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# Comparative morphology of cheliceral muscles using high-resolution X-ray microcomputed-tomography in palpimanoid spiders (Araneae, Palpimanoidea)

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## Abstract

Spiders are important predators in terrestrial ecosystems, yet we know very little about the principal feeding structures of spiders, the chelicerae, which are functionally equivalent to “jaws” or “mandibles” and are an extremely important aspect of spider biology. In particular, members of Palpimanoidea have evolved highly unusual cheliceral morphologies and functions, including high-speed, ballistic movements in mecysmaucheniid spiders, the fastest arachnid movements known thus far, and the elongated, highly maneuverable chelicerae of archaeids that use an attack-at-a-distance strategy. Here, using micro-Computed-Tomography scanning techniques, we perform a comparative study to examine cheliceral muscle morphology in six different spider specimens representing five palpimanoid families. We provide a hypothesis for homology in palpimanoid cheliceral muscles and then compare and contrast these findings with previous studies on other non-palpimanoid spiders. We document and discuss two sets of cheliceral muscles in palpimanoids that have not been previously observed in other spiders or which may represent a position shift compared to other spiders. In the palpimanoids, *Palpimanus* sp., *Huttonia* sp., and *Coloepa* sp. showed similar cheliceral muscle anatomy. In *Eriauchenius ranavalona*, which has highly maneuverable chelicerae, some of the muscles have a more horizontal orientation, and there is a greater degree of cheliceral muscle divergence. In *Zearchaea* sp. and *Aotearoa magna*, some muscles have also shifted to a more horizontal orientation, and in *Zearchaea* sp., a species with a ballistic, high-speed predatory strike, there is a loss of cheliceral muscles. This research is a first step toward understanding cheliceral form and function across spiders.

## 1 | INTRODUCTION

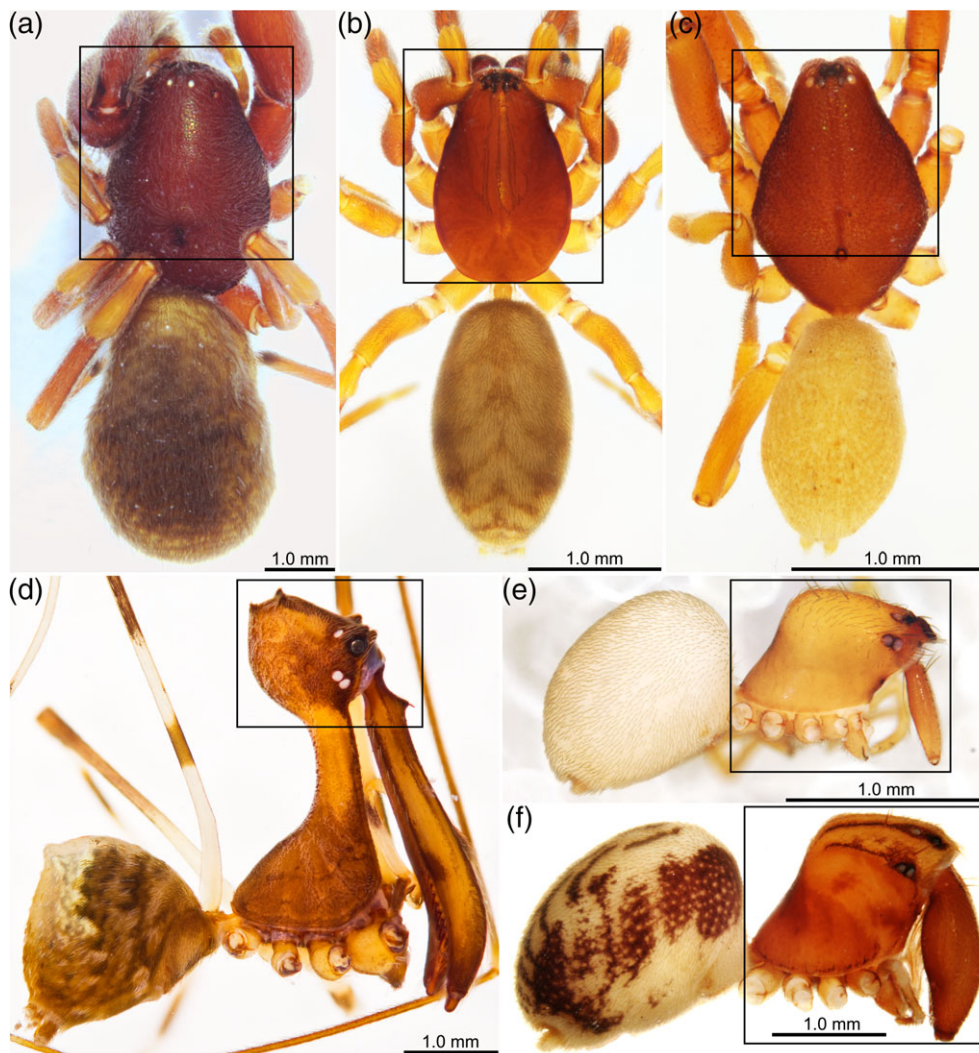
With over 47,000 described species (World Spider Catalog, 2018), spiders are notable in terms of species diversity, global ubiquity, and diversity in behavior and ecology. Yet, while great advances have been made in research on spider silk, venom, and evolutionary relationships, we know very little about the principal feeding structures of spiders, the chelicerae, which are an extremely important aspect of spider biology. Spider chelicerae are functionally equivalent to “jaws” or “mandibles” and are composed of two different sections, a basal section, hereinafter termed the paturon (after Lyonet, 1832), that articulates

with the cephalothorax, and a distal section composed of a fang that articulates with the paturon and delivers venom through a tiny opening close to the fang tip. The chelicerae are not just used for prey capture, but are also used for grasping, mastication, digging burrows, carrying egg cases, and during mating rituals (Foelix, 2011). There have been several studies that describe the internal cheliceral anatomy and musculature of spiders, but most of these studies focused on only one species (Brown, 1939; Firstman, 1954; Legendre, 1965; Whitehead & Rempel, 1959). Later, Palmgren (1978, 1980) documented the cheliceral musculature in a more comparative fashion across Araneomorphae spiders, although mostly focusing on the

Entelegynae: his combined work involved dissecting and documenting around 60 different species of spiders (at least two individuals per species were examined), comprising over 20 families.

This study documents and homologizes the cheliceral musculature of Palpimanoidea spiders for the first time using micro-Computed-Tomography ( $\mu$ CT) scanning techniques. Palpimanoidea is an ancient spider lineage that has evolved some remarkable morphologies in the carapace and chelicerae compared to other spiders (Figure 1). Their strange morphology complements their unusual and highly specialized predatory behaviors: for example, mecysmaucheniids use high-speed, ballistic, trap-jaw strikes (Wood, Parkinson, Griswold, Gillespie, & Elias, 2016), and archaeids are specialists that attack other spiders at a distance with their long, highly maneuverable chelicerae (Legendre, 1961; Wood, Griswold, & Gillespie, 2012). Phylogenetic analysis supports a monophyletic Palpimanoidea that consists of five families (Fernández et al., 2018; Wood

et al., 2012; Wood, González, Lloyd, Coddington, & Scharff, 2018): Archaeidae, Huttoniidae, Mecysmaucheniidae, Palpimanidae, and Stenochilidae. Previous research has documented the internal musculature of archaeids, yet has lacked sufficient detail: Petrunkevitch (1939) largely ignored the cheliceral musculature; Legendre (1965) noted that the “head” region was dominated by several sets of cheliceral muscles, but did not go so far as to homologize these muscles or compare them with close relatives. Here, we focus specifically on the cheliceral muscles that articulate the basal section of the chelicerae, the paturon, and we use  $\mu$ CT-scanning to compare cheliceral musculature in six different palpimanoid species that represent all five palpimanoid families. Our results are then compared with anatomical descriptions of other spiders in the literature. Our motivation is to understand homology among cheliceral muscles and to compare cheliceral musculature in different palpimanoid families.



**FIGURE 1** Habitus of different palpimanoid species, with boxed sections showing the region that was  $\mu$ CT scanned. (a) Palpimanidae, *Palpimanus* sp., female, dorsal view (CASENT9042387). (b) Huttoniidae, *Huttonia* sp., male, dorsal view (USNMENT01377163). (c) Stenochilidae, *Colopea* sp., male, dorsal view, right legs II-III-IV removed (CASENT9035143). (d) Archaeidae, *Eriauchenius ranavalona*, male, lateral view, image reversed, legs removed on one side (CASENT9010047). (e) Mecysmaucheniidae, *Zearchaea* sp., female, lateral view, legs removed on one side (USNMENT01458827). (f) Mecysmaucheniidae, *Aotearoa magna*, female, lateral view, image reversed, legs removed on one side (CASENT9028269)

## 2 | METHODS

Six specimens representing the five Palpimanoidea families were selected for  $\mu$ CT-scanning. All specimens are museum material that were preserved in 70–75% ethanol at the time of collection (Table 1). One specimen per family was scanned, except for Mecysmaucheniidae, where two specimens were scanned (Table 1): *Zearchaea* sp., a species with extremely fast, ballistic, cheliceral strikes (Wood et al., 2016), and *Aotearoa magna*, a species with slower, non-ballistic cheliceral strikes (unpublished data). For all scans, female specimens were used except for *Huttonia* sp. and *Colopea* sp., where a male specimen was used: this should not affect our results as there is not obvious sexual dimorphism in the carapace and cheliceral shape among palpimanoids. For *Aotearoa magna*, the specimen was collected over 30 years ago and thus, caution should be used when interpreting this scan as the muscles may have shrunken or become damaged. The muscles that operate the paturon that are contained within the carapace were the object of interest for the  $\mu$ CT scans (see boxes in Figure 1): for *Palpimanus*, *Huttonia* sp., and *Colopea* sp. the anterior portion of the cephalothorax was scanned with the posterior portion truncated; for *Aotearoa magna* and *Zearchaea* sp. the entire cephalothorax was scanned; and for *Eriauchenius ranavalona* only the distal, anterior portion of the elongated carapace and the cheliceral bases was scanned. Prior to scanning, specimens were stained overnight in Lugol's solution (iodine/potassium iodide), washed in water for 30 min, and then scanned in 75% ethanol. Specimens were securely placed within a pipette tip that was sealed with Sculpey® clay at either end, and this pipette was affixed to an SEM mounting stub using clay. Scans were performed using hard X-ray microtomography at the Lawrence Berkeley National Lab Advanced Light Source synchrotron, Berkeley, CA. X-ray energies of 33.5 kV were used, and images were collected with a LuAG:Ce scintillator and either a 10X or 5X objective lens and a PCO.edge CMOS detector, yielding a reconstructed 3D voxel size of  $0.65 \times 0.65 \times 0.65 \mu\text{m}$  or  $1.3 \times 1.3 \times 1.3 \mu\text{m}$ , respectively. Image stacks (TIFF format) were produced using Xi-CAM (Pandolfi et al., 2018), with the gridrec algorithm as implemented in TomoPy (Gürsoy, De Carlo, Xiao, & Jacobsen, 2014). Using Avizo software, 3D-reconstructions were created from the resulting image stacks that could be rotated and digitally sliced. The following structures were digitally labeled ("segmented") by hand, and the boundaries of these labels were converted to a surface mesh for better visualization: the carapace, the chelicerae, the inter-cheliceral-sclerite, and all cheliceral muscles that operate the paturon. Each segmented structure was color-coded and the homology of cheliceral muscles was determined based on the position of the muscle attachment to the chelicerae. Interactive 3D-files were produced by exporting individual structures as .obj files from Avizo. These files were then imported into MeVisLab (MeVis Medical Solutions AG and Fraunhofer MEVIS) using the "Scientific3DFigurePDFApp" module, reduced, colored, and exported as .u3D and .pdf files.

## 3 | RESULTS

Table 2 defines all observed muscle sets that operate the paturon in palpimanoids, and their hypothesized homology to the muscles documented by Palmgren (1978). For clarity, throughout the manuscript

whenever a muscle is mentioned we will use the terminology from Table 2 followed in parentheses by the designated number and the color used in the figures. All scanned palpimanoid specimens have the following pairs of cheliceral muscles (Figures 2–6): two pairs of muscles that attach to the lateral side of the chelicerae, (i) a more anterior lateral pair, color coded yellow in all figures, and (ii) a more posterior lateral pair, color coded magenta, both of which run to the lateral sides of the carapace; (iii) a pair that attaches to the anterior lateral corner of the chelicera, color coded red; (iv) a pair that attaches to the posterior medial corner of the chelicera and runs to the lateral sides of the carapace, color coded green; (v) a pair that attaches to the anterior medial corner of the chelicerae and runs upward and backward to the medial portion of the carapace, color coded purple; (vi–vii) two pairs of muscles, often difficult to distinguish, that attach to the anterior median corner of the chelicera and run to the anterior lateral sides of the carapace, color coded dark-blue and light-blue (absent in *Zearchaea* sp.); (viii) a set of muscles that attach to the inter-cheliceral-sclerite and run upward to the medial portion of the carapace, sometimes extending far back into the carapace, color coded aqua. Additionally, in some palpimanoids there is a pair of muscles (ix, color coded light orange) that runs from the posterior median corner of the chelicerae to the endosternite: at this time, it cannot be determined whether this muscle is truly absent in some palpimanoids or just difficult to be identified in the  $\mu$ CT-scans. All palpimanoids also have an inter-cheliceral-sclerite of various shapes (color coded orange in figures) that sits between the cheliceral bases (Figures 2–6, in particular see Figures 3j–o and 5j–o).

*Palpimanus* sp., *Huttonia* sp., and *Colopea* sp. showed similar cheliceral muscle anatomy (Figures 2 and 3). In these three specimens the lateral anterior (i, yellow) and lateral posterior (ii, magenta) muscles run upward and attach to the dorsal portion of the carapace (Figures 2a–c and 3a–f). The anterior medial inner (vi, blue) and anterior medial outer (vii, light blue) runs to the anterior and lateral portion of the carapace (Figures 2a–c, g–i and 3a–c). The anterior-medial (v, purple) and the posterior-medial (iv, green) muscles both extend far back into the carapace, but the posterior-medial (iv, green) runs to a more lateral position on the carapace compared to the anterior-medials (v, purple) (Figure 2d–f, j–l). The anterior-outer (iii, red) muscle runs from a lateral position on the chelicerae to a medial position on the carapace (Figure 2d–f, j–l). The inter-cheliceral-sclerite (orange in figures) is rod shaped in these specimens (Figure 3m–o). The inter-cheliceral-sclerite muscle (viii, aqua) is large and extends deep into the carapace, almost to the fovea (Figures 2a–c and 3j–l), and in *Colopea* sp. apodemes connect the inter-cheliceral-sclerite to the cheliceral bases (Figure 3l). Among these three specimens the endosternite muscles (ix, light orange) was only observed in *Huttonia* sp. (Figures 2e and 3b).

For the archaetid specimen, *Eriauchenius ranavalona*, the lateral anterior (i, yellow) and lateral posterior (ii, magenta) muscles attach more toward the posterior of the carapace than in *Palpimanus* sp., *Huttonia* sp., and *Colopea* sp. (hereinafter abbreviated PHC), where they attach to the dorsal part of the carapace. Additionally, each lateral anterior (i, yellow) muscle is bicipital, with an anterior bundle and a posterior bundle (Figure 5a,d). The anterior medial inner (vi, blue) muscles are larger and are also oriented more towards the posterior of the carapace (Figure 4a,g) than in PHC where they extend more dorsally and laterally.

**TABLE 1** Specimens used for microcomputed-tomography scans

Family	Species	Sex	Collection locality, year	Voucher number	Objective	Pixel size (mm)	# of images per scan	Carapace width (mm)	Chelicerae width (mm)
Palpimanidae	<i>Palpimanus</i> sp.	F	South Africa: Amanzi Game Reserve, 2011	CASENT 9042387	5X	0.0013	1,969	1.77	0.40
Huttoniidae	<i>Huttonia</i> sp.	M	New Zealand: Lewis Pass, 2018	USNMENT 01377163	5X	0.0013	1,969	1.09	0.30
Stenochilidae	<i>Colopea</i> sp.	M	Philippines: Luzon, UP los Baños campus, 2011	CASENT 9035143	10X	0.00065	1,025	0.87	0.15
Archaeidae	<i>Eriauchenius ranavalona</i>	F	Madagascar: Ranomafana National Park, 2009	USNM 01377248	10X	0.00065	2,049	1.20	0.27
Mecysmaucheniidae	<i>Zearchaea</i> sp.	F	New Zealand: Lewis Pass, 2011	USNMENT 01377164	5X	0.0013	2,049	0.59	0.14
Mecysmaucheniidae	<i>Aotearoa magna</i>	F	New Zealand: Fiordland National Park, 1986	CASENT 9028269	5X	0.0013	2,049	1.10	0.43

Abbreviations: CASENT = California Academy of Sciences Department of Entomology; USNMENT = National Museum of Natural History Entomology Department, Smithsonian Institution

The anterior medial outer (vii, light blue) are well separated from the anterior medial inner (vi, blue) and very reduced (Figure 4a,g). The anterior-medial (v, purple), the anterior-outer (iii, red), and the posterior-medial (iv, green) muscles do not differ much from those observed in PHC, although the posterior-medial muscles (iv, green) have diverged into two separate muscles with two separate apodemes that twist around each other at their attachment point on the chelicerae (Figure 5g). The inter-cheliceral-sclerite (orange in figures) is heart-shaped (Figures 4d and 5m) and there is an additional triangular sclerite present (Figure 5j,m). The inter-cheliceral-sclerite muscle (viii, aqua) has also diverged into two separate muscles (Figures 4a and 5j). The endosternite muscle (ix, light orange) was not observed in *Eriauchenius ranavalona*, but the  $\mu$ CT scan only focused on the most distal portion of the cephalothorax (Figure 1d) so we cannot say at this time whether these muscles are absent or just difficult to observe in the scans.

For the mecysmaucheniid trap-jaw spiders *A. magna* and *Zearchaea* sp., the lateral anterior (i, yellow) and lateral posterior (ii, magenta) muscles are directed more horizontally, that is, more toward the posterior of the carapace, than in PHC (Figure 5b–c,e–f). In *Zearchaea* sp., the lateral posterior (ii, magenta) muscles originate closer to the medial posterior edge of the cheliceral bases, whereas in other palpimanoids these muscles originate on the lateral posterior edge. In *A. magna*, the anterior medial inner (vi, blue) and the anterior medial outer (vii, light blue) muscles are smaller and are oriented more horizontally (Figure 4b,h) compared to PHC, where they extend more dorsally and laterally. In *Zearchaea* sp., the anterior medial inner (vi, blue) and the anterior medial outer (vii, light blue) muscles are absent (Figure 4c,f). In both *A. magna* and *Zearchaea* sp. the anterior-medial (v, purple), the anterior-outer (iii, red), and the posterior-medial (iv, green) muscles are also directed more posteriorly, oriented almost horizontally (Figure 5h–i). However, in *Zearchaea* sp., and to some extent in *A. magna*, the anterior-medial (v, purple) muscles are very large,

occupying a large proportion of the carapace dorsum (Figures 4e–f and 5h–i). Also, in *A. magna* and *Zearchaea* sp., the anterior-outer (iii, red) muscles do not run to the medial portion of the carapace, as in PHC and *Eriauchenius*, instead they run to the lateral sides of the carapace. In *A. magna*, the posterior-medial muscles (iv, green) are bicipital, and nearly completely diverged except for coming together into one attachment point at the cheliceral base. The inter-cheliceral-sclerite (orange in figures) is rod-shaped in *Zearchaea*, although with some small protrusions (Figure 5l,o), and in *A. magna* this structure has four lateral projections (Figure 5k,n). *Zearchaea* sp. has an additional triangular-shaped sclerite (Figure 5l,o). In both mecysmaucheniid species, the inter-cheliceral-sclerite muscle (viii, aqua) is smaller than in PHC, and in *Zearchaea* sp. does not extend as far posterior into the carapace (Figures 4b–c and 5k–l). The endosternite muscle (ix, light orange) was observed in *A. magna* (Figure 5b) but not in *Zearchaea*, and again, we cannot say whether these muscles are absent in *Zearchaea* sp. or just difficult to view and segment out from the  $\mu$ CT scans.

## 4 | DISCUSSION

Based solely on anatomical descriptions of muscles, previous studies have put forth hypotheses of cheliceral function (Brown, 1939; Palmgren, 1978; Whitehead & Rempel, 1959): the lateral anterior (i, yellow) muscles may abduct the chelicerae; the lateral posterior (ii, magenta) muscles may abduct and/or depress the chelicerae; the anterior medial inner (vi, blue) muscles may serve as levators and/or adductors; the anterior medial outer (vii, light blue) may serve as adductors; the anterior-medial (v, purple) muscles may be levators; the posterior-medial (iv, green) muscles may serve as depressor and/or adductors; the endosternite muscle (ix, light orange) may serve as adductors; and finally, there is a set of muscles termed the “medial retro-descendens” in

**TABLE 2** Muscle names, colors, and numbering used throughout manuscript, and the corresponding terminology and homology with muscles documented by Palmgren (1978)

Number	Color in figures	Name in present paper	Name and abbreviation in Palmgren, 1978
i	Yellow	Lateral anterior	Lateralis anterior (la)
ii	Magenta	Lateral posterior	Lateralis posterior (lp)
iii	Red	Anterior outer	Not observed in Palmgren, 1978
iv	Green	Posterior medial	Postero-medial carapacis (mc)
v	Purple	Anterior medial	Medial pro-descendens (pd)
vi	Blue	Anterior medial inner	Antero-medialis verticalis (av)
vii	Light blue	Anterior medial outer	Antero-medialis lateralis (al)
viii	Aqua	Inter-cheliceral-sclerite muscle	Not observed in Palmgren, 1978
ix	Light orange	Endosternite muscle	Postero-medial endosternalis (me)
-	-	-	Medial retro-descendens (rd), not observed in palpimanoids

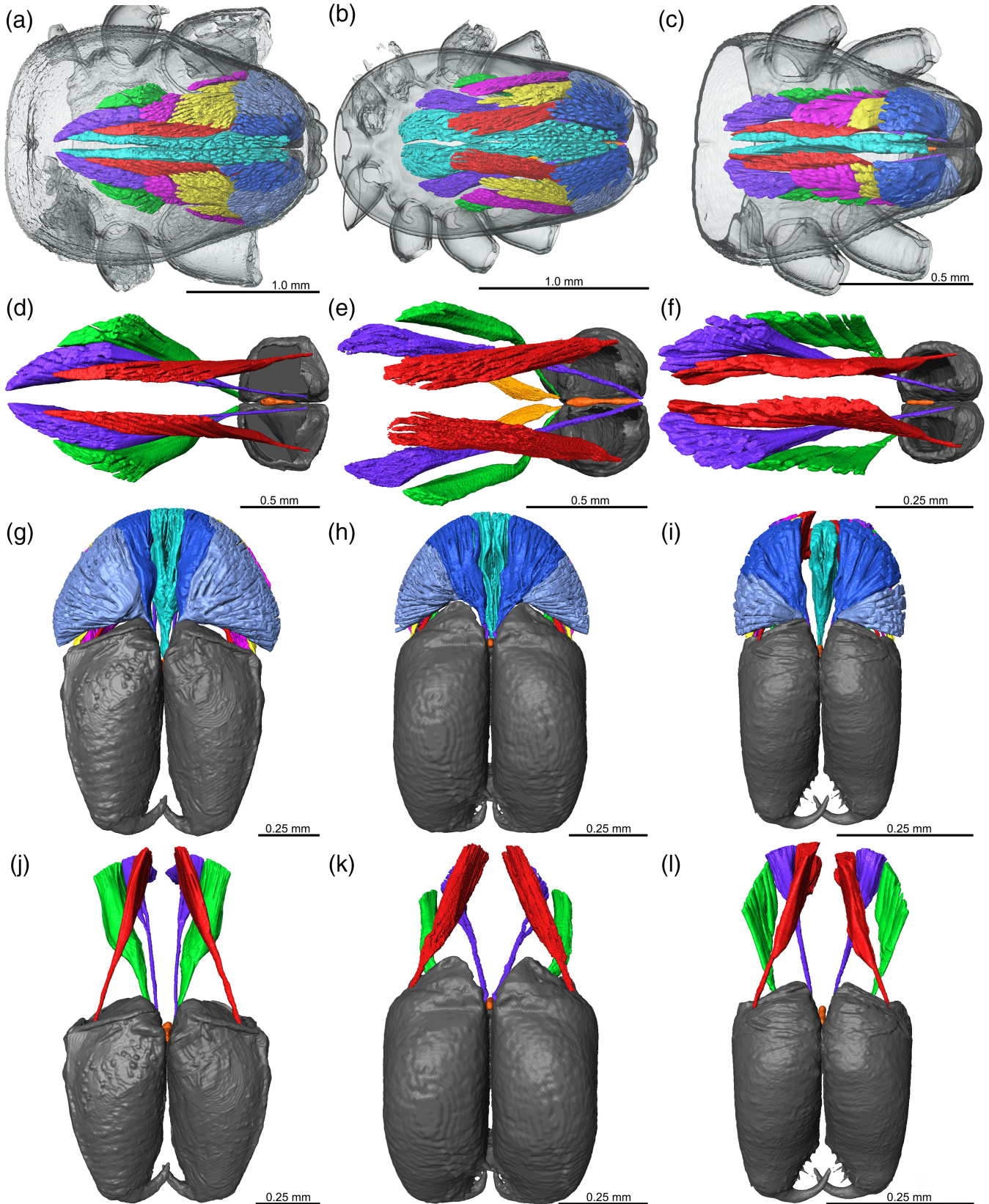
Palmgren (1978) that was not observed in palpimanoids (see Table 2) that has been hypothesized to act as depressors and/or adductors.

Our study documented two additional sets of muscles that have not been previously observed in other spiders and that thus far, have only been documented in palpimanoids (Figure 6). In this our study, homology of the cheliceral muscles was determined based on position, and we concluded that the anterior outer muscle (iii, red) is not homologous with the cheliceral muscles that were previously documented by Palmgren (1978, 1980). However, an alternative hypothesis is that the anterior medial inner and outer muscles (vi, blue, and vii, light blue) are instead both homologous to the “anterior-medialis verticalis” of Palmgren, but have diverged into two separate muscles in palpimanoids. Then, the “anterior-medialis lateralis” of Palmgren may instead be homologous to what we call here the anterior outer muscle (iii, red), which would have shifted to a more lateral position on the anterior edge of the chelicera in palpimanoids (Figure 6). Regardless of whether it is a shift in position or a de novo origination, the anterior outer muscle (iii, red), based on its orientation and position, may serve to elevate and abduct the chelicerae. This muscle may represent an innovation in the palpimanoids that set the stage for some palpimanoid lineages to extend their elongated chelicerae 90° away from their body, with a very wide gape (Wood et al., 2012; Wood et al., 2016). Archaeids and mecysmaucheniids (both palpimanoids) have shifted their cheliceral strike movements to occur mostly in the frontal plane around the dorsoventral axis (Legendre, 1961; Wood, 2008; Wood et al., 2012; Wood et al., 2016), whereas cheliceral movement in most spiders typically occurs in the transverse plane around the anteroposterior axis (Brown, 1939). Other palpimanoid lineages (palpimanids, huttoniids, stenochilids) may be morphological intermediates that do not have the extreme cheliceral movements, yet still have intermediate features that are distinct from other spiders: for example, some lineages of these spiders have a foramen surrounding the cheliceral bases as well as a space between the cheliceral bases and the endites (Wood et al., 2012). However, little is known regarding the cheliceral movements of palpimanids, huttoniids, and stenochilids. Future research should focus on documenting the cheliceral movements in all palpimanoids, as well as across spiders, and should examine which movements are produced by contraction of individual muscles.

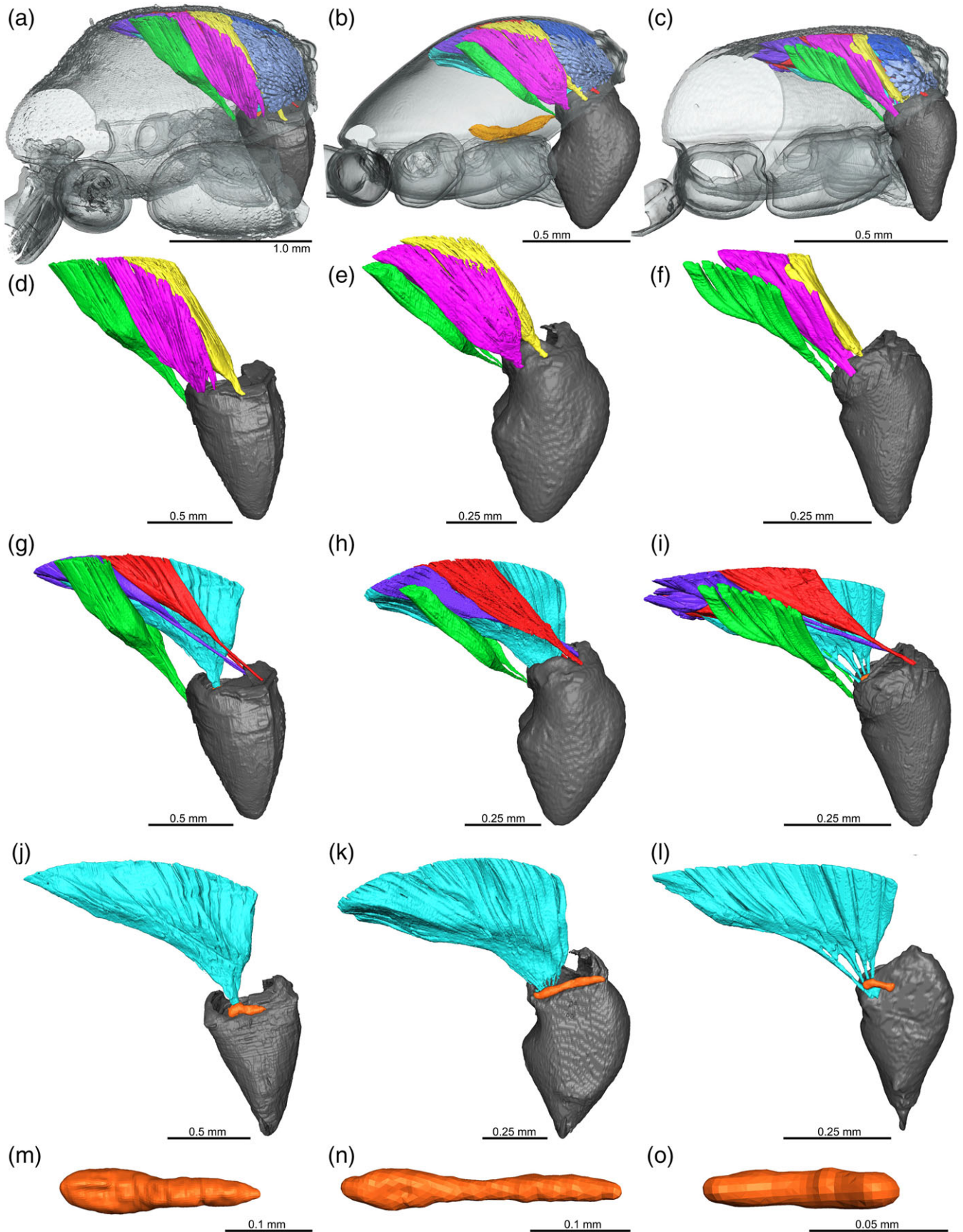
The inter-cheliceral-sclerite muscle (viii, aqua), which connects to the inter-cheliceral-sclerite (color-coded orange in figures) has also never been observed in non-palpimanoids. Brown (1939), in a detailed internal anatomy study on *Agelenopsis naevia*, documented a tiny sclerite that sat between the cheliceral bases, presumably the inter-cheliceral-sclerite, but Brown did not mention any muscle attachments to this sclerite. The inter-cheliceral-sclerite is typically reduced in other spiders compared to palpimanoids (Wood et al., 2012). Wood et al. (2012) wrongly diagnosed the triangular-shaped sclerite in archaeids as the inter-cheliceral-sclerite, however, the  $\mu$ CT scans revealed that the true inter-cheliceral-sclerite is internal in *E. ranavalona* (Figure 5j,m, and see Figure 5a in Wood et al., 2012). Furthermore, the medial retro-descendens of Palmgren (1978) was not observed in palpimanoids (Table 2, Figure 6). The medial retro-descendens muscle in other spiders (non-palpimanoids) and the inter-cheliceral-sclerite muscle (viii, aqua) of palpimanoids occur in approximately the same location, except attachment is to the cheliceral bases in non-palpimanoids and to the inter-cheliceral-sclerite in palpimanoids (Figure 6). This is suggestive that the medial retro-descendens of Palmgren and the inter-cheliceral-sclerite muscle may be homologous and may have transferred from the cheliceral bases to the inter-cheliceral-sclerite in palpimanoids. This transfer may have allowed for palpimanoids to adduct their chelicerae in unison rather than independently. It is also possible that in previous studies, which were based on traditional dissection techniques, the medial retro-descendens may have mistakenly been assigned as attaching to the chelicerae.  $\mu$ CT-scans of non-palpimanoid spider clades are necessary to make this distinction and to fully understand homology in these muscles.

Recently phylogenetic relationships among palpimanoid genera were examined using high-throughput molecular sequencing (Wood et al., 2018). In this study, a strongly supported monophyletic Palpimanoidea was recovered in some, but not all, analyses. Other studies have independently recovered a strongly supported Palpimanoidea: using morphological and molecular data (Wood et al., 2012), and using transcriptomic data (Fernández et al., 2018). Regarding relationships among palpimanoid families, most analyses in Wood et al. (2018) found Palpimanidae to be the earliest-diverging lineage, followed by Archaeidae, then Mecysmaucheniidae, and then with Huttoniidae and Stenochilidae as sister clades. No analyses recovered Palpimanidae,

morphology

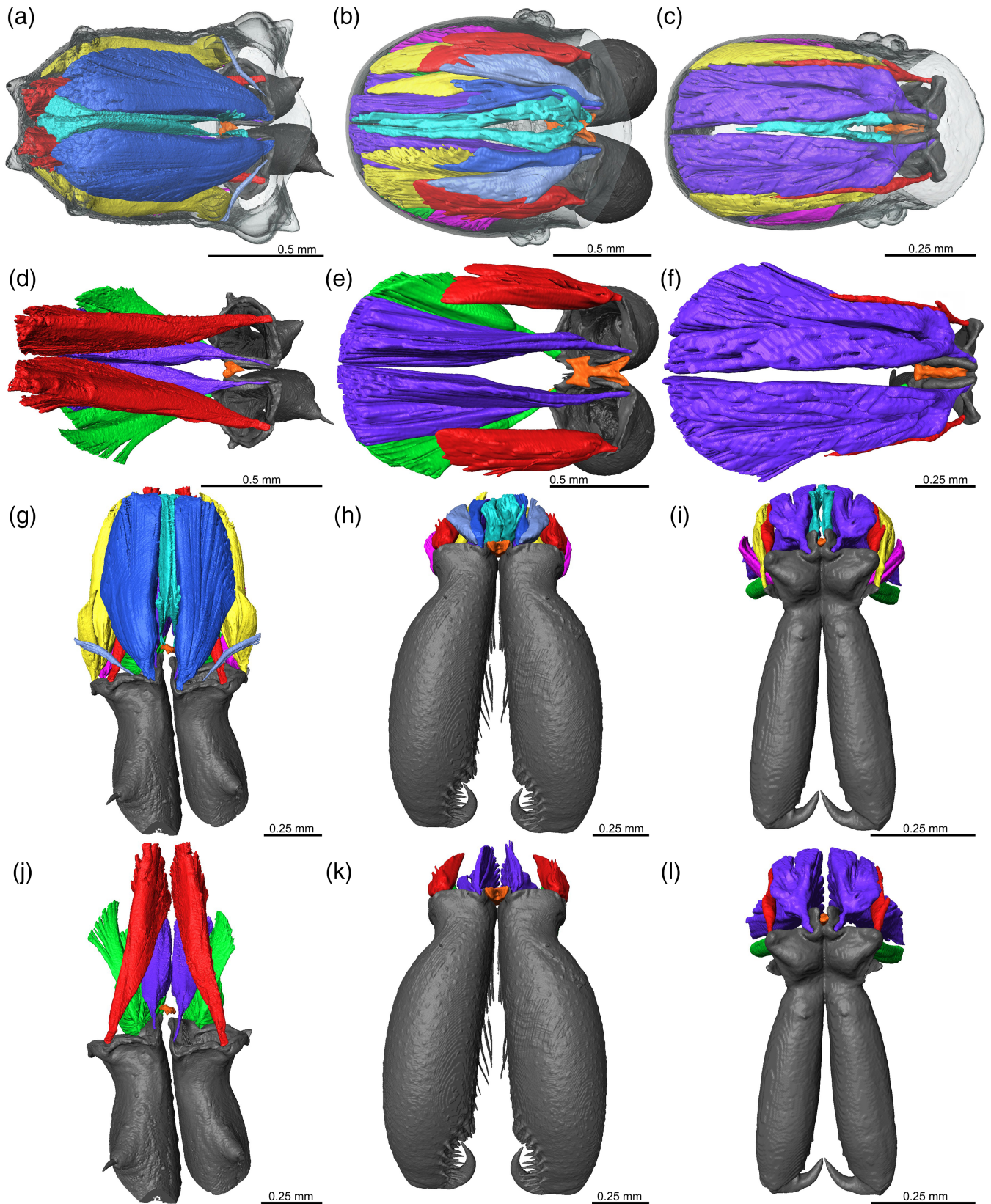


**FIGURE 2** Images of  $\mu$ CT scans with different sets of cheliceral muscles digitally segmented and color-coded following Table 2. The PDF version contains interactive 3D content that is available when using Adobe Acrobat: to activate click on (a), (b), and (c); right-click and select “show model tree” to hide different structures or make visible. Exterior of cephalothorax is shown as translucent grey and chelicerae are color-coded dark grey. Inter-cheliceral-sclerite color-coded orange. (a,d,g,j) *Palpimanus* sp. (b,e,h,k) *Huttonia* sp. (c,f,i,l) *Colopea* sp. (a–f) dorsal view. (g–l) anterior view. (d–f,j–l) only the anterior outer (iii, red), posterior medial (iv, green), anterior medial (v, purple), and endosternite (ix, light orange) muscles are shown

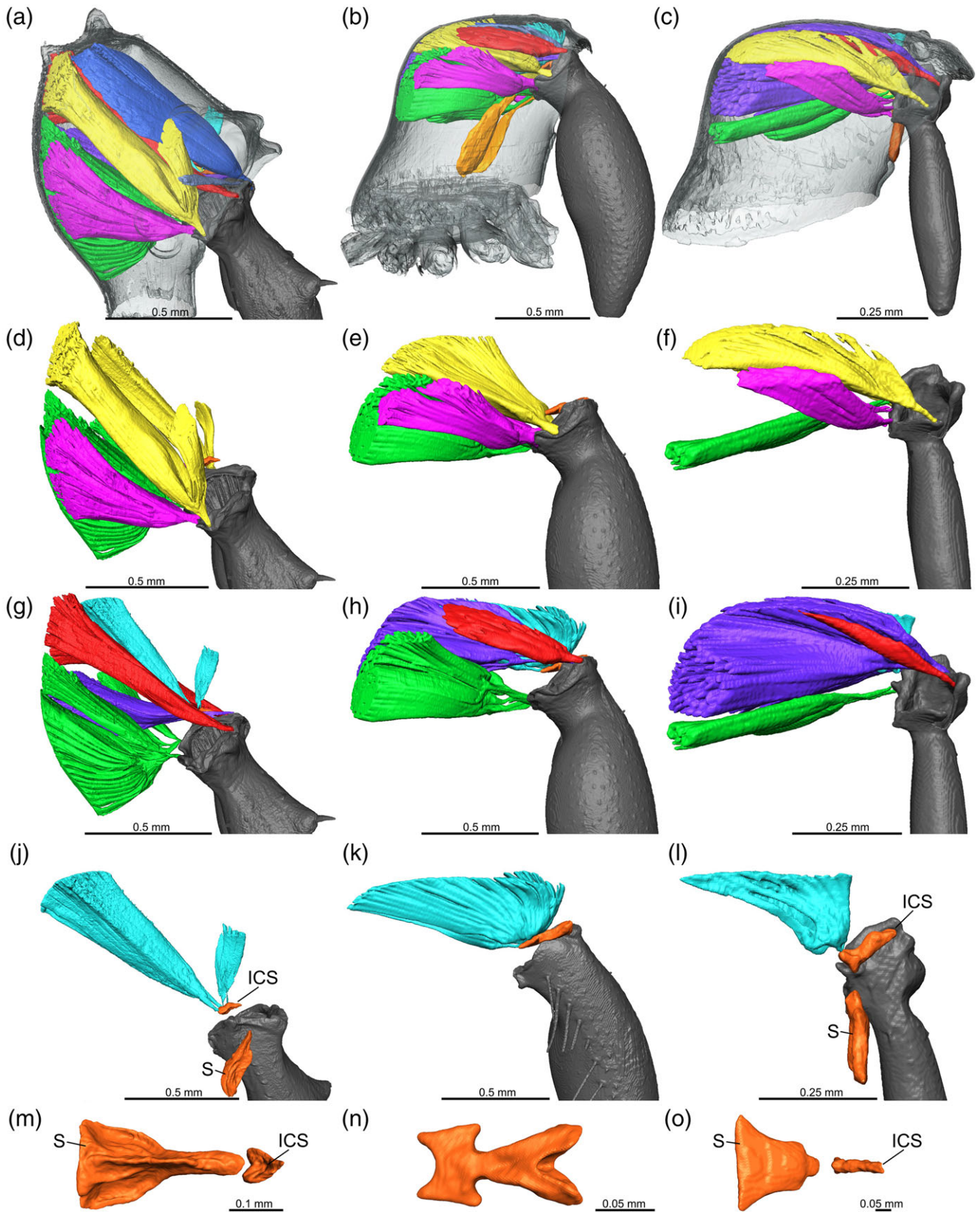


**FIGURE 3** Images of  $\mu$ CT scans with different sets of cheliceral muscles digitally segmented and color-coded following Table 2. Exterior of cephalothorax is shown as translucent grey and chelicerae are color-coded dark grey. Inter-cheliceral-sclerite color-coded orange. (a,d,g,j,m), *Palpimanus* sp. (b,e,h,k,n) *Huttonia* sp. (c,f,i,l,o) *Colopea* sp. (a–i) lateral view. (d–f) only the lateral anterior (i, yellow), lateral posterior (ii, magenta), and posterior medial (iv, green) muscles are shown. (g–i) only the anterior outer (iii, red), posterior medial (iv, green), anterior medial (v, purple), and inter-cheliceral-sclerite (viii, aqua) muscles are shown. (j–l) lateral view, with one chelicera removed showing only the inter-cheliceral-sclerite and inter-cheliceral-sclerite muscles (viii, aqua). (m–o) posterior view of inter-cheliceral-sclerite

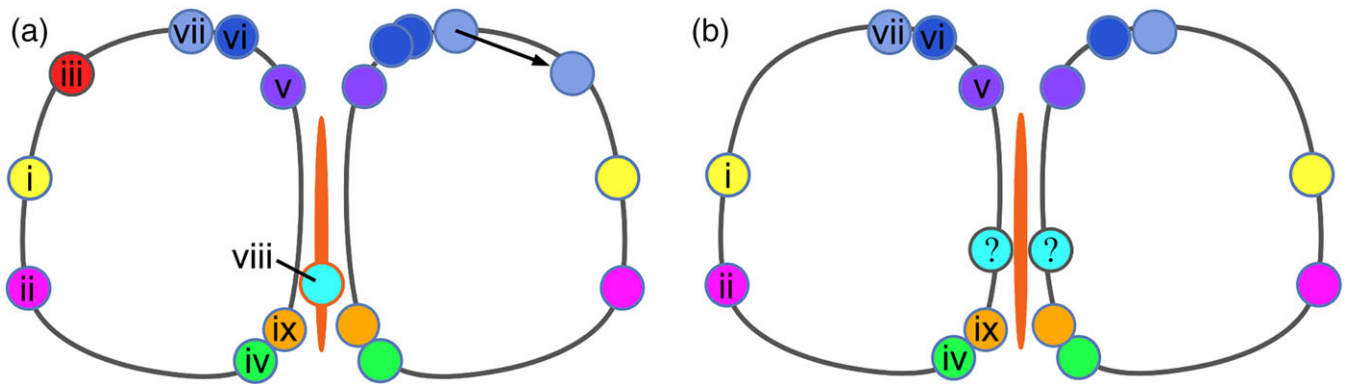




**FIGURE 4** Images of  $\mu$ CT scans with different sets of cheliceral muscles digitally segmented and color-coded following Table 2. The PDF version contains interactive 3D content that is available when using Adobe Acrobat: to activate click on (a), (b), and (c); right-click and select "show model tree" to hide different structures or make visible. Exterior of cephalothorax is shown as translucent grey and chelicerae are color-coded dark grey. Inter-cheliceral-sclerite color-coded orange. (a,d,g,j) *Eriauchenius ranavalona*. (b,e,h,k) *Aotearoa magna*. (c,f,i,l) *Zearchaea* sp. (a–f) dorsal view. (g–l) anterior view. (d–f,j–l) only the anterior outer (iii, red), posterior medial (iv, green), anterior medial (v, purple), and endosternite (ix, light orange) muscles are shown



**FIGURE 5** Images of  $\mu$ CT scans with different sets of cheliceral muscles digitally segmented and color-coded following Table 2. Exterior of cephalothorax is shown as translucent grey and chelicerae are color-coded dark grey. Inter-cheliceral-sclerite color-coded orange. (a,d,g,j,m) *Eriauchenius ranavalona*. (b,e,h,k,n) *Aotearoa magna*. (c,f,i,l,o) *Zearchaea* sp. (a–i) lateral view. (d–f) only the lateral anterior (i, yellow), lateral posterior (ii, magenta), and posterior medial (iv, green) muscles are shown. (g–i) only the anterior outer (iii, red), posterior medial (iv, green), anterior medial (v, purple), and inter-cheliceral-sclerite (viii, aqua) muscles are shown. (j–l) lateral view, with one chelicera removed showing only the inter-cheliceral-sclerite and inter-cheliceral-sclerite muscle (viii, aqua). (m–o) posterior view of inter-cheliceral-sclerite. Abbreviations: ICS, inter-cheliceral-sclerite; S, additional sclerite



**FIGURE 6** Illustration of cheliceral bases and inter-cheliceral-sclerite, dorsal view, showing hypothesized homologous muscle attachment sites in palpimanoid and non-palpimanoid spiders. Large grey circles represent the dorsal view of the cheliceral bases; small colored circles represent different muscle attachments sites and are color-coded and labeled following Table 2; orange bar between cheliceral bases represents inter-cheliceral-sclerite. (a) Muscles present in palpimanoid spiders; inter-cheliceral-sclerite muscle (viii, aqua) is a pair of muscles, but only depicted as one circle; the endosternite muscle (ix, light orange) was only found in some palpimanoids. The left chelicera depicts muscle homology based on Table 2, however, the right chelicera depicts an alternative hypothesis: the anterior medial inner muscle (vi, blue) instead diverged into two muscles, and the anterior medial outer muscle (vii, light blue) shifted to an anterior-outer position. (b) Muscles present in non-palpimanoid spiders based on Palmgren (1978); the muscles color-coded aqua and denoted by a “?” represent the medial retro-descendens from Palmgren (1978) and may be homologous with the inter-cheliceral-sclerite muscles (viii, aqua) in palpimanoids

Hutteniidae, and Stenochilidae as a monophyletic group, or Archaeidae and Mecysmaucheniidae as sister-taxa. This is of importance to our understanding of cheliceral evolution because in the current study Palpimanidae, Hutteniidae, and Stenochilidae showed similar cheliceral muscle anatomy (Figures 2 and 3). This suggests that the cheliceral muscle morphology of these three families may be symplesiomorphic, and that the unusual morphology of Archaeidae and Mecysmaucheniidae may be independently derived. Results from an ancestral character state reconstruction of the tubular-shaped carapace present in Archaeidae and Mecysmaucheniidae suggests that this trait evolved independently in each family (Wood et al., 2018). Archaeids have a thinner, more elongate “neck” and chelicerae (Figure 1d), while in mecysmaucheniids, the carapace and chelicerae are shorter and more robust and there is no constriction in the “neck” (Figure 1e–f). There are also differences in the cheliceral muscle morphology of Archaeidae compared to Mecysmaucheniidae, suggestive that these differences evolved independently.

Analysis of the  $\mu$ CT-scan of the archaeid, *Eriauchenius ranavalona*, shows that several sets of muscles have diverged compared to other palpimanoids. The lateral anterior (i, yellow) muscles are bicapital (Figure 5d), and in both the posterior-medial (iv, green) and inter-cheliceral-sclerite (viii, aqua) the original muscle appears to have diverged into two separate sets of muscles with separate attachment points (Figure 5g,j). The anterior medial inner (vi, blue) and anterior medial outer (vii, light blue) cheliceral attachment points are well-separated and easily distinguishable (Figure 4g), whereas in other palpimanoids they are difficult to separate. Furthermore, there has been a rotation in the orientation of the cheliceral muscles toward the posterior, so that some of the cheliceral muscles are oriented more horizontally compared to other palpimanoids: for example, compare the posterior-medial (iv, green) and lateral posterior (ii, magenta) muscles in Figure 5d,g with Figure 3d–i, and the anterior medial inner (vi, blue) muscles in Figure 4a with Figure 2a–c. Archaeids have highly

maneuverable chelicerae compared to most other spiders and are capable of independently extending each chelicera 90° away from their body (Forster & Platnick, 1984; Legendre, 1961; Wood, 2008; Wood et al., 2012). It has been suggested that archaeids are specialized to prey on other spiders: they have been observed in the wild preying on other spiders (Legendre, 1961; Millot, 1948; Wood et al., 2012) and in captivity, they reject non-spider potential prey (Legendre, 1961). They attack other spiders at a distance by swinging their long chelicerae out and away from their body. After the initial predatory strike, one chelicera lowers and the other chelicera remains extended 90° away from the body, with the prey impaled on the fang at the tip of the chelicera, until the prey dies (Forster & Platnick, 1984; Legendre, 1961; Wood, Griswold, & Spicer, 2007). It has been implied that this attack-at-a-distance predatory strategy may allow archaeids to successfully capture spider prey that has the potential to be injurious (Wood et al., 2012). Given the specialized movements of the highly maneuverable chelicerae, the divergences in their cheliceral musculature and the rotation toward the posterior makes sense: archaeids have shifted their cheliceral movements to occur mostly in the frontal plane around the dorsoventral axis (Wood, 2008).

Mecysmaucheniids also have highly maneuverable chelicerae, although they employ a “trap-jaw” predatory strike: prior to a strike, they open their chelicerae by extending them anterolaterally away from the body, with a wide gape, and hold them in position; then, once prey is in close proximity the chelicerae snap closed (Wood et al., 2016). Among species, there is remarkable variation in cheliceral closing speeds, with the fastest species being two orders of magnitude faster than the slowest species, and with the fastest species relying on ballistic, power-amplified, cheliceral strikes (Wood et al., 2016). For our study we  $\mu$ CT-scanned two mecysmaucheniids, *Aotearoa magna*, a species with slower, nonballistic cheliceral strikes (H. Wood, unpublished data), and *Zearchaea* sp., a species with extremely fast, ballistic, cheliceral strikes (Wood et al., 2016). Similar to the archaeids,

in the mecysmaucheniid specimens there has been a rotation in the orientation of the cheliceral muscles towards the posterior, so that some cheliceral muscles are more horizontal compared to other palpimanoids: compare the muscles in Figure 5e–f,h–i with Figure 3d–i. Similar to archaeids, mecysmaucheniids have shifted their cheliceral predatory strike movements to occur mostly in the frontal plane around the dorsoventral axis (Wood et al., 2016). *Zearchaea* sp., compared to *A. magna*, has reduced cheliceral musculature: the anterior-outer (iii, red) and posterior-medial (iv, green) muscles are smaller, and the anterior medial inner (vi, blue) and anterior medial outer (vii, light blue) are absent, although the anterior-medial (v, purple) muscles are larger, occupying a greater degree of the cephalothorax. The cheliceral predatory strikes of *Zearchaea* sp. rely on power-amplification, that is, a relatively high power output is achieved by releasing slowly stored energy almost instantaneously, resulting in movements that surpass the maximal power output of muscles. Power amplification occurs when a bow and arrow or catapult is fired, and has been documented in many organisms, particularly among the arthropods (see review by Gronenberg (1996)); examples include mantis shrimp (Patek, Korff, & Caldwell, 2004), termites (Seid, Scheffrahn, & Niven, 2008), fleas (Bennet-Clark & Lucey, 1967), and ants (Gronenberg, Tautz, & Hölldobler, 1993). The anterior medial inner (vi, blue) and the anterior medial outer (vii, light blue) may be involved in adducting the chelicerae in *A. magna*, however, these muscles are lost in *Zearchaea* sp., which may be because the chelicerae snap closed due to a release of stored energy rather than through muscle power. In mecysmaucheniids, the cheliceral bases are surrounded by membranous tissue, so there is not a stable joint between the cheliceral bases and the carapace. Clypeal tendons may anchor the cheliceral bases, and the chelicerae may pivot around this anchor to open. In the mecysmaucheniid specimens, the anterior-outer (iii, red) muscles do not run to the medial portion of the carapace, as in other palpimanoids, and instead run to the lateral sides of the carapace, making it difficult to even hypothesize which muscles abduct the chelicerae. In *Zearchaea* sp., it is possible that the energy for producing a ballistic strike is stored in the large anterior-medial (v, purple) muscles that may have elastic elements, in the clypeal tendons, or in the clypeus itself, however, the exact mechanism for energy storage is currently unknown.

## 5 | CONCLUSION

Compared to traditional dissection techniques,  $\mu$ CT-scanning may revolutionize morphological studies, allowing for fine detail to be discerned in the smallest of specimens. Furthermore, with  $\mu$ CT, 3D-computer models can be developed and input digitally or as a physical model into experimental studies that would be otherwise impossible in millimeter-sized specimens. This study documents and homologizes the muscles that operate the paturon in Palpimanoidea for the first time and represents an important step in our understanding of cheliceral anatomy. We document two muscle morphologies that have thus far never been observed in other non-palpimanoid spiders: the anterior-outer (iii, red) and the inter-cheliceral-sclerite (viii, aqua) muscles. However, to truly understand homology and to put our findings in context, future studies that use  $\mu$ CT-scanning should sample across major spider clades for comparison. In this

study, results from  $\mu$ CT-scanning are compared with previous studies that were performed using traditional dissection techniques. It is likely that future examination of cheliceral muscles across spiders using  $\mu$ CT-techniques will further transform and revise our knowledge of spider anatomy. Finally, our research has implications for future studies on cheliceral function. Hypotheses of cheliceral muscular movements made here and in previous studies are speculative and based purely on position of muscles. Future research should focus on explicitly testing what movements are produced by contractions of different muscle sets. The form, arrangement or function of muscles may be useful in the future for reconstructing phylogenetic relationships among arachnid groups.

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## CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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