



Evolutionary morphology of the antennal heart in stick and leaf insects (Phasmatodea) and webspinners (Embioptera) (Insecta: Eukinolabia)

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Abstract

The morphology of the antennal hearts in the head of Phasmatodea and Embioptera was investigated with particular reference to phylogenetically relevant key taxa. The antennal circulatory organs of all examined species have the same basic construction: they consist of antennal vessels that are connected to ampullae located in the head near the antenna base. The ampullae are pulsatile due to associated muscles, but the points of attachment differ between the species studied. All examined Phasmatodea species have a *Musculus* (*M.*) *interampullaris* which extends between the two ampullae plus a *M. ampulloaorticus* that runs from the ampullae to the anterior end of the aorta; upon contraction, all these muscles dilate the lumina of both ampullae at the same time. In Embioptera, only the australembiid *Metoligotoma* has an *M. interampullaris*. All other studied webspinners instead have a *M. ampullofrontalis* which extends between the ampullae and the frontal region of the head capsule; these species do not have *M. ampulloaorticus*. Outgroup comparison indicates that an antennal heart with a *M. interampullaris* is the plesiomorphic character state among Embioptera and the likely ground pattern of the taxon Eukinolabia. Antennal hearts with a *M. ampullofrontalis* represent a derived condition that occurs among insects only in some embiopterans. These findings help to further clarify the controversially discussed internal phylogeny of webspinners by supporting the view that Australembiidae are the sister group of the remaining Embioptera.

Keywords Circulatory organ · Phylogeny · Sister group · Polyneoptera · *Timema* · *Metoligotoma* · Australembiidae · Clothodidae

Introduction

The circulatory system of insects serves a multitude of vital transport functions within the body, including those of molecules, hemocytes, or thermal energy (reviews: Hertel and Pass 2002; Hillyer and Pass 2020; Jones 1977; Miller 1985; Miller and Pass 2009; Wirkner and Richter 2010; Wirkner et al. 2013). For this purpose, the hemolymph, which moves freely between the inner organs, is propelled through the central body cavity by rhythmic contractions of the dorsal vessel (often simply called “the heart”). However, the pumping activity of this muscular tube usually cannot circulate the hemolymph in longer hollow body appendages, such as antennae, wings, legs, cerci, and the terminal filament (Pass 1998, 2000). Only a few primarily wingless hexapods have arterial vessels that connect to the dorsal vessel and supply some of these appendages (Gereben-Krenn and Pass 1999; Pass 1991). In the course of hexapod evolution, these arteries became disconnected from the dorsal vessel

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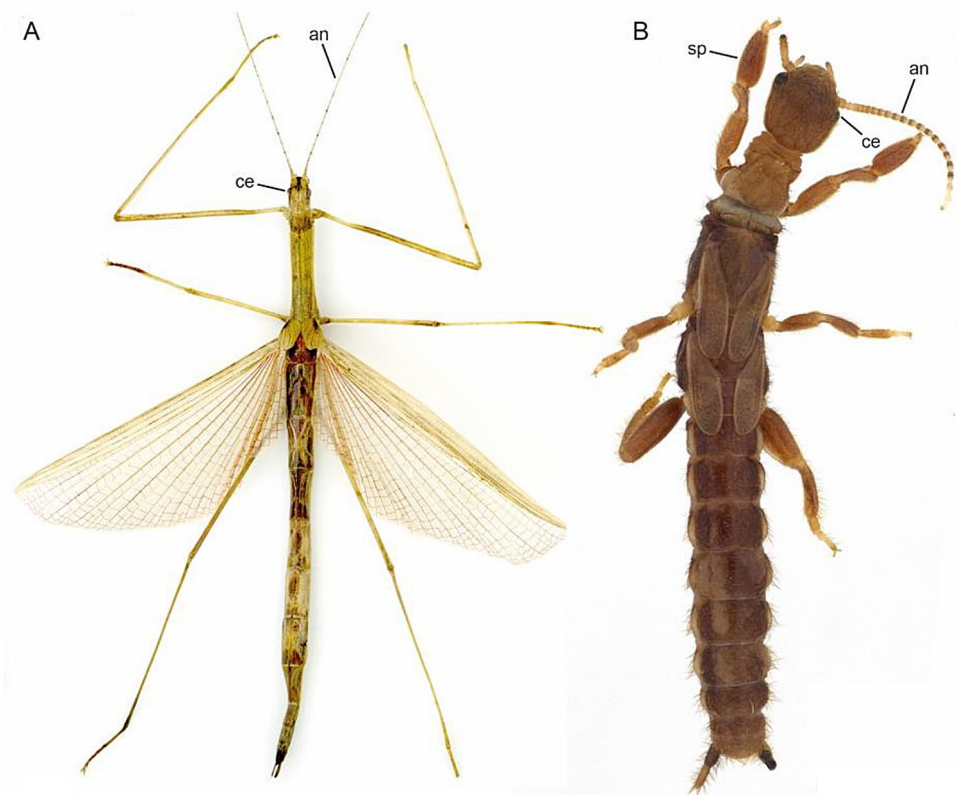
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or were reduced and independent accessory circulatory organs evolved to ensure hemolymph supply of the various body appendages. These can be either simple guiding structures that passively lead the hemolymph flow through these appendages, or accessory pulsatile organs which function as autonomous auxiliary hearts that actively pump hemolymph through these appendages (reviews: Pass 1998, 2000; Pass et al. 2006; Wirkner et al. 2013). Many of these circulatory organs are evolutionary novelties of pterygote insects, which make them prime examples to investigate the poorly understood topic of the origin of novel organs (Pass 2000; Pass et al. 2015).

Among the accessory pulsatile organs, the antennal hearts have received most attention so far (Baum et al. 2007; Hertel et al. 1985, 1997, 2012; Hertel and Pass 2002; Pass 1980, 1988, 1991; Pass et al. 1988; Pawlowa 1895; Raina et al. 2003; Sun and Schmidt 1997; Wipfler and Pass 2014). They are vital for the hemolymph supply of the numerous sensory organs located in the antennae (review: Zacharuk 1985). In addition, they may serve as neurohaemal organs which release hormones that are likely of relevance for the control of the antennal sensilla (Antemann et al. 2018; Pass 1985, 1988; Pass et al. 1988). Remarkably, the antennal circulatory organs exhibit an unusual disparity in their constructional morphology within Insecta, which makes them informative traits for phylogenetic research (Pass et al. 2006). A good example of this are the polyneopteran insects whose antennal hearts can have very different functional morphologies (Pass 1991). Within this group, three fundamentally different types can be found: I) the hemolymph can be guided into the ampullae and thus into the antennal vessel via non-pulsatile control of the cephalic hemolymph flow with septae as in some stoneflies (Pass 1991), II) active pulsatile dilation of the ampullae with several different associated muscles (in most polyneopterans: Pass 1991; Wipfler et al. 2011) or III) by compression of the ampullae by musculature in earwigs (Pass 1988). Each of these functional principles, especially the dilation of the ampulla has several morphologically strongly differing subtypes which involve a total of six different muscles (Pass 1991; Wipfler et al. 2011). The phylogenetic relationships of the high-rank polyneopteran taxa (which are classified as orders in traditional zoological systematics) remained the most puzzling part in insect phylogeny and were therefore represented long as a large unsolved polytomy (review: Kristensen 1991). Recently, however, a well-supported cladogram based on phylogenomic data has been established and phylogenies for most subgroups were provided (Misof et al. 2014; Wipfler et al. 2019). These cladograms allow for the first time a sound evolutionary interpretation of characters among the major groups of Polyneoptera, Phasmatodea, and Embioptera,

which are the focus of the present study, are groups that have been discussed controversially with regard to their phylogenetic position. Embioptera undoubtedly represent a monophyletic taxon (Kristensen 1991; Szumik et al. 2008), especially in view of unique morphological and behavioural traits. The embiopteran life is strongly dependent on silk in every postembryonic stage. These subsocial insects mainly use silk to build tube-like domiciles for themselves and their offspring; protecting them against predators and environmental influences (Edgerly 1994, 1997; Edgerly et al. 2002; Ross 2000; Stokes et al. 2018). The silk is produced in glands in their front legs (Fig. 1; spinning tarsus; Barth 1954; Büsse et al. 2015) using a “passive” pressure-induced silk spinning mechanism (Büsse et al. 2019) and a complex spinning behaviour (Edgerly et al. 2002, 2012). However, regarding their phylogenetic position within Polyneoptera, a number of different taxa were considered as closest relatives: Hennig (1969) considered a clade comprising all polyneopterans except Plecoptera and Zoraptera as potential sister group, Rähle (1970) Phasmatodea, Haas and Kukalová-Peck (2001) Plecoptera, Grimaldi and Engel (2005) Zoraptera (with Plecoptera as closest related lineage), and in the comprehensive cladistic analysis of Beutel and Gorb (2006), Embioptera were the sister to all Neoptera except Plecoptera. The same is true for phasmids: they are generally viewed as monophylum because of some unique morphological and life-history traits. Phasmids are known to be exclusively phytophagous insects, which are highly adapted to their respective habitat—they are imitating twigs or leaves for camouflage (Fig. 1; cf. Bradler 2009). This group was undergoing a fast radiation (Bradler et al. 2015), causing different lineages of phasmids inhabiting similar habitats, in an almost worldwide distribution, representing similar adaptations (cf. Buckley et al. 2009; Simon et al. 2019). These convergent adaptations are for example specialized egg deposition modes (Bradler et al. 2015; Goldberg et al. 2015; O’Hanlon et al. 2020), the loss of forewings (respectively, rudimentary forewings; cf. Zeng et al. 2020), specialized substrate-specific attachment microstructures on the tarsi (Büscher et al. 2018a, b), or the general shape of the phasmids body (cf. Buckley et al. 2009). The phylogenetic results for Phasmatodea within the Polyneoptera are, however, similarly confusing as for Embioptera, and every orthopteroid insect order has already been discussed as a potential sister group (review: Friedemann et al. 2012). In view of this controversial situation, great hopes were placed in molecular approaches. Terry and Whiting (2005) recovered Embioptera and Phasmatodea in their multigene study as sister taxa and named the clade Eukinolabia. This relationship was later supported by additional molecular (Ishiwata et al. 2011; Letsch and Simon 2013; Letsch et al. 2012; Misof et al. 2014; Simon et al. 2019; Whiting

Fig. 1 Habitus in dorsal view of **a** *Sipylodea sipylus* (adult) and **b** *Antipaluria urichi* (subadult male). *an* antennae, *ce* compound eye, *sp* spinning tarsus. Scale bars: 2.5 mm



et al. 2003; Wipfler et al. 2019) and morphological studies (Friedemann et al. 2012; Matsumura et al. 2015; Wipfler et al. 2011). In summary, today, the Eukinolabia with Embioptera and Phasmatodea as sister groups can be regarded as a well-supported hypothesis.

Against this background, the antennal hearts are of particular interest, since Embioptera and Phasmatodea have different construction types of this organ according to previous studies (Pass 1991). However, so far, only a few species which represent more recent splits in the trees of Phasmatodea and Embioptera have been investigated. Aim of the present study is to enlarge the data set and to include so-called key species, which represent early split lineages of these two polyneopteran groups.

Materials and methods

Table 1 gives an overview of the investigated species of Phasmatodea and Embioptera. The specimens were all adults and fixed in either 80% ethanol or alcoholic Bouin ("Dubosq-Brasil" mixture). For the sake of simplicity, the full scientific name of the species is given only when mentioned for the first time, later only generic names are used. For μ -computed tomography (μ -CT) scanning, the samples were critical point dried (Emitech K850 critical

point dryer) and mounted onto specific specimen holders (scanning facilities and scan parameters listed in Table 1). The data were imported into Visage Imaging Amira 5.2.2 (ThermoFisher, Waltham, USA) to perform segmentation. The final images are a combination of volume rendering (module "volren" in Amira) and surface rendering (module "surfacegen" in Amira).

The cutting parameters and embedding media for the serial sections are listed in Table 1. The sections were photographed in digitized form with an Axioskop microscope (Carl Zeiss AG, Jena, Germany) with a PixeLink PL-B686 camera (PixeLink, Ottawa, Canada) using the software PixeLink CaptureOEM 7.12 (2008). For z-stacked photography (Fig. 1), we used a custom-made 3D-printed illumination dome system (Bäumler et al. 2020) and an Olympus OMD 10mkII digital camera (Olympus K.K., Tokyo, Japan), equipped with a Leica 45 mm macro lens (Leica Camera AG, Wetzlar, Germany). The images were subsequently processed in Affinity Photo and Affinity Designer (Serif Ltd, Nottingham, United Kingdom). The histological sections and the μ -CT scans are deposited in the collection of the Alexander Koenig Museum, Bonn, Germany and can be requested from the first author. The descriptions are based on the morphological terminology of Seifert (1970), and the muscle terminology follows Wipfler et al. (2011).

Table 1 Taxon sampling and applied methods

Order/family	Species	Origin	Histological sections			μ-CT		
			Thickness	Plane	Staining	Facility	Spatial res. (μm)	Rot steps (°)
Phasmatodea/ Timematidae	<i>Timema</i> sp.	California, USA	10 μm	Cross section	Toluidine blue	DESY	3.2	0.25
Phasmatodea/ Lonchodidae	<i>Sipyloidea sipylus</i> (Westwood 1859)	Madagascar	–	–	–	BESSY	2.486	0.1
Phasmatodea/ Phylliidae	<i>Phyllium philip- pinicum</i> Henne- mann, Conle, Gottardo and Bresseel 2009	Luzon, Philip- pines	–	–	–	BESSY	2.486	0.09
Phasmatodea/ Phasmatidae	<i>Medauroidea extradentata</i> (Brunner von Wattenwyl 1907)	Annam, Vietnam	10 μm	Cross section	Azan	–	–	–
Embioptera / Embiidae	<i>Embia contorta</i> Ross 1966	India	1 μm	Cross section	Azan	–	–	–
Embioptera/Oli- gotomidae	<i>Oligotoma nigra</i> Hagen 1885	Algeria	1 μm	Cross section	Azan	–	–	–
Embioptera/Aus- tralembiidae	<i>Metoligotoma</i> sp.	Australia	–	–	–	DESY	1.74	0.25
Embioptera/ Clothodidae	<i>Antipaluria urichi</i> (Saussure 1896)	Trinidad and Tobago	–	–	–	ZFMK	2.00	0.06

BESSY BAM-Line of the Berliner Elektronensynchrotron; DESY Beamline P07 at Petra IV at the Deutsches Elektronen-Synchrotron

Results

In all studied species, the antennal hearts are autonomous circulatory organs that function independently from the dorsal vessel. The anterior end of the aorta is located behind the brain and the outpouring hemolymph flows through a kind of channel, formed by the brain, the circumesophageal connectives, and the pharynx, into the hemocoelic space in front of the supraesophageal ganglion. This is where the antennal heart is attached to the frontal cuticle.

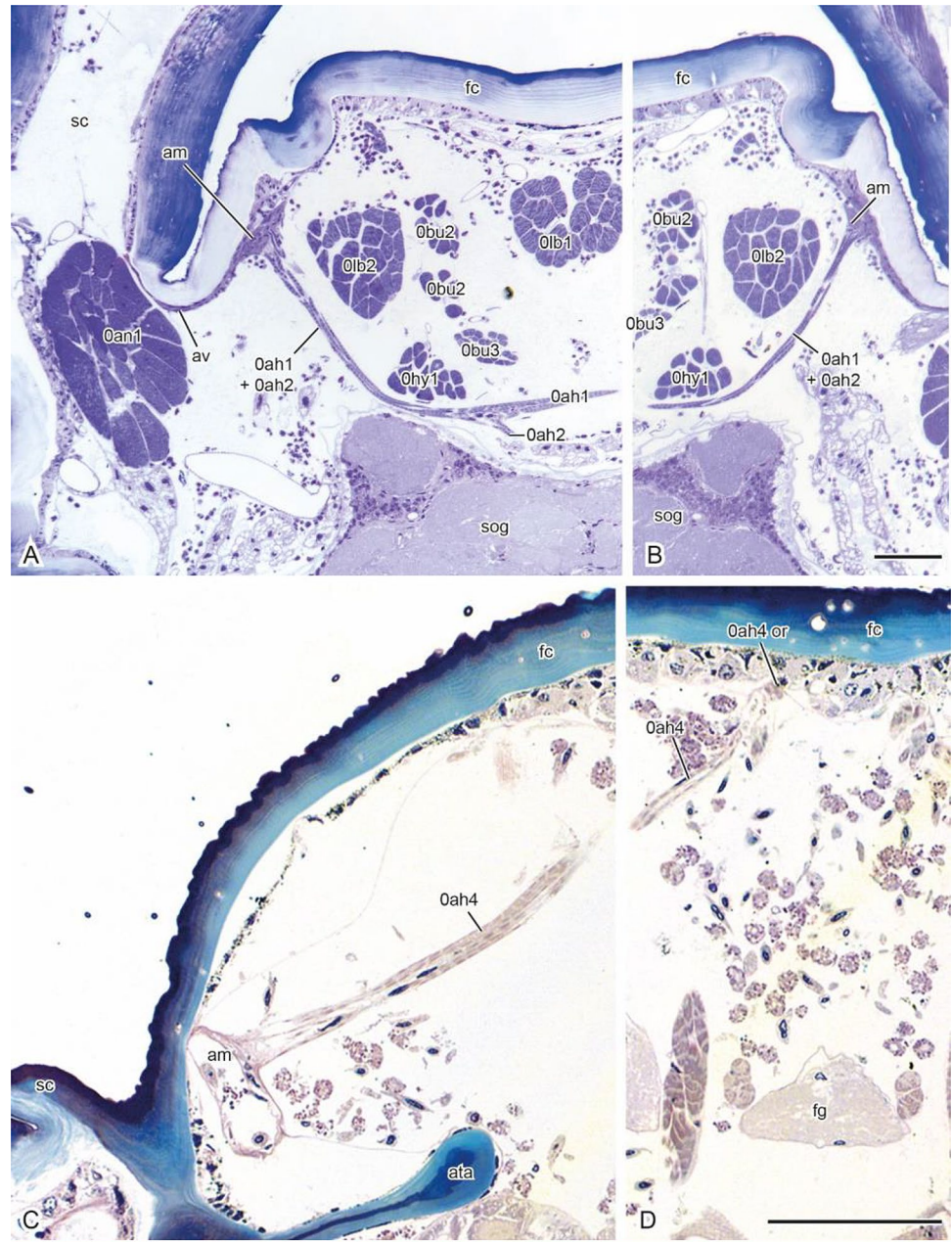
The antennal hearts of all studied species consist of thin-walled ampullary formations that are attached to the frontal cuticle close to each antenna base. The ampullae are joined to vessels which extend into the antennae. They have valved ostia through which hemolymph can enter from the hemocoelic space in front of the supraesophageal ganglion. The ampullae are pulsatile due to associated pumping muscles, which differ in their attachment points between the species studied (details are given in the following chapters). The muscles are all dilators which enlarge the ampulla lumen upon contraction. Hemolymph can then enter the ampullae through valved ostia from the hemocoelic space in front of the supraesophageal ganglion. After relaxation of the dilator muscles, the ampullae collapse due to the elasticity of the wall. Thereby hemolymph is forced through the antennal

vessels up to the tip of the antennae, where it pours out and returns back in the hemocoel of the antennae into the head capsule (see Pass 1985; Hertel et al. 1985; Boppana and Hillyer 2014).

Phasmatodea

In *Timema* sp., the ampullae are located mesally to the antennae. They are attached with their outer wall directly to the frontal cuticle and also by small connective tissue strands running laterally (Fig. 2a, b). The antennal vessels have a uniform wall of a single-layer epithelium and extend from the ampullae straight into the antennae. A *Musculus* (M.) *interampullaris* (Oah1, Figs. 2a, b and 3a, c) and a paired M. *ampulloaorticus* (Oah2, Figs. 2a, b and 3a, c) attach mesally on each ampulla. Near the mid-sagittal plane, the latter separate from the former, unite mesally and continue as very delicate muscle strands (Fig. 2a) that run underneath the supraesophageal ganglion backwards to the anterior end of the aorta (Fig. 3a). There, they are attached to the upper part of the aortic tube. From a dorsal view, the M. *interampullaris* is not stretched straight between the two ampullae, but held in a U-shaped position by the M. *ampulloaorticus*. Due to this specific anatomical arrangement, the expansion of the ampullae is apparently maximized during contraction of

Fig. 2 Horizontal semi-thin sections through the head showing the antennal circulatory organs. **a, b** *Timema* sp. (Phasmatoidea), composite image of two sections from different cutting levels. Antennal heart with *M.* interampullaris. Left section shows branching off the *M.* ampulloaorticus. **c, d** *Oligotoma nigra* (Embioptera), composite image of two sections from different cutting levels. Left hand antennal heart with *M.* ampullofrontalis; the right picture shows the origin of *M.* ampullofrontalis at the frontal cuticle. *Oah1* *M.* interampullaris, *Oah2* *M.* ampulloaorticus, *Oah4* *M.* ampullofrontalis anterior, *Oah4or* origin of *M.* ampullofrontalis anterior, *Oan1* *M.* tentorioscapalis anterior, *Obu2* *M.* frontobuccalis anterior, *Obu3* *M.* frontobuccalis posterior, *Ohy1* *M.* frontooralis, *Olb1* *M.* frontolabralis, *Olb2* *M.* frontoepipharyngalis, *am* antennal ampulla, *ata* anterior antennal arms, *av* antennal vessel, *fc* frontal cuticle, *fg* frontal ganglion, *sc* scapus, *sog* supraesophageal ganglion. Scale bars: 1 mm



these muscles. There are no connective tissue bands extending between the ampullae and the supraesophageal ganglion.

In *Phyllium philippinicum* (Fig. 3b, d), the head is much narrower and the two antenna bases are closer together. Accordingly, the proportions of the individual parts of the antennal heart are different. The configuration of the muscles associated with the ampullae however is the same as in *Timema*: There is a *M.* interampullaris and a *M.* ampulloaorticus. The same condition holds also

true for the examined species *Medauroidea extradentata* and *Sipyloidea sipyilus* (Fig. 1).

Embioptera

In *Metoligotoma* sp., (Fig. 4a, c), the antennal ampullae are positioned mesally of the antennal sockets, in the anterior corner between the anterior tentorial arms and the head capsule. They are connected by a thin *M.* interampullaris (*Oah1*,

Fig. 3 Three-dimensional visualisation of the transparent head capsule with selected inner organs of Phasmatodea. The antennal heart consists of paired ampullae to which dilator muscles (*M. interampullaris* and *M. ampulloaorticus*) are associated. **a + c** *Timema* sp.; **b + d** *Phyllium philippinicum*. **a** and **b** dorsal view; **c** and **d** frontal view. Cuticle made transparent. Blue: ampullae of antennal heart and aorta; orange: antennal heart muscles; dark grey: nervous system; green: digestive tract. *Oah1* *M. interampullaris*, *Oah2* *M. ampulloaorticus*, *am* antennal ampulla, *ao* aorta, *av* antennal vessel, *ce* compound eye, *fo* foramen occipitale, *it* intestinal tract, *sg* subesophageal ganglion; *sog* supraesophageal ganglion

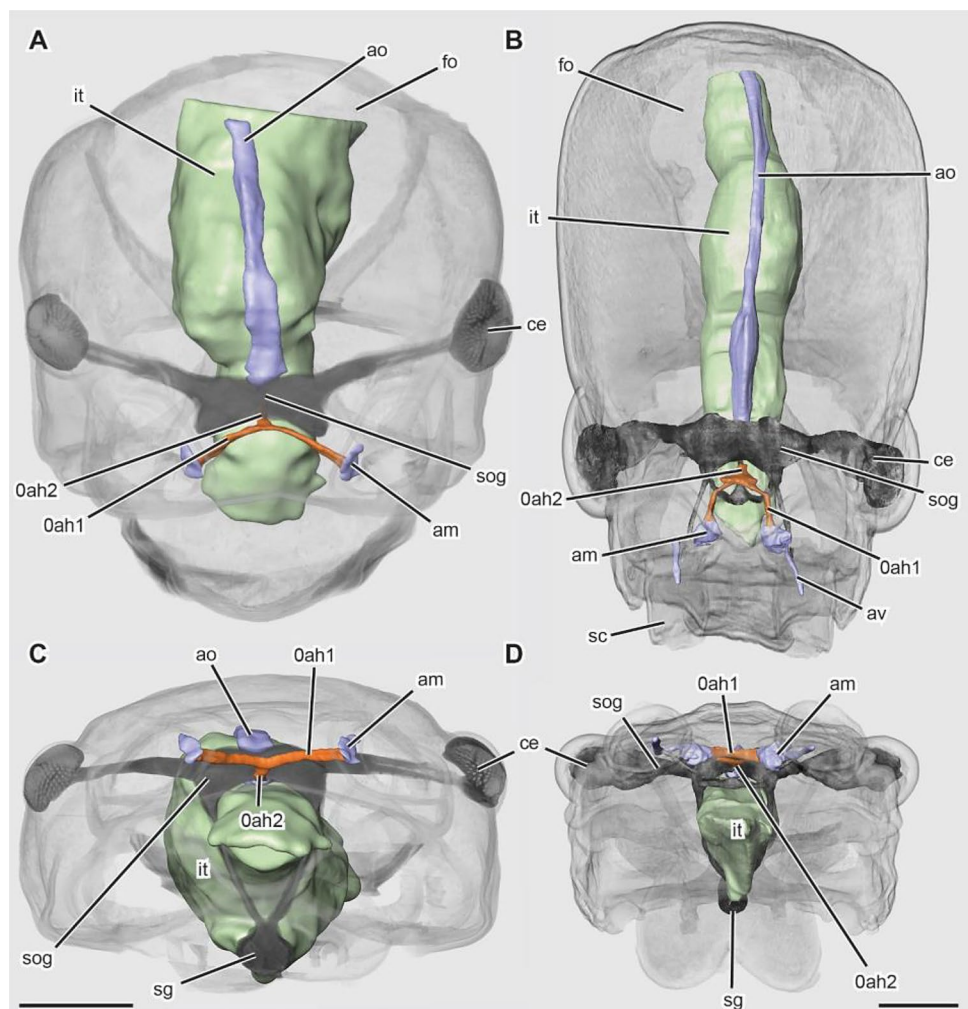


Fig. 4a, c) that extends not straight between the ampullae, but is U-shaped from the dorsal perspective (Fig. 4a). This shape of the interampullary muscle can only be explained by a few thin muscle fibers that branch off from the central area of the latter and run backwards and thus act as a counter-bearing when the interampullary muscle contracts; their second attachment point could not be unambiguously identified in the CT scan. The antennal vessel wall has a uniform structure with a single-layer epithelium along its entire length.

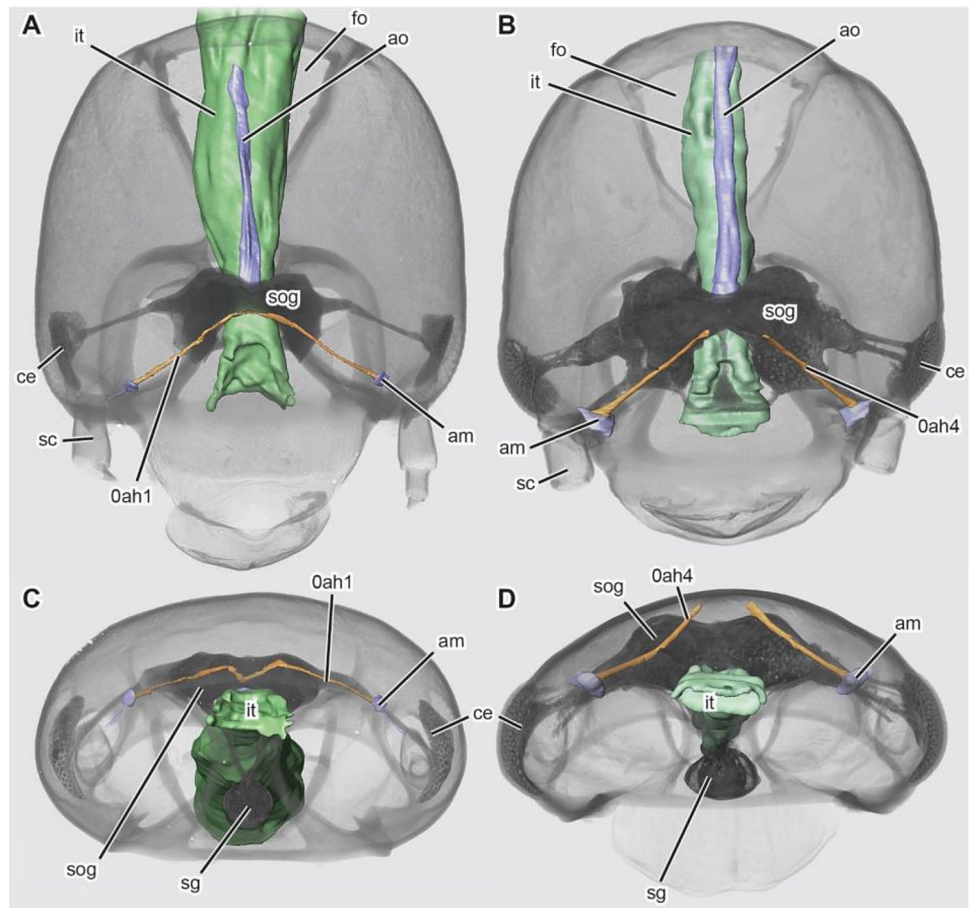
In *Oligotoma nigra* (Figs. 2c, d and 4b, d), the ampullae and the antennal vessels have exactly the same position and appearance as in *Megoligotoma*. The most important difference concerns the associated dilator muscle. In *Oligotoma*, each ampulla has its own muscle which is attached on the one end with the inner wall of the ampullae, the other attachment is mesally at the frontal cuticle (*fc* in Fig. 2c, d). The attachment sites of this paired *M. ampullofrontalis anterior* (*Oah4*, Figs. 2c, d and 4b, d) at the frontal cuticle (*Oah4* or, Fig. 1d) are close but well separated from each other. The same condition of the antennal heart as in *Oligotoma*

with a paired *M. ampullofrontalis anterior* was found in the examined *Embia contorta* and *Antipaluria urichi* (Fig. 1). In contrast to all other studied embiopterans, the ampullae of *Embia contorta* are connected to the supraesophageal ganglion by thin bands of tissue containing fibrous material and long ovoid nuclei.

Discussion

So far, only a few studies addressed the antennal heart morphology in phasmatodeans. Scholl (1969) and Pass (1991) studied *Carausius* (the first *C. morosus*, the latter unidentified *C. sp.*). In addition, there are some incomplete and unillustrated descriptions of the antennal heart muscles of *Phyllium philippinicum* (referred to as *Ph. siccifolium*), *Sipylodea sipylus* (both Friedemann et al. 2012) and *Agathemera crassa* (Wipfler et al. 2011). These descriptions are consistent with respect to the presence of paired ampullae with antennal vessels having a uniform wall and two muscles associated with the antennal heart ampulla: The unpaired

Fig. 4 Three-dimensional visualisation of the transparent head capsule with selected inner organs of Embioptera. The antennal heart consists of paired ampullae to which dilator muscles (*M. interampullaris* and *M. ampullofrontalis anterior*) are associated. **a + c** *Metoligotoma* sp.; **b + d** *Antipaluria urichi*. **a** and **b** dorsal view; **c** and **d** frontal view. Cuticle made transparent. Blue: circulatory system; dark grey: nervous system; green: digestive system; orange: musculature. *Oah1* *M. interampullaris*, *Oah4* *M. ampullofrontalis anterior*, *am* antennal ampulla, *ao* aorta, *ce* compound eye, *fo* foramen occipital, *it* intestinal tract, *sc* scapus, *sg* subesophageal ganglion; *sog* supraesophageal ganglion



M. interampullaris (*Oah1*) that interconnects the antennal ampullae and the *M. ampulloaorticus* (*Oah2*) that runs from the antennal ampullae towards the anterior end of the aorta. Our investigations confirm that this situation is also present in *Timema*, *Medauroidea*, *Sipylloidea*, and *Phyllium*. In view of this congruence and the well-supported sister group relationship between *Timema* and the remaining phasmatodeans (e.g. Ishiwata et al. 2011; Simon et al. 2019), the anatomical condition described can be regarded as the ground pattern of this organ for Phasmatodea.

With regard to Embioptera, the first description of an antennal circulatory organ goes back to Rähle (1970), who claimed that in *Embia ramburi*, the aorta is connected to each antennal ampulla by a thin vessel which, according to his description, appeared always collapsed in the histological sections. Pass (1991) investigated two species of *Embia* (*E. contorta* and *E. tyrrhenica*) and *Haploembia solieri*, and found a consistent configuration of the antennal heart in all these species: a thin muscle, the *M. ampullofrontalis anterior* (*Oah4*), is attached to the ampulla wall and on the other end at the medial part of the frontal cuticle. The results for *Oligotoma* and *Antipaluria* in this study are fully in agreement with the latter description and we again confirm the assumption of Pass (1991) that Rähle

(1970) probably misinterpreted these muscles as collapsed vessels. The re-investigation of *Embia contorta* revealed an additional band of connective tissue containing fibrous material and long ovoid nuclei between the ampulla and the supraesophageal ganglion, which was not mentioned by Pass (1991). Among insects, similar structures containing fibrous material and long ovoid nuclei are only described for *Xenonomia* (= *Grylloblattodea* and *Mantophasmatodea*) (Wipfler et al. 2011). Although this taxon is considered to represent the sistergroup to Eukinolia (Misof et al. 2014; Wipfler et al. 2019), a homology between these structures in *Embia* and *Xenonomia* would presuppose multiple losses in Embioptera and Phasmatodea, but further research is required to definitely solve this issue. The situation in *Metoligotoma* is in agreement with the other examined webspinners concerning the position of the ampulla and the uniform antennal vessel wall. However, the muscular equipment is different: the *M. ampullofrontalis anterior* (*Oah4*) is missing, and instead, there is a *M. interampullaris* (*Oah1*). The few thin muscle fibers that branch off from central area of the *M. interampullaris* and run backwards likely act as counter-bearing to hold the latter in a U-shaped position. Unfortunately, the resolution power of the CT scan was not sufficient to unambiguously

identify the second attachment site of these muscle fibers. However, we assume that they correspond to the *M. ampulloaorticus* found in other insects.

This variability of the antennal heart construction within the Embioptera is of particular interest for the intraordinal relationships as well as for the reconstruction of the ground pattern of the Eukinolabia, respectively, other parts of the polyneopteran clade.

Intraordinal relationships of Embioptera are a subject rarely tackled and have been addressed in detail only by Szumik et al. (2008) and Miller et al. (2012). Both studies used morphological and molecular data but resulted in very different phylogenies, particularly with regard to the early splits of Embioptera. Szumik et al. (2008) used the Clothodidae, an embiopteran subgroup which is believed to possess many plesiomorphic characters, to root the tree in their analyses. Miller et al. (2012), whose study is mainly based on extensive molecular data, remarkably retrieved completely different topologies within Embioptera when treating an identical data set with different methodological approaches: parsimony analyses recovered Clothodidae (which includes *Antipalura* examined in the present study) as sister group to the remaining embiopterans, likelihood analyses proposed Tetraembiidae + Oligotomidae (which includes *Oligotoma* examined in the present study) as early branch and Bayesian analyses suggest Australembiidae (which includes *Metoligotoma* examined in the present study). The transcriptomic analysis of Wipfler et al. (2019) comprises five species of Embioptera including the clothodid *Antipalura*, which appeared in the results as sister group to all other taxa (Australembiidae were not included in this study). A detailed morphological analysis of female genitalic characters reports several characters that favour *Metoligotoma* as sister to the remaining Embioptera. However, no support was found for the view that the Clothodidae are the sister group of all other Embioptera (Klass and Ulbricht 2009). This is in line with our results about the antennal hearts. Outgroup comparison with Phasmatodea indicates that the condition of *Metoligotoma* with an antennal heart having a *M. interampullaris* (most likely together with a *M. ampulloaorticus*) is the plesiomorphic character state in Embioptera. This supports the hypothesis that Australembiidae are the sister group of the remaining Embioptera. Furthermore, it is obvious to assume that this condition is the ground pattern of Eukinolabia.

Among the remaining polyneopteran, an antennal heart with a nearly identical morphology comprising a *M. interampullaris* (Oah1) and a *M. ampulloaorticus* (Oah2) is found in Zoraptera (Wipfler and Pass 2014), Dictyoptera, i.e., mantises (Mantodea) and cockroaches plus termites (Blattodea), and in some Orthoptera (Pass 1985, 1991; Wipfler et al. 2012, 2016). The only difference concerns the antennal vesicles, which in Dictyoptera have two different sections: the basal part in the head is convoluted and has a two-layered

wall with an inner transport epithelium, while the distal part in the antennae has a one-layer wall epithelium without any specific organelle equipment (Pass 1985).

The occurrence of a *M. interampullaris* plus a *M. ampulloaorticus* in Zoraptera, Orthoptera, and Dictyoptera suggests that this character state may be an older ground pattern of polyneopteran insects, but further research is needed to reconstruct the evolutionary morphology of the antennal hearts among this clade. An antennal heart with a *M. ampullofrontalis*, as found in all examined web-spinners except *Metoligotoma*, is then a derived character state. Such a condition is unique among insects and obviously an autapomorphy of a part of the embiopteran clade. Regarding the evolutionary origin of the *M. ampullofrontalis*, we assume that it developed through the displacement of some muscle fibers of the *M. interampullaris* from the attachment on the wall of the opposite ampulla to the medial area of the frontal cuticle. As a result, each ampulla has become its own dilator muscle.

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