Comparative morphology of the hemolymph vascular system in mygalomorphs (Araneae: Opisthothelae)

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Abstract. Mygalomorphs are a well-known spider group; however, astonishingly little is known about their internal anatomy. As part of a comparative survey on the circulatory system in spiders, we conducted an examination of the hemolymph vascular system (HVS) and parts of the hemolymph lacunar system (HLS) in five mygalomorph spider species. Circulatory system features were investigated using micro-computer-tomography in combination with resin injection methods and serial sectioning. Data were visualized using a 3D-reconstruction software. The HVS consists of a tubular heart, which is situated along the dorsal midline of the opisthosoma. Anteriorly, the heart gives rise to the anterior aorta. A posterior aorta system was not found. Three pairs of cardiac arteries originate laterally from the heart and the branching pattern of these arteries is visualized and described here for the first time. The anterior aorta runs through the pedicel into the prosoma where it branches to supply the muscles and particularly the central nervous system. The data on mygalomorphs are discussed in comparison to the HVS in other Araneae.

Keywords: Heart, arteries, hemolymph vascular system, anatomy, micro-computer-tomography, 3D-reconstruction

Mygalomorphs are probably one of the most investigated spider taxa and the subject of many studies on taxonomy, biogeography, toxicology, behavior or external morphology. Because of their large body size, the mygalomorph circulatory system has received considerable attention from a physiological point of view (Sherman & Pax 1970; Stewart & Martin 1974; Schartau & Leidescher 1983; Anderson & Prestwich 1985; Tiling 1986; Paul et al. 1994). Paul and his group described the circulatory system of 'Eurypelma californicum' Ausserer, 1871 (probably Aphonopelma hentzi (Girard, 1852), see Nentwig 2012) from a physiological and structural perspective; however, the latter was only superficially dealt with (Tiling 1986; Bihlmayer et al. 1989; Paul 1986, 1990; Paul & Bihlmayer 1995; Paul et al. 1989, 1994). In fact, the morphology of the circulatory system in *A. hentzi* has only been described in two unpublished diploma theses (Zahler 1990; Bihlmayer 1991).

Only two comprehensive studies dealt with the morphology of the circulatory system in mygalomorphs (Blanchard 1852; Causard 1896) and a few less detailed investigations, most of which date back almost hundred years (Petrunkevitch 1933; Gerhardt & Kästner 1938). A comprehensive comparative analysis of the circulatory system in the Mygalomorphae is lacking.

Mygalomorphs are also highly interesting from a phylogenetic perspective. Comprising 20 families of over 2900 described species (World Spider Catalog 2018), they form the monophyletic sister group (Platnick & Gertsch 1976; Raven 1985; Coddington et al. 2004) to all remaining araneomorph spiders, the Araneomorphae, which comprise over 90% percent of the spider diversity.

Therefore, as part of a comprehensive survey on the circulatory system in spiders (Huckstorf et al. 2013, 2015; Wirkner & Huckstorf 2013), we here investigate the circulatory system in five mygalomorph species belonging to the Dipluridae and Theraphosidae.

Like all other Arthropoda, spiders possess a circulatory system characterized by an open hemolymph vascular system (HVS), comprising a heart and emanating arteries (Wirkner & Huckstorf 2013). The lacunar part of this circulatory system is a complex system of sinuses and lacunae (hemolymph lacunar system), that is not directly (structurally) connected with the hemolymph vascular system (see Wirkner et al. 2013 for a review).

METHODS

Studied animals.—The spiders used in this study include Theraphosidae (*Nhandu chromatus* Schmidt, 2004; *Sericopelma angustum* (Valerio, 1980); *Lasiodora parahybana* Mello-Leitão, 1917; *Chromatopelma cyaneopubescens* (Strand, 1907) and Dipluridae (*Linothele fallax* Mello-Leitão, 1926). Additional information is listed in Table 1. All specimens were bought in Germany (www.thepetfactory.de). All species names used here follow the World Spider Catalog (2018).

Injection of resin.—In order to prepare casts of the circulatory system, we followed the procedures of Wirkner & Richter (2004). Spiders were anesthetized with a lethal dose of chloroform, and the casting resin Mercox CL-2R/2B (Vilene Comp., Tokyo, Japan) or the polyurethane Pu4ii (vasQtec, Univ. of Zurich, Switzerland) was injected into the heart. Tetric EvoFlow® (by Ivoclar Vivadent), a flow-composite with a high x-ray opacity, was added to Pu4ii to further enhance the contrast in the micro-computer-tomography (MicroCT). Immediately prior to injection, the resins were mixed with a hardener (Pu4ii) or a catalyst (Mercox). The injection setup could be finely adjusted using a mechanical micromanipulator. After injection, the specimens were left for several minutes to allow the resins to polymerize. Preparations for MicroCT were fixed in Bouin's fluid/ Duboscq-Brasil and then critical point dried (BAL-TEC CPD 030). Specimens for corrosion casting were macerated for 1–2 days through repeated baths in 10% potassium hydroxide at room temperature.

Micro-computer-tomography (MicroCT).—X-ray scans were performed using high-resolution MicroCT systems in high resolution mode: a phoenix nanotom (Baker Hughes, General Electric Company) (target: molybdenum and tungsten, mode: 0–1; performance: ca. 3–6 W; number of images: 1440; detector-timing: 1000–1500 ms; voxel size: ca. 4–8 μm) and a

Table 1.—Spiders used in this study.

Family	Species	# of specimens investigated	Scan (not injected)	Injected Scan	Injected Corrosion cast
Theraphosidae	*Nhandu chromatus*	4	+	+	+
	Sericopelma angustum	3	−	−	+
	Lasiodora parahybana	4	−	+	+
	Chromatopelma cyaneopubescens	1	+	−	−
Dipluridae	*Linothele fallax*	3	−	+	−

Zeiss XRadia 410 Versa (performance: 6–8W; number of images: 901–2101).

3D reconstruction.—To create 3D reconstructions, image stacks were produced using MicroCT. The software Imaris 6.4 and 7.0 by Bitplane was used for all 3D reconstructions. A scene was created in the program module 'Surpass'. To visualize whole data sets, the volume rendering function was selected. The contours of the studied organs were marked with a polygon on every optical cross section.

Image management.—All figure plates were arranged using Corel Graphics Suite X3. Images were embedded into CorelDRAW X3 files and edited in Corel PhotoPaint X3 after adjusting contrast and brightness.

RESULTS

Our description of the circulatory system focuses on the hemolymph vascular system (HVS), however, some aspects of the hemolymph lacunar system is also dealt with. We describe the HVS of five mygalomorph species. The morphological descriptions apply to all species we studied. Any differences in vascular morphology are described separately.

All species of Mygalomorphae possess two pairs of book lungs in the second and third opisthosomal segment. The central nerve mass in the prosoma is referred to as the prosomal ganglion (see e.g., Klußmann-Fricke et al. 2012). The terminology used in the descriptions of the HVS features is from "Ontology of Arthropod Circulatory Systems – OarCS" (online at http://oarcs.speciesfilegroup.org, Wirkner et al. 2017) and all features are described as morphemes (*sensu* Richter & Wirkner 2014), i.e., not implying homology and/or function.

Opisthosoma.—*Heart:* The shape of the hearts in the studied mygalomorph species resembles that of a convex spindle (Figs. 1 & 2) lying dorso-medially in the opisthosoma. The anterior part of the heart is broader than the posterior part (Figs. 1A, C & 2C). The heart is surrounded by a pericardial sinus into which two pairs of pulmo-pericardial sinuses merge (see below, Fig. 1B). The heart possesses four pairs of ostia and three paired cardiac artery systems (Figs. 1A & 2C). The pairs of ostia lie dorso-laterally in the heart wall. The first pair of ostia is not associated with a pair of cardiac artery systems, but the first pair of pulmo-pericardial sinuses merges into the pericardial sinus next to it (Figs. 1A, B). The second pair of ostia is the biggest (Fig. 1A). This pair is associated with the first pair of cardiac artery systems and the second pair of pulmo-pericardial sinuses merges into the pericardial sinus next to this pair of ostia. The third pair of ostia is associated with the second pair of cardiac artery systems (Fig. 2C). The

first and the third pair of ostia are of almost the same size (Fig. 1A). The fourth pair of ostia is the smallest one and is associated with the third pair of cardiac artery systems (Figs. 1A, 2C). In all mygalomorph taxa studied, the heart extends anteriorly into an anterior aorta running through the pedicel into the prosoma (aa, see below, Fig. 5). The heart ends blindly in a thin posterior tip (Figs. 2B,C), i.e., no posterior aorta system was observed in any of the investigated specimens (Figs. 2B,C).

Ventral, lateral and dorsal ligaments suspend the heart in the opisthosoma. Three pairs of ventral ligaments emanate from the ventral part of the heart between the origins of the three pairs of cardiac artery systems and run in a ventral direction to the ventral longitudinal muscles. The first pair of ventral ligaments emanates ventrally between the origins of the first pair of cardiac artery systems. The second pair of ventral ligaments emanates ventrally between the origins of the second pair of cardiac artery systems and runs postero-ventrally to the ventro-longitudinal muscles. The third pair of ventral ligaments emanates ventrally between the origins of the third pair of cardiac artery systems. The second and third pairs of ventral ligaments are associated with the anterior and posterior pair of dorso-ventral muscles and are attached next to each other on the ventral longitudinal musculature. Furthermore, the heart is suspended via five pairs of lateral ligaments. The first four pairs are associated with the four pairs of ostia. The posterior tip of the heart is suspended by the fifth pair of lateral ligaments to the postero-lateral cuticle. Additionally, many dorsal ligaments extend from the dorsal heart wall to the dorsal cuticle.

Cardiac artery systems: All studied mygalomorph specimens possess three pairs of cardiac artery systems supplying the opisthosoma. These artery systems emanate symmetrically left and right from the ventro-lateral wall of the heart and display a high degree of branching throughout the opisthosoma.

First cardiac artery system: The first cardiac artery system runs between the first pulmo-pericardial sinuses, supplying the anterior part of the gut and the midgut glands (Figs. 2A,C). Either both first cardiac artery systems are equally developed (2 specimens of *L. fallax*) or one artery displays a higher degree of branching and sends an artery in antero-ventral direction to the pedicel (right: 3 specimens of *L. parahybana*, 1 specimen of *L. fallax*). In one specimen of *N. chromatus*, both first cardiac artery systems were equally developed and sent an artery into the pedicel region.

Second cardiac artery system: The second cardiac artery system runs into the median region between both second pulmo-pericardial sinuses supplying the anterior part of the spinning glands and the median part of the gut and midgut
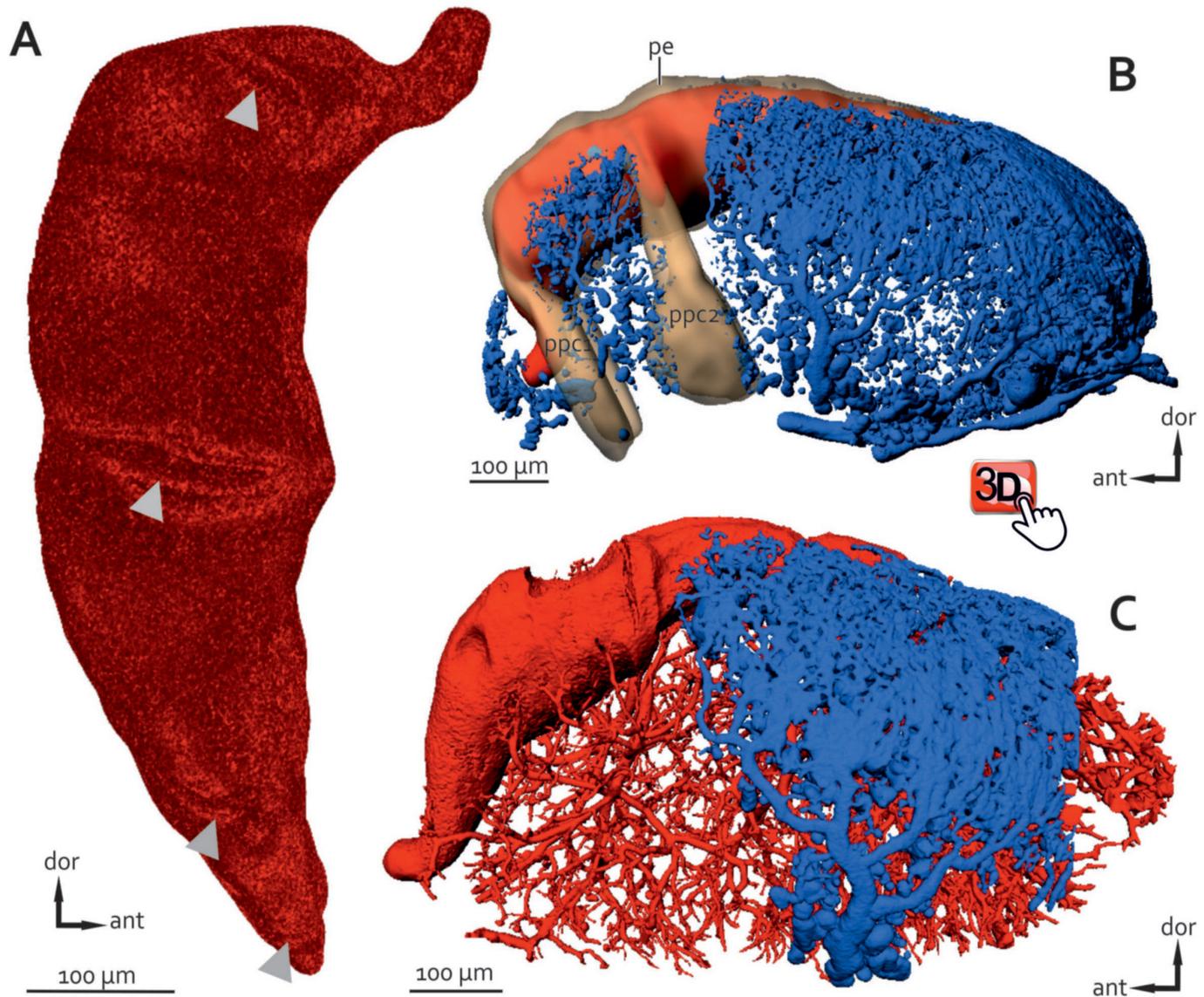
Figure 1.—Vascular and hemolymph lacunar system in the opisthosoma of *Nhandu chromatus*. PDF version contains interactive 3D content. To activate, click on the figure in Adobe Reader. **A:** Volume rendering of the heart; gray arrows mark the position of the four paired ostia; lateral view. **B:** Surface rendering showing the heart (red), parts of the lacunar hemolymph system (blue) and the pericardial sinus with the two pairs of pulmo-pericardial sinuses (brownish transparent); composed surface renderings from two specimens, lateral view. **C:** Surface rendering showing the hemolymph vascular system (heart and the three paired cardiac arteries; red) and parts of the lacunar hemolymph system, lateral view. pe, pericardial sinus; ppc1, anterior pulmo-pericardial sinus; ppc2, posterior pulmo-pericardial sinus.

glands (Figs. 2A,C). Similar to the first cardiac artery system, either both second cardiac artery systems are equally developed (1 specimen of *N. chromatus*, 1 specimen of *L. fallax*, 4 specimens of *L. parahybana*) or one artery branches more and sends an artery in an antero-ventral direction into the anterior-most region of the opisthosoma (left: 1 specimen of *N. chromatus*, 1 specimen of *L. fallax*; right: 1 specimen of *L. fallax*). In conclusion, if the first cardiac artery systems send an artery to the pedicel, the second cardiac artery systems are equally developed and vice versa.

Third cardiac artery system: The third cardiac artery system supplies the posterior opisthosoma, i.e., spinnerets, spinning glands, the posterior part of the gut including the stercoral pocket and posterior part of the midgut glands. This cardiac

artery system is paired at its origin (ol, or, Fig. 2B), with both stems emanating symmetrically from the ventro-lateral heart wall at the level of the fourth pair of ostia. Then the stems fuse into one thick main artery (3sa, Fig. 2B) which runs in a ventro-posterior direction. More posteriorly, this artery splits again into two arteries (3sar, 3sal, Fig. 3B). The further branching of this cardiac artery system exhibits intra- and interspecific variation.

In *L. fallax*, *S. angustum* and three specimens of *L. parahybana*, one artery emanates from the origins of the third cardiac artery system and runs ventro-laterally (ar, al in sketch 2, Fig. 3B). In one specimen of *N. chromatus*, one artery (al) emanates from the left origin and another artery (ar) emanates from the main artery of this cardiac artery system (3sa in
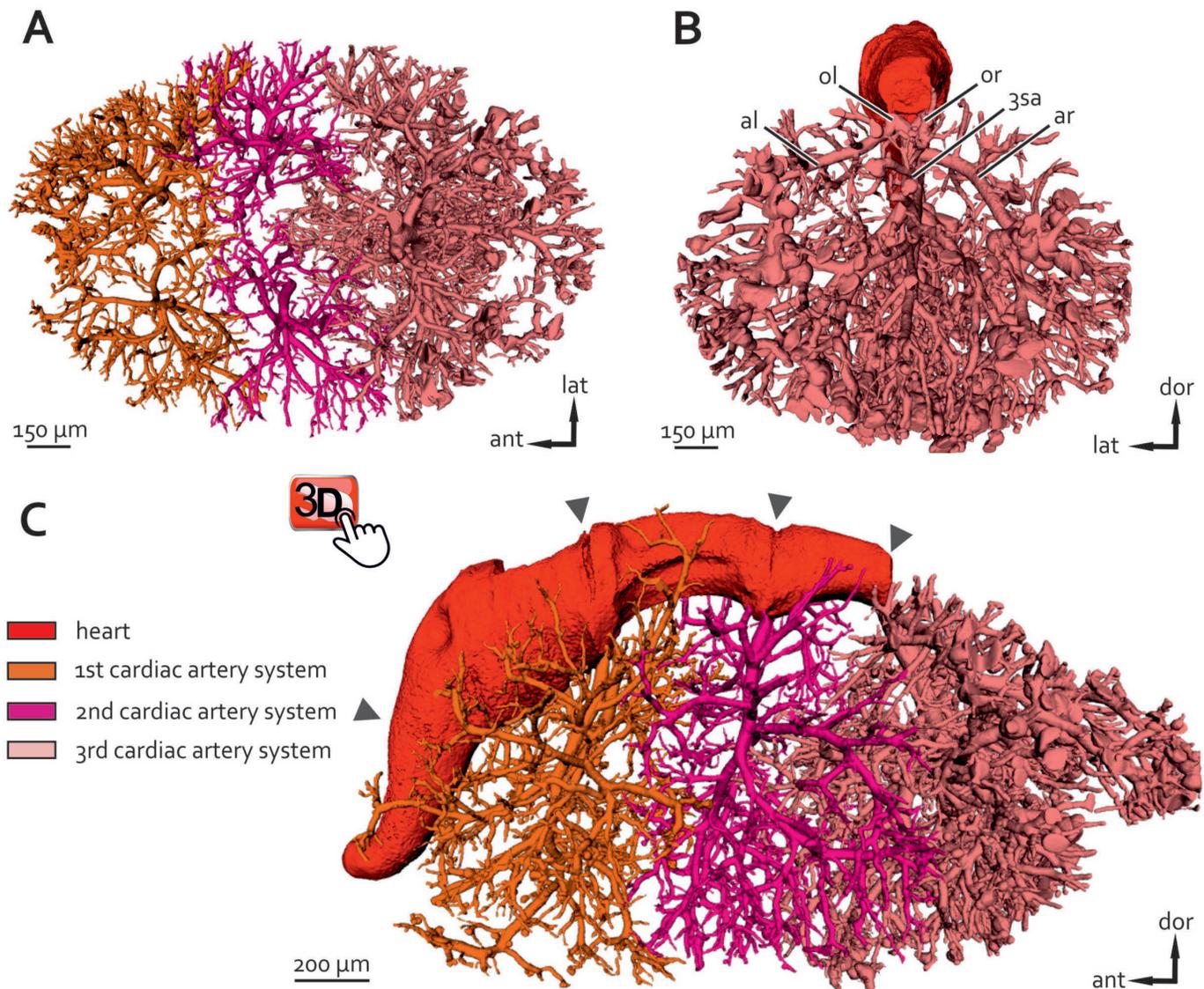
Figure 2.—Surface renderings of the hemolymph vascular system in the opisthosoma of *Nhandu chromatus*. PDF version contains interactive 3D content. To activate, click on the figure in Adobe Reader. **A:** Surface rendering showing the three paired cardiac artery systems, dorsal view. **B:** Surface rendering showing the third cardiac artery system and the heart, posterior view. **C:** Surface rendering showing the heart and the three paired cardiac artery systems; lateral view, dark grey arrows mark the position of the ostia. 3sa, main trunk of the third cardiac artery system; al, left artery emanating from the main trunk of the third cardiac artery system; ar, right artery emanating from the main trunk of the third cardiac artery system; ol, left origin of the third cardiac artery system; or, right origin of the third cardiac artery system.

sketch 1, Fig. 3B). Both arteries run ventro-laterally. In another specimen of *N. chromatus* both arteries (ar and al) emanate symmetrically from the main artery and also run ventro-laterally. In one specimen of *L. parahybana*, one artery emanates from the left origin of the third cardiac artery (al) and from the right origin, an artery emanates which runs medially into a ventro-posterior direction (ma in sketch 3, Fig. 3B). In three other specimens of *L. parahybana*, and all other studied species, artery ma emanates from the posterior side of the main artery and runs also medially above the stercoral pocket in a ventro-posterior direction (Sketches 1, 2 & 4 in Fig. 3B). Along the main artery 3sa, the origin of the artery ma can also differ.

The branching patterns of ma differ in all studied specimens. There are three to four arteries emanating from the trunk supplying the postero-medial part of the opisthosoma dorsally, laterally and posteriorly to the stercoral pocket.

Dorsally to the hindgut, the main artery divides into two nearly equally sized arteries which run initially in a ventro-lateral direction (3sar, 3sal). They supply the spinnerets, spinning glands, the hindgut (including stercoral pocket), branches of the midgut glands and the posterior part of the ventro-longitudinal musculature.

Hemolymph lacunar system in the opisthosoma: Hemolymph spaces surrounded by a membrane are called sinuses. Hemolymph spaces without a membrane are called lacunae (see discussion). Hemolymph spaces in which a membrane
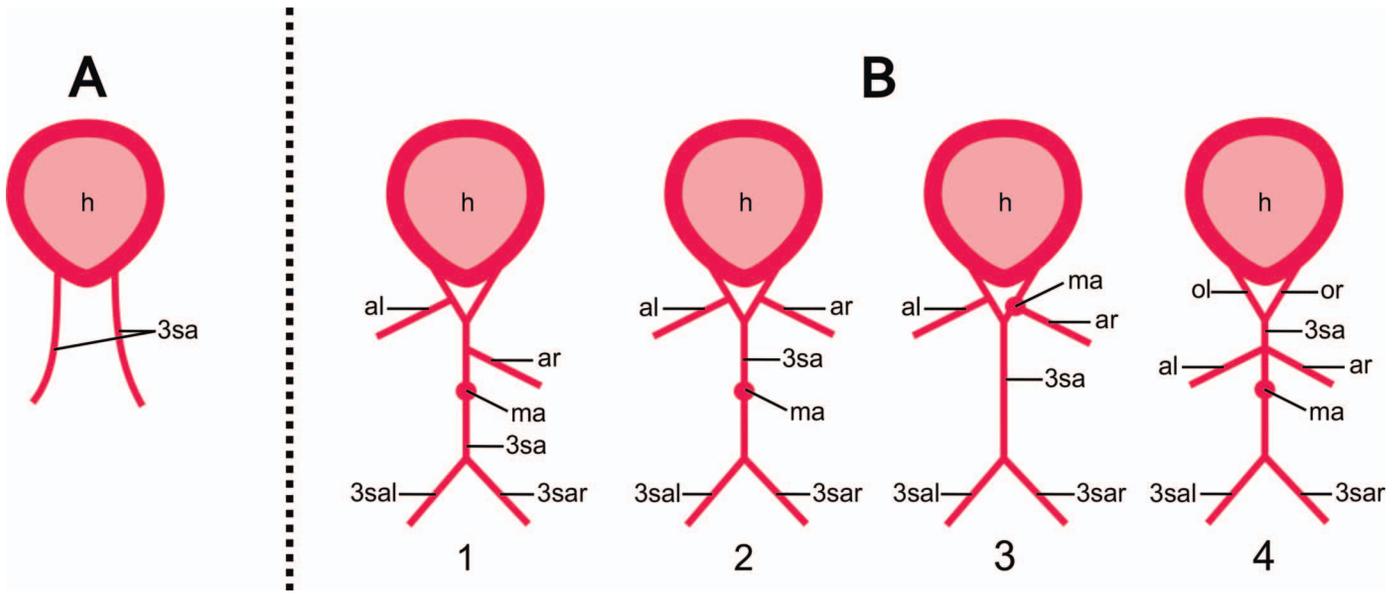
Figure 3.—Schematic drawing of the variability of the third cardiac artery system. Section level at the third cardiac artery system. **A:** Araneomorphae; **B:** 1–4: Showing the four conditions found in Mygalomorphae; 1: 1x *Nhandu chromatus*, 2: 3x *Linothele fallax*, 2x *Lasiodora parahybana*, 2x *Sericopelma angustum*; 3: 1x *L. parahybana*; 4: 1x *N. chromatus*. 3sa, main artery of the third cardiac artery system; al, left artery emanating from the left origin or from the main artery of the third cardiac artery system; ar, right artery emanating from the main artery, from the right origin or from the median running artery of the third cardiac artery system; h, heart; ma, median running artery, shown in cross section; ol, left origin of the third cardiac artery system; or, right origin of the third cardiac artery system; 3sal, left artery of the resplitting of the main artery; 3sar, right artery of the resplitting of the main artery.

could not be identified are here simply referred to as hemolymph spaces. The most voluminous sinus is the pericardial sinus, surrounding the heart entirely (pe, Fig. 1B). Next to the first pair of ostia, the first pair of pulmo-pericardial sinuses merges with the pericardial sinus (ppc1, Fig. 1B). It runs underneath the cuticle from the first pair of book lungs to the pericardial sinus. The second pair of pulmo-pericardial sinuses runs beneath the cuticle from the second pair of book lungs to the pericardial sinus and merges with the pericardial sinus next to the second pair of ostia (ppc2, Fig. 1B). The book lungs are mostly enclosed by the ventral parts of the pulmo-pericardial sinuses.

The ventral longitudinal muscles are situated in two ventral longitudinal hemolymph spaces. The two pairs of dorso-ventral muscles are surrounded by hemolymph spaces too, which insert into the ventral longitudinal hemolymph spaces.

In *N. chromatus,* we observed a highly ramified hemolymph space system near the cuticle, running to the ventral part of the opisthosoma (Fig. 1B). In the dorsal half of the opisthosoma, this system of hemolymph spaces forms a dense network. In the ventral part of the opisthosoma, the network merges into more distinct tube-like hemolymph spaces. All of these tube-like hemolymph spaces merge into even bigger tube-like hemolymph spaces: each body half has one big and at least three smaller tube-like hemolymph spaces, which merge into the ventral longitudinal hemolymph spaces. The biggest tube-like hemolymph space lies in the ventro-median part of the opisthosoma (Fig. 1C). The ramified hemolymph space system displays a similar degree of branching as some arterial systems. However, in the region of the pulmo-pericardial sinuses, this kind of hemolymph space system is absent (Fig. 1B).

Prosoma.—The anterior aorta emanates from the anterior heart and runs through the pedicel into the prosoma (aa, Fig. 5). It bifurcates behind the sucking stomach (Fig. 5). These two branches flank the stomach laterally and then bend postero-ventrally (boa, Figs. 4C,D, & 5). Before this bend, the paired cheliceral artery systems branch off (cha, Figs. 4C,D, & 5; see below). Further along the branches of the anterior aorta, four pairs of leg arteries emanate consecutively, entering the four pairs of legs (l1-l4, Fig. 5). The paired pedipalpal artery (pa, Fig. 5) emanates proximally from the first pair of leg arteries. Median transganglionic arteries (mtga, Figs. 4 & 5) and the supraneural artery (sa, Fig. 5) also exit from the branches of the anterior aorta (see "*Supply of the prosomal ganglion*", below).

Cheliceral artery systems: The paired cheliceral artery systems (cha) supply the anterior prosoma i.e., chelicerae, upper and lower lips (i.e., rostrum and labium), eyes, the dorsal part of the prosomal ganglion (adp, ldp, Fig. 5) and the musculature in the anterior part of the prosoma. The paired lip arteries emanate ventrally from the cheliceral arteries. Both arteries split into an anterior and a posterior branch. The anterior branches run into the upper lip while the posterior branches run into the lower lip supplying the musculature there. Some branches of the lower lip arteries run posteriorly under the prosomal ganglion. The unpaired optical artery emanates latero-dorsally from the right or left cheliceral artery, runs antero-dorsally to the region of the eyes and ramifies several times on its way, supplying the eyes and the musculature in the antero-dorsal part of the prosoma. An unpaired artery emanates proximally from each optical artery. It runs in a dorso-medial direction supplying the musculature in the dorso-median prosoma. The dorso-lateral musculature
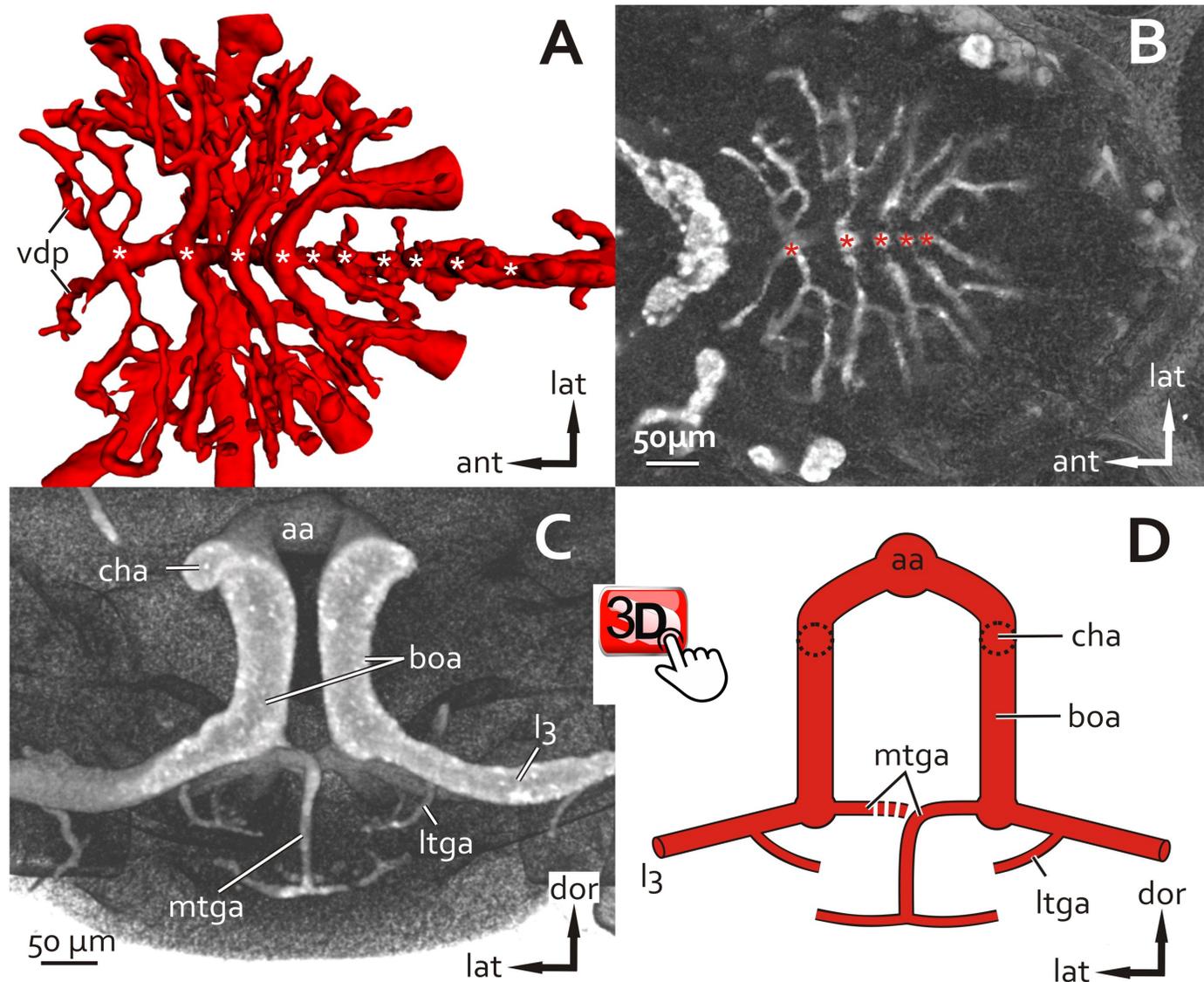
Figure 4.—Hemolymph supply of the prosomal ganglion in *L. fallax*. PDF version contains interactive 3D content. To activate, click on the figure in Adobe Reader. **A:** Surface rendering showing the ten unpaired median transganglionic arteries; ventral view. **B:** Virtual cross section showing the first five unpaired median transganglionic arteries; ventral view. **C:** Volume rendering of a part of the anterior aorta system visualizing the asymmetrically off branching median transganglionic arteries; anterior view. **D:** Schematic drawing summarizing aspects shown in C. Asterisks mark transganglionic arteries; Aa, anterior aorta; boa, branches of the anterior aorta; cha, cheliceral arteries; l3, third walking leg artery; ltga, lateral transganglionic arteries; mtgas, median transganglionic arteries; vdp, ventral supply of the dorsal part of the prosomal ganglion.

in the anterior part of the prosoma is supplied by several arteries, which branch off latero-dorsally from the cheliceral arteries. Within the chelicerae, the cheliceral arteries split into two arteries, an outer and an inner one. The outer one is bigger and supplies the dorsal and outer part of the chelicerae. In the distal part of the chelicerae, this artery divides in two. One of these branches runs into the fangs and the other one runs in a dorsal direction. The inner cheliceral artery splits and supplies the inner dorsal and ventral part of the chelicerae. In addition, two pairs of arteries, coming from the cheliceral arteries, supply the dorsal part of the prosomal ganglion (see "*Supply of the prosomal ganglion*", below).

Leg arteries: All four pairs of leg arteries emanate from the branches of the anterior aorta and run laterally into the legs (l1-l4, Fig. 5). They give rise to several small arteries supplying the leg musculature. The four pairs of lateral transganglionic arteries emanate proximally from the four pairs of leg arteries, supplying the ventral parts of the prosomal ganglion from lateral (ltga, Figs. 4 C,D, 5).

The paired pedipalpal artery emanate proximally from the first walking leg arteries and runs into the pedipalps (pa, Fig. 5). These arteries give rise to another branching artery which runs into the gnathocoxae.

Supply of the prosomal ganglion: The following descriptions only refer to two specimens of *L. fallax* and one specimen of
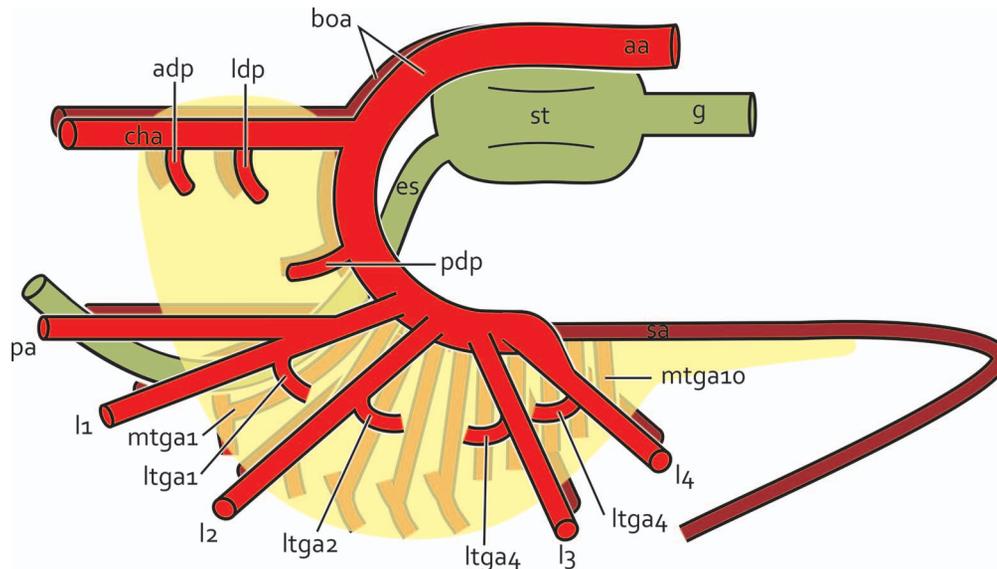
Figure 5.—Schematic drawing showing the anterior aorta system (red), the gut (green) and the prosomal ganglion (yellow transparent) in the prosoma; lateral view, anterior is left. Aa, anterior aorta; adp, paired arteries supplying the anterior and median region of the dorsal part of the prosomal ganglion; boa, branches of the anterior aorta; cha, cheliceral arteries; es, esophagus; g, gut; l1-l4, leg arteries 1-4; ldp, paired arteries supplying the lateral sides of the dorsal part of the prosomal ganglion; ltga1-4, lateral transganglionic arteries 1-4; mtga1+10, median transganglionic arteries 1+10; sa, supraneural artery; st, stomach.

N. chromatus. The ventral part of the prosomal ganglion is supplied medially by transganglionic arteries (mtga) and laterally by transganglionic arteries emanating from leg arteries (ltga). In two specimens of *L. fallax,* ten mtgas and four pairs of ltgas are present. The anterior five median transganglionic arteries emanate from the left or right branch of the anterior aorta and split terminally. In one specimen of *N. chromatus,* the first and the fourth mtga possess a bilateral origin, i.e., they emanate from both branches of the anterior aorta. The remaining five mtgas emanate from the supraneural artery. The supraneural artery emanates from the left or right branch of the anterior aorta and runs medially into a posterior direction above the ventral part of the prosomal ganglion and the opisthosomal nerve. In *L. fallax*, the anterior median transganglionic arteries split terminally into two arteries. Both these arteries run perpendicular to the mtgas into a lateral direction and split again several times, supplying the periphery of the ventral part of the prosomal ganglion.

The anterior five mtgas are more distinct and display more terminal branching than the posterior ones (Fig. 4A). The first mtga lies posteriorly next to the esophagus and splits into very short arteries flanking the esophagus laterally (mtga1, Fig. 5). These arteries split again several times. The most anterior branches of the first mtga run in a dorsal direction supplying the dorsal part of the prosomal ganglion from the ventral side (vdp, Fig. 4A). The branches of the first mtga form an anastomosis. The second mtga is the biggest, with the broadest lateral expansion (Fig. 4A,B). The arteries after the first split are longer than the original artery and they split several times. The branches of the second mtga also form an anastomosis. The third and the fourth mtga are almost of the same size and display a similar degree of branching (Fig. 4 A,B). The fifth mtga is the shortest of the anterior five mtgas. The sixth to tenth mtga are distinctly shorter than the anterior ones and display no significant or no terminal branching (Figs. 4A & 5).

In the studied specimens of *L. fallax*, *N. chromatus* and *L. parahybana*, the supraneural artery splits into two arteries. Both bend anteriorly. One of them runs to the posterior end of the prosoma and the other one splits into many arteries right after the bend. The four paired lateral transganglionic arteries emanate postero-proximally from the four leg arteries, run ventro-medially between the neuropils at half height of the ventral part of the prosomal ganglion and split terminally (ltga, Figs. 4C,D & 5).

The dorsal part of the prosomal ganglion is supplied by three pairs of arteries (Fig. 5). Two pairs emanate ventro-laterally from the cheliceral arteries and run dorsally into the prosomal ganglion (adp, ldp, Fig. 5). The posterior one supplies the lateral sides (ldp) and the anterior one supplies the anterior and median region of the dorsal part of the prosomal ganglion (adp). The third pair of arteries supplies the dorsal part of the prosomal ganglion from posterior emanating anteriorly from the branches of the anterior aorta above the esophagus (pdp, Fig. 5). Additionally, branches of the first mtga supply the dorsal part of the prosomal ganglion from the ventral side (vdp, see above, Fig. 4A).

DISCUSSION

Although in general the HVS in mygalomorph spiders shows a high level of correspondence to other studied spiders, for example to *Cupiennius salei* (Keyserling, 1877) (Huckstorf et al. 2013), basal araneomorphs (Huckstorf et al. 2015), and *Araneus diadematus* Clerck, 1757 (Runge & Wirkner 2016), some notable differences can be observed.

As a phylogenetic basis of the comparative morphological discussion below, we considered the most recent advances in spider phylogeny (Platnick & Gertsch 1976; Coddington 2005; Hedin & Bond 2006; Bond et al. 2012; Starrett et al. 2013; Wheeler et al. 2016; Fernandez et al. 2018). Although differing

in some relationships within basal araneomorph and entelegyne spiders, these hypotheses corroborate the classical view of Liphistiidae (= Mesothelae) as sister group to Opisthothelae, a taxon comprising Mygalomorphae and Araneomorphae.

Number of ostia.—All mygalomorph species studied possess four pairs of ostia. In his comprehensive work, Petrunkevitch (1933) listed the number of ostia for many spider taxa, including mygalomorphs (also data from Blanchard 1852; Causard 1896). According to Petrunkevitch, the following mygalomorph taxa have four pairs of ostia: Halonoproctidae (*Bothriocyrtum californicum* (O. Pickard-Cambridge, 1874)), Nemesiidae (*Nemesia caementaria* (Latreille, 1799)), Dipluridae (*Ischnothele guianensis* (Walckenaer, 1837)), Paratropididae (*Anisaspis tuberculata* Simon, 1892) and Theraphosidae (*Avicularia avicularia* (Linnaeus, 1758), *Cyrtopholis portoricae* Chamberlin,1917, *Eurypelma californicum* (*nomen dubium*, see Nentwig 2012), *Theraphosa blondi* (Latreille, 1804)). He also listed mygalomorph taxa with three pairs of ostia: Atypidae (*Atypus piceus* (Sulzer, 1776)), Migidae (*Poecilomigas abrahami* (O. Pickard-Cambridge, 1889)) and Barychelidae (*Sason robustum* (O. Pickard-Cambridge, 1883)). The basal araneomorph spider taxa Austrochilidae and Hypochilidae also possess four pairs of ostia (Petrunkevitch 1933; Zapfe 1955; Marples 1968; Huckstorf et al. 2015). Mesothelae, the most basal off-branching spider taxon and sister group to the Opisthothelae, possess five pairs of ostia (Bristowe 1933; Petrunkevitch 1933). We can therefore infer that four pairs of ostia constitute the basic pattern in Opisthothelae. This also implies that, within Mygalomorphae, a reduction to three pairs of ostia occurred three times: in Atypidae, Migidae and Barychelidae.

What happened to the posterior aorta system in mygalomorphs?—In all mygalomorph spiders studied, the posterior aorta system is noticeably absent: the heart ends blindly in a thin tip. In the other spider taxa, the posterior aorta system originates from the posterior tip of the heart (Mesothelae: Bristowe 1933; Araneomorphae: Huckstorf et al. 2013, 2015; Runge & Wirkner 2016). The posterior aorta runs along a slight curve in a postero-ventral direction into the anal region. There it splits in two short arteries that merge with the hemolymph lacunar system (Huckstorf et al. 2013, 2015; Runge & Wirkner 2016). The fact that all other spiders possess a posterior aorta system but Mygalomorphae do not, has to be interpreted as an apomorphic loss.

Probably as a result of the lacking posterior aorta system, the third cardiac artery system in Mygalomorphae differs from those in other spider taxa. It is paired at its origin but fuses into a thick medially running artery system supplying the posterior part of the opisthosoma. This structure is also described for the mygalomorph taxon *Eurypelma californicum* (Zahler 1990; Bihlmayer 1991; Paul et al. 1994). Something similar is also described for the fifth cardiac artery system in two mesothelean taxa *Liphistius batuensis* Abraham, 1923 and *L. malayanus* Abraham, 1923 (Bristowe 1933). However, here a posterior aorta system is present. In the mygalomorph spider *Nemesia caementaria* (Nemesiidae), there is an additional artery between both third cardiac arteries but a posterior aorta system is missing (Causard 1896). Causard interprets this additional artery as a prolongation of the heart which could be a posterior aorta that is displaced ventrally between both third

cardiac arteries. Gerhardt & Kästner (1938) state that they found similar configurations in *Nemesia* sp. and in other big mygalomorphs, but no specific taxa are named.

In summary, all five species studied here possess a third cardiac artery system with a paired origin. However, a posterior aorta system is missing. We found no further unpaired artery emanating from the heart next to the third cardiac arteries.

From an evolutionary and functional point of view, this scenario is intriguing: why would a posterior aorta system be replaced by a third pair of cardiac arteries? Mainly functional experiments could provide answers, yet such studies are technically difficult to conduct.

A ramified lacunar system within the opisthosoma.—The circulatory system in spiders comprises a hemolymph vascular system. The hemolymph, when leaving this system, enters interstices between organs, which are – in their entirety – termed the hemolymph lacunar system (HLS). This system is known to comprise not only spaces between organs, but also highly organized channels (or 'sinuses') whose primary function is to direct hemolymph currents. Wirkner et al. (2013) defined sinuses as being surrounded or bordered by a septum, while lacunae are narrow spaces between organs and muscles channeling hemolymph along distinct routes (see also OArCS, Ontology of Arthropod Circulatory Systems, Wirkner et al. 2017). In Arachnida, the major sinuses are the pericardial sinus and the pulmo(branchio)-pericardial sinuses (e.g., Xiphosura: Göpel & Wirkner 2015; Uropygi and Amblypygi: Klußmann-Fricke & Wirkner 2016; Scorpions: Wirkner & Prendini 2007, Araneae: Paul 1990, Huckstorf et al. 2013, 2015).

In our investigation, a highly ramified lacunar system beneath the cuticle in *N. chromatus* (Fig. 1B,C) was identified for the first time in Araneae. Unfortunately, we were not able prove the presence of a septum surrounding these channels, and so we are unable to say whether they are sinuses or whether they simply constitute spaces between other organs. Nonetheless, they form a complex system of tube-like spaces channeling the hemolymph in a ventral direction.

Paul et al. (1994) noted a 'subepidermal space' in *Eurypelma californicum* merging into the 'ventral sinus'. Zahler (1990, unpublished data) also refers to ramified 'hemolymph channels' merging into the 'ventral sinus'. However, these structures were not described and we can therefore only suppose that they were describing the same features as found in our study.

The precise function of these channels is not known. However, spider opisthosomata are densely packed with midgut glands and other organs, such as spinning glands and ovaries, and this might explain the necessity for a distinct supply system such as can be found, for example, in Xiphosura (see e.g., Göpel & Wirkner 2015).

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