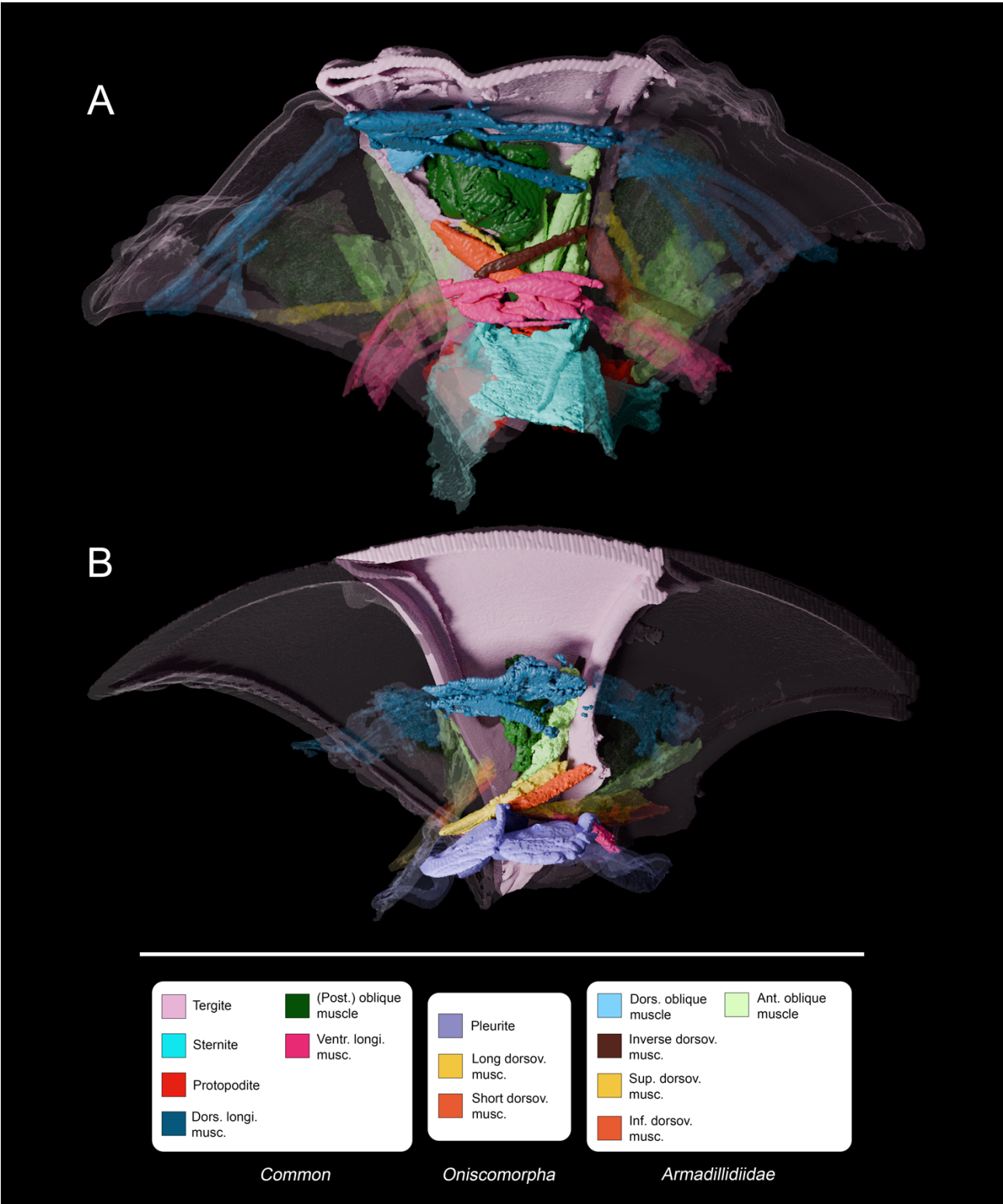


Figure 6 | Comparative anatomy of the oniscomorph (top, MCZ:IZ:165554) and armadillidiid (bottom, MCZ:IZ:90105) musculature, medial views. Individual structures color coded for each taxon.

5) to an anteriorly directed dispersion in more anterior segments (e.g., segment 3). Further, three oblique muscles extend between the prothorax and the following tergal face. The smallest of these muscles (hereafter referred to as the dorsal oblique muscle) connects the dorsal half of the prothorax to the dorsalmost portion of the tergal face. The posterior oblique muscle is the second largest, comprised of multiple thick perimysia that extend vertically between the dorsal face of the apodeme and the posterior half of the tergal face. Anterior to this muscle sits a similar structure that forms the anterior oblique muscle, connecting the protopodites and lateralmost portions of the sternites to the anterior half of the tergal face. The dorsal extremities of the anterior and posterior oblique muscles cover most of the tergal face, leaving exposed its dorsalmost third. Finally, three thin dorsoventral muscles extend between the tergal prothorax and the posterior margins of the sternites. Two of them, the inferior and superior dorsoventral muscles, run in parallel to descend from the prothorax to the posterior margin of the second anterior tergite. The third muscle, hereafter referred to as inverse dorsoventral muscle, ascends from the posterior margin of the sternite and connects to the prothorax of the corresponding tergite. Ventrally, these muscles attach to non-biomineralized structures that also support the ventral longitudinal muscles.

In the oniscomorph MCZ:IZ:165554, the ventral longitudinal muscles extend anteroposteriorly as a dorsoventrally flattened sheet of perimysia between the raised anterior margins of the pleurites (**Figure 6 B**). The dorsal longitudinal muscles extend paraxially between the prothorax, occupying a more lateral position than those found in armadillidiid isopods (**Figure 7 A-B**). The number and arrangement of the perimysia that form these muscles is unclear, as they were poorly preserved in this specimen. A single oblique muscle connects the anterior edge of the apodeme to the anterior half of the following tergal face. This structure is comparable to the isopod posterior oblique muscle in its ventral position, though its dorsal reach is limited to merely half the tergite height (**Figure 7 B**). Finally, two dorsoventral muscles connect the tergal apodeme to the anterior edges of (i) the posterior pleurite of the corresponding diplosegment (short dorsoventral muscle) and (ii) the anterior pleurite of the following (posterior) diplosegment

(long dorsoventral muscle). Both of these muscles appear to attach directly to the pleurites.

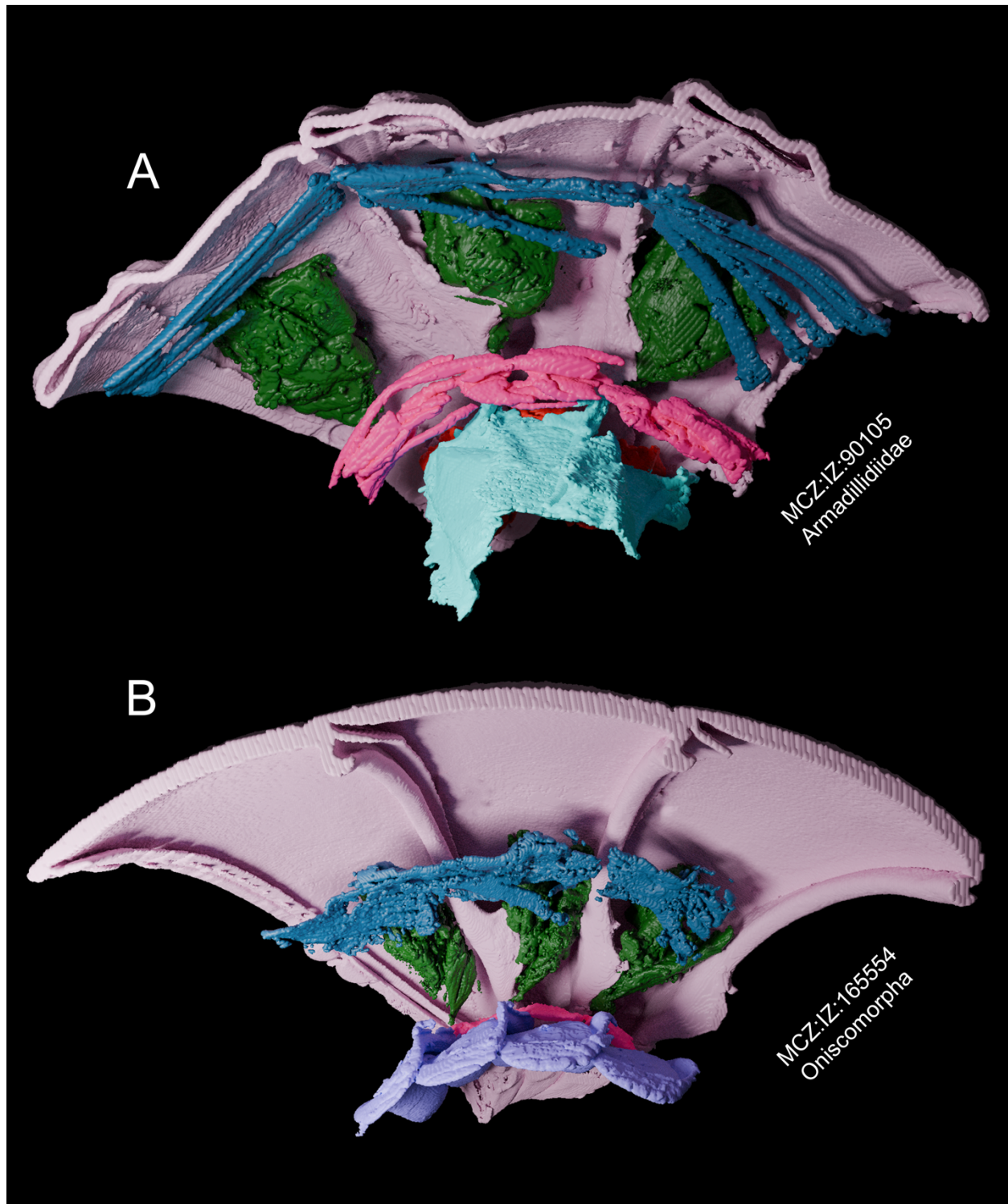


Figure 7 | Conserved muscular structures between MCZ:IZ:90105 (Armadillidiidae; top) and MCZ:IZ:165554 (Oniscomorpha; bottom). For color codes, see Figure 6.

III.4 | Interspecific variability in internal anatomy

Armadillidium depressum (MCZ:IZ:CRU-7087) and the unidentified armadillidiid isopod (MCZ:IZ:90105) exhibit important anatomical disparities in both muscular and exoskeletal structures. The tergites show the most evident differences, those of MCZ:IZ:90105 bearing dorsal ornaments in the form of parallel ridges at the posterior margins (**Figure 8B**) while this same structure is completely smooth in *A. depressum* (**Figure 8A**). In MCZ:IZ:90105, the external ridges translate to internal troughs clearly visible on the face of the tergites, these depressions serving as points of insertion for the anterior oblique muscle. The sternites of *A. depressum* are thicker than those of MCZ:IZ:90105, which are sheet-like and proved challenging to segment from tomographic slices (**Figure 8B**). In the latter species, the plates extend anteriorly and posteriorly from a median, transverse rod not conspicuous in *A. depressum*. The bundles of perimysia that make up the ventral longitudinal muscles also show distinct arrangements, those of MCZ:IZ:90105 organizing in a ventrally positioned cylinder while those of *A. depressum* (MCZ:IZ:CRU-7087) sit flat against the sternites. Finally, the anterior oblique muscles of MCZ:IZ:90105 differ in their overall appearance from those of the MCZ:IZ:CRU-7087 specimens. In the latter, they are the same general shape as the posterior oblique muscles, which in medial view form subrectangular masses bounded dorsally by the apices of the perimysia (**Figure 8A**). In MCZ:IZ:90105, the anterior and posterior sections of the anterior oblique muscles don't insert as far up the tergal face as the median section, contributing to the structure's distinctly irregular aspect (**Figure 8B**).

III.5 | Functional muscle groups and enrollment sequence.

Euarthropod body flexure is regulated by the joint action of multiple trunk muscles, which together adjust the relative positions of exoskeletal components (Losso et al., 2023). As such, the musculatures of armadillidiid and oniscomorph taxa can be divided into two main functional groups, the first responsible for enrollment and the second for unenrollment (**Figure 9**).

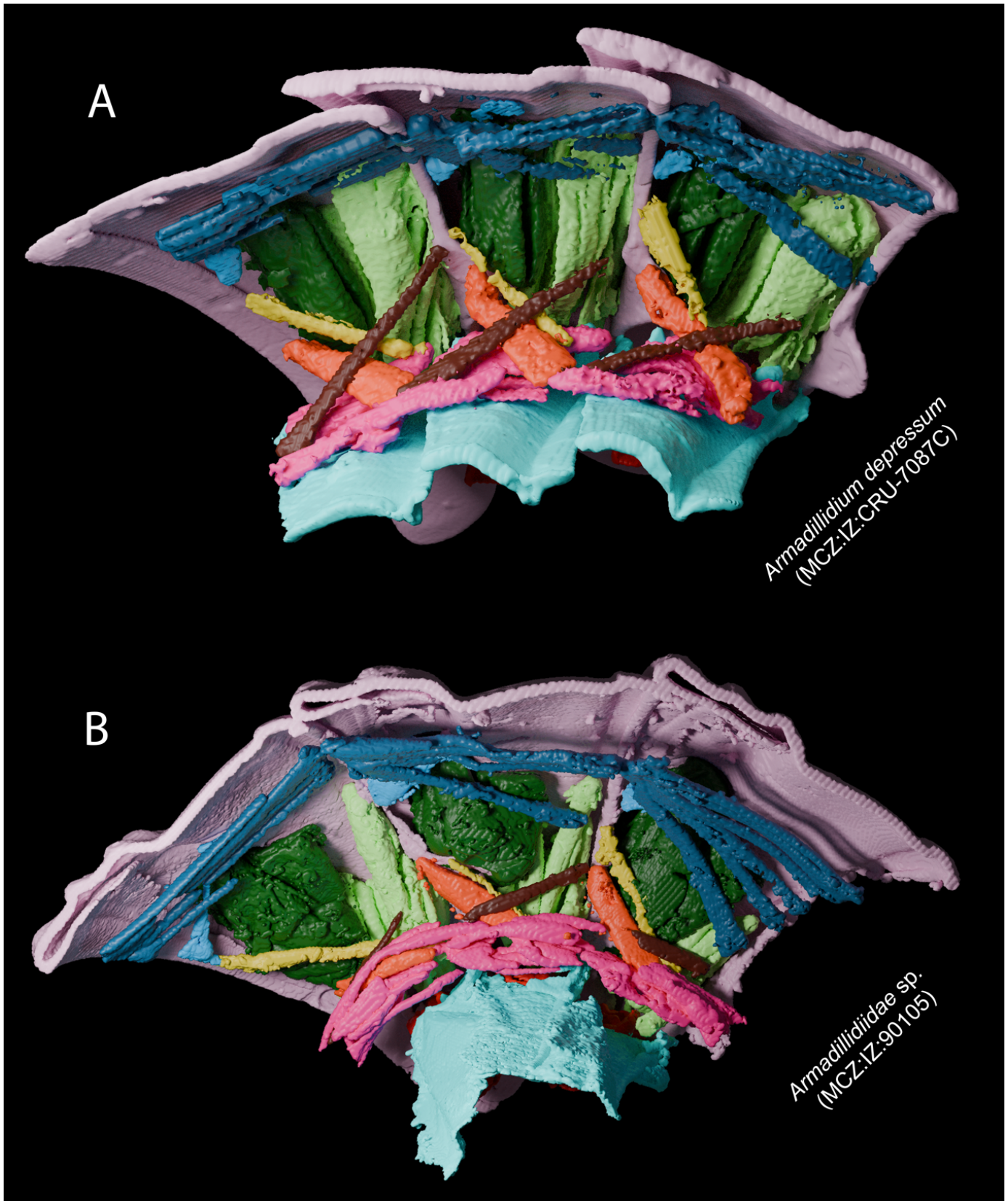


Figure 8 | Diversity of trunk anatomy between MCZ:IZ:CRU-7087C (*Armadillidium depressum*; top) and the MCZ:IZ:90105 (unidentified armadillidiid; bottom). See Figure 6 for color codes.

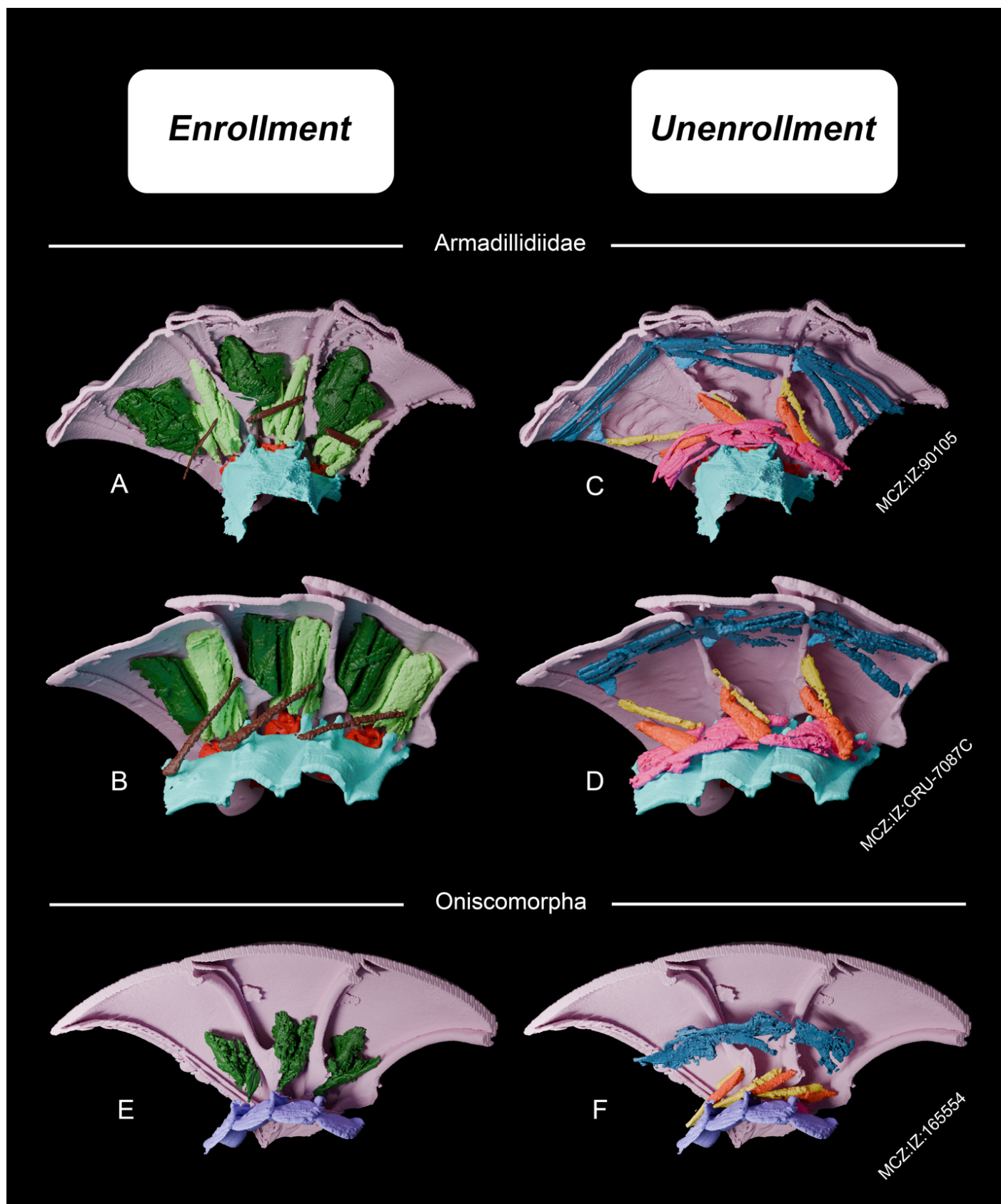


Figure 9 | Muscle groups responsible for enrollment (left) and unenrollment (right) across taxa. A-D: Armadillidiidae. A,C: MCZ:IZ:90105 (*Armadillidium depressum*); B,D: MCZ:IZ:90105 (unidentified) E-F: Oniscomorpha (MCZ:IZ:165554). For color codes, see Figure 6.

In prone armadillidiid isopods, the tergites overlap significantly due to the full contraction of the dorsal longitudinal muscles, the dorsal oblique muscles likely helping stabilize the exoskeletal plates. On the ventral side of the body, the longitudinal tension exerted by the contracted ventral longitudinal and inferior/superior dorsoventral muscles maintains the horizontal alignment of the sternites (**Figure 9 C-D**). These muscles (which constitute the “unenrollment” muscle group) relax for conglobation, with a concomitant contraction of the volvatory muscles (**Figure 9 A-B, Figure 10**). The anterior oblique muscles contract, imposing a local, forward tilting motion on the tergites while keeping them aligned parallel to one another. The posterior oblique muscles act as the main drivers of volvatory motion, pulling each prothragmal apodeme closer to the preceding tergal face. Through this contraction, the posterior oblique muscles increase the angle formed by their respective tergite and the posterior remainder of the body in an overall (*i.e.*, intertergal) tilt, thus generating body flexure. The simultaneous contraction of the inverse dorsoventral muscles angles the sternites forward by pulling anterodorsally (approx. 40° from the longitudinal axis) on their posterior margins. At the end of enrollment, the longitudinal and inferior/superior dorsoventral muscles reach their maximum extension, each segment contributing 30° (MCZ:IZ:CRU-7087B) to 48° (MCZ:IZ:90105) of flexure to the body. (**Figure 10**).

The enrollment sequence of oniscomorph diplopods is comparable to that of armadillidiid isopods, yet their distinct anatomical characteristics entail important biomechanical differences. Similar to armadillidiids, the contracted dorsal and ventral longitudinal muscles maintain the prone configuration of the exoskeletal plates. However, the horizontal arrangement of the pleurites is stabilized not by a posteriorly directed force, but by the anterodorsal tension applied to their anterior margins by the short and long dorsoventral muscles (**Figure 9 F**). The longitudinal and dorsoventral muscles relax for enrollment, whose sole driver is the contraction of the oblique muscles (**Figure 9 E**). Similar to the armadillidiid posterior oblique muscles, their contraction moves each apodeme closer to the preceding tergal face, increasing the angle formed by the two tergites. The result is an overall tergal tilt, this rotation cascading from one segment to another to generate the extreme body flexure characteristic of complete conglobation. Enrollment concludes with the longitudinal and dorsoventral muscles in a fully relaxed

state. The posterior margin of each tergite is locked in place by the transverse groove borne anteriorly by the following plate, each segment contributing approx. 30° of flexure.

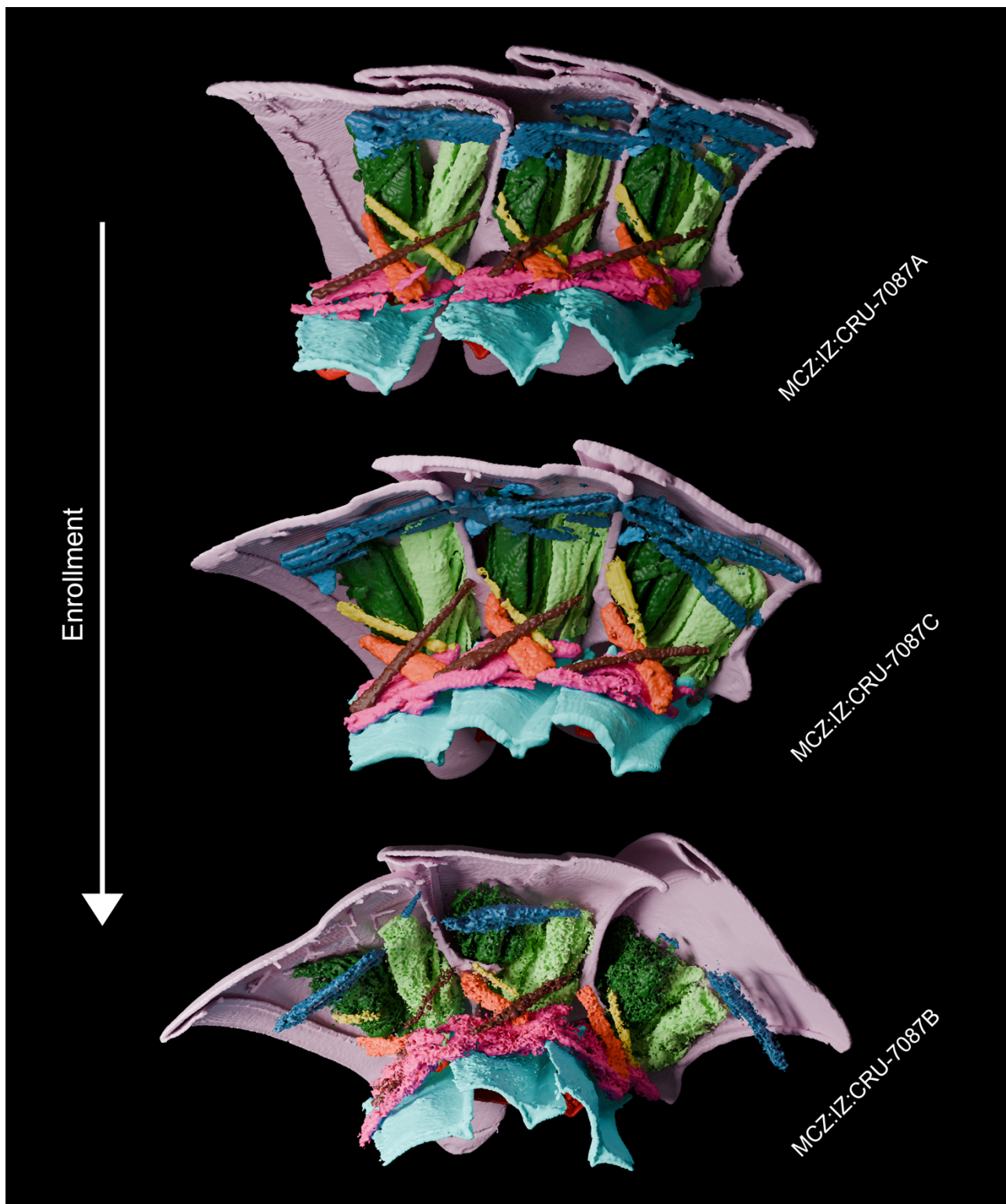


Figure 10 | *Armadillidium depressum*, enrollment sequence. Top: prone specimen, MCZ:IZ:CRU-7087A. Center: semi-enrolled specimen, MCZ:IZ:CRU-7087C. Bottom: enrolled specimen, MCZ:IZ:CRU-7087B.

III.7 | Taxon-specific investment in muscular structures

Oniscomorph diplopods and armadillidiid isopods display important differences in their levels of investment in trunk musculature, the relative volume occupied by these structures being substantially higher in Armadillidiidae (**Figure 11**). In *Armadillidium depressum*, muscles occupied between 6.4% and 11.3% of the total trunk volume (MCZ:IZ:CRU-7087B & MCZ:IZ:CRU-7087C, respectively). Similarly, the musculature accounted for 7.3% of total trunk volume in MCZ:IZ:90105. This represents a significantly higher (2.5 - 4.3 x) investment than in the oniscomorph MCZ:IZ:165554, for which musculature only accounted for 2.6% of the total volume.

Specific levels of investment in enrollment or unenrollment can also be investigated by comparing volume data across functional muscle groups. In isopods, more than three quarters (76.0 - 80.8%) of the muscular volume was dedicated to enrollment (MCZ:IZ:CRU-7087A: 76.0%; MCZ:IZ:CRU-7087B: 80.1%; MCZ:IZ:CRU-7087C: 77.3%; MCZ:IZ:90105: 80.8%). This stands in stark contrast to the muscular arrangement of MCZ:IZ:165554 (Oniscomorpha), which was more functionally balanced. In this specimen, approximately half (53.1%) of the total muscle volume belonged to the functional group responsible for enrollment.

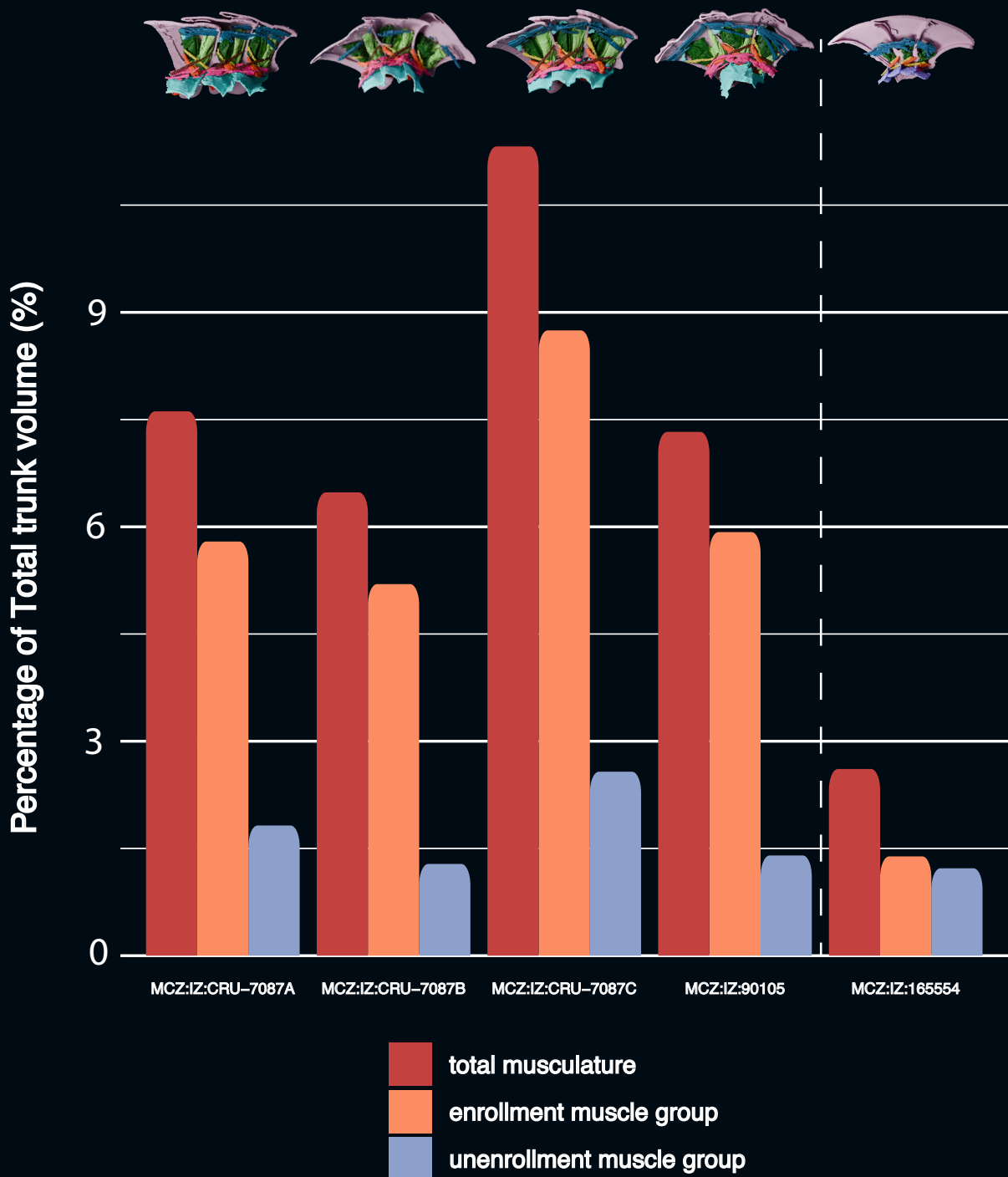


Figure 11 | Investment in trunk musculature as informed by muscular volumes. Red: proportion of the total trunk volume occupied by musculature (both muscle groups). Orange: enrollment muscle group. Blue: unenrollment muscle group.

IV. Discussion

Micro-CT imaging of natural history museum specimens provides new insights into the functional anatomy of euarthropod enrollment. This charismatic and ancient behaviour is known from several extinct and extant taxa, yet its biomechanical underpinnings have not yet been thoroughly assessed in euarthropods, where it is most prominent. Previous research has emphasized the importance of specific external features (e.g. the coaptive interlocking of exoskeletal plates (Hadrián & Šipoš, 2016)) for effective defensive volvation, but the internal structures that facilitate the extreme body flexures observed in enrolled euarthropods have never been thoroughly investigated in groups. This study directly addressed this present lack of data by generating three-dimensional models of the muscular and exoskeletal structures responsible for volvation in the two main enrolling euarthropod taxa. Furthermore, the high quality of the models obtained from century-old specimens demonstrates the suitability of museum samples for non-destructive study of delicate anatomical structures in euarthropods.

IV.1 | Long-term storage of euarthropod specimens in ethanol does not preclude their use for anatomical studies of musculature.

Data obtained from micro-CT demonstrate that century-old museum specimens retain sufficient morphological information for the direct comparison of soft internal structures across euarthropod taxa, but also confirms that long-term fluid preservation can alter the three-dimensional characteristics of muscular tissue (Leonard et al., 2022). While euarthropod muscle fibers remain understudied, those of vertebrates are known to contain approximately 70% water by mass (Reinoso et al., 1997). Ethanol being a powerful dehydrating agent (Klemm, 1990, 1998), it is likely that long-term ethanol storage leads to the dehydration of euarthropod muscular structures. The primary consequence of this phenomenon is an overall reduction in the volume of the muscle fibers (Leonard et al., 2022), constrained longitudinally by their attachment to exoskeletal structures. Osmotic dehydration of the muscles thus leads to a reduction in their cross

section and a corresponding increase in their tension. This is supported by the pattern of tissue degradation displayed by specimen MCZ:IZ:165554 (Oniscomorpha), whose long dorsoventral muscles showed signs of strong post-preservation contraction. These either separated from a single attachment point, or remained attached but tore in half, suggesting intense stresses were applied to the fibers as the exoskeletal plates remained in place. Further, the thinnest muscles (e.g., the dorsoventral muscles) showed poorer preservation than more voluminous muscles in the majority of specimens. Though these structures are likely the most delicate regardless of the source of alteration, their poor preservation may reflect an acute sensitivity to dehydration explained by high surface-to-volume ratios.

The muscle damage displayed the armadillidiid specimen MCZ:IZ:CRU-7087B, on the other hand, cannot be easily explained by ethanol-induced dehydration and contraction. In this specimen, the muscular masses appear porous, representing significant textural alteration compared to better preserved individuals of the same species and lot (MCZ:IZ:CRU-7087A, MCZ:IZ:CRU-7087C). A study comparing the effects of five different fluids for the preservation of aquatic insects showed that ethanol performed poorly with regards to maintaining tissue integrity (Begić et al., 2022). The partial loss of tissue observed in their samples is consistent with the superficial porosity of MCZ:IZ:CRU-7087B's musculature. However, the fact that all three MCZ:IZ:CRU-7087 specimens were preserved together, sharing the same fluid, raises questions as to the sole involvement of ethanol in the degradation of MCZ:IZ:CRU-7087B. Instead, the comparatively poor state of preservation of this specimen may be due to microbial activity prior to preservation, or other intrinsic factors. However, this remains difficult to ascertain due to the antiquity of the armadillidiid specimens belonging to this lot. This discrepancy reaffirms the importance of swift sample processing prior to storage and suggests that preservation fluids are not the sole determinant of soft tissue preservation in museum euarthropod specimens.

IV.2 | Micro-CT offers a compelling alternative to destructive methods for the investigation of internal euarthropod anatomy.

All but two of the euarthropod specimens processed for this study yielded high-fidelity, three-dimensional models of their internal trunk anatomy without the need for permanent alteration. Micro-CT and subsequent segmentation of the specimens that successfully underwent chemical staining consistently revealed the spatial arrangement of the trunk musculature, internal organs and exoskeletal plates, with high resolution and a rapid turnover. This represents an important development for the non-destructive study of old and oftentimes valuable museum specimens, for which destructive methods (*e.g.*, dissection) may not be acceptable. For example, study of the internal morphology of taxonomically important (*i.e.*, type) specimens may be required to disentangle complex interspecific relationships yet precluded by ethical concerns over the alteration of type material. In this regard, micro-CT likely represents the best alternative to dissection, presenting the added benefit of generating readily sharable data such as 3D mesh files. Once uploaded to online repositories, these assets represent a partial or complete digital copy of the specimens used for study, thereby participating in the conservation of museum material.

A potential roadblock for the use of micro-CT on museum specimens resides in the effectiveness of staining protocols. While our inability to segment either of the *Zoosphaerium* specimens (MCZ:IZ:81272, MCZ:IZ:83746; Oniscomorpha) was a direct consequence of poor chemical staining, the causes of this failure are unclear. The trunk musculature of all other euarthropod specimens displayed high X-ray attenuation after just five days of iodine staining, yet these larger oniscomorphs did not show satisfactory muscle detail after staining periods exceeding 20 days. The head and first diplosegment of MCZ:IZ:81272 (later nicknamed “Marie Antoinette”) were also separated from the rest of the body in an attempt to accelerate iodine diffusion, but this approach did not result in a significant improvement in image contrast. This is an unexpected result, considering that recommended stain times for significantly more massive, vertebrate taxa do not exceed two weeks in equally concentrated stain solutions (Callahan et al., 2021). The observed lack of contrast on the tomographic slices obtained from these specimens is

unlikely to be a consequence of overstaining, as CT-scanning was initially attempted after five days but abandoned due to comparably low image quality. Instead, the inefficacy of iodine staining may be a consequence of the specific histochemical characteristics of *Zoosphaerium* muscles, representing an intriguing hurdle that must be resolved to paint a fuller picture of the oniscomorph trunk architecture.

IV.3 | Convergent evolution of armadillidiid and oniscomorph volvation: parallel solutions to a common biomechanical challenge

The defensive conglobation of Armadillidiidae and Oniscomorpha represents a striking example of convergent evolution, yet the muscular structures that enable this behaviour exhibit only superficial similarities across taxa. The most evident feature uniting oniscomorphs and armadillidiids is the combined presence of ventral, dorsal, and dorsoventral trunk muscles, which together contract and relax to regulate body flexure. The trunk muscles of both taxa are anatomically comparable insofar as they attach to broadly similar exoskeletal features, such as exoskeletal plate margins (longitudinal muscles), the tergal apodeme (oblique muscles), or a combination of both (dorsoventral muscles). However, the precise characteristics of these attachments and the number of muscles involved are often distinct between armadillidiids and oniscomorphs.

For example, the unenrollment of each taxon is assisted by a pair of dorsoventral muscles, yet their orientations and ventral attachments are constrained by the exoskeletal architecture of the trunk (**Figure 9**). In Oniscomorpha, the pleurites are derived from leg-associated structures integrated into the body wall (Coulcher et al., 2015) and provide the main platform for the ventral attachment of the trunk muscles (**Figure 9 F**). In contrast, these sclerotized plates are absent from Oniscidea, and part of the armadillidiid trunk musculature instead attaches to the sternites (**Figure 9 C-D**). Beyond their distinct developmental origins, the oniscomorph pleurites and oniscidean sternites articulate in opposite patterns, with dorsal and ventral anteroposterior overlaps respectively (Losso et al., 2023). The articulatory characteristics of the oniscomorph pleurites and armadillidiid sternites thus dictate the directions of the forces required to influence body flexure, and

with them the kinematics of conglobation. Other aspects of the oniscomorph and armadillidiid trunk musculatures are outright divergent, as evidenced by the lack of clear oniscomorph analogs for certain armadillidiid muscles. For instance, the armadillidiid inverse dorsoventral muscle has no functional counterpart in Oniscomorpha, where volvation is assured by the sole contraction of the oblique muscle. Similarly, while the armadillidiid tergal face is connected to the protopodite by the anterior oblique muscle (**Figure 9 A-B**), no muscular structure attaches to the leg-associated pleurites in Oniscomorpha (**Figure 9 E**). These differences are significant, underscoring the influence of biomechanical constraints on the evolution of euarthropod body plans.

IV.4 | Mechanical inefficiency and distinct ecological pressures are likely drivers of the strong armadillidiid investment in volvatory musculature

Total armadillidiid trunk muscle volumes were 2.5 – 4.3 X higher than in Oniscomorpha, with lower proportional investment in volvatory muscles (“enrollment” muscle group). The dramatically different muscular volumes of Armadillidiidae and Oniscomorpha are an enigmatic result with unclear implications. While some of these differences can likely be explained by the higher total number of armadillidiid trunk muscles, such hypertrophy may also reflect distinct biomechanical constraints, ecological requirements, or both.

The hypertrophy of the armadillidiid trunk musculature may be a direct consequence of the general Oniscidean body plan, which is likely less suitable for volvatory adaptation than that of Diplopoda. The main driver of armadillidiid enrollment, the posterior oblique muscle, runs parallel to the tergal face and attaches to the dorsal side of the apodeme (**Figure 9 A-B**). The dorsal attachment of this muscle is likely spatially constrained by the nearby presence of the anterior oblique muscle, whose contribution to trunk flexure is less direct (**Figure 10**). In contrast, the single oniscomorph oblique muscle is free to run transversally across the tergal face and insert far down the apodeme’s anterior edge, likely generating significant leverage benefits (**Figure 9 E**). This explanation would be consistent with the disproportionate investment of Armadillidiidae in the functional muscle group responsible for enrollment, whose components may need to

compensate for relatively low mechanical efficiency. A formal analysis of the forces exerted on the exoskeleton during the enrollment of each taxon is beyond the scope of this study, however, as the field of exoskeleton biomechanics is still in its infancy (but see Dürr & Mesanovic (2023); Hecht et al. (2010); Salerno et al. (2023)). An explicit test of this hypothesis would represent an exciting research avenue.

Alternatively, the large muscular volumes characteristic of Armadillidiidae may serve as an adaptation to distinct ecological needs. An important trait of some glomerid Oniscomorpha is their ability to excrete a mixture of repellent chemicals in addition to conglobation (Meinwald et al., 1966; Schildknecht et al., 1966; Shear et al., 2011). This secretion contains quinazolinone alkaloids known to deter anuran, avian and rodent predators (Schildknecht & Wenneis, 1967) and to possess a sedative effect on spiders (Carrel & Eisner, 1984). While it represents an additional metabolic expense, a chemical repellent likely provides significant defensive benefits and limits the need for frequent and sustained conglobation. Conversely, and to our knowledge, no such adaptations have been reported from Armadillidiidae, which instead rely purely on volvatory behaviour and occasional tergal ornaments to deter would-be predators (it is worth noting that other, non-volvatory oniscideans do possess such defenses; see Gorvett (1951); Tuf & Ďurajková (2022)). In this context, the higher armadillidiid investment in volvatory muscles may simply reflect higher overall investment in this defensive strategy, to which they have no real alternative.

IV.5 | Conclusions and future directions

The present study represents the first comprehensive characterization of volvatory musculature in animals. Micro-CT imaging reveals the trunk musculatures of armadillidiid isopods and oniscomorph millipedes in unprecedented detail, highlighting distinct biomechanical characteristics separating two otherwise functionally convergent body plans. The remarkable state of preservation often displayed by historic euarthropod specimens stored in ethanol encourages their use in future CT-based anatomical studies, limiting the need for fresh specimens. Future research should focus on further deciphering the volvatory musculatures of both taxa, and developmental approaches may enable a

formal investigation of the potential homology of functionally analogous structures. Similarly, integrative approaches may help quantify the mechanical efficiency of volvation in armadillidiids and oniscormorphs, providing new insight into the evolution of this charismatic defensive strategy.

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VI. References

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