Comparative Morphology of the Hemolymph Vascular System in Scorpions–A Survey Using Corrosion Casting, MicroCT, and 3D-Reconstruction

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ABSTRACT Although scorpions are one of the better known groups of Arthropoda, detailed knowledge of their anatomy remains superficial. This contribution presents the first comprehensive investigation of the gross morphology of the scorpion vascular system, based on a survey of species representing all major lineages of the order, using classical and modern non-destructive techniques in combination with three-dimensional reconstruction. The investigation reveals that the hemolymph vascular system (HVS) of Scorpiones comprises a central pumping heart which extends the entire length of the mesosoma and is enclosed in a pericardium. Several arteries branch off the heart to supply different organs and body regions. Two different anterior aorta major branching patterns are identified among the species investigated. Arteries that branch off the anterior aorta system supply the appendages (chelicerae, pedipalps, and walking legs) and the central nerve mass with a complex arterial network. This study of the HVS of scorpions provides further evidence that the vascular systems of euarthropods can be highly complex. Use of the term "open circulatory system" within arthropods is re-emphasized, as it refers to the general organization of the body cavity (i.e. mixocoely) rather than to the complexity of the circulatory system. J. Morphol. 268:401–413, 2007. © 2007 Wiley-Liss, Inc.

KEY WORDS: Scorpiones; circulatory organs; heart; cardiac

Scorpions are one of the best known groups of arthropods. Nonetheless, their phylogenetic relationships are only now beginning to be solved, although a number of studies exist to date (e.g., Stockwell, 1989; Prendini, 2000; Soleglad and Fet, 2003). The same applies to their morphology, despite numerous studies from the classical period of morphology in the 19th Century, our knowledge of the detailed structure of the internal organ systems is still superficial.

The first comprehensive account of the circulatory system of Scorpiones was provided by Newport (1843). Newport's schematic drawing of a sagittal section of a scorpion, illustrating all major organ systems, was so influential that it was subsequently recycled several times in literature on the anatomy of scorpions (e.g., Ch'en-Pin, 1940; Gruner, 1993).

Later studies that attempted to provide a comparative assessment of this organ system only examined a few species (e.g., Schneider, 1892; Petrunkevitch, 1922; Dubuisson, 1925). Other workers concentrated on details of histology (e.g., Pawlowsky, 1922) or ultrastructure (e.g., Tjønneland et al., 1985), or focused only on specific parts of the organ system, e.g., the heart or the prosomal arteries (e.g., Randall, 1966; Locket, 2001). Textbook accounts of the morphology of the circulatory system are especially superficial as only the major arteries have been described to date, providing a simplistic picture of what is in reality a very complex arterial system (e.g., Farley, 1999). The best known aspect of the circulatory system is the structure (gross anatomy, histology, and ultrastructure) of the heart and its associated structures (e.g., Randall, 1966; Tjønneland et al., 1985). The morphology of the arterial system connected to the anterior aorta in the prosoma is poorly known, as is the arterial system of the opisthosoma. In summary, although several detailed studies of the circulatory organs of scorpions have been conducted, major components of the organ system have only been studied superficially, and an analysis of the system across the major groups of scorpions remains to be presented.

In this study, we present the first comparative overview of the morphology of the scorpion circulatory system, focusing on the gross morphology of the heart and major arterial systems, based on a study of exemplars selected from most major lineages of Scorpiones, using modern techniques for visualizing fine arteries. Our methods include a combination of corrosion-casting techniques, Micro-computer-tomography, and computer aided 3D-reconstruction, supplemented with classical semi-

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thin sectioning methods, to allow a higher degree of resolution (see also Wirkner and Richter, 2004).

MATERIALS AND METHODS
Taxon Sample

Scorpion phylogenetic research is controversial. Only a few higher-level cladistic analyses have been published thus far, and authors disagree on methods and approaches (e.g., Lamoral, 1980; Stockwell, 1989; Prendini, 2000; Soleglad and Fet, 2003; Prendini and Wheeler, 2005). All workers agree, however, that Buthidae (Buthoidea) form the sister-group of the remaining, "non-buthid" families (Coddington et al., 2004). Two major clades can be distinguished in the non-buthid lineage, the Scorpionoidea and (Chactoidea + Vaejovoidea), although (Chactoidea + Vaejovoidea) may be paraphyletic with respect to Scorpionoidea, the monophyly of which is well-established. Therefore, to obtain an overview of the main scorpion taxa for the present study, five species of Buthidae (representing both major clades within the family; marked as bold in Table 1), a species of Diplocentridae (Scorpionoidea), and a species of Iuridae (Vaejovoidea) were studied in detail (also marked bold in Table 1). Further 20 species, selected from the families Chactidae, Liochelidae, Scorpionidae, Superstitioniidae, and Vaejovidae were screened for comparison (also listed in Table 1).

Histology

Specimens were dissected and fixed in Bouin's fixative, dehydrated in ethanol and, after an intermediate step of epoxypropane, embedded in Araldit epoxy resin under vacuum. Serial semi-thin sections (1-1.5 μm) were prepared with a Microm HM 360 ultramicrotome using glass or diamond knifes, and stained with a mixture of 1% azure II and 1% methylene blue in an aqueous 1% borax solution for ~30 s at 80–90°C.

Corrosion Casting

Acrylic casting resin Mercox CL-2R/2B (Vilene Comp. Ltd, Tokyo, Japan) was injected into the heart of ether-anesthetized specimens to prepare casts of the circulatory system. Before injecting, the resin was mixed with approximately 0.05 mg MA initiator and placed in a 5 ml syringe. Micropipettes (Hilsberg pipettes, diameter 1.0 mm, thickness 0.2 mm; pulled with a KOPF Puller 720) used for injecting were filled using the syringe. The pipettes were placed on an adjustable instrument holder in a mechanical micromanipulator and the tips inserted through the intersegmental membrane between the mesosomal tergites directly into the vascular system, i.e. the heart. The resin was then injected and the specimens left for several minutes to allow polymerization and tempering of the resin. Specimens destined for the MicroCT were fixed in Bouin's fixative and then critical point dried (BAL-TEC CPD 030). Alternatively, specimens were macerated for 1-2 days by repeated immersion in 10% potassium hydroxide at ambient temperature, followed by washing in a solution of 2 g Pepsin in 10 ml of 2% HCl (Hilken, 1994).

MicroCT

X-ray imaging was performed with a SkyScan-1072 High-resolution MicroCT system in high resolution mode. See Wirkner and Richter (2004) for details of settings.

TABLE 1. *List of species in which circulatory system has been studied here and in the literature*

Superfamily	Family	Species	Structure	Reference
Buthoidea	Buthidae	**Androctonus amoreuxi** (Audouin, 1826)	AP1	This study
		Androctonus australis (Linnaeus, 1758)	AP1	This study
		Androctonus bicolor Ehrenberg, 1828	AP1	This study
		Babycurus jacksoni (Pocock, 1890)	AP1	This study
		Buthacus arenicola (Simon, 1885)	AP1	This study
		Buthus occitanus (Amoreux, 1789)	AP2	Schneider, 1892
		Centruroides exilicauda (Wood, 1863)	AP1	This study
		Centruroides insulanus (Thorell, 1876)	AP1	Petrunkevitch, 1922
		Leiurus quinquestriatus (Ehrenberg, 1828)	AP1	This study
		Lychas mucronatus (Fabricius, 1798)	AP1	This study
		Mesobuthus martensii (Karsch, 1879)	AP1	This study
		Parabuthus leiosoma (Ehrenberg, 1828)	AP1	This study
Chactoidea	Chactidae	*Brotheas granulatus* Simon, 1877	AP2	This study
	Euscorpiidae	*Euscorpius* sp.	AP2	Schneider, 1892
Scorpionoidea	Diplocentridae	*Diplocentrus spitzeri* Stahnke, 1970	AP2	This study
		Diplocentrus lindo Stockwell and Baldwin, 2001	AP2	This study
	Liochelidae	*Iomachus politus* Pocock, 1896	AP2	This study
		Opisthacanthus rugiceps Pocock, 1897	AP2	This study
	Scorpionidae	*Heterometrus laoticus* Couzijn, 1981	AP2	This study
		Pandinus cavimanus (Pocock, 1888)	AP2	This study
		Pandinus imperator (Koch, 1841)	AP2	This study
	Urodacidae	*Urodacus armatus* Pocock, 1888	AP2	Locket, 2001
		Urodacus novaehollandiae Peters, 1861	AP2	Locket, 2001
		Urodacus yaschenkoi (Birula, 1903)	AP2	Locket, 2001
Vaejovoidea	Iuridae	*Anuroctonus phaiodactylus* Wood, 1863	AP2	This study
		Hadrurus arizonensis Ewing, 1928	AP2	This study
	Superstitionidae	*Superstitionia donensis* Stahnke, 1940	AP1	This study
	Vaejovidae	*Paruroctonus silvestrii* Borelli, 1909	AP1	This study
		Serradigitus subtilimanus (Stahnke, 1940)	AP1	This study
		Serradigitus wupatkiensis (Stahnke, 1940)	AP1	This study
		Smeringurus mesaensis (Stahnke, 1961)	AP1	This study
		Uroctonus mordax Thorell, 1876	AP1	This study
		Vaejovis spinigerus (Wood, 1863)	AP1	This study

Occurrence of anterior aorta major branching patterns (AP1 and AP2) are listed in column "structure" (see also discussion). Species in bold were investigated in detail for this study.

3D Reconstruction

Stacks of virtual sections produced by MicroCT were used for 3D-reconstructions in the software Imaris 4.0.5 (Fa. Bitplane). A scene was created in the program module "Surpass", the volume rendering function was chosen to visualize the entire data set, and the contours of organs studied were marked on every virtual cross section with a polygon. Stacks of polygons were visualized by surface renderings.

Scanning Electron Microscopy

Casts for scanning electron microscopy (SEM) were air-dried, coated with gold or palladium (BAL-TEC SCD 005), and studied using a SEM (LEO 1430). See Wirkner and Richter (2003) for details.

Image Management

Figures were arranged and prepared into plates using Corel Graphics Suite 11. Bitmap images were embedded into Corel Draw 11 files and digitally edited with Coral Photo Paint 11.

RESULTS

The following descriptions of the circulatory system follow the general morphological divisions, prosoma and opisthosoma, recognized in scorpions (Hjelle, 1990). The prosoma consists of nine segments and is dorsally covered by the carapace which bears one or more pairs of lateral and a pair of median ocelli. The opisthosoma is divided into a broad, anterior mesosoma, comprising seven segments, and a slender, tail-like metasoma (or cauda) comprizing five segments and the telson terminating in a sting (aculeus).

As in all arthropods, the scorpion circulatory system comprises a system of vascular structures combined with a complex system of sinuses and lacunae, in which the movements of other organs such as the gut and muscles propel hemolymph currents to some extent. The following detailed descriptions of the circulatory system focus mainly on the vascular part of this system (hemolymph vascular system, hereafter HVS). Measurements relating to internal vessel diameters are only approximate, as the size of muscular and connective tissues depend on their state of contraction and fixation.

The central nervous system of scorpions is concentrated in the prosoma and anterior mesosoma (nomenclature following Babu, 1965; Hjelle, 1990). The system consists of the following sections: supraesophageal nerve mass (brain); subesophageal nerve mass (thoracic nerve mass), connected to the brain by short, thick circumesophageal connectives; three free mesosomal ganglia; four metasomal ganglia; and connected nerves. The nerve masses comprise the neuropils and corresponding pericaria surrounding them (Fig. 4F; sgl).

Buthidae

The following description of the circulatory system of Buthidae is based on observations of the following species: *Androctonus amoreuxi*, *Buthacus arenicola*, *Centruroides exilicauda*, and *Lychas mucronatus*. Differences among these species are noted where necessary.

Heart, Pericardial Sinus, and Associated Structures

The dorsal vessel comprises the musculated and ostiated heart that is extended anteriorly by the anterior aorta and posteriorly by the posterior aorta. The anterior end of the spindle-shaped heart lies in the first mesosomal segment, the posterior end in the middle of the seventh mesosomal segment. The musculature of the heart comprises relatively thick, spirally-arranged muscle fibers. The heart is equipped with seven pairs of incurrent ostia distributed along its entire length (Figs. 1A, 3A; os). The heart is suspended dorsally, laterally, and ventrally (Fig. 1B,F; li) to the body wall by seven groups of so-called ligaments (terminology after Petrunkevitch, 1922). The heart nerve is prominent and clearly identified as a cord extending along the dorsal surface of the heart (Fig. 1D; hn). The sac-like pericardium surrounds the entire heart (Fig. 1F; pc). Each book lung sinus is connected via a pulmono-pericardial sinus to the pericardium (Fig. 1F; ps). These sinuses are attached to the lateral cuticle (Fig. 1E; ps) and extend down to the four pairs of book lungs.

Major Arterial Systems

Several major arterial systems, including the cardiac arteries, and the anterior and posterior aortae, distribute hemolymph from the heart to different organs and regions of the body.

Eight pairs of segmental *cardiac arteries* emanate from the heart (Figs. 1C, 3A; ca1-8). The cardiac arteries exit the heart in a ventro-lateral position directly ventral to the ostia. The first pair of cardiac arteries emanates shortly posterior to the transition of the heart into the anterior aorta (Fig. 1C inset; ca1) but, as previously stated, no pair of ostia can be found in this region (compare Fig. 1A inset). Each artery is equipped with a valve at its origin. The cardiac arteries present a fine branching pattern and irrigate the organs and tissues in the mesosoma (Fig. 2A; ca1-3). The transition of the heart into the *anterior aorta* occurs at a point where the dorsal vessel passes the diaphragm (Fig. 1A,C; ao). Anterior to this point, the aorta descends obliquely toward the thin esophagus (Fig. 3B; oe). The anterior aorta is stout, with a diameter of ca. 500 μm in *Lychas mucronatus*.

The position of the anterior aorta system in relation to the cuticular structures of the prosoma can be seen in Figure 2B. The angle at which the aorta descends varies slightly in different species. For example, in *Parabuthus leiosoma* (Fig. 2C) the angle of descent is rather steep, whereas in *L.*
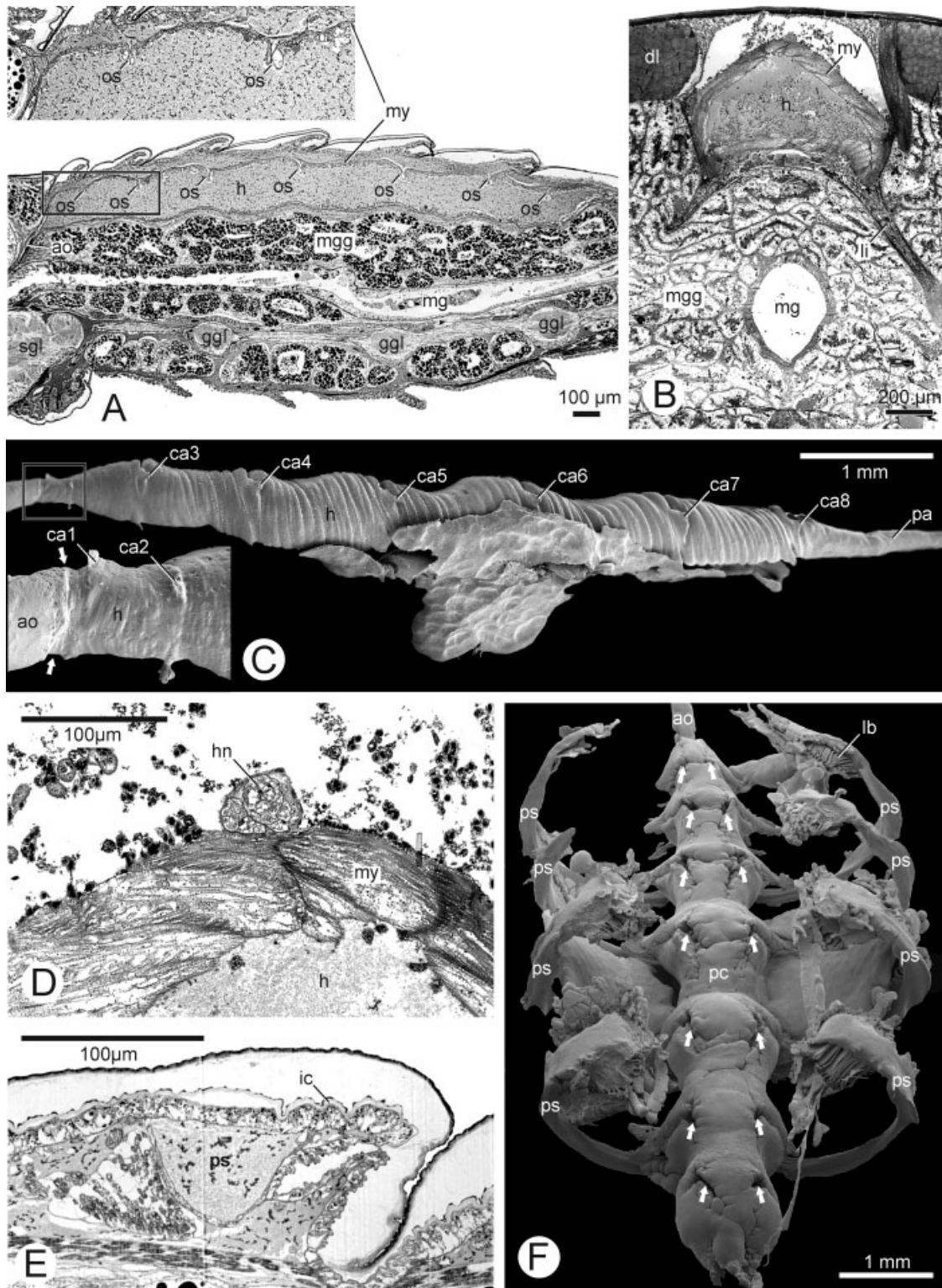

Figure 1

mucronatus (Fig. 2D), it is more gradual. The aorta splits into two large trunks which extend laterally around the esophagus. Six pairs of major vessels split off the aorta before it merges into the ventral vessel (= supraneural vessel). These are the cheliceral arteries, pedipalpal arteries, and arteries for the four pairs of walking legs (pedal arteries). The *cheliceral arteries* form a lateral arch around the supraesophageal ganglion (brain) and continue dorsally into the chelicerae (Fig. 2C,D; ch). Two branches split off the main branches antero-lateral to the brain. One of these continues to the median eyes, whereas the other supplies muscles in the posterior prosoma (Fig. 2C,D; oa, sc). Several small vessels, supplying musculature and tissues in the prosoma, emanate from these arteries. The cheliceral arteries fork to supply the cheliceral musculature. The *pedipalpal arteries* are the largest vessels branching off the aorta at the bend of the aorta trunks (Figs. 2C,D, 3B–D; pp). These arteries extend laterally around the esophageal commissures and into the pedipalps in proximity to the pedipalpal nerves. The *pedal arteries* extend straight into the corresponding legs from their origin at the aortic trunks. Along the way, these arteries produce several small vessels that supply the surrounding tissues (mainly musculature). The arteries for the first walking legs emanate from almost the same point as the pedipalpal arteries, or split off a short way along them, so that both possess a common trunk (Figs. 2C,D, 3B–D; I). After both aortic trunks have turned in a posterior direction, they produce three pairs of arteries for the second to fourth pair of walking legs (Figs. 2C,D, 3B–D; II, III, IV). Poste-

rior to the point where the arteries for the fourth pair of walking legs branch off, the two trunks of the aorta bend inwards and unite to form the supraneural *ventral vessel* (Figs. 3A,B, 4A,C; vv). This vessel continues through the entire trunk, producing lateral arteries for the ganglia in each segment. The *posterior aorta* extends into the metasoma from the posterior end of the heart.

Arterial Supply of Central Nervous System

The central nervous system in the prosoma, i.e., the supra- and subesophageal ganglion masses, is supplied by a network of vessels branching off the major arteries of the anterior aorta. The posteriorly-directed parts of the two aortic trunks are connected medially by five vessels. In the midline of the body, an artery emanates ventrally from each of these connections, and continues down through the thoracic nerve mass (Fig. 3C,D; tgl). These arteries are therefore termed transganglionic arteries (Figs. 2F, 4A; asterisks; Fig. 4F; tgl). Another four transganglionic arteries emanate from the anterior part of the ventral vessel, shortly after the two trunks of the aorta unite (Fig. 4A; last four asterisks). Each transganglionic artery descends vertically between neighboring neuromeres of the subesophageal nerve mass (Fig. 4F; tgl) before branching laterally at the border of the neuropils and the pericaria. These capillaries spread through the neuropiles and display a complex branching pattern (see Fig. 4C,D; meshwork of small arteries). The supply of the nerve masses is supplemented by small vessels branching off the cheliceral, pedipalpal, and pedal arteries (Fig. 4C,D; small arteries branching off pedal arteries I-IV). Together, these transganglionic and intraganglionic arteries form a dense meshwork of small arteries in the central nerve masses. Anterior to the thoracic nerve mass, anterior intraganglionic arteries unite and form two parallel vessels termed peribuccal vessels (Fig. 4B; pb). These vessels are medially interconnected and their dorsal branches merge with the cheliceral arteries of either side, whereas the ventral branches run parallel to the cheliceral arteries into the anterior part of the prosoma.

Fig. 1. Heart and associated structures of Buthidae. **A:** Sagittal semi-thin section through mesosoma of *Centruroides exilicauda*. Heart extends from first to last mesosomal segment. A pair of ostia occurs (os) in each segment. Inset illustrates anterior part of heart in higher magnification (anterior at left). **B:** Semi-thin cross-section through mesosomal segment of *Buthacus arenicola*. A pair of hypocardiac ligaments leading off the heart ventrally (li). **C:** Cast of heart of *C. exilicauda*. SEM. Eight pairs of cardiac arteries branch off heart ventro-laterally. Cardiac musculature is arranged spirally. Inset illustrates transition of heart into anterior aorta (arrows) and bases of first and second cardiac arteries (ca1 + ca2) (dorsal at top). **D:** Semithin section through heart nerve of *B. arenicola*. Heart nerve extends along dorsal side of heart. **E:** Parasagittal semi-thin section through mesosomal segment. Pulmono-pericaridal sinus attached to cuticle. Animal was fixed shortly before molting; therefore, new cuticle (ic) is visible as a folded membrane. **F:** Cast of pericardium and associated structures of *C. exilicauda*. SEM. Four pulmono-pericardial sinuses (ps) lead off sac-like pericardium (pc). Sites where hypocardial ligaments leave pericardium are visible (arrows). Note that lamellae of book lungs are also filled with resin (lb). ao, aorta; ca, cardiac artery; dl, dorsal longitudinal muscle; ggl, ganglion; h, heart; lb, lamellae of book lungs; li, ligament; mg, midgut; mgg, midgut gland; my, myocardium; os, ostium; pa, posterior aorta; pc, pericardium; ps, pulmono-pericardial sinus; sgl, subesophageal ganglion.

Iuridae and Diplocentridae

The circulatory system of two "non-buthid" scorpions, *Hadrurus arizonensis arizonensis* (Iuridae) and *Diplocentrus lindo* (Diplocentridae), is compared below with the system described earlier for the Buthidae. Only major details and notable differences from the buthid circulatory system are described, to avoid redundancy.
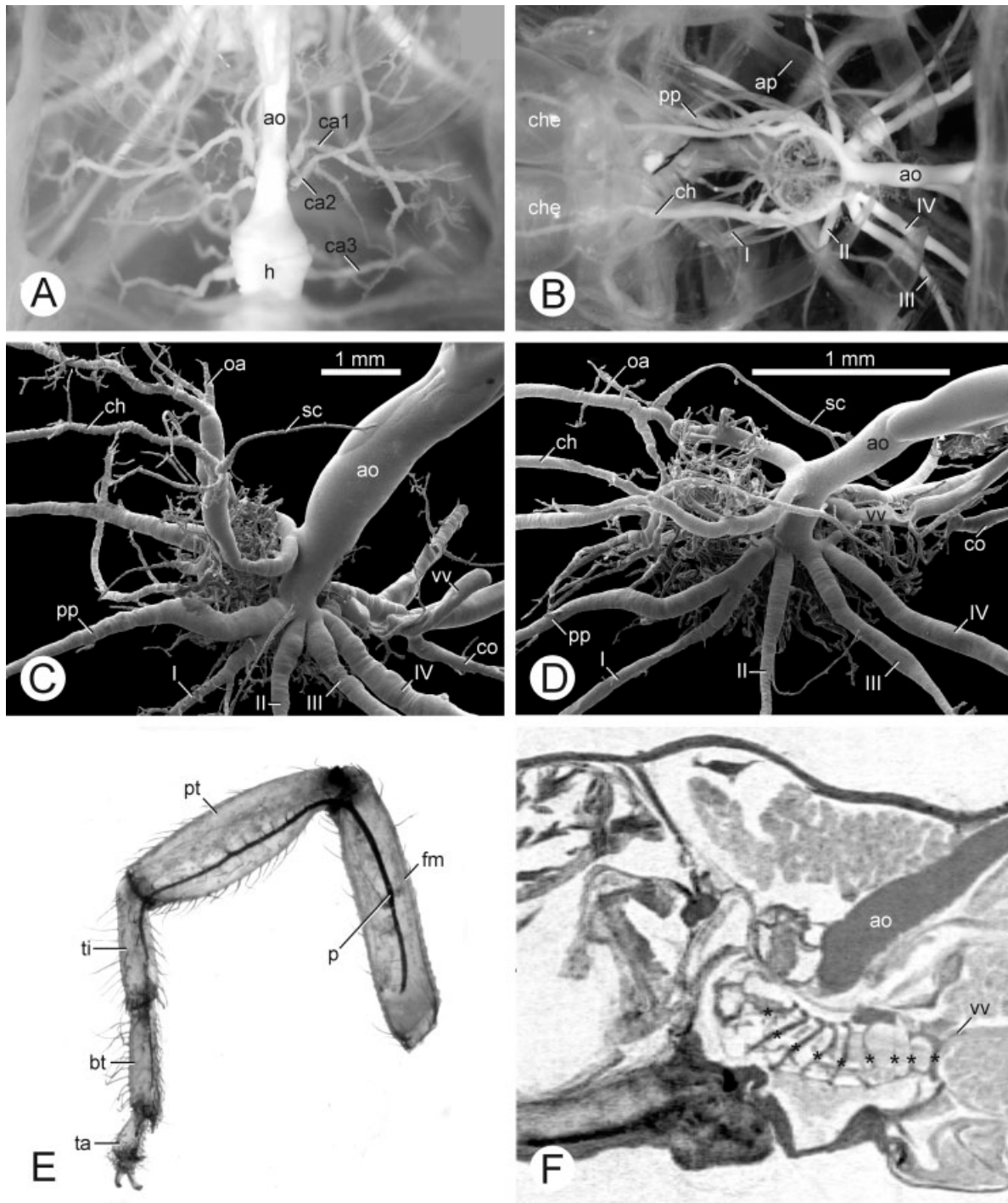
Fig. 2. Anterior aorta system of Buthidae. **A:** Photograph of cast of *Lychas mucronatus*. Soft tissues are dissolved and carapace removed to assist visualization of cast. Transition of heart into anterior aorta (ao) and first three pairs of cardiac arteries (ca) are visible (anterior at top). **B:** Photograph of cast of *L. mucronatus*. Soft tissues are dissolved and carapace removed to assist visualization of cast. Position of anterior aorta system (ao) in prosoma is visible in relation to cuticular structures, e.g. apodemes of walking legs (ap) (anterior at left). **C:** Cast of anterior aorta system of *Androctonus amoreuxi*. SEM. **D:** Cast of anterior aorta system of *L. mucronatus*. SEM. **E:** Cast of leg of *Parabuthus leiosoma*. Pedal artery is filled to tip of basitarsus (bt). Small vessels branch off leg artery mainly in patella (pt). **F:** Virtual sagittal MicroCT section through prosoma of *Buthacus arenicola*. Aorta descends in prosoma. Nine transganglionic arteries (asterisks) are discernible: first five emanate from commissural arteries, last four from anterior part of supraneural vessel. ao, aorta; ap, leg apodeme; bt, basitarus; ca, cardiac artery; ch, cheliceral artery; che, chelicera; co, coxa; fm, femur; h, heart; I-IV, pedal arteries I to IV; oa, optical artery; p, pedal artery; pp, pedipalpal artery; pt, praetarsus; sc, supracerebral artery; ta, tarsus; ti, tibia; vv, ventral vessel.
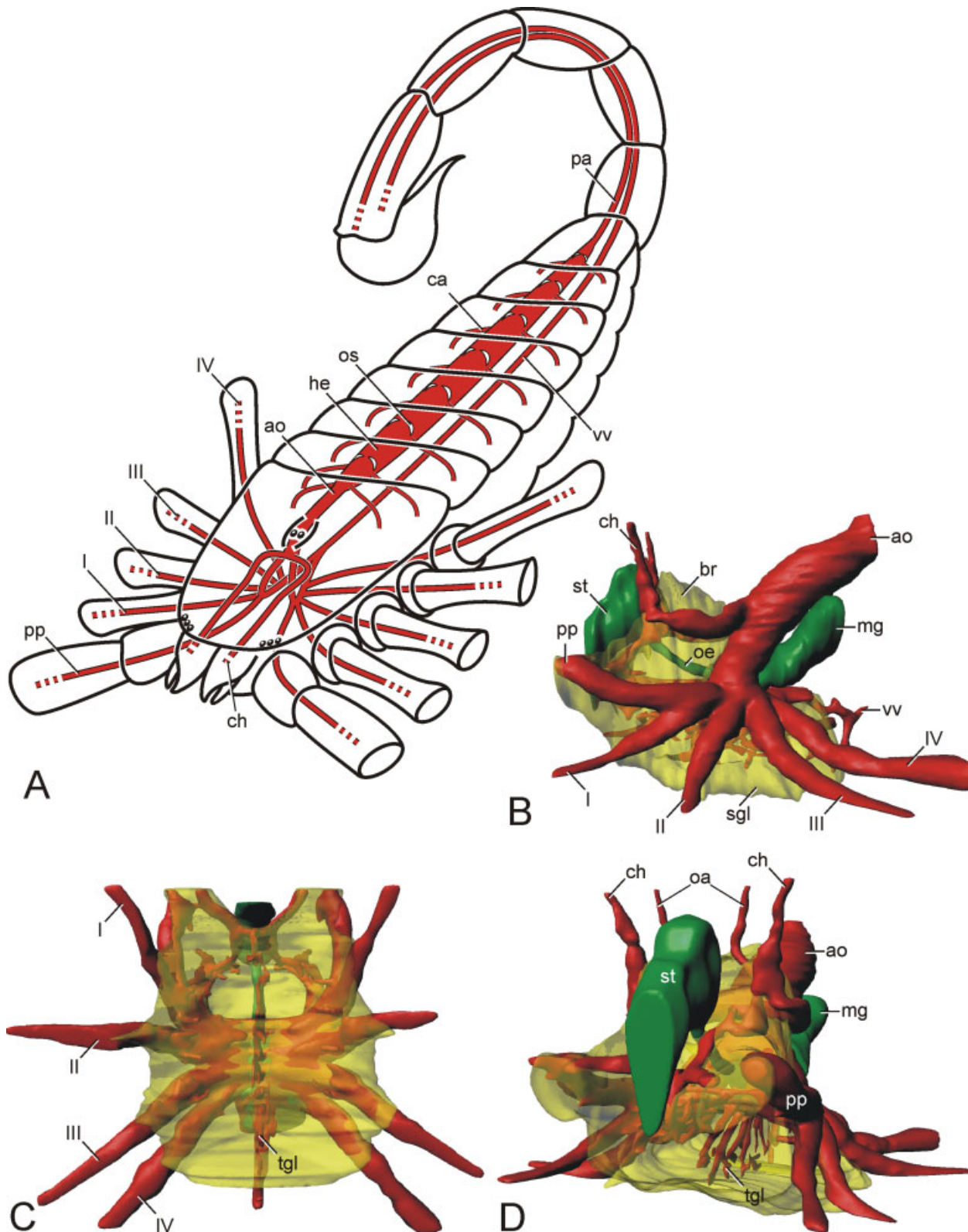
Fig. 3.   3D-reconstructions of hemolymph vascular system (HVS) of Scorpiones. **A:** Schematic diagram of major HVS of Scorpiones. **B–D:** Surface renderings of MicroCT scan of *Buthacus arenicola* (see Fig. 2F) showing major organ systems in prosoma. Color codes: red, HVS; green, digestive system; translucent yellow, CNS. B: Lateral aspect. C: Ventral aspect (anterior at top). D: Oblique anterior aspect. ao, aorta; br, brain; ca, cardiac artery; ch, cheliceral artery; he, heart; I-IV, pedal arteries I-IV; mg, midgut; oa, optical artery; oe, esophagus; os, ostium; pa, posterior aorta; pp, pedipalpal artery; sgl, subesophageal ganglion; st, stomach; tgl, transganglionic artery; vv, ventral vessel.
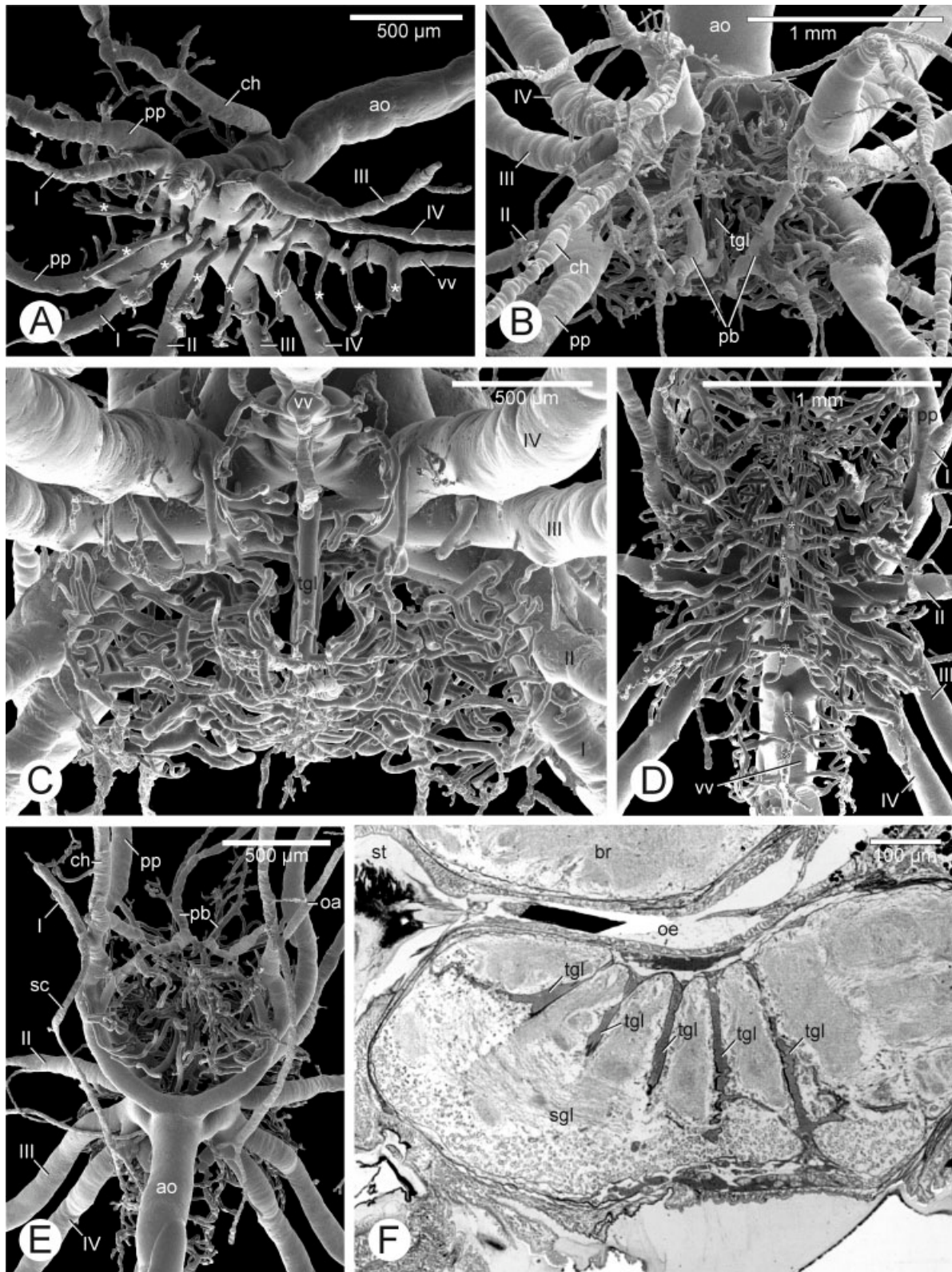
Fig. 4. Arterial supply of CNS of Buthidae. **A:** Cast of anterior aorta system of *Centruroides exilicauda*. SEM. Ventro-lateral aspect (anterior at left). Cast is incomplete as not all vessels were filled, providing view of transganglionic arteries (asterisks). **B, C:** Casts of anterior aorta system of *Androctonus amoreuxi*. SEM. B: Anterior aspect. Note dense meshwork of small arteries inside central nerve masses. C: Posterior aspect. **D, E:** Casts of anterior aorta system of *Lychas mucronatus*. SEM. **D:** Ventral aspect. **E:** Dorsal aspect. **F:** Sagittal semi-thin section through central nerve masses of *C. exilicauda* (anterior at left). ao, aorta; br, brain; ch, cheliceral artery; I-IV, pedal arteries I-IV; oa, optical artery; oe, esophagus; pa, posterior aorta; pb, peribuccal artery; sc, supracerebral artery; sgl, subesophageal ganglion; st, stomach; tgl, transganglionic artery; vv, ventral vessel.
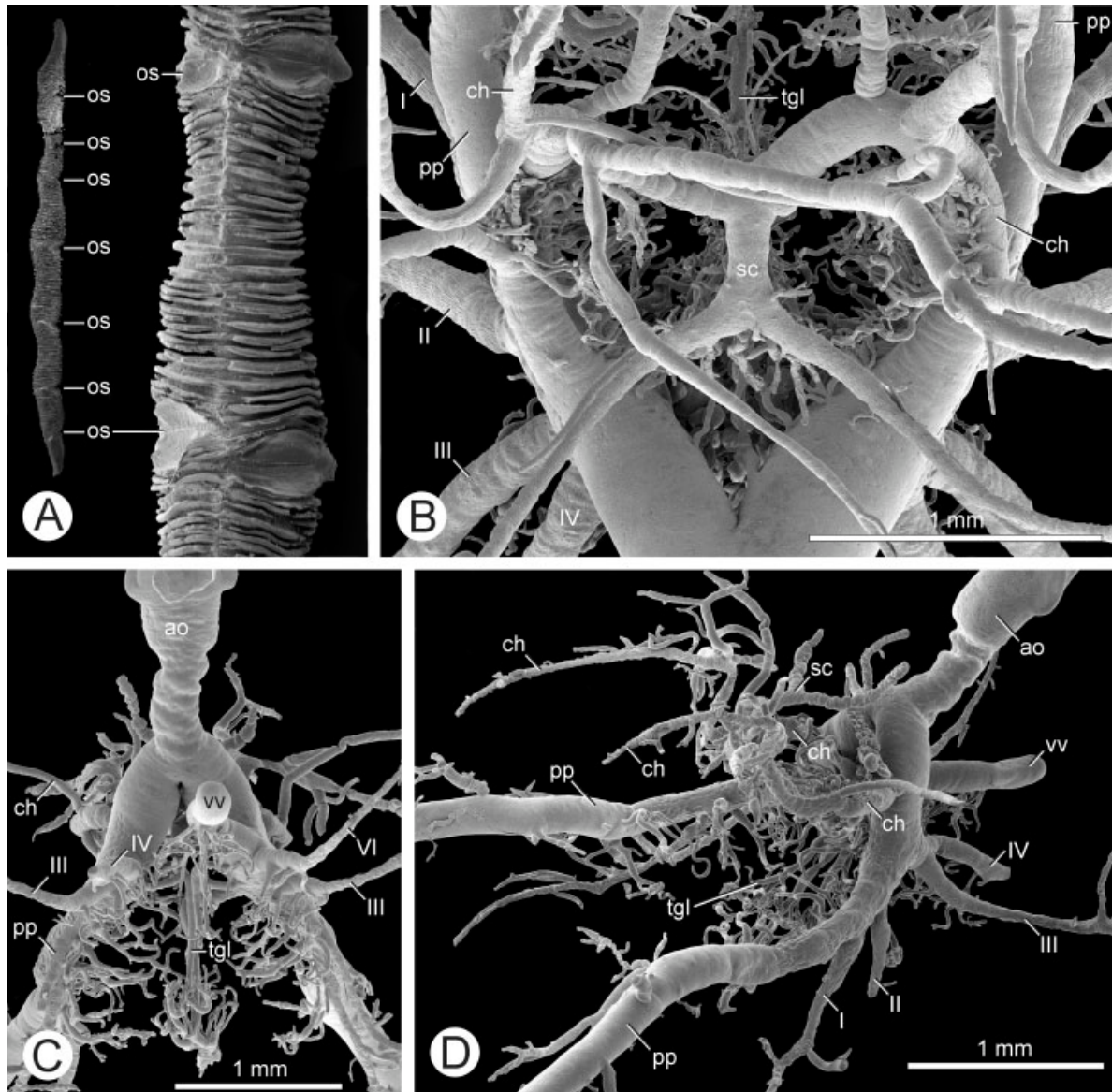
Fig. 5. HVS of Iuridae and Diplocentridae. **A:** Cast of heart of *Hadrurus arizonensis arizonensis*. SEM. Left side: complete heart (anterior at top). Right side: detail of heart showing two pairs of ostia. Note fissure in midline where semicircular muscle fibers meet. **B:** Cast of anterior aorta system of *H. a. arizonensis* (anterior at top). SEM. Note asymmetry of cheliceral arteries. **C, D:** Cast of anterior aorta system of *Diplocentrus lindo*. SEM. **C:** Posterior aspect. Transganglionic arteries bend back distally into central nerve mass. **D:** Antero-lateral aspect. Trunks of aorta continue as pedipalpal arteries. ao, aorta; ch, cheliceral artery; I-IV, pedal arteries I-IV; os, ostium; pp, pedipalpal artery; sc, supracerebral artery; tgl, transganglionic artery; vv, ventral vessel.

Heart, Pericardial Sinus, and Associated Structures

As in the buthid species described earlier, the heart of *Hadrurus arizonensis arizonensis* and *Diplocentrus lindo* extends from the first tergite to the seventh tergite, and is equipped with seven pairs of incurrent ostia, distributed along its entire length (Fig. 5A; os). Muscle fibers of the myocardium protrude into the lumen of the heart, especially in *H. a. arizonensis* (Fig. 5A). The cast shows that these fibers are semicircular and attach to one another in the midline of the heart. The pericardium encloses the complete heart. Hemolymph enters the pericardium through four pairs of pulmono-pericardial sinuses in the third to sixth mesosomal segments.

Major Arterial Systems

Eight pairs of segmental *cardiac arteries*, an *anterior aorta*, and a *posterior aorta* emanate from the heart in *Hadrurus arizonensis arizonensis* and *Dip-*
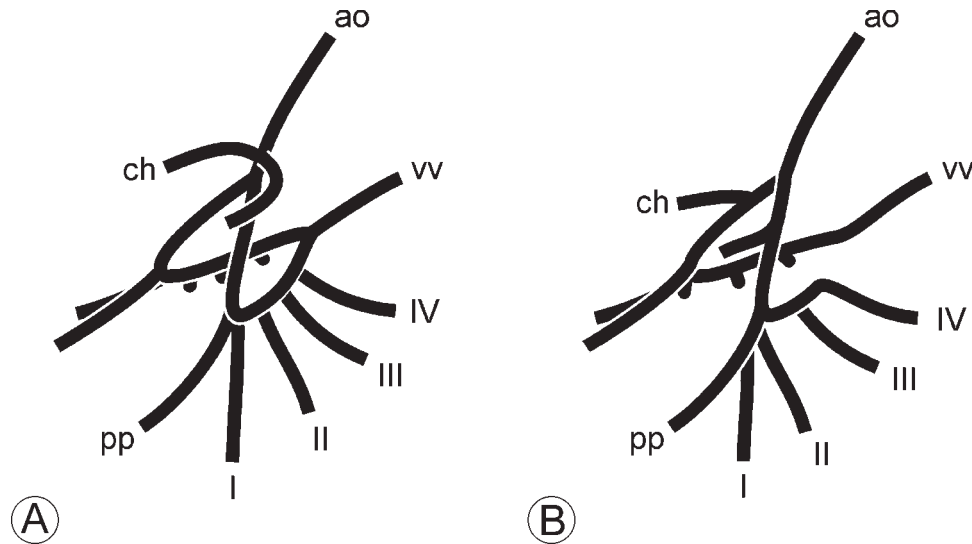
Fig. 6. Schematic diagram of two anterior aorta major branching patterns. **A:** Anterior Aorta Major Arterial Branching Pattern 1 (AP1). Cheliceral arteries share a common base (compare with Figs. 2C,D, 3A). Both aorta trunks bend backwards and unite as supraneural vessel (compare with Figs. 2D, 4A). See Table 1 and discussion for taxa in which observed. **B:** Anterior Aorta Major Arterial Branching Pattern 2 (AP2). Cheliceral arteries branch off two aorta trunks each (compare with Fig. 5B,D). Two aorta trunks merge into pedipalpal arteries (compare with Fig. 5B,D). Only one trunk of aorta continues as supraneural vessel (compare with Fig. 5C). ao, aorta; ch, cheliceral artery; I - IV, pedal arteries I to IV; pp, pedipalpal artery; vv, ventral vessel.

locentrus lindo. The cardiac arteries supply the organs in the mesosoma. The anterior aorta descends through the diaphragm slightly posterior to the brain. The *cheliceral arteries* branch off after the aorta divides and thus do not possess a common trunk (Fig. 5B,D; ch). They show asymmetry as to which regions they supply. One cheliceral artery divides into the cheliceral artery proper and another major trunk which bends posteriorly, dividing again to supply the postero-dorsal regions of the prosoma (Fig. 5B,D; sc) while the other cheliceral artery runs straight into the chelicerae, producing branches for the median eyes and the tissues in the anterior prosoma. Intraspecific variation occurs concerning which of the cheliceral arteries also supplies the posterodorsal prosoma. After the aorta divides, the aortic trunks merge directly into the *pedipalpal arteries* (Fig. 5D; pp). The first and second *pedal arteries* branch off the proximal part of the pedipalpal arteries. The third and fourth pedal arteries possess a common trunk branching off the aortic trunks posteriorly (Fig. 5D; I+II). The two aortic trunks do not unite, but one of the branches continues posteriorly as a *ventral vessel* directly above the ventral nerve cord (Fig. 5C; vv). Some intraspecific variation is evident regarding which of the trunks merge into the ventral vessel.

Arterial Supply of Central Nervous System

A fine meshwork of intraganglionic arteries is evident in *Hadrurus arizonensis arizonensis*. These arteries are minute compared to the main vessels. Nine transganglionic arteries can be

observed in *Diplocentrus lindo*, and appear to be especially long, protruding far ventrally and branching distally back into the ventral nerve mass (Fig. 5C; tgl). The supply of the ventral nerve mass is also maintained by single arteries branching off the base of the pedal and pedipalpal arteries, respectively. These vessels continue medially and branch inside the ganglia.

Comparison of the Anterior Aorta Major Branching Patterns

The distribution of the major arteries branching off the aorta differs among the species studied (compare Fig. 2C,D with Fig. 5B,D; see Table 1 in discussion section). Two patterns can be distinguished, hereafter referred to as Anterior Aorta Major Arterial Branching Pattern 1 (AP1; Fig. 6A) and Anterior Aorta Major Arterial Branching Pattern 2 (AP2; Fig. 6B). In AP1, the cheliceral arteries branch off before the aorta splits from a common trunk. Thereafter, the aorta splits into two large trunks which curve backwards around the esophagus. The pedipalpal arteries branch off anteriorly at the bends. The arteries for the first walking legs emanate from almost the same point and continue in a ventro-lateral direction in most species. However, in *Lychas mucronatus*, these arteries split off a short way along the pedipalpal arteries so that both the pedipalpal arteries and the arteries of the first walking legs possess a common trunk. After both aortic trunks have turned in a posterior direction, they produce three pairs of arteries for the second to fourth pairs of walking legs. The two trunks of the

aorta bend inwards, behind the point where the arteries of the fourth pair of walking legs branch off, and unite to form the supraneural ventral vessel. AP2 differs from AP1 in three respects (compare Fig. 6A,B). Firstly, the cheliceral arteries branch off after the split of the aorta and thus do not possess a common trunk. Secondly, the aortic trunks merge directly into the pedipalpal arteries which are similar in diameter. The arteries of the first and second pairs of walking legs branch off from the proximal part of the pedipalpal arteries. Thirdly, the two aortic trunks do not unite after bending backwards and only one of the branches continues posteriorly as a supraneural vessel directly above the ventral nerve cord. However, in *Brotheas granulatus* the two trunks of the aorta unite to form the supraneural artery.

DISCUSSION
Comparative Morphology of Vascular System in Scorpiones

The above descriptions agree favorably with previous studies on the morphology of the circulatory system in Scorpiones. The general pattern of the HVS among the different species is similar (summarized in Fig. 3A). A dorsal, pulsatile heart is extended posteriorly by a posterior aorta through the metasoma into the telson. The heart is equipped with pairs of incurrent ostia and cardiac arteries. The heart merges anteriorly with the anterior aorta, which presents a complicated branching pattern in the prosoma. Branches from these vessels supply the body appendages and tissues in the prosoma. A supraneural ventral vessel extends from the posterior part of the anterior aorta, between the gut and the ventral nerve cord, to supply ganglia in the meso- and metasoma.

Despite gross similarities in the circulatory system of the taxa studied, there are some important differences, discussed below.

The heart. The gross anatomy of the heart is very similar in the scorpion species that have been studied (Newport, 1843; Petrunkevitch, 1922; Soares, 1950; Randall, 1966; this study). The heart extends the entire length of the mesosoma and comprises spirally-arranged semicircular muscle fibers. These muscle fibers are arranged obliquely in the Buthidae (Randall 1966; this study: Fig. 1C) whereas, in two non-buthids, *Uroctonus mordax* (Randall, 1966) and *Hadrurus arizonensis arizonensis* (this study), the musculature is arranged perpendicular to the body axis. A sac-like pericardium surrounds the entire heart (e.g., Randall, 1966). The presence of seven pairs of pulmono-pericardial sinuses (Dubuisson, 1925; see also Gruner, 1993) could not be confirmed. Casts clearly show the presence of four pairs of sinuses (Fig. 1F).

Ostia. Seven pairs of ostia were observed in all species examined for the present study and have also been described in most scorpion species previously studied (Petrunkevitch, 1922; Soares, 1950; Randall, 1966). Newport (1843), however, described the heart as comprising eight consecutive heart chambers, each equipped with a pair of ostia and a pair of cardiac arteries. The chambered nature of the heart is implausible as no structures exist which could separate the heart into different chambers, (see Figs. 1C, 5A; Randall, 1966). Randall (1966) nonetheless remarked that he could not exclude the presence of a further (eighth) pair of ostia in the anterior part of the heart in the first "preabdominal" segment, because of the presence of a pair of cardiac arteries in this region (see also Fig. 1C; ca1). This additional pair of ostia could not be found in either the histological sections (Fig. 1A; inset) or the casts (Figs. 1C, 5A) prepared for the present study. We are therefore convinced that only seven pairs of ostia exist in scorpions.

Cardiac arteries. Eight pairs of cardiac arteries emanate from the heart in most scorpion species that have been studied. These vessels are equipped with valves at their origin to prevent hemolymph flow back into the heart during the systole. Randall (1966) described eight pairs of cardiac arteries in *Centruroides sculpturatus* (Buthidae), but observed only six in *Uroctonus mordax* (Vaejovidae).

Anterior aorta system. In all scorpion species studied so far, the anterior aorta splits into two lateral branches just above the esophagus. The two branches are here considered to be real vessels and not "thoracic sinuses" (e.g., Petrunkevitch, 1922; Firstman, 1973) because they are prolongations of the aorta, surrounded by a membrane.

Concerning the anterior aorta major branching patterns, type AP1 (Fig. 6A) has been observed in all buthid species studied so far (Randall 1966; this study), as well as all vaejovid species, and *Superstitionia donensis* (Superstitioniidae) (see also Table 1).

AP2 has been observed in several other nonbuthid scorpions (Fig. 6B; see also Table 1), including the chactid, iurid, diplocentrid, liochelid, and scorpionid species examined for this study, two *Urodacus* species (Urodacidae) studied by Locket (2001), and a *Euscorpius* species (Euscorpiidae) studied by Schneider (1892). Several of these observations on the cheliceral arteries and the origin of the supraneural vessel were previously noted in three *Urodacus* species by Locket (2001), who described them as asymmetries in the circulatory system. However, based on our observations, Locket's (2001) perceived asymmetries in fact conform to AP2, which has been observed in several nonbuthid scorpion families besides Urodacidae.

Arterial Supply of Central Nervous System in Arthropods

Schneider (1892) first demonstrated the presence of transganglionic arteries in the ventral nerve

mass of scorpions. However, his descriptions of 10 transganglionic arteries cannot be corroborated. In the casts prepared for the present study, only nine transganglionic arteries were observed (see Fig. 4A), a number that corresponds exactly to the number of nerves which exit the thoracic nerve mass (cf. Hjelle, 1990): a pair of pedipalpal nerves; four pairs of pedal nerves; four pairs of mesosomal nerves. Petrunkevitch (1922) also described nine transganglionic arteries but did not mention the fine meshwork of small vessels in the central nerve masses. Therefore, although previously demonstrated (Petrunkevitch, 1922; Lane et al., 1981; Locket, 2001), our study visualizes the three-dimensional complexity of this system for the first time (Figs. 4B–E, 5B–D).

Lane et al. (1981) investigated the structure of the walls of the transganglionic and intraganglionic arteries ultrastructurally and electrophysiologically, and revealed that these arteries are true vessels because they are surrounded by an endothelial cell layer and a basement membrane. These authors further demonstrated that a complex blood-brain barrier occurs between the vessels and the central nerve masses. This blood-brain barrier appears to be of comparable complexity to the blood-brain barriers of vertebrates and not as perforated as those observed in Crustacea (Lane et al., 1981).

In general, the direct hemolymph supply of the CNS appears to be required by all animals in which oxygen is distributed via the hemolymph, as evidenced by similar vascular systems described in other chelicerates (e.g., Paul et al., 1994), Crustacea (e.g., Sandemann, 1967), and Scutigeromorpha (Chilopoda, Myriapoda; Wirkner and Pass, 2002).

Complex Open Circulatory System in Euarthropods

Corrosion-casting is a useful technique for modern studies of the vascular system, the applications of which have been extensively demonstrated among a selection of invertebrates in recent years (Locket, 2001; McGaw and Reiber, 2002; McGaw, 2005; Wirkner and Richter, 2007). Very complex three-dimensional vascular structures can be readily visualized using this technique in combination with SEM. The combination of injection methods with modern noninvasive and nondestructive techniques such as MicroCT is a further advancement (see also Wirkner and Richter, 2004).

All these methodological advances have greatly enhanced our understanding of the architecture and function of the circulatory system of arthropods. Although the circulatory system of euarthropods was once believed to be rather simple (e.g., Gruner, 1993; Brusca and Brusca, 2003), we now know that complex arterial systems, of comparable complexity to the arterial systems of vertebrates, prevail in at least three major groups, Chelicerata, Myriapoda

and Malacostraca. Even in hexapods with a reduced arterial system, i.e., HVS (Jones, 1977), this reduction is compensated by a great diversity of so-called accessory pulsatile organs, addressing the need to supply diverse appendages and tissues (Krenn and Pass, 1993; Pass, 1998, 2000). Not only do the vascular structures display a high level of organization, but the non-vascular or lacunar parts of the circulatory system do so as well. As with the vascular structures, corrosion-casting methods, in particular, demonstrate that the pathways taken by the hemolymph back to the pericardial sinus are distinct and can be traced repeatedly in different specimens and taxa. Examples include the complex venous hemolymph pathways of terrestrial crabs (e.g., Greenaway and Farrelly, 1984), the podo-pericardial sinuses of assorted malacostracan Crustacea (e.g., Wirkner and Richter, 2007; in press), and the book lung sinuses of Scorpiones (Fig. 1F; ps). Additionally, the functional organization, i.e., physiology of the circulatory system, especially in those taxa with an elaborated HVS, also reveals a high level of complexity (e.g., McMahon and Burnett, 1990 and literature therein). Indeed, this high level of organization has prompted some researchers to suggest that this complex circulatory system can no longer be termed "open" but "partially closed" or something intermediate (e.g., Paul et al., 1994; McGaw and Reiber, 2002; McGaw, 2005). Others point out, however, that the term "open" (e.g., Maynard, 1960; McMahon and Burnett, 1990) does not refer to the level of organization of the circulatory system but rather to its ontogenetic development as, in all arthropods, the primary and secondary body cavities fuse during ontogeny by mixocoely (see Jones, 1977; Scholtz, 2002; Mayer et al., 2004 and literature in therein), the resulting body fluid being termed hemolymph (as opposed to blood, being contained in a closed vascular system, e.g., in Annelida; Gruner, 1993). Furthermore, no evidence exists to suggest that the HVS is a closed system in any arthropod species studied. All true arteries, i.e., tubular structures connected with the central pumping structure (i.e. heart) and surrounded by a membrane, are open-ended, allowing the hemolymph to re-enter the mixocoel. The so-called "veins" described for scorpions (e.g., Dubuisson, 1925) are membranous structures which return the hemolymph to the pericardium rather than the heart proper. Their membranes are confluent with the membrane of the pericardium. Therefore, we strongly advocate use of the term "open" for the general type of circulatory system in Arthropoda. The demonstrated complexity of this system, however, will hopefully stimulate further studies of its fascinating structure and function.

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