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# The circulatory system of Galeaspida (Vertebrata; stem-Gnathostomata) revealed by synchrotron X-ray tomographic microscopy

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## Abstract

Micro-CT provides a means of nondestructively investigating the internal structure of organisms with high spatial resolution and it has been applied to address a number of palaeontological problems that would be undesirable by destructive means. This approach has been applied successfully to characterize the cranial anatomy of *Shuyu*, a 428 million-year-old galeaspid (jawless stem-gnathostome) from the Silurian of Changxing, Zhejiang Province, China. Here, we use the synchrotron X-ray tomographic microscopy (SRXTM) to further describe the circulatory system of the head of *Shuyu*. Our results indicate that the circulatory system of galeaspid exhibits a mosaic of primitive vertebrate and derived gnathostome characters, including a number of derived gnathostome characters that are absent from osteostracans — the group conventionally interpreted as the sister lineage of jawed vertebrates. Our study provides a rich source of information that can be used to infer and reconstruct the early evolutionary history of the vertebrate cardiovascular system.

**Keywords:** synchrotron; micro-CT; galeaspid; jawless; gnathostomes; circulatory system

## 1. Introduction

The vertebrate circulatory system, also called the cardiovascular system, plays an essential role in the vertebrate body, but little is known concerning its early evolution. The vertebrate circulatory system is distinguished by a central multi-chambered heart and a body-wide vascular network of capillaries lined with endothelium (Bettex et al., 2014; Diogo et al., 2015). By circulating blood throughout the body, the cardiovascular system functions to supply the tissues with oxygen and nutrients, distribute and dissipate heat, remove metabolic waste, and assist in defending the body against disease (Jollie, 1962; Harder, 1975; Romer and Parsons, 1986). All vertebrates have a closed cardiovascular system (meaning that the blood never leaves the network of arteries, veins and capillaries) probably derived from a common ‘blueprint’ which evolved from the early chordate circulatory system with a single layered tube, as retained in tunicates and cephalochordates (Bettex et al., 2014; Stephenson et al., 2017). The vertebrate cardiovascular system has undergone significant modification, and different evolutionary configurations can be characterized in fishes, amphibians, reptiles, birds, and mammals (Monahan-Earley et al., 2013; Stephenson et al., 2017). Though the circulatory system of fishes is simple by comparison to humans and other mammals, it is critical to illustrate the early evolution of the vertebrate circulatory system. For example, the undivided two-chambered hearts in fishes, consisting of a single atrium and a single ventricle (single circuit system), are believed to be the precursor of the four-chambered heart (double circuit system). These modifications probably occurred in conjunction with the origin of tetrapods, as part of the adaptation of the vertebrate body plan to live in a terrestrial environment (Randall, 1970; Liem et al., 2001; Ishimatsu, 2012). However, the early evolution of the vertebrate circulatory system is especially difficult to trace in the transition from jawless to jawed vertebrates through fossil evidence because the heart and blood vessels are composed of soft tissues that hardly ever been fossilized. Consequently, our knowledge of the early evolution of the vertebrate circulatory system comes primarily from comparing features found in extant jawless fishes such as lampreys with those of primitive jawed vertebrates such as sharks and bony fishes

(early members of the actinopterygian and sarcopterygian lineages) (Rahmat and Gillanda, 2014).

Fig. 1 here

A primitive ‘blueprint’ for the vertebrate circulatory system probably emerged in the vertebrate stem-lineage (Monahan-Earley et al., 2013) (see Fig. 1 for the evolutionary clades and grades referred to in the text). The earliest putative fossil evidence of the vertebrate cardiovascular system is manifest as ambiguous organic films preserving aspects of the anatomy of soft-bodied primitive chordates such as *Cathaymyrus* and *Pikaia*, and vertebrates such as *Haikouichthys* and *Metaspriggina*, from the Cambrian Chengjiang Biota and Burgess Shale Lagerstätten (Shu et al., 1999, 2003; Conway Morris and Caron, 2014), but the phylogenetic position of these soft-bodied fossil taxa is hard to resolve (Sansom et al., 2010). In the Chengjiang taxa *Haikouichthys* and *Myllokunmingia* (around 521 Ma), a large organic film and loop-shaped imprints behind the gill series have been interpreted as traces of a heart and the segmental blood vessels (Shu et al., 1999, 2003). The Burgess Shale *Metaspriggina* also preserves a possible heart as an anterior organic film ventral to the oesophagus, as well as some loop-shaped imprints anterior the gill series that might represent blood vessels (Conway Morris and Caron, 2014).

The oldest examples of vascular systems in vertebrates manifest in the fossil record of stem-gnathostome ‘ostracoderms’, a paraphyletic assemblage of jawless vertebrates representing successive sister groups to jawed vertebrates (Fig. 1). These represent members of the lineage leading to jawed vertebrates after their separation from the cyclostome (hagfish and lamprey) lineage. Almost all of these extinct fishes possess a mineralized dermal skeleton that, on its visceral side, can preserve impressions of vascular system (Fig. 2). In those extinct groups, a mineralized neurocranium of calcified cartilage and/or perichondral bone can preserve the canals that once housed the arteries, veins, and indeed, the heart; which may provide direct insights into the nature of the early vascular system.

Denison (1967) described a number of vascular canals contained in the masses of globular calcified cartilage in a presumed snout of *Eriptychius* from the Upper Ordovician of North America (around 475 Ma). Silurian–Devonian heterostracans

have been described to preserve faint impressions of extrabranchial blood vessels and grooves possibly left by the facial vessels, preserved on the visceral surface of dermal headshield (Janvier and Blicek, 1979; Novitskaya, 1983). For example, Fig. 2 shows a sedimentary endocast of the visceral surface of the ventral dermoskeleton of pteraspid heterostracan *Pteraspis leathensis*, reflecting the position of the heart (Fig. 2A, B) and veins associated with the branchial pouches (Fig. 2C). Fossils such as these likely indicate that the cranial endoskeleton of heterostracans was not directly attached to the dermoskeleton, whether cartilaginous or calcified (Janvier, 1996).

Fig. 2 here

For vertebrates possessing a calcified or ossified endoskeleton attached to the exoskeleton (e.g., Galeaspida, Osteostraci, Placodermi, and Osteichthyes), numerous passages for blood vessels and nerves, as well as muscle attachment sites, were enclosed in the endoskeleton; thus, the circulatory system can be reconstructed on the basis of the vascular canals in the endoskeleton by some special methods (Janvier, 1996). For example, the circulatory system of osteostracans was inferred for the head region based on acid preparation and the traditional method of serial grinding (Stensiö, 1927; Janvier, 1981, 1985). However, the destructive nature and the extent of time and effort required by this conventional technique, have critically limited its adoption. Micro-CT is a nondestructive method that can be applied to investigate the internal structure of organisms with sufficient spatial resolution (from  $< 1 \mu\text{m}$  to  $> 100 \mu\text{m}$ ) to resolve features of fine and gross anatomy preserved, but not otherwise accessible in fossil remains (Gai and Zhu, 2013; Cunningham et al., 2014). This approach has been applied successfully to characterise the cranial anatomy of *Shuyu*, a 428 million-year-old galeaspid (jawless stem-gnathostome) from the Silurian of Changxing, Zhejiang Province (Gai et al., 2011; Gai and Zhu, 2012; Gai, 2018). Here, we use synchrotron X-ray tomographic microscopy (SRXTM) to further reveal the circulatory system of *Shuyu*. Our new material, coupled with this method, provides a rich source of information to reconstruct the early evolutionary history of the vertebrate cardiovascular system.

## 2. Geological setting

Twenty three-dimensionally preserved neurocrania of *Shuyu* were collected from the lower part of the Maoshan Formation (late Telychian, Llandovery, Silurian) in Changxing County, in northwestern Zhejiang Province, southeastern China (Fig. 3, S<sub>1m</sub>). The Maoshan Formation (originally the “Maoshan Sandstone”) is well-developed in southern Jiangsu Province and northern Zhejiang Province. The formation is mainly characterised by gray-green, purple-red lithic quartz-rich sandstones intercalated with a small amount of muddy siltstones, characteristic of a delta-fluvial facies (Bureau of Geology and Mineral Resources of Zhejiang Province, 1989). Besides brachiopods, some endemic jawless vertebrates have been found in the Formation (Bureau of Geology and Mineral Resources of Zhejiang Province, 1989). In Changxing region of Zhejiang Province, the Maoshan Formation is underlain conformably by the Kangshan Formation, and overlain unconformably by the Wutong Formation (Late Devonian) (Bureau of Geology and Mineral Resources of Zhejiang Province, 1989). The Maoshan Formation abounds with galeaspid fossils including *Shuyu*, *Anjiaspis*, *Meishanaspis*, *Changxingaspis*, fragments of *Hanyangaspis*, as well as fin-spines of *Sinacanthus* (Pan, 1986; Wang, 1991; Gai and Zhu, 2005; Gai et al., 2005; Liu et al., 2014), which are suggestive of the *Sinogaleaspis*-*Xiushuiaspis* Fauna, originally described from Xiushui, Jiangxi Province (Pan and Wang, 1980).

Fig. 3 here

### 3. Materials and methods

Seven crania of the galeaspid *Shuyu zhejiangensis* (IVPP V 14334.1–14334.7) were selected from among the 20 three-dimensionally preserved neurocrania for the SRXTM investigation to elucidate the circulatory system in head region. Specimens of *Changxingaspis* and *Meishanaspis* recovered from the same localities as *Shuyu* and *Duyunolepis*, from the Lower Devonian of Guizhou Province, were also examined. All the materials of *Shuyu*, *Changxingaspis* and *Meishanaspis* are housed in the collections of the Institute of Vertebrate Paleontology and Paleoanthropology (IVPP), Chinese Academy of Sciences, No. 142 Xi-zhi-men-wai Street, Xicheng District, Beijing 100044, China. The materials of *Duyunolepis* are housed in the collections of the Geological Museum of China (GMC), No. 15 Xisi Yangrou Lane, Xicheng District, Beijing 100034, China.

All specimens were prepared by removal of much of the surrounding matrix using an automatic cutting machine and prepared mechanically using a vibro tool with a tungsten-carbide bit or a needle. Our SRXTM investigations were performed at the X02DA TOMCAT beamline of the Swiss Light Source at the Paul Scherrer Institute (Villigen, Switzerland). SRXTM experiments followed a standard acquisition approach with the rotation axis located in the middle of the field of view and the acquisition of 1501 projections equiangularly distributed over 180 degrees of rotation. A 2x objective (resulting pixel size 3.25  $\mu\text{m}$ ), energies of 37.5 and 45 were used with exposures of 2400 ms and 5000 ms, respectively. X-ray projections were post-processed online and rearranged into flat-field and dark-field-corrected sinograms. Reconstruction was performed on a 20-node Linux computer cluster using highly optimized filtered back-projection routines. Slice data derived from the scans were then analysed and manipulated using AVIZO software (<https://www.fei.com/software/amira-avizo/>) for computed tomography on a Hewlett Packard Workstation with a 2-GHz Intel processor and 16 GB of RAM. For further details of the materials and methods refer to Gai et al. (2011) and Gai (2018). Following best practise (Davies et al., 2017), the tomographic slice data, AVIZO models, and a stereolithographic model of the vascular system of *Shuyu zhejiangensis* have been made openly available from the University of Bristol Data Repository: <https://data.bris.ac.uk/data/dataset/p34vnx48p4772ouez5a1sfoqh>, published as Gai et al. (2019). Photographs and micrographs were obtained using a NIKON D3X camera and studied under optical zoom for the comparison with the virtual 3D reconstruction data.

## 4. Description

### 4.1. Arterial system of the head

The arterial circulatory system of *Shuyu* consists of the dorsal and ventral aortic systems. The dorsal aortic system is comprised of the dorsal aorta, efferent branchial arteries, anterior cerebral artery (ac.a), middle cerebral artery (mc.a), posterior cerebral artery (pc.a), external carotid arteries (ec.a), internal carotid arteries (ic.a), hypophysial artery (hy.a), ophthalmic arteries (oph.a), anterior ethmoidal artery (ae.a), posterior ethmoidal artery (pe.a), dorsal nasal arteries (dn.a), frontal arteries (fr.a),

anterior, middle and posterior labyrinthine arteries (al.a, ml.a, pl.a). The ventral aortic system is comprised of the marginal artery (mar.v), afferent branchial artery (abr.a), dorsal surficial artery (ds.a), ventral rim artery (vr.a) and cornual artery (cor.a), but the ventral aorta and its tributaries have not been reconstructed because they are not enclosed in the endoskeleton.

Fig. 4 here

**Canal for the dorsal aorta.** A pair of large median canals for the dorsal aorta (ao, Figs. 4A, B, 5A, B, D) are located below the cranial cavity and on the roof of the oralbranchial chamber. This canal is exceptionally large and possibly encloses the esophagus and the anterior cardinal veins which probably flank the dorsal aorta ventrally. The paired dorsal aortic canals remain independent within the basicranial cartilage in the head region. Anteriorly, the canal for the dorsal aorta ascends into the neurocranium and extends forward as the canal for the internal carotid artery (ic.a, Figs. 4A, B, 5A-D, 6A, B, 7B, C, 10). Laterally, each dorsal aortic canal receives five short transverse canals for the efferent branchial arteries (ehy.a, ebr.a, Figs. 4A, B, 5A-D, 6A, B) from the branchial fossae. Caudally, the dorsal aortic canal penetrates the posterior branchial wall leading to the trunk.

Fig. 5 here

**Canal for the efferent branchial artery.** The paired dorsal aortic canals communicate laterally with the branchial fossae through the short transverse canals for the efferent branchial arteries (ehy.a, ebr.a, Figs. 4A, B, 5A-D, 6A, B). Each canal for the efferent branchial artery is located between the two adjacent branchial fossae. The first efferent branchial canal is relatively large and leads to the hyoid pouch at an inclined angle. It probably contains the efferent hyoid artery (ehy.a, Figs. 4A, B, 5A-D, 6A, B). The remaining four efferent branchial canals (ebr.a<sub>1-4</sub>, Fig. 5A) extend transversely to their respective branchial fossae. Among these canals, the last one (ebr.a<sub>4</sub>, Fig. 5A), between the fourth and fifth branchial fossae, is larger than the others (ebr.a<sub>1-3</sub>, Fig. 5A). No canal for the pseudobranchial artery is observed.



Fig. 6 here

**Canal for the external carotid artery.** The canal for the external carotid artery (ec.a, Fig. 4A, B) arises from the caudal part of the efferent hyoid canal. This canal extends laterally to join with the trigeminal chamber ventrally (Fig. 4B). Its offshoots probably supplied the structures in the velar fossa and orbital cavity, but it is difficult to trace after it converges with the trigeminal chamber.

**Canal for the internal carotid artery.** The paired canals for the dorsal aortae ascend into the neurocranium just caudal to the hypophysial pouch, and continue forward as paired canals for the internal carotid artery (ic.a, Figs. 4A, B, 5A-D, 6A, B). Inside the neurocranium, the paired canals flank the hypophysial pouch and extend antero-laterally toward the orbital cavities. The offshoots of the internal carotid artery include the canals for the ophthalmic artery (oph.a, Figs. 4A, B, 6B, 7A-D), hypophysial artery (hy.a, Fig. 6A, B) and orbitonasal artery (on.a, Figs. 4B, 6B, 7A, B) supplying blood to the orbital and the ethmoid region.

Fig. 7 here

**Canal for the hypophysial artery.** A small transverse canal branches off from the canal of the internal carotid artery to the hypophysial recess laterally. This canal penetrates the lateral wall of the hypophysial recess and must have contained the hypophysial artery (hy.a, Figs. 4A, 6A, B).

**Canal for the orbitonasal artery (palatine artery).** The canal for the orbitonasal artery (on.a, Figs. 4B, 6B) also branches off from the internal carotid artery in the hypophysial region. It extends forwards, flanks the hypophysial duct, and expands antero-laterally in the ethmoid region to the ventral part of the nasal sacs.

Fig. 8 here

**Canal for the ophthalmic artery.** The canal for the internal carotid artery extends anteriorly as the canal for the ophthalmic artery (oph.a, Figs. 4A, B, 6B, 7A-C) in the orbital region. The canal for the ophthalmic artery bifurcates into two main

rami under the optic canal: the posterior ramus ascends into the optic canal to supply the orbital cavity; the anterior ramus continues anteriorly to the ethmoid region.

**Canal for rami of the ophthalmic artery.** In the ethmoid region, the canal for the ophthalmic artery has four small rami. The most anterior ramus continues forwards to the rostral part of head and probably contains the frontal artery (fr.a, Figs. 4A, B, 6B, 7A-C). The middle ramus extends antero-dorsally to the dorsal side of the nasal sacs and must have contained the dorsal nasal artery (dn.a, Figs. 4A, B, 6B, 7A-C) irrigating the nasal sacs dorsally. The last two posterior rami ascend antero-dorsally to the fossa for the olfactory bulb, and must have contained the anterior and posterior ethmoidal artery (ae.a, pe.a, Figs. 4A, B, 6B, 7A-C) supplying the olfactory bulbs. The four rami of the ophthalmic artery are coupled with four rami of the deep ophthalmic nerve ( $V_1$ , Fig. 7A-C).

**Canal for the labyrinthic artery.** The labyrinthic cavities are probably supplied by three arteries, the anterior (al.a, Fig. 4A), middle (ml.a, Fig. 8B, C, E-G), and posterior labyrinthic arteries (pl.a, Fig. 8B-E). The canal for the anterior labyrinthic artery arises dorsally from the dorsal aorta at the level of the second branchial fossa, and extends postero-dorsally to the anterior part of vestibular division (al.a, Fig. 4A). The canal for the middle labyrinthic artery penetrates the lateral wall of the brain cavity to the middle part of the labyrinthic cavity just posterior to the acoustic fenestra (ml.a, Fig. 8B, C, E-G). The canal for the posterior labyrinthic artery is a short canal projecting anteriorly from the large vagus canal to the caudal part of vestibular division (pl.a, Fig. 8B-E). The middle and posterior labyrinthic arteries probably branch off from the posterior cerebral artery within the brain cavity.

**Canal for the middle cerebral artery.** A short canal, probably for the middle cerebral artery (mc.a, Figs. 4A, 5C), issues dorsally from the dorsal aorta canal to the brain cavity just caudal to the hypophysial pouch.

Fig. 9 here

**Canal for the posterior cerebral artery.** The floor of the myelencephalic cavity is penetrated by a pair of foramina in the occipital region. The paired foramina are located just posterior to the larger canal for the vagus and posterior lateral line nerves. They are comparatively large and probably contain the posterior cerebral arteries

(pc.a, Fig. 9A, B) which arise from the dorsal aorta dorsally to supply the posterior part of brain and labyrinths.

**Canal for the vertebro-medullar artery.** In specimen IVPP V 14334.5, the floor of the neural canal is penetrated by a pair of short canals between the fenestra of the posterior cerebral artery (pc.a, Fig. 9A, B) and the postbranchial wall, where the paired canals for the first spinal nerve project from the neural canal laterally. The paired canals probably contain the vertebro-medullar artery and vein (vm.a, vm.v, Fig. 9A, B) arising dorsally from the dorsal aorta and the anterior cardinal vein, respectively.

Fig. 10 here

**Canal for the marginal artery.** In the lateral margin of the head, the oralbranchial chamber is flanked by two pairs of dorsoventral elongate canals for the marginal vein (mar.v, Figs. 10, 11A-C) and marginal artery (mar.a, Fig. 10, 11A-C). The canal for the marginal artery is relatively small and overlies the larger marginal vein dorsomedially.

Fig. 11 here

#### **4.2. Venous system of the head**

Dorsoventrally, the cephalic venous circulatory system can be subdivided into three sets of assembly: the dorsal jugular, cardinal, and ventral jugular venous assembly. The dorsal jugular venous assembly is located on the dorsal side of the head including the dorsal jugular vein (vcl), orbitonasal vein (on.v), dorsal nasal vein, anterior cerebral vein (ac.v), pituitary vein (pit.v), middle cerebral vein (mc.v), labyrinthine vein (lab.v), posterior cerebral vein (pc.v), extrabranial vein (exbr.v) and dorsal superficial vein (ds.v). The cardinal venous assembly is located on the ventral side of the brain cavity. The anterior cardinal vein (aca.v), lateral capitis vein (lc.v), and vertebro-medullar veins (vm.v) can be referred to this assembly. The ventral jugular venous assembly is located on the ventral side of the oralbranchial chamber. The ventral jugular vein (vj.v), marginal veins (mar.v), rostral vein (ro.v),

anterior nasal vein, posterior nasal vein, lateral superficial veins, and ventral rim vein (vr.v) can be referred to this assembly.

**Canal for the lateral head vein (dorsal jugular vein).** A pair of large canals for lateral head vein, or the dorsal jugular vein (vcl, Figs. 4A, B, 10) pass through the head dorsally, flanking the labyrinthic cavity and brain cavity. The dorsal portion of the visceral arches forms the lateral wall of the dorsal jugular canal by fusing with the neurocranium laterally. Posteriorly, the dorsal jugular canal enters the body by penetrating the postbranchial wall from the pericardial area. It expands anterolaterally to orbital cavity. When it passes through the labyrinthic cavity, the large canal curves laterally to embrace the two vertical semicircular canals. The overall shape of the dorsal jugular canals is a pair of 'ω' flanking the labyrinthic cavities (Figs. 4A, B, 10). The canals for the oculomotor, V<sub>2</sub>-complex of trigeminal, facial, glossopharyngeal and vagus nerves cross the dorsal jugular canal ventrally (Fig. 4B). Dorsally, the canals for the trochlear and ophthalmic nerves extend rostrally along the dorsal surface of the dorsal jugular canal to the orbital cavity and ethmoid region (Figs. 4A, 10). Laterally this large canal receives five canals for the extrabranial veins (exbr.v, Fig. 10). It also receives a number of venous canals from the sensory organs and brain cavity.

Fig. 12 here

**Canal for the orbitonasal vein.** A short canal for the orbitonasal vein (on.v, Figs. 4A, B, 10, 12A-D) penetrates the frontal wall of the orbital cavity paralleling the canal for the profundus nerve (V<sub>0</sub>, Fig. 12). This canal is probably the anterior extension of the dorsal jugular vein to the ethmoid region. Rostrally, it bifurcates into two rami: one ramus for the dorsal nasal vein (dn.v, Figs. 4A, B, 10, 12A-D), which curves anteroventrally to the dorsal surface of the nasal sacs; the other ramus for the frontal vein (fr.v, Figs. 4A, B, 12A, B, D) continues forward to the rostral part of the head.

**Canal for the anterior cerebral vein.** The lateral head canal anteriorly connects to the brain cavity through the transverse canals for the anterior cerebral vein (ac.v, Figs. 4A, 7B). The canal for the anterior cerebral vein (ac.v, Figs. 4A, 7B) issues anterodorsally from the lateral wall of the diencephalic cavity. It extends antero-

laterally at a slight angle to the orbital cavity, inside which it probably amalgamated with the dorsal jugular vein. This canal overlies the canal for the optic nerve dorsally. In some specimens, they form one large canal called the optic canal (opt.c, Fig. 7A, C).

**Canal for the middle cerebral vein.** The canal for the middle cerebral vein (mc.v, Figs. 4A, 7A) is very large, issuing dorso-laterally from the metencephalic division of the brain cavity. It extends antero-laterally a short distance to the canal for the dorsal jugular vein. In the left part of specimens IVPP V 14334.3 and IVPP V 14334.4, numerous small canals arise dorsally from the middle cerebral vein to the dorsal portion of the head. These canals probably contained the dorsal superficial veins (ds.v, Fig. 4A), draining the vascular plexi to the dorsal jugular vein.

**Canal for the pituitary vein.** A short canal, probably for the pituitary vein, (pit.v, Figs. 4A, B, 7A) separates from the dorsal jugular canal. It is positioned ventral to the canal for the oculomotor nerve (III, Fig. 4A), and extends medially a short distance to the hypophysial recess. This short canal must have contained the pituitary vein draining the hypophysial organ to the dorsal jugular vein.

Fig. 13 here

**Canal for the anterior cardinal vein.** There is no independent canal for the anterior cardinal vein. The anterior cardinal vein was probably contained in the large dorsal aortal canal flanking the dorsal aorta.

**Canals for the lateral capitis vein.** The canal for the lateral capitis vein (lc.v, Figs. 12A, 13C) probably bifurcates from the anterior cardinal vein in the orbital region. It crosses the interorbital wall and the suborbital shelf to the ventral part of the ethmoidal region (Fig. 11A). The canal for the lateral capitis vein can be traced ventral to the orbital cavity in the specimen IVPP V 14334.22 (lc.v, Fig. 13C).

Fig. 14 here

**Canal for the labyrinthine vein.** In the otic region, a short transverse canal for the labyrinthine vein (lab.v, Figs. 4A, B, 10, 14A, B) separate from the dorsal jugular canal to the vestibular chamber of the labyrinthine cavity. In the middle part of the

labyrinthine cavity, the canal for the labyrinthine vein bifurcates into two rami for the anterior labyrinthine vein (al.v, Figs. 4A, B, 10, 14A, B) and the posterior labyrinthine vein (pl.v, Figs. 4A, B, 10, 14A, B).

Fig. 15 here

**Canal for the extrabranchial vein.** The roof of each branchial fossa is overlain by an elongate transverse canal for the extrabranchial vein (exbr.v, Figs. 10, 15B, C). The canal for the extrabranchial vein branches off from the dorsal jugular vein and extends anterolaterally to the lateral margin of the branchial fossa. Its distal end probably joins with the canal for the marginal vein draining the branchial fossa towards the dorsal jugular vein. Anterolaterally, each extrabranchial vein projects a short canal for the dorsal superficial veins (ds.v, Figs. 10, 15B, C) to the endoskeleton draining vascular plexi to the dorsal jugular vein.

**Canal for the vertebro-medullar vein.** The floor of the neural canal is penetrated by another short canal for the vertebro-medullar vein (vm.v, Fig. 9B) rostral to the postbranchial wall. This canal is closely adjacent to the canal for the vertebro-medullar artery and probably contained the vertebro-medullar vein draining the neural canal towards the anterior cardinal vein.

**Canal for the ventral jugular vein.** The canal for the ventral jugular vein or inferior jugular vein (vj.v, Fig. 12B, D) penetrates the roof of the velar chamber of the oralbranchial chamber. It is connected with the canal for the marginal vein and rostral veins (ro.v, Fig. 12A, B) between the nasal sacs and hyoid pouch. Inside the oralbranchial chamber, the canal for the ventral jugular vein cannot be traced and can only be inferred.

**Canal for the rostral vein.** The canals for the rostral veins (ro.v, Fig. 12A-C) are a pair of elongate canals flanking the ethmoidal region ventrally. The canal continues posteriorly as the marginal vein and joins with the ventral jugular vein medially in the suborbital region (vj.v, Fig. 12D). Along its course, the rostral vein receives two transverse canals from the nasal sacs. The anterior one is for the anterior nasal vein (an.v, Fig. 12A, B, D), and the posterior one is the posterior nasal vein (pn.v, Fig. 12A, B, D). It also receives a series of small canals for the ventral rim vein and dorsal

superficial vein from the ventral rim and lateral margin of the head (vr.v, Fig. 11B, C).

**Canal for the marginal vein.** The canals for the rostral vein continue posteriorly as a pair of elongate canals for the marginal vein (mar.v, Figs. 10, 11A-C, 13B, C) flanking the oralbranchial chamber ventrally. Along its course, the canal for the marginal vein projects numerous small canals for the lateral superficial vein (sf.v, Fig. 11A), the ventral rim vein (vr.v, Fig. 11B, C), and the efferent branchial vein (ebr.v, Fig. 12B, C). The canals for the lateral superficial vein (sf.v, Fig. 11B, C) extend dorso-laterally to the lateral parts of the head. The canals for the ventral rim vein (vr.v, Fig. 11C) extend ventro-laterally to the ventral rim. The canals for the efferent branchial vein (ebr.v, Fig. 12B, C) extend medially to the branchial fossae. Posteriorly, a series of elongate transverse canals branch off from the marginal vein to the cornual process (cor.v, Fig. 11C, D). The canal for the marginal vein is probably connected with the dorsal jugular vein through the six canals for the extrabranial veins.

**Canal for the cornual vein.** Among the ventro-lateral canals from the marginal vein to the ventral rim, the last three canals are especially well-developed and extend postero-laterally to the distal end of the cornual process. We regard the three subbranches of marginal vein as the cornual vein (cor.v, Figs. 10, 11C, D). The middle cornual vein further bifurcates into two large branches (cor.v<sub>3</sub>, Fig. 11D). Each cornual vein had numerous small rami.

### 4.3. Subcutaneous vascular plexuses

There exists a rich network or plexus of subcutaneous vascular canals (va.p, Fig. 16A-D) at the boundary between the exo- and endo-skeletons. The subcutaneous vascular plexi are irrigated by the dorsal superficial artery from the marginal artery, and drained by the dorsal superficial veins from the dorsal jugular, orbitonasal, extrabranial, middle cerebral and marginal veins.

Fig. 16 here

## 5. Discussion

### 5.1. Evolution of the dorsal aorta

The dorsal aorta, as the main artery in vertebrate body, is a long tube running the length of the body. There is only a single ventricle in fishes. The de-oxygenated blood is transmitted from the conus arteriosus towards gills by the ventral aorta, and the oxygenated blood is transmitted from the gills towards all parts of the body by the dorsal aorta. It is probably homologous with the descending aorta of tetrapods (Randall, 1970). Two features of the dorsal aorta of galeaspids are noteworthy.

Firstly, the dorsal aorta of galeaspids is paired as in gnathostomes. The dorsal aorta of lampreys (*Petromyzon*), whether in the embryo or adult, is unpaired throughout the branchial region. During development, there is no sign of a fusion of paired vessels into the unpaired tube in this region (Holmgren, 1946). In osteostracans, the dorsal aorta is also unpaired and housed in a large median groove on the roof of the oralbranchial cavity together with the oesophagus, a condition resembling that of lampreys (Janvier, 1981, 1996). Placoderms consistently exhibit evidence of paired lateral aortae extending to the posterior limit of the neurocranium (Stensiö, 1963; Young, 1986). In crown chondrichthyans, including holocephalans, the dorsal aorta divides into paired lateral aortae at or behind the posterior limit of the neurocranium. The lateral aortae remain separated until just behind the hypophysis where they enter the basicranium to form the median cephalic sinus (Schaeffer, 1981). Our new data indicate that galeaspids also possess paired dorsal aortae in the head region like gnathostomes. In *Branchiostoma*, a median dorsal aorta lies in the mid-line immediately beneath the notochord behind the pharynx, but it divides into paired dorsal aortae at its anterior end (Jefferies, 1986). Holmgren (1946) noted the presence of paired dorsal aortae in hagfishes and considered that in its embryonic development, the dorsal aorta of hagfishes is closer to the gnathostome pattern than that of lampreys. Therefore, paired dorsal aortae probably represent a plesiomorphic condition for vertebrates since they are also present in amphioxus and hagfishes (Jefferies, 1986), whereas the unpaired dorsal aorta in osteostracans is probably convergent with that of lampreys (Janvier, 1996).

Secondly, the dorsal aorta runs through the basicranial cartilage in galeaspids. A dorsal aorta enclosed within the basicranial cartilage is present in primitive chondrichthyans (Schaeffer, 1981; Coates and Sequeira, 1998), few placoderms such as *Radotina* (Gross, 1959; Stensiö; 1963, 1969, Young, 1980), certain early actinopterygians such as *Mimipiscis* (Gardiner, 1984), but absent in acanthodians



(Miles, 1973), most early actinopterygians (Gardiner, 1984; Coates and Sequeira, 1998), and in most placoderms (Young, 1986; Janvier, 1996). Brazeau (2009) considered that this feature may be a gnathostome symplesiomorphy or simply highly variable. There is no basicranial cartilage enclosing the dorsal aorta in hagfishes and lampreys. Romer (1937) assumed that the dorsal aorta and related structures are probably enclosed in the base of the braincase from the internal carotid region back into or beyond the aortic arch region in the primitive gnathostomes. In osteostracans, the dorsal aorta is half-enclosed by the basicranial cartilage and housed in a large median groove on the roof of the oralbranchial cavity together with the oesophagus. In this regard, galeaspids are more similar to jawed vertebrates than osteostracans.

## **5.2. Homology of lateral head vein and dorsal jugular vein**

The most striking resemblance between galeaspids and osteostracans in the anatomy of the cardiovascular system is the very large lateral head vein that is one of the major dorsal head veins draining the blood from the anterior part of the head towards the anterior cardinal vein and the atrium of the heart via the ductus Cuvieri (Janvier, 2007). In osteostracans, the canal was regarded as the homologue of the lateral head vein of larval lampreys (Stensiö, 1927), or the dorsal jugular vein of placoderms and crown gnathostomes (Forey and Janvier, 1993). However, the lateral head vein is greatly reduced or modified in adult lampreys and hagfishes. Whether or not it existed in heterostracans, anaspids, and “thelodonts” remains unclear. In heterostracans, only a faint impression on the internal surface of the cranial headshield was referred to a lateral head vein (Janvier and Blicek, 1979), but this interpretation is dubious. In crown gnathostomes, most blood from the head, the eyes and the brain drains into a large longitudinal vein, the lateral head vein or vena capitis lateralis on either side of the braincase (Wake, 1992). In amphibians and reptiles, the lateral head vein remains but becomes less important, and the blood from the more superficial head structures is collected by an external jugular vein that probably represents one or more of the finer anterior branches of the anterior cardinal (Wake, 1992). It is reasonable to consider the lateral head vein as a primitive trait of vertebrates, and the large lateral head vein is most likely to be a derived character shared (at least) by galeaspids, osteostracans, and gnathostomes.

## **5.3. Orbitonasal artery and vein in galeaspids**

The identification of orbitonasal artery and vein in galeaspids has important significance. In gnathostomes, the orbitonasal vein can be regarded as the anterior extension of the lateral head vein penetrating the anterior wall of the orbital cavity and connecting with the nasal capsule (de Beer, 1937). No corresponding canal has been described in hagfishes, lampreys, and osteostracans. In osteostracans, a short canal penetrates the anterior wall of the orbital cavity to the ethmoid region. Wängsjö (1952, fig. 21B) suggested the canal to be “for the anterior, or pre-orbital division of dorsal jugular vein (lateral head vein)”. Janvier (1975) proposed that this canal corresponds to the facial vein of lampreys (Lindström, 1949), but he rejected this interpretation later because the facial vein of lampreys is derived from the anterior cardinal veins, rather than the anterior extension of the lateral head vein (Janvier, 1981). Although this canal can be compared to the orbitonasal vein of gnathostomes topologically, it does not lead to nasal sacs in osteostracans because the nasal sacs are located high on the dorsal surface of the head, rather than anterior to the orbital cavity as in gnathostomes. As such, Janvier (1981) attributed a new name “frontal vein” for this canal in osteostracans. In contrast, the nasal sacs of *Shuyu* are located laterally in the braincase and rostral to the orbital cavity (as in jawed vertebrates), the short canal of *Shuyu* apparently leading to the nasal sacs. Thus, this canal in *Shuyu* can be homologised with the orbitonasal vein of gnathostomes. The orbitonasal vein bifurcates into two branches: one is the dorsal nasal vein leading to the nasal sacs, the other continues rostrally as the frontal vein corresponding to the frontal vein of osteostracans.

In gnathostomes, the orbitonasal artery arises from the internal carotid artery just before it enters the cranium and runs anteriorly along the medial wall of the orbital cavity (Meng and Su, 1987; Ostrander, 2000). In a typical teleost, it gives off two branches: one branch is the optic artery to the extrinsic muscles, the retina and lentiform body of the eye (Harder, 1975), and the other continues to the nasal sacs where the arteries of the two sides meet in the rostro-nasal region (Bertin, 1958; Ostrander, 2000). The courses and pattern of the orbitonasal artery in galeaspids resemble the condition in jawed vertebrates. No corresponding orbitonasal artery has been identified in lampreys or osteostracans. The appearance of the orbitonasal artery and vein is probably related to the separation of nasohypophysial complex and the lateral displacement of the nasal placodes (in development and evolution) which were regarded as fundamental prerequisites for the development of jaws and diplorhiny

(Gai et al., 2011; Gai and Zhu, 2012). Therefore, the presence of the orbitonasal artery and vein might be regarded as two characters uniquely shared by galeaspids and gnathostomes.

#### **5.4. Homology of marginal artery and vein**

Galeaspids and osteostracans uniquely possess a large marginal vein and artery, a vascular system located in the margin of the orobranchial chamber. The problem of homology of the marginal vein in osteostracans was discussed in detail by Stensiö (1927), Wängsjö (1952) and Janvier (1975, 1981). Stensiö (1927) considered it homologous to the dorsal superficial longitudinal vein. However, Wängsjö (1952) pointed out that the dorsal superficial longitudinal vein of lampreys is connected with anterior cardinal vein. The marginal vein of osteostracans is connected (at least anteriorly) with the ventral jugular vein and lacks a connection with the anterior cardinal vein. Janvier et al. (1991) considered that the marginal sinus/vein on each side of the body plays the same role as the anterior cardinal vein of lampreys, and may be considered the anterior cardinal vein in an aberrant position. However, the anterior cardinal veins of lampreys flank the dorsal aorta dorsal to the branchial pouches, whereas the marginal veins are ventral to the branchial pouches. In addition, the anterior cardinal veins probably continue anteriorly as the lateral capitis vein, which has been clearly identified in *Shuyu*. Janvier (1981) expressed difficulty in comparing the marginal veins with the known veins in other vertebrates. To some extent, the function of the marginal vein in galeaspids and osteostracans may be similar to that of the rim of anterior cardinal vein or to that of the ventral jugular vein which drains the extrabranchial vessels and superficial veins of the ventral face of the head-shield.

Since the canals for the ventral jugular vein and ventral aorta have not definitely been identified in both groups, one possible interpretation is that the marginal vein and artery are a modified ventral jugular vein and ventral aorta uniquely adapting to the laterally expanded skull of galeaspids and osteostracans. The marginal vein and artery are always coupled together in both groups and are located ventral to the branchial pouches as the ventral jugular vein and ventral aorta in other vertebrates. In addition, they also issue numerous transverse canals, probably for the efferent branchial vein and afferent branchial artery to the branchial pouches, as in the ventral jugular vein and ventral aorta in other vertebrates. Compared to that of osteostracans,

the marginal vein of *Shuyu* is probably connected with the dorsal jugular vein through the extrabranchial vein. As in osteostracans, it also has three large subbranches for the cornual vein leading to the cornual process. In osteostracans, the marginal artery and vein are also connected with the brachial artery and vein of the paired fins, which are absent in galeaspids.

### **5.5. Cerebral vascular supply in galeaspids and osteostracans**

The vascular supply to specific brain regions has been reviewed in various vertebrate groups (Rahmat and Gillanda, 2014), but still remains poorly known on the fossil stem-gnathostome groups.

No independent canal for the anterior cerebral artery has been identified in *Shuyu*. The anterior cerebral artery probably enters the hypophysial pouch via the foramen for the hypophysial artery, and traverses the hypophysial pouch vertically to the forebrain as in extant vertebrates. The anterior cerebral artery of osteostracans was assumed to connect to the forebrain via the optic fenestra (Wängsjö, 1952). However, Janvier (1981) thought that the anterior cerebral artery did not traverse the orbital fenestra but, rather, follows the canal that was attributed by Stensiö (1927) to a sensorial root of the trigeminal nerve. In *Shuyu*, a small foramen for the hypophysial artery penetrates the lateral wall of the hypophysial pouch. Thus, the anterior cerebral artery of osteostracans is more likely to connect to the hypophysial pouch via the foramen for the hypophysial artery, before ascending to the forebrain, as in *Shuyu*, rather than following the optic fenestra.

In osteostracans, the floor of myelencephalic division is penetrated by a pair of foramina just rostral to the vagus canals (Stensiö, 1927; Janvier, 1981, 1985). The paired foramina were assumed to contain the posterior encephalic artery (Stensiö 1927) or the encephalic occipital artery (Janvier, 1981, 1985), which corresponds to the posterior cerebral artery of *Shuyu*. In addition, the ventral face of the myelencephalic division of osteostracans shows two longitudinal furrows perhaps for the rhombencephalic arteries (Janvier, 1985, fig. 37B, s.a.rh.) as in lampreys (Sterzi, 1904; Janvier, 1975, fig. 3A, a.rh). However, no similar longitudinal furrow on the ventral face of the myelencephalic division has been observed in our specimens of *Shuyu*.

The canal for the anterior cerebral vein has been clearly identified in galeaspids. However, the identification of the anterior cerebral vein in osteostracans remains controversial. According to Wängsjö (1952), the anterior cerebral vein passes through a wide canal from the mesencephalic division. After crossing the trigeminal cavity, it opens to the lateral head vein inside the orbital cavity. However, no such canal has been identified in *Norselaspis glacialis* (Janvier, 1981). Janvier (1981) thought that the anterior cerebral vein passed through the optic fenestra (fig. 5.1B, opt.f) directly and emptied into the venous sinuses surrounding the eyeball. In *Shuyu*, the anterior cerebral vein is clearly resolved. It follows an independent canal dorsal to the optic nerve to the telencephalon. In the orbital cavity, it probably amalgamates with the dorsal jugular vein, or the venous sinuses surrounding the eyeball. The condition of the anterior cerebral vein in *Shuyu* corroborates Janvier's interpretation (1981) for the anterior cerebral vein in osteostracans.

There is a large canal issuing from the metencephalic division antero-dorsally just posterior to the root of ophthalmic nerve in both osteostracans and galeaspids. This large canal extends antero-laterally to the posterior division of the orbital cavity in osteostracans, but leads directly to the lateral head vein in galeaspids. In osteostracans, the large canals were interpreted as a postorbital superficial artery (Stensiö, 1927, figs. 22, 23A, a.dsm) or a dorsal median superficial artery (Janvier, 1975, fig. 3B, C, a.dsm) as numerous rami (a.dsm1-3) arises from this canal to the headshield dorsally. The superficial artery was assumed to bifurcate from the encephalic occipital arteries underneath the brain cavity and traverse the brain cavity to the roof (Janvier, 1975). However, this interpretation is less acceptable in *Shuyu* for two reasons. Firstly, the encephalic occipital arteries are located more posteriorly on the opposite side of the brain cavity in our specimens. There is no evidence of a connection between the large canal and the encephalic occipital arteries inside the cranial cavity (even under tomographic examination). Secondly, the large canal leads directly to the lateral head vein in *Shuyu*. According to its position relative to the trigeminal and facial nerve, this large canal is more likely to contain a middle cerebral vein draining blood from the metencephalic division to the lateral head vein, rather than an artery. In the left part of specimens IVPP V 14334.3 and IVPP V 14334.4, numerous rami emerge from the middle cerebral vein and lateral head vein to the dorsal side of the headshield. Accordingly, these rami must have housed the dorsal

superficial veins, rather than arteries because they all eventually return to the lateral head vein. We suggest that the large canal for the dorsal median superficial artery in osteostracans probably contains the middle cerebral vein as well, which joins with the lateral head vein inside the orbital cavity. Our interpretation for the middle cerebral vein is consistent with the condition in crown gnathostomes. For example, in the Pennsylvanian actinopterygian *Lawrenciella* the middle cerebral veins are preserved as ridges along the anterodorsal part of the cerebellar auricles and extend down to the anteroventral corners of the cerebel (Hamel and Poplin, 2008).

There is no evidence in our material for an independent canal for the posterior cerebral vein draining the posterior portion of the brain. The posterior cerebral vein of galeasids probably leaves the cranial cavity via the jugular foramen along with the vagus nerve as that of living fishes or the internal jugular vein of tetrapods (Wake, 1992).

#### **5.6. New interpretation for the controversial canals in osteostracans**

In osteostracans, the internal carotid artery emits two main branches lateral to the ethmoid region: the dorsal one is interpreted as the facial artery, and the other is the adorbital artery (Stensiö, 1927; Janvier, 1981). However, the facial artery or the external maxillary artery is actually a branch of the external carotid artery that supplies to the structures of the face in humans. In *Shuyu*, the internal carotid artery have three offshoots including the canals for the ophthalmic artery, hypophysial artery and orbitonasal artery supplying blood to the orbital and the ethmoid region. Thus, the ‘facial artery’ of osteostracans is probably corresponding to a subbranch of the ophthalmic artery of *Shuyu*.

In osteostracans, the posterior part of the brain cavity is irrigated by a pair of small arteries arising from the dorsal aorta which were named the occipital cranial arteries, and were probably modified vertebro-medullar arteries (Stensiö, 1927; Janvier, 1981).

In osteostracans, a small canal arises a little posterior to the hypophysial pouch between the oculomotor and trochlear nerve. Stensiö (1927) thought it corresponded to the pituitary vein of fishes even though it does not leave the brain cavity at the level of the hypophysial pouch. Wängsjö (1952) rejected this homology and considered this canal a branch of the anterior cerebral vein. A similar canal is identified in *Shuyu* just

ventral to the oculomotor nerve. This canal clearly separates from the dorsal jugular vein and leads to the hypophysial pouch caudally. Thus, it must have housed a pituitary vein. In addition, the anterior cerebral vein has been shown to pass through the optic fenestra.

In osteostracans, the dorsal jugular vein receives a number of small tributaries of the labyrinthine vein (Stensiö, 1927, text-figs. 18, 19, 27, v.lab<sub>1-5</sub>) from the vestibular division of the labyrinthine cavity. These small tributaries can correspond to the anterior and posterior labyrinthine vein of *Shuyu*, which converges into the labyrinthine vein. The so-called posterior labyrinthine vein of *Cephalaspis* (Wängsjö, 1952, fig. 12, vlabp) has been shown to be the canal for the glossopharyngeal nerve by Janvier (1981).

The *dx* canal was identified in various osteostracan genera (Stensiö, 1927, 1963; Wängsjö, 1952; Janvier, 1981). Stensiö (1927) thought that the canal contained an artery or a nerve to the posterior part of the dorsal sensory field or for both an artery and a nerve, and further identified it as the supratemporal branch of the vagus, which may have reached the dorsal side of the cranium to innervate the dorsal sensory field (Stensiö, 1963, figs. 9A, 11, 13, dx). Wängsjö (1952), however, thought that it contained an artery arising from the occipital encephalic artery to the posterior part of the dorsal sensory field rather than a vein or nerve, because the occipital encephalic artery pierced the floor of the brain cavity in this region. Janvier (1981) considered the homolog of the canal undetermined, perhaps containing a blood vessel. A strikingly similar canal to the *dx* occurs also in galeaspids. This fairly narrow canal (*dx*, Fig. 8E, G) issues dorsally from the large vagus canal, ascends vertically, and bends anteriorly to meet the endolymphatic duct (*en.d*, Fig. 8E, G) just rostral to the dorsal commissure sensory canal (*dcm*, Fig. 8F). One possible interpretation is that the canal contained a ramus of the posterior lateral line nerve innervating the lateral dorsal sensory canal or the dorsal commissure sensory canal. However, as described previously, the posterior cerebral vein was probably also housed by the large vagus canal (X). The condition in *Shuyu* suggests that the *dx* canal perhaps contained the dorsal superficial vein of the posterior cerebral vein because the posterior cerebral vein is probably contained in the vagus canal and there is a similar canal for the dorsal superficial vein issuing from the middle cerebral vein to the dorsal side of head-shield.

There is an undetermined canal *t* just dorsal to the canal of the glossopharyngeal nerve in various osteostracan taxa such as *Cephalaspis hoeli* (Stensiö, 1927, fig. 17, *t*), *Kiaeraspis auchenaspidoides* (Stensiö, 1927, fig. 22, *t*), “*Cephalaspis*” *excellens* (Wängsjö, 1952, pl. 15, fig. 2), *Norselaspis glacialis* (Janvier, 1981, fig. 18A, d.x.), but its interpretation remains controversial. Stensiö (1927) considered that it perhaps contained a branch of the acoustic nerve or a blood vessel. Janvier (1981) suggested that it was likely to contain a sensory branch of the first spinal nerve or a blood vessel. A similar canal (pl.a, Fig. 8B-E) in the same position is identified in *Shuyu*. Here, we follow the latter interpretation, and suggest that it probably contains the posterior labyrinthic artery that is separated from the posterior cerebral artery within the brain cavity.

## 6. Conclusion

Synchrotron radiation X-ray tomographic microscopy has allowed us to reconstruct the cardiovascular system of galeaspids in three-dimension and in high resolution. Our results indicate that the circulatory system of galeaspids exhibits a mosaic of primitive vertebrate and derived gnathostome characters, including many otherwise gnathostome characters that are absent from osteostracans — the presumed sister-lineage to the jawed vertebrates.

Fig. 17 here

The paired dorsal aortae in amphioxus, hagfishes, galeaspids and jawed vertebrates (gnathostomes) probably represent a plesiomorphic condition for vertebrates (Fig. 17), and the unpaired condition in osteostracans and lampreys is probably due to convergent evolution. A dorsal aorta completely enclosed within basicranial cartilage can be regarded as a derived character uniquely shared by galeaspids and gnathostomes (Fig. 17). The dorsal aorta housed in a large median groove together with the oesophagus in osteostracans probably represents an intermediate condition between that of lampreys versus galeaspids and jawed vertebrates (Fig. 17).

The lateral head vein in jawless fishes is probably homologous with the dorsal jugular vein of jawed vertebrates. It is reasonable to consider the presence of the



lateral head vein as a plesiomorphic condition for vertebrates, but the large lateral head vein canal is one of the most conspicuous similarities of internal anatomy among osteostracans, galeaspids, placoderms, and crown gnathostomes (Fig. 17). It is worth noticing that extrabranchial draining is effected by the dorsal jugular vein and marginal vein in galeaspids, whereas in osteostracans it is effected by the marginal veins. A dorsal jugular vein receiving numerous extrabranchial veins is probably a unique character for galeaspids.

The presence of an orbitonasal artery and vein is another derived character uniquely shared by galeaspids and gnathostomes (Fig. 17). The evolution of the orbitonasal artery and vein was probably coupled with the phylogenetic separation of nasohypophysial complex and the lateral migration of nasal placodes, which are regarded as fundamental prerequisites for the development of jaws and diplorhiny.

The marginal vein and arteries uniquely shared by galeaspids and osteostracans, are probably the modified ventral jugular vein and ventral aorta, corresponding to the extensive lateral expansion of head-shield in both groups (Fig. 17). Extrabranchial draining is effected by the dorsal jugular vein and marginal vein in galeaspids, whereas by the marginal veins in osteostracans. The dorsal jugular vein receiving numerous extrabranchial veins is probably a unique character for galeaspids.

The new advances in galeaspid cardiovascular system also shed new light on some long-standing controversial issues on the cardiovascular system of osteostracans.

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Fig. 1. Phylogenetic relationships among living and fossil chordates. Resolved relationships are based on Keating and Donoghue (2016). *Metaspriggina* (Conway Morris and Caron, 2014) and *Haikouichthys* (Shu et al., 1999, 2003) have been interpreted as vertebrates while *Cathaymyrus* (Shu et al., 1996) and *Pikaia* (Conway Morris and Caron, 2012) have been interpreted as cephalochordate-like invertebrates; however, the precise phylogenetic position of these taxa remains unclear.

Fig. 2. Impressions of the heart and arteries preserved in a natural endocast of the visceral surface of the ventral dermoskeleton of the pteraspid heterostracan *Pteraspis leathensis* from the Early Devonian of Stanton Lacy, Shropshire, and housed in the Natural History Museum, London, UK, NHMUK PV P34277. (A) Overview, rostral

to the top, caudal to the bottom. (B) Position of the heart and caudally directed veins. (C) Branches of the larger veins ramify in association with the outer lateral position of the branchial pouches. Scale bar in millimeters.

Fig. 3. The fossil sites of *Shuyu* in northwestern Zhejiang, China. (A) Geological map of Changxing County, showing two main occurrences of *Shuyu* (redrawn from Bureau of Geology and Mineral Resources of Zhejiang Province, 1989). (B) Location map, showing three main fossil sites of galeaspids in Zhejiang Province, China.

Fig. 4. 3D virtual reconstruction of vascular system of *Shuyu*, reconstructed from specimen IVPP V 14334.3. (A) Virtual endocast in dorsal aspect after removing the endocast of brain cavity to show the arterial circulatory system. (B) Virtual endocast in ventral aspect after removing the oralbranchial chamber and the left part of dorsal aorta. All vascular canals are marked in black text, others in grey text.

Fig. 5. Dorsal aorta and efferent branchial artery. (A) A horizontal CT section of specimen IVPP V 14334.1. (B) A natural endocast of specimen IVPP V 14334.24, in ventral view. (C) The 512th of 1024 CT sagittal slices of specimen IVPP V 14334.3. (D) A sagittal section through the endocast of braincase (V14334.3).

Fig. 6. The vascular system in the naso-hypophysial region. (A) Natural cast of the naso-hypophysial region of specimen IVPP V 14331.5, in dorsal view. (B) Virtual cast of the naso-hypophysial region of specimen IVPP V 14331.5, in dorsal view. All vascular canals are marked in black text, others in grey text.

Fig. 7. The vascular system in the ethmoid region, showing the coupled assembly of ophthalmic artery and profundus nerve. (A, B) Virtual endocast of specimen IVPP V 14434.3, after removing the endocast of orbital cavity, antero-lateral view; (A) left part; (B) right part. (C) An interpretive drawing of virtual endocast of specimen IVPP V 14434.3. All vascular canals are marked in black text, others in grey text.

Fig. 8. The vascular system in the occipital region. (A) Virtual endocast (dorsal view) of *Shuyu*, reconstructed from specimen IVPP V 14334.4, in which the boxed region is



magnified to illustrate the detailed anatomy of the occipital region (B-G). (B) Ventral view (right half). (C) Dorsal view (right half). (D) Lateral view (from right). (E) Front view. (F) Lateral view (from right), after removing the cast of dorsal jugular vein. (G) Lateral view, from left. All vascular canals are marked in black text, others in grey text.

Fig. 9. Vascular canals and cranial nerves in the occipital region. (A) Photo of a natural endocast (IVPP V 14334. 5), in ventral view. (B) Interpretive drawing of a natural endocast (IVPP V 14334. 5). All vascular canals are marked in black text, others in grey text.

Fig. 10. Diagrammatic restoration (A) and interpretive drawing (B) of endocast of *Shuyu*, in dorsal view. All vascular canals are marked in black text, others in grey text.

Fig. 11. The vascular canals for marginal artery and vein. (A) A complete natural endocast preserving the marginal and internal carotid arteries, V 14334.26A. (B) The right part of the head, specimen IVPP V 14334.25. (C) The right margin of the head, specimen IVPP V 14334.5. (D) The cornual process of specimen IVPP V 14334.27, showing the cornual artery from the marginal artery. All vascular canals are marked in black text, others in grey and white text.

Fig. 12. Venous canals in the ethmoidal region. (A, B) The virtual endocast reconstructed from specimen IVPP V 14334.2; (A) antero-lateral view from the right side; (B) antero-lateral view from the left side. (C) A virtual endocast of the nasal region reconstructed from specimen IVPP V 14334.3, in antero-lateral view. (D) The virtual endocast reconstructed from specimen IVPP V 14334.1, in antero-lateral view from the left side. All vascular canals are marked in black text, others in grey text.

Fig. 13. Venous canals in the optic region. (A) The virtual endocast of the orbital region reconstructed from specimen IVPP V 14334.1, in dorsal view. (B, C) The natural cast of the optic cavity of specimen IVPP V 14334.22; (B) the left eye; (C) the right eye. All vascular canals are marked in black text, others in grey text.

Fig. 14. Venous canals in otic region. (A) Virtual endocast of labyrinthic cavity reconstructed from specimen IVPP V 14334.3, in dorsal view. (B) The natural cast of the right labyrinthic cavity of specimen IVPP V 14334.5. All vascular canals are marked in black text, others in grey and white text.

Fig. 15. Extrabranial and dorsal superficial vein, all in dorsal view. (A) A natural endocast, specimen IVPP V 14334.5. (B) Close-up of the panel of (A) showing the extrabranial vein. (C) Close-up of the panel of (B), showing the dorsal superficial vein arising from the extrabranial vein. All vascular canals are marked in black text, others in white text.

Fig. 16. Network of subcutaneous vascular plexus of galeaspids. (A, B) *Shuyu zhejiangensis*; (A) IVPP V 14330.3; (B) IVPP V 14334.28. (C) *Meishanaspis lehmani*, IVPP V 8298.b. (D) *Duyunolepis paoyangensis*, GMC V 1324. All vascular canals are marked in black text, others in white text.

Fig. 17. The early evolution of vertebrate circulatory system. Red node A–D, G–I showing the evolutionary sequence of circulatory system from stem chordates to tetrapods; green node E showing the characters uniquely shared by osteostracans and gnathostomes; blue node F and red node G showing the characters uniquely shared by galeaspids and gnathostomes.

chordates

olfactores

jawless

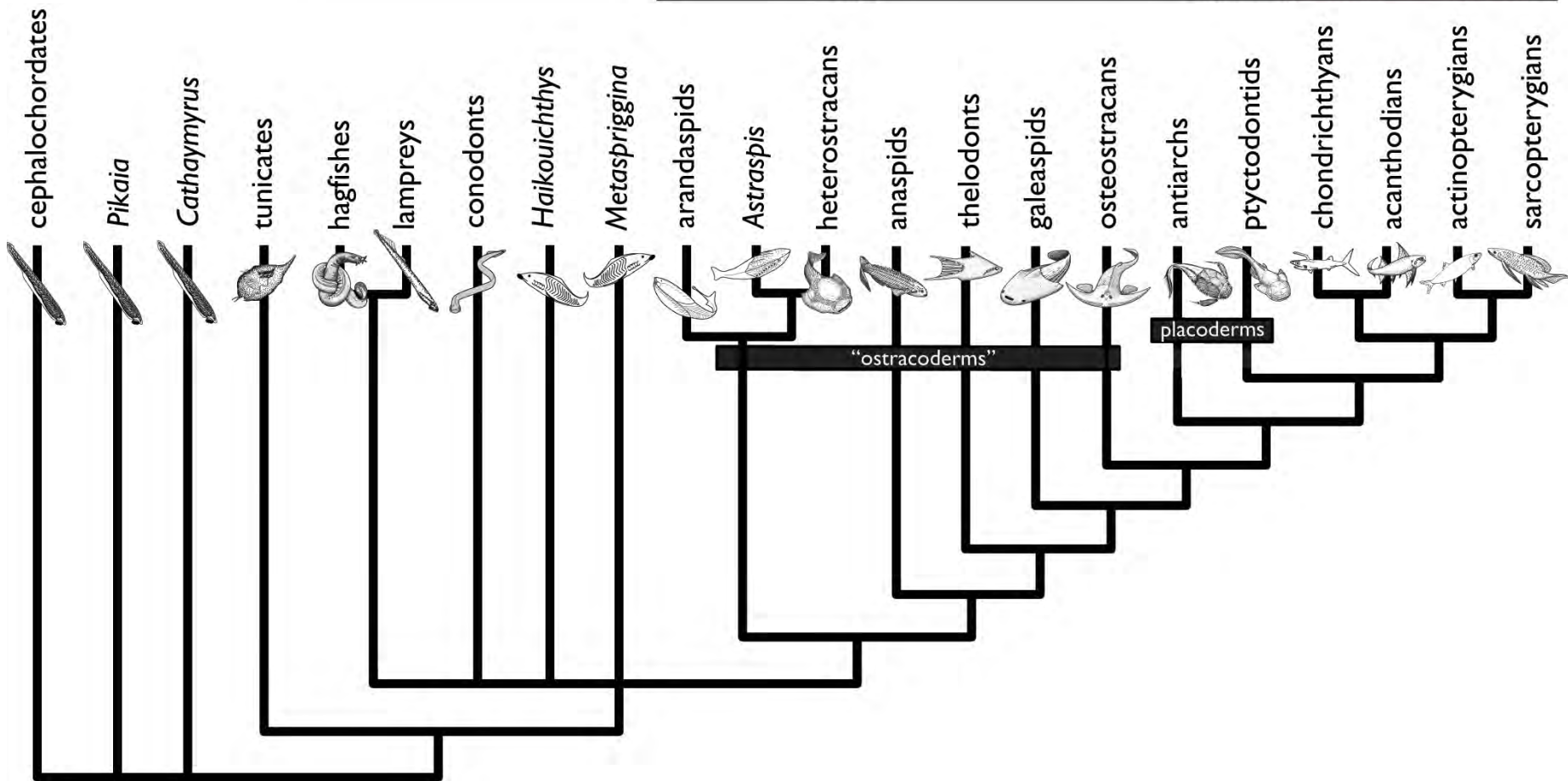
vertebrates

jawed

stem

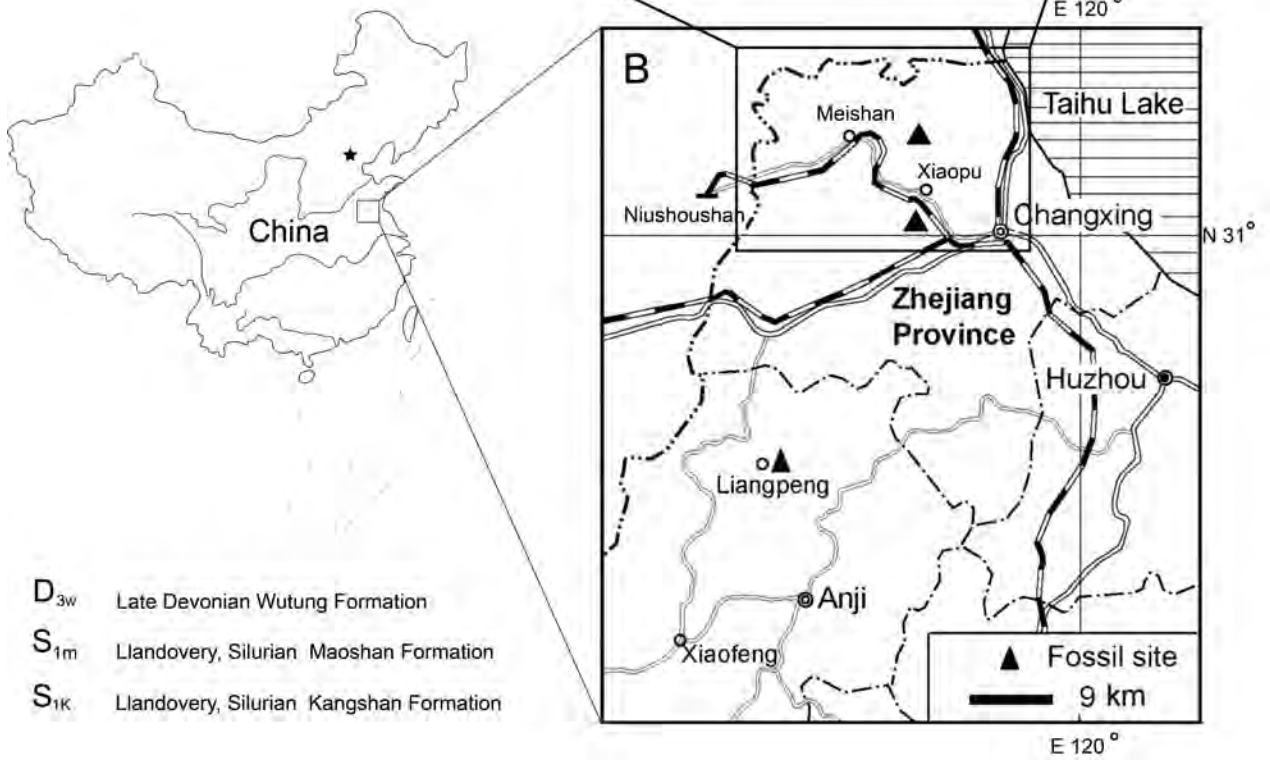
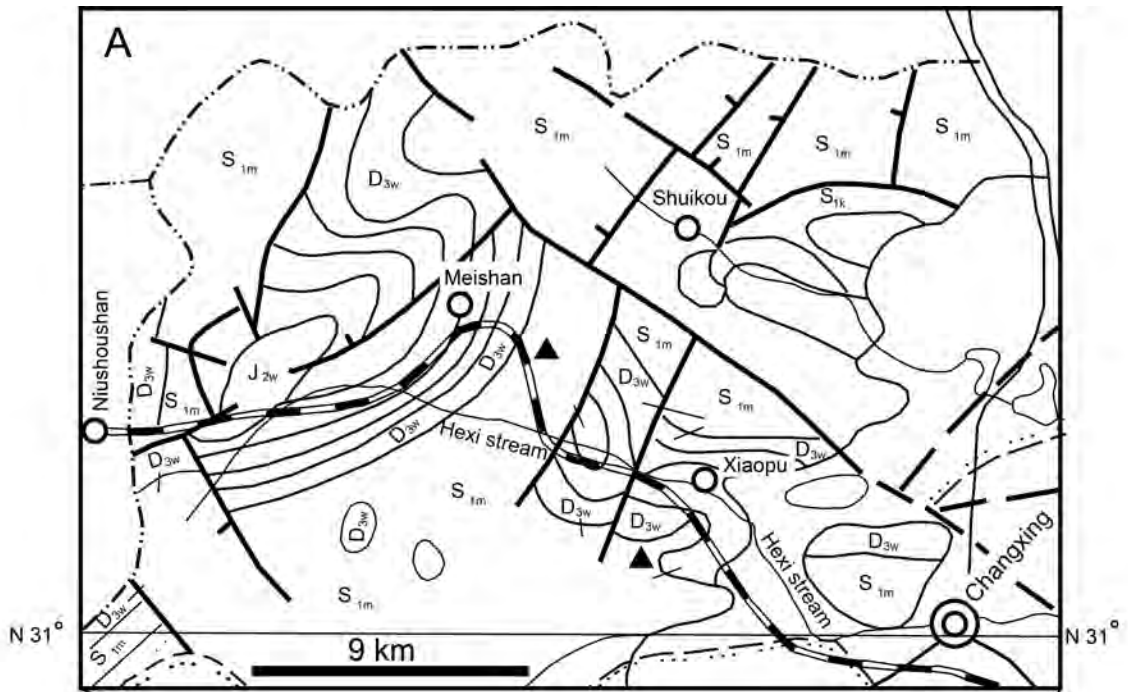
gnathostomes

crown

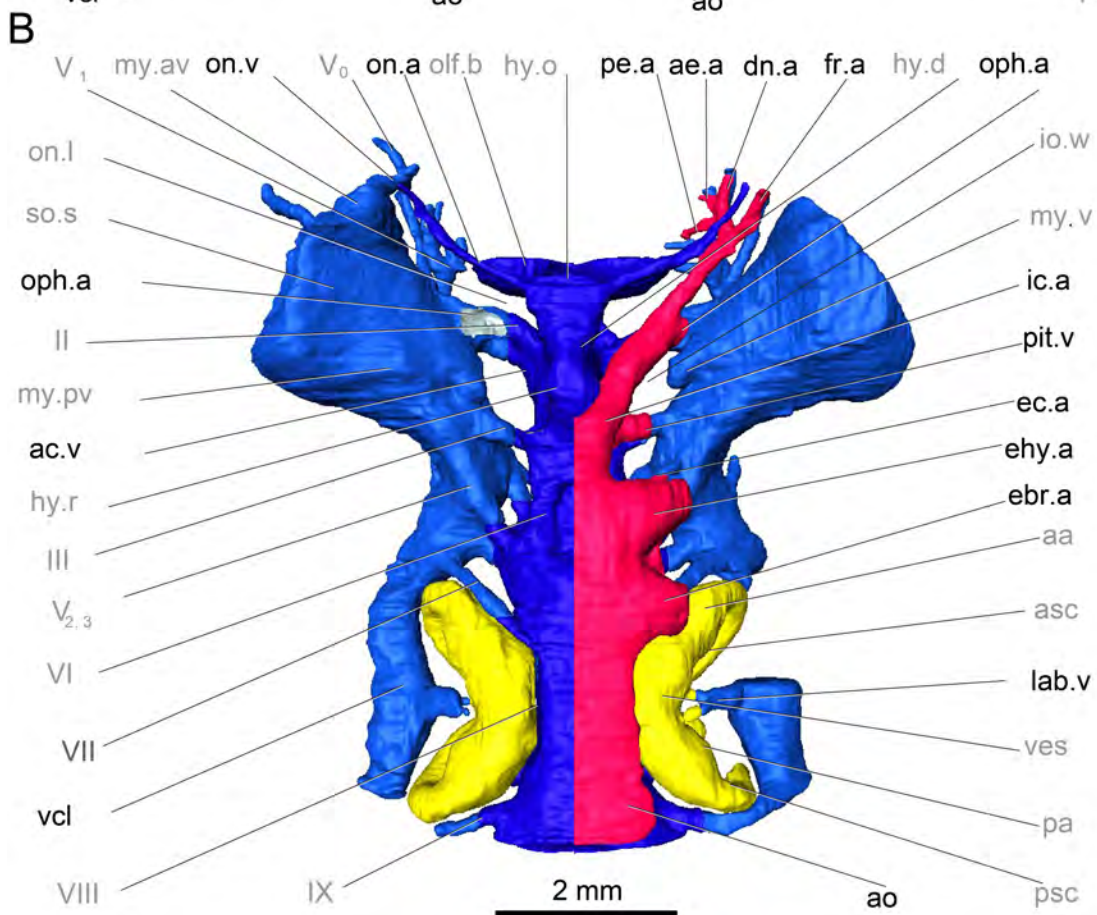
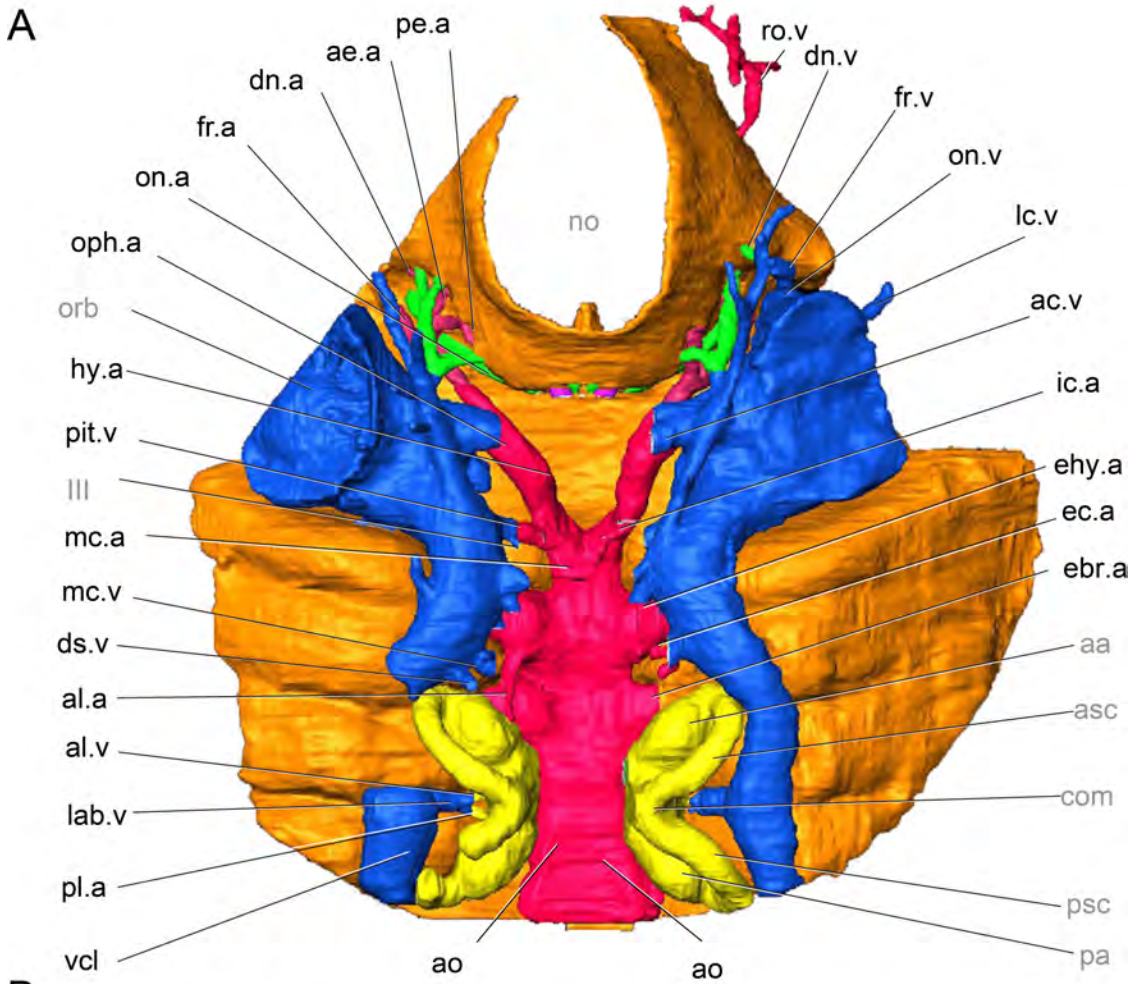


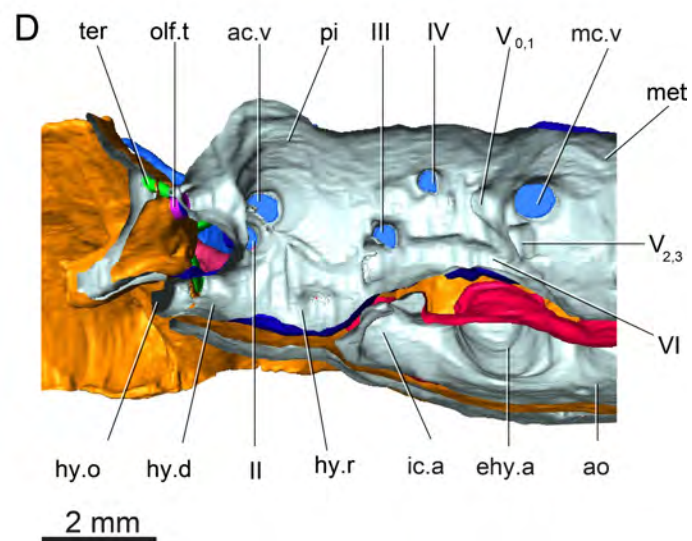
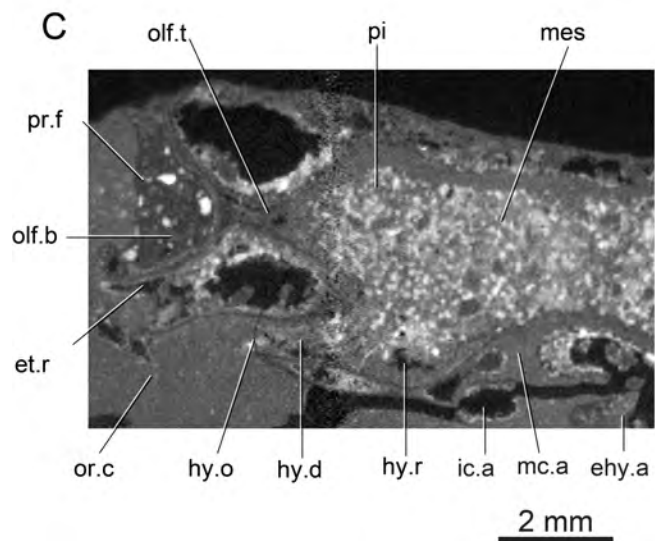
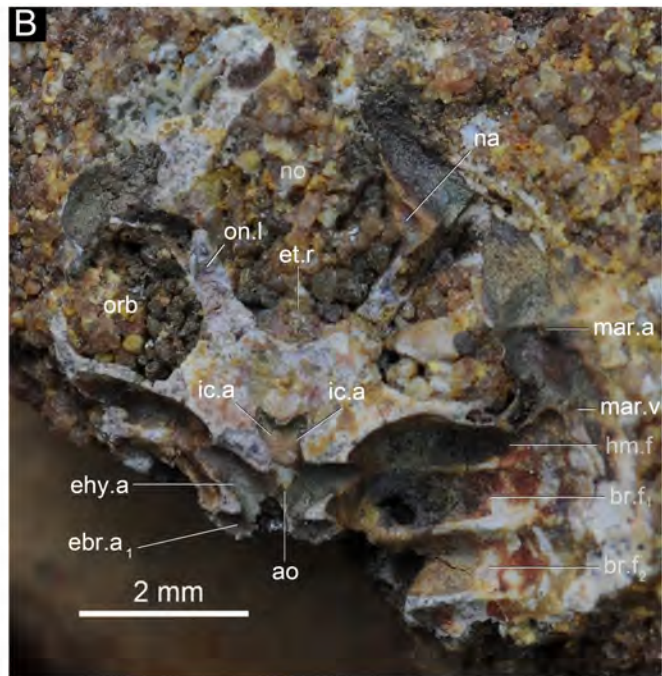
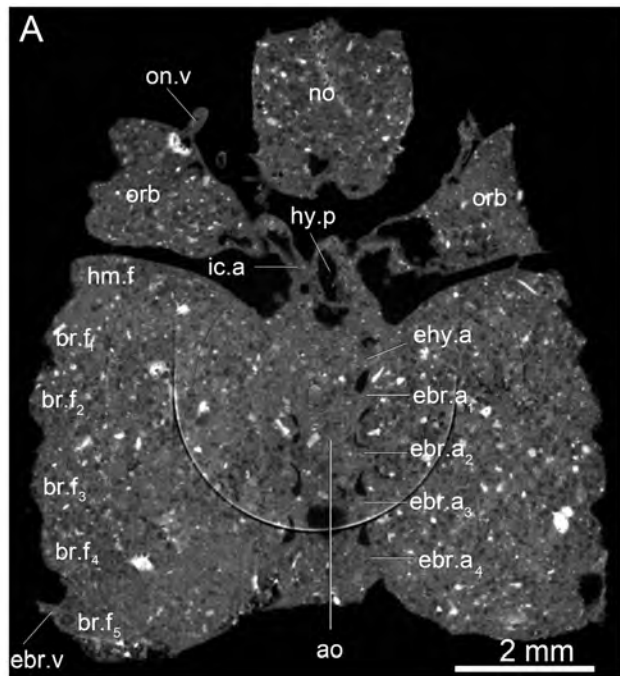










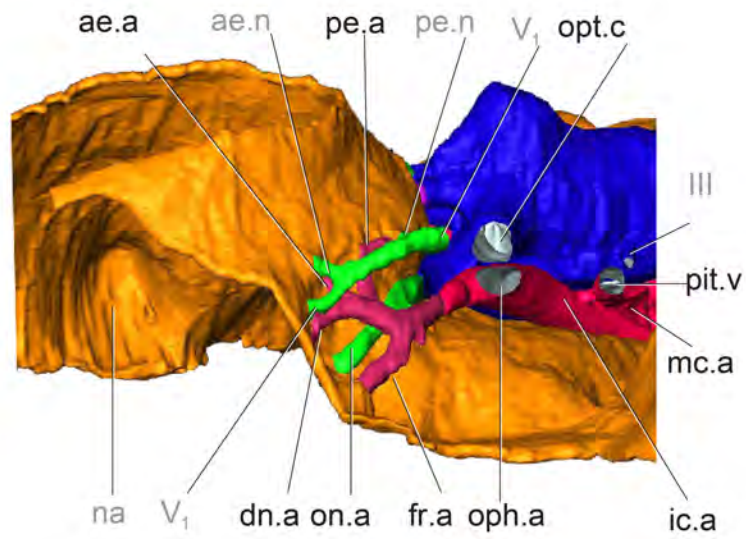




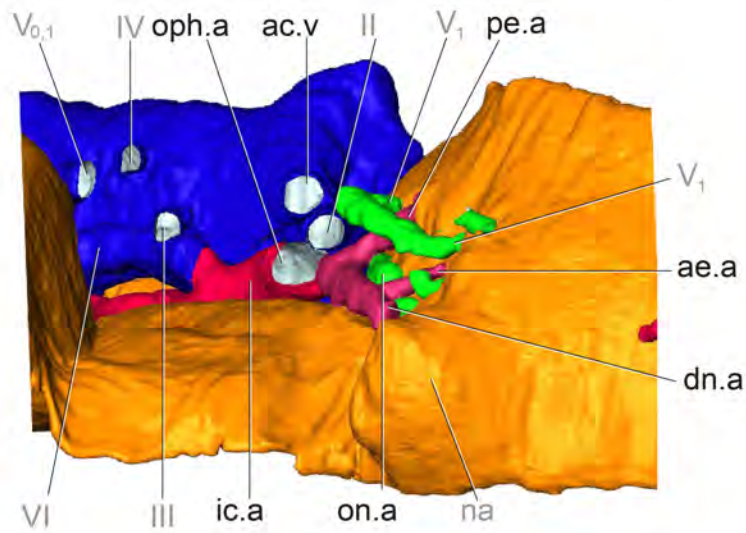




A

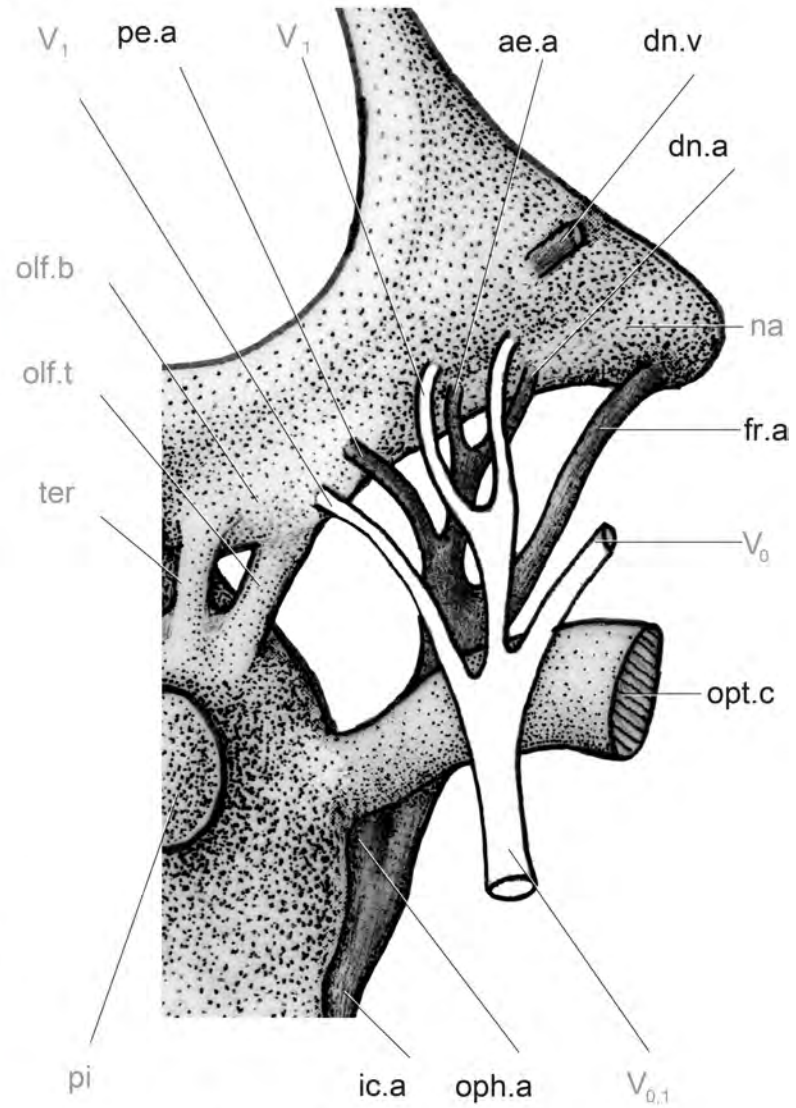


B

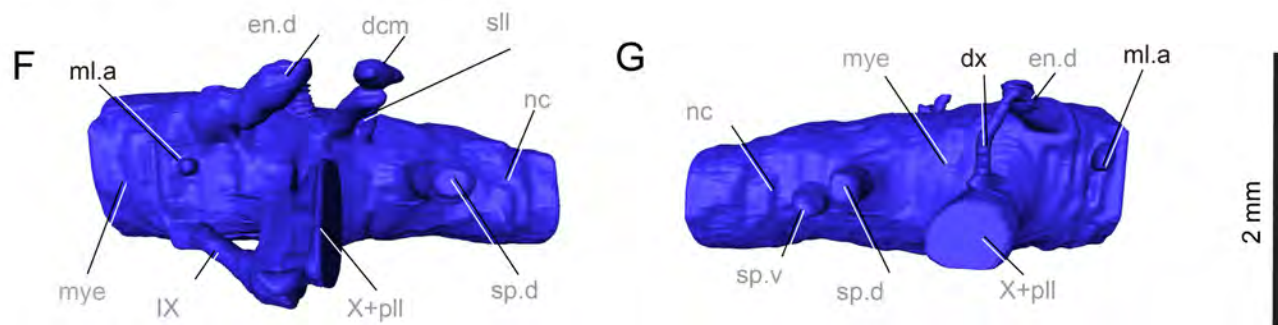
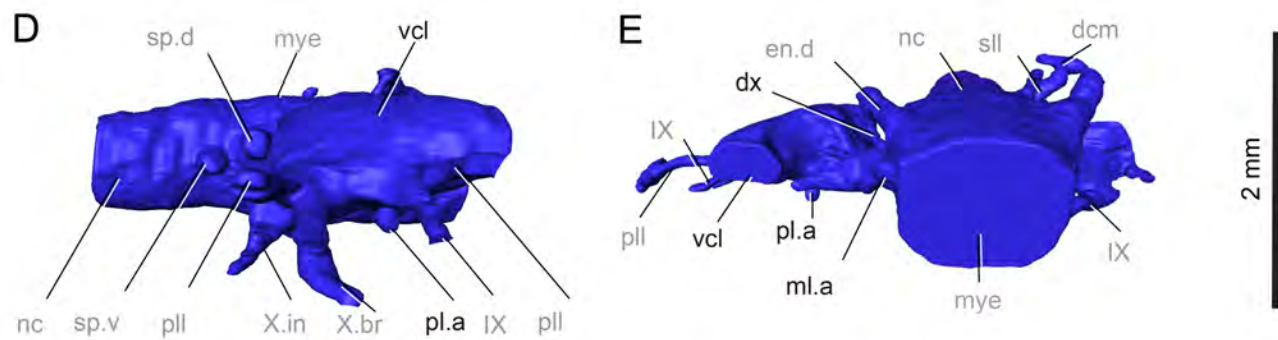
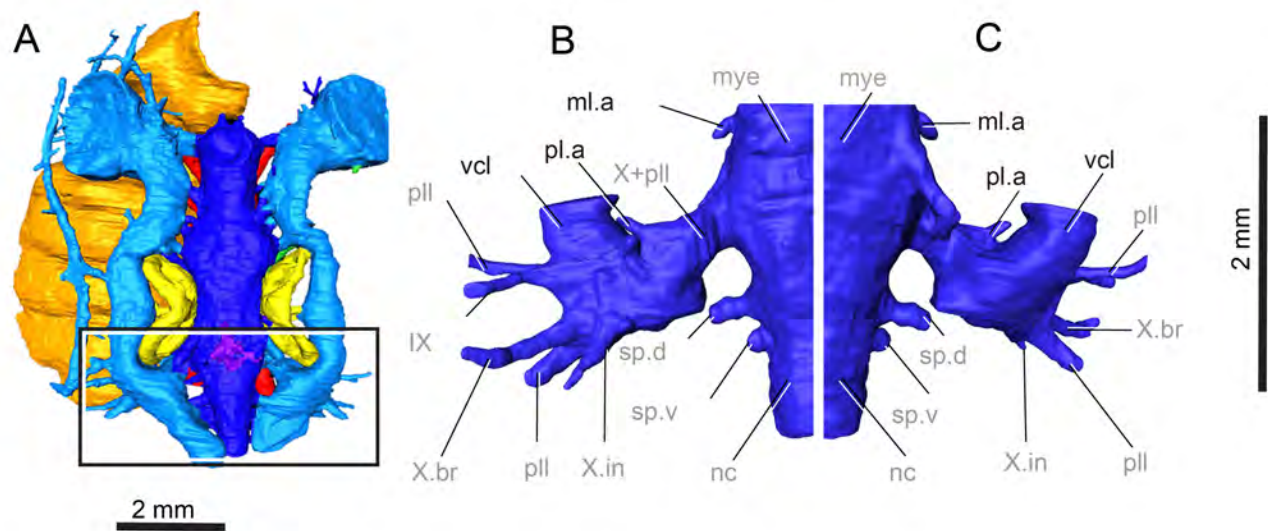


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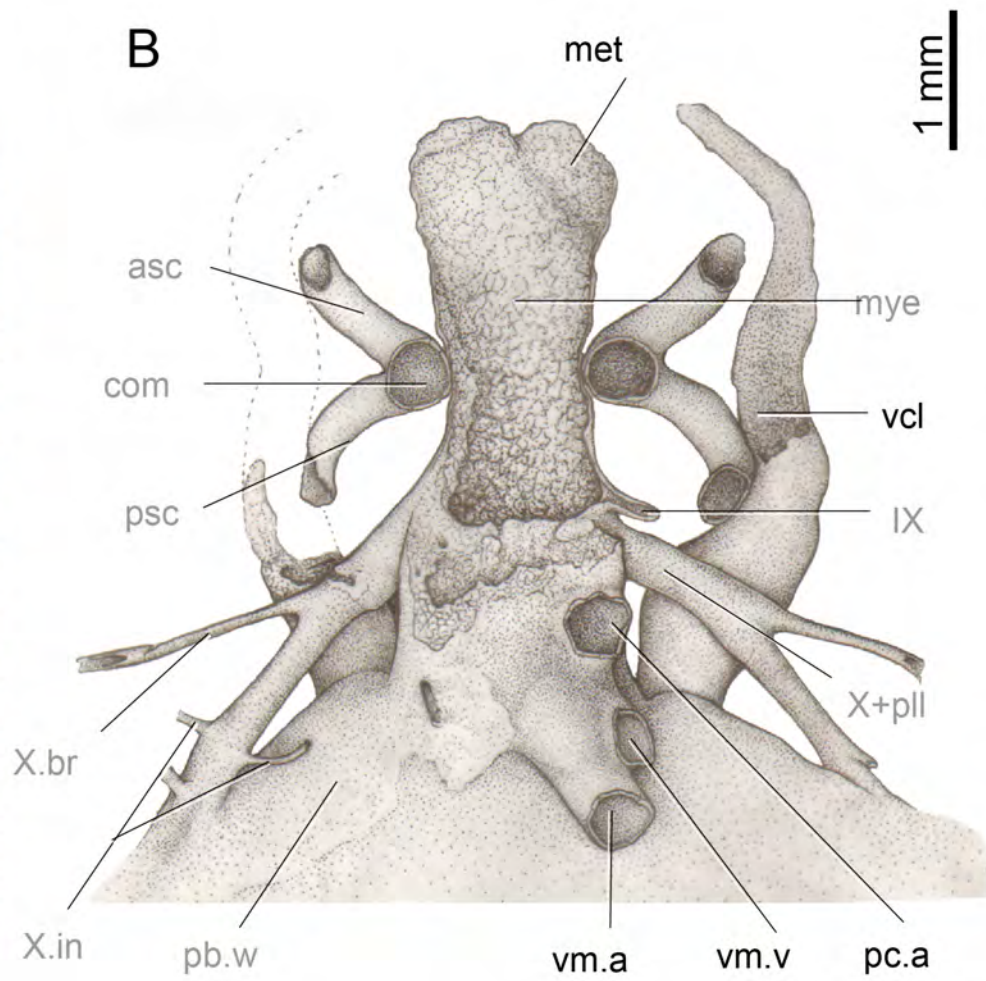
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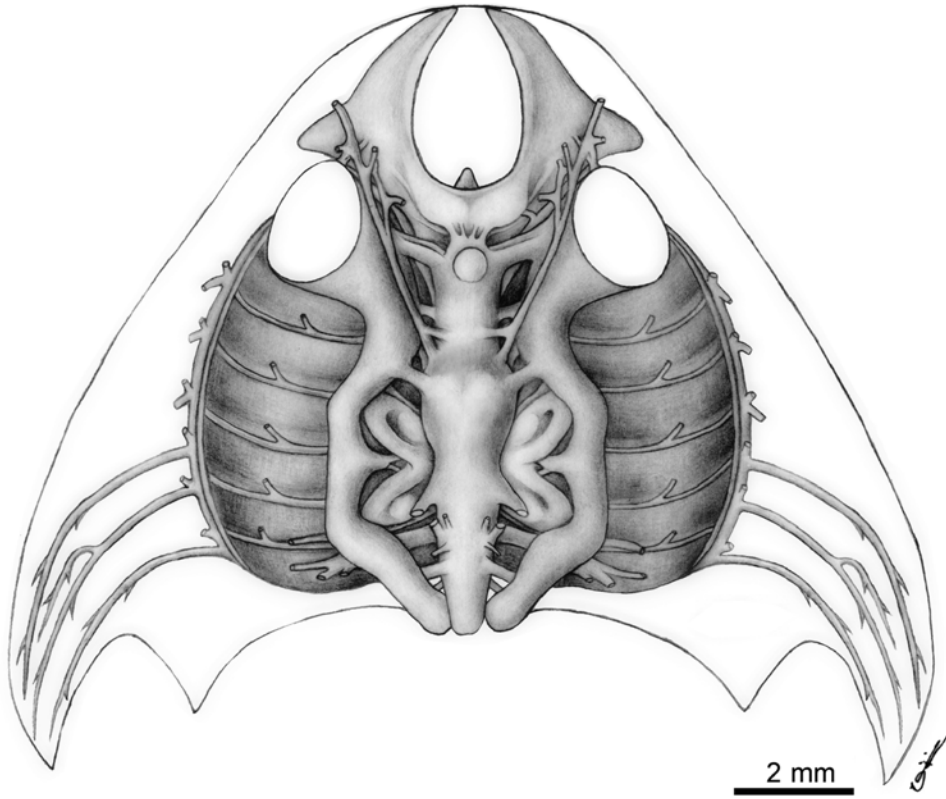
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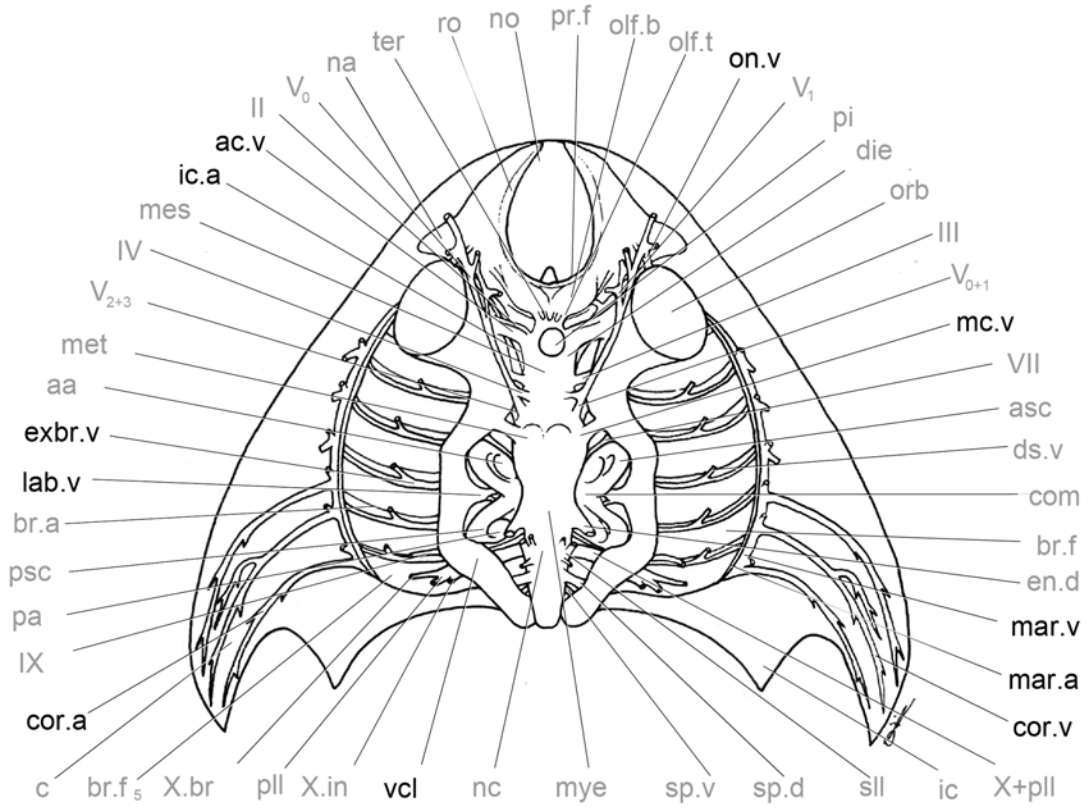




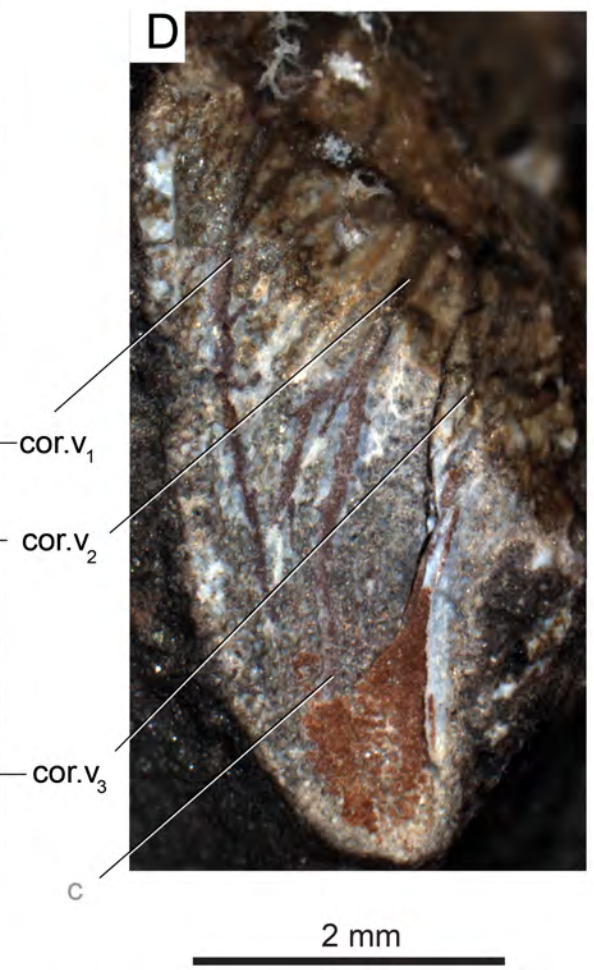
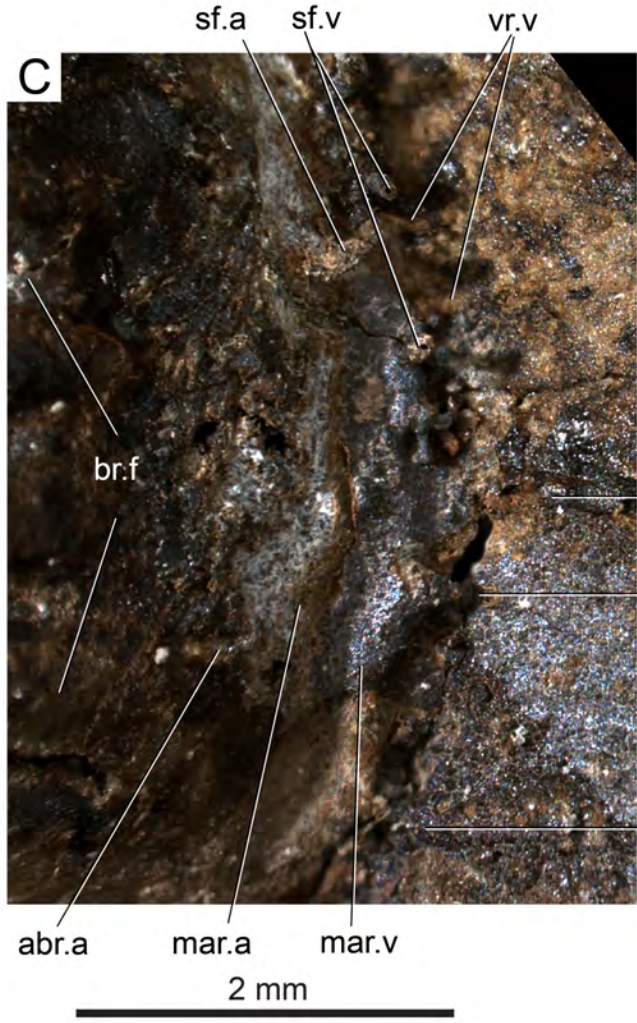
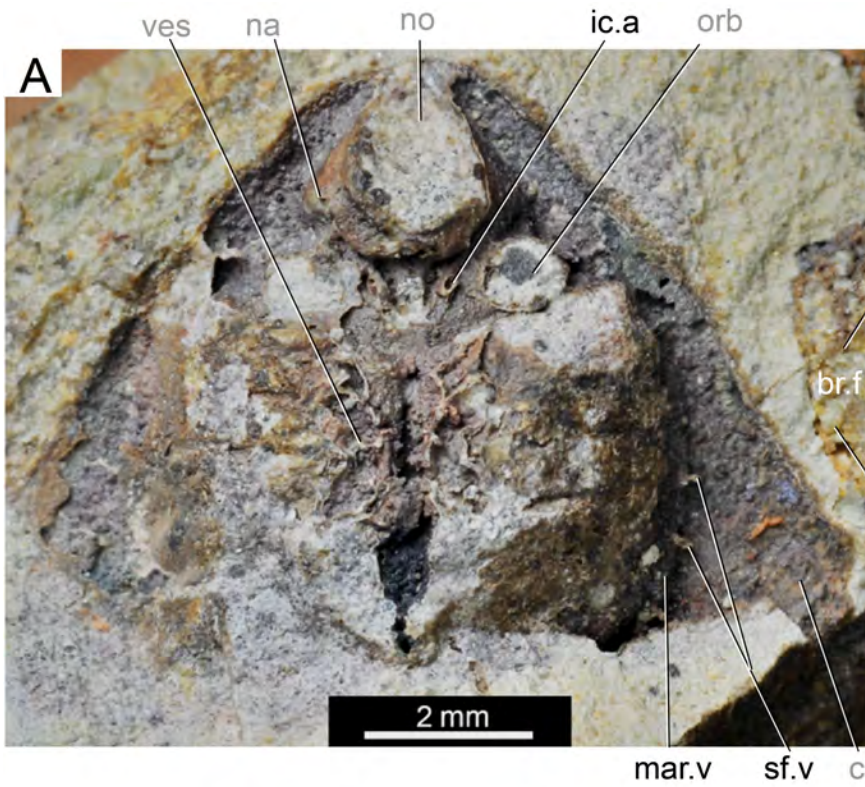
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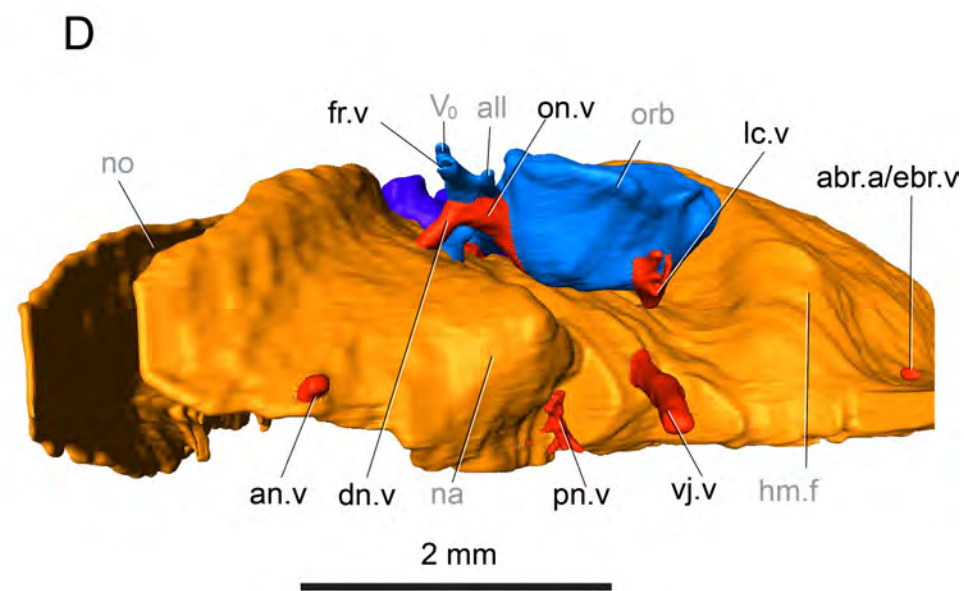
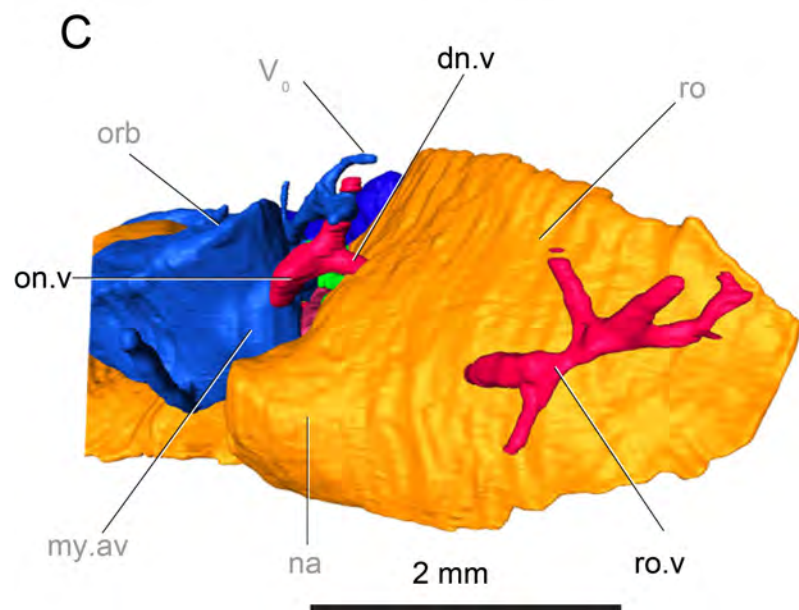
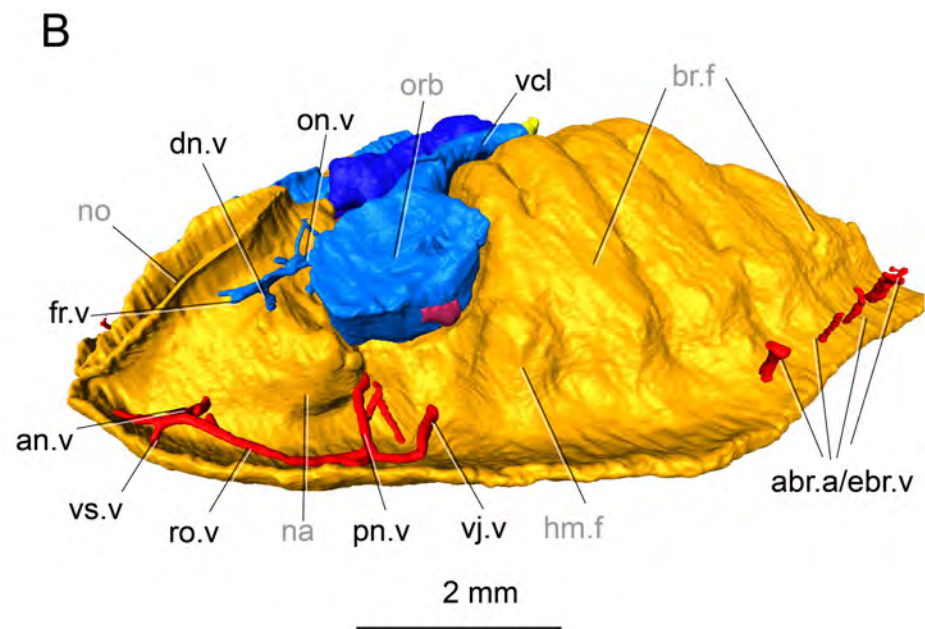
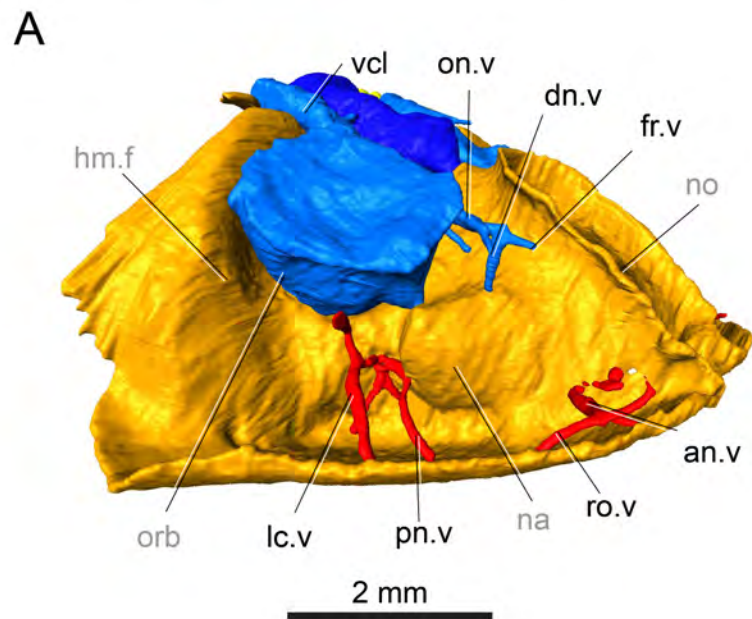
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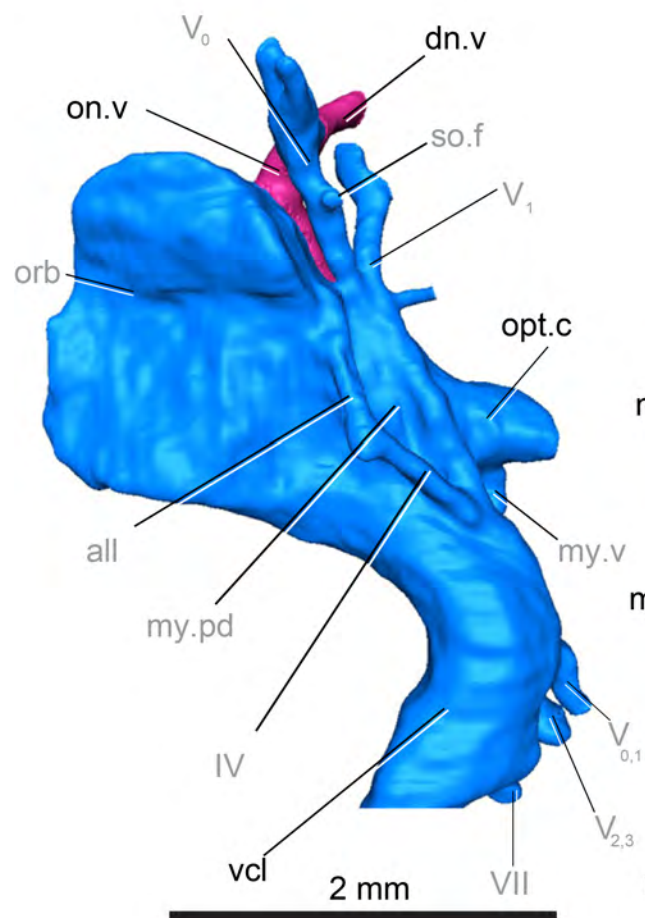
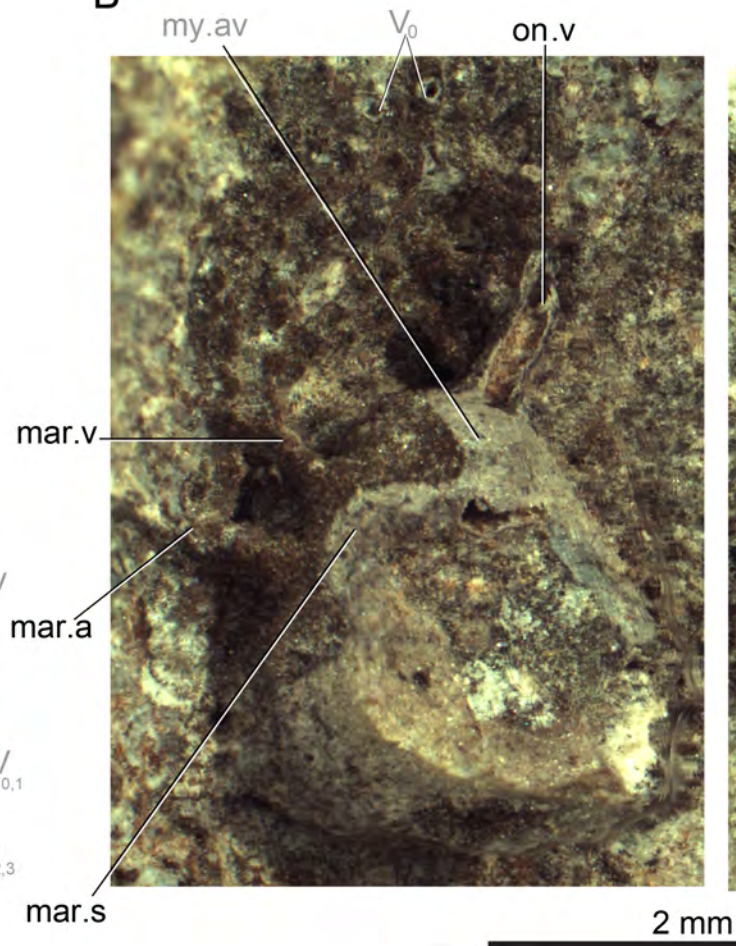
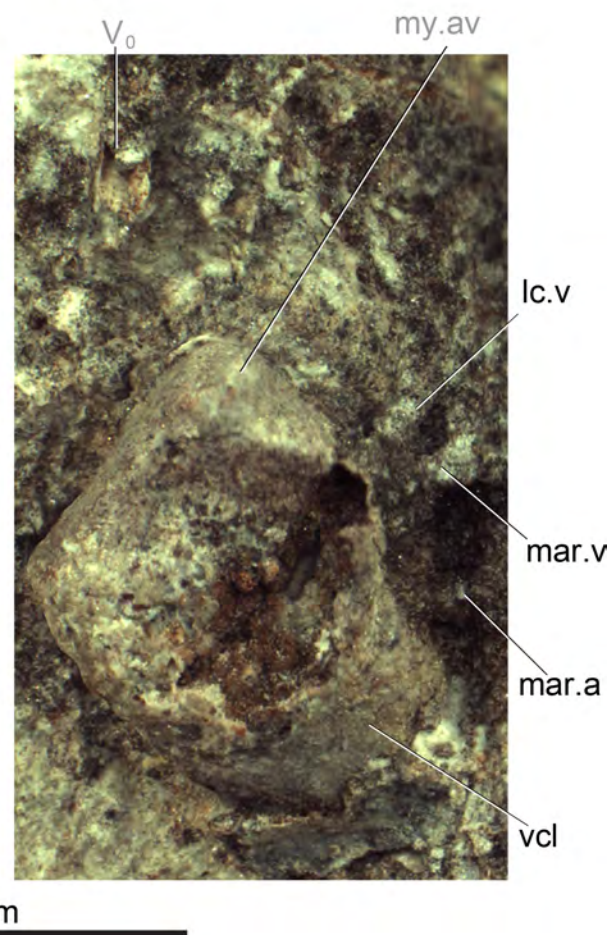






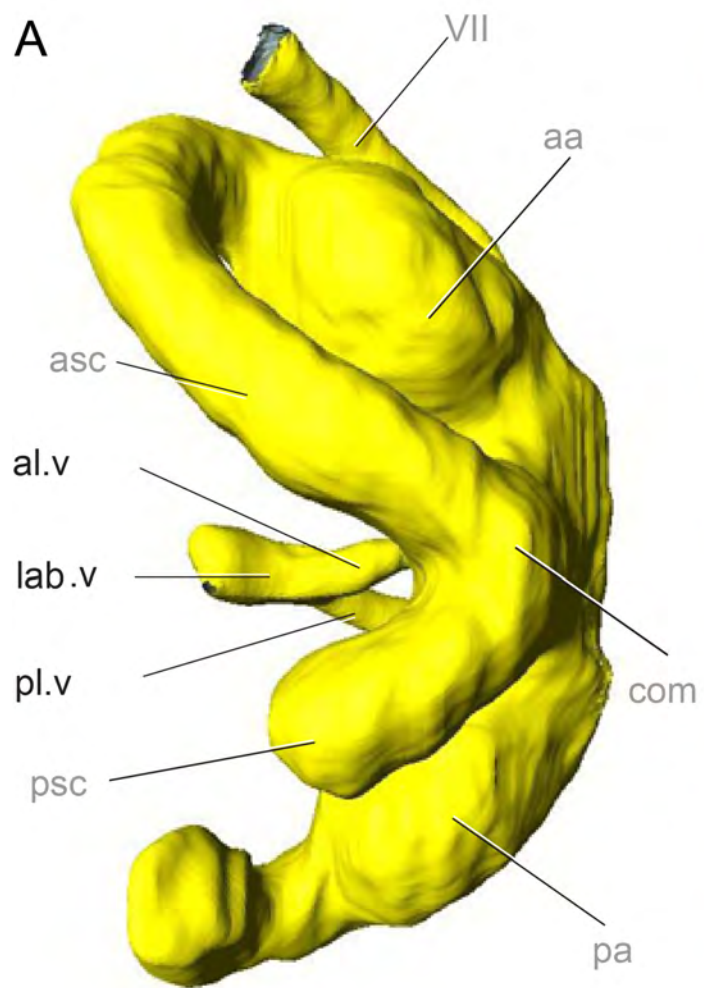




**A****B****C**

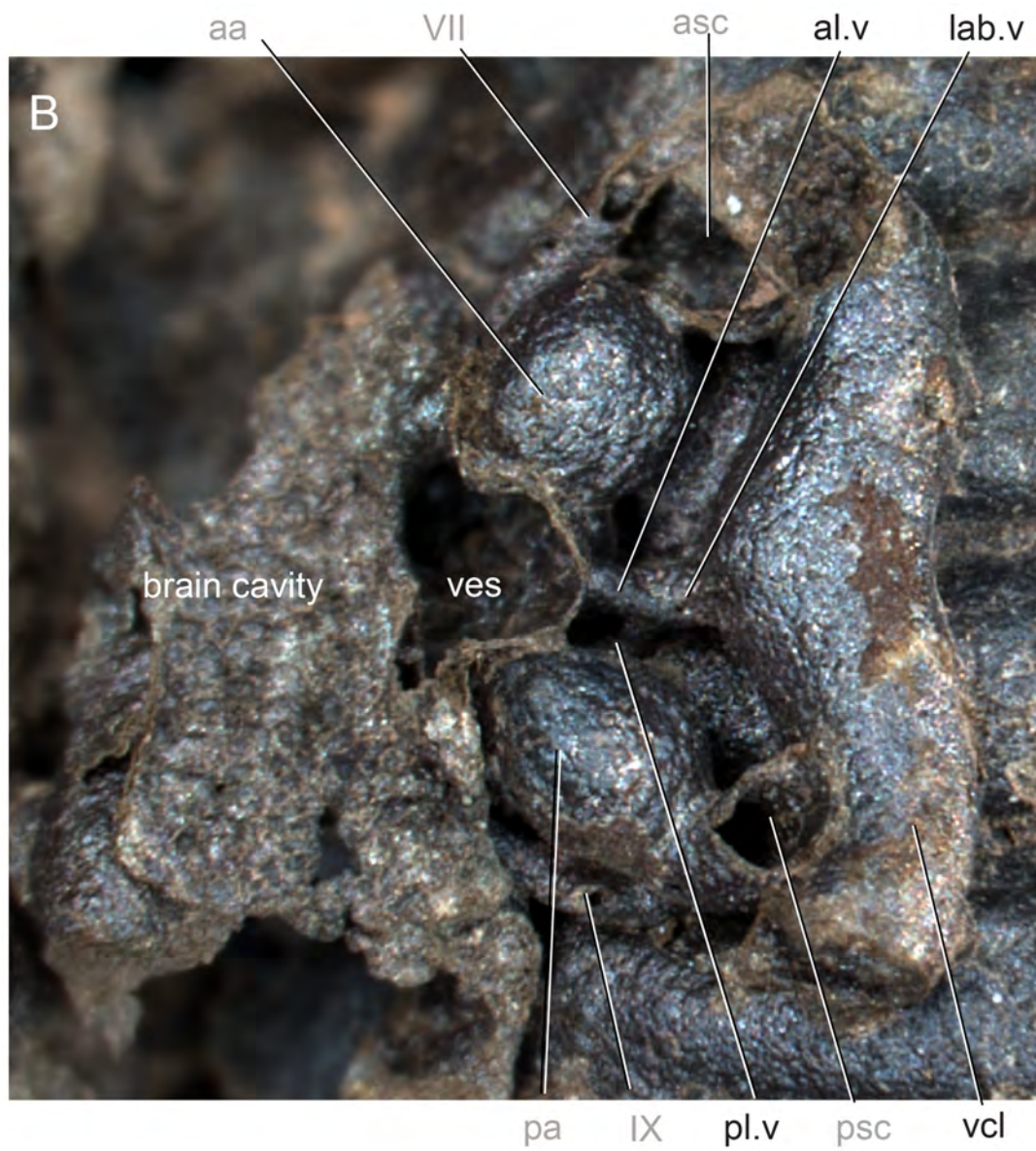


A



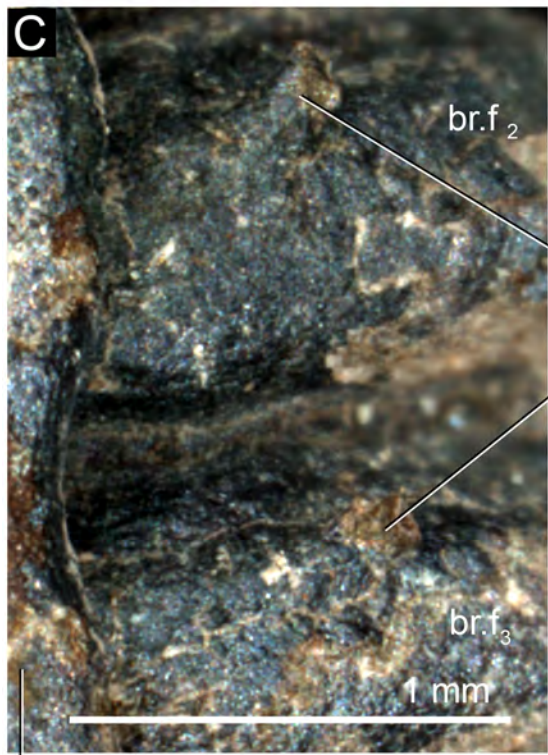
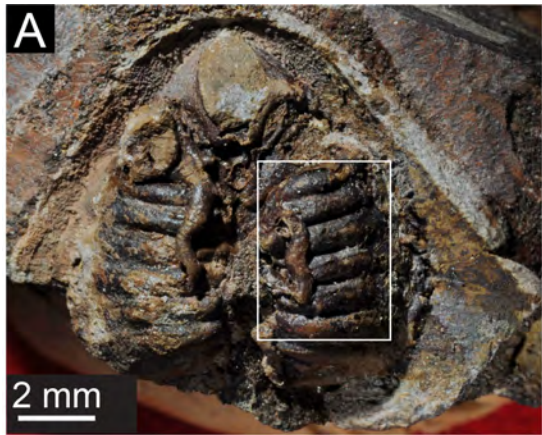
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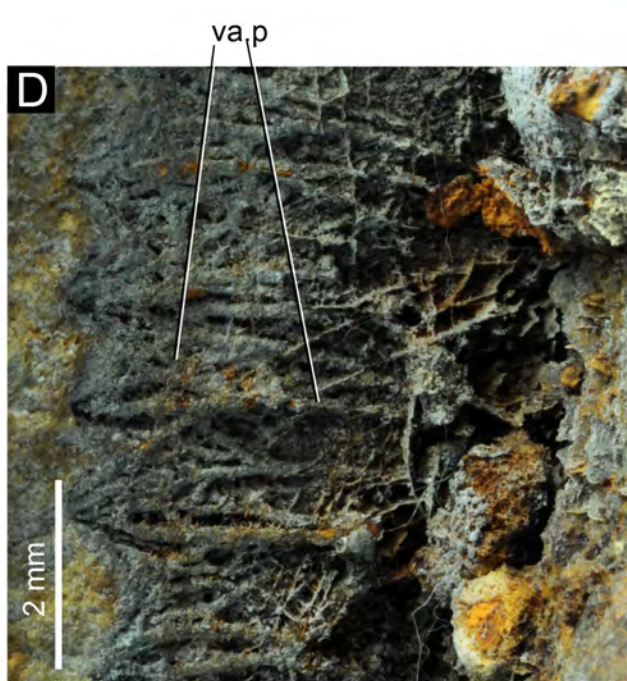
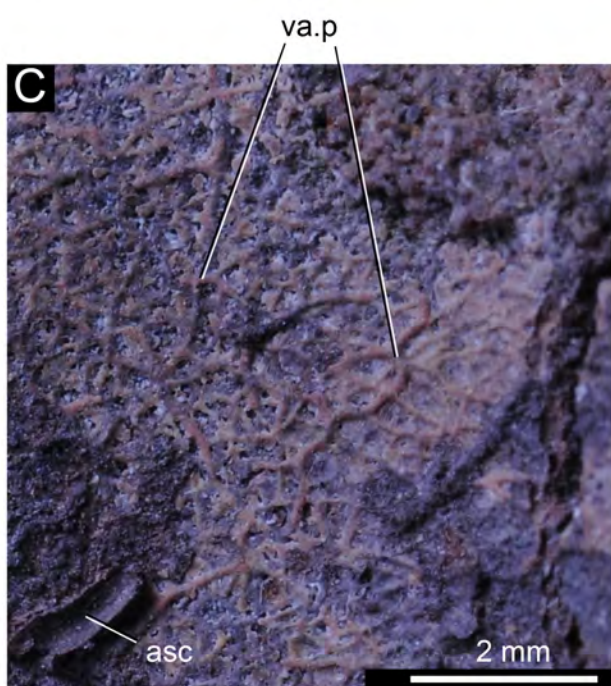
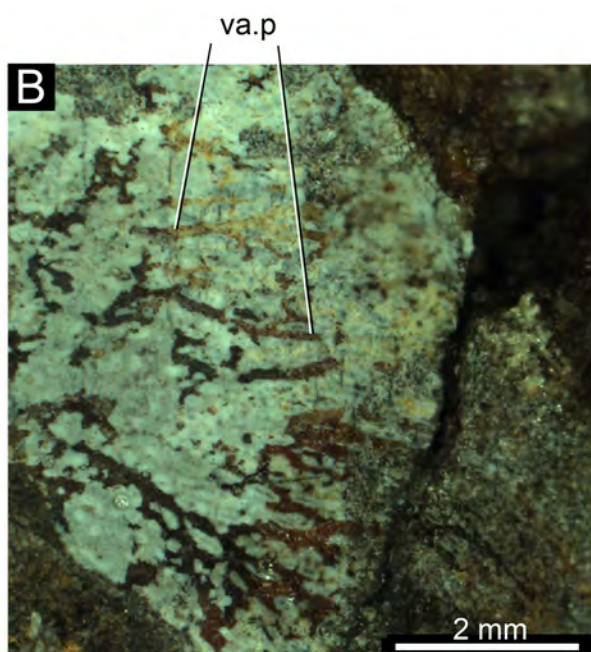
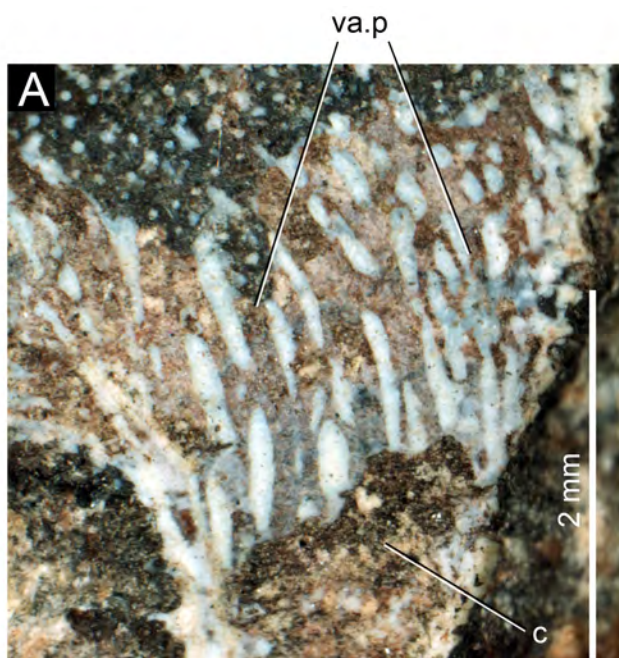




vcl

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Chordata

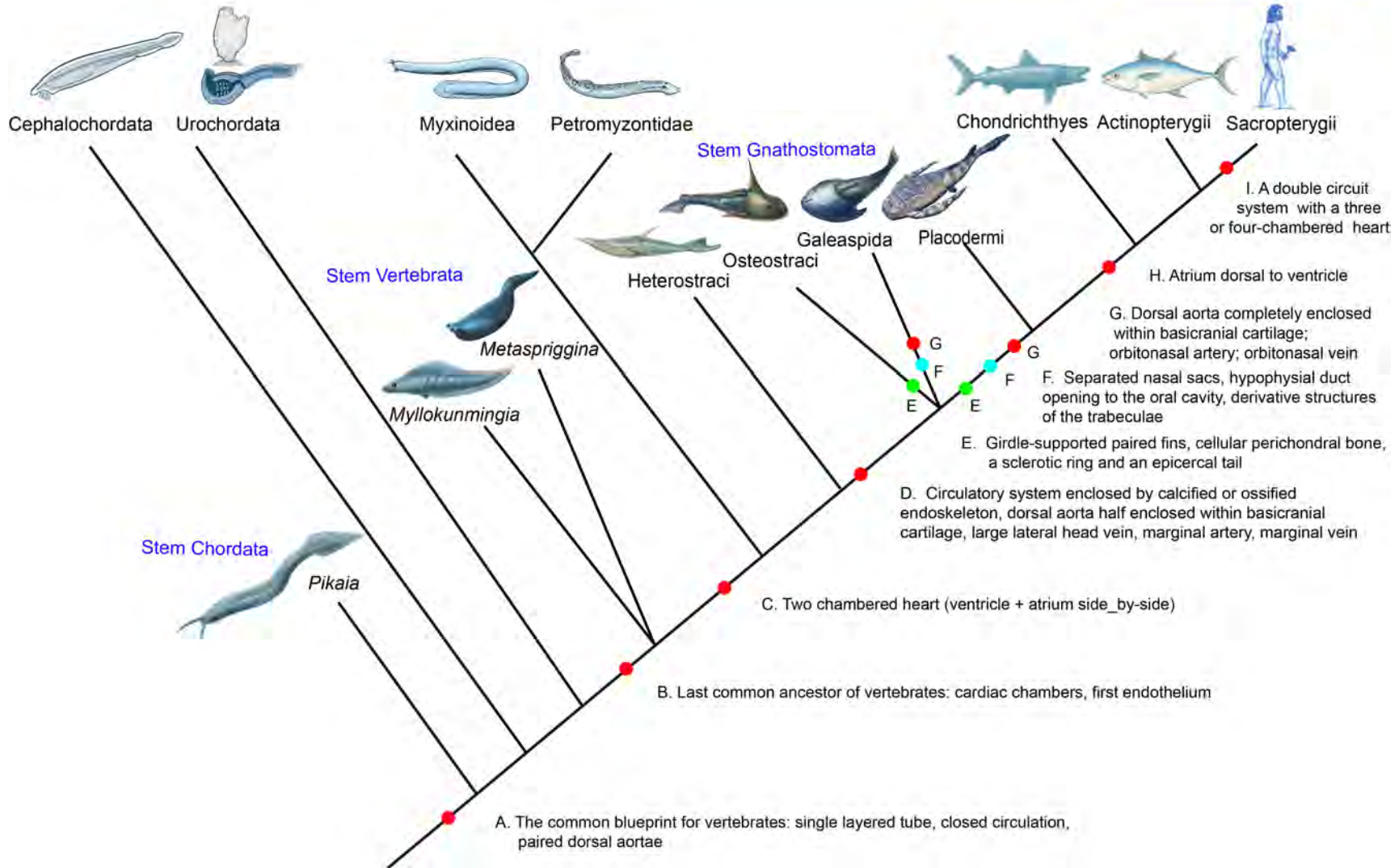
Olfactores

Vertebrata

Cyclostomata

Gnathostomata

Osteichthyes



Appendix A. Anatomical abbreviations used in text and figures.

aa, anterior ampulla  
abr.a, afferent branchial artery  
abr.v, afferent branchial vein  
ac.v, anterior cerebral vein  
ae.a, anterior ethmoidal artery  
al.a, anterior labyrinthine artery  
al.v, anterior labyrinthine vein  
all, anterior lateral line nerve  
an.v, anterior nasal vein  
ao, dorsal aorta  
asc, anterior semicircular canal  
br.a, branchial arch (interbranchial ridge)  
br.c, branchial chamber  
br.f, branchial fossa  
c, cornual process  
com, crus commune  
cor.a, cornual artery  
dn.a, dorsal nasal artery  
dn.v, dorsal nasal vein  
ds.v, dorsal superficial vein  
dx, canal for a nerve or an artery, or for both  
e, eye  
ebr.a, efferent branchial artery  
ebr.v, efferent branchial vein  
ec.a, external carotid artery  
ehy.a, efferent hyoid artery  
en.d, endolymphatic duct  
et.r, ethmoid rod  
exbr.v, extrabranial vein  
fa.a, facial artery  
fr.a, frontal artery  
fr.v, frontal vein

hm.f, hyoid fossa  
hy.a, hypophysial artery  
hy.d, hypophysial duct  
hy.o, hypophysial opening  
hy.r, hypophysial recess  
ibr.r, interbranchial ridge  
ic, inner cornual process  
ic.a, internal carotid artery  
lab.v, labyrinthic vein  
lc.v, lateral capitis vein  
mar.a, marginal artery  
mar.s, marginal sinus  
mar.v, marginal vein  
mc.a, middle cerebral artery  
mc.v, middle cerebral vein  
mes, mesencephalic division  
met, metencephalic division  
ml.a, middle labyrinthic artery  
mye, myelencephalic division  
my.av, anterior ventral myodome  
my.pd, posterior dorsal myodome  
my.pv, posterior ventral myodome  
my.v, ventral myodome  
na, nasal sacs  
nc, neural canal  
no, nostril  
nt, notochord  
oc.a, occipital artery  
oc.v, occipital vein  
oc.s, occipital sinus  
olf.b, olfactory bulb  
olf.t, olfactory tract  
on.a, orbitonasal artery  
on.c, oronasal chamber



on.v, orbitonasal vein  
oph.a, ophthalmic artery  
opt.c, optic canal  
opt.f, optic fenestra  
orb, orbital opening  
or.c, oral cavity  
pb.w, postbranchial wall  
pe.a, posterior ethmoidal artery  
pha, pharynx  
pi, pineal organ  
pit.v, pituitary vein  
pl.a, posterior labyrinthic artery  
pl.v, posterior labyrinthic vein  
pll, posterior lateral line nerve  
pn.v, posterior nasal vein  
psc, posterior semicircular canal  
pr.c, precerebral cavity  
pr.f, precerebral fenestra  
ro, rostrum  
ro.v, rostral vein  
sf.a, superficial artery  
sf.v, superficial vein  
sll, supratemporal lateral line nerve  
sp.d, dorsal spinal nerve  
sp.v, ventral spinal nerve  
t, canal probably for vessels  
tel, telencephalic division  
ter, terminal nerve  
tr.c, trigeminal chamber  
va.p, vascular plexuses  
vao, ventral aorta  
vcl, lateral head vein or dorsal jugular vein  
vcl.s, dorsal jugular vein sinus  
ves, vestibular division

vm.a, vertebro-medullar artery  
vm.v, vertebro-medullar vein  
vr, ventral rim  
vr.a, ventral rim artery  
vr.v, ventral rim vein  
X.br, branchial branch of vagus nerve  
X.in, intestinal branch of vagus nerve  
II, optic nerve  
III, oculomotor nerve  
IV, trochlear nerve  
V<sub>0</sub>, superficial ophthalmic branch of trigeminal nerve  
V<sub>1</sub>, deep ophthalmic or profundus branch of trigeminal nerve  
V<sub>0,1</sub>, common canal of V<sub>0</sub> and V<sub>1</sub>  
V<sub>2</sub>, maxillary branch of trigeminal nerve  
V<sub>3</sub>, mandibular branch of trigeminal nerve  
V<sub>2,3</sub>, V<sub>2</sub>-complex of trigeminal nerve or maxillo-mandibular nerve  
VI, abducens nerve  
VII, facial nerve  
VIII, acoustic nerve  
IX, glossopharyngeal nerve  
X, vagus nerve