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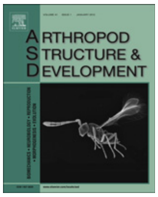

Evolutionary morphology of the hemolymph vascular system in scorpions: A character analysis

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ABSTRACT

Phylogenetically informative characters from the internal anatomy of scorpions were first reported more than 150 years ago, but the subject received little attention after the mid-1920s. Recent investigations, using traditional dissection, illustration and histological sectioning, microscopy, and innovative new methods for investigating complex soft tissue anatomy identified a new wealth of variation. Additionally, these investigations confirmed the phylogenetic significance of previously identified structures. Building on earlier work we present a more detailed description of the hemolymph vascular system (HVS) in scorpions, based on comparison of the vascular structures of the heart and the branching pattern of the prosomal arteries among 45 exemplar species representing most of the major scorpion lineages. Using corrosion-casting, MicroCT in combination with computer-aided 3D-reconstruction, and scanning electron microscopy, we conceptualize a series of phylogenetically informative characters for the anterior aorta system and characters of the heart and associated structures (e.g. arrangement of the ostia) of scorpions. Furthermore, we optimize the possible evolution of these new characters on a previous hypothesis of scorpion phylogeny, and discuss alternative character state transformations, their evolutionary consequences, and possible underlying evolutionary mechanisms acting on the HVS.

1. Introduction

Although scorpions are among the better-known orders of arachnids, their phylogenetic relationships continue to be debated. A thorough analysis, using up-to-date methods, all available sources of data, and including representatives of all major scorpion lineages remains to be presented (Prendini and Wheeler, 2005). The morphological characters thus far employed in analyses of scorpion phylogeny have been based almost entirely on external structures (e.g. trichobothrial patterns, spiniform setae on leg tarsi) although the potential importance of internal anatomy has long been known.

Phylogenetically informative characters from the internal anatomy of scorpions were first reported more than 150 years ago (Newport, 1843; Lankester et al., 1885; Laurie, 1890, 1891, 1896a, b; Schneider, 1892; MacClendon, 1904; Buxton, 1913, 1917; Pavlovsky, 1913, 1915a, b, c, d, 1917, 1922, 1924a, b, c, d, 1925, 1926; Petrunkevitch, 1922; Dubuisson, 1925; Pavlovsky and Zarin, 1926), but the subject received little attention after the mid-1920s. Early studies on scorpion internal anatomy were based on a fairly limited sample of taxa, however, and have not been thoroughly confirmed. Sometimes questionable observations were later incorporated into phylogenetic analyses, and occasionally generalized to taxa in which these findings had not been directly observed (Volschenk et al., 2008).

Recent investigations using traditional dissection, histological sectioning, microscopy (light microscopy, SEM, and TEM), and innovative new methods for investigating complex soft tissue anatomy, combining confocal laser-scanning microscopy with immunohistochemistry, corrosion-casting, MicroCT, and 3D-reconstruction, not only confirmed the importance of previously identified structures, but identified a wealth of new variation in the circulatory system (heart, arterial branching patterns), digestive system (digestive glands), lymphatic system (lateral lymphoid organs), nervous system (particularly the *corpora pedunculata* or 'brain'), respiratory system (book lung fine structure), male reproductive system (paraxial organ; hemispermatophore; sperm packages; sperm ultrastructure), female reproductive system (ovariuterus; genital atrium; mating plug), and venom glands (Farley, 1984; Horn and Achaval, 2002; Wolf and Harzsch, 2002; Jacob et al., 2004a, b; Prendini et al., 2006; Shultz, 2007; Wirkner

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and Prendini, 2007; Kamenz and Prendini, 2008; Lautié et al., 2008; Vignoli et al., 2008; Volschenk et al., 2008; Wolf, 2008; Michalik and Mercati, 2010; Vrech et al., 2011). A new, comparative understanding of scorpion anatomy will provide a revised terminology and additional characters, consistent with homology, for systematic studies of scorpions at all levels.

To this end we studied the hemolymph vascular system (HVS) to identify and conceptualize characters from the enormous complexity of vessels outlined by Wirkner and Prendini (2007), who presented the first comparative overview of the scorpion HVS based on representatives of most of the major lineages.

The hemolymph vascular system (HVS) is one of three major components of the hemolymph circulatory system (HCS), representing the entire flow system within the body of an arthropod. The other components of the HCS are the hemolymph lacunar system (HLS), consisting of sinuses and lacunae, and the hemolymph itself (see Wirkner, 2009 for details). The HVS comprises a central pumping structure, the heart, from which several arteries usually emanate. These arteries lead to different body regions and supply various tissues and organ systems with oxygenated hemolymph. The HLS consists of so-called lacunae, tissue-free spaces (i.e. the mixocoel) between organs, and sinuses, that are bordered by a membrane and function exclusively as hemolymph channels, but are not structurally connected to the HVS (e.g. Wirkner and Prendini, 2007, and citations therein).

Building on the work of Wirkner and Prendini (2007), we present a more detailed description of the vascular structures of the heart and the branching pattern of the prosomal arteries, and conceptualize a series of phylogenetically informative characters for the HVS. We optimize the possible evolution of these new characters on a previous hypothesis of scorpion phylogeny (Coddington et al., 2004), discuss alternative character state transformations, their evolutionary consequences, and possible underlying evolutionary mechanisms acting on the HVS.

2. Materials and methods

2.1. Taxon sample

Data were gathered from forty-five exemplar species (94 adult or subadult individuals of both sexes), representing thirteen families, thirty-five genera and most of the major lineages of scorpions (Table 1). The taxon sample included fourteen buthids, nine vaejovids, four scorpionids, four liochelids, three iurids, two

Table 1
Matrix of characters concerning the hemolymph vascular system scored in 45 exemplar scorpion species. Character states for *Urodacus* based on Locket (2001), states of characters 7, 9 and 13 for *Euscorpius carpathicus* on Kästner (1940), and of character 13 for *Uroctonus mordax* on Randall (1966). Character 6 in *B. keyserlingi* polymorphic: *=1,2.

Family	Species	Individuals (*n*)	`Characters`
Bothriuridae	*Bothriurus keyserlingi* Pocock, 1893	3	`0110?*100110?0`
Buthidae	*Androctonus amoreuxi* (Audouin, 1826)	2	`10000000000000`
Buthidae	*Androctonus australis* (Linnaeus, 1758)	2	`1000000?0000?0`
Buthidae	*Androctonus bicolor* Ehrenberg, 1828	2	`100000000000?0`
Buthidae	*Babycurus jacksoni* (Pocock, 1890)	2	`1000000?0000?0`
Buthidae	*Buthacus arenicola* (Simon, 1885)	2	`100000000000?0`
Buthidae	*Centruroides exilicauda* (Wood, 1863)	3	`1000000??00000`
Buthidae	*Centruroides gracilis* (Latreille, 1804)	1	`1??????????0??`
Buthidae	*Centruroides vittatus* (Say, 1821)	2	`1000000??000?0`
Buthidae	*Hottentotta conspersus* (Purcell, 1901)	2	`10????????0???`
Buthidae	*Leiurus quinquestriatus* (Ehrenberg, 1828)	2	`100000000000?0`
Buthidae	*Lychas mucronatus* (Fabricius, 1798)	2	`10000000000??0`
Buthidae	*Mesobuthus caucasicus* Nordmann, 1840	1	`1000000??00???`
Buthidae	*Mesobuthus martensii* (Karsch, 1879)	2	`1000000??000?0`
Buthidae	*Parabuthus leiosoma* (Ehrenberg, 1828)	2	`?00000000000?0`
Chactidae	*Brotheas granulatus* Simon, 1877	2	`00101110011??0`
Chactidae	*Broteochactas orinocensis* Scorza, 1954	1	`001011100?1??0`
Chaerilidae	*Chaerilus celebensis* Pocock, 1848	3	`0000?11?0000?0`
Diplocentridae	*Diplocentrus lindo* Stockwell & Baldwin, 2001	2	`?111122111110?0`
Diplocentridae	*Diplocentrus peloncillensis* Stahnke, 1970	1	`01111?2??????0`
Euscorpiidae	*Euscorpius carpathicus* (Linnaeus, 1767)	5	`0010111?011000`
Iuridae	*Anuroctonus phaiodactylus* Wood, 1863	2	`01111?2?2?1??0`
Iuridae	*Hadrurus arizonensis* Ewing, 1928	5	`0111112?2110?0`
Iuridae	*Iurus kraepelini* von Ubisch, 1922	1	`01101110011??0`
Liochelidae	*Hadogenes paucidens* Pocock, 1896	1	`?111122111 1??0`
Liochelidae	*Iomachus politus* Pocock, 1896	2	`?111?221111??0`
Liochelidae	*Opisthacanthus madagascariensis* Kraepelin, 1894	1	`?1111221111??0`
Liochelidae	*Opisthacanthus rugiceps* Pocock, 1897	1	`0111?22???1???`
Pseudochactidae	*Pseudochactas ovchinnikovi* Gromov, 1998	3	`0000??????????`
Scorpionidae	*Heterometrus laoticus* Couzijn, 1981	2	`0111112110?0?0`
Scorpionidae	*Opistophthalmus boehmi* (Kraepelin, 1896)	4	`01111121 1010?0`
Scorpionidae	*Pandinus cavimanus* (Pocock, 1888)	2	`0111112 11010?0`
Scorpionidae	*Pandinus imperator* (C.L. Koch, 1841)	2	`01111121 1010?0`
Scorpionidae	*Scorpio maurus* Linnaeus, 1758	1	`?111??2?10???0`
Superstitioniidae	*Superstitionia donensis* Stahnke, 1940	3	`00101?1001?0?0`
Urodacidae	*Urodacus* sp.	–	`?111112??1????`
Vaejovidae	*Paruroctonus silvestrii* Borelli, 1909	2	`011011???110?0`
Vaejovidae	*Serradigitus gertschi* (Williams, 1968)	1	`0110???????0??`
Vaejovidae	*Serradigitus joshuaensis* (Stahnke, 1940)	2	`?11??????1????`
Vaejovidae	*Serradigitus subtilimanus* (Williams, 1970)	2	`011011???????0`
Vaejovidae	*Smeringurus mesaensis* (Stahnke, 1961)	2	`011011???11???`
Vaejovidae	*Uroctonus mordax* Thorell, 1876	2	`0?101?100?1010`
Vaejovidae	*Vaejovis coahuilae* Williams, 1968	1	`0??????????0?0`
Vaejovidae	*Vaejovis confusus* Stahnke, 1940	2	`0110111?01?0?0`
Vaejovidae	*Vaejovis spinigerus* (Wood, 1863)	6	`01101110011 0?0`

diplocentrids, two chactids, a bothriurid, chaerilid, euscorpiid, pseudochactid and superstitioniid. Scorpion higher classification follows Prendini and Wheeler (2005).

2.2. *Corrosion casting*

In order to prepare casts of the circulatory system, the acrylic casting resin Mercox CL-2R/2B (Vilene Comp. Ltd, Tokyo, Japan) was injected into the heart of ether-anaesthetized specimens, using micropipettes (Hilsberg pipettes, diameter 1.0 mm, thickness 0.2 mm; pulled with a KOPF Puller 720). The resin was mixed with approximately 0.05 mg MA initiator (benzoyl peroxide) prior to injecting, and placed in a 5 ml syringe used to fill the pipettes. 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 heart. The specimens were left for several minutes after injecting, to allow polymerization and tempering of the resin. Specimens destined for the MicroCT were fixed in Bouin's fixative, contrasted with iodine (Metscher, 2009) and then critical-point-dried (BAL-TEC CPD 030; EMITECH K850). Alternatively, tissues were digested by immersion for 1–2 days 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).

2.3. *MicroCT*

X-ray imaging was performed at the University of Rostock with a Phoenix nanotom® (Phoenix|x-ray, GE Sensing & Inspection Technologies) High-resolution MicroCT system, in high-resolution mode, using the program *datos|x acquisition* (Target: Molybdenum, Mode: 0–1; Performance: ca. 8–13 W; Number of projections: 1080–1440; Detector-Timing: 1000–1500 ms; Voxelsize ca. 2–10 μm). A volume-file was generated using the software *datos|x reconstruction* and a stack of virtual sections exported with the software *VGStudio max*. See Wirkner and Richter (2004) for further details.

2.4. *3D reconstruction*

Stacks of virtual sections produced by MicroCT were used for 3D-reconstruction with *Imaris 7.0.0* software (Bitplane©). A scene was created in the program module "Surpass", and the volume-rendering function chosen to visualize the entire data set. The contours of organs studied were marked with polygons on each virtual cross-section, using the "Surfaces" function. The resulting 3D reconstructions are therefore termed "surface renderings". Different functions ("Isoline" and "Distance") were used for segmentation. Stacks of polygons were visualized by surface renderings. In order to assign colors to different objects, 3D-reconstructions were further subdivided into smaller objects (e.g. different arteries; Fig. 1) using the "Edit" module.

2.5. *Light-microscopy*

Some casts of large specimens were photographed with a Zeiss AxioCam ICc 1 CCD camera attached to a Zeiss Discovery.V12 stereomicroscope, using *AXIOVision 4.7.1* software. In each case, a z-stack, comprising ca. 30–50 images, was used to generate a fully-focused image (Mode: Wavelets; Quality: very high).

2.6. *Scanning electron microscopy (SEM)*

Casts for SEM were air-dried, coated with gold or palladium (BAL-TEC SCD 005; Leica SCD500), and studied using a SEM (LEO 1430; Zeiss DSM 960A), as described by Wirkner and Richter (2004). Corrosion casts were mounted on a specimen holder (Pohl, 2010) to achieve a homogeneous black background.

2.7. *Image processing*

Figures were arranged and prepared into plates using *Corel Graphics Suite X3*. Bitmap images were embedded into *Corel Draw X3* files and digitally edited with *Corel Photo Paint X3*.

2.8. *Character conceptualization and optimization*

By comparing the 3D visualizations obtained for different taxa, we were able to conceptualize characters concerning the branching pattern of prosomal arteries and the orientation of ostia and score them in a character matrix (Tables 1 and 2). These characters represent hypotheses of homology among taxa allocated the same character state (Hennig, 1966). Character states assigned to the genus *Urodacus* are based on Locket (2001). Some character states concerning *Euscorpius carpathicus* and *Uroctonus mordax* are based on Kästner (1940) and Randall (1966), respectively. Various characters could not be scored in particular taxa and therefore were assigned missing entries (?; see Table 1). In order to trace the transformation from one character state to another on the hypothesis of scorpion phylogeny presented by Coddington et al. (2004), we created a character matrix (Table 1) and optimized the characters with parsimony using *Mesquite Version 2.74* (Maddison and Maddison, 2010) and *WinClada Version 1.00.08* (Nixon, 2002).

3. Results

3.1. *The scorpion HVS*

As in all arachnids, the scorpion body is divided into the anterior prosoma and posterior opisthosoma, separated from one another by a muscular diaphragm (Hjelle, 1990). The opisthosoma comprises an anterior mesosoma and posterior metasoma (or 'cauda'). A telson with aculeus (sting) is attached to the metasoma posteriorly. The prosoma comprises nine segments, bears six pairs of appendages, and is covered by a carapace.

The main organ systems of the prosoma are the anterior digestive system, i.e. esophagus and stomach, a number of sensory organs such as the lateral and median eyes, and the prosomal ganglion. In accordance with Richter et al. (2010) we use the term "prosomal ganglion" instead of "cephalothoracic nerve mass" *sensu* Babu (1965). The term "cephalothoracic" may introduce confusion between the prosoma and the cephalothorax of certain crustaceans and the term "ganglion" is more precise than "nerve mass" (see Richter et al., 2010). The prosomal ganglion combines the dorsal supra- and the ventral subesophageal ganglia, which are connected by a broad pair of circumesophageal connectives (Horn and Achaval, 2002).

The tubular heart of scorpions is located in the dorsal midline of the mesosoma and surrounded by a sac-like pericardium. It is equipped with seven pairs of valve-like ostia and six or eight pairs of cardiac arteries (Kästner, 1940; Randall, 1966; Wirkner and Prendini, 2007), which supply the various organs of the mesosoma. Hypocardial ligaments suspend the heart from the ventral body wall. Eight (not seven; Wirkner and Prendini, 2007) pairs of ligaments were observed in all taxa studied thus far. The heart is connected posteriorly to the posterior aorta, which runs through the entire metasoma and telson to the aculeus. The heart extends anteriorly into the anterior aorta. The former is separated from the latter by a flap-like valve at the position where the dorsal vessel passes through the diaphragm, and descends obliquely towards the thin esophagus.

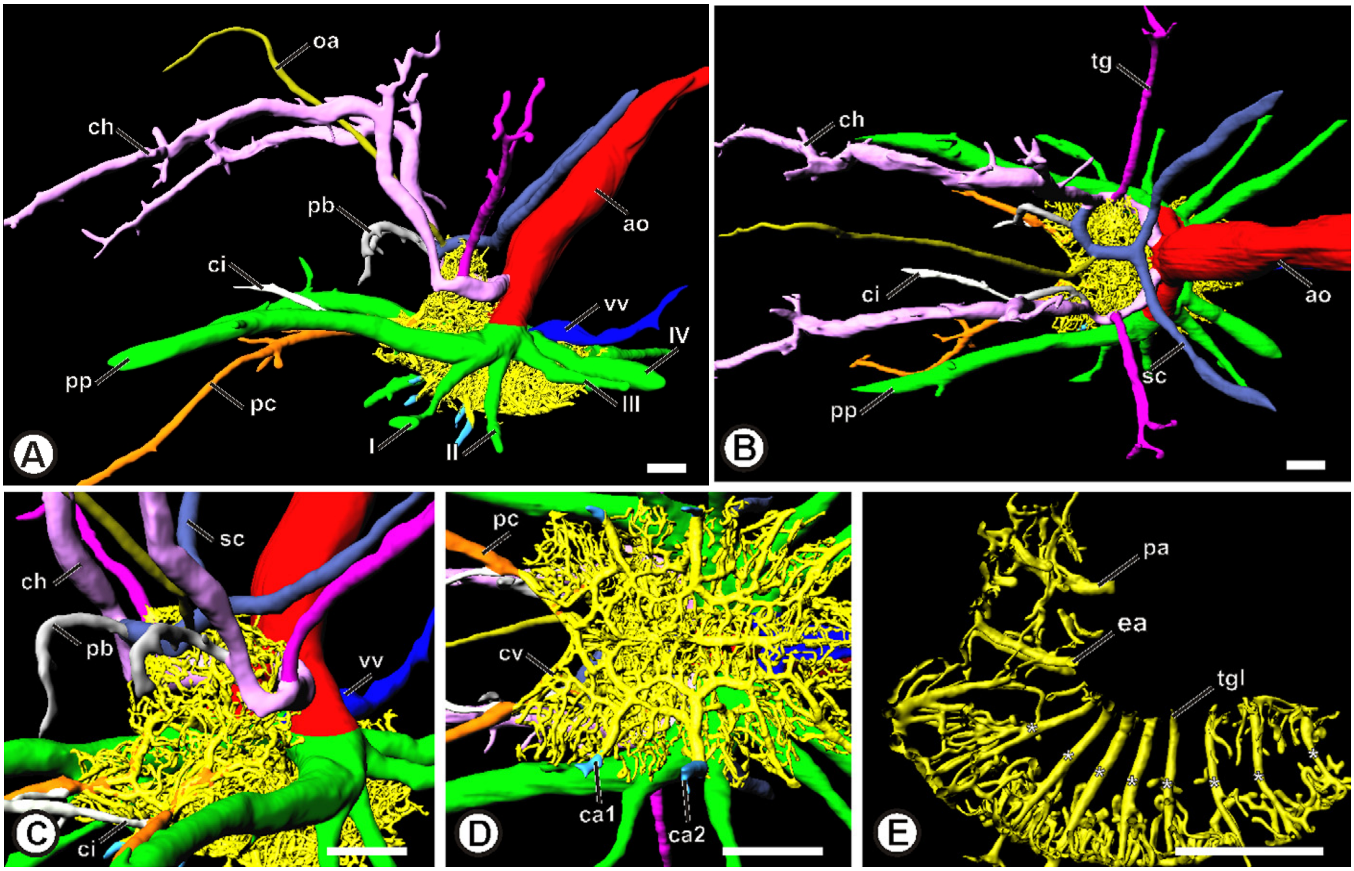


Fig. 1. General morphology of prosomal arteries in *Brotheas granulatus*, Chactidae. Scale bars: 500 μm. **A**–**D**. Surface renderings of anterior aorta system. **A**. The web version of this article contains interactive 3D content. Please click in the PDF file on the figure to activate the content and then use the mouse to rotate the objects. Use the menu in the activated figure to use further functions. Lateral view, anterior at left. Different arterial subsystems indicated with different colors. **B**. Dorsal view. **C**. Anterolateral view. **D**. Ventral view. Numbers indicate coxapophyses to which each artery corresponds. **E**. Surface rendering of median prosomal ganglionic vasculature, lateral view. Asterisks indicate eight transganglionic arteries (tgl); the rest of the cerebral vasculature (cv) has been removed. Abbreviations: ao, aorta; ca, coxapophysal artery; ch, cheliceral artery; ci, coxinternal artery; cv, cerebral vasculature; ea, esophageal artery; oa, optic artery; pa, protocerebral artery; pb, peribuccal artery; pc, pedipalpocoxal artery; pp, pedipalpal artery; sc, supracerebral artery; tg, tegumental artery; tgl, transganglionic artery; vv, ventral vessel; I–IV, arteries of legs I–IV.

Table 2
List of characters concerning the hemolymph vascular system scored in 45 exemplar species representing most of the major lineages of extant scorpions.

Character 1. Ostia, orientation: opposite (0); alternating (1).
Character 2. Cheliceral arteries, origin: shared (0); separate (1).
Character 3. Arteries of pedipalps and first two legs, branching pattern: common trunk on pedipalps and first pair of walking legs (0); common trunk on pedipalps and first two pairs of walking legs (1).
Character 4. Ventral vessel, connection: connection to both lateral trunks (0); connection to one lateral trunk (1).
Character 5. Supracerebral artery, branching pattern: paired (0); unpaired (1).
Character 6. Optic arteries, branching pattern: cheliceral arteries bifurcate (0); branch off supracerebral artery (1); branch off one cheliceral artery (2).
Character 7. Pedipalpocoxal arteries, origin: in cerebral vessel meshwork with direct connection to cheliceral arteries (0); in cerebral vessel meshwork without connection to cheliceral arteries (1); branch off pedipalpal arteries (2).
Character 8. Coxinternal artery, origin: branches off pedipalpocoxal arteries (0); emanates from pedipalpal artery (1).
Character 9. Coxapophysal arteries, origin: emanates from cerebral vessel meshwork (0); branches off from arteries of first pair of walking legs (1); branches off from arteries of pedipalps and first pair of walking legs (2).
Character 10. Tegumental arteries: absent (0); present (1).
Character 11. Peribuccal arteries: absent (0); present (1).
Character 12. Ostia, number of pairs: 7 (0)
Character 13. Cardiac arteries, number of pairs: 8 (0); 6 (1)
Character 14. Transganglionic arteries, number: 9 (0)

The following description focuses on the branching pattern of the prosomal arteries in the chactid, *Brotheas granulatus*, as an example of the arterial subsystems of the anterior aorta. We used this species as a representative because all described arterial systems in the prosoma (e.g. tegumental arteries; see below) were present in the specimens examined.

Cheliceral arteries (ch) branch off the anterior aorta at the level of the supraesophageal ganglion, extending into the chelicerae in a stretched S-shape (Fig. 1A and B). Along the way, these arteries give rise to several other arteries, which supply the dorsal musculature, anterior parts of the prosomal digestive glands, and the median ocelli. Tegumental arteries (tg) branch off laterally to the supraesophageal ganglion and travel in a dorsolateral direction (Fig. 1B), ramifying distally into many smaller vessels that supply diverse muscles and the tegument.

The supracerebral artery branches off either from the right or left cheliceral artery at the point where the cheliceral arteries bend upwards. It then turns and extends to the top of the supraesophageal ganglion in a posterior direction before splitting into two lateral branches anterior to the aortic trunk. The two lateral branches of the supracerebral artery (sc) travel in a posterodorsal direction to supply the posterodorsal musculature of the prosoma and the prosomal digestive glands (Fig. 1B). The dilation musculature of the stomach is supplied by two arteries, one emanating from the cheliceral artery, the other from the supracerebral artery

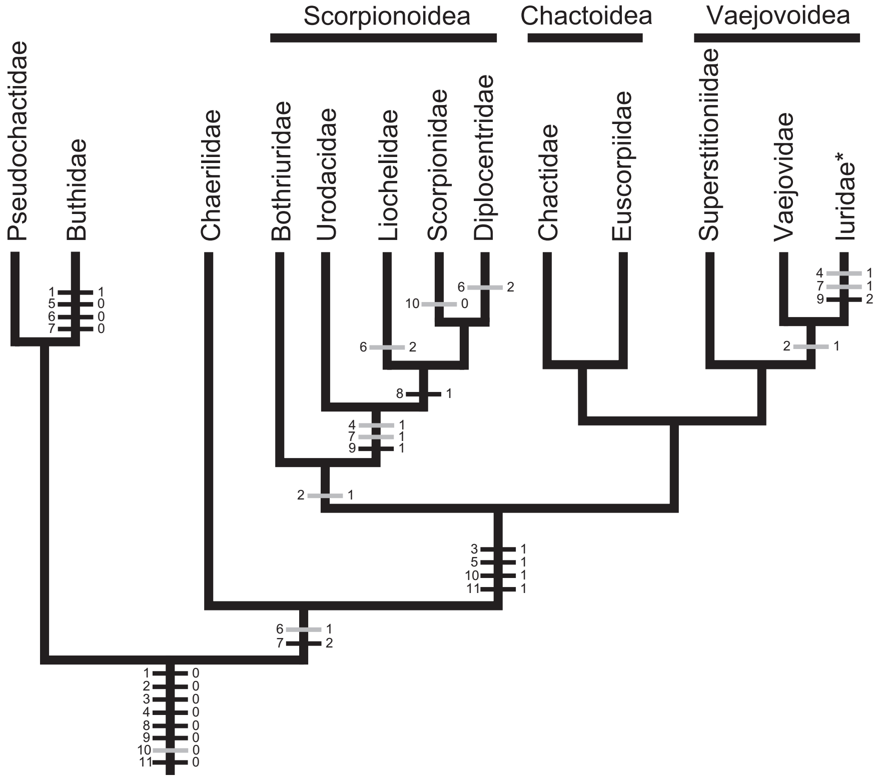


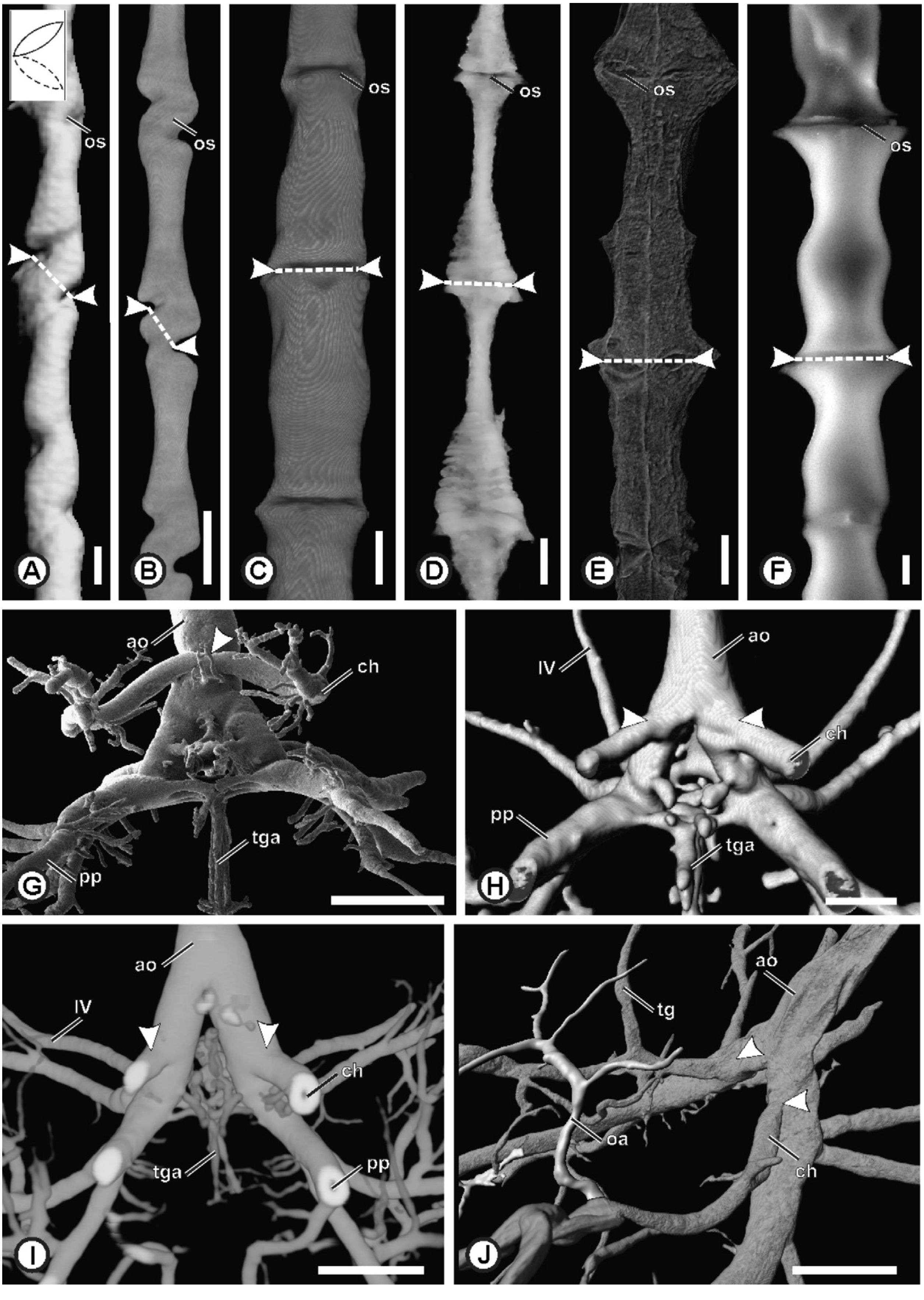
Fig. 2. Hypothesis of scorpion phylogeny modified from Coddington et al. (2004), with eleven characters concerning hemolymph vascular system optimized. Thin horizontal black bars at nodes indicate uniquely derived apomorphic character states, whereas thin horizontal gray bars indicate parallel derivations of apomorphic states. Numbers to left and right of bars indicate characters and states, respectively (see Table 1 for details). *Character statements for Iuridae correspond to Hadrurinae *sensu* Stockwell (1989).

(Fig. 1D; pb). The peribuccal arteries travel in a semi-circle over the dilation musculature of the stomach which is arranged horizontally between each side of the stomach and the epistome. The optic arteries (oa) branch off from the supracerebral artery, travel to the median ocelli, and supply the retinae (Fig. 1A).

After the cheliceral arteries have branched off, the anterior aorta splits over the thin esophagus into two lateral trunks, which subsequently give rise laterally to the arteries of the pedipalps and walking legs (Fig. 1A; pp, I–IV). After these arteries have branched off, the lateral trunks of the aorta curve backwards and give rise to the ventral vessel (vv). This vessel extends along the entire opisthosoma, on the dorsal side of the ventral nerve cord (Fig. 1A), and is therefore sometimes called the “supraneural artery” (Locket, 2001). Along the way, smaller vessels branch off from the ventral vessel to supply the free ganglia of the ventral nerve cord. At each ganglion, four vessels branch off the ventral vessel, two surrounding it laterally and two surrounding it anteriorly and posteriorly (see supplement).

The prosomal ganglion (PG), is supplied by a dense vascular network (Fig. 1D; cv, cerebral vasculature in the figures) that emanates from the anterior aorta system at various points. These fine vessels reticulate within the PG forming numerous anastomoses (for a detailed description of the ultrastructure of the cerebral vasculature see Lane et al., 1981). The protocerebral artery (Fig. 1E; pa) in the supraesophageal ganglion and the nine transganglionic arteries (Fig. 1E; tgl) in the subesophageal ganglion are the most prominent arteries traveling into the PG. The protocerebral artery branches off medially between the origin of the cheliceral arteries and travels anteriorly into the protocerebrum. Paired additional arteries emanate from the lateral trunks of the anterior aorta and travel anteriorly along the esophagus (Fig. 1E; ea). The posteriorly directed parts of the two aortic trunks are connected medially by five vessels, each of which gives rise to an artery that travels ventrally in the midline of the subesophageal ganglion. Four more posterior transganglionic arteries branch off the ventral vessel. The posterior-most of these arteries does not travel through the subesophageal ganglion but travels ventrally at the posterior border of the ganglion. This relatively thick artery gives rise to the paired pectinal arteries which travel posteroventrally into the pectines (not shown). Several other arteries also branch off the cerebral vasculature. The pedipalpocoxal arteries (pc) emanate from the cerebral vascular network (Fig. 1A, D) and travel anteriorly to the anteroventral parts of the coxa, supplying the musculature located there. The coxinternal artery (ci) branches off from these arteries (Fig. 1B, C), supplying the horizontally-arranged musculature between the coxae of the pedipalps. The coxapophysal arteries (ca 1 + 2) emanate from the ventral side of the cerebral vascular network (Fig. 1D) and travel into the coxapophyses of the first and second pairs of walking legs, where the so-called maxillary glands (Hjelle, 1990; p. 51) are located.

The above descriptions agree favorably with previous work on the anatomy of the scorpion circulatory system (e.g. Schneider, 1892; Petrunkevitch, 1922; Dubuisson, 1925; Kästner, 1940; Locket, 2001; Wirkner and Prendini, 2007). Wirkner and Prendini (2007: p. 410) described two general branching patterns (their AP1 and AP2) of the anterior aorta system, but noted some particular variations. We confirm their findings, but the characteristics of the two patterns occur in different combinations. For example, cheliceral arteries without a shared origin and the ventral vessel with a connection to both of the lateral trunks may be present in the same taxon. We coded characteristics of the aortic branching patterns described by Wirkner and Prendini (2007) into phylogenetic characters, and renamed several arteries. The peribuccal arteries of Wirkner and Prendini (2007) were renamed


os
A
os
B
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C
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D
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"pedipalpocoxal arteries", because they supply musculature in anterior parts of the pedipalpal coxa. Arteries supplying the dilation musculature of the stomach were termed "peribuccal arteries".

3.2. Characters and optimizations

In this section, we conceptualize ten new characters pertaining to the branching pattern of the prosomal arteries, two characters concerning the orientation and number of the ostia, and one character concerning the number of heart arteries (Tables 1 and 2), and discuss their optimization on the phylogenetic hypothesis of Coddington et al. (2004), indicated in Fig. 2. For simplicity we use supraordinal taxon names for groupings of studied terminals, e.g. Vaejovidae for all the studied *Paruroctonus, Serradigitus, Smeringurus, Uroctonus,* and *Vaejovis* species (see Table 1).

3.2.1. Orientation of ostia

Ostia (os) are valve-like openings, located on the dorsal surface of the heart, and through these ostia the hemolymph enters the heart lumen during diastole. All scorpion taxa studied thus far exhibit seven pairs of ostia. Randall (1966) and Wirkner and Prendini (2007) described two different arrangements of the ostia with respect to one another in each pair along the body axis (Character 1). In Buthidae, the ostia of each pair alternate along the body axis (Fig. 3A, B) whereas, in all non-buthid taxa examined, the ostia of each pair are opposite one another (Fig. 3C–F).

When optimized on the phylogeny of Coddington et al. (2004), ostia situated opposite each other are resolved as ancestral. Alternating ostia are synapomorphic for Buthidae.

Character 1. Ostia, orientation: opposite (0); alternating (1).

3.2.2. Cheliceral arteries

The cheliceral arteries (ch) supply the musculature within these appendages. Two distinct branching patterns have been identified. The first pattern, observed in buthids, pseudochactids, chaerilids and chactoids, is characterized by a single common base at the anterior aorta splitting into the two cheliceral branches. Afterwards, the anterior aorta splits into two lateral trunks (Fig. 3G). In the second pattern, observed in iurids, most vaejovids and scorpionoids, the anterior aorta splits directly into two lateral trunks, each of which gives rise to one cheliceral artery (Fig. 3H–J). The second pattern can be expressed in two ways: in the vaejovid exemplars studied, the arteries emanate at the point where the aorta splits (Fig. 3H) whereas in the iurid and scorpionoid exemplars, the arteries emanate after the aorta splits. In both cases, the arteries have no shared origin.

In the phylogenetic optimization (Fig. 2), cheliceral arteries with a shared origin are plesiomorphic in scorpions. Cheliceral arteries without a shared origin evolved independently on two occasions, first in the clade comprising Iuridae and Vaejovidae, and second in Scorpionoidea s. l. (i.e. including Bothriuridae). The transition between the two character states is ambiguous in Vaejovidae, because the condition in *U. mordax* is difficult to determine. *Uroctonus mordax* was scored unknown (?) in the character matrix (Table 1), because the origins of the cheliceral arteries are very close together in this species.

Character 2. Cheliceral arteries, origin: shared (0); separate (1).

3.2.3. Branching pattern of arteries in the pedipalps and first two pairs of legs

The branching pattern of the first three arteries supplying the prosomal extremities differs regarding whether only two, or all three share a common trunk. In the first condition, the arteries of the pedipalps (pp) and first pair of walking legs (I) share a common origin whereas the artery of the second pair of legs branches off the lateral trunk independently (Fig. 4A). This condition, observed in Buthidae, Pseudochactidae, and Chaerilidae, is plesiomorphic in scorpions (Fig. 2). In the second condition, observed in all other taxa examined, all three arteries (pp, I + II) share a common origin (Fig. 4B–D). In the scorpionoid exemplars studied, the arteries of the pedipalps appear to extend the lateral trunks of the aorta directly into the pedipalps and the arteries of the walking legs branch off them (Fig. 4D). In Scorpionoidea s. str. (i.e. excluding Bothriuridae), the arteries of the third and fourth pairs of walking legs also share a common trunk. The shared origin of the arteries of the pedipalps and first two pairs of legs is synapomorphic for the clade including all non-buthid taxa except Chaerilidae and Pseudochactidae (Fig. 2).

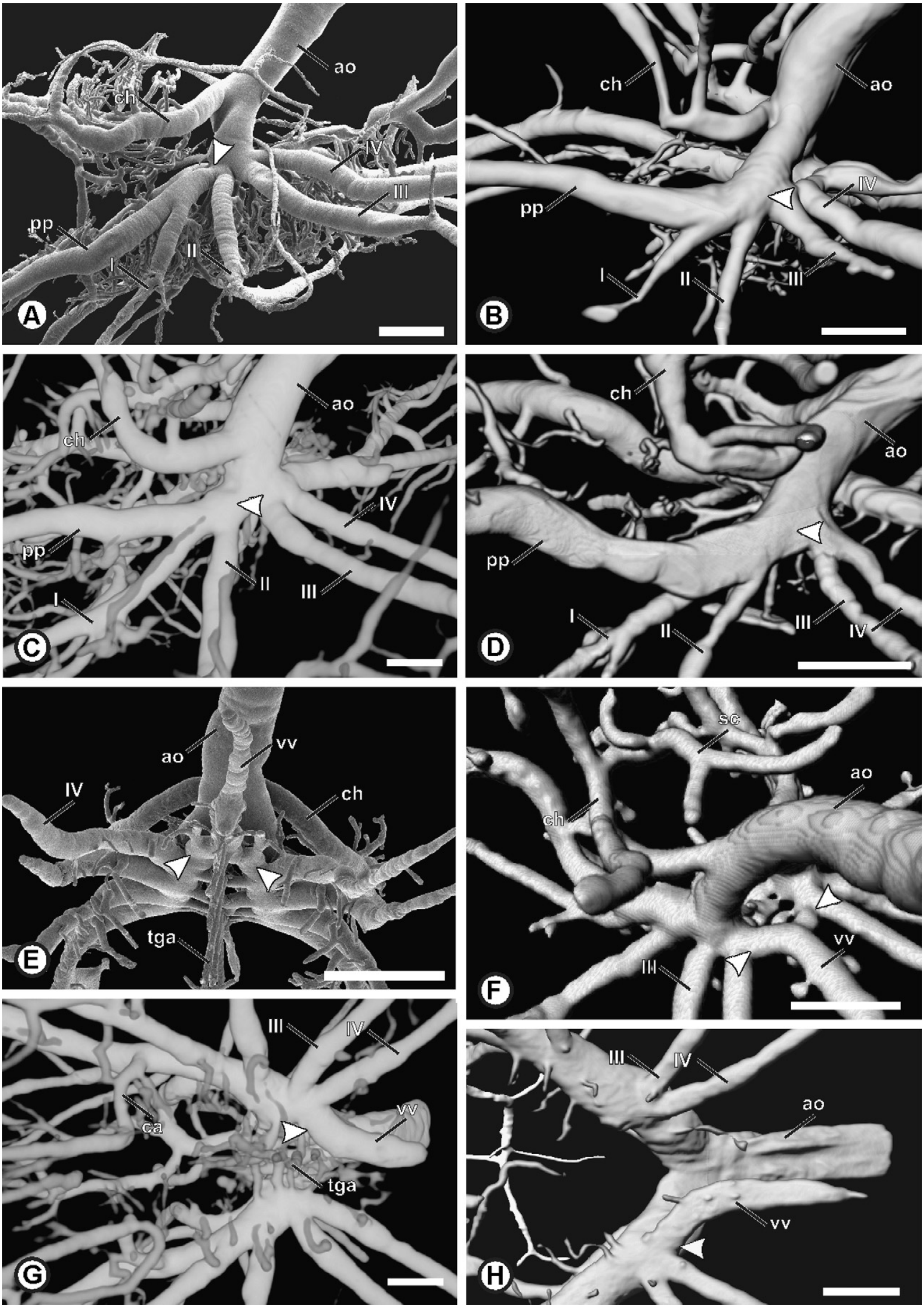
Character 3. Arteries of pedipalps and first two legs, branching pattern: common trunk on pedipalps and first pair of walking legs (0); common trunk on pedipalps and first two pairs of walking legs (1).

3.2.4. Connection of the ventral vessel

The ventral vessel (vv) travels in a posterior direction throughout the entire opisthosoma, above the ventral nerve cord which it supplies. After the lateral trunks of the anterior aorta have given rise to the arteries of the pedipalps and walking legs, two different patterns may be observed. Either both lateral trunks reunite, forming an unpaired ventral vessel that extends posteriorly, as observed in buthids, pseudochactids, chaerilids, chactoids, vaejovids and bothriurids (Fig. 4E, F), or only one lateral trunk gives rise to the ventral vessel, as observed in *Anuroctonus phaiodactylus*, *Hadrurus arizonensis*, diplocentrids, liochelids, scorpionids and urodacids (Fig. 4G, H). Which of the lateral trunks makes this connection may vary even within one species. Locket (2001) first described this variation in a species of *Urodacus* and we confirmed his observations in other scorpionoid genera. Despite this variation, the ventral vessel consistently emanates from only one of the lateral trunks of the anterior aorta.

A ventral vessel originating from both trunks is plesiomorphic in extant scorpions and the loss of one connection evolved twice independently, first in *A. phaiodactylus* and *H. arizonensis*, providing a potential synapomorphy for Hadrurinae (Stockwell, 1989), and second in Scorpionoidea s. str. (Fig. 2). The double-sided connection of the ventral vessel in bothriurids supports the widely accepted

Fig. 3. Comparison of ostial orientation (**A**–**F**; os) and origin of cheliceral arteries (**G**–**J**; ch) in selected scorpion taxa. Scale bars: 500 μm. **A**–**F**. Dorsal view. Anterior at top. Dashed line indicates orientation of one pair of ostia; arrowheads indicate positions of ostia. **A**. *Leiurus quinquestriatus*, Buthidae, surface rendering of heart lumen. **B**. *Centruroides vittatus*, Buthidae, volume rendering of heart lumen. **C**. *Uroctonus mordax*, Vaejovidae, volume rendering of heart lumen. **D**. *Brotheas granulatus*, Chactidae, corrosion cast of heart lumen. **E**. *Bothriurus keyserlingi*, Bothriuridae, volume rendering of the heart. **F**. *Pandinus imperator*, Scorpionidae, corrosion cast of heart lumen. **G**–**J**. Arrowheads indicate origins of cheliceral arteries (ch). **G**. *Centruroides exilicauda*, Buthidae, scanning electron micrograph of corrosion cast of anterior aorta system. Arrowhead indicates shared origin of cheliceral arteries. **H**. *Paruroctonus silvestrii*, Vaejovidae, surface rendering of anterior aorta system. **I**. *Hadrurus arizonensis*, Iuridae, volume rendering of anterior aorta system. Parts anterior to viewing-plane sliced away. **G**–**I**. Anterior view. **J**. *Iomachus politus*, Liochelidae, volume rendering of anterior aorta system, including surface rendering of optic artery, anterolateral view. Abbreviations: ao, aorta; ch, cheliceral artery; oa, optic artery; os, ostium; pp, pedipalpal artery; tg, tegumental artery; tga, transganglionic arteries; IV, artery of leg IV.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opinion that the katoikogenic taxa (Scorpionoidea s. str.) are monophyletic (Prendini, 2000; Coddington et al., 2004).

Character 4. Ventral vessel, connection: connection to both lateral trunks (0); connection to one lateral trunk (1).

3.2.5. Supracerebral artery

Several arteries branch off the cheliceral arteries *en route* to the chelicerae. Among these, one or more supracerebral arteries (sc) extend into the posterodorsal region of the prosoma, supplying the musculature and prosomal digestive glands. Two distinct branching patterns can be observed in the supracerebral arteries. In Buthidae, there is a pair of arteries, one of which branches off each cheliceral artery at the dorsal-most point, curves backwards, and then travels posteriorly through the prosoma (Fig. 5A; arrowheads). In the non-buthid taxa thus far studied, the artery is unpaired, branching off one of the cheliceral arteries (Fig. 5B, C), either the left or right, which may vary within one species. In the unpaired condition, the supracerebral artery initially makes a U-turn and travels dorsal to the supraesophageal ganglion in a posterior direction (Fig. 1A) but then, just anterior to the anterior aorta, it splits and each branch runs dorsolaterally alongside the anterior aorta.

The ancestral condition for scorpions is ambiguous: paired supracerebral arteries are reconstructed as synapomorphic for Buthidae, unpaired arteries as synapomorphic for non-buthids excluding Chaerilidae and Pseudochactidae (Fig. 2), in which the condition is unknown (Table 1).

Some unique differences in the branching pattern of the supracerebral artery were observed in three taxa. In one scorpionid, *Scorpio maurus*, an artery branches off the cheliceral artery in the same position, and follows the same course as described for the unpaired condition, but without splitting anterior to the aortic trunk. Additionally, a second artery emanates from the same lateral trunk of the anterior aorta from which the other artery branched off, curves backwards and runs posterodorsally on the same side as it branched off. Unfortunately, we examined only one individual of this species and therefore coded *S. maurus* unknown (?) for this character (Table 1).

Another pattern was observed in one specimen of *Bothriurus keyserlingi*: at the point where the supracerebral artery emanates in other species, another artery also emanates but travels anteriorly. An optic artery emanates further along this artery (see below). No supracerebral artery was observed in the other specimens examined, however, because the resin did not fill the artery sufficiently. We therefore scored this character unknown (?) in the character matrix (Table 1), pending further investigation.

Additionally, a tendency towards reduction of the supracerebral artery was observed in Liochelidae. The artery was unmodified in *Hadogenes paucidens* but greatly reduced, only a small vessel branching off one cheliceral artery and traveling in a posterior direction, in *Iomachus politus* (Fig. 5D). Neither condition was differentiated into a distinct state, however, pending further investigation.

Character 5. Supracerebral artery, branching pattern: paired (0); unpaired (1).

3.2.6. Optic arteries

The arteries of the median ocelli (oa) are always associated with the cheliceral arteries or the arteries branching off them (e.g. the supracerebral artery). They supply the median ocelli and optic nerves which extend from the ocelli into the optic lobes. Three branching patterns of the optic arteries were identified. In the first pattern, observed in Buthidae, each cheliceral artery bifurcates lateral to the brain into the vessel that extends into the chelicerae and the optic artery (Fig. 5E). The inner vessel first curves medially and then dorsally to the median ocelli. In the second pattern, observed in chactoids, vaejovids, some iurids, urodacids and scorpionids, the optic arteries (one or two) branch off the supracerebral artery dorsal to the supraesophageal ganglion (Fig. 5F) and, depending on the position of the ocelli, travel dorsally or anterodorsally. In the third pattern, observed in diplocentrids and liochelids, one optic artery branches laterally off the cheliceral artery opposite the supracerebral artery and extends to the median ocelli (Fig. 5D). The optic arteries travel anteromedially through the median ocelli, in the second and third patterns.

The ancestral state of this character is again ambiguous: the first state was reconstructed as synapomorphic for Buthidae, the second as synapomorphic for non-buthids (Fig. 2).

The second state is plesiomorphic for Scorpionoidea, while the third state evolved independently in Diplocentridae and Liochelidae.

Both character states were observed in one specimen of *B. keyserlingi*. One optic artery branched off the supracerebral artery (see above), traveling posteriorly to the median ocelli, and another branched off one of the cheliceral arteries. No supracerebral artery could be observed in the other specimens examined because the resin did not fill the artery sufficiently. We therefore scored the character polymorphic in *B. keyserlingi* (Table 1).

A further unique condition was observed in *S. maurus*, where one optic artery branches off each cheliceral artery on the posterior side, but we refrained from defining an additional state for this taxon pending observation in additional specimens.

Character 6. Optic arteries, branching pattern: cheliceral arteries bifurcate (0); branch off supracerebral artery (1); branch off one cheliceral artery (2).

3.2.7. Pedipalpocoxal arteries

The pedipalpocoxal arteries (pc) ("peribuccal arteries" *sensu* Wirkner and Prendini, 2007) travel into the anteroventral parts of the pedipalpal coxa, supplying the musculature located there. There are three different branching patterns concerning these arteries. In Buthidae, the pedipalpocoxal arteries emanate from the cerebral vascular network (cv) and are directly connected to the cheliceral arteries. The pedipalpocoxal arteries branch off at the position where the cheliceral arteries bend dorsally, and travel mid-ventrally before uniting (Fig. 6A, arrow). This common trunk is connected to the cerebral vasculature and gives rise to the arteries of the pedipalpal coxa, which travel in an anterolateral direction (Fig. 6A). In chactoids, vaejovids, and bothriurids, the pedipalpocoxal arteries also originate in the cerebral vasculature but are not directly connected to the cheliceral arteries (Fig. 6B, C). The

Fig. 4. Comparison of branching pattern of arteries of pedipalps and first two pairs of walking legs (**A**–**D**) and connection of ventral vessel (**E**–**H**; vv) in selected scorpion taxa. Scale bars: 500 μm. **A**–**D**. Lateral view, anterior at left. Arrowheads indicate shared origin of arteries. **A**. *Lychas mucronatus*, Buthidae, scanning electron micrograph of corrosion cast of anterior aorta system. **B**. *Brotheas granulatus*, Chactidae, volume rendering of anterior aorta system. **C**. *Hadrurus arizonensis*, Iuridae, volume rendering of anterior aorta system. **D**. *Diplocentrus lindo*, Diplocentridae, volume rendering of anterior aorta system. **E**–**H**. Arrowheads indicate connection of ventral vessel (vv). **E**. *Centruroides exilicauda*, Buthidae, scanning electron micrograph of corrosion cast of anterior aorta system, posteroventral view. **F**. *Paruroctonus silvestrii*, Vaejovidae, surface rendering of anterior aorta system, posterolateral view. **G**. *H. arizonensis*, volume rendering of anterior aorta system, ventral view. **H**. *Iomachus politus*, Liochelidae, volume rendering of anterior aorta system, ventral view. Abbreviations: ao, aorta; ch, cheliceral artery; pp, pedipalpal artery; sc, supracerebral artery; tga, transganglionic artery; vv, ventral vessel; I–IV, arteries of legs I–IV.

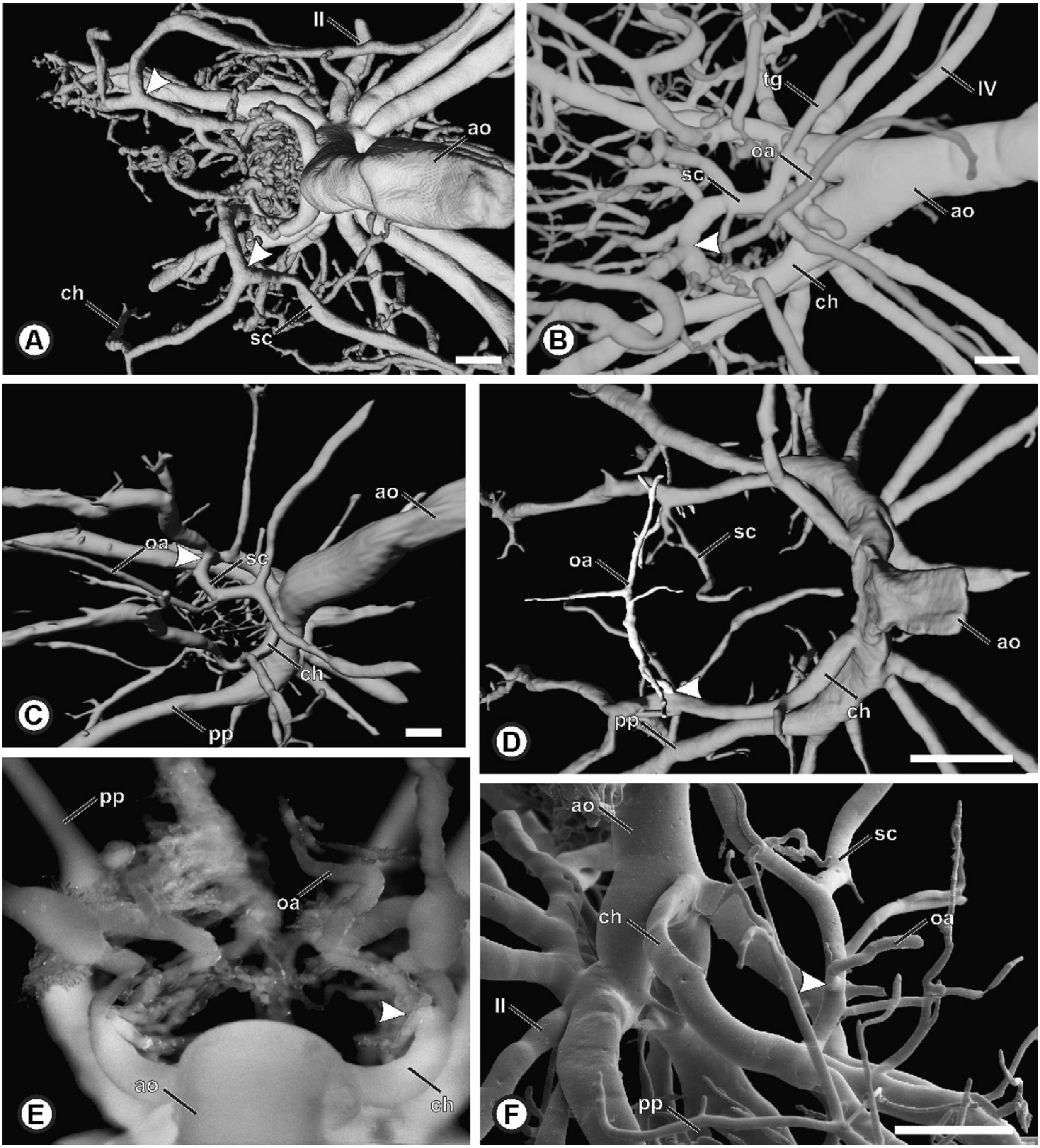

Fig. 5. Comparison of branching patterns of supracerebral arteries (**A**–**C**; sc) and optic arteries (**D**–**F**; oa) in selected scorpion taxa. Scale bars: 500 µm. **A**–**C**. Arrowheads indicate branching point of supracerebral arteries (sc). **A**. *Parabuthus leiosoma*, Buthidae, volume rendering of anterior aorta system, dorsal view. **B**. *Hadrurus arizonensis*, Iuridae, volume rendering of anterior aorta system, dorsal view. **C**. *Brotheas granulatus*, Chactidae, volume rendering of anterior aorta system, dorsolateral view. **D**–**F**. Arrowheads indicate branching point of optic arteries (oa). **D**. *Iomachus politus*, Liochelidae, volume rendering of anterior aorta system, dorsal view. **E**. *Leiurus quinquestriatus*, Buthidae, light microscopy image of a corrosion cast of anterior aorta system, dorsal view. **F**. *Euscorpius carpathicus*, Euscorpiidae, scanning electron micrograph of corrosion cast of anterior aorta system, lateral view, anterior at right. Abbreviations: ao, aorta; ch, cheliceral artery; oa, optic artery; pp, pedipalpal artery; sc, supracerebral artery; tg, tegumental artery; I–IV, arteries of legs I–IV.

arteries of the pedipalpal coxa branch off the pedipalpal arteries in *A. phaiodactylus*, *H. arizonensis* and the scorpionoids (excluding bothriurids) thus far examined (Fig. 6D).

The plesiomorphic condition of the origin of the pedipalpocoxal arteries could not be resolved unambiguously (Fig. 2). Pedipalpocoxal arteries emanating from the cerebral vasculature and directly connected to the cheliceral arteries was reconstructed as synapomorphic for Buthidae; pedipalpocoxal arteries emanating from the cerebral vasculature without a direct connection to the cheliceral arteries as synapomorphic for the clade comprising most non-buthids (the condition is unknown in Pseudochactidae); and pedipalpocoxal arteries branching off the pedipalpal arteries as synapomorphic for Scorpionoidea s. str. with an independent derivation in the iurid exemplars (*A. phaiodactylus* and

H. arizonensis), providing a potential synapomorphy for Hadrurinae (Stockwell, 1989).

Character 7. Pedipalpocoxal arteries, origin: in cerebral vessel meshwork with direct connection to cheliceral arteries (0); in cerebral vessel meshwork without connection to cheliceral arteries (1); branch off pedipalpal arteries (2).

3.2.8. Coxinternal arteries

The coxinternal arteries (ci) supply the strong horizontally-arranged musculature between the coxae of the pedipalps, located inside the so-called labrum (*sensu* Shultz, 2007), and are always associated with the pedipalpocoxal arteries. The coxinternal arteries either branch off the arteries of the pedipalpal coxa, as observed in buthids, chactoids and vaejovids (Fig. 6B, C; arrow), or emanate from one pedipalpal artery at the same position as one of the pedipalpocoxal arteries, as observed in diplocentrids, liochelids and scorpionids (see above; Fig. 6D–F). In the latter case, the coxinternal artery is thicker, with a more defined course, traveling in a stretched S-shape between the two coxae, than in the former.

Coxinternal arteries emanating from the pedipalpocoxal arteries are plesiomorphic in scorpions, whereas coxinternal arteries branching off the pedipalpal artery are apomorphic in Scorpionoidea s. str., having evolved once in the clade comprising diplocentrids, liochelids and scorpionids, and perhaps other katoikogenic scorpionoid taxa, but could not be resolved unambiguously because the condition of this character is presently unknown in Urodacidae (Table 1).

Character 8. Coxinternal artery, origin: branches off pedipalpocoxal arteries (0); emanates from pedipalpal artery (1).

3.2.9. Coxapophysal arteries

The coxapophyses are outgrowths of the coxae of the first and second pairs of walking legs that enclose the preoral chamber (i.e. stomotheca *sensu* Shultz, 2007) ventrally. They contain the so-called maxillary glands (Hjelle, 1990; p. 51), supplied by vessels that branch off from three different arterial systems. Three distinct patterns were identified. In the first pattern, observed in buthids, chactoids, vaejovids, and bothriurids, the two pairs of coxapophysal arteries (ca) emanate from the ventral vascular network inside the subesophageal ganglion (Fig. 7A; cv), the start of these arteries lies generally ventral to the nerve of the corresponding leg (i.e. legs I and II), and each artery runs anteroventrally into the apophyses. In the second pattern, observed in scorpionoids (excluding bothriurids), the coxapophysal arteries branch off the arteries of the first pair of legs at the point where the coxa inserts at the prosoma (Fig. 7C), and possess a common trunk which splits immediately on one side, and after a short distance on the other, into a pair of branches each extending into the corresponding apophyses (Fig. 7C; arrowheads). A third condition, where the arteries of the coxapophyses branch off the arteries of the pedipalps and first pair of legs, is observed in both iurid exemplars, *A. phaiodactylus* and *H. arizonensis* (Fig. 7B).

Coxapophysal arteries emanating from the cerebral vascular network are resolved as plesiomorphic in scorpions (Fig. 2). Coxapophysal arteries emanating from the arteries of the first pair of legs only are apomorphic in the exemplars of Scorpionoidea s. str., having evolved once in the clade comprising diplocentrids, liochelids and scorpionids, and perhaps other katoikogenic scorpionoid taxa, but could not be resolved unambiguously because the condition of this character is presently unknown in Urodacidae (Table 1). Coxapophysal arteries emanating from the arteries of the pedipalps, in addition to the first pair of legs, are restricted to the iurid exemplars (*A. phaiodactylus* and *H. arizonensis*), providing another potential synapomorphy for Hadrurinae (Stockwell, 1989).

Character 9. Coxapophysal arteries, origin: emanates from cerebral vessel meshwork (0); branches off from arteries of first pair of walking legs (1); branches off from arteries of pedipalps and first pair of walking legs (2).

3.2.10. Tegumental arteries

The tegumental arteries (tg) supply the dorsolateral musculature and tegument anterior to those parts of the tegument supplied by the supracerebral artery. In all non-buthid scorpions examined, except Chaerilidae and Scorpionidae, the tegumental arteries are present, branching off the cheliceral arteries at the position where they surround the brain, traveling dorsolaterally (Fig. 7D, F), and ramifying terminally into several smaller vessels to supply the organs located there. The tegumental arteries curve backwards, supplying the posterodorsal parts of the prosoma, in *I. politus*. Tegumental arteries are absent in Buthidae, Chaerilidae and Scorpionidae (Fig. 7E).

The absence of tegumental arteries is plesiomorphic in scorpions (Fig. 2), their presence synapomorphic for the clade comprising all non-buthids (Coddington et al., 2004), although the condition in Pseudochactidae is unknown. The absence of tegumental arteries in Scorpionidae is optimized as a secondary loss (reversal), synapomorphic for this family (Fig. 2).

Character 10. Tegumental arteries: absent (0); present (1).

3.2.11. Peribuccal arteries

Paired peribuccal arteries (pb) supply the stomach dilation musculature between the stomach and the epistome (*sensu* Shultz, 2007). These arteries are either arranged in a semi-circular pattern anteriorly over the dilation musculature, or one of them travels over and the other one under the musculature. Many smaller branches emanate from the peribuccal arteries, and split into smaller vessels that travel between the fibers of the dilation musculature (Fig. 8D). Peribuccal arteries are absent in Buthidae and Chaerilidae. A few smaller vessels emanate from the dorsal-most point of the cheliceral arteries in some larger specimens (Fig. 8A), but these small vessels are not considered to be a distinct arterial subsystem of the anterior aorta. Peribuccal arteries are present, and branch off different arterial systems, including the cheliceral arteries and the supracerebral artery (Fig. 8B), in all non-buthid taxa examined, except Chaerilidae. The condition in Pseudochactidae is unknown. Some variation was observed in this character. For example, in one specimen of *H. arizonensis*, both peribuccal arteries branch off the supracerebral artery at the point where it performs the U-turn (Fig. 8C).

When this character is optimized on the phylogenetic hypothesis of Coddington et al. (2004), the absence of peribuccal arteries is resolved as ancestral in scorpions (Fig. 2) and the presence of the peribuccal arteries is synapomorphic for non-buthid taxa except Chaerilidae.

Character 11. Peribuccal arteries: absent (0); present (1).

3.3. Additional characters

The following additional characters were not optimized on the tree because data are only available for a few taxa or were observed to be invariant in the exemplar species studied and may prove potentially synapomorphic for scorpions. Further investigation is

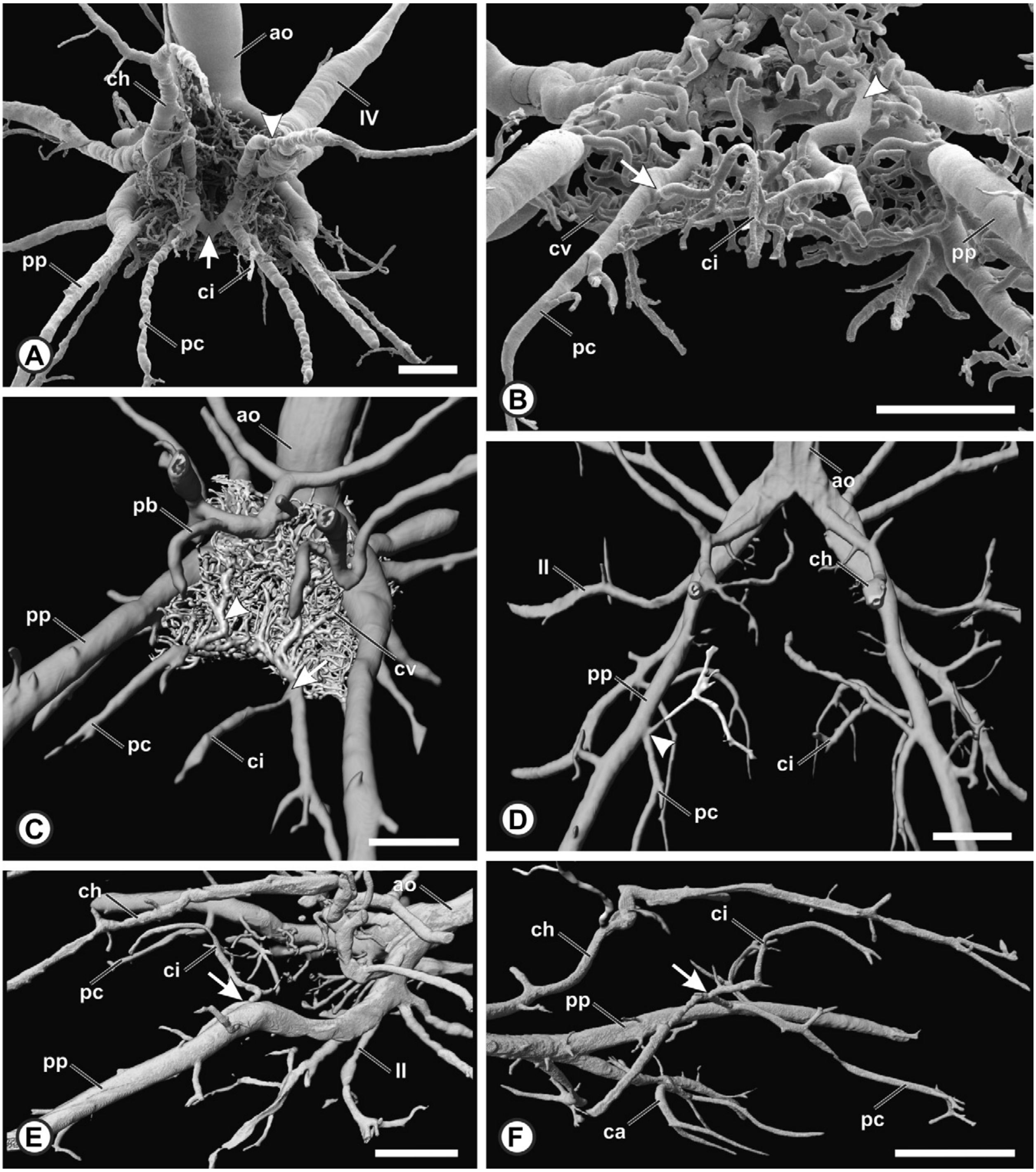

Fig. 6. Comparison of branching patterns of pedipalpocoxal arteries (pc) and coxinternal arteries (ci) in selected scorpion taxa. Scale bars: 500 μm. **A**–**D**. Arrowheads indicate origin of pedipalpocoxal arteries (pc). **A**. *Androctonus amoreuxi*, Buthidae, scanning electron micrograph (SEM) of corrosion cast of anterior aorta system, anterior view. Arrow indicates fusion of pedipalpocoxal arteries. **B**. *Uroctonus mordax*, Vaejovidae, SEM of corrosion cast of anterior aorta system, anterior view. Arrow indicates origin of coxinternal arteries. **C**. *Brotheas granulatus*, Chactidae, volume rendering of anterior aorta system, anterolateral view. **D**. *Iomachus politus*, Liochelidae, volume rendering of anterior aorta system, dorsal view. **E**. *Diplocentrus lindo*, Diplocentridae, volume rendering of anterior aorta system, anterolateral view. Arrow indicates origin of coxinternal artery. **F**. *I. politus*, volume rendering of anterior aorta system, lateral view, anterior at right. Arrow indicates origin of coxinternal artery. Abbreviations: ao, aorta; ca, coxapophysal artery; ch, cheliceral artery; ci, coxinternal artery; cv, cerebral vasculature; pb, peribuccal artery; pc, pedipalpocoxal artery; pp, pedipalpal artery; II, artery of leg II.

needed to check the states of these characters in different scorpion taxa.

The first character concerns the number of pairs of ostia. Seven pairs of ostia, located on the dorsal surface of the heart, were observed in the exemplar species investigated for this character (see Wirkner and Prendini, 2007 for discussion).

Character 12. Ostia, number of pairs: 7 (0).

Randall (1966) and Wirkner and Prendini (2007) mentioned taxon-specific differences in the number of cardiac arteries (six or eight pairs). These arteries are in most cases associated with the ostia, emanate ventral to the ostia, and supply the mesosomal

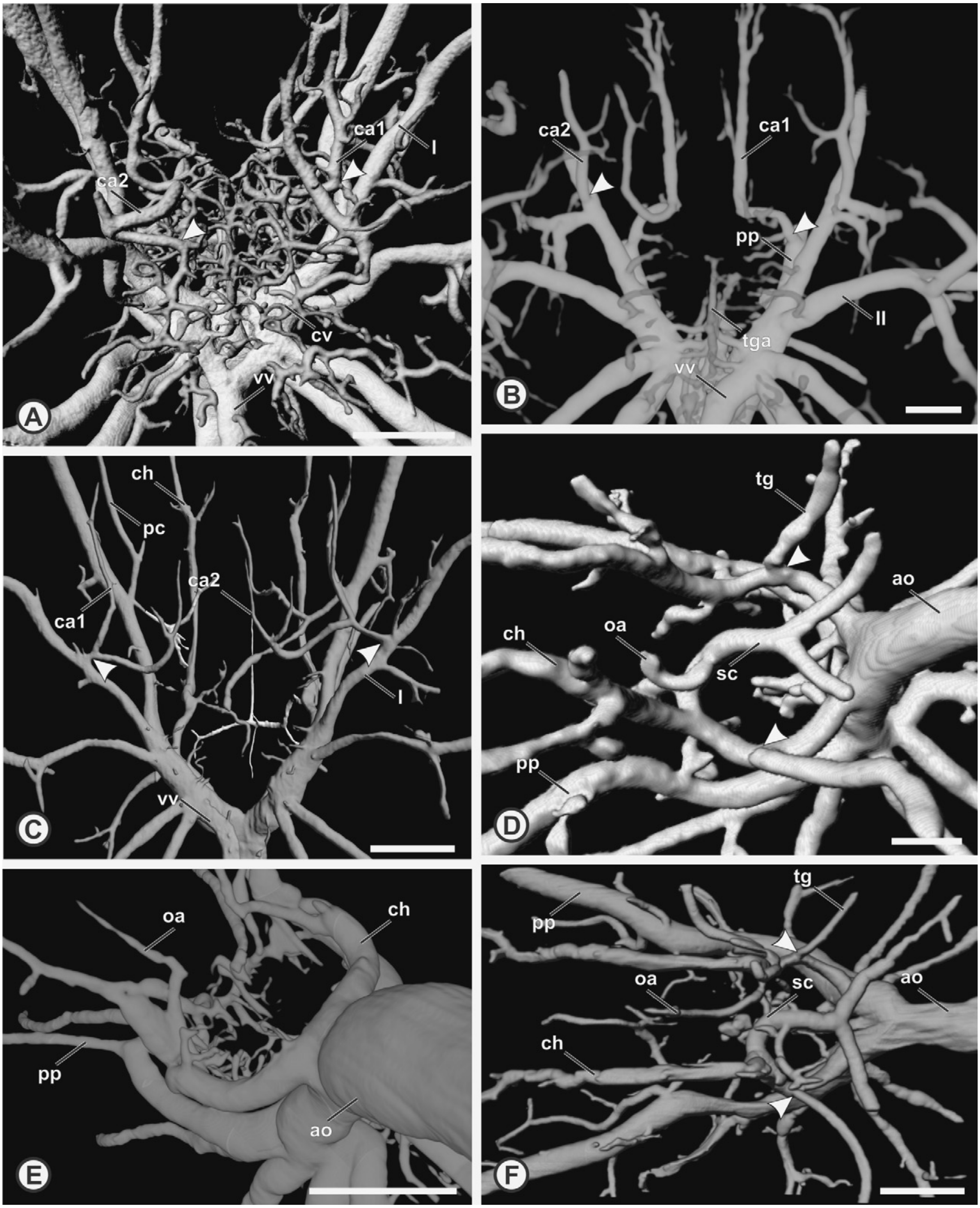

Fig. 7. Comparison of branching patterns of coxapophysal arteries (**A**–**C**; ca) and tegumental arteries (**D**–**F**; tg) in selected scorpion taxa. Arrowheads indicate origin of arteries. Scale bars: 500 μm. **A**–**C**. Ventral view. Numbers indicate coxapophyses to which each artery corresponds. **A**. *Uroctonus mordax*, Vaejovidae, volume rendering of anterior aorta system. **B**. *Hadrurus arizonensis*, Iuridae, volume rendering of anterior aorta system. **C**. *Iomachus politus*, Liochelidae, volume rendering of anterior aorta system. **D**. *Paruroctonus silvestrii*, Vaejovidae, surface rendering of anterior aorta system, dorsolateral view. **E**. *Buthacus arenicola*, Buthidae, volume rendering of anterior aorta system, dorsolateral view. **F**. *Diplocentrus lindo*, Diplocentridae, volume rendering of anterior aorta system, dorsolateral view. Abbreviations: ao, aorta; ca, coxapophysal arteries; ch, cheliceral artery; cv, cerebral vasculature; oa, optic artery; pc, pedipalpocoxal artery; pp, pedipalpal artery; tg, tegumental artery; tga, transganglionic artery; sc, supracerebral artery; vv, ventral vessel.

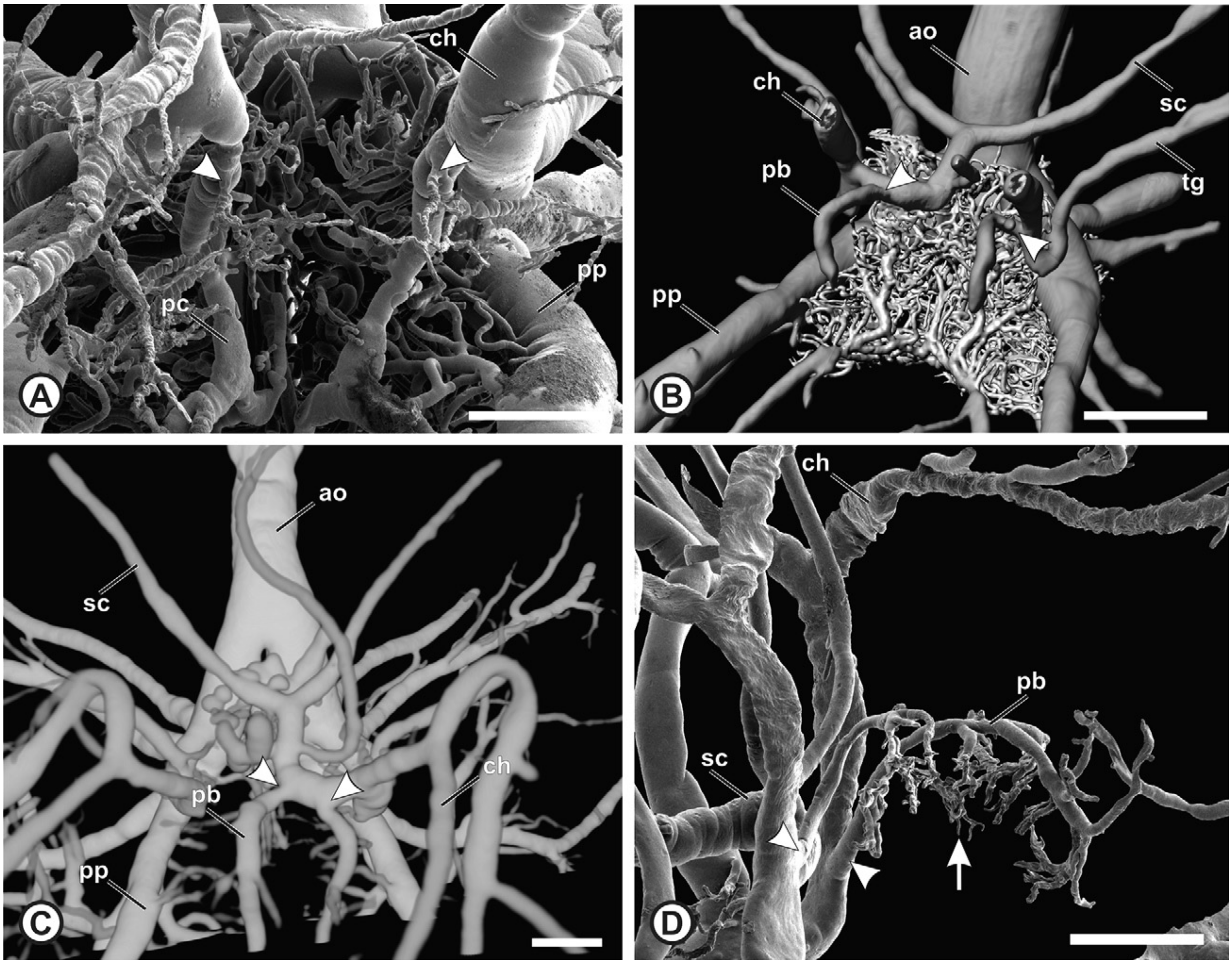

Fig. 8. Comparison of branching patterns of peribuccal arteries (pb) in selected scorpion taxa. Scale bars: 500 μm. **A**. *Androctonus amoreuxi*, Buthidae, scanning electron micrograph (SEM) of corrosion cast of anterior aorta system, anterior view. **B**. *Brotheas granulatus*, Chactidae, volume rendering of anterior aorta system, anterolateral view. **C**. *Hadrurus arizonensis*, Iuridae, volume rendering of anterior aorta system, dorsal view. **D**. *Opisthacanthus rugiceps*, Liochelidae, SEM of corrosion cast of anterior aorta system, lateral view, anterior at right. Arrow indicates small vessels that travel between fibers of dilation musculature of stomach. **A**–**D**. Arrowheads indicate origin of arteries. Abbreviations: ao, aorta; ch, cheliceral artery; pb, peribuccal artery; pc, pedipalpocoxal artery; pp, pedipalpal artery; sc, supracerebral artery; tg, tegumental artery.

organs. Unfortunately, resin did not fill the cardiac arteries sufficiently to allow this character to be evaluated in most of the exemplar species examined.

Character 13. Cardiac arteries, number of pairs: 8 (0); 6 (1).

Nine transganglionic arteries (Fig. 1E; tgl), which run midventrally through the subesophageal ganglion, were observed in all exemplar species studied. Each transganglionic artery descends vertically between neighboring neuromeres of the subesophageal nerve mass before branching laterally at the border of the neuropils and the somata. The number of these arteries corresponds with the number of ganglia incorporated in the subesophageal ganglion.

Character 14. Transganglionic arteries, number: 9 (0).

4. Discussion

4.1. Evolution of the HVS in scorpions

The HVS of scorpions is a complex organ system comprising a tubular heart, from which numerous arteries arise. The most prominent of these arteries is the anterior aorta system, which runs into the prosoma and branches in a highly complex pattern supplying the various organ systems located there (for details and a discussion concerning “open” vs. “closed” circulatory systems, see Wirkner and Prendini, 2007). During the evolution of the HVS, different transformations occurred within the heart and the branching pattern of the anterior aorta system. Based on the evidence presented above, it is clear that characters of the HVS are valuable for phylogenetic inference in scorpions as the states observed are more or less taxon-specific and not randomly distributed over the tree presented by Coddington et al. (2004).

It is noteworthy that ecologically different yet phylogenetically related species (e.g. the buthids *Leiurus quinquestriatus* and *Lychas mucronatus*, inhabiting arid and humid habitats, respectively) possess the same branching pattern of vessels in the anterior aorta system, suggesting a strong evolutionary constraint on this organ system.

The evolutionary mechanisms (selection pressures) acting on the vascular system of scorpions are unknown. Although there is no obvious advantage in altering the branching pattern of the arterial subsystems of the anterior aorta, some constraints may be considered in particular cases. For example, the supracerebral artery is reduced, its function apparently having been replaced by the tegumental arteries, in *I. politus*. This may perhaps be associated with the extreme dorsoventral compression of the body of these so-called "flat rock-scorpions", which hide in the cracks and crevices of rocks. Whereas change in shape or form may affect the evolution of the HVS, as illustrated by *I. politus*, change in size (e.g. miniaturization) appears to play little if any role, because all arterial subsystems of the anterior aorta are present in relatively small species (e.g. *E. carpathicus*).

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.asd.2012.06.002.

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