



Body tagmatization in pseudoscorpions[☆]

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ABSTRACT

The opisthosoma of pseudoscorpions carries 12 dorsal and 11 ventral sclerites. The external genital opening is between first and the second ventral sclerite, a position that contrasts to the arachnid body organization in which the reproductive organs always open on/behind the second opisthosomal segment. The mismatch of dorsal and ventral opisthosomal sclerites, and the unconventional position of the genital opening traditionally have been explained by the loss of the ventral sclerite of the first opisthosomal segment, so that the morphologically second sclerite becomes the first visible on the ventral side of the opisthosoma, and a mismatch between dorsal and ventral sclerites emerges. We study the serial pattern of opisthosomal musculature to analyze the segmental (re)organization of the opisthosoma in two species of pseudoscorpions. We use micro-computed tomography (μCT) and light microscopic serial sections to reconstruct the segmental musculature. We test five explicit hypotheses about changes in the pattern of musculature that are predicted from the possible changes in sclerite morphology. By analyzing the serial pattern of dorso-ventral and intersegmental musculature including their origin and insertion, we document borders between body tagmata and segments, and assign dorsal and ventral sclerites to specific opisthosomal segments. The results of the study offer an interpretation of the muscle topography that is in contrast to the current textbook paradigm, i.e., the first opisthosomal sternite being reduced. In contrast, our results on the segmental musculature of the opisthosoma support the idea that the first opisthosomal segment carries dorsal and ventral sclerites. The second opisthosoma segment carries a dorsal sclerite, but its ventral sclerite is internalized and forms the cuticular wall of the genital atrium. The third opisthosoma segment and all following segments possess dorsal and ventral sclerites.

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1. Introduction

Pseudoscorpions (*Pseudoscorpiones* de Geer, 1778) are a small group of arachnid chelicerates (ca. 3000 species), their origin dating back to the Devonian (Dunlop 2010; Edgecombe and Legg 2014; Harms and Dunlop, 2017). Since then, they maintained a largely unchanged body organization. Most recent phylogenies consider pseudoscorpions and solpugids (Solifugae) as sistergroups (Haplochlemata). This sistergroup relationship is consistently supported by morphological analyses (Weygoldt and Paulus, 1979a, 1979b; Weygoldt 1998; van der Hammen 1989; Shultz 1990, 1991, 2007a) combined morphological and molecular data (Wheeler and Hayashi, 1998) or molecular analysis (Giribet et al., 2002; for a contrasting hypothesis see Alberti and Peretti 2002, or Garwood and

Dunlop 2014). Many morphological features of the pseudoscorpions are plesiomorphic, e.g., body tagmatization into a prosoma (7 segments; i.e., 1 ocular segment and 6 segments with appendages) and an opisthosoma (12[?] segments), and the genital opening supposedly positioned on the second opisthosomal segment (9th body segment). Autapomorphic features of extant pseudoscorpions are large silk glands that open at the tip of the movable finger of the chelicerae, and the rostrosoma, i.e., the labrum, the labium, and morphological derivatives from the coxae of the pedipalps (but see Dunlop 2000 for a different interpretation) are drawn out into an elongate functional tube around the mouth opening.

The prosoma of pseudoscorpions is dorsally covered by a large, undivided shield; ventrally the large coxae of the pedipalps and the four pairs of walking legs meet in the midline. A sternum is not present; however, in few species (Chthoniidae, Tridenscothoniidae) a rudimentary sclerotized tubercle between the coxae of the third and fourth walking legs may be interpreted as a residual sternum. Other species (Cheliferinea) have a soft skinned “pseudosternum” between the coxae of the second and third walking legs (Vachon 1949; Gruner et al., 1993).

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The situation is more complicated for the opisthosoma where the number of dorsal and ventral sclerites differs, i.e., it has 12 dorsal plates but only 11 ventral plates (Fig. 1). Several hypotheses are possible explaining this specific morphology. The most frequent explanation and current textbook paradigm assumes that dorsal and ventral sclerites on the opisthosoma are tergites and sternites *sensu strictu*, i.e., represent the sclerotized dorsal or ventral cuticle of the body wall of a segment, respectively. This paradigm assumes that the sternite of the first opisthosomal segment (pregenital segment) is reduced and that the genital operculum is formed by the (morphological) second sternite (Chamberlin 1931; Vachon 1949; Weygoldt 1969; Gruner et al., 1993; Dunlop and Lamsdell 2016) which has moved into first position on the opisthosoma. However, besides the position of the genital opening, no morphological evidence supports this paradigm. Somewhat related to this idea, but with different morphological implications, is the suggestion by Vachon (1949) that the sternite of the first opisthosomal segment is internalized and fused to the endosternite. Alternative hypotheses consider that the anterior region of the opisthosoma might be shaped by processes that involve other skeletal elements than just sternites in a strict sense. For example, the genital operculum may be formed from modified opisthosomal appendages as described for megoperculate arachnids (Shultz 1993, 1999). It is also possible that the second opisthosomal sternite was reduced and the genital operculum is formed by the first sternite in its original position. Finally, one can hypothesize that none of the sclerites is reduced, but that the ventral sclerite of the second opisthosomal segment is internalized and forms the genital atrium.

The association of certain opisthosomal sclerites with segments cannot be analyzed by external examination only, because independent landmarks are missing. It requires explicit morphological landmarks for identifying the origin of sclerites from specific segments. Van der Hammen (1986; p. 5) and Shultz (1993) suggested that chelicere segmentation should be confirmed by analyzing the segment specific pattern of the opisthosomatic musculature. However, only limited information is available on the musculature of arachnids. – The extensive work by Shultz (1990, 1991, 1993, 1999, 2000, 2001, 2007b) on the musculature of Xiphosurida, Scorpiones, Amplypygi, Uropygi and Opiliones substantially contributed to the reconstruction of the ground pattern of the arrangement of the musculature in arachnids, and provides important comparative information for the analysis of pseudoscorpion tagmatization. The arachnid ground pattern of musculature is derived from the ancestral arthropod box-truss model (Shultz, 2001, Fig. 9; Shultz, 2007b Fig. 6). For the prosoma, it suggests serially arranged dorsal suspensor muscles that originate from the endosternite and insert on the dorsal prosoma shield, and ventral suspensor muscles that originate ventrally from the endosternite and insert on the ventral sclerites (coxae of walking legs). In the opisthosoma, it comprises paired dorsal longitudinal (intersegmental) muscles connecting the tergites of adjoining segments, paired ventral longitudinal (intersegmental) muscles connecting adjoining sternites, posterior oblique muscles reaching from sternites to the lateral pleural wall, and a pair of dorso-ventral muscles extending from tergite to sternite in each segment (Shultz 2001, 2007b; Fig. 2A).

For each of the hypotheses above, we predict specific changes in the arrangement of the topography of the opisthosomal musculature. Our predictions are based on the assumption that muscle origin and insertion are conservative with respect to the specific sclerites; thus changes in sclerite morphology may result in associated changes of the muscles. Hypothesis 1: “loss of the first sternite” implies the correlated loss of the dorso-ventral muscles in the first opisthosoma segment, and loss of the ventral longitudinal muscles associated with the first opisthosomal sternite. The dorso-ventral muscle of the second opisthosomal segment would

reach from the second dorsal sclerite to the first ventral sclerite, i.e., the genital operculum formed by the (morphologically) second opisthosomal sternite (Fig. 2B). Hypothesis 2: “first sternite merged to the endosternite” predicts that the dorso-ventral musculature of the first opisthosomal segment might be preserved, but, since the first sternite is merged to the endosternite, it is now spanning between the first dorsal sclerite and the posterior margin of the endosternite. Equally, the ventral longitudinal muscle originally spanning between the first and second opisthosomal segment might be maintained, now originating from the posterior margin of the endosternite and inserting on the next following opisthosomal sternite (formally opisthosomal sternite II; Fig. 2C). Hypothesis 3 “genital operculum formed by other sclerotization than sternite” assumes that the sternites of the first two opisthosomal segments are displaced or completely reduced (the prosomal tubercle/soft sternum in some species of pseudoscorpions might be a rudiment of such originally opisthosomal sternites). The hypothesis predicts the loss of dorso-ventral musculature in the first and second opisthosomal segment because the original sternites are missing (Fig. 2D). It also predicts the loss or shift of the associated ventral longitudinal muscles. Hypothesis 4: “second opisthosomal sternite reduced”, predicts the loss of the dorso-ventral and ventral intersegmental musculature associated with the second opisthosomal segment; dorso-ventral musculature would be expected in the first and the third opisthosomal segments (Fig. 2E). Hypothesis 5: “Internalization of the second sternite” predicts that all sclerotized elements and associated muscles were maintained, but their topographic relationship changed (Fig. 2F).

In this study, we analyze the tagmatization of the pseudoscorpion body aiming to explain the unequal number of dorsal and ventral sclerites on the opisthosoma. We test the hypotheses above by analyzing the pattern musculature in the posterior region of the prosoma and the entire opisthosoma of two species of pseudoscorpions. We use µCT-imaging and serial histology as complementary methods to document the pattern of musculature in prosoma and opisthosoma of pseudoscorpions.

2. Material and methods

For light microscopy, we used two specimens (male and female) of *Neobisium carcinoides* (Hermann, 1804) preserved in 4% paraformaldehyde in 0.1 mol l⁻¹ phosphate buffered saline at pH 7.4. For µCT-imaging, we used one female of *Chernes hahni* (C.L. Koch, 1839) preserved in 2.5% glutardialdehyde in 0.1 mol l⁻¹ phosphate buffered saline at pH 7.4. Pseudoscorpions were collected under permit of the Bavarian State collection of Zoology Munich and species were determined according to Drogla and Lippold (2004), Mahnert (2004) and Hörweg (2014).

2.1. Macrophotography

Images of external morphology (Fig. 1) were taken in the fixative using cross-polarized light microscopy (Keyence BZ-9000 epifluorescence microscope with a 4x objective). Image stacks of approximate thirty images were processed with CombineZP (Version 2010) resulting in high resolution images, which were merged with Adobe Photoshop CC (Version 14.0). Additionally photographs of *Neobisium carcinoides* were taken in cross polarized light using a Canon EOS Rebel T3i camera (Canon U.S.A., Inc., Melville, NY, USA) with a Canon MP-E 65 mm macro lens and two external flash-units.

2.2. Light microscopy

For light microscopy, male and female *Neobisium carcinoides* were rinsed in phosphate buffer (0.1 mol l⁻¹) overnight, dehydrated in increasing ethanol (EtOH) concentration (30%, 50%, 70% 80%, 90%

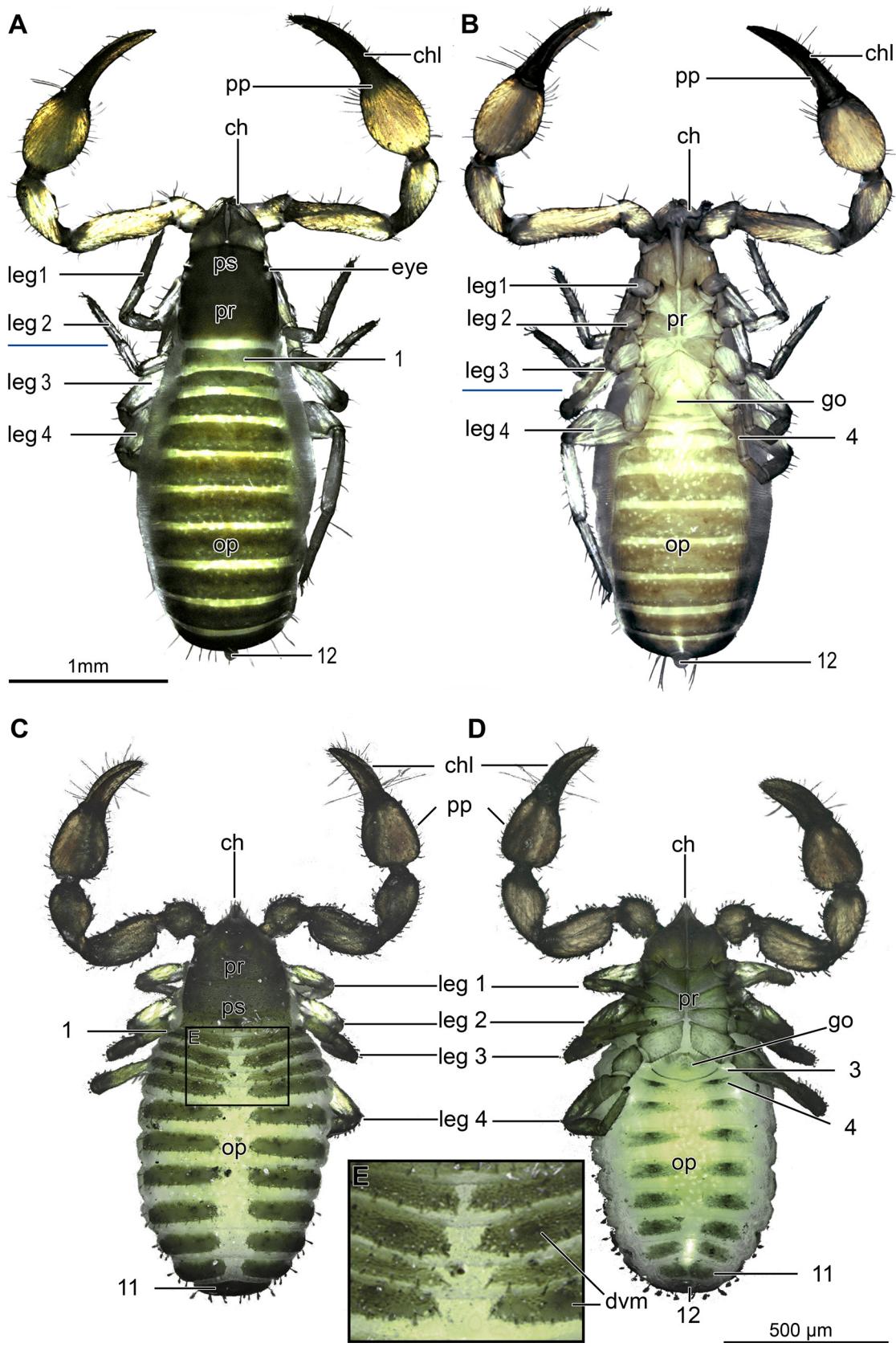


Fig. 1. External morphology of two species of Pseudoscorpiones; *Neobisium carcinoides* (A) in dorsal view, and (B) in ventral view. (C) *Chernes hahni* in dorsal view, and (D) in ventral view. (E) Close-up of the surface of the opisthosomal tergites (black box in C). Abbreviations: ch, chelicerae; chl, chela; dvm, insertion points of dorso-ventral musculature; go, genital operculum; op, opisthosoma; pp, pedipalp; ps, prosomal shield; pr, prosoma; 1–11, dorsal and ventral sclerites of the opisthosoma, respectively; 12, anal cone.

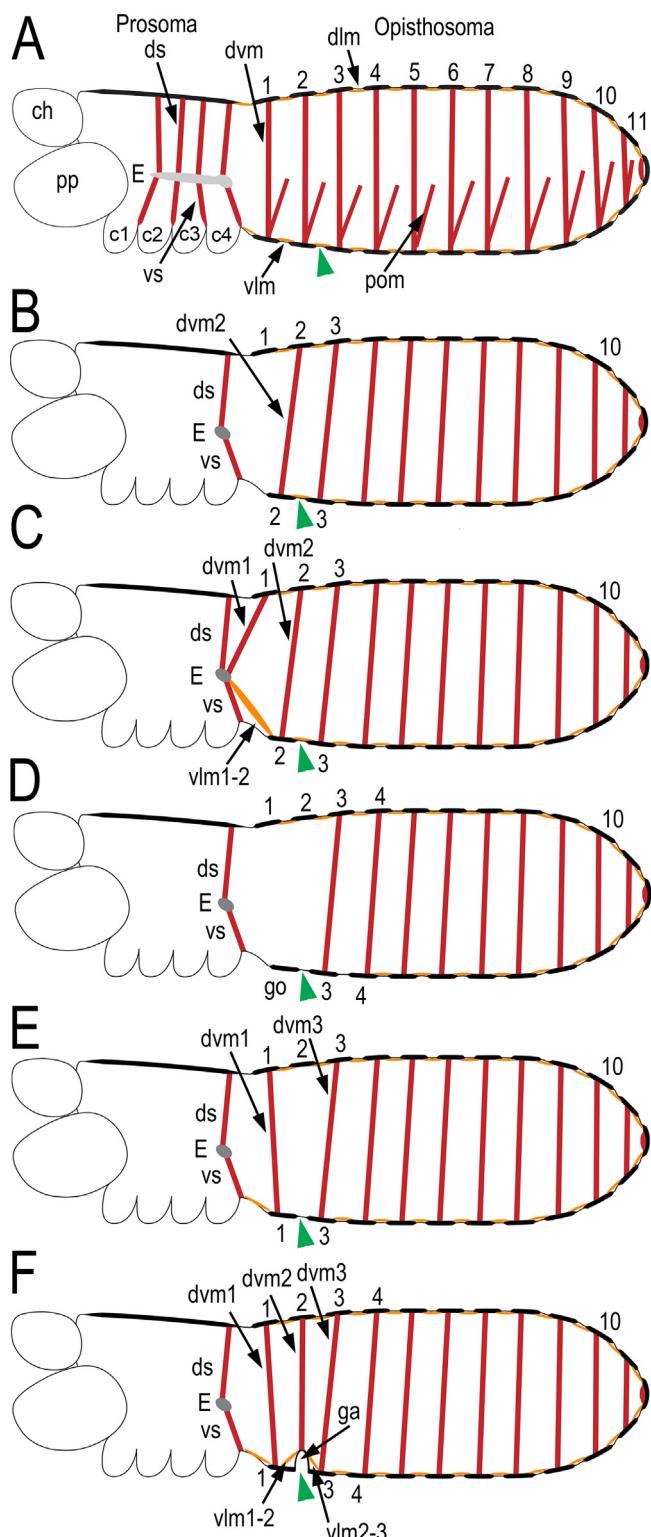


Fig. 2. Arachnid ground pattern of musculature (A) and predicted changes of muscle topography (B-F) for the hypotheses tested in this study. (A) The arachnid ground pattern of musculature comprises dorsal and ventral suspensor muscles in the prosoma, serially arranged, paired dorso-ventral musculature in the opisthosoma and posterior oblique muscles spanning from the ventral sclerites to the lateral pleural wall. Dorsal and ventral longitudinal muscles span between neighboring dorsal and ventral sclerites, respectively. (B) Hypothesis 1: "loss of the first sternite". (C) Hypothesis 2: "first sternite merged to the endosternite". (D) Hypothesis 3: "genital operculum formed by other sclerotization than sternite". (E) Hypothesis 4: "second opisthosomal sternite reduced". (F) Hypothesis 5: "Internalization of the second sternite". Abbreviations: c1-c4, coxae of walking legs 1–4; ch, chelicera; ds, dorsal

and 96%) for 2 h each, embedded in hydroxyethyl methacrylate (Historesin; Leica Microsystems, Wetzlar, Germany) and polymerized for two days. Semi-thin cross (female) and longitudinal (male) sections (2 µm) were cut with a HM 340 E electronic (Thermo Fisher Scientific Inc., Waltham, MA, USA) and a AO Spencer No. 820 rotary microtome. Sections were stained with Rüdeberg solution (0.1% methylene blue, 0.1% thionin and 0.1 mol l⁻¹ Na₂HPO₄ in distilled water; Rüdeberg, 1967). Microphotographs were taken with an Olympus BX51 microscope and Industrial Digital Camera ToupeTek DCM 510 (UCMOS camera, ToupeTek Photonics, Hangzhou, P.R. China) with ToupeView Software (ToupeTek Photonics, Hangzhou, P.R. China) for capturing.

2.3. Image processing

All stacks were processed with CombineZM/ZP to constantly sharp images and merged with Adobe Photoshop CC, version 14.0 × 64 bit (Adobe Systems Incorporated, San Jose, CA, USA) to compositions. Editing via Photoshop includes color and contrast adjustment/enhancement, scale bar, label and background addition as well as image cropping.

2.4. Micro-computed tomography (µCT)

Two specimens of *Chernes hahnii* fixed in GDA were dehydrated through graded series of EtoH (30%, 50%, 70%, 90%, 96% and absolute) and transferred in 1% iodine solution (in 99.5% EtoH). Critical point drying of the specimens was performed using Leica EM CPD300 (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). Only one specimen was used for µCT-imaging.

For micro-computed tomography, an XRadia MicroXCT-200 x-ray microscope (Carl Zeiss Microscopy GmbH, Jena, Germany) equipped with scintillator-objective lens unit was used. The scan was performed with a 4x objective with X-ray source setting at 40 kV and 8 W for 1 s acquisition time.

The recorded 1600 projections per tomography were reconstructed with the XMReconstructor software (Carl Zeiss Microscopy GmbH, Jena, Germany), resulting in TIFF format image stacks. The scan was performed using Binning 2 and subsequently reconstructed using Binning 1 (full resolution) to avoid information loss. The reconstruction resulted in system based calculated pixel size of 3.13 µm, 1014 × 1014 px. Consequently, the spatial resolution of the images is optimally c. 6.5 µm for clear borders or c. 10 µm for individual structures.

The complete sets of µCT-images are deposited with MorphoBank (O'Leary and Kaufman, 2011) at <http://morphobank.org>; project 2802.

2.5. 3D-reconstruction

Image stacks were cropped in three overlapping parts (pedipalps, prosoma and opisthosoma) using Fiji (ImageJ Version: 2.0.0-rc-43/1.50e). The outer body surface and segmental musculature of the opisthosoma were reconstructed in OsiriX (v.3.9.2, 32-bit). Images were rendered in Blender (Version 2.49b, Blender Foundation, Amsterdam, Netherlands). Finally, the three partial image were merged again to a full 3D-reconstruction of the animal.

suspensor muscles; dvm, dorso-ventral muscles; dlm, dorsal longitudinal (intersegmental) muscles; E, endosternite; ga, genital atrium; pom, posterior oblique muscles; pp, pedipalp; vlm, ventral longitudinal (intersegmental) muscles; vs, ventral suspensor muscles. Numbers on opisthosoma indicate dorsal and ventral sclerites, respectively; green arrowhead, genital opening.

3. Results

3.1. External morphology

Prosoma and opisthosoma are clearly distinct body regions. In both species (Fig. 1), the prosoma carries a single, large sclerite, the prosomal shield on its dorsal side. The ventral side is occupied by the coxae of the pedipalps and the 4 walking legs. No sternal elements are found in the two studied species of pseudoscorpions. The opisthosoma is broadly attached to the prosoma, but is externally distinguished from the prosoma by its segmented organization. In *Neobisium carcinoides* (Fig. 1A,B), the dorsal and ventral sclerites of the opisthosomal segments are unpaired; in *Chernes hahni* (Fig. 1 C–E) the opisthosomal segments carry paired dorsal and ventral sclerites. In both species, the dorsal view shows 12 sclerotized (paired) elements (including the anal cone). In high magnification, the dorsal sclerites of all opisthosomal segments, except the first and the third, show external markings which are interpreted as insertions of the dorso-ventral musculature (Fig. 1E). The ventral view shows only 11 sclerotized elements (including the anal cone). The genital pore is found on/beyond the topographically first ventral opisthosomal sclerite.

3.2. Micro-computed tomography and 3D-reconstruction

Micro-computed tomography produces stacks of in-situ images that can be used for three-dimensional reconstructions of the internal anatomy. However, using the specific scanner settings for overview images of the entire morphology of the animal, μ CT-imaging is limited by relatively low resolution and lack of structural (cytological) detail (i.e., muscle origin and insertion cannot be analyzed, fine muscle strands are not recognized and differentiation between tissues is possible only on grey level differences but not on cytological detail). The precision of any 3D-reconstruction built upon μ CT-image stacks depends on the correct interpretation of structures. Fig. 3 presents a direct comparison of the recorded imaged based on μ CT and microscopic images of histological sections in longitudinal and cross sections. The topography of the dorso-ventral musculature and its association with dorsal and ventral sclerites can be traced with both methods. However, the lower spatial resolution of the μ CT-images is evident, limiting the detection of muscle strands to a minimum size of 30 μ m in the present case, as the scan was performed as overview of the entire specimen. Small muscle strings as well as details of origin and insertion points of musculature were therefore analyzed using light microscopy.

Our 3D-reconstruction of the muscle anatomy of *Chernes hahni* (Fig. 4) shows one string of dorso-ventral muscle originating from the endosternite and attaching to the posterior region of the prosomal shield (see also Fig. 6A, B). Additional muscles originate from the endosternite reaching anteriorly and ventrally. The insertion of these fine muscles strands cannot be resolved with μ CT because of the low spatial resolution in this case, but the ventral muscle strings point to the posterior margin of the coxae of the 4th walking leg.

The first dorsal opisthosomal sclerite is associated with the genital operculum on the ventral side (Fig. 4). However, we found no dorso-ventral musculature spanning between these two elements. The second dorsal opisthosomal sclerite is attachment for a pair of dorso-ventral muscle strands that extend through the segment and insert ventrally on the roof of the sclerotized genital atrium (genital apodeme (Vachon 1949)). The third dorsal and ventral opisthosomal sclerites have no dorso-ventral muscles attached to them. From the fourth dorsal opisthosomal sclerite on, we find serially arranged dorso-ventral muscles that extend to the ventral sclerite of the respective element. The serial arrangement of the dorso-ventral musculature extends to the 11th opisthosomal tergite. The resolution of the recorded μ CT-images does not allow recognizing

muscular elements in the anal cone. Also, the very fine muscle strands of the dorsal and ventral longitudinal musculature are not recorded in the tomographic data.

3.3. Histological serial sections

Histological serial sections in longitudinal and cross-sectional orientation were used to determine origin, insertion and topographic position of the strands of the dorso-ventral and intersegmental musculature of the opisthosoma (Figs. 5–8). From the analysis of complete serial sections we projected the musculature observed in the slices in a two-dimensional diagram (Fig. 8). – The most anterior, dorso-ventral muscle found spans between the posterior margin of the prosomal plate and the endosternite. Another muscle band spans from the posterior margin of the endosternite (Figs. 7 and 8) to the intersegmental membrane between the coxae of the 4th walking leg and the first ventral opisthosomal sclerite (=genital operculum).

No dorso-ventral musculature was found spanning between the first dorsal opisthosomal sclerite and the genital operculum. However, pairs of dorsal and ventral intersegmental muscle strands were present. The dorsal intersegmental muscles span between the anterior margin of the first and the anterior margin of the second dorsal sclerite. The ventral intersegmental muscles span between the anterior margin of the genital operculum and the roof of the sclerotized genital atrium.

The anterior margin of the second dorsal opisthosomal sclerite is origin of dorso-ventral muscles that extend across the segment and insert on the roof of the sclerotized genital atrium. We also found dorsal and ventral intersegmental musculature associated with the second dorsal sclerite as well as the sclerotized genital atrium (Fig. 7). The dorsal intersegmental musculature of the second dorsal opisthosomal sclerite spans between its anterior margin and the third sclerite. The ventral intersegmental musculature spans between the roof of the genital atrium (right behind the attachment site of the dorso-ventral muscle) and the posterior margin of the 3rd and anterior margin of the 4th ventral sclerite (Figs. 7 and 8).

No dorso-ventral musculature was detected connecting the third dorsal sclerite and its associated ventral sclerite, but the dorsal intersegmental musculature was present. From the 4th to the 11th opisthosomal dorsal sclerites, we found serially arranged paired dorso-ventral muscles spanning from a middle position of the dorsal to a middle position on the ventral segmental sclerite (Fig. 5). Between all dorsal and ventral sclerites, we found also paired strands of intersegmental muscles. In the anal cone, we found thin strands of dorso-ventral musculature.

4. Discussion

4.1. Pattern of musculature in pseudoscorpions

For pseudoscorpions, a cursory description of the segmental dorso-ventral muscles and dorsal and ventral intersegmental muscles of the opisthosoma was given by Beier (1931). Vachon (1949) described the detailed topography of the musculature of the endosternite (p. 450, Fig. 209), showing that the endosternite is largely reduced and only two small residues, i.e., anterior and posterior endosternite, provide the origin for prosomal musculature that consists of (1) muscles spanning to the dorsal plate of the prosoma, and (2) of muscles, spanning to the coxae of the walking legs. For the opisthosoma, he presented a generalized scheme of the topographic anatomy of *Chernes hahni* (p. 461, Fig. 221) documenting dorso-ventral as well as dorsal and ventral longitudinal (intersegmental) musculature in opisthosomal segments 4–11. Vachon (1949) stated explicitly that the dorso-ventral muscula-

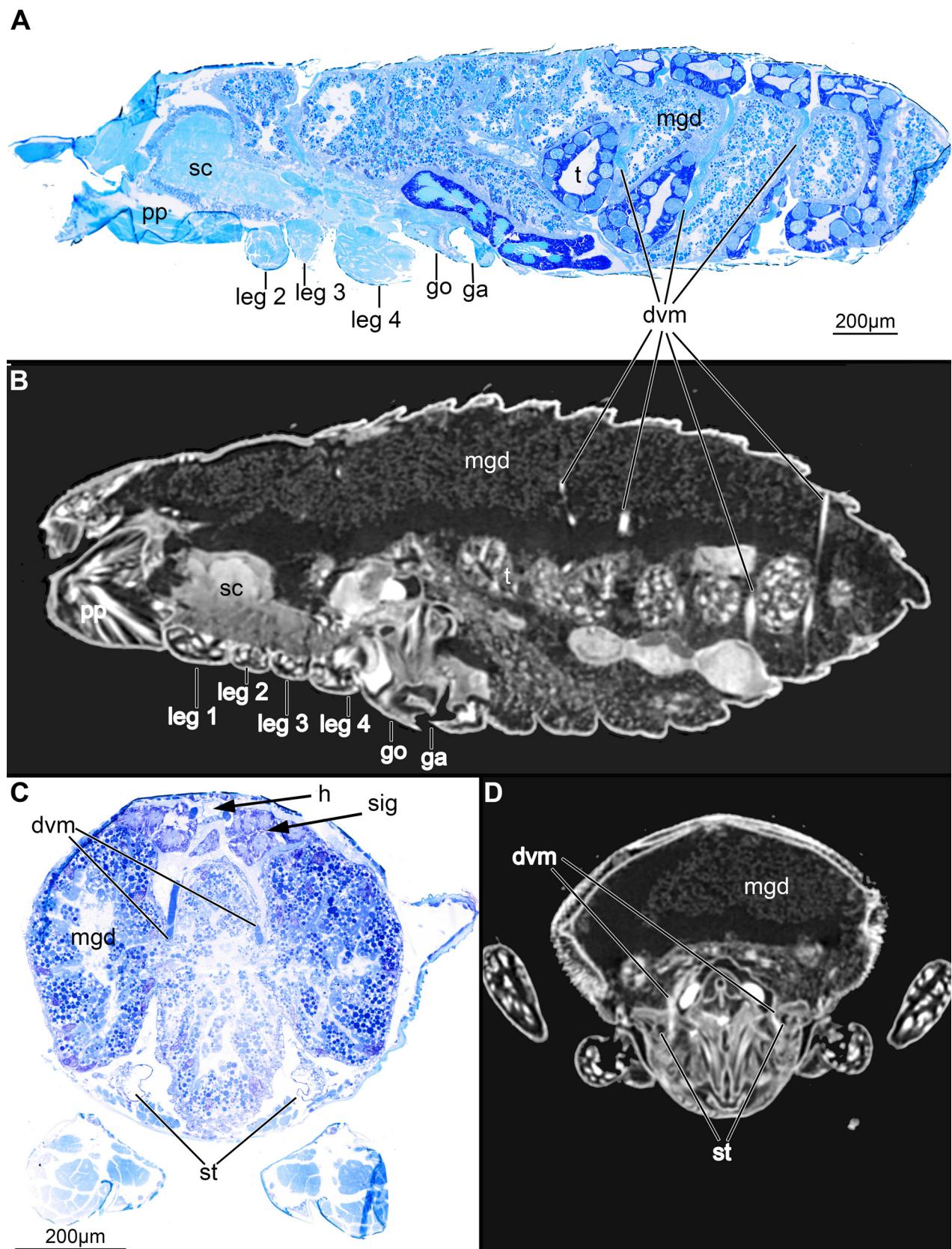


Fig. 3. Comparison of imaging details with light microscopy and μ CT. (A) *Neobisium carcinoides*, mid-sagittal section, light microscopy. (B) *Chernes hahnii*, μ CT-image, mid-sagittal section. (C) *Neobisium carcinoides*, light microscopy cross-section through the 4th opisthosomal segment. (D) *Chernes hahnii*, μ CT-imaging, cross-section through 2nd opisthosoma segment. Abbreviations: dvm, dorso-ventral musculature; ga, genital atrium; go, genital operculum; h, heart; mgd, midgut diverticulum; op, opisthosoma; pp, pedipalp; sc, syncerebrum; sig, silk gland; st, stem trachea; t, testicles.

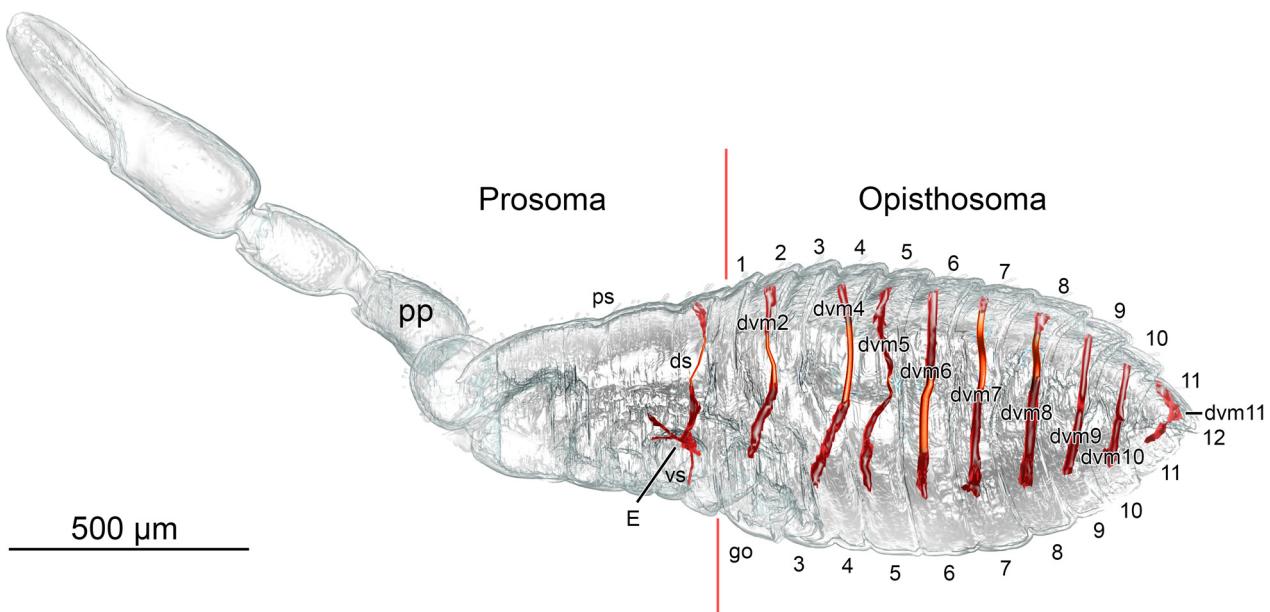


Fig. 4. Three-dimensional reconstruction of the external body contour and the musculature of *Chernes hahni* based on μ CT-imaging. Abbreviations: E, endosternite; go, genital operculum; ps, prosomal shield; ds, dorsal suspensor muscle; dvm, dorso-ventral musculature; pp, pedipalp; vs, ventral suspensor muscle. Numbers on opisthosoma indicate dorsal and ventral sclerites, respectively.

ture is missing in the first and the third opisthosomal segment, and that the second opisthosomal segment contains dorso-ventral musculature spanning from the dorsal sclerite to the roof of the genital atrium (genital apodeme). His drawing of the topographic anatomy has occasionally been reproduced in textbooks (Gruner et al., 1993) and represents the currently most detailed documentation of body musculature of pseudoscorpions. Weygoldt (1964, 1969) presented a similar schematic of the topographic anatomy of a protonymph of *Pselaphochernes* sp. that also contains basic information on the body musculature. – Our results confirm the descriptions by Vachon (1949), i.e., dorsal and ventral muscles originating from the posterior endosternite; the ventral muscles reaching to the intersegmental membrane between the coxae of the forth walking leg and the first ventral opisthosomal sclerite (=genital operculum). We also find that dorsal-ventral musculature associated with the first and the third dorsal opisthosomal sclerite is missing, but is present on the second dorsal opisthosomal sclerite from where it reaches to the roof of the genital atrium. – A possible interpretation of this observed topography is that the muscles originating from the (posterior) endosternite represent dorsal and ventral suspensor muscles, respectively (but see below for an alternative interpretation). As such, they mark the border between prosoma and opisthosoma. Because of the largely reduced prosomal musculature and the small size of the endosternite, we cannot assign this musculature to specific segment numbers; instead we assume that they represent the most posterior suspensor muscles. – Vachon (1949) suggested that the first opisthosomal sternite were internalized and merged with the endosternite. If correct, this would result in an alternative identification of these muscles as ventral intersegmental muscle. However, this interpretation would require the assumption that the ventral suspensor muscle and the first intersegmental muscle (spanning between the posterior margin of the prosoma and the first ventral sclerite) were reduced.

4.2. Junction between prosoma and opisthosoma

The muscle topography at the junction between prosoma and opisthosoma is a landmark for the border between the two tag-

mata. However, it is complex and variable among various groups of extant chelicerates. Shultz (1993, 1999, 2001, 2007b) describes the dorso-ventral musculature of the first opisthosomal segment as originating from the posterior margin of the endosternite and inserting on the first opisthosomal tergite as *dorsal endosternal suspensor muscle* [muscle #13, Shultz (1993); muscle #17, Shultz (1999)] and as originating from the posterior margin of the endosternite and inserting on the first opisthosomal sternite as *ventral endosternal suspensor muscle* (muscle #15, Shultz (1993); #19 Shultz (1999)]. He suggests serial homology with the dorso-ventral muscles of the following opisthosomal segments (muscle #17 (Shultz 1993) muscle #21 (Shultz 1999)). With all due respect to his meticulous anatomical work and the great amount of detail provided in his studies, we are concerned about this homologization. In clear contrast to all segmental, dorso-ventral opisthosoma muscles that extend as a single string of muscle between tergite and sternite of the same segment, the described muscles are intersegmental, i.e., originating from the endosternite (=element of prosoma) and inserting on opisthosomal sclerites. Also, dorsal and ventral suspensor muscles are two distinct muscles, but they together are homologized with just one muscle. We therefore think, that the described muscles cannot easily be homologized with dorso-ventral opisthosoma muscles, but represent the most posterior suspensor muscles of the prosoma and mark the border between prosoma and opisthosoma.

4.3. First opisthosoma segment

In many extant chelicerates, the first opisthosomal segment has been reduced to various degrees or morphologically modified (van der Hammen, 1986). Therefore, the transition between prosoma and opisthosoma does not easily compare to the ground plan of serially arranged segments and associated musculature. Other groups, however, show a presumably ancestral segmental pattern, but lack the dorso-ventral musculature in the first opisthosomal segment (e.g., Palpigradi [[Kästner, 1931](#); *Koenenia mirabilis*; own unpublished data confirm this observation], and Solifugae [[Kästner 1931](#) [the musculature of *Galeodes granti*, Solifugae, is doc-

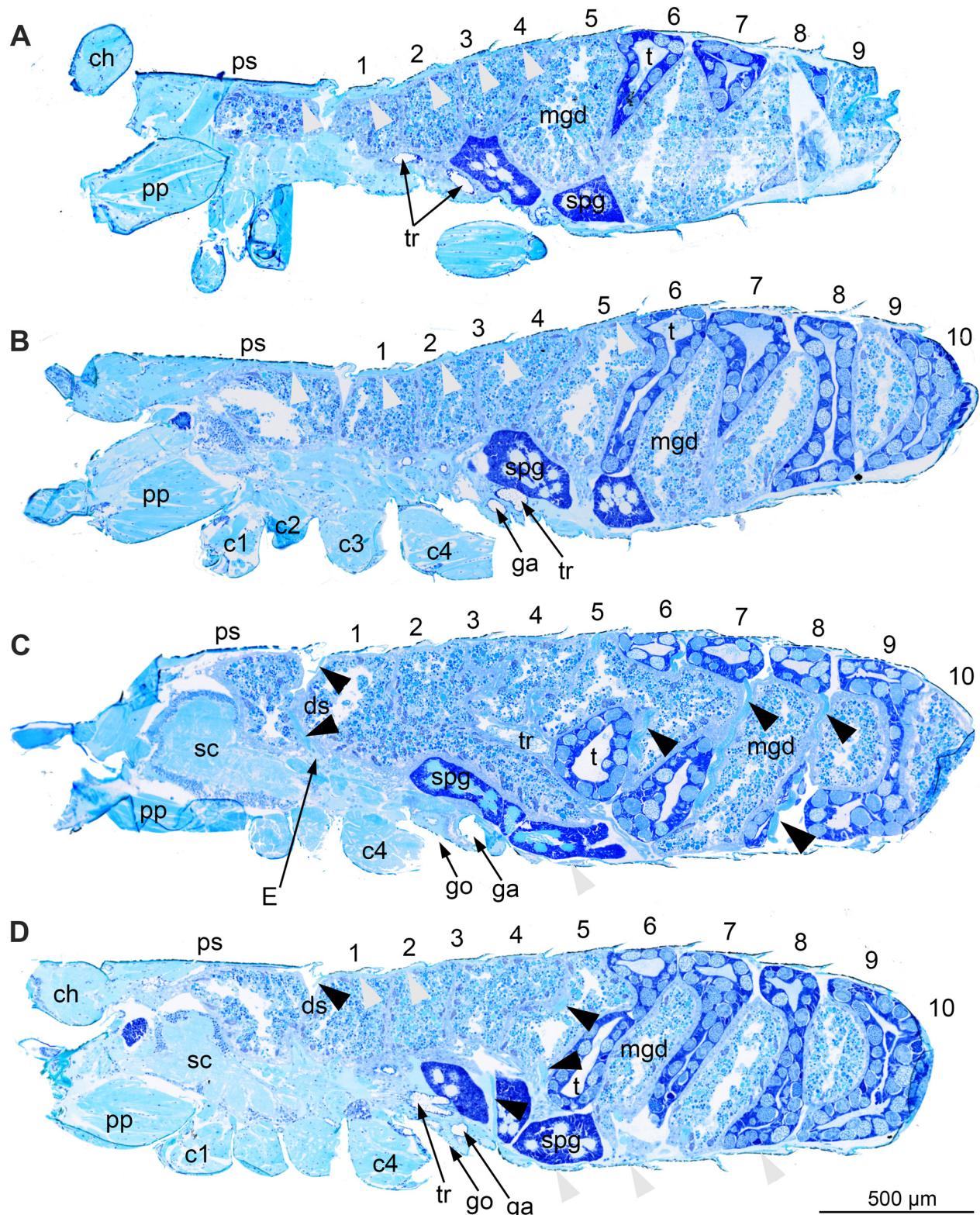


Fig. 5. Light micrographs of selected longitudinal sections of *Neobisium carcinoides*. The section plane proceeds from lateral (A) to medial (D). In each of the images, parts of the intersegmental longitudinal muscles or the dorso-ventral muscles can be seen. Because no muscle is sectioned from origin to insertion, the section images are patchy. The complete topographic anatomy of the musculature can only be visualized by reconstructing the dorso-ventral and the intersegmental musculature by following each individual muscle strand through all slides (the results of the reconstruction are documented in Fig. 8). In (C) and (D) the anterior arrowheads point to parts of the dorsal suspensor muscle. Abbreviations: ch, chelicerae; c1-c4, coxae of walking legs 1-4; ds, dorsal suspensor muscle; E, endosternite; go, genital operculum; leg 1-4, walking legs 1-4; mgd, midgut diverticulum; pp, pedipalp; ps, prosomal shield; sc, syncerebrum; spg, spermatophore gland; t, testicle; tr, trachea; numbers on opisthosoma indicate dorsal sclerites. Black arrowheads, dorso-ventral muscles; grey arrowheads, dorsal and ventral intersegmental, longitudinal muscles.

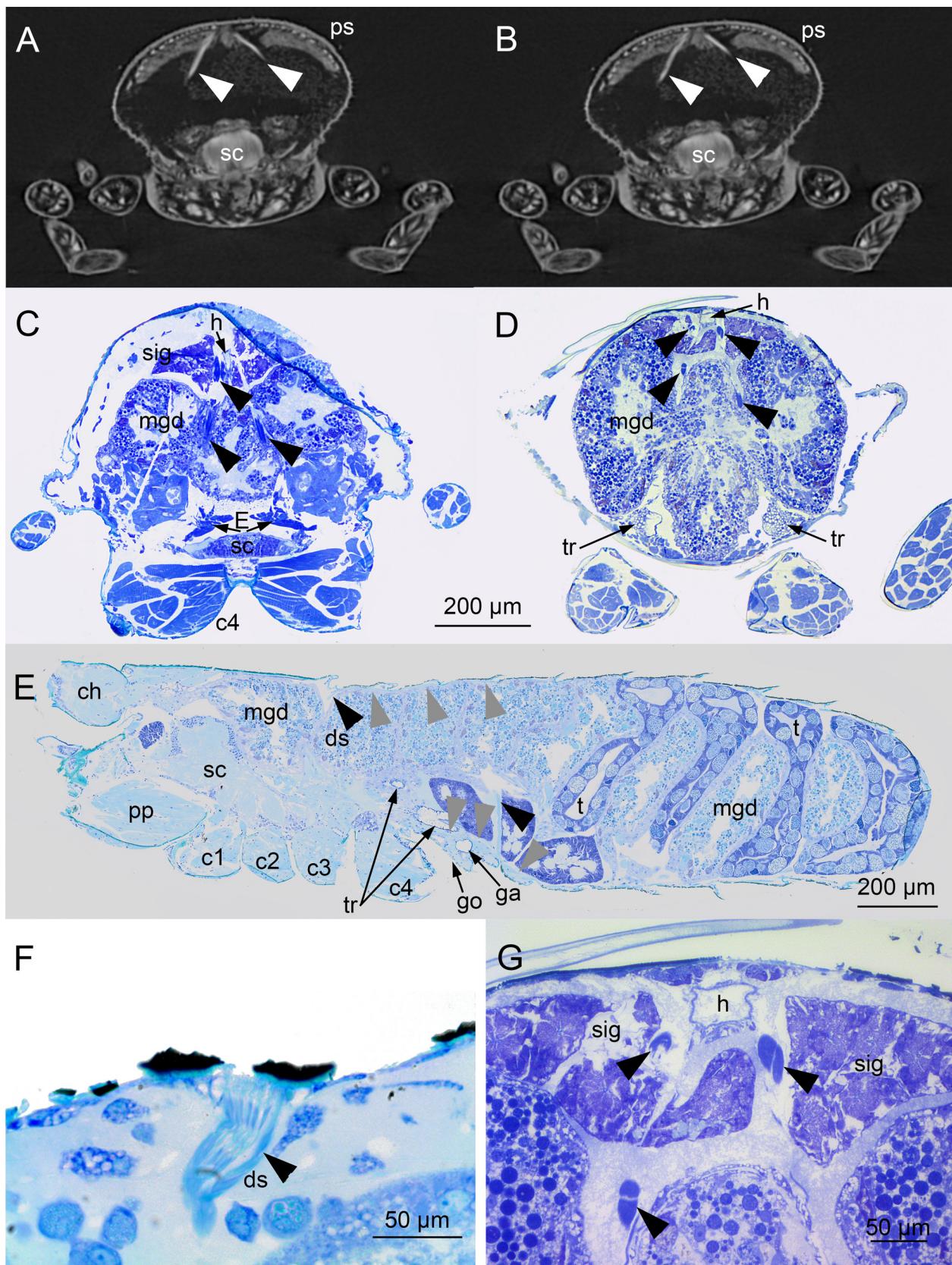


Fig. 6. Details of muscle attachment and course of musculature. (A, B) *Chernes hahnii*; two consecutive µCT images through the posterior region of the prosoma documenting the attachment of the dorsal suspensor muscle to the prosomal shield. (C) *Neobisium carcinoides*, histological cross section through the posterior region of the prosoma. The black arrowheads indicate the course of the dorsal suspensor muscle. The origin of the dorsal suspensor muscles from the endosternite is documented on this slide. Like in all other sections, individual sections document only parts of the course of the muscles and it requires the reconstruction of the complete topography from serial sections. See Fig. 8 for a complete graphical reconstruction of the muscle topography. (D) *Neobisium carcinoides*, histological cross section through the forth opisthosoma segment. The black arrowheads indicate the course of the dorsal-ventral muscles. (E) *Neobisium carcinoides*,

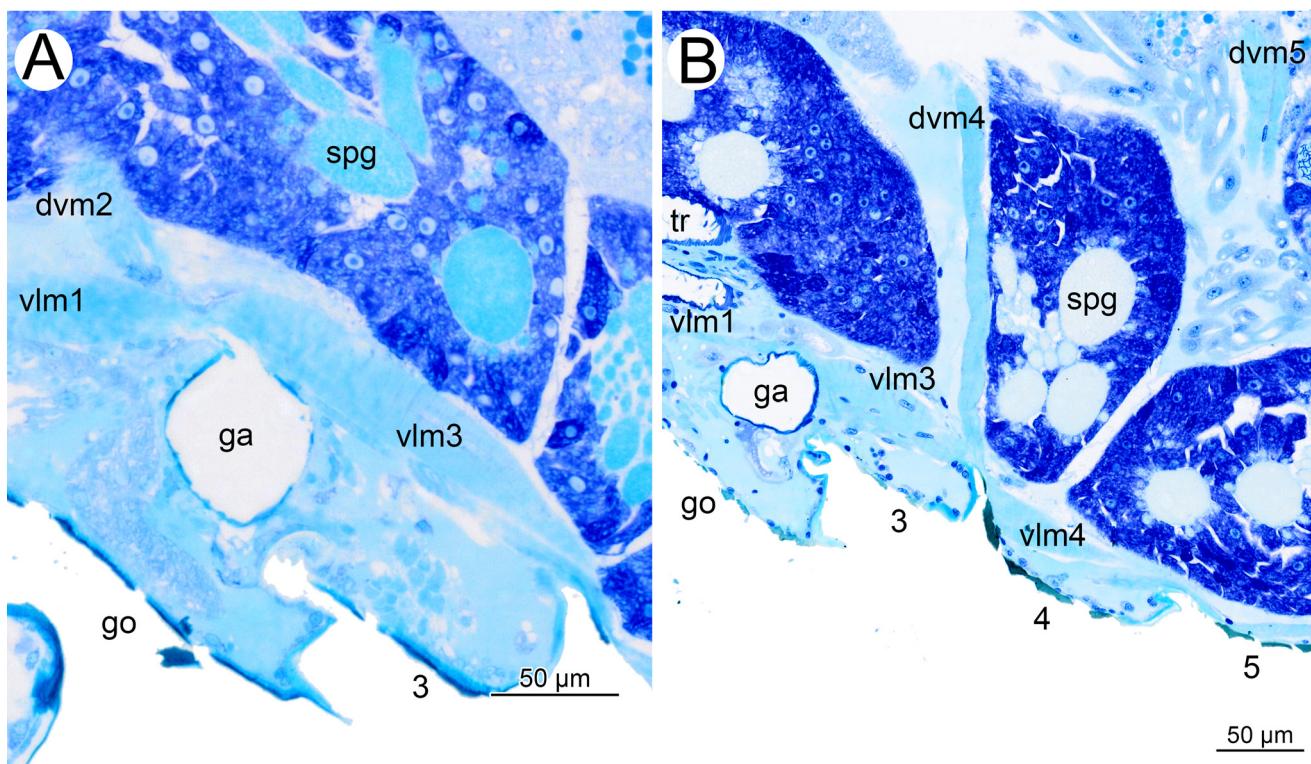


Fig. 7. *Neobisium carcinoides*, light microscopic micrographs documenting the attachment of the ventral longitudinal muscles to the genital atrium. (A) The micrograph documents a section where the first and the third ventral longitudinal muscles attach to the roof of the genital atrium, and the dorso-ventral muscle of the second sclerite reaches to the roof of the genital atrium. (B) the micrograph is from a neighboring section to a but now documenting the direct attachment of the ventral longitudinal muscles to the roof of the genital atrium. The image also documents the dorso-ventral muscle 4with its attachment to the 4th ventral sclerite. Abbreviations: dvm, dorso-ventral muscle; ga, genital atrium, go, genital operculum; spg, spermatophore gland; vlm ventral intersegmental, longitudinal muscle; tr, trachea; numbers indicate position of sclerites.

umented by μCT-imaging accessible through <http://morphobank.org/permalink/?P2422>).

In the pseudoscorpions, we did not find dorso-ventral muscles associated with the first dorsal opisthosomal sclerite and the genital operculum. However, intersegmental muscles are present, dorsally spanning between prosoma and the first dorsal sclerite, the first and the second dorsal sclerite, and ventrally spanning between the genital operculum and the roof of the sclerotized genital atrium. Our results concur with the documentations by Vachon (1949) and Weygoldt (1964, 1969). Both authors do not show any dorso-ventral musculature in association with the first dorsal and ventral opisthosomal sclerite (i.e., genital operculum), but also document the dorsal and ventral longitudinal muscles with the topography described here.

4.4. Second opisthosoma segment

The second dorsal opisthosomal sclerite is origin of dorso-ventral musculature. The paired muscle strands insert on the roof of the sclerotized genital atrium, a condition also documented by Vachon (1938, 1949), Gruner et al. (1993) and Weygoldt (1964, 1969). We also find dorsal and ventral pairs of longitudinal, intersegmental muscles. Dorsally, they span between the second and the third dorsal sclerite; ventrally they span between the roof of the

sclerotized genital atrium and the third ventral sclerite. We suggest that this represents a full set of ground pattern muscles; if this interpretation is correct, the second ventral sclerite is internalized and forms the genital atrium. This is supported by the internal cuticula cover of the genital atrium suggesting that epidermal material has been internalized to form the atrium.

4.5. Third and following opisthosoma segments

The third opisthosomal segment has, no dorso-ventral musculature, but is clearly marked by the dorsal and ventral intersegmental musculature. Its dorsal sclerite is partially involved in forming the posterior margin of the genital opening. From the 4th to the 11th opisthosomal segment the dorso-ventral and the intersegmental musculature is well developed and equals the pattern of the ground pattern.

4.6. Interpretations

The pattern of musculature observed in the two species of pseudoscorpions is simplified and reduced as compared to the ground pattern. Therefore, interpretations are not straightforward and some of the original hypotheses require additional assumptions to become compatible with the results. The lack of dorso-ventral

parasagittal histological section, documenting the dorsal and ventral, intersegmental longitudinal muscle system. The insertion of the dorsal suspensor muscle is indicated by the anterior black arrowhead. (F) *Neobisium carcinoides*, high power magnification of a histological parasagittal section documenting the insertion of the dorsal suspensor muscle to the posterior margin of the prosoma. This micrograph is from a consecutive section to (E). (G) *Neobisium carcinoides*, high power magnification of a histological cross section through the posterior region of the prosoma, documenting several sections through the dorsal suspensor muscle. Abbreviations: c1-c4, coxae of walking legs 1–4; ch, chelicera; ds, dorsal suspensor muscle; E, endosternite; ga, genital atrium; go, genital operculum; h, heart; mgd, mid gut diverticulum; ps, prosoma shield; sc, syncerebrum; sig, silk gland; t, testis; tr, trachea; black arrowheads, dorso-ventral muscles; grey arrowheads, dorsal and ventral longitudinal, intersegmental muscles.

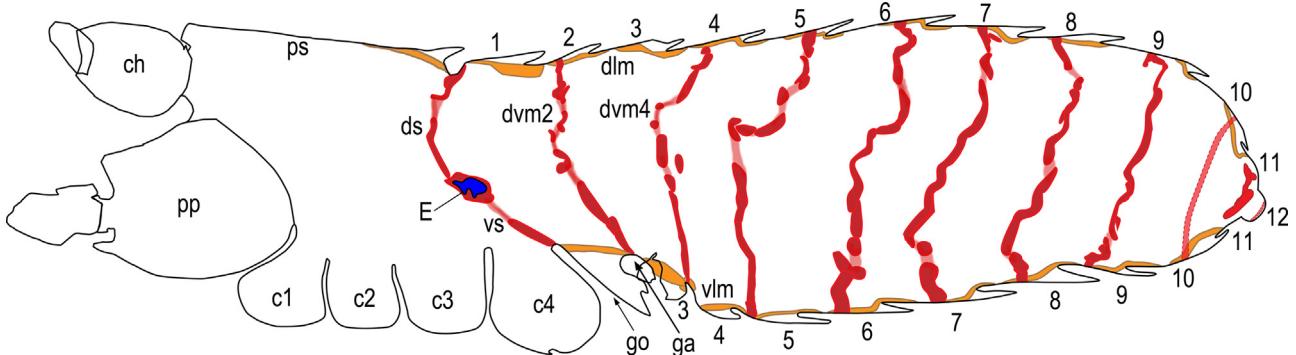


Fig. 8. Graphical reconstruction of the dorso-ventral and intersegmental musculature of *Neobisium canceroides* from serial sections. Dorso-ventral musculature is shown in red, intersegmental musculature in orange; the endosternite is blue. Parts of the dorso-ventral musculature that were missing from serial slides because of missing slides or damaged parts of a section were reconstructed in light red. Abbreviations: ch, chelicerae; c1–c4, coxae of walking legs 1–4; dlm, dorsal longitudinal, intersegmental muscles; ds, dorsal suspensor muscle; E, endosternite; ga, genital atrium; go, genital operculum; pp, pedipalp; ps, prosomal shield; vlm, ventral longitudinal, intersegmental muscles; vs, ventral suspensor muscle; numbers on opisthosoma indicate dorsal and ventral sclerites, respectively.

musculature associated with the first and the third dorsal sclerite of the opisthosoma might be unrelated to the unequal number of dorsal and ventral sclerites, because we observe a similar topography in other chelicerate taxa with the complete set of opisthosomal segments.

Hypothesis 1. “loss of the first sternite”: we observed the predicted loss of the dorso-ventral muscles in the first opisthosoma segment, but find musculature that we interpreted as the ventral longitudinal muscle spanning between the posterior margin of the prosoma and the first opisthosomal sternite. Also, the dorso-ventral muscle of the second opisthosomal segment does not reach from the second dorsal sclerite to the first ventral sclerite, i.e., the genital operculum, but to the roof of the genital atrium. The observation of longitudinal ventral muscles and the insertion of the dorso-ventral musculature of the second dorsal opisthosomal sclerite on the genital atrium reject hypothesis #1.

Hypothesis 2. “first sternite merged to the endosternite” predicts that the dorso-ventral musculature of the first opisthosomal segment spans between the first dorsal sclerite and the posterior margin of the endosternite. The (probably unrelated) loss of the dorso-ventral musculature associated with the first dorsal opisthosomal sclerite makes it impossible using this predicted topography in favor or against this hypothesis. The hypothesis also predicts that the ventral longitudinal muscle originally spanning between the first and second ventral opisthosomal sclerites would originate from the posterior margin of the endosternite and insert on the next following opisthosomal sternite (genital operculum). If we interpret the observed muscle as part of the ventral longitudinal system, we would, indeed, find partial support for this hypothesis; however, we would have to assume that the ventral suspensor muscles were reduced (or overlooked) and that the insertion had switched from intersegmental to the posterior margin of the prosoma. This hypothesis, would also require assuming that the ventral suspensor muscles were lost, and that the longitudinal muscles spanning between the posterior margin of the prosoma and the first ventral opisthosomal sclerite were also lost.

Hypothesis 3. “genital operculum formed by other sclerotization than sternite” predicts that dorso-ventral muscles and muscles of the ventral longitudinal system were missing because sternites s. str. are missing. This hypothesis cannot be excluded because dorso-ventral muscles are missing in the first segment, but one would need to assume that the ventral longitudinal muscle system had at least partially changed its origin and insertion points. Also, we would need to explain where and why the new sclerites were derived from (both difficult) and assume that the original sternites

were lost or moved anteriorly. However, with few species having a sternal tubercle or others having a “soft sternum”, the pattern in pseudoscorpions is ambiguous and requires more comparative morphological studies.

Hypothesis 4. “second opisthosomal sternite reduced”, predicts the loss of the dorso-ventral and ventral intersegmental musculature associated with the second opisthosomal segment; thus it can pretty safely be rejected. Hypotheses 1–4 would require the assumption that the sclerotized genital atrium were formed from the intersegmental fold between sclerites 2 and 3, and that the dorso-ventral musculature of the second dorsal sclerite had changed its insertion to the roof of the genital atrium.

Hypothesis 5. “Internalization of the second sternite” predicts that all sclerotized elements and associated muscles were maintained, but the sternite of the second opisthosomal segment internalized as genital atrium. While the dorso-ventral musculature of the first and third dorsal opisthosomal sclerite is reduced, all other muscles are present, at least in a simple and straightforward interpretation without additional assumptions of changed muscle origin and insertion. However, this hypothesis requires the assumption that the loss of dorso-ventral musculature in segment 1 occurred unrelated (or with unrecognized function) to the internalization of the second sternite. The dorso-ventral muscle attached to the genital atrium and the segmental ventral longitudinal muscles between the segments provide some support to this idea. However, because the pattern of segmental musculature is reduced and obviously rearranged in response to other constructural needs not considered in our original hypotheses, the interpretations of the pattern remain necessarily somewhat arbitrary.

4.7. Conclusions

We used μ CT-imaging and standard histology to analyze the body tagmatization of pseudoscorpions. Both methods complement each other and together provide explicit data that can be used for reconstructing the muscle topography and derive a new interpretation of the body tagmatization.

Axial muscle topography in pseudoscorpions is simplified as compared to the arachnid ground plan derived from the box-truss model. Anterior and posterior oblique muscles are completely lacking. In our interpretation, dorsal and ventral muscles originating from the endosternite are dorsal and ventral suspensor muscles, respectively, and mark the posterior border of the prosoma. Dorso-ventral muscles are missing in the first and third opisthosomal segment, however, the associated dorsal and ventral longitudinal muscle systems are present in these two segments. The results

do not unequivocally support one hypothesis. For all hypotheses we need additional assumptions, but weighted by the number of additional assumptions Hypothesis 5 “internalization of the second opisthosomal sclerite” provides the most simple, i.e., parsimonious explanation. – Most parsimonious does not necessarily mean that evolution was not more complex and that the observed morphology did not evolve on different pathways, but, preference to the explanation with the least number of necessary assumptions is based on a reproducible concept and renders interpretations that can be challenged when additional evidence becomes available.

Author contributions

LM prepared all histological slides, made light microscopic micrographs, and drew the schematic reconstruction of the musculature. MH prepared samples for µCT and conducted µCT-imaging. YD prepared the 3D-reconstruction of the musculature. JMS initiated the study, supervised the project, and wrote the first draft of the manuscript and provided a critical revision of the manuscript. All authors contributed to discussions of the manuscript.

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