The evolution and functional morphology of hemipteran vibrational organs

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To my mother, and George McGavin

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Appendix

The evolution and functional morphology of hemipteran vibrational organs

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Abstract

Acoustic and vibrational signals are among the prevalent modes of arthropod communication, exploited by at least 230,000 species (Cocroft and Rodríguez, 2005). Vibroacoustic signals dominate hemipteran communication, being generated by stridulation, wing buzzing, percussion, membrane buckling and shaking of the body relative to the legs (tremulation). The last two mechanisms are produced by basi-abdominal organs known as tymbals in Cicadomorpha and tergal plates in Heteroptera, respectively, whose systematic distribution and homologies are poorly understood. Other groups, such as the planthoppers (Fulgoromorpha) and moss bugs (Coleorrhyncha), are more enigmatic, as they generate vibrations with mechanisms which have so far remained unexplored. In spite of limited available morphological evidence, it has been suggested that all hemipteran basi-abdominal organs are homologous and evolved once (Tymbalia hypothesis) (Wessel et al, 2014). The aim of this dissertation is to elucidate the evolution of hemipteran vibroacoustic organs by describing their biomechanics, morphology and systematic distribution, and to use the resulting data to test the claims of the Tymbalia hypothesis.

I used a combination of state-of-the-art methods such as X-ray synchrotron microtomography and confocal laser scanning microscopy with more traditional techniques

(e.g. scanning electron microscopy), in order to describe the morphology of vibroacoustic organs from taxa selected from across hemipteran phylogeny. For experiments with living specimens, I used laser Doppler vibrometers to record their vibrational signals and high speed cameras to film the motion of the organs responsible for their production.

I find that Fulgoromorpha produce vibrational signals with a novel mechanism I term the "snapping organ", which is biomechanically and morphologically distinct from tymbals. Furthermore, re-examination of supposed stridulatory organs in derbid planthoppers reveals that they are instead more likely to be used in chemical signalling. I show that tymbals are ubiquitous in Cicadomorpha, and that their segmental affinities were misinterpreted by most previous studies. Finally, I document the tergal plate in a systematically important group of Heteroptera, and I challenge the evidence used to support the Tymbalia hypothesis. Overall, this dissertation documents the morphology and systematic distribution of hemipteran vibroacoustic organs in unprecedented detail, and provides a significant step towards resolving their evolutionary origins.

Life is short, the art long, opportunity fleeting, experience treacherous, judgment difficult.

Hippocrates, *Aphorisms*

Chapter 1: Introduction to thesis

1. Introduction

From microbial eukaryotes to animals with complex nervous systems, communicating information to others represents a fundamental need. Insects, the world's most diverse multicellular animals, communicate using an array of different signals, typically chemical, visual, tactile or acoustic (Chapman, 2013). A newly-discovered but widespread means of signalling is vibrational communication, with minimum estimates of at least 230,000 arthropod species using this form of communication (Cocroft and Rodríguez, 2005; Hill and Wessel, 2016; Virant-Doberlet and Čokl, 2004). Within insects, vibrational communication has been discovered in most neopteran Orders, where it frequently is the dominant mode of signalling (Cocroft and Rodríguez, 2005). Hemiptera display the greatest diversity in the structures responsible for the production of vibrational signals, as they use stridulation (Čokl et al, 2006), wing buzzing (Kavčič et al, 2013), percussion (Žunič et al, 2008), and basi-abdominal organs such as tymbals and tergal plates (Gogala, 1984; Young and Bennet-Clark, 1995). The evolutionary origins, homologies, biomechanics and systematic distribution of hemipteran basi-abdominal organs have remained the most controversial topics in hemipterology (e.g. Wessel et al, 2014), and forwarding our understanding on these long-standing problems is the objective of this thesis.

I begin by introducing the reader to the latest classification of Hemiptera and their main suborders, which form the basis of this dissertation. This section is then followed by a brief review on the organs responsible for the generation of acoustic and substrate-borne vibrational signals (collectively referred to as vibroacoustic organs) in Hemiptera, and the

theories attempting to explain their evolution. Finally, I outline the main objectives of this dissertation, along with a description of the structure of its comprising chapters.

1.1. Hemipteran classification

The Hemiptera, characterised by their modified piercing sucking mouthparts, are the richest hemimetabolous insect Order, with more than 100,000 described species (Beutel et al, 2014). Hemiptera are divided into four suborders – the Auchenorrhyncha (cicadas, leafhoppers, spittlebugs, treehoppers, and planthoppers), the Coleorrhyncha (moss bugs), the Heteroptera (true bugs) and the Sternorrhyncha (aphids, scale insects and white flies) (Fig. 1).

The monophyly of hemipteran suborders is generally well supported, with the "classic" scheme classifying Sternorrhyncha as the sister group to a lineage known as the Euhemiptera (= Auchenorrhyncha + Coleorrhyncha + Heteroptera) (Fig. 1) (Zrzavý, 1990; Cryan and Urban, 2011; Song and Liang, 2013). However, within Euhemiptera, the relationships of Coleorrhyncha to the remaining suborders have been highly debated. Some morphological and molecular studies support Coleorrhyncha as the sister group to Heteroptera, jointly forming the suborder known as the Heteropterodea (Fig. 1A) (Schlee, 1969; Zrzavý, 1990; Cryan and Urban, 2011; Song and Liang, 2013; Friedemann et al, 2014). However, several phylogenomic studies and one morphological study have recovered Coleorrhyncha as sister to Auchenorrhyncha (Fig. 1B), rendering Heteropterodea paraphyletic (Misof et al, 2014; Yoshizawa, Ogawa and Dietrich, 2017; Johnson et al, 2018). Although we have tentatively adopted the concept of Heteropterodea in some parts of this dissertation, due to the larger number of published studies supporting this classification, we stress that the systematic position of Coleorrhyncha may change in the near future.

In the sections below, I treat each suborder separately, reviewing our knowledge on their vibroacoustic organs, with a focus on those located on the abdomen base (basi-abdominal

organs). Note that Sternorrhyncha have not been included in this study due to time constraints, and because they have not been part of the hypotheses attempting to explain hemipteran signalling evolution, which form the crux of this dissertation. However, as indicated in Chapter 8, abdomen-based vibrations have been recorded from certain Sternorrhyncha (e.g. in Aleyrodidae, Kanmiya & Sonobe, 2002), and future examination of their abdominal morphology may provide valuable insights on the evolutionary origins and the morphological transformations that led to the development of euhemipteran vibrational organs.

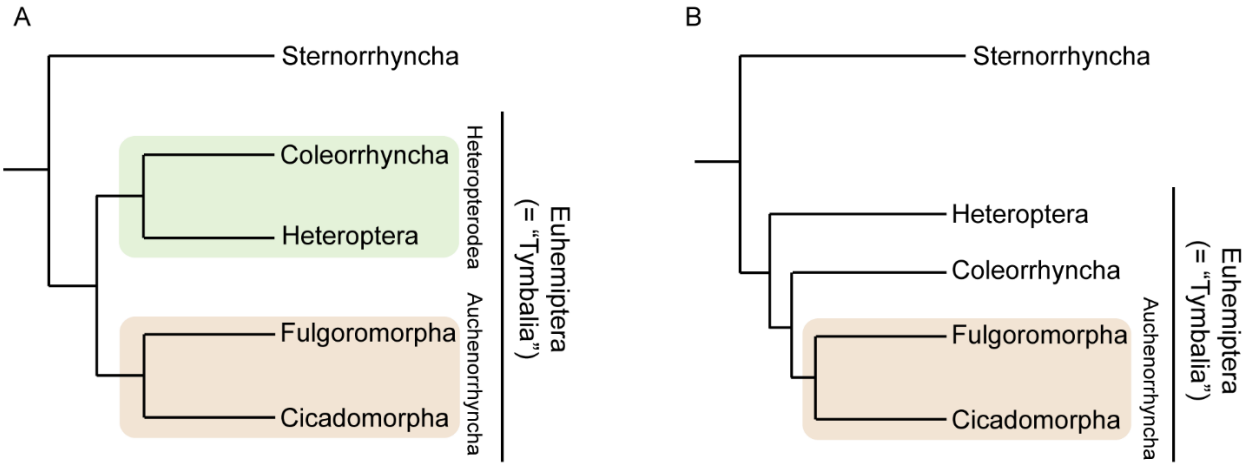

Figure 1. Hypotheses on the relationships of hemipteran infraorders. A) "Classic" topology consistent with the findings of Schlee (1969) and Cryan and Urban (2011), with a monophyletic Heteropterodea; B) Topology consistent with the recent studies of Misof et al. (2014), Yoshizawa, Ogawa and Dietrich (2017), and Johnson et al. (2018), with Heteropterodea recovered as paraphyletic. Note that Euhemiptera (sensu Zrzavý, 1990) are supported in both topologies.

1.2. Auchenorrhyncha

1.2.1. Cicadomorpha

Cicadomorpha is one of the most biodiverse hemipteran infraorders, and includes the cicadas, the froghoppers, the leafhoppers and the treehoppers. Cicadas (Cicadidae) are

undoubtedly the most renowned cicadomorphans, due to their loud acoustic songs (Pringle, 1954). To attract mates, male cicadas produce acoustic signals using paired organs known as the tymbals (tb), which are located on abdominal segment one (Fig. 2A, black arrows) (Pringle, 1954). Tymbals are externally composed of a flexible tymbal membrane (mb), which possesses several "ribs" (rb) on its external surface (Fig. 2C). This membrane is internally connected to a pair of greatly enlarged dorsoventral tymbal muscles (tbm; Fig. 1B). The mechanism of sound production in cicadas is complex, but I provide a simplified description here, based on Bennet-Clark and Young (1992) and Young and Bennet-Clark (1995): contraction of the tymbal muscle causes the tymbal membrane to buckle inwards by deformation of the tymbal ribs, resulting in the generation of a loud click. An additional click is generated when the membrane buckles outwards, due to the restoring force of the elastic protein resilin present on its surface. The complex songs of cicadas result from an interplay of sequential buckling of the tymbal membrane and its ribs, and their manipulation by additional muscles (Pringle, 1954; Fonseca et al, 2008), while the whole system is tuned by specific abdominal movements (Young and Bennet-Clark, 1995). In order to amplify the sound of their songs, the abdomen of cicadas is filled by airsacs (as), which terminate into two ventral apertures, the tympana (tmp; Fig. 2B). Due to a tymbal-tympana interaction, the abdomen of cicadas forms a Helmholtz resonator and radiates sound from the tympana, allowing them to communicate over large distances (Bennet-Clark and Young, 1992; Young and Bennet-Clark, 1995).

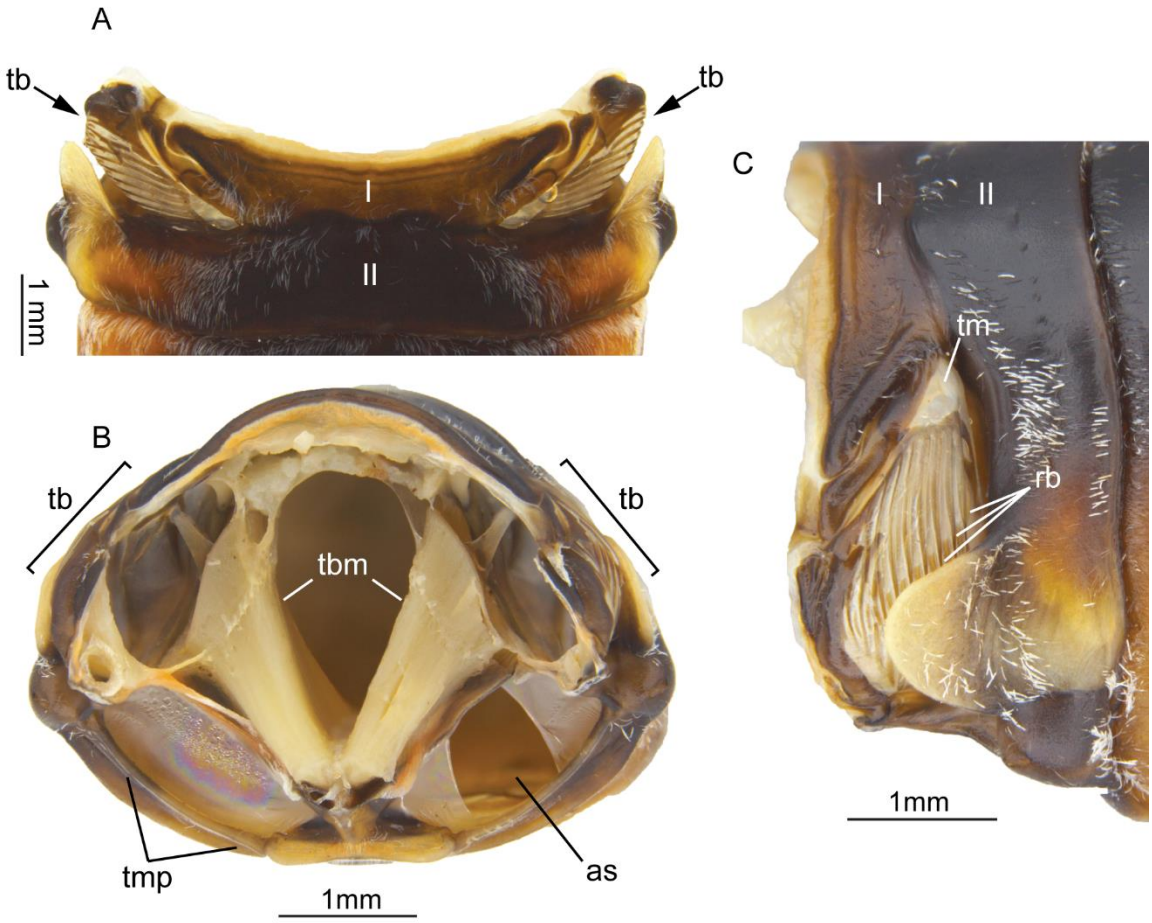

Figure 2. Morphology of a cicadid tymbal, exemplified here by a male *Oligoglena flaveola* (Brullé, 1832). A) Dorsal view of abdominal segments 1–2 (metathorax omitted), black arrows indicating the location of the tymbals (tb); B) Cross-section of the anterior portion of abdominal segment 2, showing the hypertrophied dorsoventral tymbal muscles (tbm), the tympana (tmp) and the airsacs (as) which occupy most of the abdominal cavity; C) Lateral view of abdominal segments 1–2 (metathorax omitted), with the long tymbal ribs (rb) and tymbal membrane (mb) being clearly visible.

The acoustic songs of cicadas, however, represent only one of the two signalling modalities used in cicadomorphan communication. In fact, many non-cicadid Cicadomorpha use substrate borne vibrational signals in mating behavioural contexts (e.g. Ossiannilsson, 1949; de Groot, Čokl & Virant-Doberlet, 2011; Rodríguez et al, 2012), and so do the hairy cicadas (Tettigarctidae) (Claridge et al, 1999), the sister group of Cicadidae (Cryan and Urban,

2011). This observation makes sense from a biomechanical perspective, as there are several advantages in communicating with low frequency substrate-borne vibrations over acoustic signals. Substrate-borne vibrations are typically confined to their medium, so most signals propagate over a two dimensional area (Arnason, Hart and O'Connel-Rodwell, 2002). In contrast, sound expands in a three dimensional manner, which increases the rate of signal attenuation (Arnason, Hart and O'Connel-Rodwell, 2002). As a result, acoustic signals require more powerful oscillatory movements for efficient long-range transmission, which small insects with limited muscle power, such as the majority of non-cicadid Cicadomorpha, are generally unable to produce (Bennet-Clark, 1998).

Although many Cicadomorpha are thought to generate these vibrational signals by tymbals similar to those of the cicadas (Ossiannilsson, 1949), their systematic distribution within the infraorder, their biomechanics, and their homologies to the vibrational organs of other Hemiptera remain poorly understood (Wessel et al, 2014). Furthermore, certain Cicadomorpha such as the Typhlocybinae (Cicadellidae) generate vibrations using organs which morphologically appear very unlike tymbals (Vondráček, 1949), and their homologies to the latter are also unknown.

1.2.2. Fulgoromorpha

Fulgoromorpha also communicate using low frequency substrate borne vibrations in mating contexts, although their songs are generally characterized by simpler and more uniform structure (with the exceptions of certain Delphacidae and Caliscelidae) compared to the songs of Cicadomorpha (Tishechkin, 2003, 2008).

Our knowledge on the morphology of the fulgoromorphan vibrational organ has been poor, as its musculoskeletal system has been illustrated for only four species, all belonging to the family Delphacidae (Ossiannilsson, 1949; Mitomi, Ichikawa and Okamoto, 1984; Asche,

1985). Regarding the possibility of homology of the delphacid vibrational organ with the tymbals of many Cicadomorpha, Ossiannilsson (1949) was uncertain, and described the condition of the former as "deviating". Mitomi, Ichikawa and Okamoto (1984) stated: "[…] *the vibration-producing organ in Delphacidae does not seem to be a strictly homologous organ with the sound-producing organ in Cicadoidea, because the vibration-producing organ in Delphacidae apparently functions mainly through the action of a pair of dorsal longitudinal muscles* [...] *None of the other families of Fulgoroidea seems to have been studied in as great detail as Delphacidae for the mechanism producing their vibration signals. Therefore it is necessary to obtain more information on other Fulgoroidea species than Delphacidae to clarify the evolutionary process of this mechanism in this group.*".

Unfortunately, until now, more information was never obtained, and the morphology, biomechanics, systematic distribution and homologies of fulgoromorphan vibrational organs have remained unstudied. In spite of this obvious gap in our knowledge, most subsequent studies on planthopper biotremology assumed that all Fulgoromorpha possessed tymbals (e.g. Virant-Doberlet and Čokl, 2004), and some even proposed that the latter represent an autapomorphy of Auchenorrhyncha (Hennig, 1981; Wessel et al, 2014).

Acoustic communication has been reported in the family Derbidae, and among delphacids, in the genus *Perkinsiella*, both of which supposedly stridulate (Muir in Kirkaldy, 1907). The morphology of these organs is poorly studied, and acoustic communication in any fulgoromorphan remains experimentally unconfirmed.

1.3. Coleorrhyncha

The Coleorrhyncha, or moss bugs, are a relict group of elusive insects, which are thought to have diverged from other bugs prior to the breakup of Gondwanaland, approximately 230 million years ago (Hennig, 1981). In terms of their mode of communication, Hoch, Deckert

and Wessel (2006) discovered the emission of low frequency (82 Hz) substrate borne vibrational signals in the moss bug *Hackeriella veitchi*. Although they did not examine the morphology of this species, these authors assumed that a tymbal produced the observed vibrational signals, and suggested that tymbals are ancestral for the Hemiptera (excluding Sternorrhyncha). Hoch, Deckert and Wessel (2006) based this assumption on the study of Sweet (1996), who reported large apodemes on the first and second abdominal dorsal sclerites, which he hypothesized functioned as tymbals. However, Sweet (1996) never illustrated these sclerites, nor did he describe them in detail. Consequently, the morphology and biomechanics of moss bug vibrational organs have remained unknown.

1.4. Heteroptera

Heteropteran communication is more generalized compared to Auchenorrhyncha, in that it uses chemical, acoustic and vibrational signals (Aldrich, 1995; Virant-Doberlet and Čokl, 2004). To generate acoustic signals, Heteroptera use wing buzzing, appendage percussion, and stridulation (Žunič et al, 2008; Kavčič et al, 2013). Substrate borne vibrations are generated by shaking the body relative to the legs, typically by means of an organ consisting of the dorsal part of the first two abdominal segments (terga), which are closely associated and form a plate-like structure (Fig. 3). This plate is operated primarily by dorsal longitudinal muscles, which raise the entire abdomen in an up-and-down manner in order to generate low frequency vibrations (Leston, 1954; Jordan, 1958).

The heteropteran vibrational organ was named a “tergal plate” (tp), due to its plate-like shape and its superficial dissimilarity to the cicadid tymbal organs (Fig. 3) (Gogala, 1984). However, in studies of heteropteran biotremology, the terms tergal plate and tymbal have been used somewhat interchangeably, the latter usually in quotation marks (e.g. Gogala, 2006). Indeed, certain authors (e.g. Gogala, 1986) have explicitly expressed uncertainty on

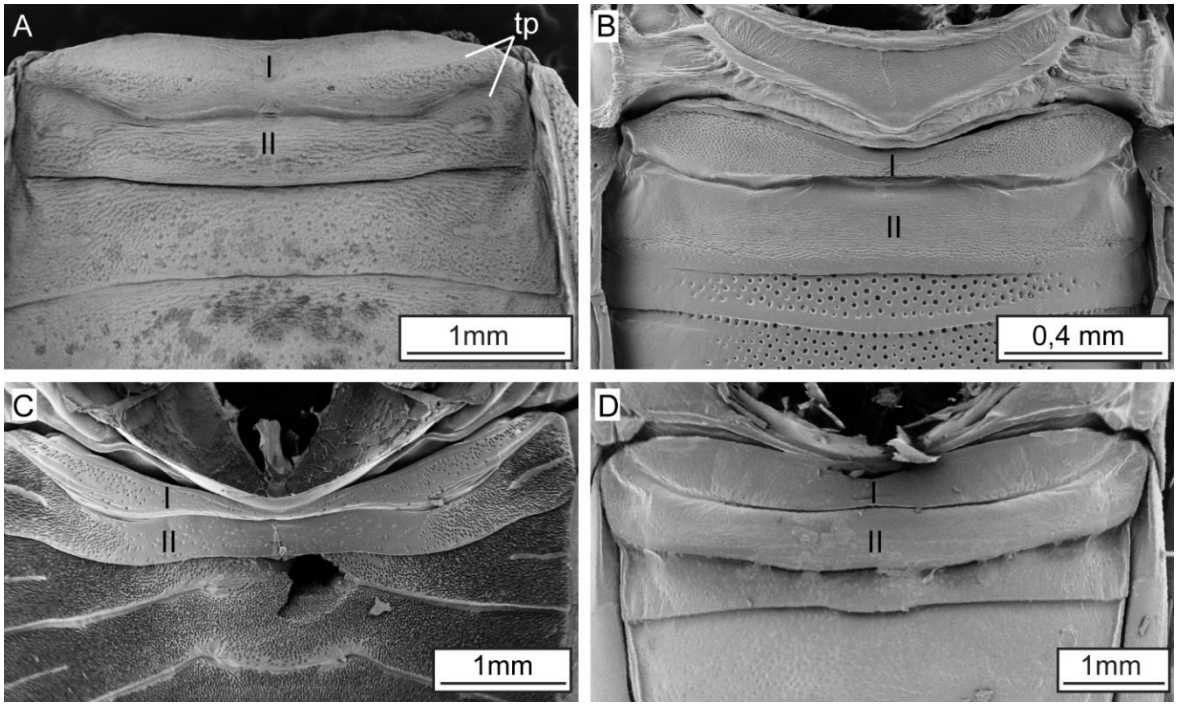

Figure 3. The tergal plate (tp) in representatives of various Pentatomomorpha, showing its conservative, easily identifiable morphology, consisting of partly fused abdominal terga I–II. A) *Coreus marginatus* (Linnaeus, 1758) (Coreidae); B) *Geocoris erythrocephalus* (Lepeletier and Serville, 1825) (Geocoridae); C) *Carpocoris mediterraneus* Tamanini, 1958 (Pentatomidae) and D) *Cydnus aterrimus* (Forster, 1771) (Cydnidae).

whether the tergal plate can be classified as a "tymbal", i.e. the vibroacoustic organ found in many Cicadomorpha.

The detailed morphology of the tergal plate is known from only one pentatomomorphan: the pentatomid *Nezara viridula* (Malouf, 1933), which is a model organism in the field of biotremology (Virant-Doberlet and Čokl, 2004). Regarding their systematic distribution, vibrational organs in Heteroptera have been found in several families of Pentatomomorpha (Leston, 1954; Jordan, 1958; Virant-Doberlet and Čokl, 2004), Cimicomorpha (Reduviidae) (Gogala, 2006), and Nepomorpha (Belostomatidae) (Barber, 1971) (Fig. 4). Gogala (1984) stated that "*a systematic search for such structures should be made and would probably show that such a vibration-producing mechanism was an old acquisition in heteropteran*

evolution. […] *Accordingly, one has to search for primitive forms of a tymbal in conservative groups of both Heteroptera and Homoptera* (= an obsolete suborder which included what is now defined as Auchenorrhyncha, Coleorrhyncha and Sternorrhyncha).".

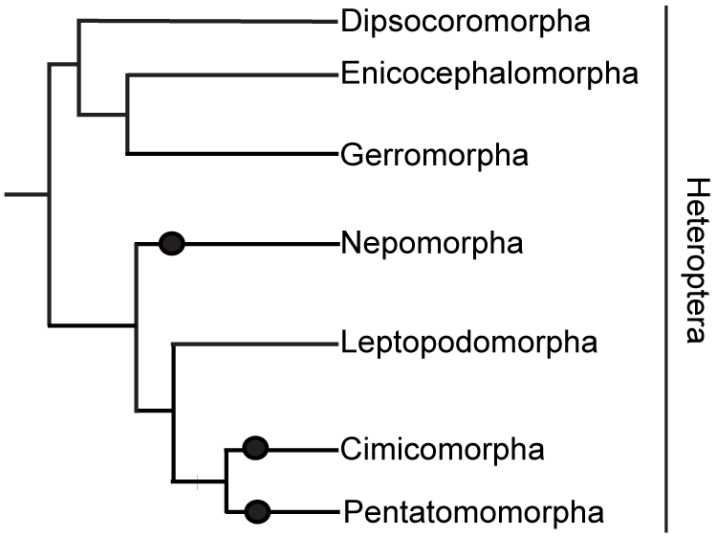

Figure 4. The known distribution of the tergal plate in Heteroptera. Phylogeny based on Wang et al. (2017).

In spite of major advances in our understanding of heteropteran vibrational communication (e.g. Virant-Doberlet and Čokl, 2004; Žunič et al, 2008; Kavčič et al, 2013), a systematic search for tergal plates and their morphology in the early diverging lineages of Heteroptera, (e.g. such as the Enicocephalomorpha and Dipsocoromorpha), has never been undertaken (Fig. 4), and their homology to the basi-abdominal vibroacoustic organs of other Hemiptera remains ambiguous (section 1.5. below).

1.5. Current theories on the origins of hemipteran vibroacoustic organs

From the account I have presented above, it is evident that there are major gaps in our knowledge regarding the morphology, systematic distribution, and homologies of the different types of hemipteran vibroacoustic organs. Snodgrass, one of the fathers of

arthropod morphology, stated in (1931) that: "*little has been done on the abdominal musculature of Hemiptera*". Nearly a century later, this situation has hardly changed. However, the paucity of morphological information has not prevented the emergence of hypotheses on the origins of hemipteran vibroacoustic signalling.

Hennig (1981) was the first to suggest that tymbal organs were an autapomorphy (or more correctly, a synapomorphy) of the Auchenorrhyncha. This view was expanded by Sweet (1996), who suggested that tymbals defined the Auchenorrhyncha, the Coleorrhyncha and the Heteroptera, a grouping which was later referred to by Senter (2008) as the "tymbaled superclade". This theory was further elaborated by the review of Wessel et al. (2014), who claimed to have identified a homologous set of muscles which defined the tymbal organs across the tymbaled superclade, which they renamed into "Tymbalia" (Fig. 1). The same study suggested that the different types of hemipteran abdominal vibroacoustic organs are all homologous, that they should be renamed into "tymbalian tymbal organs", and postulated that they originated at the root of the "Tymbalia", 300 million years ago (Fig. 1). Although the interpretations of Wessel et al. (2014), referred to throughout the present study as the Tymbalia hypothesis, have remained untested, they were quickly adopted by the entomological community (e.g. Miles et al, 2017).

2. Aims and rationale of thesis

The evolution of hemipteran vibroacoustic organs offers a fruitful area of study, with implications on several fields. Hemipteran phylogeny is a hotly debated topic (Schlee, 1969; Zrzavý, 1990; Cryan and Urban, 2011; Song and Liang, 2013; Friedemann et al, 2014; Misof et al, 2014; Yoshizawa, Ogawa and Dietrich, 2017; Johnson et al, 2018), and advances in our knowledge on the morphology of their vibroacoustic mechanisms may provide novel character systems for phylogenetic studies. Furthermore, studying the functional

morphology of hemipteran vibroacoustic mechanisms may lead to the discovery of novel biomechanical systems, which in turn might be useful for the development of bioinspired technologies (Flammang and Porter, 2011). Finally, many Hemiptera are important pests, and the exploitation of their vibrational signals is proving increasingly effective as a means of pest control (Krugner and Gordon, 2018; Laumann et al, 2018). Advances in our knowledge of the organs responsible for their production may therefore offer new targets for biocontrol.

In spite of the abovementioned potential, hemipteran abdominal vibroacoustic organs have remained largely unstudied, and there has been no attempt to validate the claims of the Tymbalia hypothesis. This is understandable, as tymbal organs are among the morphologically most complex vibroacoustic organs (Wessel et al, 2014), hemipteran abdominal musculature is almost a *terra incognita*, and the small size of many of these insects makes it technically challenging to study their morphology.

However, in recent years, the use of X-Ray microcomputed tomography (micro-CT) has revolutionised morphological, developmental, taxonomic and palaeontological studies, allowing non-invasive 3D imaging and analysis of virtually any biological specimen, including rare species and types (Metscher, 2009; Friedrich et al, 2013; Garcia et al, 2017). Using micro-CT, one can therefore investigate the morphology of even the smallest insects in unprecedented detail, and the vibroacoustic organs of Hemiptera offer a suitable system to demonstrate the utility of CT-scanning for the study of long-standing problems in arthropod morphology and biomechanics.

Considering the above, I aim to achieve the following goals in this dissertation:

1) Build a dataset of hemipteran CT scans, which will be used to document the morphology of their vibroacoustic organs, in conjunction with more traditional methods, such as scanning electron microscopy and confocal laser scanning microscopy.

2) Use the resulting CT scan dataset together with biotremological experiments on live specimens (i.e. recording their vibrational signals using laser Doppler vibrometry), in order to identify the morphology and mechanics of previously understudied taxa such as the Fulgoromorpha.

3) Reconstruct the evolution of hemipteran vibroacoustic organs, and test the validity of the Tymbalia hypothesis.

The above-mentioned aims are largely met, and the results of this dissertation are summarised below for each chapter:

Chapter 2: Material and methods

In this chapter, I summarise the depositories from which all the examined specimens originate, the techniques used for specimen collection, and the methods used to document the morphology and biomechanics of the hemipteran vibroacoustic organs examined in this thesis.

Chapter 3: Planthopper bugs use a fast, cyclic elastic recoil mechanism for effective vibrational communication at small body size

To tackle the question of how planthoppers (Fulgoromorpha) produce vibrational signals, I conducted biotremological experiments on living specimens and examined the morphology of preserved material using SRμ-CT and other techniques. By combining these two approaches, I was able to describe the biomechanics and morphology of a new type of vibrational mechanism which I termed the snapping organ, and is nearly ubiquitous in

planthoppers. The new organ is mechanically and morphologically distinct from the tymbal organs of cicadas, although the question of homology between the two is not addressed in this chapter.

Chapter 4: On the morphology and possible function of two putative vibroacoustic mechanisms in derbid planthoppers (Hemiptera: Fulgoromorpha: Derbidae)

An outstanding question on planthopper vibroacoustic behaviour is an old report of acoustic signals from the family Derbidae (Muir in Kirkaldy, 1907), which are thought to be produced by a stridulatory mechanism involving an interaction between a modified surface on the metathoracic wing and specialised hairs on the third abdominal segment. By examining the detailed morphology and systematic distribution of this supposed stridulatory mechanism for the first time, I suggest that it is highly unlikely to be stridulatory. I instead propose a function in spreading chemical secretions, which has also evolved independently in certain tephritid flies.

Chapter 5: Response to "On the evolution of the tymbalian tymbal organ: Comment on "Planthopper bugs use a fast, cyclic elastic recoil mechanism for effective vibrational communication at small body size" by Davranoglou et al. 2019"

Following the description of the snapping organ as the dominant vibrational mechanism in planthoppers, a subset of the authors of the Tymbalia hypothesis (Wessel et al, 2014) published a comment where they suggested that snapping organs are homologous to the tymbals of Cicadomorpha, and should be renamed into "tymbalian tymbal organs with a snapping mechanism". In this chapter, I demonstrate that the homology between snapping organs and tymbals remains an open question, and that the defining criteria used in the formulation of the Tymbalia hypothesis were based on morphological misinterpretations.

Chapter 6: On the morphology and evolution of cicadomorphan tymbal organs

The systematic distribution, segmental identity and evolutionary origins of cicadomorphan tymbal organs have puzzled scientists for more than a century. In this chapter, I examine the morphology and systematic distribution of tymbals across cicadomorphan phylogeny. I find that tymbal organs are ubiquitous in this infraorder, and provide evidence that most studies have misinterpreted the identity of tymbal muscles as belonging to the first abdominal segment, when they in fact belong to the second. I also propose homologies between the abdominal musculature of Cicadomorpha with that of Fulgoromorpha, and I compare the morphology of tymbals to the newly described snapping organs, in order to forward our understanding of their evolutionary affinities.

Chapter 7: The pregenital abdomen of Enicocephalomorpha and morphological evidence for different modes of communication at the dawn of heteropteran evolution

Moving on to Heteroptera, I address the question of the systematic distribution and origins of chemical and vibrational communication in these insects, by examining the metathoracic and abdominal morphology of the systematically important infraorder Enicocephalomorpha. I find that they possess tergal plates which are morphologically capable of generating vibrations, and chemical organs which operate using the same muscle as other Heteroptera, although the way the chemical secretions are disseminated is distinct. Based on this evidence, I suggest that these modes of communication likely originated at the root of heteropteran phylogeny. I also discuss the homologies of tergal plates to the mechanisms of other hemipterans.

Chapter 8: Conclusions

In this chapter I summarise my findings and indicate areas that require further study. I show that the question of the evolutionary origins of hemipteran vibroacoustic mechanisms remains unsettled, and should be further investigated using a cladistic and developmental

approach. The discovery of a new mechanism from an otherwise well-studied and diverse lineage, the Fulgoromorpha, shows that many biomechanical surprises are likely to be found in currently unexplored lineages, such as most Cicadomorpha and the Coleorrhyncha. Finally, I provide a list of further areas of study, which will be essential in answering key evolutionary questions regarding the origin of vibroacoustic communication in the Hemiptera.

3. Thesis structure

This thesis largely comprises chapters in the form of published or submitted manuscripts to peer reviewed journals. Details regarding the publication status and links to the relevant journal websites and full-size images and illustrations are provided at the beginning of each chapter, while a statement of authorship is inserted at the end. In accordance to the University's copyright guidelines, the author-submitted version is used for all published chapters. Consequently, the chapter structure and reference style is not uniform throughout this dissertation, due to the different formats required by each journal.

Supplementary materials cited in chapters 3, 4 and 6 are included in the Appendix section at the end of the dissertation.

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Chapter 2: Material and methods

2.1. Material

The bulk of the material examined in this study was obtained from the following institutions: the Natural History Museum, London, UK (BMNH); the Oxford University Museum of Natural History (OUMNH); the Moravian Museum, Brno, Czech Republic (MMBC); the National Museum, Prague, Czech Republic (NMPC); the Paleontological Institute, Russian Academy of Sciences, Moscow, Russia (PI); and the University of Gdańsk (UG).

The hemipteran taxa collected by the author are referred to as part of the Davranoglou Personal Collection (DPC). Specimens were collected by the author in various locations in Greece between the years 2008-2014. The primary techniques used were sweep-netting of vegetation (Fig. 1A), hand-searching for specimens in their particular habitats (e.g. under tree bark, Fig. 1B), and by sifting leaf litter (Fig. 1C), with subsequent extraction of the insects from the sifted debris by means of a Winkler apparatus (Fig. 1D). All samples used for morphological studies were euthanized by placing them into a tube with ethyl acetate, and were subsequently fixed in 70% ethanol. The description of the collection and maintenance of live samples are described in detail in the relevant sections of Chapter 3 (4.1. Insects; S1 Methods).

Figure 1. Collecting techniques employed for this thesis. A) The author sweep-netting at a xerothermic disturbed habitat in Konitsa village, Greece (photo by Zestin Soh); B) Hand-searching under the bark of *Pinus halepensis* (Miller, 1768) in Athens, Greece, revealed a fifth instar larva of *Reduvius personatus* (Linnaeus, 1758) (Heteroptera: Reduviidae) (photo by Amaia Salazar); C) The author sifting leaf litter at Steni village, Euboea; D) Extraction of insects from sifted-leaf litter using a Winkler apparatus.

2.2. Methods

2.2.1. High speed cameras

To record the rapid motions of the vibrational organs of Fulgoromorpha, a high-speed camera was used (Grasshopper3 2.3 MP Colour USB3 Vision, Sony Pregius IMX174; Point Grey, Richmond, BC, Canada), mounted on a Leica S8 AP0 stereomicroscope, recording at a rate of 100 frames s−1, at the Oxford Flight Group, Department of Zoology, University of Oxford. Videos were recorded directly to a computer using Spinnaker SDK-1.3.0.21

software (Point Grey). A detailed description of this method can be found in Chapter 3 (4.4. High-speed video recordings).

2.2.2. Laser confocal scanning microscopy

I used laser confocal scanning microscopy to visualise the detailed morphology of the pregenital abdomen and its musculature in minute, poorly sclerotized specimens, which are otherwise difficult or impossible to observe. I placed dissected specimens of *Agalmatium bilobum* (Fieber, 1877) (Chapter 3) and intact specimens of *Lomagostus* sp.nov. and *Proboscidopirates* sp.nov. (Chapter 7) between two cover slips in 70% ethanol. Images were taken at the Light Microscopy Facility of the Sir William Dunn School of Pathology, University of Oxford, with an Olympus FV1000, at a laser wavelength of 488 nm.

2.2.3. Laser Doppler vibrometry

To record the vibrational signals of *A. bilobum*, which are otherwise inaudible to the human ear, I used a laser Doppler vibrometer (Polytec PDV-100; Polytec, Waldbronn, Germany), at a sampling frequency of 9.6 kHz and a gain of 100 mm/s/V. The technique is described in more detail in Chapter 3 (4.3. Laser Doppler Vibrometry; S1 Methods).

2.2.4. Microtome sections

To ensure the reliability of SR-μCT in the documentation of morphological characters of extremely small size, two ethanol-preserved *Cocles* sp. nov. (Chapter 7) were stained and sectioned using a Leica Biosystems DSC1 microtome at the Sir William Dunn School of Pathology, University of Oxford. The results of both techniques were compared and found to be congruent.

2.2.5. Photomicrography

Specimens from the various depositories were imaged using the following equipment: 1) a Leica M165c microscope equipped with a Leica DFC490 camera (at OUMNH); a Keyence VHX-5000 digital microscope with VH-Z20T and VH-ZST objectives (at MMBC); and 3) a Canon EOS 700D camera attached to a Leica MZ125 microscope (at BMNH). All resulting stacked images were combined using Helicon Focus (Helicon Soft, Kharkiv, Ukraine) or VHX-5000 system software.

2.2.6. Resilin visualisation

To investigate the possible presence of resilin in the vibrational organs of Fulgoromorpha, dissected specimens of *A. bilobum* were placed in excavated microscope slides and viewed through a Leica DM2000 LED under ultraviolet (UV) illumination at 365 nm, using a general blue (465/20)/green (530/30)/red (640/40) bandpass filter and a MC120 HD camera, at the School of Biological Sciences, University of Bristol. Images captured at the same focal planes under UV and visible light were superimposed using Photoshop CS6. For more details refer to Chapter 3 (S1 Methods).

2.2.7. Scanning electron microscopy

To observe microscopic external structures (e.g. sensillae, setae, microsculpture), specimens were examined in a JEOL Neoscope JCM-5000 (SEM, JEOL, Ltd) at 15 kV high vacuum, following coating for 150 s at 18 mA with gold/palladium (Quorum Technologies SC7620), giving a coating of 12.5 nm, at the Oxford Silk Group laboratory, Department of Zoology, University of Oxford. Three specimens studied in Chapter 4 (*Cedusa* sp., *Omolicna joi* Wilson, Halbert & Bexine, 2014, and *Kaha* sp.) were examined by Igor Malenovský without coating by a Hitachi S-3700N environmental electron microscope at 15 kV high vacuum at the NMPC.

2.2.8. Synchrotron radiation microcomputed tomography (SR-μCT)

Synchrotron radiation microcomputed tomography (SR-μCT) was conducted at the TOMCAT beamline, Swiss Light Source (SLS), Paul Scherrer Institute, Switzerland. The author visited SLS three times (August 2015, 2016, 2017), together with the research group he belongs to (Taylor lab, Oxford Flight Group, Department of Zoology, University of Oxford). The duration of each visit lasted approximately 7 days, and the author was scanning specimens during 7-8 hour shifts, twice daily, with an average of 4 specimens scanned per hour. Most specimens were scanned with a beam energy of 15.99 keV (pixel size 1,625 μm), but for smaller specimens and for detailed scans of particular surfaces, a beam energy of 12 keV (pixel size 0, 325 μm) was used. For scanning, ethanol-preserved and critically point-dried specimens were used. A total of approximately 300 insect specimens were scanned, which resulted in the largest dataset of SR-μCT scans, to my knowledge.

Three-dimensional reconstruction of the SR-μCT scans was conducted using Amira 6.1 software (Mercury Systems, Andover, MA, USA). Colouration and labelling of figures were performed in Adobe Illustrator CS6, and image brightness adjustment was performed in Adobe Photoshop.

2.2.9. Terminology

The terminology used to describe hemipteran exoskeletal morphology, innervation, and musculature, is provided in detail in the relevant sections of each chapter. It should be noted, however, that there is a small inconsistency in the use of the terms “larva” and “nymph”. Although the term “nymph” has traditionally been associated with the immature stages of hemimetabolan insects, I adopted the opinion of Rédei and Štys (2016), which states that if one is to use a homology-based terminology, the term “larva” would be most accurate (for more details, refer to this study). I therefore use this term to describe immature Hemiptera throughout this thesis, and use the term “nymph” only for Chapter 7 (which was the first

published chapter, in 2017), as I was not aware of the study of Rédei and Štys (2016) at that time.

Acknowledgements

I would like to thank Amaia Salazar and Zestin Soh for providing the photographs of the author in the field, which are reproduced here with their permission.

References

Rédei, D. and Štys, P. (2016) ‘Larva, nymph and naiad – for accuracy's sake’ *Systematic Entomology*, 41(3), p505–510.

Chapter 3: Planthopper bugs use a fast, cyclic elastic recoil mechanism for effective vibrational communication at small body size

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Abstract

Vibrations through substrates are an important source of information for diverse organisms, from nematodes to elephants. The fundamental challenge for small animals using vibrational communication is to move their limited mass fast enough to provide sufficient kinetic energy for effective information transfer through the substrate whilst optimising energy efficiency over repeated cycles. Here, we describe a vibratory organ found across a commercially important group of plant-feeding insects, the planthoppers (Hemiptera: Fulgoromorpha). This elastic recoil snapping organ generates substrate-borne broadband vibrations using fast, cyclical abdominal motion that transfers kinetic energy to the substrate through the legs. Elastic potential energy is stored and released twice using two different latched energy-storage mechanisms, each utilising a different form of elastic recoil to increase the speed of motion. Comparison to the acoustic tymbal organ of cicadas (Hemiptera: Cicadomorpha) reveals functional convergence in their use of elastic mechanisms to increase the efficacy of mechanical communication.

1. Introduction

Information transfer involving substrate-borne vibrations along surfaces or through materials is important to a wide variety of taxa, from elephants to nematode worms [1]. The key challenge for successful vibration generation lies in balancing energy-efficient motion for repeated signalling [2] with effective and robust information transfer [3]. Signalling efficiency can be optimised by minimising the frequency of active muscle contraction [4], whereas signalling efficacy is optimised by maximising the kinetic energy transferred to the substrate. One solution to this tradeoff, as we show here, is to make use of elastic recoil mechanisms in which elastic energy is stored slowly and then quickly released. This is sometimes referred to informally as power amplification because the time over which work is performed is reduced [2], although this is not true amplification in the sense of adding energy into the system from an outside source. This rapid release of energy is essential because kinetic energy scales with mass and with speed squared such that signalling efficacy is increased by producing faster, higher-amplitude motions that improve the chances of the signal reaching and stimulating its potential receivers. A further reason for favouring faster motions is that broadband signals are more robust to frequency-based filtering and environmental noise than are narrowband signals [5]: for mechanical impulses, or taps, a higher speed of motion increases the frequency content of the signal by producing a sharper impulse [6]. Frequency filtering and noise level will vary with the physical properties of the substrate [5].

Achieving the fast motions needed for effective vibrational communication is a particular challenge for smaller animals. Other things being equal, their lower mass means that faster speeds are needed to transfer kinetic energy to the substrate. Smaller animals also have shorter lever arms that limit output speed and amplitude for a given motor input, and their smaller muscles have limited potential for high motor input through direct muscle action [7]. Natural mechanisms for increasing the speed of motion, especially in smaller animals,

involve elastic recoil mechanisms in which energy is elastically stored slowly and released quickly. This is particularly well studied for one-off ballistic motions such as the closing of ant jaws [8] or mantis shrimp claws [9], the projecting of toad tongues [10], or the jumping of froghoppers [11]. Much less is known about whether and how biological systems use elastic recoil to achieve very fast cyclical motions, in which the added challenge is to accommodate this within an efficient cycle of multidirectional motion. Perhaps the only good example of elastic recoil cyclical motion is the buckling of the drum-like tymbal organ of cicadas, which can generate loud acoustic vibration through an efficient bistable motion [12]. Insect flight provides another example of elastic energy storage in a fast oscillatory system, but the cyclical motions of the flight motor are mainly optimised for smooth transfer of kinetic and potential energy through the cycle, producing a nearly sinusoidal motion of the wingtips in a typical insect such as a hoverfly [13]. In contrast, vibration generation in the cicada's buckling tymbal organ relies on the sudden release of energy [2]. Good examples from other contexts are lacking, meaning that general insights into how biological systems overcome these challenges have yet to be drawn. This leads to the fundamental research question that we set out to answer in this study: how do very small animals achieve the very fast motions needed for effective and efficient vibration generation?

Hemiptera, or true bugs, have expanded the use of vibrational signalling more than any other insect order [14]. Although there is a large and growing body of research into the behavioural ecology of vibrational communication [1], there are few studies detailing the mechanisms by which these enigmatic vibrations are generated. Hemiptera are known to generate vibrations in various ways, ranging from the use of buckling tymbals (ribs that pop between bent and straight conformations) [15] or stridulatory structures (body parts that are rubbed together, often as a scraper and a file) [16] to the use of wing buzzing [17], leg drumming [18], and tremulation (vibration of the body relative to legs) [17, 19]. With the exception of

tremulation, which does not generate much acoustic vibration, these various mechanisms all emit acoustic and substrate-bound vibrations simultaneously [20]. Here, we report a novel, to our knowledge, vibratory organ, the snapping organ, in planthoppers (Hemiptera: Fulgoromorpha). These bugs are a speciose infraorder comprising over 12,500 described species [21] and containing several economically important crop pests [22, 23]. Planthoppers generate vibrations primarily for mate localisation and courtship [24, 25], and their vibrational signals are remarkably consistent across taxa, with the exception of planthoppers in the family Delphacidae [25], at least some of which generate unusual vibrations using so-called 'drumming' organs [26]. Planthopper vibrations have previously been assumed to be generated by tymbal-like organs, homologous to those of cicadas [19, 27], or by the highly specialised delphacid 'drumming' organs [26, 28]. Yet morphological evidence from a range of planthopper taxa was lacking, and their vibration-generation mechanism was unknown. Here, we use a state-of-the-art morphological investigation of all 21 families of planthoppers (S1 Table) to study the vibration generation organs that are present throughout the group. We combine this analysis with experimental measurement of behavioural kinematics and the vibrations they produce to describe the remarkable mechanism of vibration generation in planthoppers and to explore the use of fast cyclical motions in the hidden world of substrate-borne vibrational communication.

2. Results

2.1. Snapping organ morphology

We begin by characterising the morphology of the newly-described snapping organ in our model species, *Agalmatium bilobum* (Fulgoromorpha: Issidae). The snapping organ can be found dorsally on each side of the body at the junction between the metathorax and the abdomen, spanning the first two abdominal segments (Fig 1A and 1B and S1 Movie). The

organ has a W shape; a ridge (Fig 1B) articulates at its base with the thorax (first ‘V’) and fuses at its tip to the anterior arm of a Y-lobe (Fig 1B), which has resilin (Fig 1B) between its arms (second ‘V’; Figs 1B and S1). The posterior arm of the Y-lobe is fused with the second segment, tergum 2 (tg2; Fig 1B) of the abdomen. The Y-lobe is linked at its base to an internal spine (sp; Fig 2C) of the second segment via a membranous connector (Figs 1B, 2A and 2C). Eight muscle pairs are directly associated with the snapping organ (Fig 2 and S2 Table and S1 Text), comprising three pairs of dorsal longitudinal muscles (DLMs) and five pairs of dorsoventral muscles (DVMs). Four other muscle pairs are indirectly associated with the snapping organ (ventral longitudinal muscles [vlms] IIIvlm2, Ivlm1, and IIvlm2 and intersegmental dorsoventral muscle [IIisdvm]) (Fig 2A). The snapping organ is not sexually dimorphic.

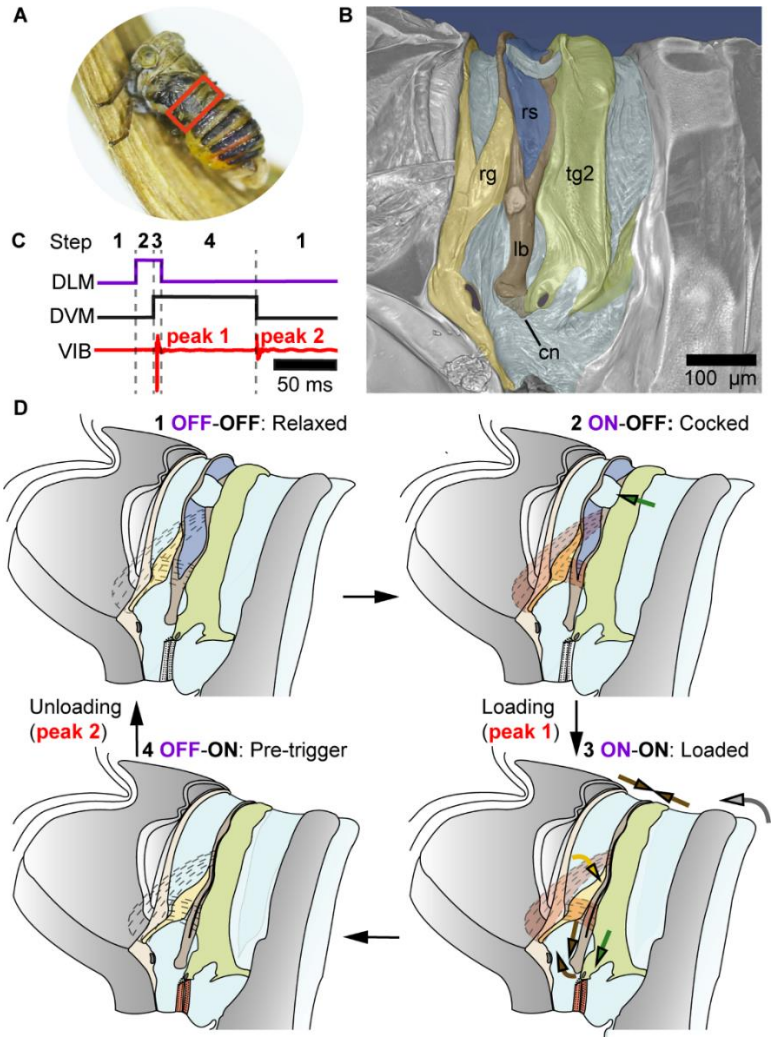

Fig 1. Vibration generation in planthoppers, using *A. bilobum* as a model. (A) Red box marks the snapping organ location. The forewings of this live specimen were removed to expose the snapping organ and its location on the abdomen. (B) False-colour SR-μCT scan of the snapping organ of *A. bilobum*, lateral view (scans deposited at CXIDB: http://cxidb.org/id-93.html). (C) Measured VIB for one sample recording and inferred activity of DLMs Idlm1-Idlm2 (purple) and DVMs IIedvm1-IIedvm2 (black) of the snapping organ during one cycle of vibration. (D) Schematic of the proposed four steps of the snapping organ required to generate one cycle of vibration. Muscles assumed to be in a relaxed state are transparent and labelled OFF, whereas those contracted are filled in red and labelled ON. Purple text refers to DLMs and black to DVMs. Loading and unloading result in the vibrational peaks seen in panel C. Structures and arrows colour-coded as follows: yellow, rg; brown, lb; light brown, cn (panel B only); dark blue: membrane with rs; green: tg2. Arrows indicate the direction of motion of these parts, whereas grey arrow denotes motion of abdomen. Latin numerals for muscles indicate segmental identity, whereas Arabic numerals indicate muscle set. cn, membranous connector; CXIDB, Coherent X-ray Imaging Data Bank; DLM, dorsal longitudinal muscle; DVM, dorsoventral muscle; edvm, external dorsoventral muscle; lb, Y-lobe; rg, ridge; rs, resilin; SR-μCT, synchrotron radiation microcomputed tomography; tg2, tergum 2; VIB, velocity of midabdomen in dorsoventral direction. Image link.

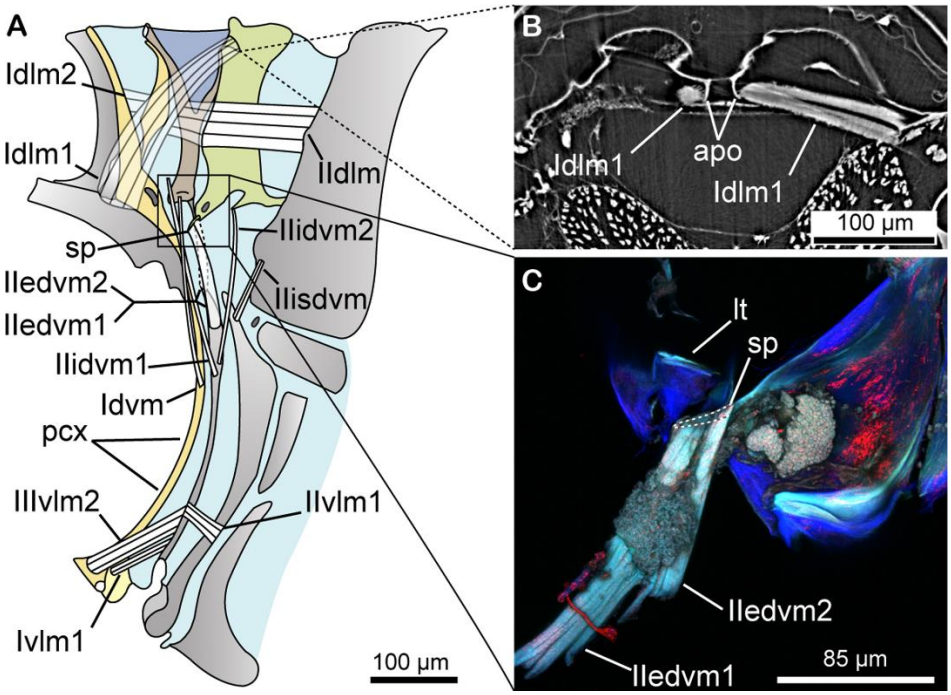

Fig 2. Generalized schematic of internal structure and musculature of the snapping organ. (A) Complete musculature of the first two abdominal segments. Square inset marks ventral junction of the lb base and tg2. (B) Transverse SR-μCT section of muscle-bearing apodeme of segment two, with hypertrophied Idlm1 inserting on it. (C) Confocal laser scanning microscopy image of lateral view of lb base-tg2 junction, with primary DVMs IIedvm1 and IIedvm2 inserting on sp (interrupted line). The angle of muscles IIedvm1 and IIedvm2 is somewhat distorted because of the fact that their ventral attachments have been severed. Colour coding of structures: yellow, rg; brown, lb; purple, rs membrane; green, tg2. Latin numerals for muscles indicate segmental identity, whereas Arabic numerals indicate muscle set. apo, apodeme of tergum 2; DLM, dorsal longitudinal muscle; DVM, dorsoventral muscle; edvm, external dorsoventral muscle; idvm, internal dorsoventral muscle; isdvm, intersegmental dorsoventral muscle; lb, Y-lobe; lt, list of base of Y-lobe; pcx, postcoxale; rg, ridge; rs, resilin; sp, spine of tergum 2; SR-μCT, synchrotron radiation microcomputed tomography; tg2, tergum 2; vlm, ventral longitudinal muscle. Image link.

Homologous vibrational organs are present throughout the entire planthopper clade (Fig 3 and S1 Table). The defining features of the musculature (Fig 2 and S2 and S3 Tables), innervation (S2 Fig), and external morphology (the ridge, Y-lobe, and connector) of the snapping organ are consistent and identifiable, despite variation in its proportions and shape across the planthoppers (Fig 3B–3E and S1 Text). Two groups deviate from this general picture: part of the family Delphacidae, in which the exoskeleton and musculature have been drastically reorganized to form an entirely different type of vibrational organ (S3 Fig and S1 Text), and part of Derbidae, which have an externally obscure snapping organ and also possess tentative stridulatory structures (Fig 3 and S4 Table). Based on their phylogenetic position, the deviations observed in these two groups are likely to be derived (Fig 3).

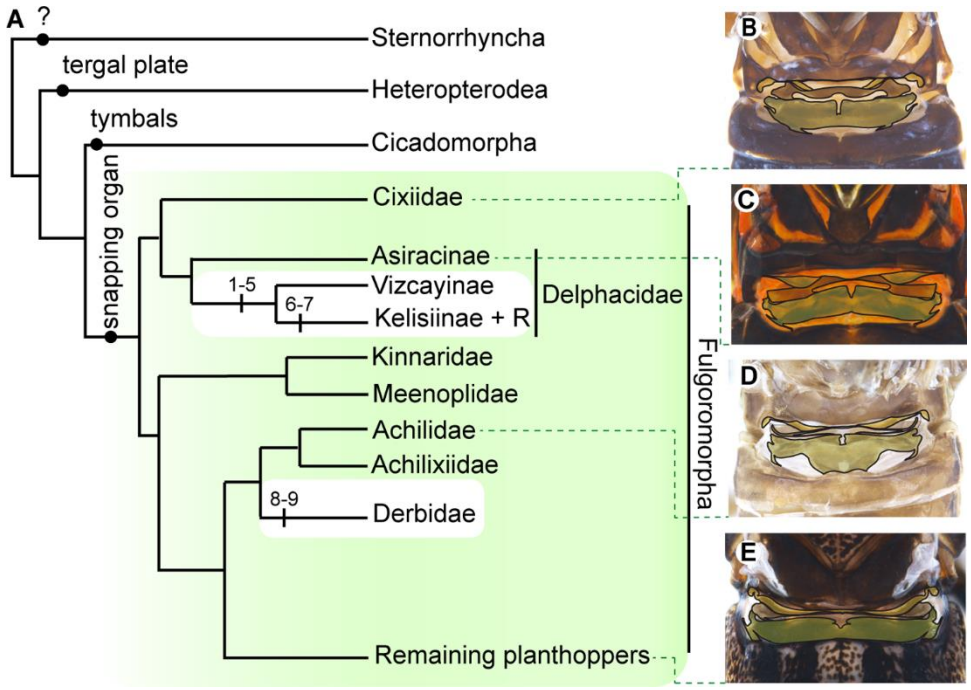

Fig 3. The snapping organ likely evolved once in the planthoppers. (A) Systematic distribution of the snapping organ (green, Fulgoromorpha) indicates a single origin at the root of planthopper phylogeny. White spaces within planthoppers indicate modification of snapping organs in the non-Asiracinae delphacids and the Derbidae. Numbers within the white spaces represent character states underlying the morphological transformations of the snapping organs in these planthoppers (see S4 Table). Other types of known abdominal vibrational organs are shown in the outgroups. Dorsal views (not to scale) of the snapping organs of (B) male *Pentastira* sp. (Cixiidae), (C) male *Asiraca clavicornis* (Delphacidae: Asiracinae), (D) male *Cixidia skaloula* (Achilidae), and (E) female *Caliscelis wallengreni* (Caliscelidae). Green dashed lines link snapping organs to their respective families; the branch of the tree labelled 'remaining planthoppers' also includes our model species *A. bilobum* (Issidae). Phylogenetic reconstruction is based on previous studies [29, 30] . R, remaining delphacid planthoppers. Image link.

2.2. Snapping organ biomechanics

To determine the kinematics of the snapping organ, we used high-speed videography and laser vibrometry on our model species, *A. bilobum* (Fig 4 and S1 Movie and S1 Data). Each vibrational cycle began with the snapping organ in its relaxed position (Figs 1D and 4A).

Subsequently, the thorax/midabdomen was raised over a 15 ms timescale (Fig 4B). The first mechanical impulse followed (loading vibrational peak), which resulted in closed Y-lobe arms, extended ridge, and the base of the Y-lobe pulled down and rotated clockwise (Fig 4C). The system resonated in response, giving a jagged waveform over a 15–20 ms timescale (Fig 4D). The cycle was completed by a second mechanical impulse (unloading vibrational peak), in which the Y-lobe arms reopened, the ridge retracted, and the base of the Y-lobe rose and rotated back (Fig 4E). This resulted in whole-system resonance ultimately returning the organ to the same relaxed position as at the beginning of the cycle. Each vibration generation cycle takes place within 120 ms, and the mechanism does not generate any audible acoustic noise [27].

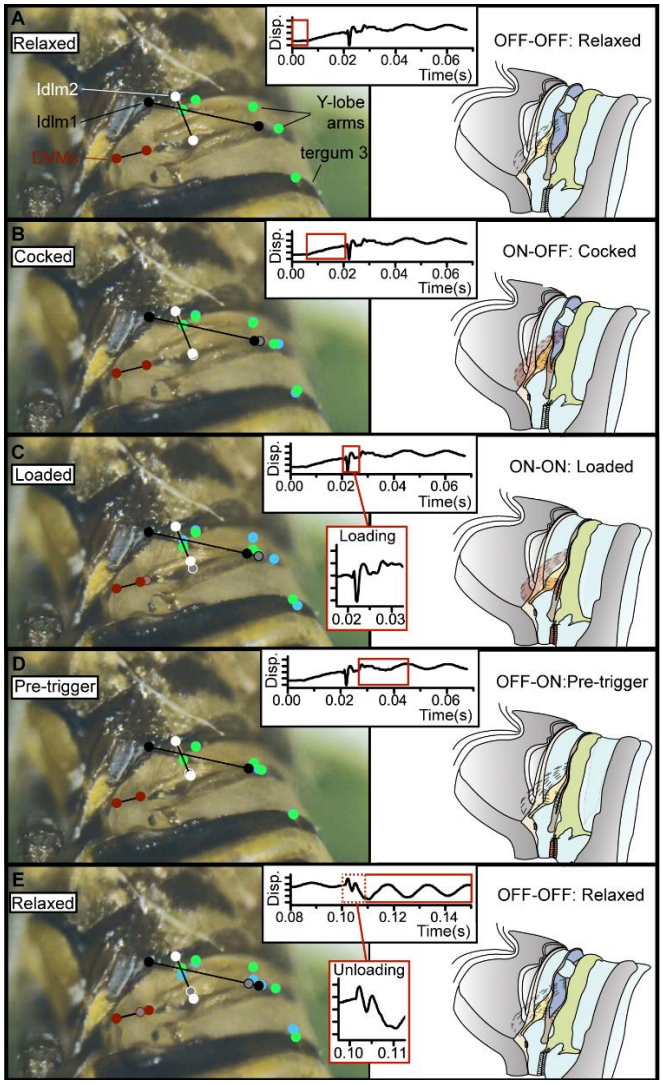

Fig 4. Stages of snapping organ mechanism in a male *A. bilobum*, illustrating external landmarks used to infer muscle strains internally (left) with corresponding stages of proposed mechanism shown diagrammatically (right). Muscle action was inferred from high-speed videography and laser vibrometry in conjunction with a separate microscopic study of the musculoskeletal anatomy to identify their origins and insertions (centre inset: Disp. of prothorax against time in seconds for one sample recording; identical axes on each panel): (A) relaxed, (B) cocked, (C) loaded, (D) pretrigger, (E) relaxed. (C) and (E) also have insets showing vibrometry recording for loading and unloading, respectively (Disp.–time). Origins and insertions of snapping organ muscles are symbolised by coloured circles (black: Idlm1; white: Idlm2; red: DVMs IIedvm1-IIedvm2). Muscle IIdlm is not included because the area it occupies does not undergo any noticeable change during stages (A)–(E) and is unlikely to contribute to the snapping organ mechanism. Unfilled coloured circles mark position of the respective muscle attachment in the previous panel; the change in distance between the points of muscle attachment indicates the extent of the muscle strain. Green and blue circles indicate position of other selected areas of the snapping organ in the current and previous panel, respectively. Red box on laser vibrometry inset panel indicates vibrational activity associated with the stage of motion represented in that panel. The underlying vibrometry data can be found within S1 Data. Disp., displacement; DLM, dorsal longitudinal muscle; DVM, dorsoventral muscle; edvm, external dorsoventral muscle. Image link.

We propose that each cycle of vibration generation consists of four main steps (Figs 1D and 4). Transition from the relaxed state to the cocked state was comparatively slow (on a timescale of 15 ms), and the movements of landmarks on the external exoskeleton suggest that this phase of the cycle was driven directly by DLM contraction (Figs 2 and 4B). Whilst we do not have direct recordings of muscle activity, the distance between the origin and insertion points of both DLMs shortens at this point in the cycle (Fig 4B), and there is no other muscle whose action could produce this strain. The distance between these points shortens even further at the transition from the cocked state to the loaded state (Fig 4C), but

this change occurs too quickly to be explained by direct muscle action alone. Specifically, the rate of change in the kinetic energy of the abdomen during loading implies energy release at a much higher power density than the DLMs and DVMs combined (Idlm1, Idlm2, IIedvm1, IIedvm2) could possibly supply through contraction (7,080 W kg−1, which is nearly 15 times the typical 500 W kg−1 power density for a muscle [31]; see S1 Methods and S1 Data). It follows that some form of elastic recoil, which acts as a kind of mechanical power amplifier, must be involved in the transition between the cocked and loaded states. This fast phase (0 to peak velocity taking 0.35 ms), which we term loading, is responsible for producing the first mechanical impulse transferring vibrational energy to the substrate. The distance between the origin and insertion points of the DVMs also shortens at this point in the cycle (Fig 4C), but contraction of these muscles alone cannot supply the mechanical energy at a high enough rate to explain the rapidity of the loading phase. Instead, the events at this transition are consistent with DVM contraction serving as an unlatching mechanism that triggers the rapid pulling down of the abdomen, followed by system resonance (Fig 4C).

The next phase of the cycle, in which the system transitioned to its pretrigger state, was a slow phase, probably involving muscle relaxation, over a 15–20 ms timescale. The subtle shift of exoskeleton positions, and particularly the lengthening of the distance between the points of origin and insertion of the DLMs (Fig 4D), is consistent with the DLMs relaxing during this phase. In contrast, the distance between the points of origin and insertion of the DVMs remain constant through this phase of the cycle, suggesting that they remain in their contracted state. The final transition in the cycle was from the pretrigger state to the relaxed state. This second fast phase, which we term unloading, is responsible for producing the second mechanical impulse transferring vibrational energy to the substrate. The associated increase in distance between the points of origin and insertion of the DVMs (Fig 4E) suggests that unloading is triggered by DVM relaxation, which causes the rapid return of the

snapping organ to its relaxed conformation through a second release of stored elastic potential energy. There is no evidence for muscle contraction at this phase of the cycle, and we therefore infer that this elastic potential energy is likely to be stored in the deformed exoskeletal elements of the snapping organ.

To verify whether passive release of elastic potential energy could be responsible for the fast unloading phase, we built a simplified mathematical model of the snapping organ, in which we replaced the ridge and the anterior arm of the Y-lobe with a pair of rigid bars connected in series to the thorax by a pair of torsional springs (Figs 5A and S4). The stiffness constants of these torsional springs were determined experimentally in a static loading experiment (S1 Methods). The abdomen and posterior arm of the Y-lobe were modelled as a mass-spring–damper system attached to the free end of the second rigid bar (Figs 5A and S4), and the spring constants and damping coefficients of this system were fitted as free parameters (S1 Methods). Quantitative comparison of the measured and modelled motion supports our supposition that the unloading phase can be explained through passive recoil of the Y-lobe, in which mechanical energy is stored elastically (Fig 5B and S1 Methods). When released, the elastic potential energy of these stiff springs acts to move the mass of the abdomen back to its relaxed state, resulting in resonant motion of the abdominal mass. More harmonic content is apparent in the measured vibrations than the modelled ones, which is not surprising given the simplicity of the model, but importantly from the perspective of information transfer, both the measured and the modelled spectra involve a broad range of different frequencies (Fig 5C and 5D).

2.3. Snapping organ elastic recoil and transformation

The motion generated by the snapping organ during the two fast loading and unloading phases was on a timescale that would not have been possible through direct muscle action

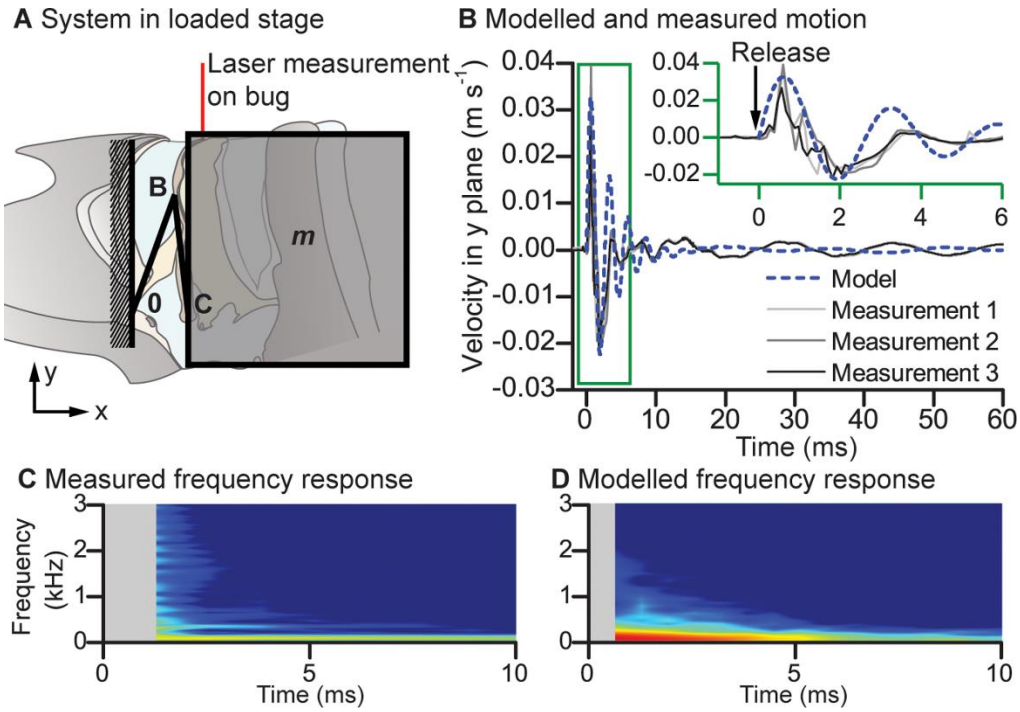

Fig 5. Modelled and measured motion of the snapping organ during unloading. A) Schematic of the mathematical model and location of the laser vibrometry measurement in relation to the snapping organ. The model comprised two stiff beams in series representing the rg and anterior arm of the lb and could rotate at points 0 (junction of thorax and rg), B (junction of rg and lb), and C (base of lb). The thorax was fixed, but point C was connected to tg2 and the rest of the abdomen's mass (*m*). Springs and damping elements not shown; see S4B Fig). Modelled (dashed blue line) and measured unloading motions in the dorsoventral direction (black, dark grey, and grey lines; measurements from the midabdomen of the same bug over three different cycles). The inset gives the same data over a shorter timescale, as indicated by the green box. (C and D) Frequency response from measured and modelled outputs, respectively, in which the colour scale gives relative magnitude in arbitrary units on an identical scale from low (blue) to high (red). The underlying data can be found within S1 Data. lb, Y-lobe; rg, ridge; tg2, tergum 2. Image link.

alone. The snapping organ instead uses two distinct elastic recoil mechanisms, each of which involves storing energy in springs, then releasing the stored energy quickly [8–11]. During the loading phase, the obvious candidate locations for elastic energy storage are the DLMs themselves, given that the exoskeleton itself deforms very little during loading (Fig 4B).

This would mean that these muscles act both as engines, actively generating the force required for loading, and as springs, storing elastic energy within their deformed structure when subject to resistance against shortening from the exoskeleton. Muscles have previously been suggested to act as springs [10], and here the elastic energy storage is in the range achievable by the cross-bridges (energy density for paired Idlm1 and Idlm2 conservatively c. 2.47 J kg−1) [32]. We therefore suggest that resistance to shortening of the contracted DLMs allows these muscles to act as an elastic spring during the loading phase [31], storing energy slowly, then releasing this quickly when triggered. A latch must be involved to prevent early release of energy, and a mechanical constraint at the base of the Y-lobe could act as a latch that is removed when the DVMs contract, acting to trigger the release of elastic potential energy stored in the DLMs.

During the unloading phase, a more straightforward passive elastic recoil is the likely mechanism, as captured by our mathematical model (Fig 5). Energy is stored in stiff springs within the W-shaped exoskeleton linkage system that are deformed and therefore loaded during the loading phase (Fig 4C), but which return to their resting position and are therefore unloaded following the unloading phase (Fig 4E). The first elastic recoil event during the active loading phase thereby stores the energy that is released during the second elastic recoil event, which is the passive unloading phase. DVM relaxation is the likely trigger, with the membranous connector and acting as a possible cuticular latch preventing early release (Fig 2). Rapid recoil is made possible by DLM relaxation during the pretrigger step, and resilin between the Y-lobe arms (S1 Fig) will act to limit damage during recoil. Additional muscles may modulate the vibration during unloading (e.g., IIIvlm2), but the muscles are far too small to account for the power density during unloading (c. 765,000 W kg−1 if normalising the mechanical power by IIIvlm2 mass; Fig 2A and S1 Data).

In summary, the snapping organ uses two muscle contraction events per cycle and typically repeats its cycle every 0.3–1 s [33], giving a muscle contraction frequency of under 5 Hz (S5A Fig). In contrast, the frequencies of the mechanical impulses resulting from this motion as measured on the midabdomen were broadband under 3 kHz (shown for recoil in Fig 5C and 5D). Crucially, from a communication perspective, the complete system also acts to transfer mechanical motion from the snapping organ to the substrate. This represents another form of mechanical power transformation, albeit one that is modulated by the substrate. For motion vertical to the plant stem for one individual, the velocity ratio of motion measured on the plant relative to motion measured on the insect midabdomen indicates that the velocity of motion is attenuated by 83% (average −15.5 ± 6.2 dB), with lower attenuation in velocity of motion between the prothorax and plant at 71% attenuation (average −10.5 ± 5.5 dB, S1 Data and S5 Fig).

3. Discussion

The consistency of the snapping organ's morphology, and its systematic distribution across planthoppers indicates that this most likely represents a conserved mechanism for generating abdominal vibrations across the Fulgoromorpha. Previous studies have only examined delphacid vibrational organs [24, 26, 34], but our analysis of their peculiar morphology indicates that the drumming organs of delphacids are the exception and not the rule. The consistency of snapping organ morphology across the rest of the planthoppers provides a clear mechanistic explanation for the observed uniformity of their vibrational signals [25, 33]. These findings reflect the fundamental importance of vibrational signals in planthopper communication.

The functional morphology of the snapping organ also reveals some remarkable functional convergences and some equally remarkable mechanistic differences between the mechanical

communication mechanisms of planthoppers and their close relatives, the cicadas [12, 24]. Both make use of paired elastic recoil mechanisms and low-frequency active muscle contractions to enhance the efficiency and efficacy of communication, using exoskeletal integration to transform mechanical impulses into substrate vibration [2, 12]. Driven by a single muscle, the cicadas' tymbal organs use buckling instability of multiple stiff ribs to store and release elastic energy, turning slow muscle contraction into fast motion as the ribs buckle [12]. Muscle relaxation and the release of energy stored in resilin pads causes the ribs to restraighten again, leading to a second step involving elastic energy release [15]. In contrast, the snapping organ uses two different energy-storage mechanisms for paired elastic recoil: elastic storage in contracted muscle for loading and elastic storage in the deformed exoskeleton for unloading. Instead of buckling like the ribs of a tymbal, the arms of the Y-lobe in the snapping organ use snapping motions similar to those used in fast raptorial strikes by jaws and claws [8, 9]. Finally, whereas tymbal vibrations in most cicadas are often associated with resonant chambers that act to transform motion into loud acoustic signals [12], the snapping organ is specialised for substrate-borne vibration generation, with comparable muscle contraction rates that act to transfer mechanical energy into vibrations of the substrate [12].

In conclusion, the unique biomechanics of the snapping organ demonstrate the general importance of elastic recoil mechanisms in the fast motions of small arthropods, extending our knowledge of such mechanisms beyond the simpler one-off ballistic motions that characterise jumping, predatory strikes, and feeding. Elastic recoil is a very general mechanism allowing small animals to overcome the limitations of their size and enabling robust vibrational communication.

4. Materials and Methods

4.1. Insects

Individuals of *A. bilobum*, the model planthopper species used in this study, were collected in large numbers (n = 250) in late April 2017 as fourth/fifth-instar larvae or adults from Lycabettus Hill, Athens, Greece, and were imported to Oxford, UK under DEFRA Plant Health Licence no. 52972/198417/6. Larvae were reared into adulthood in mesh cages (47.5 cm × 47.5 cm × 47.5 cm) kept at 22–29°C, 50% humidity, with a 16:8 photoperiod (light/dark). In addition, the morphology of specimens from more than 130 taxa were examined, covering the entire phylogenetic spectrum of Fulgoromorpha. S1 Table details the techniques used to examine the morphology of the snapping organ for each species, along with its preservation method.

4.2. Morphological analysis

Planthoppers belonging to 12 families (including three specimens of *A. bilobum*: adult male, female, and larva) were used for synchrotron radiation microcomputed tomography (SR-μCT) at the TOMCAT beamline, Swiss Light Source (SLS), Paul Scherrer Institute, Switzerland (S1 Table). All specimens were scanned at a beam energy of 15.99 keV with a final pixel size of 1.625 μm, allowing visualisation of even the smallest muscles and nerves of the snapping organ (Figs 1B and 2B and S2 and S3B–S3D), which were otherwise not detected by other techniques. Three-dimensional reconstruction was carried out using Amira 6.1 software (Mercury Systems, Andover, MA, USA). All shown tomographic data (reconstructed TIFFs) for the two imaged species (*A. bilobum* and *Stenocranus minutus*) are freely available at CXIDB (http://cxidb.org/id-93.html) [35]. Colouration and labelling of figures were performed in Adobe Illustrator CS6. In order to reveal the primary DVMs operating the snapping organ in *A. bilobum*, the ventral junction between the Y-lobe and tg2 were excised from an ethanol-preserved (70%) male (Fig 2C). The dissected sample was

placed between two cover slips in 70% ethanol and was imaged with a laser confocal scanning microscope (Olympus FV1000; Olympus, Tokyo, Japan) at a laser wavelength of 488 nm. The morphologies of specimens belonging to all 21 planthopper families were examined under light microscopy. Images of the snapping organ of four species of planthoppers shown in Fig 3 were taken using a Leica M165c microscope equipped with a Leica DFC490 camera (Leica, Wetzlar, Germany). The final, stacked images were combined using Helicon Focus (Helicon Soft, Kharkiv, Ukraine). Image brightness adjustment was performed in Adobe Photoshop, and drawings were generated in Adobe Illustrator CS6.

4.3. Laser Doppler vibrometry

To record vibrational signals, planthoppers were placed on a dried grass (*Schedonorus giganteus*) stem (17 cm in height). The base of the stem was inserted inside an empty c. 1-cm–diameter tube and was held in place by aluminium foil. Vibrational signals were recorded by a laser Doppler vibrometer (Polytec PDV-100; Polytec, Waldbronn, Germany), focussed at different positions approximately orthogonal to the stem and bug. A sampling frequency of 9.6 kHz was used for recordings at a gain of 100 mm/s/V. Recording started immediately once the planthoppers were placed on the stem. Each recording lasted 6 minutes and was repeated until the animal either ended its vibrational call or after four recordings if no songs were present.

A total of 61 recordings were made, 31 on single planthoppers, 26 on male–female groups, and four on male–male groups, using a total of 19 individuals (12 males, 7 females). Recordings from two individuals are included in S1 Data, in which the laser was focussed on the plant stem (individual 1), bug prothorax (individual 1), bug genitalia (individual 2), or bug midabdomen (individual 1). All vibrometry recordings were similar in the type and pattern of motion observed, so the data presented in S1 Data and S5 Fig are assumed to be

representative. Attenuation of motion during loading and unloading from the midabdomen to the plant stem and prothorax to the plant stem was calculated in decibels (S1 Data). Vibrometry figures were drawn using Raven Lite 2.0 (Cornell Lab of Ornithology, Ithaca, NY, USA) and OriginPro 8. To stimulate vibration generation, we used playback tracks of recorded songs. The stem was vibrated 7.3 cm from the base by a pin glued to a small piezo disc (RS Components, Corby, UK), which was glued on an inverted plastic cup. Playback songs consisted of prerecorded and amplified vibrational signals of both sexes. All males responded to the playback by emitting a series of pulses for several minutes.

4.4. High-speed video recordings

The motion of the snapping organ in *A. bilobum* was captured with a high-speed camera (Grasshopper3 2.3 MP Colour USB3 Vision, Sony Pregius IMX174; Point Grey, Richmond, BC, Canada) mounted on a Leica S8 AP0 stereomicroscope, recording at a rate of 100 frames s−1. Videos were recorded directly to a computer using Spinnaker SDK-1.3.0.21 software (Point Grey). A total of three males were video recorded, and a movie and still frames from one male are given in Fig 4 and S1 Movie. The males were filmed over multiple cycles, frames were classified into the different stages of the mechanism, and the clearest frames were chosen from these classified groups within Fig 4. Pixel coordinates of three points on the bug prothorax were quantified for each frame used in Fig 4 to check for alignment of the bug within the video frame over time. Standard deviation over the five frames for each of the three points was within the order of 0.01 pixels, suggesting the bug has limited movement within the video frame over successive cycles (also supported by S1 Movie). Prior to recording, it was necessary to expose the snapping organ by removing the fore and hind wings with a scalpel. The males were then left on their host plant for one hour to recover after wing removal before playback recordings were started to stimulate vibration

generation. Based on our observations, the motion captured in S1 Movie is representative of the vibration-generation mechanism across different individuals.

4.5. Calculations and modelling

The vibrometry recordings were analysed to calculate the peak energy and power of the loading and unloading motions (S1 Data). Maximum and minimum peak velocities and the timings of the peaks were extracted from the vibrometry data. The peak kinetic energy of the motion was calculated from the speed of the measured dorsoventral translation of the abdominal mass, and the corresponding mechanical power was determined by dividing this peak kinetic energy by the time taken to reach it from rest. The muscle power density that would be required to generate this motion through direct muscle contraction was calculated by dividing these values by the relevant muscle mass, as measured from SR-μCT measurements of *A. bilobum*, modelling muscles as cylinders with a density of 1,060 kg m−3 [36].

A mathematical model was developed to support the interpretation that unloading was due to elastic recoil of the system (Figs 5 and S4). The model included the abdomen as a mass attached to two rigid bars in series (anterior Y-lobe arm and ridge, respectively), each with a stiff rotational spring at their junctions. The anterior bar was fixed to a surface, representing the thorax. Springs and dampers acting on the mass of the abdomen modelled the combined action of the muscles, resilin, other exoskeletal components, and interior morphology on the motion of the mass in the dorso–ventral and anterior–posterior planes. Full details of the model are given in S1 Methods.

Author Contributions

L.-R. D conceived the study, secured funding, undertook the experiments, analysed and interpreted data, prepared figures, and co-wrote the paper. A.C. developed the mathematical

model and wrote the relevant sections in the manuscript and supporting information. G.K.T. supervised the study, advised on methodology, contributed to data interpretation and co-edited the manuscript. B.M. supervised the study, analysed and interpreted data, prepared figures, and co-wrote the paper.

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Student Confirmation

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By signing the Statement of Authorship, you are certifying that the candidate made a substantial contribution to the publication, and that the description described above is accurate.

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Chapter 4: On the morphology and possible function of two putative vibroacoustic mechanisms in derbid planthoppers (Hemiptera: Fulgoromorpha: Derbidae)

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Abstract

A mechanism involving interaction of the metathoracic wing and third abdominal segment of derbid planthoppers was first discovered over a century ago, and interpreted as a stridulatory organ for sound production. Although referred to occasionally in later taxonomic works, the detailed morphology, systematic distribution, and behavioural significance of this structure have remained unknown, and its proposed use in sound production has never been corroborated. Here we examine the distribution and morphology of the supposed stridulatory organ of Derbidae and the recently-described vibratory mechanism of planthoppers – the snapping organ, across 168 species covering the entire taxonomic spectrum of the family. We find that many derbids possess snapping organs morphologically similar to those of other planthoppers, and find no evidence for the presence of tymbal organs, which were previously thought to generate vibrational signals in derbids. We find the supposed stridulatory mechanism to be widespread in Derbidae, and conclude

that it provides several systematically and taxonomically important characters. Nevertheless, its morphology appears unsuitable for the production of sound, and we instead speculate that the mechanism plays a role in spreading chemical secretions or wax. Finally, we observe wax production by tergal glands in derbid larvae, and illustrate their external morphology in adults.

1. Introduction

Hemiptera, or true bugs, have expanded the use of acoustic and substrate-borne vibrational signalling more than any other insect Order (Cocroft & Rodriguez, 2005). Hemipterans are known to generate vibrations in various ways, using: (i) stridulation, where a scraper (plectrum) and a file (stridulitrum) are moved against one another to generate sound (Čokl et al., 2006); (ii) buzzing (Kavčič et al., 2013), in which the wings are vibrated to generate sound; (iii) percussion, where the legs tap against a surface (Žunič et al., 2008); (iv) tremulation, where the body vibrates relative to the legs (Žunič et al., 2008; Kavčič et al., 2013); (v) tymbal buckling, which is a bistable mechanism involving a buckling membrane, usually with ribs that pop bent and straight (Young & Bennet-Clark, 1995); and (vi) abdominal snapping, in which the abdomen is jerked up and down using a recently-discovered snapping organ found across planthoppers (Davranoglou et al., 2019).

Derbids are among the most taxonomically diverse planthoppers, with ca. 1,700 described species occurring primarily in tropical and subtropical areas (Bartlett et al., 2014; Bourgoin, 2019). Their morphology is similarly diverse. Defining traits of derbids include a small and tapered apical labial segment; a row of spines on the second hind tarsal segment and parameres that greatly extend beyond the abdomen (Wilson, 2005; Bartlett et al., 2014). Peculiar modifications in some taxa include forward-facing expansions of the pronotum known as subantennal processes; greatly enlarged, sexually dimorphic antennae; and unique

antennal appendages (Emeljanov, 1996; Bourgoin & Yap, 2010; Bartlett et al., 2014). In terms of their habits, derbid larvae are considered mycophagous, and can be found in rotting logs and leaf litter (O'Brien & Wilson, 1985; Yang & Yeh, 1994; Howard et al., 2001; Gossner & Damken, 2018), whereas adults typically feed on monocot plants (often palms) and woody dicot plants (Wilson et al., 1994; Howard et al., 2001), often forming large aggregations under their leaves (Kirkaldy, 1907; O'Brien, 2002). Approximately 20 species are potential agricultural pests, and some may transmit phytoplasmas (Wilson, 2005; Brown et al., 2006; Halbert et al., 2014).

Regarding communication among the Derbidae, substrate-borne vibrational signals have been recorded from *Cedusa* spp., which were attributed to as yet unknown tymbal organs (Tishechkin, 2003, 2008). Although acoustic signalling has not yet been experimentally demonstrated in planthoppers, the eminent hemipterist F.A.G. Muir reported noise emanating from hundreds of individuals of the derbid *Muiria stridula* Kirkaldy, 1907, which were aggregating under a palm. Based on his observations of live animals, Muir identified the sound as being stridulatory in origin, and proposed a mechanism where a part of the metathoracic (hind) wing is modified into a stridulitrum and strikes against a field of hairs on the abdomen, supposed to act as a plectrum. Since Muir's original observations and illustrations (reported in Kirkaldy, 1907), the supposed stridulitrum has occasionally been used in descriptive taxonomy and classification of derbids (e.g. Fennah, 1952; Emeljanov, 1996; Banaszkiewicz & Szwedo, 2005), albeit that its detailed morphology, systematic distribution, homologies, and function have remained unstudied. The abdominal hairs that Muir interpreted as functioning as a plectrum have been neglected by most subsequent studies, and their structure and distribution has remained undocumented. In addition, there have been no subsequent observations of acoustic signalling in derbids that would confirm

Muir's observations, and the function of this unusual mechanism has not been examined in a behavioural context.

In order to gain a better understanding of the morphological basis of communication in derbids, we examined the external morphology of the pregenital abdominal segments and putative stridulatory structures of 168 species of Derbidae, covering almost the entirety of the currently recognised subfamilies, tribes, and subtribes of the family. We also investigated the internal morphology of one species using synchrotron-based micro computed tomography (SR-μCT). Our findings present novel morphological information which may be important for reconstructing the systematics and taxonomy of Derbidae and provide a new perspective on the functional morphology and behavioural significance of the supposed stridulatory mechanism of the group. We also show wax production from tergal glands in derbid larvae, and describe their external morphology in adults for the first time, discussing the relevance of these observations to functional interpretations of the putative stridulatory mechanism.

2. Material and Methods

2.1. Material

2.1.1. Stereomicroscopy

We examined dry-mounted specimens of 168 species under a stereomicroscope (Table S1), using material deposited in the Natural History Museum, London, UK (BMNH) and the Moravian Museum, Brno, Czech Republic (MMBC).

2.1.2. Additional specimens

Given that the methods of sections 2.2.–2.5. below involved manipulation, partial destruction or imaging of specimens using different techniques, we analysed additional

species not necessarily included in Table S1, based on material deposited at the BMNH, MMBC, the Oxford University Museum of Natural History, UK (OUMNH), and specimens in the wild:

Adults

1. *Alara fumata* (Melichar, 1914). Male holotype, Indonesia, Java, Goenoeng Oengaran, xii.1909, E. Jacobson leg. (coll. Melichar, MMBC).

2. *Cedusa* sp. Two males, Peru, Callanga (coll. Melichar, MMBC).

3. *Derbe* sp. One male, Ecuador, Pichincha, Nambillo Valley near Mindo, 1450 m, 15.viii. 1987, M. Cooper leg. (BMNH).

4. *Kaha* sp. One male, Philippines, Luzon, Los Banos, P. I. Baker leg. (coll. Melichar, MMBC).

5. *Malenia bosnica* (Horváth, 1907). One male, Bulgaria, gara Kresna railway station, Kresna Gorge near Struma River, 230–300 m, 20–21.viii.1972, P. Lauterer leg. One female, Bulgaria, Lilyanovo, towards Sandanski, Sandanska Bystrica valley, 13.vii.1971, P. Lauterer leg. (both MMBC).

6. *Mysidia* sp. One male, Ecuador, Shushufindi, ii.1987, ex palms in forest, B. Pertnuis leg., CIE A18814, sp. 235 (BMNH).

7. *Omolicna joi* Wilson, Halbert & Bextine, 2014. One male and one female, USA, Florida, Highlands County, Venus, Archbold Biological Station, 27°10′53″N, 81°20′54″W, 45–69 m, 1–2.x.2016, scrub with *Sabal etonia* and *Serenoa repens*, I. Malenovský leg. (MMBC).

8. *Paraphenice mawae* Wilson, 1987. One male paratype, Tanzania, Chambezi, iv.1984, on *Cocos nucifera*, M. Schuiling leg., C.I.E. AI5996 (BMNH).

9. *Proutista* sp. One male, Uganda, Unyoro, Bugoma “oserdo” 3700, vii. 1914, Kittenberger leg. dd ’19, C.A. Wiggins (OUMNH).

10. *Zoraida pterophoroides* (Westwood, 1851). One female, India, Assam, Khasia (coll. Melichar, MMBC).

11. *Zoraida* sp. 1. One male, SUNII, Indonesia, Sulawesi, Toraut, viii.1985, S. Greenwood leg. (OUMNH-2010-089).

12. *Zoraida* sp. 2. One female, same data label as above (OUMNH).

Larvae

1. *Pamendanga* sp. Fifth instar, Singapore, Zhenghua park, 21.x.2016, photographed by N. Bay.

2. *Zoraida* sp. Fifth instar, Singapore, 14.ix.2014, photographed by N. Bay.

2.2. Photomicrography

The metathoracic wing and abdomen of *Proutista* sp. and *Derbe* sp. were imaged using a Leica M165c microscope with a Leica DFC490 camera, while stacked images were combined using Helicon Focus (Helicon Soft, Kharkiv, Ukraine). The slide-mounted metathoracic wings of *Alara fumata*, *Malenia bosnica* and *Omolicna joi* and a spread metathoracic wing of a dry-mounted specimen of *Zoraida pterophoroides* were imaged using a Keyence VHX-5000 digital microscope with VH-Z20T and VH-ZST objectives.

2.3. Macrophotography

The two derbid larvae were photographed in the wild by Nicky Bay (see Acknowledgments) using a Nikon D800 camera equipped with a Tamron 90mm F2.8 SP Di Macro Lens.

2.4. Scanning Electron Microscopy (SEM)

Two critical point-dried specimens of Zoraidinae (*Zoraida* sp. 1 & sp. 2) and a dry-mounted specimen of *Mysidia* sp. were examined in a JEOL Neoscope JCM-5000 (SEM, JEOL, Ltd) at 15 kV high vacuum, following coating for 150 s at 18 mA with gold/palladium (Quorum Technologies SC7620), giving a coating of 12.5 nm. Scanning electron micrographs of *Cedusa* sp., *Omolicna joi* and *Kaha* sp. were taken from dry-mounted specimens without coating by a Hitachi S-3700N environmental electron microscope at 15 kV high vacuum.

2.5. SR-µCT

A specimen of a critical-point dried *Zoraida* sp.1 was scanned at the TOMCAT beamline, Swiss Light Source (SLS), Paul Scherrer Institut, Switzerland, at a beam energy of 15.99 keV with final pixel size of 1.625 mm. Three-dimensional reconstruction was carried out using Amira 6.1 software (Mercury Systems). Image labelling and illustrations were generated in Adobe Illustrator CC/CS6 (Adobe Systems Incorporated, San Jose, California, USA).

2.6. Terminology

We follow the terminology of Dworakowska (1988) regarding metathoracic wing venation. Although we describe a posterobasal sclerotisation/pigmentation of the metathoracic wing membrane that Dworakowska (1988) identified as the posterior jugal vein (JP), we are uncertain on whether it represents a true vein. This structure may in fact be a secondarily detached thoracic sclerite, in particular from the posterior notal wing process – a transformation which frequently occurs in other insects as well (e.g. Snodgrass, 1935; as a fourth axillary sclerite). According to the same author, the posterior anal (AP) and anterior jugal (JA) veins fuse basally in most Auchenorrhyncha. We indeed observe a close basal association of the two veins, but given that the situation is unclear, we will simply refer to them as AP and JA respectively. When referring to structures of the metathoracic wing, inner

indicates its dorsal surface, and outer the ventral surface. Wessel et al. (2014) used the term tymbalian tymbal to describe the vibroacoustic organs of all non-sternorrhynchan Hemiptera (Tymbalia), which they suggested were homologous. In this study, our definition of a tymbal is strictly biomechanical, i.e. to describe an organ that uses paired buckling membranes with or without buckling ribs, in order to generate vibroacoustic signals. This definition encompasses the primary vibrational organs of most Cicadomorpha, which are characterised morphologically by the presence of paired, buckling membranes on the lateral margin of tergum 1, which may or may not possess buckling ribs (Ossiannilsson, 1949). The snapping organ described for the first time by Davranoglou et al. (2019), is mechanically and structurally distinct from cicadomorphan tymbals, and its external morphology consists of three main defining characters: 1) a ridge arising from the part of the first abdominal tergum which is fused to the thorax; 2) a Y-shaped lobe with two "arms" which snap shut upon muscle contraction; and 3) a membranous connector at the base of the Y-lobe which link it to tergum 2. We use these traits, along with their associated musculature, to identify the presence or absence of the snapping organ in Derbidae. We followed Bourgoin (2019) for taxonomic nomenclature and classification. The derbid larvae on Fig. 10A, B were tentatively identified using the keys in Yang & Yeh (1994).

Abbreviations used in the figures: **AA:** Anterior Anal; **ajf:** anojugal fold; **AP:** Posterior Anal; **axc:** axillary cord; **C:** Costa; **CuA:** Anterior Cubitus; **CuP:** Posterior Cubitus; **cv:** cross-vein; **dm:** dense material; **gl:** tergal gland; **gr:** groove of tergal gland; **Idlm1:** group I dorsal longitudinal muscle of tergum 1; **JA:** Anterior Jugal; **JP:** Posterior Jugal; **l:** lumen; **lb:** Y-lobe of snapping organ; **lp:** loop of dense material; **MA:** Anterior Media; **MP:** Posterior Media; **mb:** membrane; **o:** ostiole of tergal gland; **rg:** ridge of snapping organ; **Pc:** Postcubitus; **plc:** plectrum; **po:** pore of stridulitrum; **R:** Radius; **ScA:** Anterior Subcosta; **ScP:** Posterior Subcosta; **sct:** scale-like tubercle; **set:** seta; **sp:** spiracle; **spn:** stridulitral

spine; **sst:** short seta; **std:** stridulitral depression; **stl:** stridulitral line; **str:** stridulitrum; **t:** tergum; **tc:** seta-bearing tubercle

3. Results

We first describe the condition found in the Cedusinae and Cenchreini: groups in which all or most species lack the putative stridulatory mechanism, and which reflect a more generalised condition of the wings and abdomen, similar to non-derbid planthoppers. Subsequently, we examine the conditions in all other derbid subfamilies, describing the various modifications of the metathoracic wing and pregenital abdomen that have taken place. The descriptions are based on the species cited in the figures, unless explicitly stated otherwise.

3.1. Abdominal organs and metathoracic wing structures of Cedusinae

The first two abdominal segments of all examined tribes of Cedusinae display modifications characteristic of a snapping organ (Davranoglou et al., 2019). The first abdominal segment is subdivided into two distinct units. The proximal portion of the first abdominal segment is strip-like, followed by a broad membrane which is entirely deflated in the examined dry-mounted specimens, obscuring the ridge which is present but not visible in Fig. 1A. A Y-lobe is present (lb; Fig. 1A), its base being extremely elongate, with no visible external connector linking the Y-lobe base to tergum 2. The anterior arm of the Y-lobe is strongly pronounced (Fig. 1A), separated from the posterior arm by a membrane (mb; Fig. 1A). The posterior arm is much shorter, fusing to tergum 2 (Fig. 1A). Tergum 2 (t2) is narrow, poorly sclerotized and separated from tergum 3 by a membrane in the anterolateral parts of its posterior margin. In addition to possessing these features characteristic of a snapping organ, the first three abdominal segments also display several other anatomical features of note: terga 1–2 are surrounded by membranous cuticle, and the more sclerotized tergum 3 (t3)

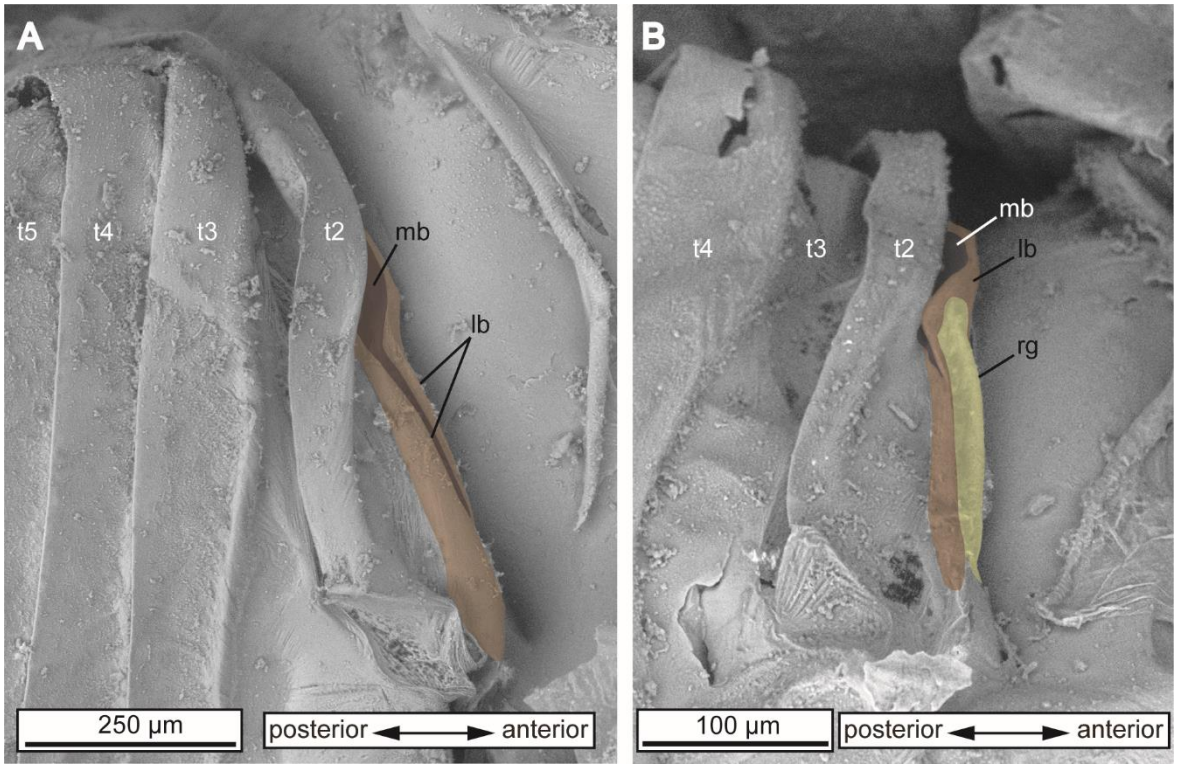

Fig. 1. External morphology of the snapping organ and pregenital abdomen of Cedusinae and Cenchreini (SEM, false-coloured). A: Abdomen of *Cedusa* sp. (Cedusinae), male, dorso-caudal view, lb, Y-lobe of snapping organ; mb, membrane; t, tergum; B: Abdomen of *Omolicna joi* (Cenchreini), female, dorso-caudal view, rg, ridge of snapping organ. Image link.

lacks distinct setae on its surface (Fig. 1A); spiracle one is about two times larger than the remaining abdominal spiracles.

The metathoracic wing lacks any structures that would indicate the presence of a stridulitrum (Fig. 2A). The anterior jugal vein (JA) is present beneath the anojugal fold (ajf) and the posterior jugal vein (JP) is indistinct except for a pigmentation along the jugal margin, immediately above the axillary cord (axc; Fig. 2A).

3.2. Abdominal organs and metathoracic wing structures in part of Cenchreini (Derbinae)

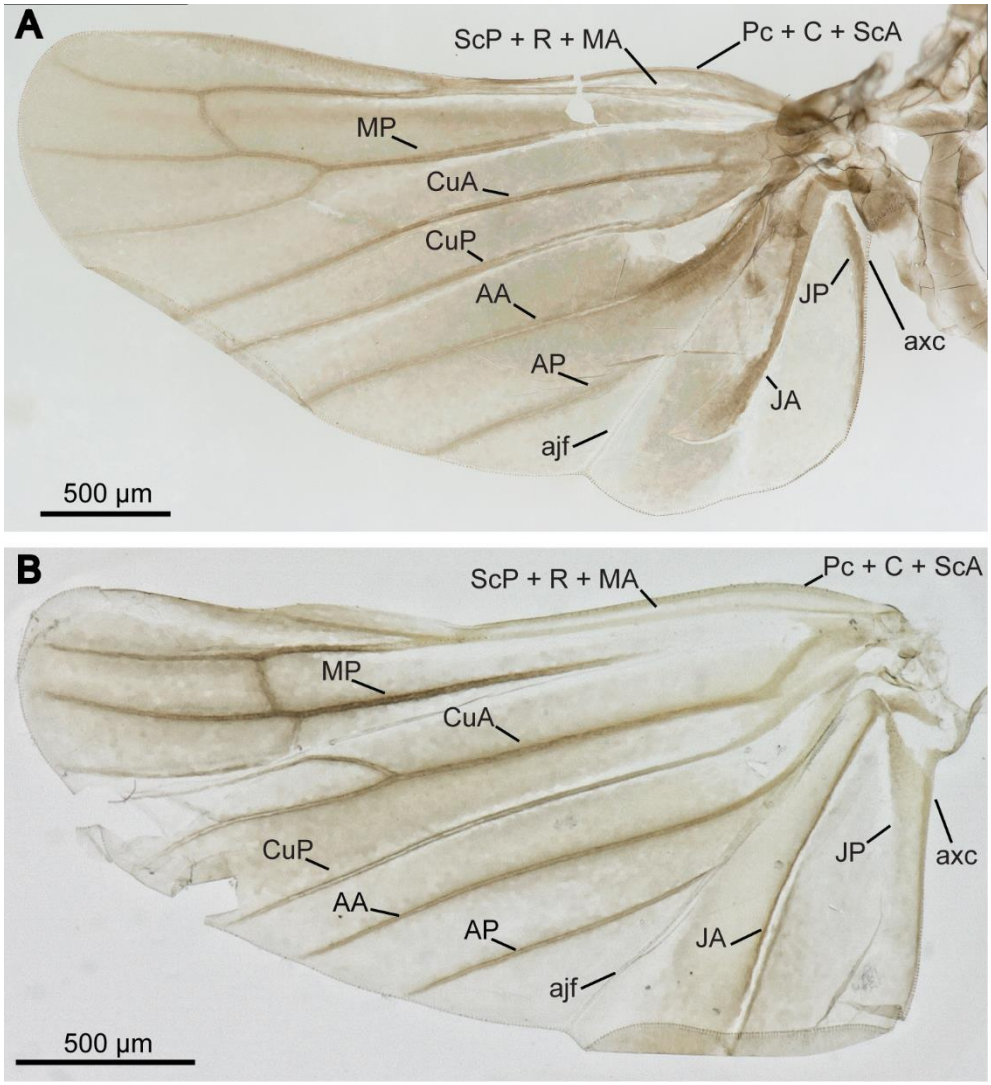

Fig. 2. Metathoracic wing morphology of Cedusinae and Cenchreini (slide-mounted, dorsal view). A: *Malenia bosnica* (Cedusinae), AA, Anterior Anal; ajf, anojugal fold; AP, Posterior Anal; axc, axillary cord; C, Costa; CuA, Anterior Cubitus; CuP, Posterior Cubitus; JA, Anterior Jugal; JP, Posterior Jugal; MA, Anterior Media; MP, Posterior Media; Pc, Postcubitus; R, Radius; ScA, Anterior Subcosta; ScP, Posterior Subcosta; B: *Omolicna joi* (Cenchreini). Image link.

Exoskeletal components defining a snapping organ such as a Y-lobe and ridge (rg) are present in *Nesorhamma badia* (Muir, 1927) (Table S1) and *Omolicna joi* (Fig. 1B), very similar to those of Cedusinae (Fig. 1A). Structural details of the first three abdominal segments could not be observed in all specimens, but tergum 3 lacks distinct setae on its surface in most Cenchreini (Table S1).

The metathoracic wing lacks any structure identifiable as a stridulitrum in most species (Fig. 2B; Table S1), JA is present and JP forms a pigmented area at the jugal margin (Fig. 2B), as in Cedusinae (exceptions mentioned in section 3.3.1).

3.3. Abdominal organs and metathoracic wing structures in Derbidae (excluding Cedusinae, Cenchreini and part of Derbini)

3.3.1. Metathoracic wing stridulitrum

The axillary cord in the jugal area of the metathoracic wing bears in its posterior margin a thickened structure, which we refer to anatomically as a stridulitrum (str; Figs. 3A, 5A–C, 6A, B, D), without prejudice to its function. The stridulitrum is composed of multiple transverse elevated areas termed stridulitral lines (stl), each line succeeded by a deep stridulitral depression (std; Figs. 3B, C, 4A, 5A, B). The number of stridulitral lines per stridulitrum ranges from 16 to 40, with smaller species generally having a higher number of stridulitral lines (e.g. *Synavea* spp., 35–40 lines) than larger species (e.g. *Phenice* spp., 20 lines). The surface of both the stridulitral lines and their depressions appears membranous and deflated in critical-point dried specimens (Fig. 3B), and is covered with a distinctive microsculpture, composed of thousands of small (<1 μm) pores (po; Fig. 3C, D). Given that the surface of the remainder of the axillary cord is covered by a different microsculpture (Fig. 3F), and that the stridulitrum is morphologically continuous with the latter, it is likely that the stridulitrum represents a modified part of the axillary cord.

Both proximal and distal ends of the stridulitrum are continuous with the axillary cord, and SR-μCT scans show a large lumen (l1) in cross sections of the organ (Fig. 4C, D). Given that the axillary cord transfers haemolymph towards the scutellum (Kukalová -Peck, 1983) it is plausible that haemolymph flows through this lumen l1. The stridulitrum in cross-section is composed of optically dense material (dm; light colour in 4C) of uncertain origin,

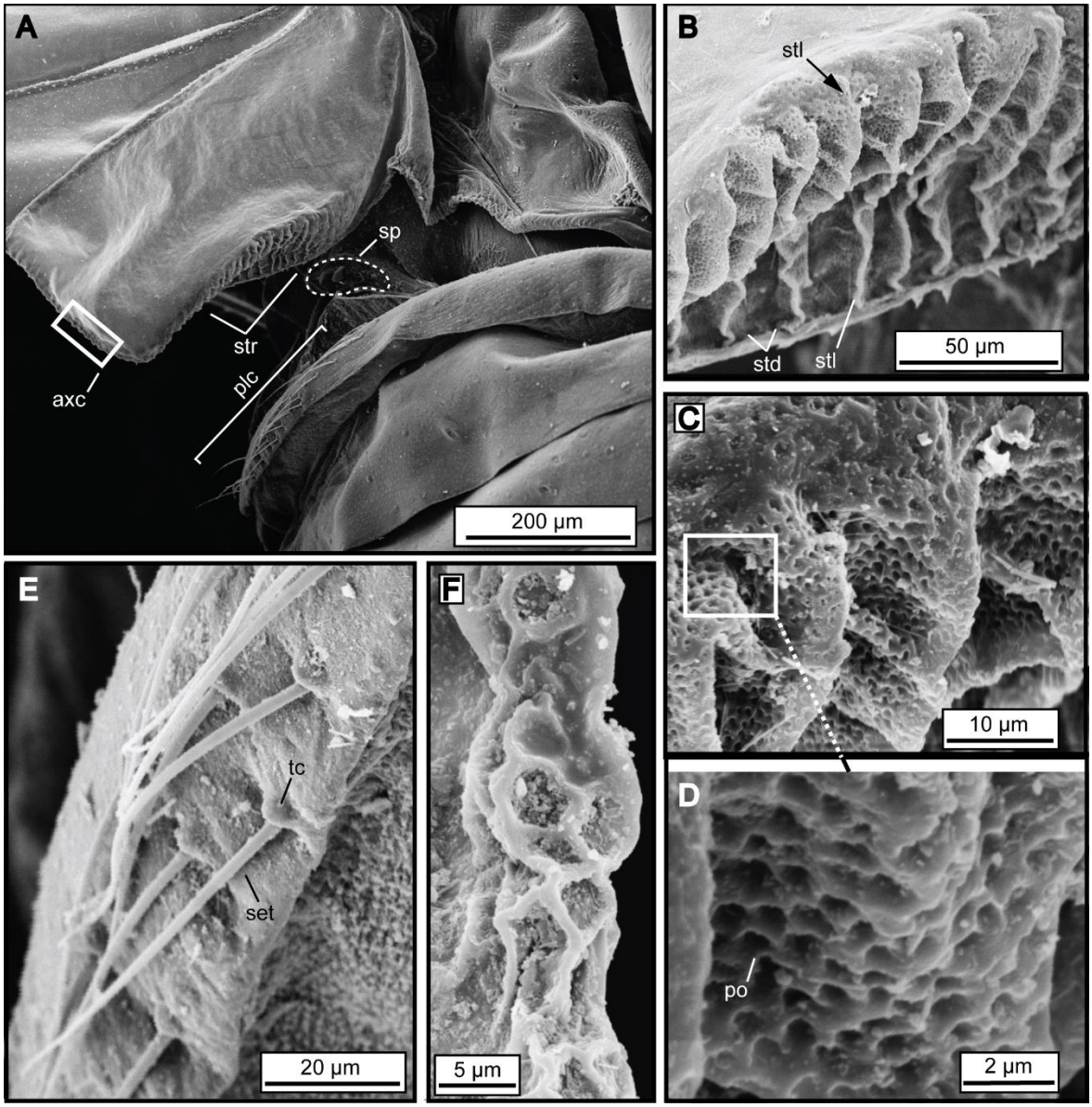

Fig. 3. Morphology and ultrastructure of stridulatory mechanism in *Zoraida* sp.1 (SEM). A: Dorso-caudal view of mechanism, white dashed line indicates outline of spiracle 1, axc, axillary cord; plc, plectrum; sp, spiracle; str, stridulitrum; B: Hind wing stridulitrum, std, stridulitral depression; stl, stridulitral line; C: Higher magnification view of stridulitrum; D: Enlarged view of white inset of previous panel, showing the porous surface of the stridulitrum, po, pore of stridulitrum; E: Surface of plectrum (tergum three) (note that hairs are artificially appressed in CPD-treated specimens), set, seta; tc, seta-bearing tubercle; F: Enlarged view of white inset in panel A, demonstrating the ultrastructure of the axillary cord. Image link.

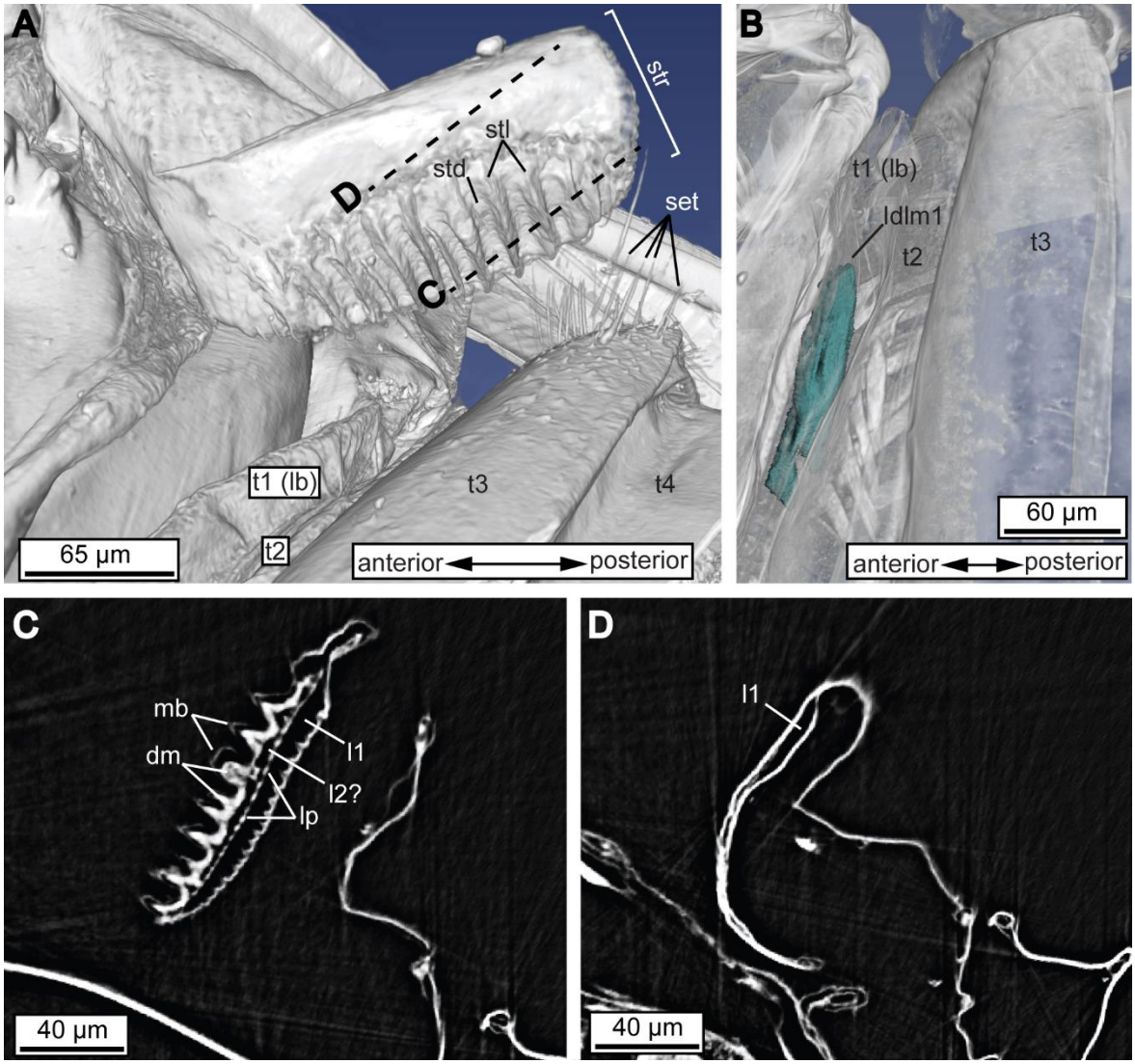

Fig. 4. SR-µCT, volume-rendered 3D reconstruction of stridulatory mechanism in *Zoraida* sp.2. A: Dorsolateral view of metathoracic wing and proximal pregenital segments, dashed lines indicating planes of section of SR-µCT tomograms in panels C and D, lb, Y-lobe of snapping organ; set, setae, std, stridulitral depression; stl, stridulitral line; str, stridulitrum; t, tergum; B: Lateral view of thorax and pregenital segments, Idlm1 (coloured green), group I dorsal longitudinal muscle of tergum 1; C: cross-section of stridulitrum, illustrating its internal morphology, dm, dense material; l, lumen; lp, loop of dense material; mb, membrane; D: Same, more anterior section. Image link.

which is not fully attached to its external membrane (mb; Fig. 4C), possibly due to poor preservation. This dense material forms a loop (lp), its ventral portion being much thinner

than its dorsal counterpart (Fig. 4C). The loop may possibly form a secondary lumen (labelled l2 in Fig. 4C). The outer surface of the stridulitrum is strongly rugose, and the many fine stridulitral lines are clearly visible in the SR-μCT scans (Fig. 4A).

The size and thickness of the stridulitrum is highly variable across taxa. The stridulitrum may be short (e.g. most Rhotanini; Table S1) or very long, occupying the entire length of the jugal lobe (Sikaianini, Zoraidini; Figs. 3A, 4A, 5B and 6C, D). It may be situated close (Otiocerini) or far (Rhotanini, Phenicini) from the wing base, depending on the size of the jugal lobe and the stridulitrum (Table S1). The stridulitrum may be very thin, appearing as a small, thickened strip of the axillary cord (some Otiocerinae; Table S1), moderately thick in others (e.g. Phenicini) or strongly expanded, occupying most of the jugal lobe (Kamendakini, Sikaianini, Zoraidini; Figs. 3A, 4A, 5B and 6C, D). The outer surface of the stridulitrum is generally concave, although in Aquaeliciini, Neocyclokarini, Phenicini, Sikaianini, and Zoraidini, it is straight and slightly convex (Table S1).

The metathoracic wing of non-Cedusinae derbids also varies in terms of wing venation, the shape of the jugal area, and the morphology of the stridulitrum. In the majority of derbids [part of Otiocerinae: Otiocerini, Kamendakini, most Rhotanini and some Cenchreini (Derbinae): *Dawnaria*, *Muirileguatia*, *Aethocauda*], the jugal area is very large and both JA and JP are present (Table S1). In others, primarily in the Otiocerinae [Phenicini, part of Rhotanini (*Alara*), all Sikaianini and Zoraidini] and part of Derbinae [Dawnarioidini (*Neodawnaria*), Derbini (*Dysimia*, *Symidia*)], the jugal area is reduced to a small quadrangular lobe which bears the stridulitrum, and vein JA is absent (Fig. 5A, B). Among taxa possessing this metathoracic wing type, JP may be short and thick in some species (e.g. *Alara*, *Raizoda*; Fig. 5A), or long and slender in others (most species), depending on the size of the jugal lobe and consequently its distance from the axillary sclerites (Table S1).

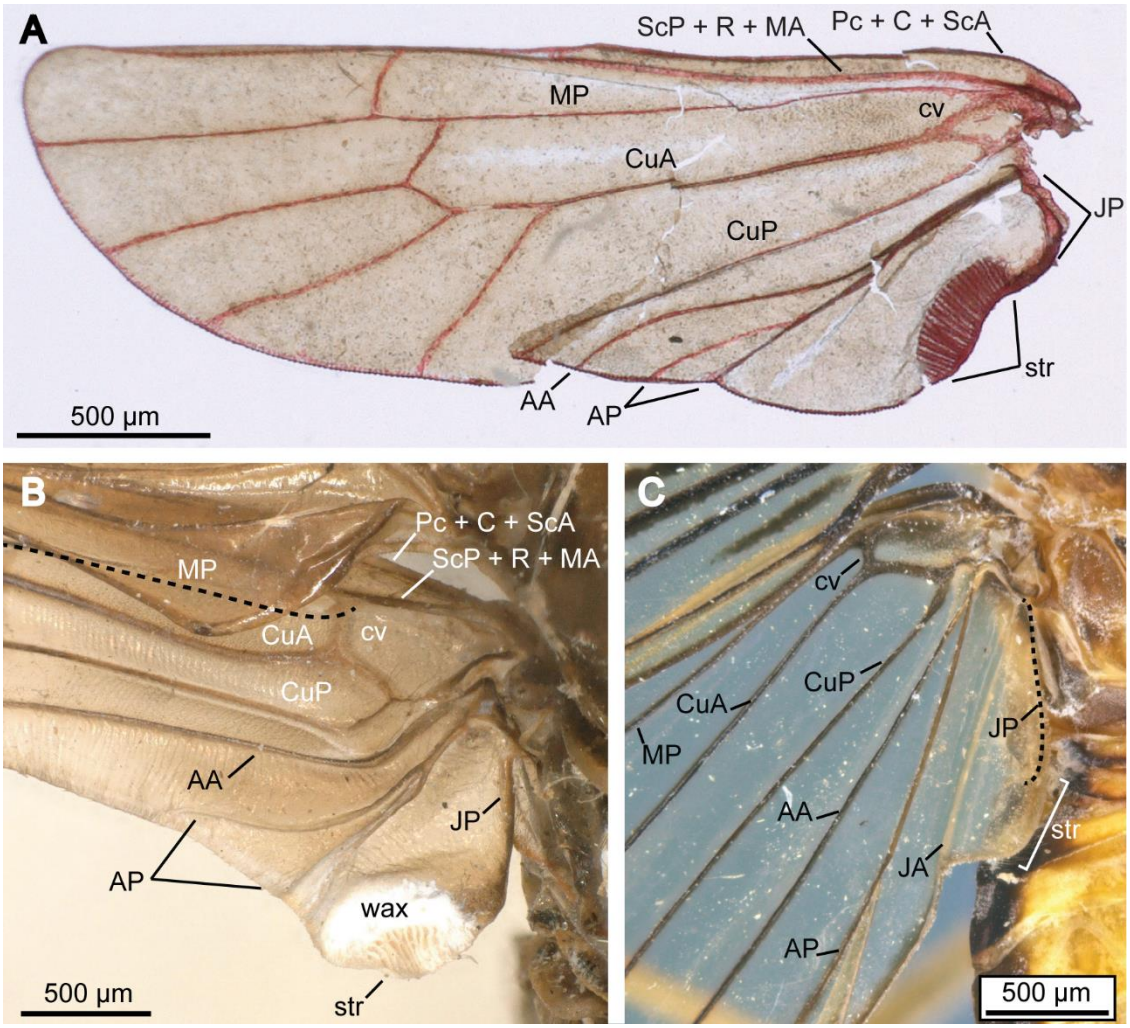

Fig. 5. Main variations of metathoracic wing stridulitrum (dorsal view). A: *Alara fumata* (Otiocerinae: Rhotanini), slide mounted, AA, Anterior Anal; AP, Posterior Anal; C, Costa; CuA, Anterior Cubitus; CuP, Posterior Cubitus; cv, cross-vein; JP, Posterior Jugal; MA, Anterior Media; MP, Posterior Media; Pc, Postcubitus; R, Radius; ScA, Anterior Subcosta; ScP, Posterior Subcosta; str, stridulitrum; B: *Zoraida pterophoroides* (Otiocerinae: Zoraidini), wax visible immediately above stridulitrum, dashed line indicates outline of MP (obscured by mesothoracic wing); C: *Derbe* sp. (Derbinae: Derbini), dashed line indicates outline of JP, JA, Anterior Jugal. Image link.

There is no evidence of sexual dimorphism in the structure of the stridulitrum in any of the examined species.

3.3.2. Structure of the first three abdominal segments

The first abdominal segment is very narrow, proximally fused to the metathorax, its distal part being membranous. At least in *Zoraida* sp. 1 and *P. mawae* (both Zoraidini), several components typical of a snapping organ are present: a pair of ridges distinctly fuse on the anterior arm of the Y-lobe, which is also present (Fig. 6A), and the principal muscle of the snapping organ (Idlm) was also observed (Fig. 6B; refer to the Musculature section below). The structure of the Y-lobe differs slightly from that of other planthoppers, in that it is dorsoventrally flattened and extremely narrow (Figs. 4A, B and 6A). Other morphological features of note, not obviously associated with the snapping organ, are the following: abdominal spiracle 1 is greatly enlarged (sp; Fig. 3A), at least 20 times larger than the remaining abdominal spiracles; segment 2 (t2) is very narrow, also largely membranous (Figs. 4A, B and 6A); both segments 1 and 2 are strongly desiccated in most dried specimens and almost impossible to observe (Table S1).

Tergum 3 is greatly expanded laterally into a broad, spatulate plate, becoming much narrower medially (Fig. 6 C, D). The posterolateral margin of tergum 3 is distinctly raised, exposing a large membrane, which links it to tergum 4. The remaining segments are not as raised and the membrane is not visible. This conformation is present in all derbid taxa examined, except for Cedusinae, Cenchreini (part) and Derbini (part). In all taxa possessing a stridulitrum, a field of erect setae (set) is invariably present on tergum three, representing the structure that would constitute the plectrum (plc) of the supposed stridulatory mechanism (Figs. 3A, E, 4A and 6 A–D; Table S1). The area covered by the setae, and their length and density are all variable: the setae may be long or short (some *Zoraida*; Table S1); they may form a dense field occupying the entirety of the first half of the abdomen (e.g. *Swezeyia*; Table S1), or confined to the posterior margin of tergum 3 (e.g. *Rhotanini*, some Zoraidini; Fig. 7C; Table S1). In others (e.g. *Phenice*), they may be concentrated in only a small area of tergum 3 (Table S1). The setae are usually densest towards the base of tergum 3, gradually

narrowing into only a few rows of sparse setae towards the dorsal part of tergum 3 (Fig. 7C; Table S1). Among dried specimens, it is not unusual for the setae to be clumped together by the crystallised remnants of a presumably viscous secretion, or to be scraped off entirely (Table S1).

Distinctly raised tubercles (tc) form the bases for these setae (Figs. 3E, 7 A–C) and in at least one species two additional ultrastructures can be observed at high magnification: sparse, very short setae (sst) which insert in less raised tubercles, and conical, scale-like tubercles (sct) lining the posterior margin of tergum three (Fig. 7A, B). The remaining abdominal segments are typically glabrous (Fig. 3A).

We did not observe sexual dimorphism in the morphology of tergum 3 in any of the examined derbids.

Musculature: Largely not preserved in SR-μCT scans, although the main snapping organ muscle Idlm1 is distinct (Fig. 4B): this is the largest muscle of the pregenital abdomen, Origin – apodeme on metaphragma; Insertion – apodeme on antecosta of tergum two; Function – principal muscle of snapping organ mechanism (Davranoglou et al., 2019).

3.4. Abdominal organs and metathoracic wing structures in the rest of Derbini

3.4.1. Metathoracic wing stridulitrum

The metathoracic wing in part of Derbina (*Derbe*) and Mysidiina (*Mysidia*, *Pseudomysidia*) is characterised by a smaller jugal lobe, the presence of JA and a modified stridulitrum (Figs. 5C, 8A–C; Table S1). The stridulitrum of most Derbini is extremely reduced and narrow, appearing as a small, thickened flap of the axillary cord (Figs. 5C and 8A–C; Table S1). The typical components of the stridulitrum which are otherwise found in most other derbids (stridulitral lines and depressions) are not present, and the stridulitral surface is instead rather flat and covered with short cuticular spines (spn; Fig. 8B, C).

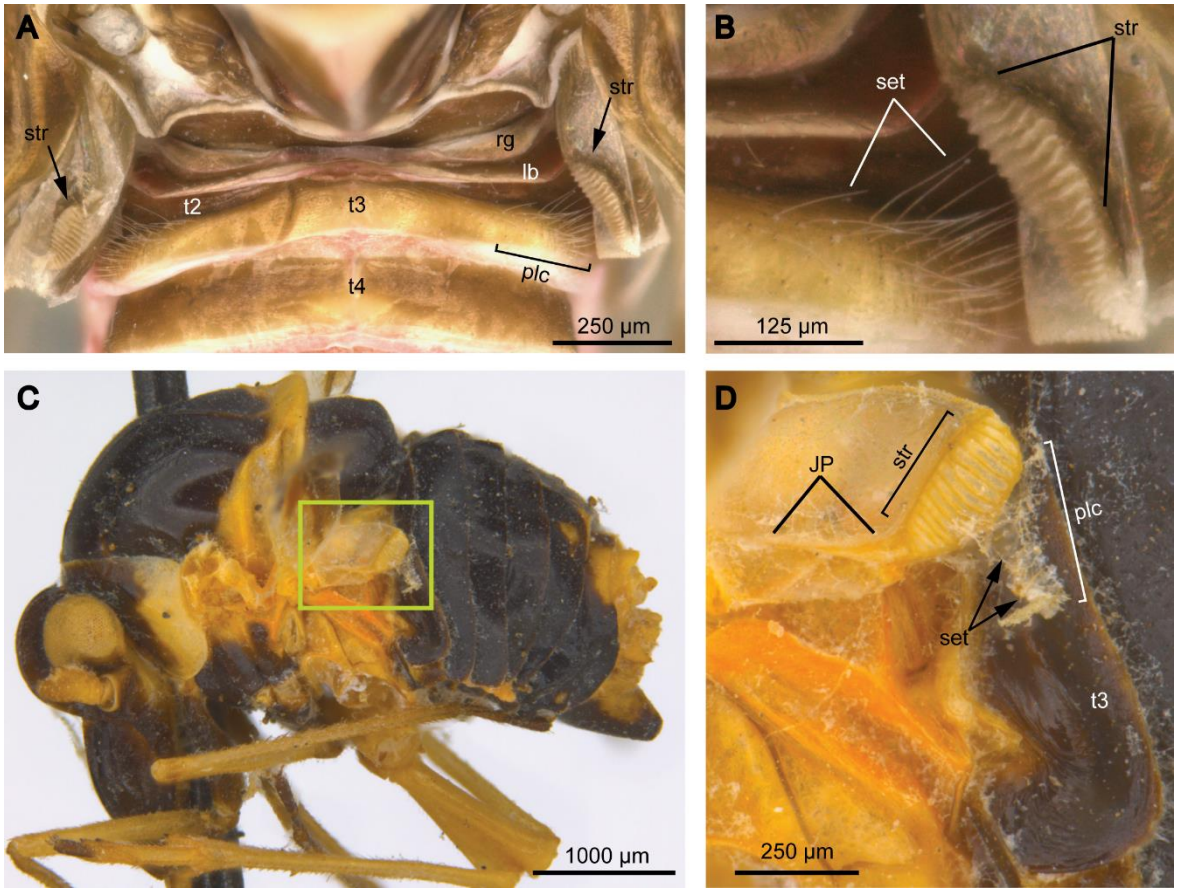

Fig. 6. Different views of stridulitrum-plectrum interactions at various stages of contraction (dry-pinned specimens). A: *Paraphenice mawae*, dorsal view, with snapping organ Y-lobe arms snapped shut, lb, Y-lobe of snapping organ; plc, plectrum; rg, ridge of snapping organ; str, stridulitrum; t, tergum; B: same, higher magnification of stridulitrum-plectrum, set, setae; C: *Proutista* sp., lateral view, yellow inset marking position of the stridulatory mechanism; D: Same, higher magnification view of yellow inset in previous panel, the stridulitrum making contact with the plectrum, JA, Posterior Jugal. Image link.

3.4.2. Structure of the first three abdominal segments

The first two abdominal segments display the typical modifications of a snapping organ (in *D. strigipennis* Westwood, 1840; Table S1): a ridge is present, dorsally obscuring the Y-lobe arms. Tergum 2 is unmodified. Tergum 3 is not distinctly convex or broader than tergum 2, and possesses a small field of sparse, short or long plectral hairs, at the narrow

contact zone between stridulitrum and plectrum in Derbina (*Derbe*) and at least some taxa of Mysidiina (*Dysimia*, *Pseudomysidia*, *Symidia*; Table S1).

3.5. Systematic distribution of the plectrum and stridulitrum

The supposed stridulatory mechanism is common but not ubiquitous among derbids (Table S1). It is absent in all examined taxa of Cedusinae. Among the Derbinae, it is lacking in most Cenchreini we examined (Fig. 2B), with the exceptions of *Aethocauda rubella* Williams, 1978, *Dawnaria atroterminata* (Distant, 1911) and *Muirileguatia fernandesi* (Muir, 1925) (Table S1). The mechanism is absent or strongly reduced in most, but not all Mysidiina, and part of Dawnarioidini [*Dawnarioides sordidulus* (Muir, 1918)] (Table S1). Within Otiocerinae, it is absent from all examined Nicertina (Table S1).

3.6. Interaction of the plectrum and stridulitrum

The stridulitrum and plectrum are positioned in a way that maximises their contact when the metathoracic wings move by a sudden jerk of the mesothoracic wings (Kirkaldy, 1907; Fig. 6C) which are functionally and morphologically connected with the former. The stridulitrum in each species has a size that correlates with the width and position of the plectrum, and the two structures can be seen in varying degrees of interaction in dry-mounted specimens (6A–D). In addition, in several taxa with smaller or more compact stridulitra (e.g. *Dawnaria*, *Derbe*), the plectrum is confined to the small area where the two structures can come into contact in a precise manner (Table S1). All taxa lacking a stridulitrum also lack a plectrum (Figs. 1A–B and 2A–B; Table S1), further supporting a functional correlation between the two structures.

The deflated appearance of the stridulitrum in dried specimens (Fig. 3B) suggests that in living specimens, the membranes may be more voluminous (possibly eversible?) and filled

with haemolymph. The JP probably acts as a strengthening bar and adds structural support to the stridulitrum when it contacts the abdomen (Fig. 6C, D).

3.7. Systematic distribution of snapping organs in Derbidae

Snapping organs were found in all examined Cedusinae (Table S1; Fig. 1A), most Cenchreini (Table S1; Fig. 1B), some Derbina, Nicertina, Phenicini (Table S1) and Zoraidini (Fig. 4A, B). We were unable to observe the structure of the first two abdominal segments (which contain the snapping organ) in most dry-mounted species that we examined (Table S1), as their membranous nature caused them to shrivel significantly, obscuring any view of their morphology. However, the presence of the snapping organ in these major derbid tribes suggests that it is widespread in the family.

3.8. Tergal glands

We observed two pairs of large glands (gl) on the dorsolateral surface of tergum 6, and one pair on tergum 7 in *Zoraida* sp. 1 & sp. 2. The glands possess characteristic crescent-shaped grooves (gr; Fig. 9B–D), with a median slit-like ostiole (o; Fig. 9C, D). The internal structure of the glands could not be reconstructed in the SR-μCT scans. These glands are also present in the same locations in larval Zoraidini and exude long, thick filaments of wax (Fig. 10A, B). The larval glands form distinctly raised tubercles (Fig. 10A, B), whereas the adult counterparts are flattened (Fig. 9A–D).

The condition of the examined Zoraidini is not universal among derbids, as some Cedusinae, Cenchreini, Otiocerini and Rhotanini may possess only one gland on tergum 7, and *Basileocaphalus germanus* Yang & Wu, 1993 (Cenchreini) is unique in that tergites 5-7 each possess a single tergal gland (Yang & Yeh, 1994). Given that all known larval Zoraidini possess the same number of tergal wax glands (Yang & Yeh, 1994) with the adults of the

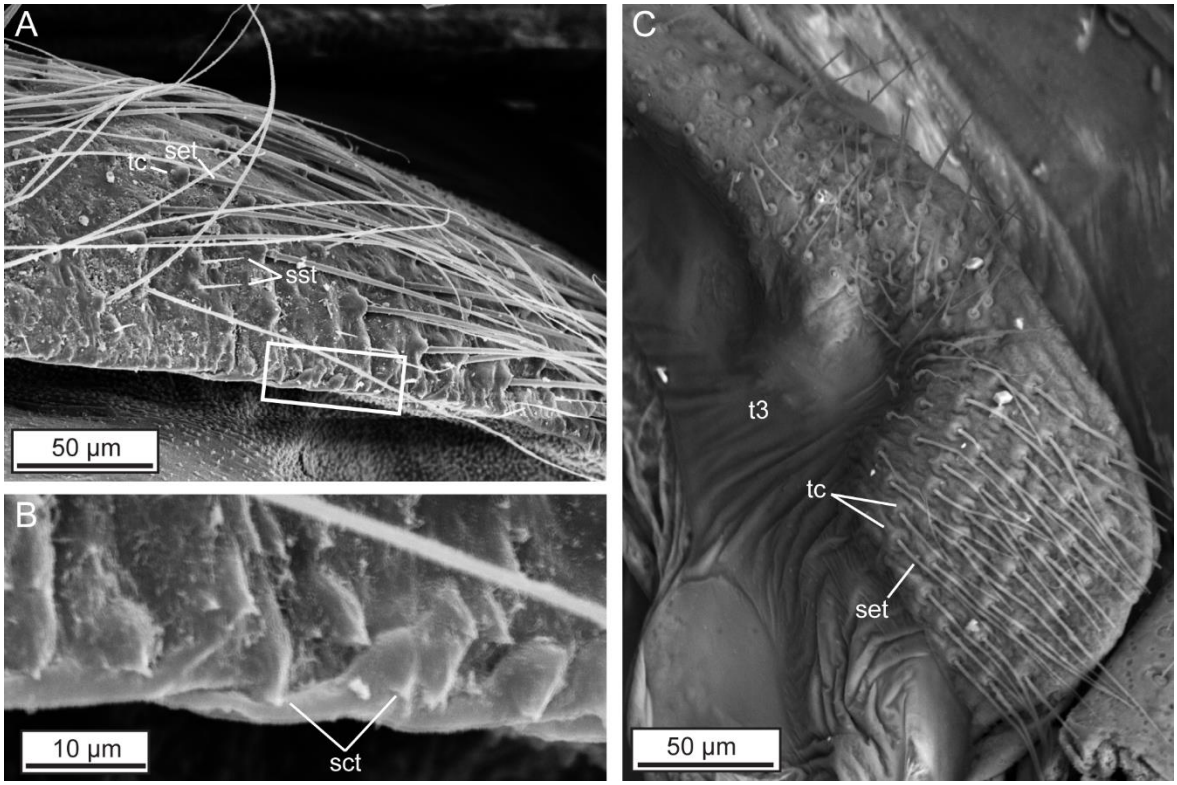

Fig. 7. Surface ultrastructure of plectrum (tergum 3) in two derbid species (SEM; hairs artificially appressed in CPD-treated specimens). A: *Zoraida* sp.1 (Otiocerinae: Zoraidinae) posterior view of posterior margin of tergum 3, set, seta; sst, short seta; tc, seta-bearing tubercle; B: Enlarged view of white inset of previous panel, sct, scale-like tubercle; C: *Kaha* sp. (Otiocerinae: Otiocerini), lateral view of tergum three, t, tergum. Image link.

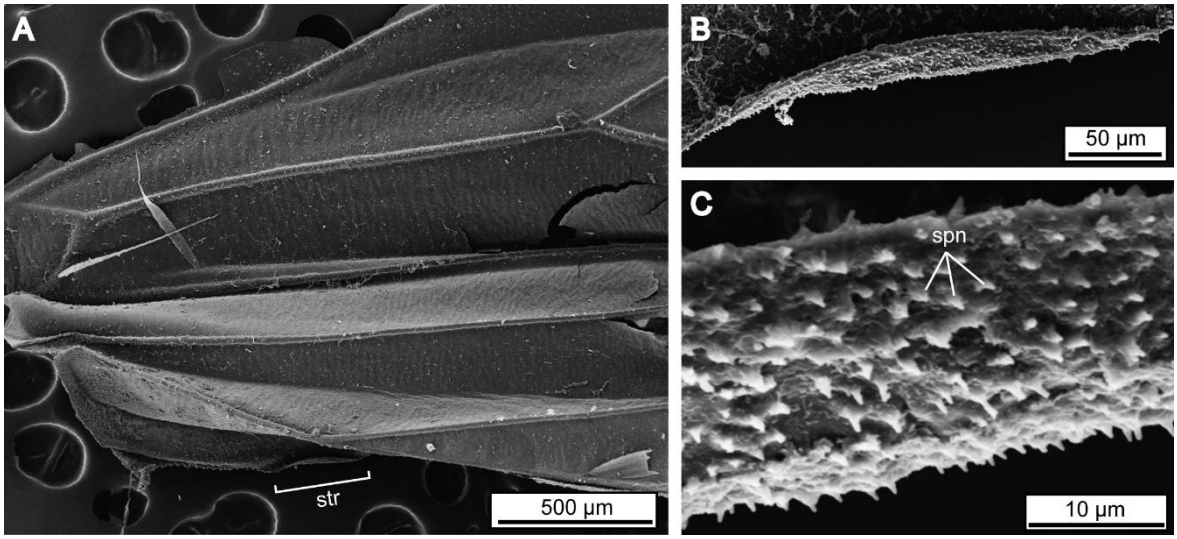

Fig. 8. External morphology and ultrastructure of stridulitrum of *Mysidia* sp. (SEM). A: Metathoracic wing, dorsal view, str, stridulitrum; B: Higher magnification of stridulitrum; C: Surface ultrastructure of stridulitrum, which is covered in spines, spn, spines. Image link.

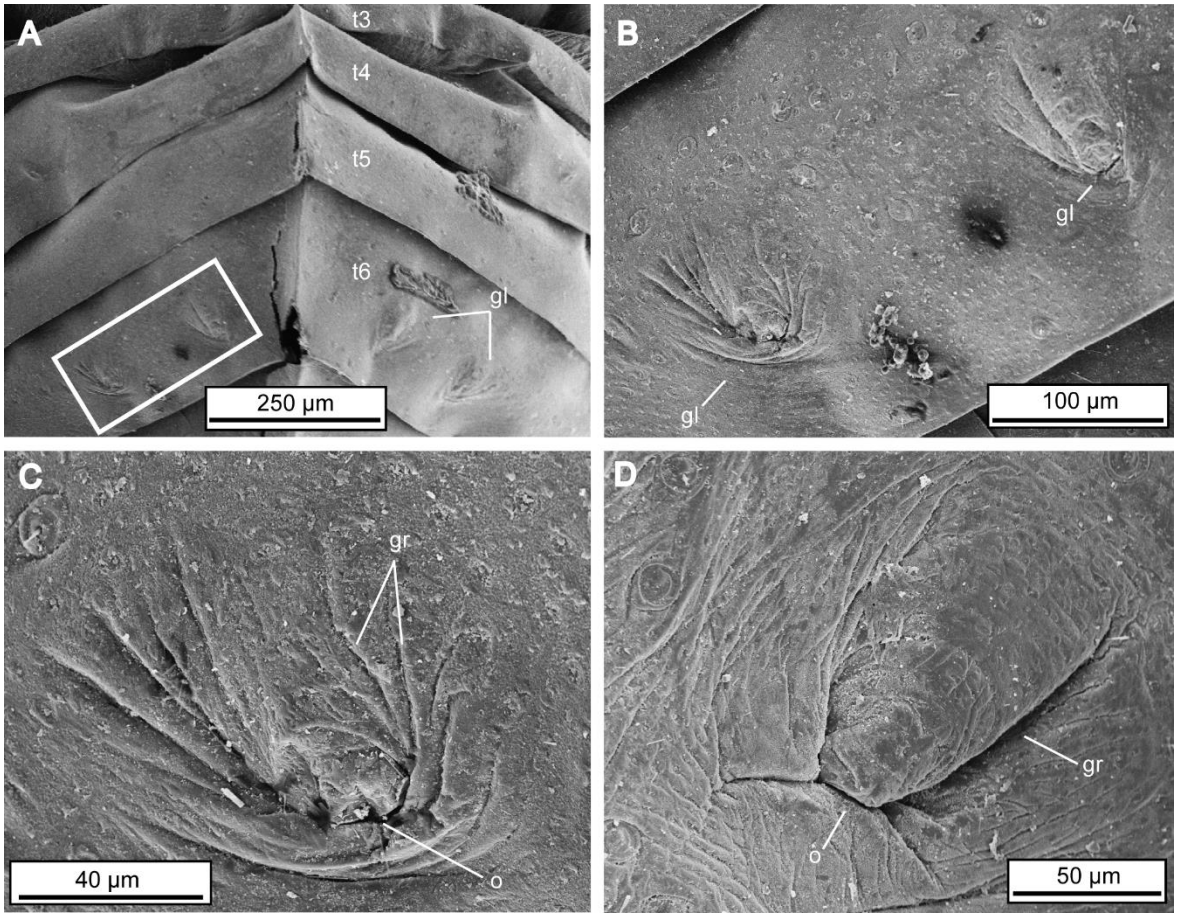

Fig. 9. External morphology of derbid tergal glands (SEM). A: *Zoraida* sp.1 (Otiocerinae: Zoraidinae), abdomen, caudal view, demonstrating the position of tergal glands, gl, tergal gland; t, tergum; B: Same, enlarged view of white inset in previous panel; C: Same, higher magnification view of tergal gland, illustrating its detailed morphology, gr, groove of tergal gland; o, ostiole of tergal gland; D: *Zoraida* sp.2, tergal gland, demonstrating interspecific variation in the morphology of this structure. Image link.

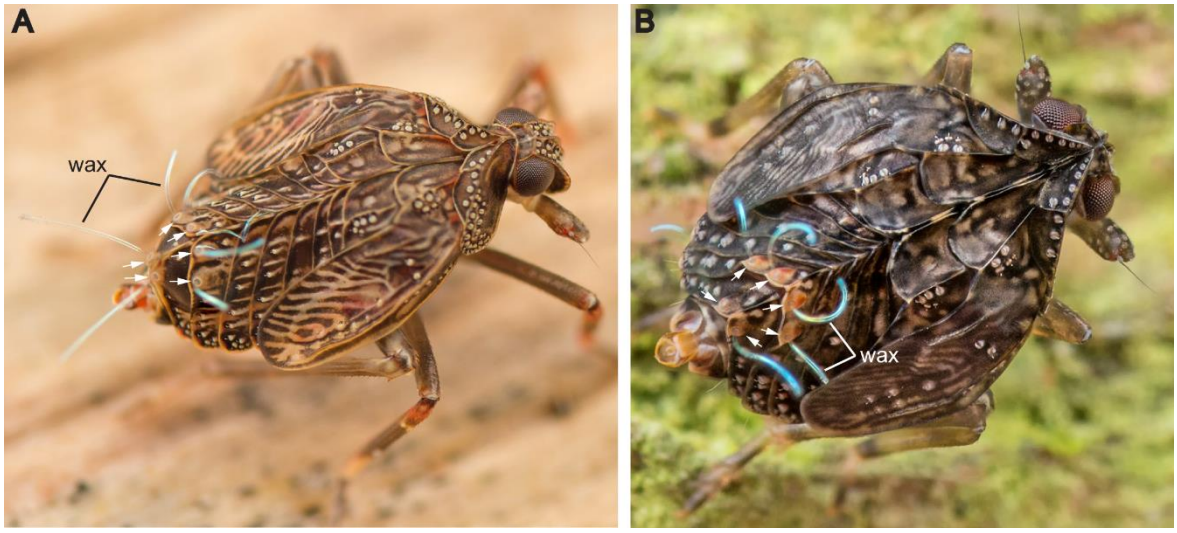

Fig. 10. Wax filaments produced by abdominal tergal glands in larval derbids. A: *Zoraida* sp., white arrows indicating position of abdominal tergal glands; B: *Pamendanga* sp. Reproduced by kind permission of the photographer Nicky Bay. Image link.

two examined species (Fig. 9), it seems that at least in these taxa, the number of tergal glands does not change during the larva to adult transition.

4. Discussion

4.1. Is a distinction between snapping organs and tymbals justified?

Recently, Hoch et al. (2019) published a criticism of our latest discovery of the snapping organ, a vibrational organ found throughout Fulgoromopha (Davranoglou et al., 2019). This critique did not focus on the soundness of our research, but mostly on whether a distinction between tymbalian tymbals of Wessel et al. (2014) and snapping organs was justified (Hoch et al., 2019). We briefly respond here to some of the comments relevant to this study.

The focus of Davranoglou et al. (2019) was to describe the biomechanics, morphology and systematic distribution of the snapping organ, and not its homologies with other hemipteran vibroacoustic organs. In Davranoglou et al. (2019) and the current study, we have adopted mechanistic definitions for distinguishing between snapping organs, tymbals (Young &

Bennet-Clark, 1995) and heteropteran tergal plates (Jordan, 1958), due to their experimentally tested biomechanical differences. Furthermore, we believe that the mechanical distinction between tymbals and snapping organs is warranted, as the latter do not involve vibrating or clicking sclerites as defined in Wessel et al. (2014): instead of buckling like the membrane and ribs (if present) of a tymbal, the arms of the Y-lobe in the snapping organ use a fundamentally different snapping motion (Davranoglou et al., 2019).

We adhere to biomechanical definitions, as the concept of a tymbalian tymbal of Wessel et al. (2014) remains an untested hypothesis, with a number of causes for concern. Previous research of ours (Davranoglou et al., 2017; Davranoglou et al., 2019), showed that based on innervation and exoskeletal structure, the segmental identity of muscles (Iadvm1+2, IIIvlm) used in the definition of tymbalian tymbal organs was misidentified. Furthermore, previous studies (Kramer, 1950; Snodgrass, 1933) were congruent with our interpretations on the segmental identity of auchenorrhynchan sterna (S1 Text in Davranoglou et al., 2019), and not with those of Ossiannilsson (1949) and Weber (1928), on which Wessel et al. (2014) were based upon. Position is one of the main criteria of homology (Patterson, 1982), and thus confusion over segmental identity between different hemipteran groups cast doubt on the accuracy of the homologies proposed by Wessel et al. (2014).

Based on the above, we believe that the homologies between hemipteran vibroacoustic organs are far from resolved, and we would therefore be reluctant to assume homology of all these organs prior to a more detailed examination of the abdominal morphology of additional hemipteran taxa. We therefore suggest that the biomechanical and morphological distinctions between tymbals, snapping organs and tergal plates should be maintained until more evidence is available. An extensive response to the comments by Hoch et al. (2019) is underway (Davranoglou et al., in preparation).

4.2. Vibrational communication in Cedusinae and other derbids

Tishechkin (2003) recorded vibrational signals from *Cedusa sarmatica* (Anufriev, 1966), which he attributed to tymbal organs yet to be identified. However, the published oscillograms of *C. sarmatica* (Tishechkin, 2003) as well as two other *Cedusa* spp. (Tishechkin, 2008) have a structure comprising a prolonged succession of syllables, sometimes paired, that is characteristic of other planthopper signals that have since been shown to be produced by the snapping organ (Davranoglou et al., 2019). In addition, our examination of the external morphology of the abdomen of Cedusinae failed to find any tymbal-like organ (i.e. comprising of buckling membranes with or without buckling ribs) (Fig. 1A), yet a snapping organ was invariably present (Fig.1A, Table S1). This suggests that the snapping organ of Cedusinae (Fig. 1A), at least, is fully functional and responsible for producing their recorded vibrations. Although there are to our knowledge no recordings of vibrational signals in any other derbid subfamily, the presence of components defining a snapping organ (ridge, Y-lobe) and its principal muscle, the hypertrophied Idlm1, in species of Cenchreini (Derbinae, Table S1) and Zoraidinae (Fig. 4B; Table S1), suggests that the snapping organ may be capable of generating vibrations in these subfamilies as well.

4.3. Taxonomic and systematic implications

The internal phylogenetic relationships of Derbidae are poorly understood. The only comprehensive phylogenetic hypothesis is that of Emeljanov (1996), based on morphology. Although this served as a basis for the current classification of the family and remains a very influential work, it was not derived through cladistic methods, which would properly test the homology of the individual characters used by Emeljanov (1996) to define higher taxa, their monophyly, and sister-group relationships. As a result, we can make only limited inferences on the evolutionary implications of our findings.

The absence of a putative stridulitrum or plectrum in Cedusinae and part of Cenchreini (Fig. 2; Table S1) and the similarity in their metathoracic wing venation are treated here as possible symplesiomorphies shared with other planthoppers. Indeed, Cedusinae and part of Cenchreini may be closely related (Fennah, 1952) and the former have been considered the sister group to all other Derbidae (Emeljanov, 1996). This may suggest that the placement of Cenchreini within Derbinae or Otiocerinae as the sister group to Nicertina (Emeljanov, 1996) is artificial. The presence of a stridulitrum and plectrum in three genera (*Aethocauda*, *Dawnaria*, *Muirileguatia*) of Cenchreini (Table S1), which is similar to that of Otiocerinae, suggests that these taxa may be taxonomically misplaced. This being so, either the tribe as currently defined may be polyphyletic, or it may contain the same stridulatory structures as other derbids evolved independently, which we consider less parsimonious.

Characters present in the remaining Derbidae, such as the external reduction of the snapping organ (covered almost entirely by the ridge; Fig. 6A, B), the further enlargement of abdominal spiracle one and the presence of the stridulitrum and plectrum could represent possible synapomorphies that unite all derbids excluding Cedusinae and Cenchreini. The significant reduction of the jugal area and its modification into an expanded quadrangular lobe in Sikaianini and Zoraidini (Figs. 3A, 4A, 5B, 6C, D) may indicate a close relationship between the two tribes, as suggested by Emeljanov (1996).

The unambiguous presence of the stridulitrum and plectrum in several taxa of Derbinae (Figs. 5C and 8A–C) contradicts Emeljanov (1996) and Banaszkiewicz & Szwedo (2005), who key the subfamily as lacking this trait. At present, we cannot determine whether these supposed stridulatory structures of Derbini are homologous to those of other derbids. The morphology of the stridulitrum in some taxa (*Dysimia*, *Symidia*) is very similar to that of Otiocerinae (Table S1), while in *Derbe* spp. and some *Mysidia* spp. the stridulitrum is extremely small, narrow and has unique ultrastructure found in no other derbid group (Fig.

8A–C). Other Derbini (e.g. some *Mysidia* spp.) lack the mechanism altogether (Table S1). Given that Derbini are a well-defined group (Broomfield, 1985), these differences may stem from varying degrees of reduction of the stridulatory mechanism, or due to convergent evolution.

Our extensive microscopic examination of nearly 200 species revealed considerable variation in the components of the supposed stridulatory organ among different groups of derbids (Table S1). We are confident that detailed examination of both macro-and-microscopic features of the derbid stridulatory organ will reveal many taxonomically and systematically useful characters, and we recommend their use in future studies on this group of insects. It is likely that the unbalanced systematic distribution of many of these characters may challenge our current understanding of derbid subfamilies, tribes and subtribes. An in-depth phylogenetic analysis including the details of the supposed stridulatory mechanism and other morphological and molecular characters is needed.

4.4. Evolution of supposed stridulatory organs in Derbidae

Due to insufficient knowledge of derbid phylogeny, not much can be deduced regarding the evolutionary trajectory of derbid stridulatory organs. Based on our current work, we suggest the following preliminary conclusions: 1) Cedusinae and Cenchreini communicate primarily via surface vibrations, using a snapping organ, which is thought to represent the ancestral condition in planthoppers as a whole (Davranoglou et al., 2019); 2) in all other derbids, an area of the axillary cord expands and becomes morphologically modified, forming the stridulitrum (this process perhaps taking place more than once); based on the current tribal classification, this supposed stridulatory organ was secondarily lost at least three times independently (Nicertina, part of Dawnarioidini and Mysidiina).

4.5. Is the stridulatory mechanism actually involved in sound production?

There is sufficient morphological (e.g. Fig. 6) and behavioural (Kirkaldy, 1907) evidence to conclude that a mechanical interaction between the stridulitrum and the plectral hairs takes place, but whether and how this could produce loud acoustic sound remains enigmatic. Although the stridulitrum is somewhat more rigid than the surrounding wing surface and receives structural support from the JP (Fig. 6C, D), it is still membranous, lacks sclerotisation in any part, and has a porous surface (Fig. 3C, D) unlike the typical file-like texture characterising most known stridulitra (e.g. Jansson, 1972; Čokl et al., 2006). Moreover, its assumed flexibility would make the stridulitrum unsuitable for generating the normal force and friction force necessary for stridulation involving a scraper and file. Concerning the supposed plectrum, its structure is also atypical compared to other known plectra. Setae forming part of a plectrum are not unknown, but they are usually much shorter, robust and strongly sclerotized (Jansson, 1972). The soft, easily removable plectral hairs of derbids (Table S1) do not appear to offer sufficient resistance against strikes by the stridulitrum – it is thus unlikely that they would produce appreciable sound in this way.

4.5.1. A role in chemical communication?

In light of these observations, we suggest that the stridulitrum and plectrum may serve in the spreading of a chemical secretion (e.g. a pheromone) rather than in the production of sound, although this hypothesis should be treated as speculative in the absence of observations on live derbids. We base our hypothesis on a remarkably similar mechanism which exists in the tephritid fly *Bactrocera cucurbitae* (Coquillett, 1849) and its relatives (Kuba & Sokei, 1988), which was also initially thought to be a stridulatory organ (Monro, 1953; Kanmiya, 1988). In *Bactrocera*, the anal vein of the male mesothoracic wing is characterised by distinct microtrichia, opposed by long, erect hairs on the posterior margin of tergum 3, in a position identical to that of the derbid plectrum. When the male fly signals to females, it collects a chemical secretion from its anus with its hind legs, spreads it on the

modified area of the metathoracic wing, and then rapidly strikes the latter against the tergal hairs, creating a "pheromone cloud" (Kuba & Sokei, 1988). Our observation of clumped plectral hairs, covered by crystallised secretions in several dried derbids (Fig. 6D; Table S1), was also found in male tephritids (Kanmiya, 1988). This may support our speculative hypothesis that the organ may actually play a role in chemical signalling, although we cannot exclude that the "crystals" in derbids are minute wax particles. Furthermore, a role of wing-flicking in pheromone dissemination has also been suggested in crickets as well (Heinzel & Dambach, 1987; Kämper & Dambach, 1979), and may have formed the behavioural-preadaptation necessary for the evolution of infrasound stridulation in these insects. Other morphological features of derbids, such as elaborate, sexually dimorphic antennae and antennal appendages (Emeljanov, 1996; Bourgoin & Yap, 2010; Bartlett et al., 2014) may also indicate the presence of chemical signalling in this family, which remains unconfirmed from all Auchenorrhyncha. If our hypothesis is confirmed by future experimental studies, it may suggest that derbids use bimodal signalling, involving chemical signals produced by certain as yet undiscovered glands, perhaps distributed by the mechanical interaction of the plectrum and stridulitrum, and vibrational signals produced using the abdominal snapping organ.

4.5.2. Possible dissemination of wax and the role of tergal glands

Another possible function of the derbid metathoracic wing-abdomen mechanism is the production and dissemination of wax, which sometimes covers the bodies of derbid planthoppers. Support for this hypothesis stems from the porous surface of the stridulitrum which resembles the wax glands found on the abdomen of some non-derbid planthoppers, particularly the Cixiidae (Holzinger 2002; Holzinger et al., 2002).

However, this similarity may be superficial, as the pores of the stridulitrum are much smaller (<1 μm) compared to the abdominal wax glands of Cixiidae and Flatidae (ca. 2 μm; Sforza et al., 1999; Holzinger, 2002; Lucchi & Mazzoni, 2004), their shape is more irregular and they seem to be less deeply impressed on the cuticle, implying that there is no underlying secretory unit. It is thus unlikely that the stridulitrum itself secretes wax, although this does not exclude it from being involved in its dissemination from another source.

However, 1) there is no apparent correlation between the possession of the stridulatory mechanism and wax – among ca. 55 species photographed in the wild, the abdomen and stridulitra of only six species were at least partly covered by wax (Bay, 2019); 2) species with reduced or absent stridulitra (e.g. *Cedusa*, *Mysidia*, Nicertina) may or may not be covered with wax; 3) the stridulitrum of Derbinae, a group frequently covered by wax, lacks the distinctive porous ultrastructure and bears no resemblance to a wax gland.

Although certain authors (e.g. Hamilton, 2011) claim that derbids lack abdominal wax glands altogether, our observation of derbid larvae with obvious wax filaments secreted from the tergal glands (Fig. 10A, B) contradict this notion. The few wax filaments of larval derbids are peculiar, since in some other planthoppers, extensive fields of wax glands exude hundreds or thousands of wax filaments that may coat the entire animal (e.g. Hamilton, 2011). Their morphology is also distinctive, since in other planthoppers, the wax gland pores are much smaller, form extensive honeycomb-like aggregations and are often provided with distinct secretory tubules (e.g. Sforza et al., 1999; Holzinger, 2002; Lucchi & Mazzoni, 2004). The function of tergal wax filaments in planthopper larvae remains unknown, with untested hypotheses suggesting roles in cleaning, crypsis and defence (Hamilton, 2011).

It is unclear whether tergal glands remain functional in adult derbids, which are generally covered with a powder-like layer of wax (if at all), instead of long filaments (Bay, 2019). It

is thus possible that other, yet unknown glands are responsible for wax production in adult derbids.

4.6. Conclusions

In the present study, we documented the detailed morphology and systematic distribution of supposed vibroacoustic mechanisms of Derbidae for the first time. We find that snapping organs (Davranoglou et al., 2019) are present in many derbids (Table S1), and propose that these may be responsible for the vibrational signals of Cedusini that were previously attributed to unknown tymbal organs (Tishechkin, 2003). This being so, we expect that the various other derbids possessing a snapping organ mechanism (Table S1) will also turn out to have the capacity for generating substrate-borne vibrations. Regarding the supposed stridulatory mechanism of Muir (Kirkaldy, 1907), we offer novel morphological information on the anatomy of the stridulitrum and plectrum that will be of use to derbid systematics and taxonomy, and challenge the long-held view that this structure functions as a stridulatory organ. Instead, we hypothesise that the stridulitrum and plectrum may make contact mechanically for the purposes of spreading a chemical secretion. However, the precise function of these structures remains ambiguous in the absence of detailed behavioural observations, and experimental work using live animals will be needed to elucidate the behavioural function of the enigmatic derbid metathoracic wing-abdomen mechanism and test our speculative hypotheses. If the organ is indeed a stridulatory mechanism as originally proposed, then its mechanism may be unique from a biomechanical and bioacoustic perspective. If our alternative, speculative hypothesis that the organ is used in chemical communication is experimentally confirmed, it will be the first known instance of any auchenorrhynchan using this signalling modality, paving the way for the design of pheromone traps for potential pest species in several areas of the world (Brown et al. 2006; Wilson, 2005).

Author contributions

L.-R.D. conceived and designed the study, collected and interpreted the data, performed the analysis, prepared figures, and wrote the paper. B.M and G.K.T collected the SR-μCT data with L.-R.D., and contributed to their analysis. I.M. contributed to the collection of other morphological data with L.-R.D., including photomicrography and scanning electron microscopy, and contributed to data interpretation and analysis. All authors contributed to critical revision of the draft manuscript, and gave final approval of the version to be published.

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By signing the Statement of Authorship, you are certifying that the candidate made a substantial contribution to the publication, and that the description described above is accurate.

Supervisor name and title: Professor Graham K. Taylor

Supervisor comments

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Date 04/11/2019

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Chapter 5: Response to "On the evolution of the tymbalian tymbal organ: Comment on "*Planthopper bugs use a fast, cyclic elastic recoil mechanism for effective vibrational communication at small body size*" by Davranoglou *et al.* 2019"

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Abstract

Vibrational communication is ubiquitous in planthoppers (Hemiptera: Fulgoromorpha), but its mechanism remained unknown until a recent paper by Davranoglou *et al*. (2019) describing the functional morphology, behavioural biomechanics, and systematic distribution of a widespread vibrational mechanism that they termed a "snapping organ". The mechanism of the snapping organ differs fundamentally from the only comparably well-known vibroacoustic organs of Hemiptera – the tymbal organs of cicadids (Cicadomorpha). Shortly after, Hoch *et al*. (2019) argued that it was "*unnecessary, if not misleading*" to call the mechanism a snapping organ, which they asserted should instead be identified as a "*tymbalian tymbal organ with snapping mechanism*". This identification refers to the "Tymbalia" hypothesis of Wessel *et al*. (2014), who proposed that the known abdominal

vibroacoustic organs of Hemiptera represent modifications of an abdominal vibrational organ hypothesised to have been present in the last common ancestor of Fulgoromorpha, Cicadomorpha, and Heteropterodea over 300mya. Here, we demonstrate that the criteria that Wessel *et al*. (2014) used to define the tymbalian tymbal organ are based on segmental misidentifications of the key muscles. The "Tymbalia" hypothesis is therefore in need of re-evaluation. We further demonstrate that the muscle terminology used by Davranoglou *et al*. (2019) is standard in the field, and provide morphological evidence that supports our identification of all of the snapping organ muscles as muscles, and not as scolopidial organs. We suggest that the distinctions between the snapping organs of Fulgoromorpha and the tymbals and tymbal-like organs of Cicadomorpha should be maintained on biomechanical grounds, and conclude that it would be at best premature – and at worst false – to describe the snapping organ as a "tymbalian tymbal organ" until the "Tymbalia" hypothesis has been tested formally using cladistic methods.

1. Introduction

Davranoglou *et al*. (2019) recently described the functional morphology and biomechanics of a fast, cyclic elastic recoil mechanism used for vibrational communication by planthoppers (Hemiptera: Fulgoromorpha). This structure, which they called a "snapping organ", spans the first two abdominal segments, and is located dorsally at the junction of the metathorax and abdomen. The mechanism was described in detail for a model species, *Agalmatium bilobum* (Fulgoromorpha: Issidae), but Davranoglou *et al*. (2019) identified similar structures with homologous musculature and innervation throughout the entire planthopper clade, with the exception of some Delphacidae and Derbidae, in which the snapping organ is highly modified. The snapping organ is defined by the presence of a Y-shaped cuticular lobe, the arms of which snap together suddenly following a slow contraction of the hypertrophied dorsal longitudinal muscles of the 1st abdominal segment

(Idlm1-2). Contraction of Idlm1-2 cocks the system, storing elastic-potential energy that is transferred to the arms of the Y-lobe when a change in the conformational state of the system is triggered by contraction of the small dorsoventral muscles of the 2nd abdominal segment (IIedvm1-2; see below for discussion of the identification of these muscles). This action raises the abdominal mass, producing a rapidly decaying vibration that is communicated to the substrate through the legs. This is followed a short time later by a second vibrational transient as the abdomen is thrust downward by the sudden re-opening of the Y-lobe arms. This release of the elastic-potential energy now stored in the Y-lobe is triggered by relaxation of IIedvm1-2.

It will be apparent from this description that the focus of Davranoglou *et al*. (2019) was on the functional morphology and biomechanics of the snapping organ, which differ fundamentally from the functional morphology and biomechanics of the only other comparably well-described hemipteran vibroacoustic organs: the tymbal organs of modern cicadas (Cicadomorpha: Cicadidae). Prior work on the mechanism of the tymbal organ was neatly summarised by Young & Bennet-Clark (1995): "*the tymbal membrane forms a convex dome, which is set in a ring of sclerotised cuticle. Posteriorly on the tymbal, there is an irregularly shaped region of sclerotised cuticle, the tymbal plate, onto which the tymbal muscle attaches dorsally. Anteriorly, there are a number of long sclerotised ribs, which alternate with short ribs arranged in a line. When the tymbal muscle contracts, the tymbal buckles inwards along the line of short ribs in a stepwise manner; each step results in a sound pulse and is due to the buckling of one or more long ribs, beginning with the most posterior*." The tymbal organ itself acts as a resonator, and is coupled to a second resonator comprising the large abdominal air sac and the paired tympanal membranes on the anterior part of the abdomen, which together function as a Helmholtz resonator, rather like the air

cavity of a guitar. Different forms of tymbal organs are found in other Cicadomorpha, but their mechanisms have not yet been described in detail.

With this biomechanical background in mind, Davranoglou *et al*.'s (2019) statement that the mechanism of the snapping organ of planthoppers differs fundamentally from the mechanism of the tymbal organ of cicadids ought to have been uncontroversial. We are therefore surprised that Hoch *et al*. (2019), who assume that the snapping organ of planthoppers is homologous with the tymbal organ of cicadids, conclude that it is "*unnecessary, if not misleading*" to introduce a new name for what they regard as "*a particular configuration in a long and complex chain of evolutionary transformation*". Instead, Hoch *et al*. (2019) recommend using the rather less snappy name "*tymbalian tymbal organ with a snapping mechanism*". This recommendation refers to the proposal by some of the same authors (Wessel *et al*., 2014) of the name "Tymbalia" for "*the taxon comprising Cicadomorpha, Fulgoromorpha, and Heteropteroidea* [and to be strictly correct, all descendants of the last common ancestor thereof], *based on the possession of a tymbal apparatus as an autapomorphic* [or correctly, synapomorphic] *character*". Wessel *et al*.'s (2014) "Tymbalia" hypothesis (Fig. 1) echoes earlier work by Sweet (1996) who proposed that "*tymbals may be an important synapomorphy relating the Coleorrhyncha to the Auchenorrhyncha* [i.e. Cicadomorpha and Fulgoromorpha] *and the Heteroptera*", and by Senter (2008) who referred to the same taxa as "*the tymbaled superclade*".

To call the vibrational organ of planthoppers a "*tymbalian tymbal organ with a snapping mechanism*" (Hoch *et al*., 2019) is to assert or assume homology of this organ with the tymbal organs of cicadids. This represents a logically stronger claim than referring to it merely as a "*snapping organ*" (Davranoglou *et al*., 2019), which makes no claim either way (Fig. 1). The onus is therefore upon Hoch *et al*. (2019) to demonstrate homology of the structure that we have described to the tymbal organs of cicadids. But do their claims bear

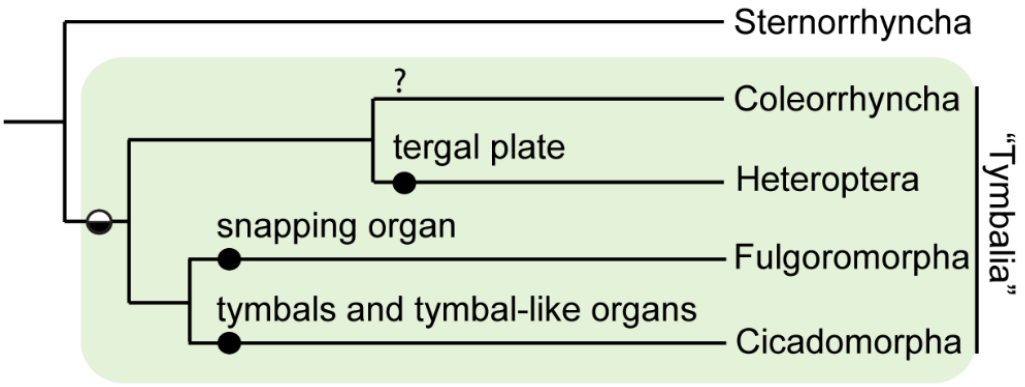

Figure 1. Systematic distribution of abdominal vibroacoustic organs in the Hemiptera, modified from Davranoglou *et al.* (2019). The clade within the green shaded area corresponds to the "Tymbalia" of Wessel *et al*. (2014), previously named the Euhemiptera. The half-filled circle denotes the ambiguous ancestral state of this clade: the main tenet of the "Tymbalia" hypothesis is that abdominal vibroacoustic organs were present as the ancestral state. Davranoglou *et al.* (2019) did not show this ambiguous ancestral state, but the present figure is intended to show that their presentation is compatible with either the presence or absence of abdominal vibroacoustic organs as the ancestral state. The question mark on the branch for Coleorrhyncha indicates that the detailed morphology of their vibroacoustic organs is not yet elucidated.

scrutiny? As we explain below, there is no reason yet to reject outright the "Tymbalia" hypothesis that the tymbal organs of cicadids and the snapping organs of planthoppers both represent modifications of an abdominal vibrational organ that was already present in their last common ancestor (i.e. just as the wings of a hummingbird and the wings of an albatross each represent modifications of the functional wings that were present in the last common ancestor of modern birds). Equally, there is no reason yet to reject the alternative hypothesis that the tymbal organs of cicadids and the snapping organs of planthoppers represent independent origins of abdominal vibroacoustic organs, involving some of the same musculoskeletal elements (i.e. just as the wings of a bird and the wings of a bat represent modifications of the pentadactyl limb of tetrapods, but are independently evolved as wings).

Until such time as these hypotheses have been tested formally using rigorous cladistic methodology, it would be premature to conclude either way. It would be equally premature – and perhaps false – to describe the snapping organ as a "tymbalian tymbal organ" as Hoch *et al*. (2019) would have us do. We respond to their more detailed comments on our work below.

2. The criteria for identifying a "tymbalian tymbal organ" require re-evaluation

The monophyly of the taxon that Wessel *et al*. (2014) called the "Tymbalia" is not at issue here, although as Wessel *et al*. (2014) note, the name Euhemiptera has priority as a name for the same proposed clade. Nevertheless, as we discuss below, the identification of several of the key muscles that Wessel *et al*. (2014) used to homologise the "tymbalian tymbal organ" is highly questionable. Wessel *et al*.'s (2014) literature review, which Hoch *et al*. (2019) treat as authoritative, relied entirely on the anatomical interpretations of earlier authors. Our own research using state-of-the-art morphological techniques (Davranoglou *et al*., 2017, 2019) challenges the muscle identifications of Ossiannilsson (1949) and Weber (1928, 1930), upon which Wessel *et al*. (2014) based their conclusions. These new findings do not necessarily contradict the "Tymbalia" hypothesis, but they do cast serious doubt on the validity of the morphological criteria that Wessel *et al*. (2014) used to homologise the "tymbalian tymbal organ" across taxa, and they further reinforce our primary conclusion here that it would at best be premature to describe the snapping organ as a "*tymbalian tymbal organ with a snapping mechanism*", as Hoch *et al*. (2019) recommend.

The first error in the muscle identifications of Wessel *et al*. (2014) was inherited from Ossiannilsson (1949), who frequently misidentified the ventral longitudinal muscle of the first abdominal segment as a metathoracic muscle, IIIvlm2 (Davranoglou *et al*., 2017). This error was repeated by Wessel *et al*. (2014), such that their metathoracic IIIvlm1

(confusingly, Ossiannilsson's IIIvlm2) should in fact be identified as Iavlm following their convention of using an "a" to indicate a muscle of abdominal origin (Davranoglou *et al*., 2019). The second error in the muscle identifications of Wessel *et al*. (2014) also appears to have been inherited from Ossiannilsson (1949). By studying the innervation and location of the primary dorsoventral muscles of the vibrational organs of Fulgoromorpha, we demonstrated that Ossiannilsson's (1949) assignment of these muscles to the first abdominal segment was erroneous, and that they instead belong to the second abdominal segment (see S1 Text in Davranoglou *et al*., 2019). Again, this error was repeated by Wessel *et al*. (2014), whose Iadvm1-2 should therefore be identified as IIadvm1-2, following their own naming conventions.

In Davranoglou *et al*. (2019), the same dorsoventral muscles of the second abdominal segment are labelled IIedvm1-2, where the "e" is an abbreviation of "external". Rather than recognizing that the key scientific issue here is one of muscle segmental identity, Hoch *et al*. (2019) again take issue with our terminology, commenting that "*it is mistaken to speak of "external muscles"".* In fact, use of the term "external muscles" has been standard in insect morphology since at least the foundational work of Snodgrass (1935), who wrote: "*With respect to the dorsal and ventral muscles the most general departure from the simple plan, in which the fibers all lie in a single plane against the body wall, consists of a differentiation of the fibers in each group into external muscles and internal muscles. Thus it is found in nearly all insects that the dorsal and ventral muscles comprise each two layers, there being, namely, internal dorsals (di) and external dorsals (de), and internal ventrals (vi) and external ventrals (ve). ... The lateral* [i.e. dorsoventral] *muscles are more subject to irregularities of position than are the dorsal and ventral muscles, but they likewise are often divided into internal laterals (Fig. 142, li) and external laterals (le).*" As this terminology has since been used consistently among insect morphologists (e.g. Albrecht, 1953; Klug &

Klass, 2006; Klug & Bradler, 2006), we see no error in using it to distinguish IIedvm1-2 from the distinct internal dorsoventral muscles of the same segment (IIidvm1-2).

Hoch *et al*. (2019) also hypothesised that some of the structures identified as dorsoventral muscles by Davranoglou *et al*. (2019) might be scolopidial organs, which they asserted are hard to distinguish from muscles in CT scans. Synchrotron-based μCT (SR-μCT) is in fact among the most accurate of all methods of morphological investigation (Friedrich *et al*., 2013), and its exceptional resolution has already allowed us to reconstruct the neuronal and muscular anatomy of another hemipteran abdomen in a previous study (Davranoglou *et al*., 2017). Of course, SR-μCT tissue contrast depends on various factors, including sample preparation, sample preservation, and beam energy (Friedrich *et al*., 2013), and for this reason Davranoglou *et al*. (2019) also used a suite of other methods including laser confocal microscopy, manual dissection, and microtome sectioning (unpublished) to verify that the structures that they had identified using SR-μCT were indeed muscles. This is particularly straightforward in this case, because ethanol-induced desiccation causes planthopper muscle fibres to split in a characteristic manner (Fig. 2A-E, white and black arrows) that is visible in both the 3D-volume rendered SR-μCT reconstructions (Fig. 2A, C, D) and the original SR-μCT tomograms (Fig. 2B, E). This clearly distinguishes these structures from neuronal tissue, which does not degrade similarly owing to the obvious absence of muscle fibres. Finally, the structures that we identified as dorsoventral muscles (Davranoglou *et al*. 2019) insert in identical positions (Fig. 2F, G) to known auchenorrhynchan dorsoventral muscles from previous studies (e.g. Mitomi, 1984; Ossiannilsson, 1949), leaving little doubt as to their identity.

It follows that Figure 20.5 of Wessel *et al*. (2014), which summarises the configuration and segmental identity of the muscles involved in their hypothesised “tymbalian tymbal organ”, is in error. Specifically, the muscles labelled therein as IIIvlm1 [which represents IIIvlm2

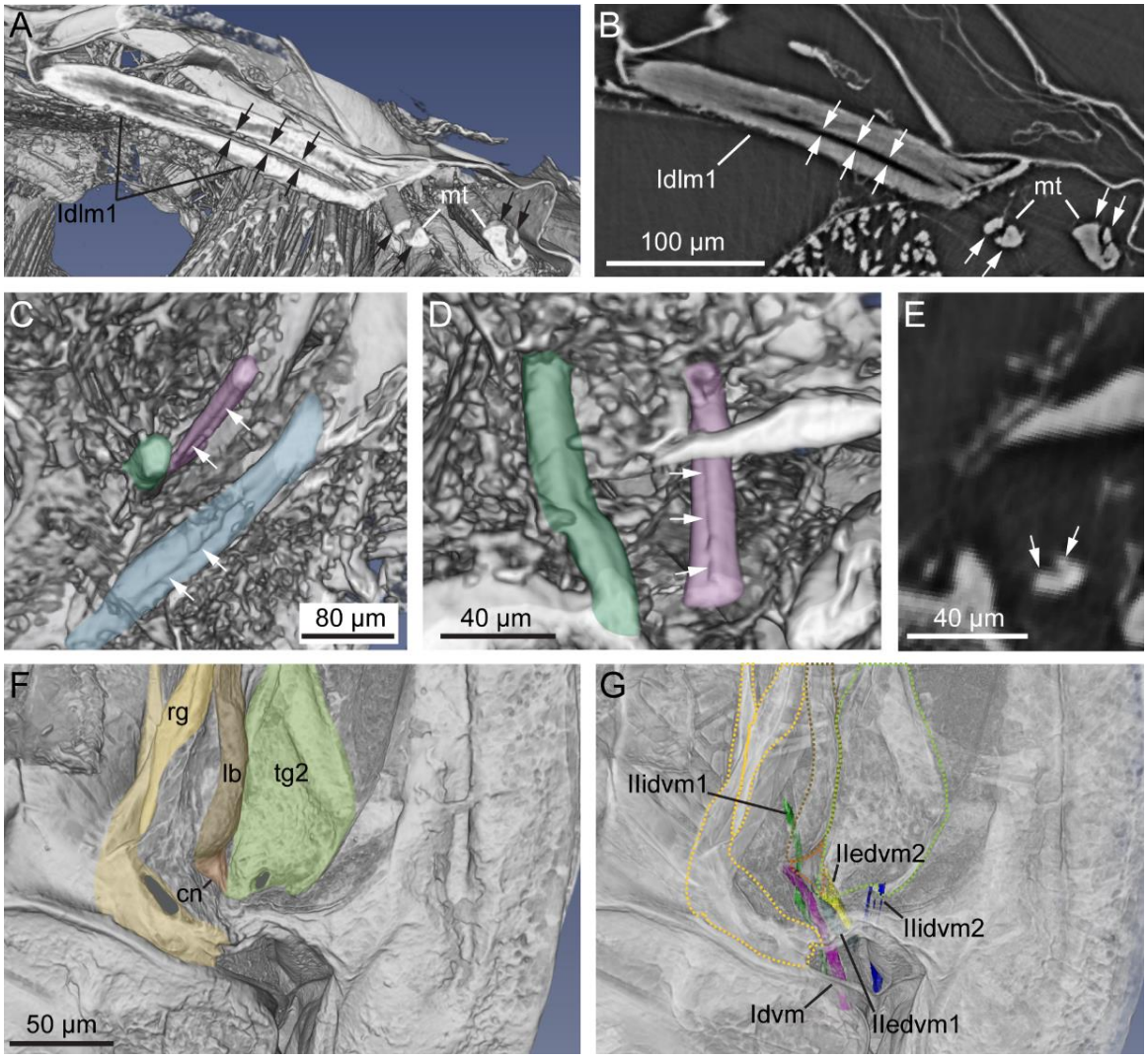

Figure 2. Effects of ethanol-induced desiccation as a guide to identifying muscle tissue in synchrotron-based µCT-scans, using snapping organ musculature as an example. A) *Elasmoscelis* sp. (Lophopidae) volume-rendered reconstruction, showing the primary snapping organ muscle (Idlm1) and metathoracic muscles (mt), both of which exhibit distinct gaps (black arrows) due to the separation of muscle fibres; B) Same, SR-µCT scan cross-section, where the effects of alcohol-induced desiccation are visible (white arrows); C) Same, false-coloured reconstruction of snapping organ dorsoventral muscles IIedvm1 (light blue), Iadvm (purple) and IIidvm1 (green) exhibiting distinct grooves due to the splitting of muscle fibres following ethanol-induced desiccation; D) Same, different view, with IIedvm1 not shown; E) Same, SR-µCT scan cross-section, showing Iadvm1 splitting into two groups (white arrows); F) Volume-rendered, false-coloured reconstruction of *Phantia subquadrata* (Flatidae), showing the defining components of a snapping organ: a ridge

(rg), a Y-lobe (lb; snapped shut), and a connector (cn) linking it to tergum 2 (tg2; green); G) Same, faded, to show the tergal insertions of the snapping organ dorsoventral muscles: Iadvm (purple); IIedvm1 (light blue); IIedvm2 (yellow); IIidvm1 (green) and IIidvm2 (dark blue).

of Ossiannilsson (1949)] and Iadvm are incorrectly identified as belonging to the metathorax and first abdominal segment, respectively, and should instead have been labelled Iavlm and IIadvm. These muscles are of key importance to the argument of Wessel *et al.* (2014), who wrote "*If we want to describe in short the "close similarity in the basic plan" (Pringle, 1957: p. 154) of the tymbalian tymbal organs, we must refer first and foremost to a homologous set of muscles (I a dlm + II a dlm + I a dvm + III vlm + II a vlm, see Fig. 20.5), working together in order to produce vibrations for communication purposes*." This confusion over muscle segmental identity is understandable, given that Wessel *et al*. (2014) did not examine muscle innervation, but it is obviously problematic in identifying muscle homology. Wessel *et al.* (2014) did not refer to the foundational works of Snodgrass (1933), Kramer (1950), and Wohlers & Bacon (1980), whose interpretations of abdominal segmentation and musculature in Auchenorrhyncha are congruent with our own (see S1 Text in Davranoglou *et al*., 2019), but not with those of Weber (1928) and Ossiannilsson (1949), upon which Wessel *et al.* (2014) relied. Wessel *et al.'s* (2014) criteria for identifying a "tymbalian tymbal organ" therefore require careful re-evaluation before they are used to draw any further conclusions regarding the homology or otherwise of the various hemipteran vibroacoustic organs.

Wessel *et al*. (2014) also attempted to homologise the muscles of "tymbalian" and "non-tymbalian" Hemiptera. For example, in their Figure 20.2, Wessel *et al*. (2014) highlight two pairs of dorsoventral muscles labelled Iadvm1-2 (but see above) in *Aphis fabae* (Sternorrhyncha) and *Platypleura capitata* (Auchenorrhyncha). The identification of the muscles in *Aphis* followed Weber (1928), but a later paper by the same author (Weber, 1935)

examining *Aleyrodes proletella* (Sternorrhyncha) demonstrated that sternorrhynchan abdominal dorsoventral musculature can be considerably more complex than suggested by his earlier work on *Aphis* (Weber, 1928). Wessel *et al*. (2014) did not refer to the later work by Weber (1935), and they did not explain how they were able to homologise these 2 pairs of muscles in Sternorrhyncha to any of the 6 or more pairs of dorsoventral muscles of the first and second abdominal segments of Auchenorrhyncha (see Ossiannilsson, 1949; Davranoglou *et al*., 2019). In fact, the complex musculature of *Aleyrodes* is difficult to homologise with that of its fellow sternorrhynchan *Aphis* – let alone with that of a more distantly-related auchenorrhynchan (Fig. 1).

To summarise, our findings challenge the validity of the criteria that Wessel *et al*. (2014) used to define the "tymbalian tymbal organ", and demonstrate that the homologies of the musculature defining hemipteran vibroacoustic organs are far from resolved – just as Wessel *et al*. (2014) themselves cautioned. We are therefore unwilling to follow Hoch *et al*. (2019) in assuming homology of the snapping organs of Fulgoromorpha with the tymbal organs of Cicadomorpha and the tergal plates of Heteroptera (i.e. specialised terga of abdominal segments 1-2, used to generate vibrations), pending more detailed examination of the abdominal morphology of a broad range of Hemiptera. Our upcoming work (Davranoglou *et al*., in preparation) re-examines the homologies of the relevant abdominal musculature across Auchenorrhyncha, and will expand on the summary information that we have presented here. We do not wish to pre-judge the outcome of this analysis, but until the "Tymbalia" hypothesis is tested formally using cladistic methods of ancestral state reconstruction, we must respectfully disagree with Hoch *et al*.'s (2019) comment that "*it must be at least considered doubtful that vibration producing structures evolved three times independently*" in Cicadomorpha, Fulgoromorpha, and Heteropterodea. We stress that what appears to be the most parsimonious explanation of a given evolutionary pattern does not

always reflect actual evolutionary events. Notable examples include the stridulatory wings of crickets and allies, and the jumping mechanisms of Cicadomorpha and Fulgoromorpha – each of which are thought to have evolved independently (Desutter-Grandcolas *et al*., 2017; Ogawa & Yoshizawa, 2017).

3. Recommended terminology

Even if the vibroacoustic organs of Cicadomorpha, Fulgoromorpha, and Heteropterodea do turn out to be derived from a vibroacoustic organ that was already present in their last common ancestor – and for the avoidance of doubt, we reiterate that Davranoglou *et al*. (2019) makes no claim either way (see Fig, 1) – it is self-evident that we will still require a clear, agreed functional terminology to make sense of the diversity of hemipteran vibroacoustic mechanisms. We routinely describe the forelimb of a bird as a "wing"; not as a "pentadactyl limb with a feather mechanism", though both descriptions are factually correct. In the same way, it is neither unnecessary nor misleading, as Hoch *et al*. (2019) claim, to describe the vibroacoustic mechanism of planthoppers as a snapping organ, in contradistinction to the tymbal organ of a cicadid. The former involves the snapping instability of a single pair of Y-shaped lobes; the latter involves the buckling instability of multiple curved ridges. These are fundamentally different mechanisms, and they each merit their own terminology.

We agree with Hoch *et al.*'s (2019) comment that there is considerable variation in the types of vibroacoustic organs present in Cicadomorpha. In fact, the systematic distribution, morphology, and homology of these structures is the subject of an upcoming study of ours (Davranoglou *et al*., in preparation). However, there is little disagreement in the literature that most Cicadomorpha possess tymbal or tymbal-like organs, defined with reference to their musculature and to the form of their exoskeletal components (Ossiannilsson, 1949).

The term tymbal-like is certainly useful here (Wessel *et al*., 2014; Davranoglou *et al*., 2019; cf. Hoch *et al*., 2019), because it points to the observed morphological similarity between the tymbal organs of cicadids and the vibroacoustic organs of non-cicadid Cicadomorpha, whilst simultaneously highlighting the fact that their biomechanics are likely to differ in light of their key structural differences. For example, Deltocephalinae and Typhlocybinae possess vibrational mechanisms that have diverged greatly from those of other Cicadomorpha. Even so, there is little doubt that these mechanisms originate from a more generalised tymbal or tymbal-like condition (Ossiannilsson, 1949). We cannot yet say the same of the snapping organs of Fulgoromorpha, but nor do we rule out this out as a possibility.

4. Conclusions

Here and in our previous work (Davranoglou *et al*., 2017, 2019), we have shown that the muscle homologies that Wessel *et al*. (2014) used to identify a "tymbalian tymbal organ" relied on misinterpretations by previous authors. To stand the test of time, the defining criteria of the "tymbalian tymbal organ" will need to be re-evaluated, and the "Tymbalia" hypothesis tested formally using cladistic methodology. Until then, it would be premature – and perhaps incorrect – to conclude as Hoch *et al.* (2019) would have us do, that the snapping organs of Fulgoromorpha are homologous with the tymbal and tymbal-like organs of Cicadomorpha, and with the tergal organs of Heteropterodea. We firmly believe that a multidisciplinary approach, combining functional morphology, behavioural biomechanics, developmental biology, and phylogenetic systematics will be necessary to elucidate the origins of hemipteran vibroacoustic organs – key aspects of which we have studied in our own work both past (Davranoglou *et al*., 2017, 2019) and current (Davranoglou *et al*., in preparation). We have chosen not to engage with Hoch *et al*.'s (2019) discussion of

"visibility" and "attention" as the "currency" of the "scientific market", because we do not recognise their comments as having anything to say about our own research ethics.

5. Author contributions

L.-R.D. analysed data, prepared figures, co-wrote this paper, and was first author of the original paper commented on by Hoch et al. (2019); A.C. was a co-author of the original paper; B.M. was the senior author of the original paper; G.T. co-wrote this paper, and was co-author of the original paper. All authors commented on and approved the final draft of this paper.

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Title of Paper	Response to "On the evolution of the tymbalian tymbal organ: Comment on "Planthopper bugs use a fast, cyclic elastic recoil mechanism for effective vibrational communication at small body size" by Davranoglou et al. 2019"
Publication Status	✓□**Published** □ Accepted for Publication □Submitted for Publication □Unpublished and unsubmitted work written in a manuscript style
Publication Details	Davranoglou, L.R., Cicirello, A., Taylor, G.K. and Mortimer, B. (2019) 'Response to "On the evolution of the tymbalian tymbal organ: Comment on "Planthopper bugs use a fast, cyclic elastic recoil mechanism for effective vibrational communication at small body size" by Davranoglou et al. 2019"', *Cicadina*, 18, 17–26.

Student Confirmation

Student Name:	Leonidas-Romanos Davranoglou
Contribution to the Paper	Analysed data, prepared figures, co-wrote the paper, and was first author of the original paper commented on by Hoch et al. (2019)

Signature

Date 1/11/2019

Supervisor Confirmation

By signing the Statement of Authorship, you are certifying that the candidate made a substantial contribution to the publication, and that the description described above is accurate.

Supervisor name and title: Professor Graham K. Taylor

Supervisor comments

Signature

Date 04/11/2019

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Chapter 6: On the morphology and evolution of cicadomorphan tymbal organs

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Abstract

Cicadas and many of their relatives (Hemiptera: Cicadomorpha) generate vibroacoustic signals using tymbal organs located on their first two abdominal segments. Although tymbals are well-studied in Cicadidae, their systematic distribution in other Cicadomorpha and their possible homologies to the vibroacoustic mechanisms of other Hemiptera have been debated for more than a century. In the present study, we re-examine the morphology of the musculoskeletal system of cicadomorphan vibroacoustic organs, and we document their systematic distribution in 78 species drawn from across the phylogeny of Cicadomorpha. We also compare their morphology to the recently-described snapping organ of planthoppers (Fulgoromorpha). Based on the structure and innervation of the metathoracic and abdominal musculoskeletal system, we find that several key elements of cicadomorphan vibroacoustic organs have been incorrectly assigned by previous studies to the first abdominal segment, when in fact they belong to the second. We find that tymbal organs are nearly ubiquitous in Cicadomorpha, and conclude based on their phylogenetic distribution, that they are likely to be synapomorphic. The unusual tymbal-like organs of the Deltocephalinae and Typhlocybinae, represent derived modifications. Finally, we propose a

standardised terminology for sternal components of the cicadomorphan vibrational organs, which can be used in future taxonomic descriptions.

1. Introduction

Cicadas are well-known for their often loud acoustic songs, which have captured the attention of scientists and naturalists since Aristotle first tried to understand their mechanism of sound production (Weiss, 1929). All Cicadas (Cicadoidea, comprising the families Cicadidae and Tettigarctidae) produce their songs using paired buckling structures on the first abdominal tergum, known as tymbals, whose structure was summarized by Young & Bennet-Clark (1995) as follows: "*the tymbal membrane forms a convex dome, which is set in a ring of sclerotised cuticle. Posteriorly on the tymbal, there is an irregularly shaped region of sclerotised cuticle, the tymbal plate, onto which the tymbal muscle attaches dorsally. Anteriorly, there are a number of long sclerotised ribs, which alternate with short ribs arranged in a line. When the tymbal muscle contracts, the tymbal buckles inwards along the line of short ribs in a stepwise manner; each step results in a sound pulse and is due to the buckling of one or more long ribs, beginning with the most posterior*". Cicadas are not the only hemipterans to communicate using tymbal organs, however, as many of their cicadomorphan relatives, including the leafhoppers (Cicadellidae), treehoppers (Membracidae), and froghoppers (Cercopoidea) also produce substrate-borne vibrational signals using anatomically similar structures with muscles arranged to pull inward on a distinct area of convex and possibly ribbed or striated cuticle (Ossiannilsson, 1949). Although the anatomical details vary between species, notably in the presence or absence of ribs, the buckling dynamics of a tymbal organ are ultimately what define it as such biomechanically.

The morphology, evolution, and biomechanics of the vibroacoustic tymbal organs of cicadas have received considerable attention (e.g. Pringle 1954, 1957; Simmons & Young, 1978; Young & Bennet-Clark, 1995; Moulds, 2005; Nahirney et al., 2006; Marshall et al., 2018), but the same cannot be said of the vibrational organs of other cicadomorphans. These have been examined comparatively in only a handful of studies (Ossiannilsson, 1949; Vondráček, 1949; Smith & Georghiou, 1972; Shaw & Carlson, 1979; Strübing & Schwarz-Mittelstaedt, 1986; Miles et al., 2017; Kuhelj et al., 2018), perhaps owing to their small size, the subtlety of their structure, and the fact that they produce songs that are inaudible to the human ear without specialized equipment. Indeed, the systematic distribution of vibroacoustic organs in Cicadomorpha has remained unstudied since the pioneering work of Ossiannilsson (1949). Our limited knowledge of the comparative morphology of tymbal organs contrasts with our extensive knowledge of the signals they produce, as their behavioural significance and possible use in pest control has been well investigated (e.g. Cocroft & McNett, 2006; de Groot et al., 2011; Kuhelj & Virant Doberlet, 2017; Mazzoni et al., 2017; Takanashi et al., 2019). It also hinders our understanding of the origins of vibroacoustic behaviour in Cicadomorpha, and prevents any formal test of the homology of cicadomorphan tymbals to their counterparts in other Hemiptera.

In light of their complex structure, and the lack of recent comparative studies, even the most fundamental aspects of tymbal organ evolution remain the subject of continuing debate. In particular, a recent review by Wessel et al. (2014) and later commentary by Hoch et al. (2019) conclude that the vibroacoustic organs of all Hemiptera excluding Sternorrhyncha are homologous to the tymbal organs of cicadas. According to this hypothesis, all such vibroacoustic organs should be called “tymbals”, and the clade possessing them the “Tymbalia” (Wessel et al., 2014). We have argued elsewhere that this conclusion is premature (Davranoglou et al., 2019a), and have suggested that the criteria which Wessel et

al. (2014) used to define the “tymbalian tymbal organ” require additional morphological information, and a careful reassessment of muscle segmental identity and homology. Such a reappraisal is necessary, because previously authors have variously assigned the sternal components and principal musculature of the tymbal organ to either the first abdominal segment (Ossiannilsson, 1949; Vondráček, 1949), the second abdominal segment (Snodgrass, 1933; Kramer, 1950; Wohlers & Bacon, 1980), or the flight muscles of the metathorax (Pringle, 1954). Nevertheless, in the 70 years since Ossiannilsson (1949) first assigned most of the tymbal muscles to the first abdominal segment, his conclusions have gone largely unchallenged, and have formed the basis of most subsequent opinion in the study of hemipteran vibroacoustic organs (e.g. Pringle, 1954, 1957; Wessel et al., 2014; Miles et al., 2017; Kuhelj et al., 2018). In summary, the evolution of hemipteran vibroacoustic organs remains a hotly debated topic, with a diversity of theories and interpretations, but until the morphology of cicadomorphan vibroacoustic organs is explored in greater detail across a wider variety of taxa, we can expect little substantive progress in this debate.

In the present study, we analyse the morphology and systematic distribution of cicadomorphan vibroacoustic organs by combining our own new morphological observations with a critical reappraisal of the published literature. Furthermore, we identify similar structures, where present, in the vibroacoustic organs of Cicadomorpha and Fulgoromorpha, providing new insight into questions regarding their evolutionary origin. We also update the terminology used for the description of the taxonomically-important sternal sclerites of cicadomorphan vibroacoustic organs, in accordance with our own reinterpretation of their segmental identity.

This new perspective on the morphology and homologies of cicadomorphan vibroacoustic organs aims: (i) to provide the necessary framework for future studies aimed at identifying

vibrational organs in Cicadomorpha; (ii) to provide new characters for incorporation into future phylogenetic and taxonomic studies; and (iii) to elucidate the evolutionary origins of vibroacoustic organs more broadly in Hemiptera.

2. Material and methods

2.1. Material

We described the distribution of tymbal and tymbal-like vibroacoustic organs in 78 species of Cicadomorpha, drawn from 11 of the 12 currently-recognised extant families except Epipygidae (Table 1). We examined material from 67 species deposited in the Natural History Museum, London, UK (BMNH); the Oxford University Museum of Natural History (OUMNH); the Moravian Museum, Brno, Czech Republic (MMBC); the National Museum, Prague, Czech Republic (NMPC); the Paleontological Institute, Russian Academy of Sciences, Moscow, Russia (PI); and the Davranoglou Personal Collection (DPC). We also supplemented our sampling of the non-cicadid Cicadomorpha by making use of a small number of earlier literature records for species we could not examine directly (Ossiannilsson, 1949; Vondráček, 1949; Kuhelj et al., 2018). The observational methods, mode of preservation, and depository of each examined species are summarised in Table 1. For most species, we documented the external morphology only. Our description of the corresponding musculature (Table 2) and innervation (Table 3) is based on a much narrower sample, which was examined using manual dissections and microtomography, supplemented by earlier observations from the literature (Table 1).

2.2. Photomicrography

Specimens from the different depositories were imaged using the following equipment: 1) a Leica M165c microscope equipped with a Leica DFC490 camera (DPC-OUMNH); a Keyence VHX-5000 digital microscope with VH-Z20T and VH-ZST objectives (MMBC,

NMPC); and 3) a Canon EOS 700D camera attached to a Leica MZ125 microscope (BMNH). All resulting stacked images were combined using Helicon Focus (Helicon Soft, Kharkiv, Ukraine) or VHX-5000 system software.

2.3. Synchrotron microcomputed tomography (SR-μCT)

A specimen of an ethanol-preserved *Cercopis vulnerata* was scanned at the TOMCAT beamline, Swiss Light Source (SLS), Paul Scherrer Institut, Switzerland, at a beam energy of 15.99 keV with final pixel size of 1.625 mm. Three-dimensional reconstruction of the phase-contrasted scans was undertaken using Amira 6.1 software (Mercury Systems).

2.4. Inference of homology

We used the three criteria of Patterson (1982) to infer homology between the different exoskeletal and muscular components of the vibroacoustic apparatus: 1) similarity (i.e. homologous structures should display topographic correspondence of the musculature, muscle attachment points, and muscle innervation); 2) conjunction (i.e. two supposed homologues cannot be homologous if they are found together in the same organism); and 3) congruence (i.e. the phylogenetic distribution of a homology should be consistent with the phylogenetic distribution of other homologies). For all of our phylogenetic inferences, we consulted the latest molecular phylogenies of Cicadomorpha (Cryan, 2005; Song et al., 2017; Dietrich et al., 2017; Skinner et al., 2019). We found that most muscles could be readily identified because of their conserved patterns of attachment and innervation, although some were difficult to homologise, especially when the neurological data were absent (see Section 3.7).

2.5. Terminology

2.5.1. Tymbals and tymbal-like organs

Here, as in our previous works (Davranoglou et al., 2019a, b, c), we use the term “tymbals” in its original functional, morphological, and biomechanical sense. That is, we take the term “tymbal” to describe any hemipteran organ that uses (or is supposed to use) paired areas of buckling cuticle, with or without buckling ribs, in order to generate vibroacoustic signals. We should stress, however, that our inferences are based solely on morphology, such that cuticular buckling to produce vibroacoustic signals is assumed on the basis of their observed structure, or inferred through manipulation. We expressly avoid use of the extended definition of a “tymbalian tymbal” originating from the Tymbalia hypothesis of Wessel et al. (2014), which encompasses the abdominal vibroacoustic organs of all hemipterans excluding the Sternorrhyncha, and which therefore presupposes the homology that the present study provides the necessary basis for testing (see Davranoglou et al., 2019a for further discussion). Our definition of a tymbal organ is useful in being based on the characteristic morphology thereof, which always includes a distinct area of buckling cuticle, typically convex in its relaxed conformation, and often structured with ribs that can be identified even in preserved specimens. Ossiannilsson (1949) used the term “striae” and “striated tymbals” to describe what we describe here as ribbed tymbals, although his terminology is slightly more inclusive, as it includes the barely visible, faint wrinkles on the tymbal or tymbal-like organs of some cicadellids, the biomechanical significance of which is uncertain. In contrast, we use the term “rib” to describe sclerotized, rod-like structures which provide stiffening on the otherwise deformable cuticle of the tymbal.

The term “tymbal membrane” itself merits further discussion, because although the area of cuticle between the tymbal ribs is transparent and appears membranous in cicadas, it is actually quite hardened, and is clearly not the same as the soft, flexible membrane surrounding terga 1-2 in most Cicadomorpha. These structural properties make sense biomechanically, because the tymbal “membrane” must resist deformation sufficiently to

buckle suddenly under muscular load, and because the consequent production of vibrations would not be as efficient in soft cuticle. We maintain use of the term “tymbal membrane” for consistency with previous studies (e.g. Young & Bennet-Clark, 1995), but stress that it is somewhat imprecise morphologically. Moreover, in non-cicadid Cicadomorpha, there is usually no membranous area as such between their tymbal ribs, if indeed ribs are present at all. Rather, these ribs exist on a recognisably modified area of deformable cuticle, arranged such that it buckles inwards upon contraction of the associated muscles. This modified area of cuticle contrasts with the remainder of tergum 1, which is stiff and less readily deformable. To identify tymbals in ethanol-preserved and dry mounted specimens, we used some or all of the following characters: 1) presence of a distinct area of ribbed cuticle on tergum 1; 2) presence of a distinct area of depigmented cuticle on tergum 1, deformable under manipulation with forceps in ethanol-preserved specimens; 3) presence of distinct attachment points for the principal tymbal muscles; and 4) separation of the tymbal area from the remainder of tergum 1 by a membranous bridge or cuticular elevation.

For species which do not possess readily identifiable buckling or ribbed structures, but which presumably represent modifications of a tymbal bauplan, we use the term “tymbal-like organs”.

2.5.2. Musculature

Previous studies have used a variety of different terminologies for the musculature of Cicadomorpha, which can make it difficult to confirm which muscle is which across the different species being examined in each study. Here we adopt the terminology of Tröger et al. (2019) for the abdominal musculature, and that of Friedrich & Beutel (2008) for the metathoracic musculature. Unlike the muscle classification scheme used in our own previous studies of Fulgoromorpha (Davranoglou et al., 2019b, c), these schemes do not

classify the dorsoventral muscles as “internal” or “external” based on their position (see e.g. Snodgrass, 1935), which would be difficult to determine consistently across all auchenorrhynchan taxa, because of the exoskeletal transformations of their sterna, and the poorly-understood homologies of their dorsoventral muscles with those of other hemipterans. Based on our homologisation of auchenorrhynchan musculature, we attempt to standardize our terminology with the designations used by previous authors to describe the muscles operating auchenorrhynchan vibroacoustic organs (Table 2), which we do by listing their attachments and innervation (Table 3). We do the same for the different terminologies of the nervous system in Table 4.

2.5.3. Abbreviations

Abbreviations used in the figures: **apo:** apodeme; **apoph:** apophysis; **bs:** basisternum; **cl:** coxal cleft; **cn:** connector of terga 1–2; **cV:** chitinous V; **cVa:** chitinous V arm; **ds:** drop-shaped sclerite; **dvm:** dorsoventral muscle; **em:** epimeron; **es:** episternum; **fm:** folded membrane; **fu:** furca; **gs:** gamma-shaped sclerite; **ia:** internal apodeme of deltocephaline organ; **lb:** Y-lobe of snapping organ; **lr:** long tymbal rib; **lt:** laterotergite; **ma:** muscle attachment of stn2a for dvmII1; **mb:** membrane; **ms:** metapostnotal sclerite; **pl:** flap-like plate of tergum 1; **pm:** proximal margin of tymbal; **pocx:** postcoxale; **prc:** process of stn2b; **pt**: pit of tymbal plate; **rb:** tymbal rib; **rg:** ridge; **rs:** resilin; **sa:** sternal arm of stn2b; **scl:** sclerite of typhlocybine organ; **sp:** spiracle; **sr:** short tymbal rib; **st,** stem of chitinous V; **stn:** sternum; **stn1a:** anterior subdivision of sternum 1; **stn1b:** posterior subdivision of sternum 1; **stn2a:** anterior subdivision of sternum 2; **stn2b:** posterior subdivision of sternum 2; **tb:** tymbal; **tc:** tymbal cover; **td:** tendon of tymbal muscle dvmII1; **tg:** tergum; **tm:** tymbal membrane; **tp:** tymbal plate; **tr:** transverse sclerite of deltocephaline organ; **vb:** v-bar; **vlm:** ventral longitudinal muscle; **ty:** tympanum. Arabic numerals indicate segmental identity of the structure involved.

3. Results

In the following sections, we describe the musculoskeletal system and innervation of cicadomorphan vibroacoustic organs, based on sampling of 79 species directly or through wider examination of the available literature (Table 1). We begin by introducing the "classic" tymbal organs of Cicadoidea, whose conformation as reinterpreted here forms the basis of the proceeding comparison with the vibrational organs of Cercopoidea and Membracoidea. Our examination does not aim to be taxonomically exhaustive, but to describe in detail the main components of known tymbal and other abdominal vibrational organs, and their homologies in each cicadomorphan superfamily.

Note that our interpretation of the segmental identity of auchenorhynchan sternal sclerites and their musculature contrasts with those of most published studies. For this reason, we provide evidence to support our interpretations in our description of the cicadid condition, which is applicable across Auchenorrhyncha, due to the consistent morphology of the relevant parts. Our reinterpretation is explained in more detail in the Discussion (Section 4).

3.1. Tymbal morphology of the Cicadoidea

3.1.1. Tergal structures

The following description is based specifically on *Cicada orni* Linnaeus, 1758, unless explicitly stated otherwise. Tergum 1 is characteristically bipartite: its proximal portion fuses to the metathorax and is membranous (mb; Fig. 1A–C), whereas its distal portion is typically strongly sclerotized (t1; Fig. 1A–C) and forms a broad, convex plate. The tymbal organs are paired structures located on either side of the base of tergum 1 (tb; Fig. 1A), and are distinctly separated from the latter by a narrow membrane (white arrows; Fig. 1B, C). Each tymbal possesses long (lr) and short (sr) ribs, which are located on the tymbal membrane (tm; Fig. 1A–C), although in the Tettigarctidae examined (Table 1), we could

only observe long ribs. The proximal margin (pm) of the tymbal consists of a sclerotized U-shaped lobe (Fig. 1B, C). A metapostnotal (?) ridge (rg) fuses to the proximal margin of the tymbal (Fig. 1B, C). The ventral surface of the tymbal is formed by a sclerotized connector [cn; Figs. 1C, 2A–D; Fig. 2D showing the condition in *Oligoglena flaveola* (Brullé, 1832)] from the second abdominal segment, which incompletely fuses to the tymbal proximal margin (Fig. 2C). This connector is known as the t-bar (Young & Hill, 1977). The distal margin of the tymbal possesses the tymbal plate (tp; Fig. 1B, C), whose distinct pit (pt; Fig. 1B) internally forms the insertion of the main tymbal muscle tendon (td; Fig. 4A). In many species, the tymbal is protected by a flap-like extension of tergum 2, known as the tymbal cover (partially excised in most figures, complete only in *O. flaveola*, Fig. 2D).

From the above description, it can be deduced that tymbals are composite organs, consisting of exoskeletal elements pertaining to the metathorax and abdominal segments 1–2.

3.1.2. Sternal structures

Sternum 1

The sterna of the first two abdominal segments are particularly complex, as each is subdivided into two morphologically dissimilar components. The anterior subdivision of sternum 1 (stn1a) is sclerotized and fused to the metathorax on the postcoxale (pocx), and bears the first abdominal spiracle (Figs. 2A–C, 3, red). Internally, two apodemes are present on each side of stn1a: an anterio-median one (apo1; Fig. 3), on which chordotonal organs insert (Young, 1975), and a posterio-lateral one (apo2), which forms the attachment for several muscles (Figs. 2A–C, 3; Table 3). Stn1a is succeeded by a folded membrane (fm; Fig. 2A, C), which actually represents the posterior subdivision of sternum 1 (stn1b; Fig. 3, blue). The conformation of stn1b as a folded membrane allows the first two segments to fold inwards and outwards upon muscle action, much like an accordion (Pringle, 1954).

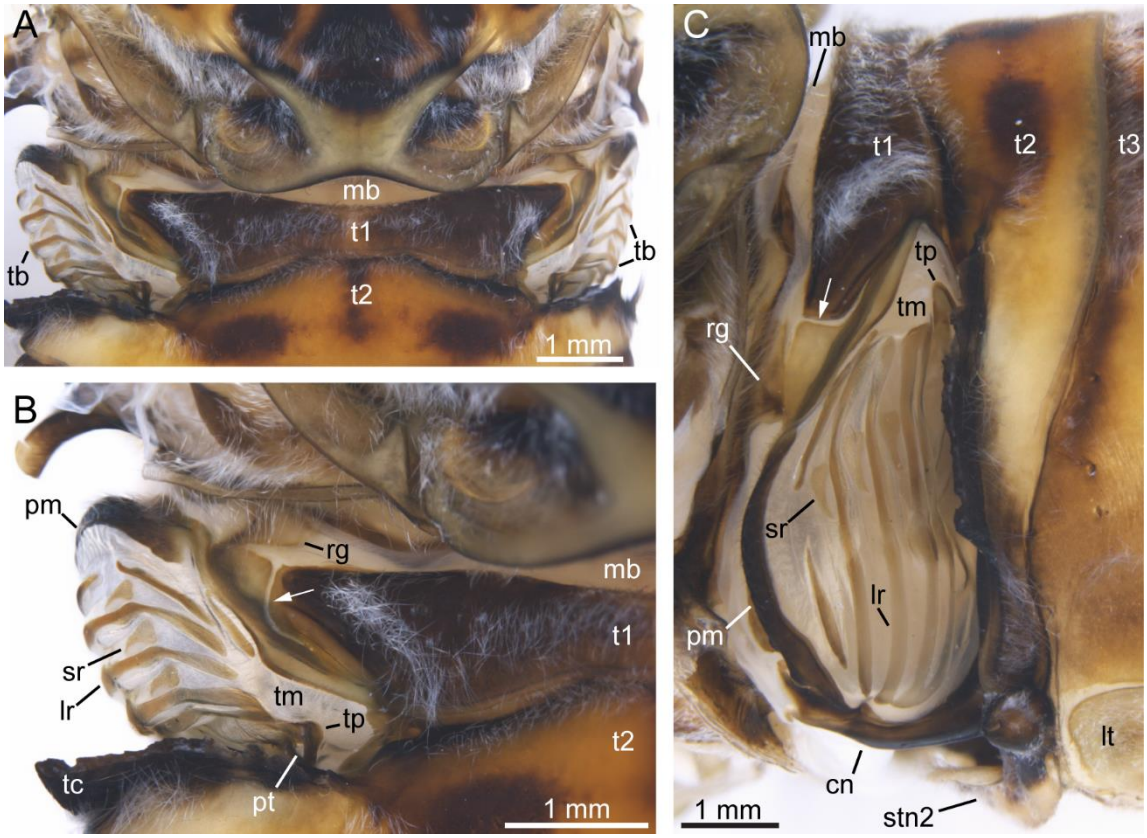

Fig. 1. External morphology of the tymbals of *Cicada orni*. A) Dorsal view of metathorax and abdominal segments 1–2, mb, membrane; tb, tymbal; t, tergum; B) Magnified dorsal view of left tymbal, white arrow indicating the membranous boundary between the main body of tergum 1 and the tymbal, lr, long tymbal rib; pm, proximal margin of tymbal; rg, ridge; pt, pit of tymbal plate; sr, short tymbal rib; tm, tymbal membrane; tc, tymbal cover; tp, tymbal plate; C) Lateral view of tymbal, white arrow indicating the membrane separating the tymbal from tergum 1, cn, connector of terga 1–2; lt, laterotergite; stn, sternum. Note that the tymbal cover has been removed to enable observation of the tymbal.

Justification for segmental interpretation: Stn1a has been previousy interpreted as the metathorax (Vogel, 1923; Ossiannilsson, 1949). We interpret this as the first abdominal segment which is fused to the metathorax, as: 1) its fusion line to the metathorax is distinctly visible (Figs. 2, 3); 2) it possesses the first abdominal spiracle (Figs. 2, 3); and 3) its muscles (vlmI1, dvmI1–3) receive innervation from the nerve of the first abdominal segment (section 3.1.3.; Table 2).

Sternum 2

In Cicadidae, the first subdivision of sternum 2 (stn2a) is represented by a V-shaped structure (in cross-section) known as the chitinous V (after Myers & Myers, 1928) (cV; Fig. 2B), the base of which is formed by a narrow stem (st; *O. flaveola*, Fig. 2D). The chitinous V is given its shape by paired, wing-like structures which expand dorsolaterally, known as the chitinous-V arms (cVa; Figs. 2A–D, including *O. flaveola*; 3). These arms represent greatly enlarged sternal apodemes which are completely fused to each other, their boundaries being clearly visible in macerated specimens (black dashed line; Fig. 2B). The median, internal portion of each chitinous-V arm is provided dorsally with a semi-transparent, cup-like chitinous muscle attachment (ma), which forms the origin for the principal tymbal muscle (*O. flaveola*, Fig. 2D; Fig. 3; Table 3). The chitinous-V arms fuse dorsally with an internal expansion of tergum 2 known as the vertical bar (vb; Fig. 4A, B). The dorsolateral boundary of each chitinous-V arm is fused to a tergal lobe bearing the second abdominal spiracle and the sclerotized connector of the tymbal (*O. flaveola*, Fig. 2D). In non-cicadid taxa, the muscle attachments forming the main body of the chitinous V are less strongly fused (if at all), their boundaries being clearly visible (Fig. 4C). This is also the case in the Tettigarctidae, where the muscle attachments are not fused to each other into a chitinous V, but have the form of small, widely separated apodemes (Evans, 1941).

The second subdivision of sternum 2 (stn2b) is represented by a narrow strip which is fused to sternum 3, its boundaries marked by the antecosta of sternum 3 (Fig. 2A, B, white dashed line). At its extreme lateral margin, stn2b possesses a small apodeme for a dorsoventral muscle (Fig. 2B).

The tympanic organs (ty) are located in the space between stn2a and stn2b (Fig. 3), and are composite structures, formed ventrally by sternum 2, dorsally by the chitinous V arms, and laterally by the vertical bar (Fig. 4B).

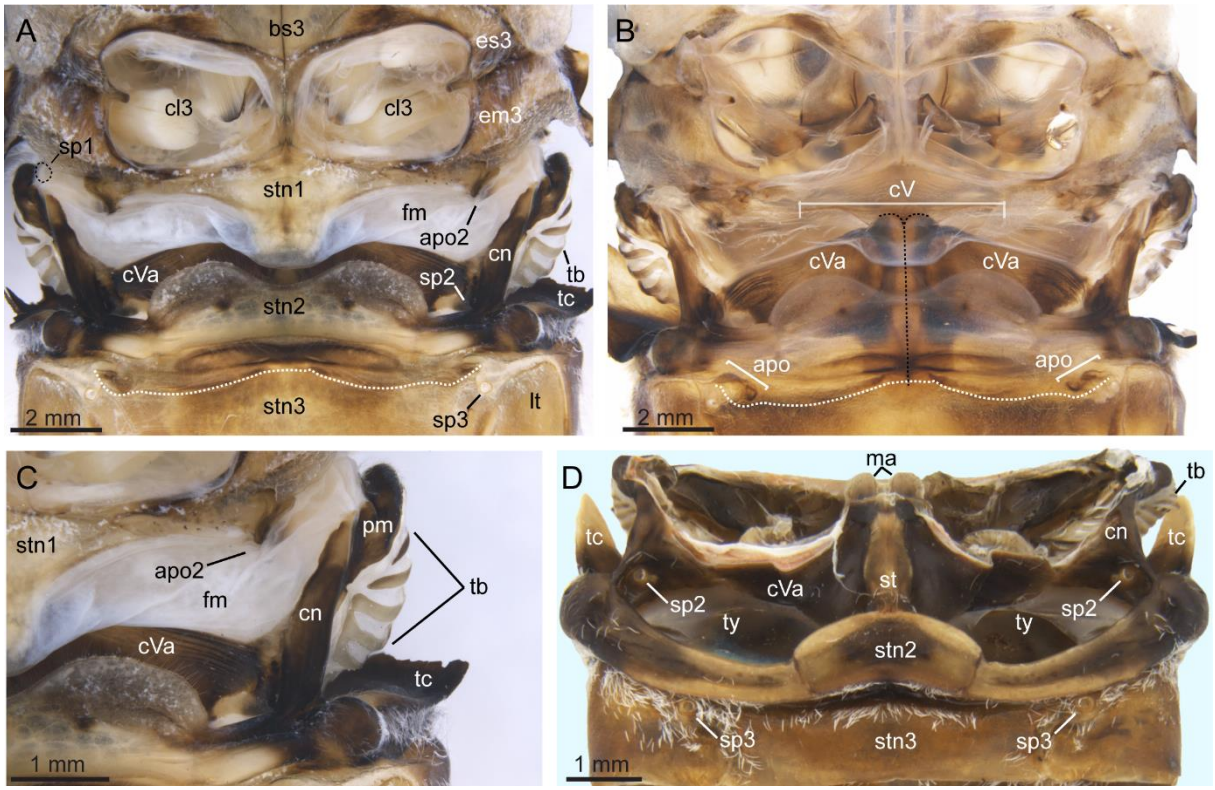

Fig. 2. Ventral abdominal segmentation in Cicadidae. A) Metathorax and abdominal sterna 1–3 of a male *Cicada orni*, the white dashed line indicating the posterior boundaries of sternum 2 (stn2); apo, apodeme; bs, basisternum, cl, coxal cleft; cn, connector of terga 1-2; cVa, chitinous-V arm; em, epimeron; es, episternum; fm, folded membrane; lt, laterotergite; sp, spiracle; stn, sternum; tb, tymbal; tc, tymbal cover; B) Same, KOH-treated specimen, showing the intact structure of chitinous V (cV), the black dashed line showing the points of fusion of the chitinous-V arms; C) Magnified view of left tymbal connector, showing how it fuses with the proximal tymbal margin (pm); D) Detailed morphology of sternum 2 and its components in a male *Oligoglena flaveola*, ma, muscle attachment of stn2a for dvmII1 (main tymbal muscle); st, stem of chitinous V, ty, tympanum. Note that the tympanum is visible only in *O. flaveola*, as it is obscured in *C. orni* by the flap-like extensions of sternum 2.

Justification for segmental interpretation: The chitinous V and the tymbal and muscle have been interpreted by most authors as parts of sternum 1 (e.g. Ossiannilsson, 1949; Pringle 1954, 1957), while Vogel (1923) considered their adjacent spiracle as the first abdominal one. We interpret these structures as part of the anterior sclerite of the second abdominal segment (stn2a), based on the following characters: 1) the origin of the metathoracic muscle IIIvlm2 marks the anterior border of segment 2 across neopteran insects (Friedrich & Beutel, 2008). In Auchenorrhyncha, its origin immediately beneath the chitinous V identifies this structure as part of the second abdominal segment; 2) muscle vlm1 inserts on stn1a and originates beneath the chitinous V, showing that the boundaries of segment 1 are located anterior to the latter structure; 3) spiracle 2 is fused to this sclerite; and 4) the tymbal muscle and all other muscles of what we identify as stn2a-b are innervated by the second abdominal nerve (section 3.1.3.; Table 2)

3.1.3. Tymbal musculature

Our proposed homologies of cicadid dorsoventral muscles with those of other Cicadomorpha are summarised in Table 2, whereas their attachments and innervation are provided in Tables 3, 4. In this section, we only mention the musculature associated with cicadid tymbals, which we observed in the male of *C. orni*.

Metathorax:

IIIvlm2 (Tables 2, 3; Fig. 3): Large and sheet-like muscle, I (=Insertion): metafurca; O (=Origin): median region of antecosta of abdominal sternum 2 (stn2a), beneath muscle attachments of chitinous V; In. (=Innervation): nerve of first abdominal segment (n. ab. 1; Tables 3, 4; Vasvary, 1966); F (=Function): Contracts the abdomen inwards, towards the metathorax (Pringle, 1954).

First abdominal segment:

vlmI1 (Tables 2, 3; Fig. 3): Small muscle, beneath IIIvlm2, I: median region of antecosta of abdominal sternum 1 (stn1a); O: median region of antecosta of stn2a, beneath muscle attachments of chitinous V; In.: n. ab. 1 (Tables 3, 4; Vasvary, 1966); F: Contracts the abdomen inwards, towards the metathorax (Pringle, 1954).

dvmI1 (Tables 2, 3; Fig. 3): Large muscle, I: distal apodeme of stn1a; O: In front of tymbal; In: n. ab. 1 (Tables 3, 4; Vasvary, 1966; Young, 1975; Wohlers et al., 1979; Wohlers & Bacon, 1980); F: Increases song amplitude by making tymbals more convex (Simmons & Young, 1978).

Muscles dvmI2–3 (Tables 2, 3), were not observed in this species.

Second abdominal segment:

vlmII1 (Tables 2, 3; Figs. 3, 4B): small, broad muscle, I: posterior margin of chitinous V base and tympanum; O: antecosta of abdominal sternum 3; In.: nerve of second abdominal segment (n. ab. 2; Tables 3, 4; Vasvary, 1966; Young, 1975; Wohlers et al., 1979); F: Creases the auditory tympanum, protecting it from acoustic damage (Pringle, 1954).

dvmII1 (Tables 2, 3; Figs. 3, 4A, B): Principal tymbal muscle, the biggest abdominal muscle, I: muscle attachment of chitinous V, on stn2a; O: posterior margin of tymbal, on tymbal plate, via a tendon; In.: n. ab. 2 (Tables 3, 4; Vasvary, 1966; Young, 1975; Wohlers et al., 1979; Wohlers & Bacon, 1980); polyneuronal innervation known from one species, where this muscle also receives a metathoracic nerve (Table 3; Vasvary, 1966); F: Buckles tymbal (Simmons & Young, 1978); Comment: Receives trachea of first abdominal spiracle.

dvmII5 (Tables 2, 3; Fig. 3): Thin, elongate muscle, I: apodeme on stn2b (apo; Figs 2B, 3); O: posterior margin of v-bar; In.: n. ab. 2 (Tables 3, 4; Vasvary, 1966); F: Unknown.

Additional muscles are known from this segment in both sexes of cicadids. Muscle dvmII6 was not observed in this species, but is known from other cicadas (Tables 3, 4; Vasvary, 1966). Tensor tympani muscles 1–3, also not observed here, are of uncertain homology and are usually resorbed in males after molting (Vogel, 1923; Pringle, 1954).

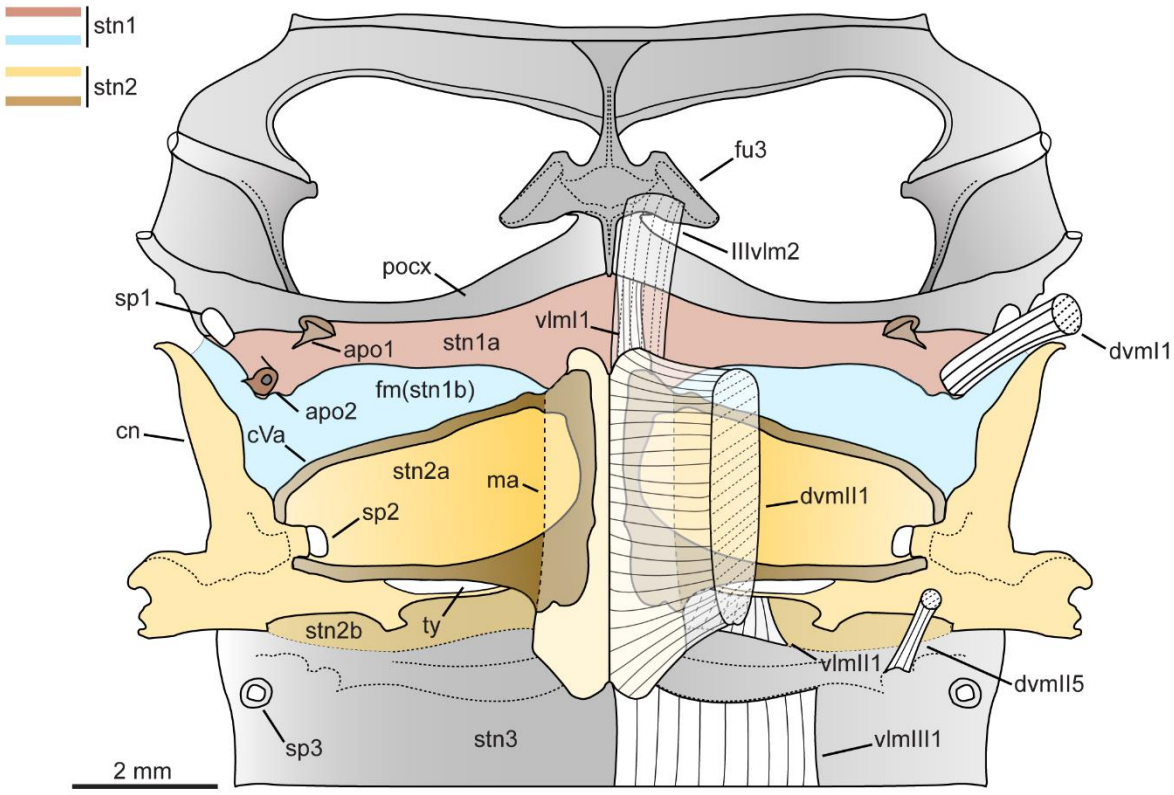

Fig. 3. Ventral musculoskeletal system of the tymbal organ of a male *Cicada orni*, sectioned just above the connector of terga 1–2. Red and blue colours demarcate components of sternum 1, while brown and yellow indicate components of sternum 2. Muscles are shown only for the right side of the abdomen, apo, apodeme; cn, connectors of terga 1–2; cVa, chitinous-V arm; dvm, dorsal longitudinal muscle; fm, folded membrane; fu, furca; ma, muscle attachment of stn2a for dvmII1 (main tymbal muscle); pocx, postcoxale; sp, spiracle; stn, sternum; stn1a: anterior subdivision of sternum 1; stn1b: posterior subdivision of sternum 1; ty, tympanum; vlm, ventral longitudinal

muscle. Note that cn, although of tergal origin, is coloured for clarity as belonging to sternum 2, due to its fusion to the latter.

3.2. Tymbals in Cercopoidea

Most exoskeletal components of the cicadid tymbal can be found in the tymbals of Cercopoidea. The following description is based on the families Aphrophoridae [species *Lepyronia coleoptrata* (Linnaeus, 1758) and *Neophilaenus campestris* (Fallén, 1805)] and Cercopidae (*Cercopis vulnerata* Rossi, 1807 and *Phymatostetha* spp.). In the Cercopoidea, tergum 1 is also bipartite: its proximal portion is membranous (mb; Fig. 5A, B), whereas its distal portion is typically sclerotized (t1; Fig. 5A, B). The sclerotized portion of tergum 1 is very narrow, especially medially (Fig. 5A). Tergum 1 is continuous with the tymbals (Fig. 5A–E), or is separated from these by a cuticular elevation (in the aphrophorid *N. campestris*, Fig. 6A). Tymbal ribs were observed in all Aphrophoridae and Cercopidae examined (Figs. 5, 6), and were arranged closely together, with no membrane-like cuticle between them (Figs. 5B, C, 6A–C), in contrast to those of cicadids (Fig. 1A–C). Ribs can be readily visible under an optical microscope (Figs. 5A–C, 6A–C), or can be very faint (in *C. vulnerata*, Fig. 5E), only visible under special illumination in dry specimens or in SR-µCT scans (Fig. 5E). We observed distinct tymbals in the examined Clastoperidae and Machaerotidae, although we were unable to observe tymbal ribs with light microscopy, possibly due to the small size of the specimens (Table 1). Tymbal plates are present in the form of flap-like plates (pl; Fig. 5B), but these are not universally present in either cercopids or aphrophorids.

A connector linking terga 1–2 is also present (Figs. 5B, E; 6A), but unlike the condition in the Cicadidae, it is not fused with the second abdominal sternum. A ridge is usually present, fused with tergum 1.

The first two abdominal sterna are similarly subdivided to those of the Cicadidae, although the apodemes for muscle dvmII1 are much smaller and not fused to each other; dvmII1 is usually the largest muscle of the abdomen, and inserts in the posterior margin of the tymbal (Fig. 4D), although other muscles may also be enlarged (e.g. dvmII5; Fig. 4D).

3.3. Tymbals in Membracoidea (excluding Deltocephalinae and Typhlocybinae)

There is considerable diversity in tymbal structure in the Membracoidea, although most of the exoskeletal components are shared with those of other cicadomorphans. Some membracoid families such as the Aetalionidae and Membracidae, possess cercopid-like

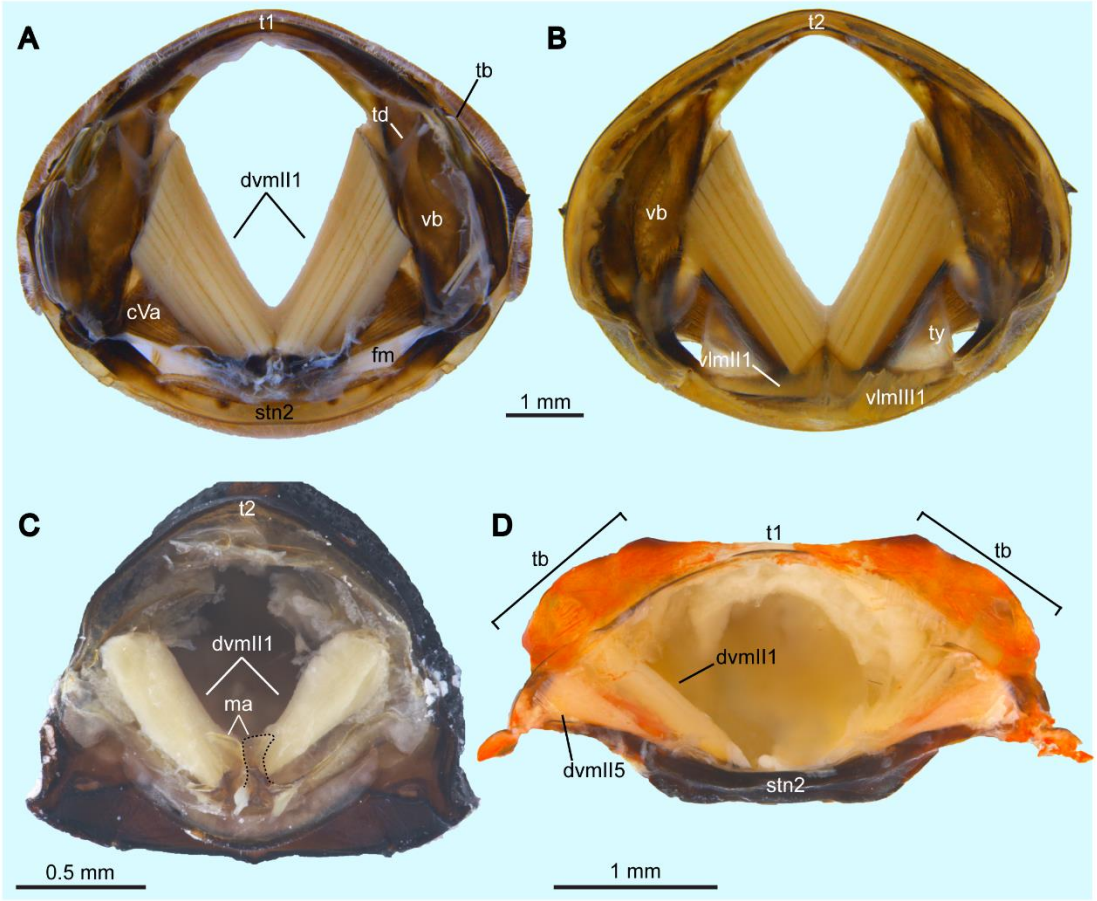

Fig. 4. Tymbal sections of various Cicadomorpha, showing the enlargement of the principal tymbal muscle dvmII1, and the modifications of its muscle attachments. A) Anterior view of the tymbal section of *Cicada orni*, showing the dorsal fusion of the chitinous-V arms (cVa) to the v-bar (vb), and the enormous dvmII1 originating from the tymbals (tb) via a tendon (td). Muscle fibres visible

on the left tymbal are part of dvmI1, which is otherwise not shown in this image, fm, folded membrane; t, tergum; stn, sternum; B) Same, posterior view, showing the tympana (ty) and the ventral longitudinal muscle of sterna 2 (vlmII1) and 3 (vlmIII1); C) Anterior view of the sectioned tymbal of *Gargara genistae* (Fabricius, 1777), showing the enlarged dvmII1 and its muscle attachments (ma), which are incompletely fused (white dashed line marking the outline of the left muscle attachment), in contrary to their cicadid homologue (the chitinous V); D) Posterior view of the sectioned tymbal of *Cercopis vulnerata*, showing the insertion of dvmII1 on the posterior margin of the semi-transparent tymbal cuticle; dvmII5 is also enlarged.

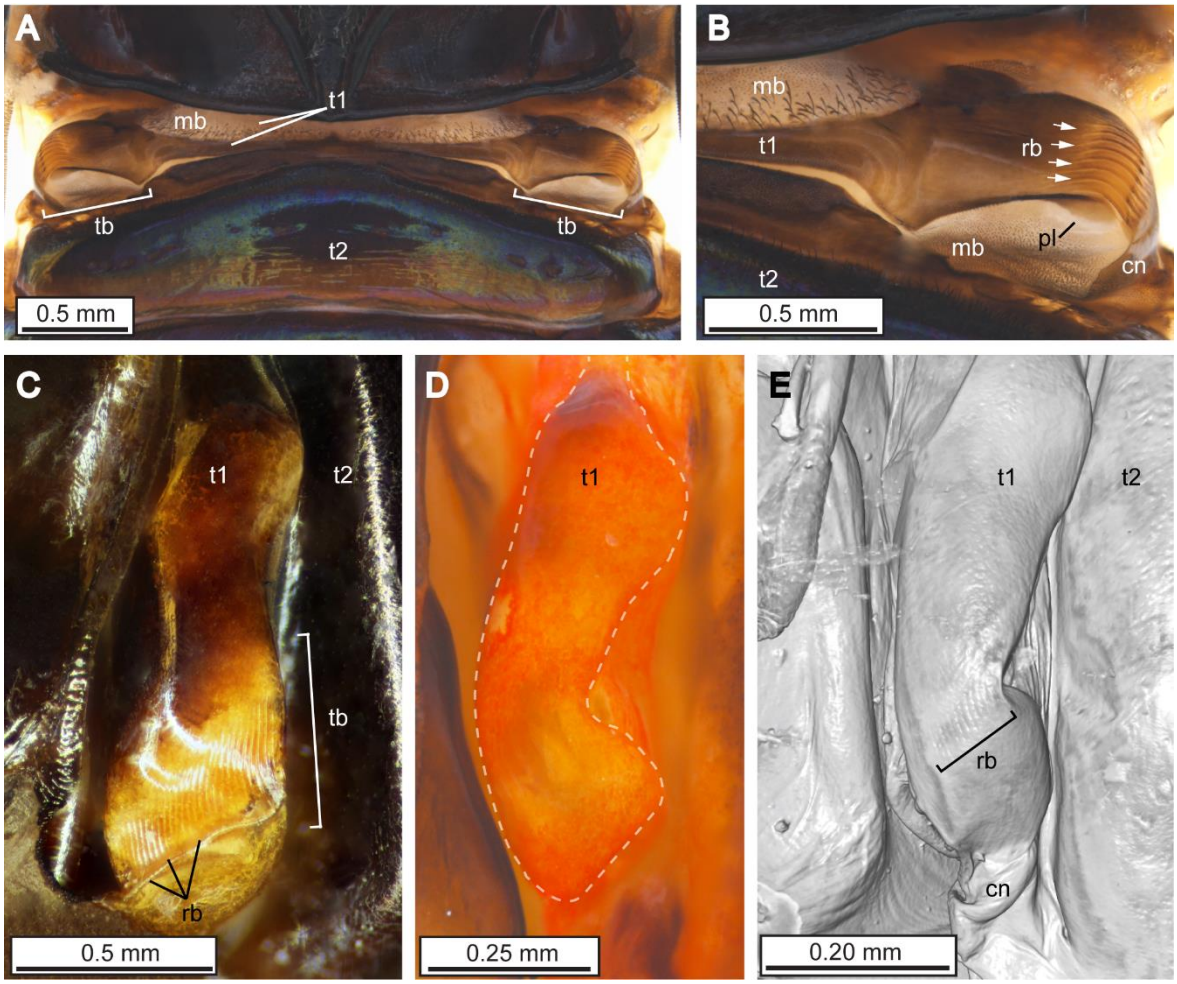

Fig. 5. Variation in tymbal structure in the Cercopidae. A) *Phymatostetha* sp., male, dorsal view, showing tymbal (tb) position, mb, membrane; t, tergum; B) Same, enlarged view of right tymbal, which possesses distinct ribs (white arrows, rb) and a flap-like plate (pl) on which dvmII1 originates; the connector (cn) of terga 1–2 is also visible; C) Lateral view of terga 1–2 in male *Ph. signifera*

(Walker, 1851), showing the large number of tymbal ribs and the continuity between the tymbal and tergum 1; D) Lateral view of tergum 1 in male *Cercopis vulnerata* under light microscopy, where the tymbal ribs are not visible; E) Same, synchrotron microtomography, volume-rendered 3D reconstruction of tergum 1, showing the tymbal ribs, which are inconspicuous in this species.

tymbals, which are ribbed and continuous with tergum 1 (*Aetalion* sp., Fig. 7A; Table 1). We note that although we documented tymbals with distinct ribs in all the membracids we examined (Table 1), Miles et al. (2017) did not find such structures in *Umbonia crassicornis* (Amyot & Serville, 1843). We also observed distinct tymbals in a family related to the Membracidae, the Melizoderidae, but we could not observe distinct tymbal ribs by using light microscopy (Table 1), although this does not mean they are not present. Other membracoid families such as the Cicadellidae are more variable. For example, the subfamily Cicadellinae contain examples of tymbals lacking obvious ribs (e.g. *Proconia* sp., Fig. 7C), and separated from tergum 1 by a narrow membrane (white arrow; Fig. 7C). A similar conformation exists in some Ledrinae, although the medial part of tergum 1 is flat and membranous, and the tymbal is continuous with the sclerotized portion of tergum 1 [*Ledra mutica* (Fabricius, 1803), Fig. 7D]. On the other hand, the condition of certain other Cicadellinae [*Cicadella viridis* (Linnaeus, 1758)] is unique, with tergum 1 differentiated into distinct lobes (Fig. 7B), which lack distinct tymbal membranes. The lateral lobe possesses faint wrinkle-like structures (dashed lines, Fig. 7B), but these cannot be identified definitively as being homologous to the ribs of other Membracoidea, and due to their small size, it is uncertain whether they are of functional importance. Tergal muscle attachments for dvmII1 are present in a form identical to the cicadid tymbal plate in the Membracidae (present study, not shown; Ossiannilsson, 1949), yet in other families, their structure is similar to the plates found in Cercopoidea, if at all present. A connector linking terga 1–2 is

usually present, and as in Cercopoidea, it is not fused to the abdominal sternum (cn; Fig. 7A, B).

In terms of musculature, the same muscles as those of Cercopoidea and Cicadoidea are present in Membracoidea (Tables 2, 3); dvmII1 is frequently the largest abdominal muscle and inserts in distinct sternal apodemes (Fig. 4C), although other muscles may be enlarged and become the principal operators of the vibrational mechanism (Ossiannilsson, 1949; Miles et al., 2017; Kuhelj et al., 2018). At least some Cicadellinae (*C. viridis*), Eurymelinae [*Idiocerus lituratus* (Fallén, 1806)] and Deltocephalinae (e.g. certain Opsiini, El-Sonbati, Wilson & Al-Dhafer, 2018) combine a tymbal-like mechanism with elongated apodemes on stn2a that support an enlarged vlmII1 (Table 1; Fig. 11A; Ossiannilsson, 1949). Some other taxa (e.g. Megophthalminae and Aphrodinae) also possess well-developed apodemes on

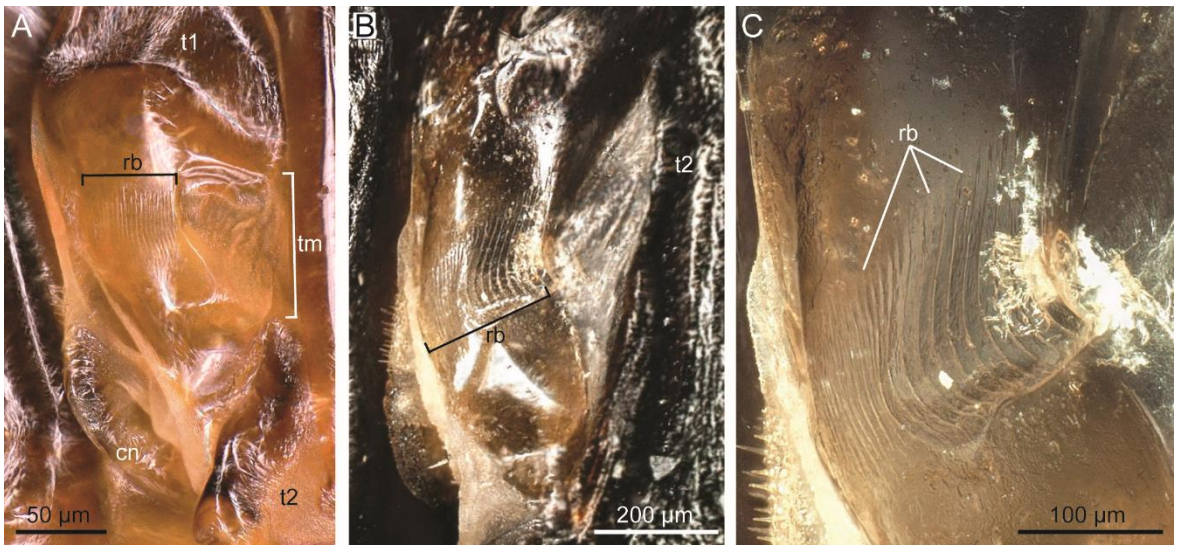

Fig. 6. Variation in the tymbals of Aphrophoridae. A) Tymbal of male *Neophilaenus campestris*, lateral view, showing a distinct tymbal membrane (tm), and a row of inconspicuous ribs (rb) confined in a small region of the latter, t, tergum; cn, connector of terga 1–2; B) Lateral view of tymbal of male *Lepyronia coleoptrata*, showing distinct ribs which cover most of its surface; C) Same, enlarged view of tymbal ribs.

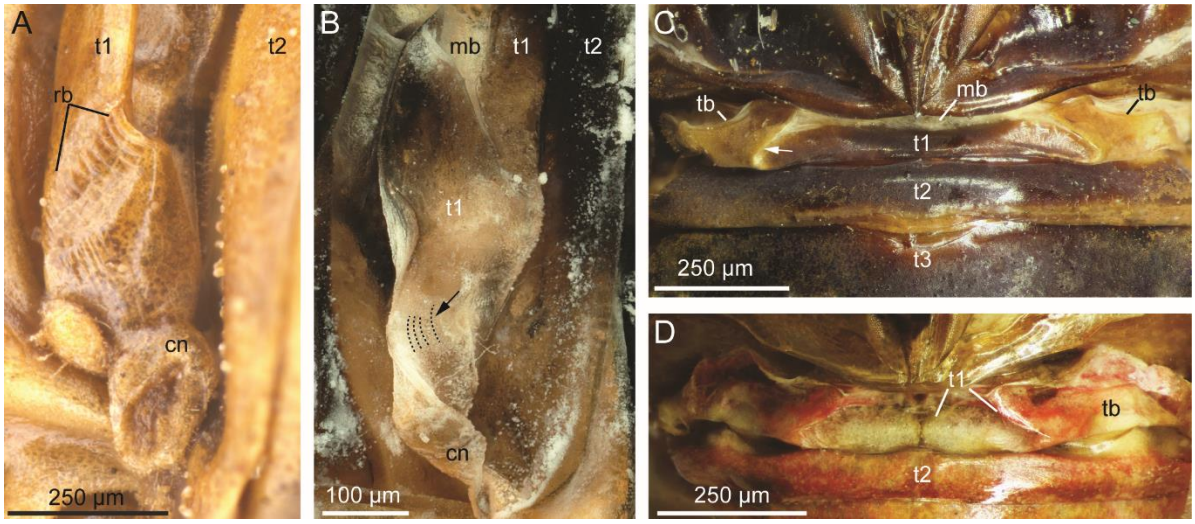

Fig. 7. Tymbals in the Membracoidea. A) Lateral view of the tymbal of *Aetalion* sp., showing the presence of distinct ribs (rb); cn, connector of terga 1–2; t; tergum; B) Lateral view of the bilobed tymbal-like organ of a male *Cicadella viridis*; the outline of faint, nearly imperceptible wrinkled structures is marked by dashed lines, and their location is indicated by a black arrow, mb, membrane; C) Dorsal view of terga 1–3 of a male *Proconia* sp., whose tymbals consist of a tymbal membrane devoid of ribs, separated from the main body of tergum 1 by a distinct membrane (white arrow); D) Dorsal view of terga 1–2 of *Ledra mutica*, whose tymbals are similar to *Proconia*, but they are continuous with tergum 1, and the median surface of the latter is membranous.

stn2a but not to the degree with those of the previously mentioned taxa, and their vlmII1 is less voluminous. The vibrational organs of the phylogenetically important Myerslopiidae are described separately (in section 3.4. below), as they have remained undocumented to date, and so merit a more extensive account.

3.4. The tymbals of Myerslopiidae

Our description provided below is based on the examination of five species of Myerslopiidae (Table 1), and provides the first documentation of the vibrational organs of this family. Although our description cites particular species to link them to the relevant photographs, it

is applicable to all the species we have examined, whose pregenital abdominal morphology is very similar.

3.4.1. Tergal structures

All pregenital terga are composed of relatively soft cuticle, as they are protected by the mesothoracic wings, which are strongly sclerotized and take the form of tegmina. The proximal portion of tergum 1 has the form of two flap-like sclerites (t1; possibly homologous to the metapostnotal sclerite of Deltocephalinae), whereas most of its median surface is membranous (mb), with a circular sclerite close to its anterior portion [*Pemmation bifurca* (Knight, 1973); Fig. 8A, black arrow]. Laterally, unribbed tymbals are present in the form of a leaf-like lobe [tb; Fig. 8A in *P. bifurca*, dashed line; 8B, in *P. variabile* (Knight, 1973)]. A connector linking tergum 1 to tergum 2 is also present (*P. variabile*; cn; Fig. B). A ridge was not observed.

3.4.2. Sternal structures

Sternum 1 could not be studied due to poor preservation. Sternum 2 is subdivided into a narrow, transparent stn2a, and a broad, strongly sclerotized stn2b [*Mapuchea chilensis* (Nielson, 1966); Fig. 8C]. Pregenital sterna 3–6 are strongly sclerotized, separated from each other by narrow membranes and are hard to tear apart using forceps in non-macerated specimens.

3.4.3. Musculature

Our reconstruction of the myerslopiid musculature is incomplete, due its poor preservation in the specimens examined, although the main muscles operating the tymbal could be observed. Our description is based on *M. chilensis*.

vlmII1 (Fig. 8D): largest abdominal muscle observed, I: posterior margin of median apodeme of stn2a; O: antecosta of abdominal sternum 3; F: unknown.

dvmII1 (Fig. 8C, D): enlarged dorsoventral muscle (but not to the degree observed in other Cicadomorpha), I: on lateral apodeme of stn2a; O: base of tymbal lobe; F: tymbal buckling (?).

dvmII3 (Fig. 8C, D): short and compact dorsoventral muscle, I: extreme lateral margin of stn2a; O: slightly lower than tymbal base; F: unknown.

dvmII5 (Fig. 8F): long and very thin muscle, I: lateral portion of stn2b; O: antecosta of tergum 2, immediately behind tymbal base; F: unknown.

Overall, the myerslopiid tymbal and its musculature, although somewhat reduced in size, do not depart significantly from the condition found in other Membracoidea.

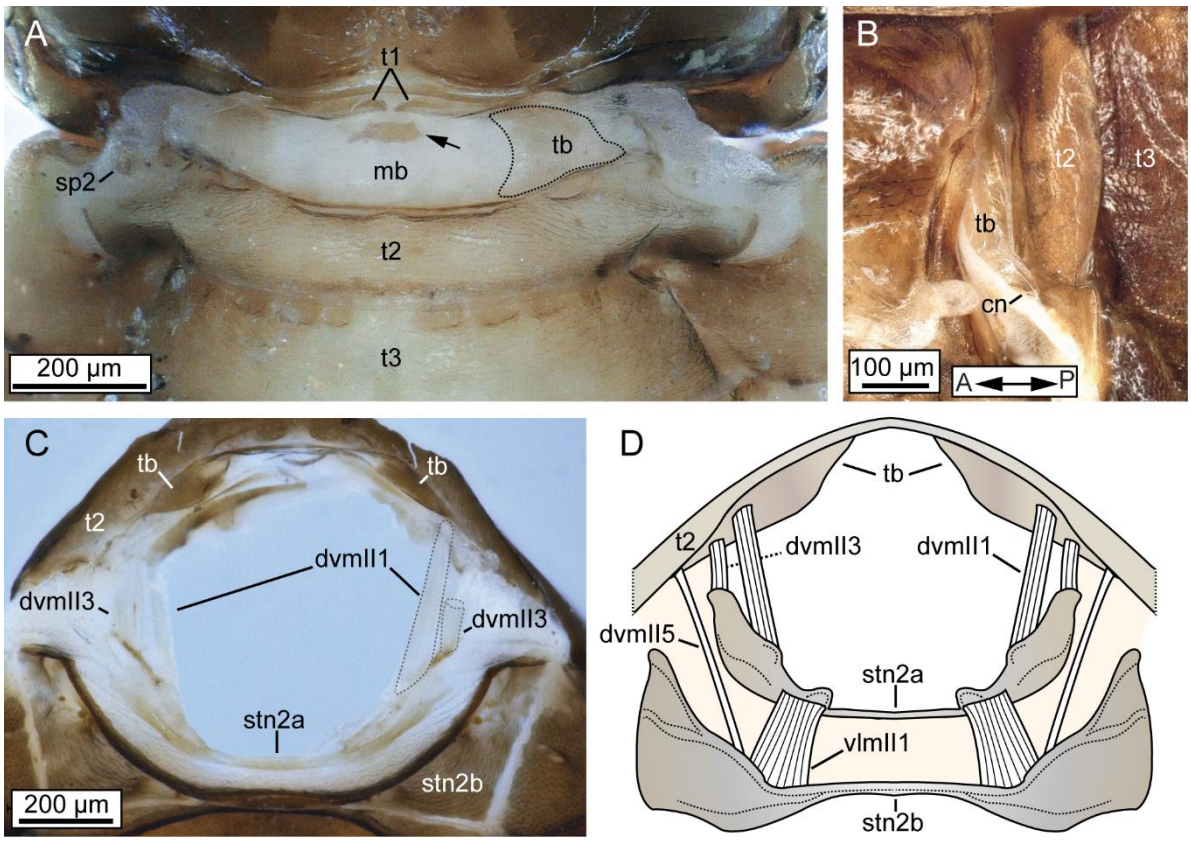

Fig. 8. The vibrational organs of Myerslopiidae. A) Dorsal view of terga 1–3 of *Pemmation bifurca*, t, tergum; tb, tymbal; mb, membrane; sp, spiracle; dashed line indicates outline of otherwise pale tymbal; arrow indicates sclerite of tergum 1; B) Lateral view of abdominal segments 1–3 in *Pemmation variabile*, cn, connector of terga 1–2; arrows indicate anterior (A) and posterior (P) parts of the body; C) Anterior section of abdominal segment 2 of *Mapuchea chilensis*, showing the tymbal musculature, dvm, dorsoventral muscle; stn2a, anterior subdivision of sternum 2; stn2b, posterior subdivision of sternum 2; dashed line of one side indicates the outline of the main dorsoventral muscles; D) Illustration of posterior section of abdominal segments 1–2 of *M. chilensis*, showing the tymbal musculature. Roughly same scale as panel C.

3.5. The vibrational organs of Deltocephalinae

The vibrational organs of Deltocephalinae are considerably modified compared to other, more generalised Membracoidea. Taxa from the deltocephaline tribes Chiasmini (*Doratura*), Eupelicini (*Eupelix*) and Penthimiini (*Penthimia*) exhibit a simpler condition, described here from *D. stylata* (Boheman, 1847). In this species, tergum 1 is largely membranous, subdivided into three basic components: 1) a median, drop-shaped sclerite (ds; Fig. 9A); 2) on the main body of tergum 1 (t1; Fig. 9A); and 3) a lateral tymbal-like lobe of tergum 1 (tbl; Fig. 9A, B), whose lower portion bears faint but distinct rib-like structures (white arrows; Fig. 9C; no inference of homology with the ribs of other Cicadomorpha). The ridge is also modified into an elongate metapostnotal sclerite (ms), which terminates close to the drop-shaped sclerite (Fig. 9A). A similar condition can be found in the genus *Aphrodes* (Ossiannilsson, 1949), where a drop-shaped sclerite and a similar rib-bearing lobe is present, contrary to Ossiannilsson (1949); see Kuhelj et al. (2018). A modified version of the deltocephaline organ can be found in the tribes Athysanini (*Euscelis*, *Macustus*), Macrostelini (*Macrosteles*), Opsiini (*Circulifer*, *Opsius*) and Selenocephalini (*Selenocephalus*). In these taxa, exemplified here by a *Selenocephalus* sp., the ridge is

modified into a Y-shaped sclerite (ms) (Fig. 9D, E), also in close contact with the drop-shaped sclerite (Fig. 9D, E). The main body of tergum 1 is strongly reduced, represented by a transverse sclerite with two spines (tr; Fig. 9E), which is connected posteriorly with a transparent portion of tergum 1. Spiracle 2 is present as a sclerite fused at the base of the tymbal-like lobe (tbl), the latter being modified into possessing gamma-shaped arms (gs) which are in close contact with the Y-shaped sclerite of tergum 1 (Fig. 9E). Tergum 2 is narrow, and its proximal margin possesses two internal apodemes (ia; Fig. 9E). There is no indication of any ribbed structure, although this could have been missed in optical microscopy. However, a SEM examination of a species of *Euscelis* did not reveal ribs (Strübing & Schwarz-Mittelstaedt, 1986).

The principal muscles operating the deltocephaline organ are the same as those of cicadomorphan tymbal organs (Table 2), and are found in similar positions (Table 3). The largest muscle is dvmII1, originating on the internal apodemes of tergum 2 (white asterisk, Fig. 9D; Ossiannilsson, 1949; Strübing & Schwarz-Mittelstaedt, 1986). The use of the deltocephaline organ in vibrational communication has been inferred based on the presence of a tymbal-like lobe which bears enlarged dorsoventral muscles, as well as recordings in biotremological studies (Ossiannilsson, 1949; Tishechkin & Burlak, 2013).

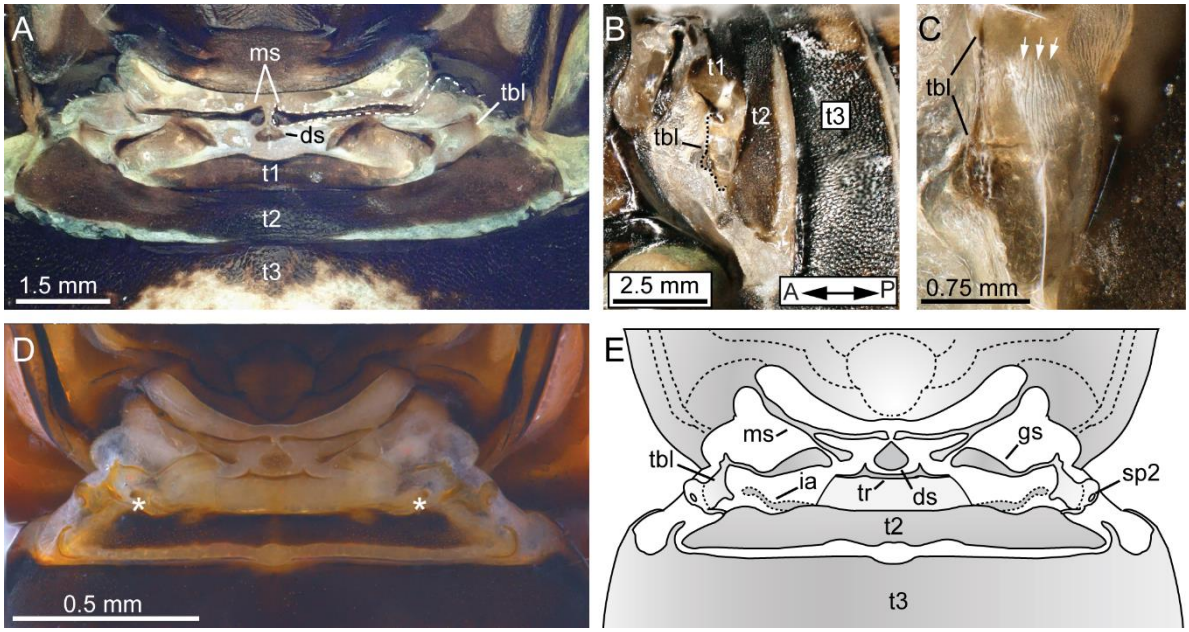

Fig. 9. External morphology of the highly modified vibrational organ of the Deltocephalinae. A) Dorsal view of terga 1–3 of *Doratura stylata*, ds, drop-shaped sclerite; ms, metapostnotal sclerite; t, tergum; white dashed line demarcates outline of ms; B) Same, lateral view of terga 1-3, black dashed line highlighting the outline of tymbal-like sclerite (tbl), arrows indicating anterior (A) and posterior (P) parts of the body; C) Same, enlarged view of tymbal-like sclerite, with white arrows indicating the position of ribs; D) Dorsal view of terga 1–3 of *Selenocephalus* sp., white asterisks indicating the insertion of dvmII1, as inferred from dissection; E) Same, illustration of the sclerites composing the deltocephaline organ, which are obscure in the light microscopy photomicrograph, gs, gamma-shaped sclerite; ia, internal apodeme of deltocephaline organ; sp, spiracle; tr, transverse sclerite of deltocephaline organ.

3.6. The vibrational organs of Typhlocybinae

The structure of typhlocybine abdominal terga 1–2 is described in Ossiannilsson (1949), who could not observe any of the defining components of tymbal organs in this subfamily. However, Shaw & Carlson (1979) suggested based on muscle attachments that the tymbal is a thin, unstriated region of the first abdominal tergite lying between a thickened region of this tergite and the thickened antecostal region of the second. Our account differs from that

of Ossiannilsson (1949), Vondráček (1949) and Shaw & Carlson (1979) mainly in the segmental identity of the ventral musculoskeletal components of the typhlocybine abdomen (Fig. 10), which are briefly described below.

3.6.1. Abdominal apodemes

The anterior part of the first abdominal sternum (stn1a) is represented by enlarged triangular apodemes fused to the metathoracic postcoxale (red; Fig. 10), whereas its posterior part (stn1b) is entirely membranous, its boundaries with sternum 2 being undefined. The anterior part of the second abdominal sternum (stn2a) is transverse, provided with a dorsad-facing apophysis, whose proximal margin is less sclerotized and flexible (stn2a apoph; Fig. 10). A small, rounded sclerite of uncertain segmental identity (scl; Fig. 10) is closely associated with stn2a. Stn2b is linked to stn2a by a pair of sternal arms (sa; Fig. 10), and is adjacently provided with a pair of dorsad-facing triangular apophyses (stn2b apoph; Fig. 10). The posterior margin of stn2b is greatly expanded posteriorly to form two enormous tongue-like apodemes (apo; Fig. 10), which can extend down to the 6th abdominal segment. The inner proximal margin of each apodeme is modified into an acute process (prc; Fig. 10).

The size and structure of the apodemes of stn2b exhibit considerable interspecific variation, being extremely reduced in some taxa (e.g. *Luodianasca*, *Zygina*) (Ossiannilsson, 1981; Qin et al., 2014).

3.6.2. Musculature

The musculature operating the typhlocybine vibrational organ is shown in Fig. 10, and the homologies and attachments of the dorsoventral muscles are provided in Tables 2 and 3. Most of the dorsoventral muscles are reduced, whereas the ventral longitudinal muscles of stn2b (vlmII1) are greatly enlarged (Fig. 10). The principal tymbal muscle dvmII1 of other cicadomorphans is reduced in typhlocybines and is present only in the females of the

examined species (its position marked by a black arrow in Fig. 10; Table 2). The use of the typhlocybine organ as well as the dorsoventral muscles in vibrational communication has been inferred by the enlargement of certain muscles (dvmII4; vlmII1; Fig. 10) and actual recordings of their songs (Shaw, Vargo & Carlson, 1974; Tishechkin & Burlak, 2013).

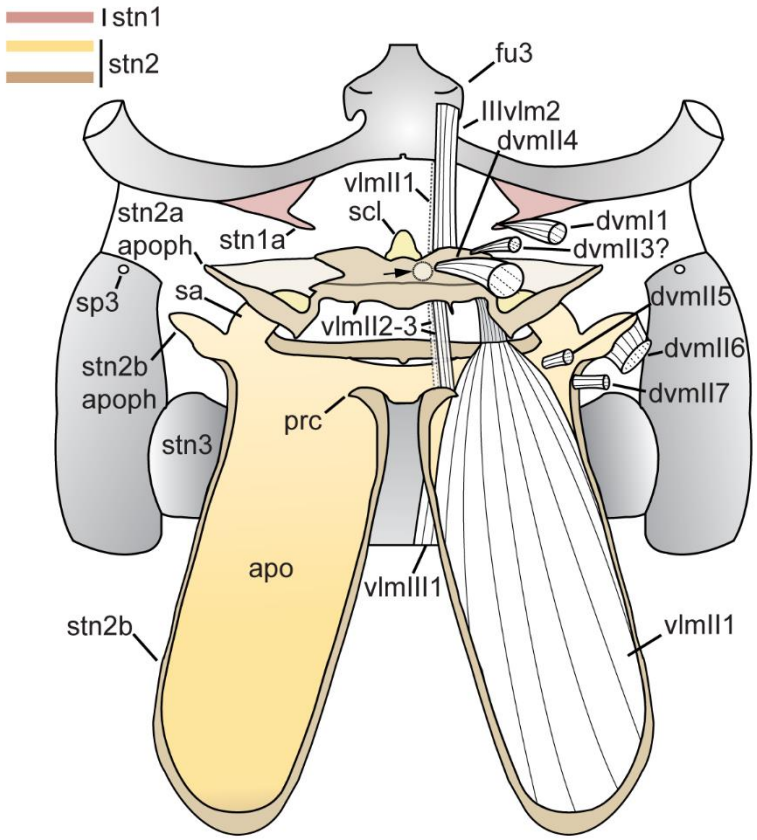

Fig. 10. Ventral musculoskeletal system of the typhlocybine organ, here shown in a male *Eupteryx atropunctata* (modified from Ossiannilsson, 1949). Red colour demarcates components of sternum 1, while brown and yellow indicate components of sternum 2. The black arrow indicates the position of dvmII1, which is present only in females. Muscles are shown only for the right side of the abdomen, apo, apodeme; apo, apophysis; dvm, dorsoventral muscle; fu, furca; prc, process of stn2b; sa, sternal arm of stn2b; scl, sclerite of typhlocybine organ; sp, spiracle; stn, sternum; vlm, ventral longitudinal muscle.

3.7. Muscle homologies across Cicadomorpha and Fulgoromorpha

The homologies of auchenorrhynchan abdominal muscles with those recognized and illustrated by previous authors are shown in Table 2, while their attachments and innervation are provided in Tables 3, 4. Although we based our homologisation on conspicuous similarities in muscle attachment and innervation (Fig. 12; Table 3), comparative studies across different taxa and developmental stages will still be needed to confirm our interpretations.

Homologisation of the ventral longitudinal muscles was fairly straightforward (Table 2), although muscles vlmII2–3 of Typhlocybinae could not be found in any other auchenorrhynchan. The study of dorsoventral muscles was particularly challenging, as they may be poorly preserved, hard to observe, or absorbed in different taxa in the adult stage (e.g. tensor tympani muscles are usually absorbed in male cicadas after their final molt; Pringle, 1957).

We documented a maximum of 3 dorsoventral muscles from stn1a (dvmI1–3), 4 from stn2a (dvmII1–4), and 3 from stn2b (dvmII5–7) (Table 2). Due to their complexity, the homologies of dorsoventral muscles are described in more detail in the sections below, with the terminology of Ossiannilsson (1949) and Davranoglou et al. (2019c) in brackets (O, D, respectively):

3.7.1. Sternum 1 (stn1a)

dvmI1 (O: Iadvm3; D: Idvm): In both Cicadomorpha and Fulgoromorpha, it originates on the anterior margin of the base of tergum 1, i.e. the tymbal (Fig. 12A) and the snapping organ Y-lobe (Fig. 12B). The muscle Ossiannilsson (1949) termed Iadvm3 in *Neophilaenus* is not the same with the muscles of the same name found in all other Cicadomorpha he examined (Table 2).

Note: Muscles dvmI2–3 (not shown here) have so far been found only in Cicadidae (Table 2).

3.7.2. Sternum 2 – stn2a

dvmII1 (O: Iadvm1; D: IIedvm1): In Cicadomorpha it typically originates on the posterior margin of the tymbal area at the base of tergum 1 (Fig. 12A), while in Fulgoromorpha it originates posteriorly to the anterior margin of the connector (Fig. 12B; Davranoglou et al., 2019c).

dvmII2 (O: Iadvm2; D: IIidvm1): In Cicadomorpha it invariably originates on the ridge (Fig. 12A). In Fulgoromorpha, this muscle originates on the membrane in front of the base of tergum 1 (Table 3; Fig. 12B; Davranoglou et al., 2019c).

dvmII3 (O: Iadvm/Iadvm4; D: IIedvm2): It originates at the base of tergum 1 next to the connector in Cicadomorpha (Fig. 12A), while in Fulgoromorpha it originates on the posterior margin of the connector, next to dvmII1 (Fig. 12B).

dvmII4 [O: Iaism; D: IIidvm2 (?)]: It has been found only in the Typhlocybinae in Cicadomorpha, where it originates on the antecosta of tergum 2, above spiracle 2 (Ossiannilsson, 1949; Table 3). A possible homologue of this muscle originates on the posterior margin of tergum 2 of Fulgoromorpha (Fig. 12B; Davranoglou et al., 2019c), although its identity remains ambiguous.

3.7.3. Sternum 2 – stn2b

dvmII5 (O: IIadvm; D: -): This is an easily identifiable muscle across Cicadomorpha, invariably found on the posterior margin of tergum 2, close to spiracle 2. This muscle has not been found in any of the Fulgoromorpha examined by Davranoglou et al. (2019c).

dvmII6 (O: IIaism/IIaism2; D: IIisdvm): An intersegmental muscle, which invariably originates at the base of the antecosta of tergum 3, in both Cicadomorpha and Fulgoromorpha (Fig. 12A, B; Table 3; Davranoglou et al., 2019c).

dvmII7 (O: IIaism1; D: -): Among Cicadomorpha, it has only been found in Typhlocybinae, where it originates on the antecosta of tergum 3, above the origin of dvmII6. A muscle which has been found in all SRμ-CT-examined fulgoromorphan specimens from the dataset of Davranoglou et al. (2019c), but was not described in that study, originates on a similar position, but above the insertion point of dvmII6 (Fig. 12B), is tentatively homologised with the typhlocybine dvmII7. However, the segmental identity of this muscle is ambiguous, as it inserts on the membrane separating stn2b from sternum 3 (Fig. 12B).

Note: In the Cicadidae, dorsoventral muscles other than dvmI1, dvmII1 and dvmII5 are difficult to homologise with those of other Cicadomorpha, due to the extreme expansion of the chitinous V (Fig. 2B; Fig. 3), which has drastically altered the structure and attachment sites of stn2a (Fig. 3). As a result, the tensor tympani muscles of cicadas are of uncertain homology, and were not mentioned in the above list. Tensors tympani 2–3 are present only in females, while tensor tympani 1 is present in both sexes (Vogel, 1923; Wohlers & Bacon, 1980). Based on their innervation, tensor tympani 3 is considered to represent the highly reduced dvmII1 of the females (Wohlers & Bacon, 1980), whereas tensor tympani 2–3 could be homologous with any of dvmII2–4.

4. Discussion

4.1. Segmental reinterpretation of auchenorrhynchan vibroacoustic organs

The fusion of the first abdominal sternum to the metathorax, and the subdivision of sterna 1 and 2 into distinct subunits (stn1a, stn1b, stn2a, stn2b), have been the source of considerable confusion for the determination of the segmental identities of the tymbal organ: Vogel

(1923) ignored stn1a, which he considered to be a part of the metathorax, and misidentified the chitinous-V (stn2a) and spiracle 2 as the first abdominal segment and spiracle

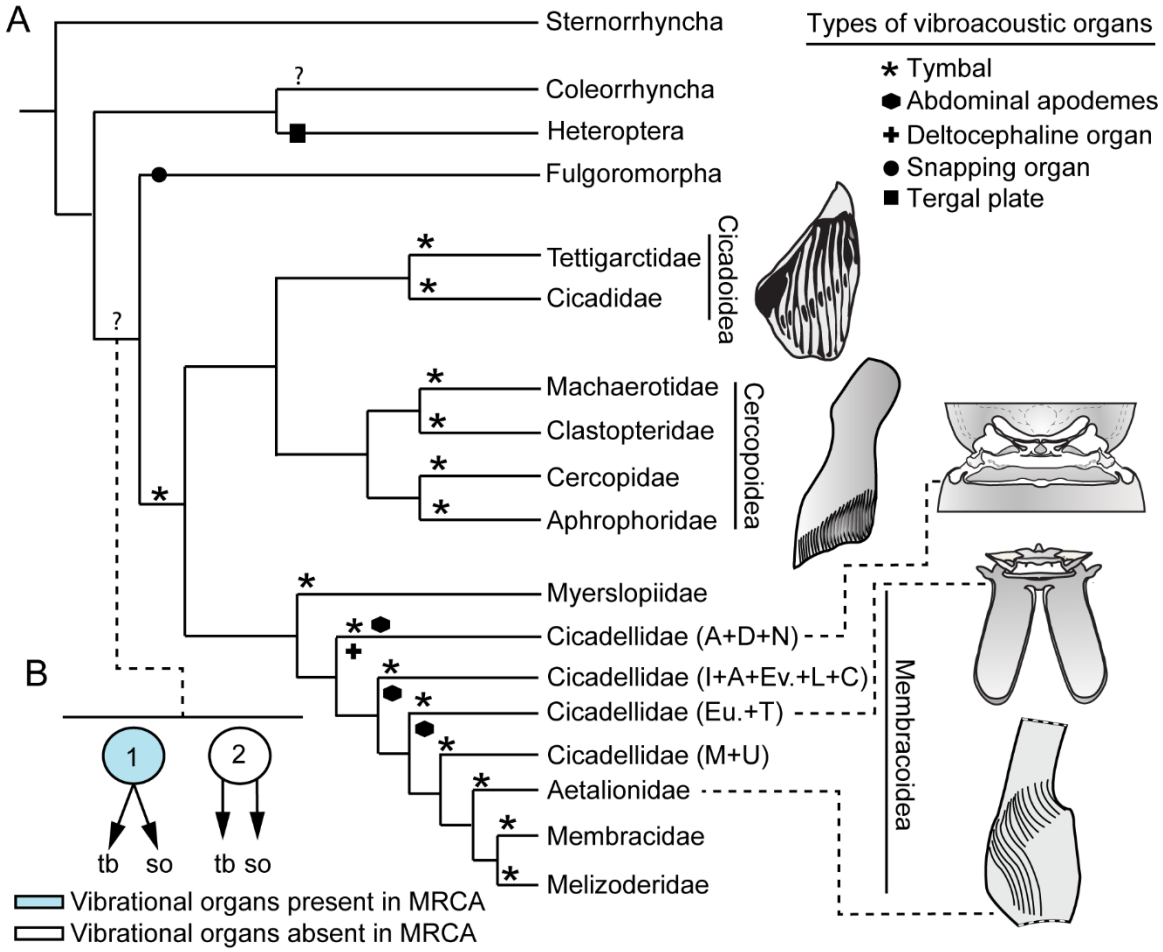

Fig. 11. The systematic distribution of hemipteran vibroacoustic organs and hypotheses on their evolution in the Auchenorrhyncha. A) Phylogeny of Hemiptera, with an emphasis in Cicadomorpha (Epipygidae not included). The systematic distribution of different types of hemipteran vibroacoustic organs is shown using different symbols (without an inference of homology). The order of each symbol does not imply an evolutionary sequence. The question marks (?) in the root of Auchenorrhyncha and Coleorrhyncha show the ambiguity regarding their vibrational organs. The phylogeny of Membracoidea is not exhaustive, only showing the major subfamilies. A, Aphrodinae; C, Cicadellinae; D, Deltocephalinae, Ev., Evacanthinae; Eu, Eurymelinae; I, Iassinae; L, Ledrinae; M, Megophthalminae; N, Neocoelidiinae; T, Typhlocybinae; U, Ulopinae. Note that Aphrodinae, Cicadellinae, and Ledrinae (the last two not shown in this simplified phylogeny) are polyphyletic.

The phylogenetic tree was constructed based on Cryan (2005), Song et al. (2017), and Skinner et al. (2019) for the relationships between the cicadomorphan superfamilies (Cercopoidea, Cicadoidea, Membracoidea) and with the other hemipteran infraorders, and Dietrich et al. (2017) for the internal phylogeny of Membracoidea. Note that the systematic position of Coleorrhyncha is ambiguous, with some studies placing them as sister to Heteroptera (Cryan, 2005), and others sister to Auchenorrhyncha (Skinner et al., 2019); B) Two mutually exclusive hypotheses on the evolution of vibroacoustic organs in the Auchenorrhyncha, based on their systematic distribution on panel A: 1) Tymbals (tb) and snapping organs (so) evolved from the same abdominal vibrational organ already present in the most recent common ancestor (MRCA) of Auchenorrhyncha; 2) The MRCA of Auchenorrhyncha lacked an abdominal vibrational organ, so tymbals and snapping organs therefore represent independent developments.

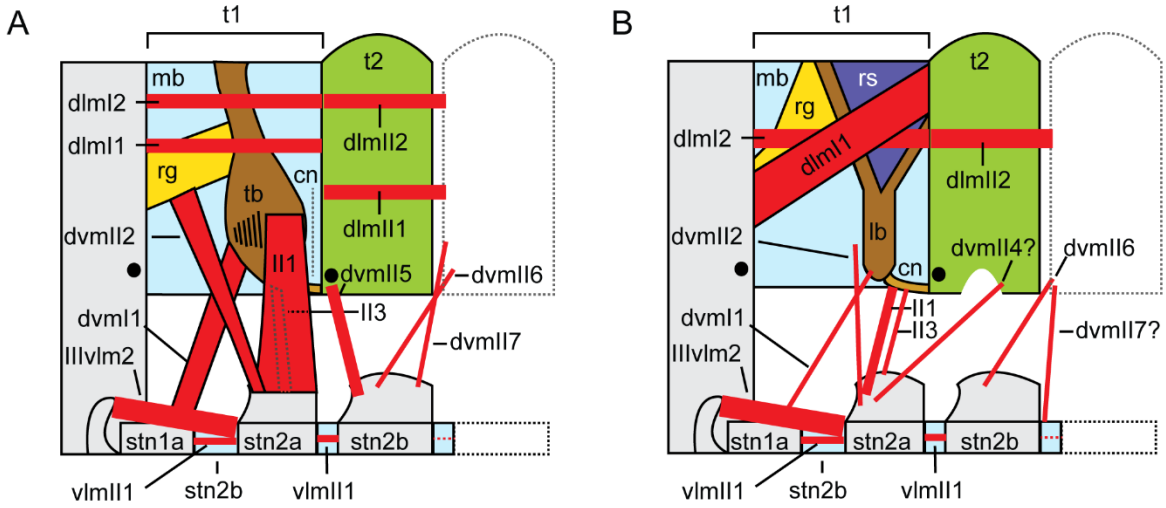

Fig. 12. Schematic illustration comparing the morphology of vibroacoustic organs of generalised Cicadomorpha and Fulgoromorpha. A) Exoskeleton and musculature of a cicadomorphan tymbal organ, showing the enlargement of dorsoventral muscles, cn, connector of terga 1–2; dvm, dorsoventral muscle; mb, membrane; rg, ridge; stn1a, anterior subdivision of sternum 1; stn1b, posterior subdivision of sternum 1; stn2a, anterior subdivision of sternum 2; stn2b, posterior subdivision of sternum 2; t, tergum; tb, tymbal; vlm, ventral longitudinal muscle; B) Exoskeleton and musculature of a fulgoromorphan snapping organ, showing the enlargement of dorsal

longitudinal muscles, rs, resilin. Note that muscle dvmII4 is not shown in Cicadomorpha. Muscles dvmII1, 3 are mentioned as II1, II3, for clarity.

respectively, stn2b as the second abdominal segment, and spiracle 3 as spiracle 2. Ossiannilsson (1949) and Vondráček (1949) considered IIIvlm2 and vlmI1 as metathoracic muscles and interpreted their insertion (antecosta 2) as the base of sternum 1, when we show here that the latter in fact represents stn2a. As a result, all muscles present on stn2a, including the primary tymbal muscle dvmII1, were misinterpreted as pertaining to the first abdominal segment (Table 2). This error was adopted and reproduced by the overwhelming majority of subsequent studies (e.g. Pringle, 1957; Wessel et al, 2014; Miles et al., 2017; Kuhelj et al., 2018). Pringle (1954) suggested that the tymbal muscles and the chitinous V represented a posteriorly transposed portion of the metathorax, although he later reappraised this theory and adopted the interpretation of Ossiannilsson (1949).

In this study, following a detailed examination of the exoskeleton, musculature and innervation, we have provided substantial evidence for a re-evaluation of the segmental affinities of cicadomorphan vibroacoustic organs. We have shown that cicadomorphan sterna 1–2 are subdivided, their anterior-most components (stn1a, stn2a) receiving the spiracle corresponding to their ascribed segmental identity (spiracles 1 and 2, respectively). Furthermore, our segmental interpretations are supported by the innervation of the muscles and sensory organs: nerves of the first abdominal segment (n. ab. 1) supply the muscles (Table 3) and chordotonal organs (Pringle, 1954; Young, 1975) of stn1a, and the second abdominal nerve (n. ab. 2) supplies its corresponding segment as well (Table 3). The nerves can be reliably identified as belonging to segments 1–2, as their sensory fibres are arranged in the mesothoracic ganglion in a way that reflects their metameric organization, i.e. they lie in the neuromere of the segment they innervate (Wohlers et al., 1979; Wohlers & Bacon, 1980). Pringle (1954) stated that dvmII1 shares a nerve which innervates the first abdominal

spiracle, although this was disproven by Vasvary (1966), who found that spiracle 1 is clearly innervated by n. ab. 1, whereas dvmII1 and the second abdominal spiracle are innervated by n. ab. 2. In terms of musculature, metathoracic muscle IIIvlm2 is a reliable indicator of segmental identity, as in most insects, it inserts on sternal antecosta 2, which marks the posterior boundary of sternum 1 (Friedrich & Beutel, 2008, supplementary table; Davranoglou et al., 2017, 2019c). The insertion of this muscle on the anterior margin of the chitinous V suggests that it is indeed the proximal portion of sternum 2 (stn2a), providing further support for our interpretations.

We should note that the (largely ignored) studies of Snodgrass (1933) and Kramer (1950) on the musculoskeletal system, and Wohlers & Bacon (1980) on innervation, are congruent with the conclusions drawn from this study, and with Davranoglou et al. (2019c) on Fulgoromorpha.

4.2. Ultrastructure of cicadid tymbal muscles

Abdominal muscles in insects typically contain a 6:1 actin:myosin ratio (Toselli & Pepe, 1968; Chapman, 2013), while wing muscles are characterized by a 3:1 ratio (Nahirney et al, 2006). Interestingly, the main tymbal muscle dvmII1 in cicadas (whether synchronous or asynchronous) exhibits the 3:1 actin:myosin ratio characteristic of wing muscles and expresses wing-like myofibrillar proteins (Josephson & Young, 1981; Nahirney et al, 2006; Iwamoto, 2017), which could be interpreted as providing support for Pringle's (1954) hypothesis which states that this muscle is of metathoracic origin. However, based on our rejection of this hypothesis on musculoskeletal grounds, we instead suggest that this wing-like ratio represents a convergent adaptation, indicating that muscle ultrastructure may exhibit substantial evolutionary plasticity when required to fulfil biomechanical demands which differ from its original function (Iwamoto, 2017).

4.3. Systematic distribution of cicadomorphan vibroacoustic organs and evolutionary implications

By mapping the distribution of vibroacoustic organs on the cicadomorphan phylogeny, we found that tymbal or tymbal-like organs are ubiquitous in the infraorder, and that specialised mechanisms such as the deltocephaline organ likely originate from a tymbal-like precursor (Fig. 11A). An exception to this may be the typhlocybine organ, whose sternal abdominal apodemes are unlikely to have derived from tymbals, although they may be supplemented by a tymbal-like mechanism, based on certain enlarged dorsoventral muscles (dvmII4; Fig. 10) and reports of a tymbal membrane (Shaw & Carlson, 1979). Further support stems from the systematic position of Typhlocybinae, which are nested within "tymbaled" clades, and it is likely that their common ancestor possessed a tymbal or tymbal-like organ, which later became lost or modified, with the sternal abdominal apodemes becoming the principal vibrational mechanism. Some Cicadellinae (in conjunction with a tymbal mechanism; Table 1) and Deltocephalinae also possess enlarged abdominal apodemes (Fig. 11A), although it remains to be determined whether they evolved independently from those of the Typhlocybinae, as they are present in only a subset of the taxa in these subfamilies (Table 1), their musculature has not been examined in a comparative manner, and Cicadellinae have been shown not to be monophyletic (Dietrich et al., 2017). It should also be noted that the Eurymelinae (= Idiocerinae), which were placed as the sister-group to Typhlocybinae in the recent molecular phylogeny by Dietrich et al., 2017, also possess relatively well-developed apodemes (see Ossiannilsson 1949, 1981).

Based on parsimony, the presence of a tymbal or tymbal-like organ in the common ancestor of Cicadomorpha is likely (Fig. 11). This finding raises the question of the evolutionary relationships between two biomechanically distinct mechanisms, the cicadomorphan

tymbals and the fulgoromorphan snapping organs – specifically, did these two distinct organs evolve independently from each other, or from a shared ancestral mechanism?

To answer this question, it is necessary to reassess the similarities and differences between the two mechanisms. If our proposed dorsoventral muscle homologies are accurate (Table 2), then it is clear that the two infraorders share a similar bauplan in terms of the sets of muscles on the first two abdominal segments (Fig. 12), which reflects their likely sister-group relationship (Cryan & Urban, 2012; Song et al., 2017; Skinner et al., 2019). However, the two infraorders have exploited the morphology of the same segment in fundamentally different ways: in Cicadomorpha the *lateral* portion of tergum 1 has been modified into a typically ribbed tymbal which contains resilin at least in cicadas (rs; Figs. 13A, 14A) (Young & Bennet-Clark, 1995, or a depigmented deformable area with rib-like wrinkles, which is primarily operated by enlarged *dorsoventral muscles* (Fig. 12A). In contrast, in Fulgoromorpha, the *entirety* of tergite 1 is modified into a Y-shaped lobe (lb; Figs. 12B, 13B, 14B), the arms of which are separated by a resilin membrane and snap together suddenly following contraction of hypertrophied *dorsal longitudinal muscles* (Fig. 12B; note that the dorsoventral muscles are not enlarged) of the first abdominal segment, thereby

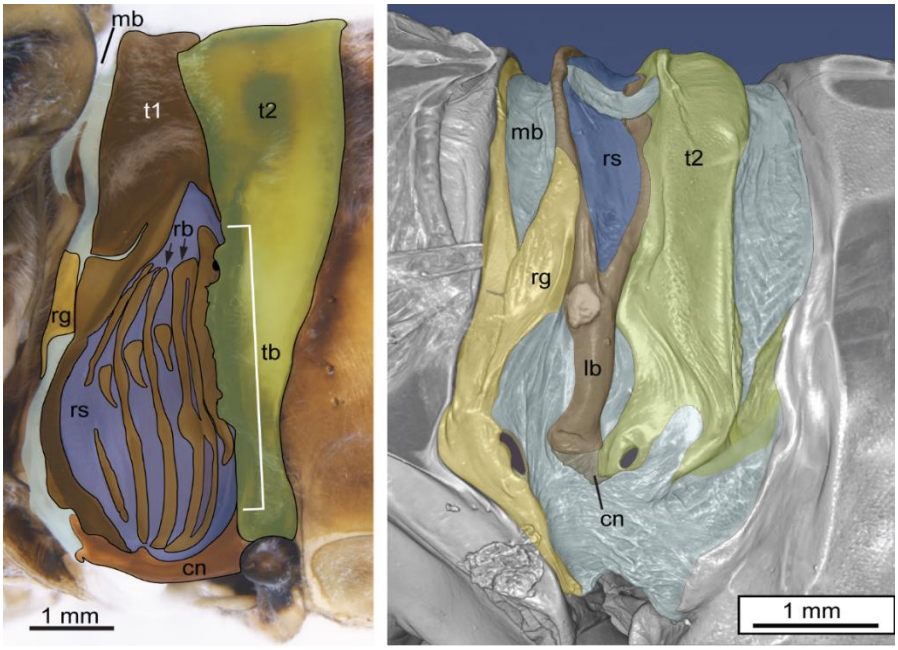

Fig. 13. Lateral view comparison of the vibroacoustic organs of Cicadomorpha and Fulgoromorpha, false-coloured SR-µCT 3D volume-rendered reconstruction. A) Tymbals of *Cicada orni*, cn, connector of terga 1–2 (light brown); mb, membrane; rb, tymbal ribs (indicated by arrow; light brown); rg, ridge (yellow); rs, resilin; t, tergum (tergum 2 green); tb, tymbal; B) Snapping organ of *Agalmatium bilobum* (Fieber, 1877), lb, Y-lobe of snapping organ.

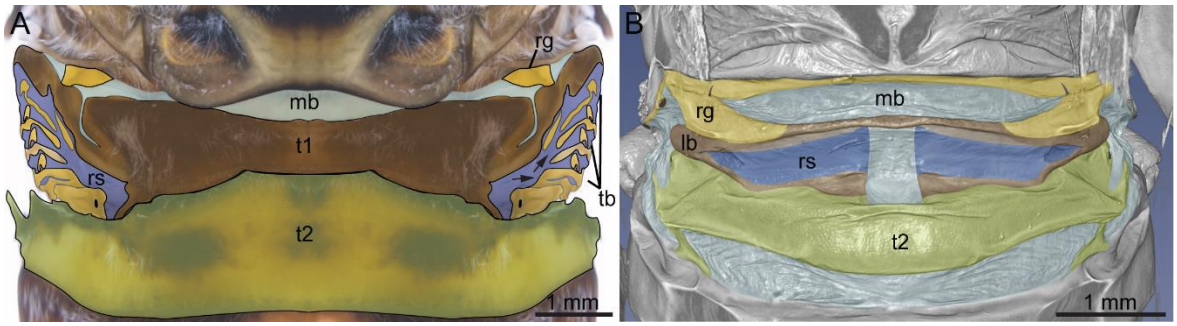

Fig. 14. Dorsal view comparison of the vibroacoustic organs of Cicadomorpha and Fulgoromorpha, false-coloured SR-µCT 3D volume-rendered reconstruction. A) In Cicadomorpha, the lateral lobe of tergum 1 (t1) is modified into a vibroacoustic tymbal organ, exemplified here by *Cicada orni*, mb, membrane; rb, tymbal ribs (indicated by arrow; light brown); rg, ridge (yellow); rs, resilin; t2, tergum 1, green); tb, tymbal; B) In Fulgoromorpha, the entirety of tergum 1 is modified into a Y-shaped lobe (lb), known as a snapping organ, exemplified here by *Agalmatium bilobum* (Fieber, 1877).

raising the entire abdomen in a snapping motion that is biomechanically distinct from the more localised buckling mechanism of a tymbal organ (Davranoglou et al., 2019c).

Certain taxa oppose the general pattern of muscle development observed within their respective infraorder (i.e. Fulgoromorpha versus Cicadomorpha), although these exceptions are rare, and likely represent secondary modifications (Fig. 11). For example, whereas it is usually the dorsoventral muscles that are hypertrophied in the cicadomorphan tymbal organ, the ventral longitudinal muscles are hypertrophied in Typhlocybinae. Conversely, whereas it is only the dorsal longitudinal muscles that are usually hypertrophied in the fulgoromorphan snapping organ, the dorsoventral muscles are also hypertrophied in most

Delphacidae (Davranoglou et al., 2019c). Furthermore, in certain Cicadomorpha, tergum 1 is medially membranous and looks superficially similar to a snapping organ Y-lobe (e.g. Fig. 7D, Ledrinae). It is unlikely to function as such, however, because the associated dorsal longitudinal musculature is of normal size, whereas the dorsoventral muscles are enlarged, as expected from a tymbal-like mechanism (Fig. 7D; Ossiannilsson, 1949). Moreover, the membrane between abdominal terga 1–2 in these Cicadomorpha does not appear to have the same functionality with the resilin membrane of Fulgoromorpha (rs; Figs. 12B; 13B; 14B), which is a key functional element of the snapping organ, accommodating wholesale pitching motion of the abdomen (Davranoglou et al., 2019c).

In terms of similarities, both Cicadomorpha and Fulgoromorpha are characterised by having abdominal sterna 1–2 subdivided into distinct subunits (st1a, stn1b, stn2a, stn2b; Fig. 12A–B). In addition, the first abdominal tergum is linked to the metathorax by a distinct ridge (rg; Figs. 12–14), and the lateral margins of terga 1–2 are linked by connectors (cn; Figs. 12–13). However, some of these characters display subtle differences, which suggests that their homology should be further investigated. In Fulgoromorpha, the incorporation of the abdomen onto the metathorax is more complete than in Cicadomorpha, as stn1a, spiracle 2, the ridge, and the proximal portion of tergum1 are joined together into a ring-like sclerite which is entirely fused to the metathorax (yellow, Fig. 13B) (Davranoglou et al., 2019c). In the Cicadomorpha that we have examined, the situation is more variable, with the distinction between the tergal and sternal components of segment 1 which are fused to the metathorax being more clear (e.g. Figs. 1– 3), while spiracle 1 may be independent from the metathorax, or fused to it by means of a spine-like process (Ossiannilsson, 1949).

Although we have presented morphological similarities and differences between the vibroacoustic organs of Cicadomorpha and Fulgoromorpha, it is clear from the above account that the homologies of their basic components are far from resolved. The

homologies of auchenorrhynchan vibrational organs to those of their sister groups, Heteroptera and/or Coleorrhyncha, are even murkier. Heteroptera have a strongly reduced sternum 1, their abdominal sterna are much broader, and the musculature is greatly simplified; terga 1–2 are closely associated and form a simple tergal plate, which is devoid of either a buckling membrane or a Y-lobe (Davranoglou et al., 2017). The condition of Coleorrhyncha is poorly known (Hartung, 2007; Wessel et al., 2014), although an investigation of their morphology is currently under way (Davranoglou et al., in preparation). We conclude that it would be premature to homologise the snapping organs of Fulgoromorpha with the tymbal organs of Cicadomorpha, in the sense of claiming that both represent direct modifications of a single vibrational organ present in the most recent common ancestor (MRCA) of the two groups, although we expressly do not exclude this hypothesis.

On the basis of the currently available information, it is difficult to reconstruct the evolution of auchenorrhynchan vibroacoustic organs. Based on their phylogenetic distribution, we propose two alternative hypotheses, which now await testing (Fig. 11B):

Hypothesis 1. The MRCA of Cicadomorpha and Fulgoromorpha possessed a well-developed abdominal vibrational mechanism, perhaps used for abdominal tremulation, that was modified differently in each descendant infraorder (Fig. 11). Whereas Cicadomorpha became increasingly reliant on the dorsoventral muscles of this mechanism during the evolution of their tymbal organ (Fig. 12A; with some secondary reversals), Fulgoromorpha became increasingly reliant on their dorsal longitudinal muscles during the evolution of their snapping organ (Fig. 12B).

Hypothesis 2. The MRCA of Cicadomorpha and Fulgoromorpha lacked a well-developed abdominal vibrational mechanism, and tymbals and snapping organs evolved independently

(2; Fig. 11B). Given that the two infraorders are sister-groups, this is arguably a less parsimonious hypothesis. Nevertheless, parsimony does not always predict actual evolutionary pathways correctly. For example, the highly elaborated jumping mechanisms of Cicadomorpha and Fulgoromorpha are now thought to have evolved independently (Ogawa & Yoshizawa, 2017), albeit that some limited form of jumping ability may also have been present in their MRCA.

To test between these two hypotheses, it now needs to be confirmed whether the shared similarities between the two infraorders (ridge, connector, subdivided sterna 1–2) have arisen independently or only once. This could in principle be determined by more detailed morphological studies on each of these components across Auchenorrhyncha, at different stages of development, supplemented by the use of molecular markers in evolutionary developmental studies. Fossil evidence from early hemipterans could also prove pivotal in resolving their origins.

4.4. Implications for the Tymbalia hypothesis

Tymbals were traditionally associated only with the Cicadomorpha, until Wessel et al. (2014) expanded the term to include all vibroacoustic organs of Euhemiptera [a name used for the clade Auchenorrhyncha + Coleorrhyncha + Heteroptera since Zrzavý (1990), = Tymbalia of Wessel et al. (2014); see the latter paper for a detailed discussion on nomenclature], which they suggested evolved once at the root of the clade, and were operated by a set of homologous muscles.

Although our results neither confirm nor reject the Tymbalia hypothesis, we have shown both in the present study and in our own past studies (Davranoglou et al., 2019, a, c) that the muscles of Wessel et al.'s (2014) "tymbalian tymbal organ" were misidentified, and that their hypothesis would have to be re-formulated accordingly. It might be argued that

segmental misidentification of a few key muscles does not alter the main argument of Wessel et al. (2014). We would disagree with this argument, however, because if homology requires demonstration of the topographical similarity criterion of Patterson (1982), then it is clearly impossible to test the homology of ostensibly similar structures across different taxa whilst their segmental nature remains uncertain. Furthermore, segmental identity is important in determining muscle type, which may also be crucial for inferences of homology. For example, Ossiannilsson (1949) considered muscle "Iaism" (our dvmII4; Table 2) intersegmental, as it inserted on what he interpreted as sternum 1, but originated on tergum 2 (Table 3). Our finding that sternum 1 of Ossiannilsson (1949) in fact represents stn2a, renders the identification of this muscle as intersegmental erroneous, and consequently not homologous with the true intersegmental muscles found in some Heteroptera (Davranoglou et al., 2017) and Sternorrhyncha (Weber, 1935).

Based on the above, and consistent with our previous studies (Davranoglou et al., 2019a), we recommend that use of the term "tymbal organ" be restricted to Cicadomorpha for the time being, in light both of its own biomechanical distinctness and its currently unproven homology with the vibroacoustic organs of other Euhemiptera.

4.5. Towards a unifying terminology

The morphology of cicadomorphan tymbal organs (especially their sternal components) is used as standard in defining the taxonomy of the infraorder, as they usually display clear species-specific differences (e.g. Hamilton, 1980; Le Quesne & Payne, 1981; Ossiannilsson, 1981; Qin et al., 2014). In most cases, some of their components have been identified as belonging to the first abdominal segment, as indicated by the most commonly used terminology: sternal apodemes 1S and 2S (e.g. Ross, 1959; Catalano et al., 2012; Cao et al., 2019), male sternal apodemes 1 and 2 (e.g. Stiller, 2016), first sternal complex and second

sternal complex (Catalano et al., 2011), and Ia and IIa apodemes (Ossiannilsson, 1949). Hamilton (1980, 1985) correctly identified stn2a as the second abdominal sternum, but interpreted stn2b as the third sternum. In this work, we have shown that all of these apodemes actually belong to the second abdominal segment, overwhelmingly on stn2a, where they support enlarged muscles dvmII1-dvmII3, or the posteriorly enlarged apodemes on stn2b, which support hypertrophied ventral longitudinal muscles.

Based on our reinterpretation of the cicadomorphan abdomen, the aforementioned terminology should be updated as follows: sternal apodemes 1S and 2S become sternal apodemes 2Sa-2Sb; male sternal apodemes 1 and 2 become male sternal apodemes 2a and 2b; the first sternal complex becomes the second sternal complex; and Ia apodemes becomes IIa apodemes 1 (for the apodemes of stn2a, as IIa apodemes is already preoccupied) and IIa apodemes 2 (for the apodemes of stn2b). Even so, we consider that the use of multiple terms to describe homologous and morphologically similar structures is unnecessary, and can be avoided by taking the following re-interpreted terms of Ossiannilsson (1949) as standard: 1) apodeme of sternum 2a (stn2a apod.): typically medially located, cup-shaped muscle attachments for muscle vlmII1, which sometimes may be overtaken by dvmII1–2. Muscles IIIvlm2–vlmI1 also insert on their anterior surface; 2) apophysis of sternum 2a (stn2a apoph.; Fig. 10): spine-like apodeme at the edge of stn2a, its extreme apex serving as the attachment for muscles dvmII3–4; 3) apodeme of sternum 2b (stn2b apod.): mediolateral apodeme, invariably serving as the attachment for vlmII1; 4) apophysis of sternum 2b (stn2b apoph.; Fig. 10): spine-like process, typically found in Typhlocyinae, where dvmII5–7 attach.

4.6. Biomechanical function

Our assumption that the tymbal and tymbal-like organs of non-cicadid Cicadomorpha utilise a cuticular buckling mechanism must be considered speculative in the majority of cases, pending behavioural studies to confirm this (but see Kuhelj et al., 2018). In any case, given the extensive morphological variation that we have observed across the Cicadomorpha, we should clearly not expect their tymbal biomechanics to be similar across the clade. This is particularly true of the tymbal organs of non-cicadid cicadomorphans: in the few cases for which their biomechanics are known, these are very different to those of the Cicadidae, even though they may share the property of producing tymbal-like clicks (Miles et al., 2017) or involve visible deformation of their tymbal membrane (Kuhelj et al., 2018). Moreover, even within Cicadidae, the biomechanics of the tymbal organs may vary considerably between taxa (Fonseca & Popov, 1994). Finally, the presence of tymbals does not exclude the participation of other parts of the body in the generation of the vibroacoustic signal (e.g. tremulation of the entire abdomen, use of enlarged abdominal apodemes, participation of additional muscles, etc.).

5. Conclusions

In the present work, we have shown that the segmental identity of key musculoskeletal components of the tymbal and ther vibrational organs of Cicadomorpha have been misidentified in most previous studies, including those that have proposed evolutionary theories regarding the origins of hemipteran vibroacoustic organs (Wessel et al., 2014). Our study provides support for a previously neglected view on the debate regarding the segmental identity of the tymbal musculoskeletal system – a topic which has puzzled scientists for more than a century. This will in turn allow future studies to make progress on fundamental evolutionary questions regarding the homologies of different hemipteran

vibroacoustic organs. Although we have documented the basic morphology of vibroacoustic organs across Cicadomorpha, we fully expect that other modifications of these organs remain to be documented, especially in the megadiverse Membracoidea. The characters that we have presented concerning the morphology of sclerites and muscles at the base of the abdomen have been largely neglected in previous morphological classifications and phylogenetic analyses (e.g. Davis, 1975; Dietrich & Deitz, 1993; Zahniser & Dietrich, 2008, 2010). Their inclusion in future studies may help to diagnose higher taxa within Cicadomorpha, and may assist in finding support for the phylogenetic relationships between them. These relationships still remain largely unresolved, despite the impressive amount of recently-acquired molecular phylogenomic data (Dietrich et al., 2017; Skinner et al., 2019). The biomechanics of non-cicadid tymbals and other vibrational organs are still poorly known (Miles et al., 2017; Kuhelj et al., 2018), and will undoubtedly be a fruitful subject for future studies.

Author contributions

L.-R. Davranoglou conceived the study, secured funding, undertook the measurements and observations, analysed and interpreted data, prepared figures, and wrote the paper. B. Mortimer and G. Taylor supervised L.-R. Davranoglou; I. Igor Malenovský contributed to the collection of morphological data with L.-R.D., including photomicrography, and contributed to data interpretation and analysis. All authors contributed to manuscript editing.

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Table 1. Species list of all 78 analysed taxa, where the type of vibroacoustic organ used is indicated for each species, along with data on individual mode of preservation, observation method, and depository. For species with tymbals, we describe whether these are ribbed or not ribbed, using a question mark (?) to indicate when the presence or absence of ribs could not be verified conclusively. We use (S#) to denote species whose photomicrographs are not shown in the main text but are provided in the Supplementary Material in the Appendix. Literature records are drawn from

Ossiannilsson (1949), Vondráček (1949), and Kuhelj et al. (2018); all other records are based on our own examination of specimens from the following depositories: BMNH, Natural History Museum, London; DPC, Davranoglou Personal Collection; MMBC, Moravian Museum, Brno; NMPC, National Museum, Prague; OUMNH, Oxford University Museum of Natural History; PI, Paleontological Institute, Moscow. Other abbreviations: SR-μCT, synchrotron radiation microcomputed tomography.

Taxon	Type of vibroacoustic organ	Preservation method	Observation method	Depository
Cercopoidea				
Aphrophoridae				
Aphrophora alni (Fallén, 1805)	tymbal (ribbed)	ethanol; dry mounted	Microscopy; Photomicrography (S1)	MMBC
Lepyronia coleoptrata (Linnaeus, 1758)	tymbal (ribbed)	ethanol; dry mounted	Microscopy; Photomicrography; Dissection	DPC; MMBC
Neophilaenus campestris (Fallén, 1805)	tymbal (ribbed)	ethanol	Microscopy; Photomicrography; Dissection	DPC; MMBC
Philaenus spumarius (Linnaeus, 1758)	tymbal (ribbed)	ethanol	Microscopy; Photomicrography (S2); Dissection	DPC
Plinia marginalis (Schmidt, 1919)	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Poophilus adustus (Walker, 1851)	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Sphodroscarta trivirgata (Amyot & Serville, 1843)	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Cercopidae				
Amberana elongata Distant, 1908	tymbal (ribbed)	dry mounted	Microscopy	BMNH
A. dimidiata (Signoret, 1860)	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Cercopis vulnerata (Rossi, 1807)	tymbal (ribbed)	ethanol	Microscopy; Photomicrography; SR-μCT; dissection	DPC
Colsa costaestriga Walker, 1857	tymbal (ribbed)	dry mounted	Microscopy	BMNH

Kanaima katzensteinii (Berg, 1879)	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Korobona lineata Distant, 1909	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Mahanarva bicolor (Signoret, 1862)	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Phymatostetha sp. Sabah_OUMNH-2013-056	tymbal (ribbed)	ethanol	Microscopy; Photomicrography	OUMNH
Phymatostetha borneensis Butler, 1874	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Ph. pahangana Lallemand, 1930	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Ph. signifera (Walker, 1851)	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Ph. stellata (Guérin-Méneville, 1844)	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Clastopteridae				
Clastoptera rufescens Fowler, 1898	tymbal (?)	dry mounted	Microscopy	BMNH
C. semivitrea Fowler, 1898	tymbal (?)	dry mounted	Microscopy	BMNH
Machaerotidae				
Blastacaena rugiceps Maa, 1963	tymbal (?)	dry mounted	Microscopy	BMNH
Chaetophyes compacta (Walker, 1851)	tymbal (?)	dry mounted	Microscopy	BMNH
Cicadoidea				
Cicadidae				
Cicada orni Linnaeus, 1758	tymbal (ribbed)	ethanol	Dissection; Microscopy; Photomicrography	DPC
Magicicada septendecim (Linnaeus, 1758)	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Oligoglena flaveola (Brullé, 1832)	tymbal (ribbed)	ethanol	Dissection; Microscopy; Photomicrography	DPC
Pomponia sp.	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Tacua speciosa (Illiger, 1800)	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Tibicen plebejus (Scopoli, 1763)	tymbal (ribbed)	ethanol	Dissection; Microscopy; Photomicrography (S3)	DPC
Tettigarctidae				
Tettigarcta crinita Distant, 1883	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Membracoidea				
Aetalionidae				

Aetalion sp.	tymbal (ribbed)	dry mounted	Microscopy; Photomicrography	OUMNH
Darthula hardwickii (Grey, 1932)	tymbal (ribbed)	dry mounted	Microscopy	BMNH
<u>Cicadellidae</u>				
Aphrodinae				
Aphrodes spp.	tymbal (ribbed)	literature record	Kuhelj et al. (2018);Ossiannilsson (1949)	-
Cicadellinae				
Acrobelus reflexus (Signoret, 1855)	tymbal (not ribbed)	dry mounted	Microscopy	BMNH
Cicadella viridis (Linnaeus, 1758)	tymbal-like organ; abdominal apodemes	ethanol	Microscopy; Photomicrography	MMBC
Dichrophleps symmetrica Young, 1968	tymbal (not ribbed)	dry mounted	Microscopy	BMNH
Proconia sp.	tymbal (not ribbed)	dry mounted	Microscopy	BMNH
Propetes compressa Walker, 1851	tymbal (?)	dry mounted	Microscopy	BMNH
Coelidiinae				
Docalidia bifurcata Nielson, 1979	tymbal (not ribbed)	glycerol	Microscopy	BMNH
Deltocephalinae				
Doratura stylata (Boheman, 1847)	deltocephaline organ with ribs	ethanol	Microscopy; Photomicrography	MMBC
Eupelix cuspidata (Fabricius, 1775)	deltocephaline organ with ribs	dry mounted	Microscopy	DPC
Macustus grisescens (Zetterstedt, 1828)	deltocephaline organ	literature record	Ossiannilsson (1949)	-
Macrosteles cristatus (Ribaut, 1927)	deltocephaline organ	literature record	Ossiannilsson (1949)	-
Opsius stactogallus Fieber, 1866	deltocephaline organ	literature record	Ossiannilsson (1949)	-
Penthimia caliginosa Walker, 1870	deltocephaline organ with ribs	dry mounted	Microscopy	BMNH

Selenocephalus bytinskii Lindberg, 1953	deltocephaline organ	dry mounted	Microscopy	BMNH
Selenocephalus sp.	deltocephaline organ	ethanol	Dissection; Microscopy; Photomicrography	DPC
Evacanthinae				
Evacanthus interruptus (Linnaeus, 1758)	tymbal (not ribbed)	ethanol	microscopy; photomicrography (S4)	MMBC
Iassinae (=Gyponinae)				
Gypona (Marganalana) signoreti Stal, 1864	tymbal (not ribbed)	dry mounted	Microscopy	BMNH
Eurymelinae (=Idiocerinae)				
Idiocerus lituratus (Fallén, 1806)	tymbal; abdominal apodemes	literature record	Ossiannilsson (1949)	-
Ledrinae				
Chatura nigella (Distant, 1908)	tymbal (not ribbed)	dry mounted	Microscopy	BMNH
Ledra aurita (Linnaeus, 1758)	tymbal (not ribbed)	dry mounted	Microscopy	BMNH
L. mutica Fabricius, 1803	tymbal (not ribbed)	dry mounted	Microscopy; Photomicrography	BMNH
Ledromorpha planirostris Donovan, 1805	tymbal (not ribbed)	dry mounted	Microscopy	BMNH
Ledropsis cancroma White, 1844	tymbal (ribbed)	dry mounted	Microscopy	BMNH
L. rubromaculata Laidlaw, 1930	tymbal (not ribbed)	dry mounted	Microscopy	BMNH
Thlasia corona (Linnavuori, 1972)	tymbal (not ribbed)	dry mounted	Microscopy	BMNH
Tituria planata (Fabricius, 1794)	tymbal (not ribbed)	dry mounted	Microscopy	BMNH
Macropsinae				
Oncopsis flavicollis (Linnaeus, 1758)	tymbal	literature record	Ossiannilsson (1949)	-
Megophthalminae				

Agallia brachyptera (Boheman, 1847)	tymbal	literature record	Ossiannilsson (1949)	-
Megophthalmus scanicus (Fallén, 1806)	tymbal	literature record	Ossiannilsson (1949)	-
Typhlocybinae				
Empoasca fabae (Harris, 1841)	abdominal apodemes	literature record	Ossiannilsson (1949)	-
Eupteryx atropunctata (Goeze, 1778)	abdominal apodemes	literature record	Ossiannilsson (1949)	-
Kybos virgator (Ribaut, 1933)	abdominal apodemes	literature record	Ossiannilsson (1949)	-
Ribautiana ulmi (Linnaeus, 1758)	abdominal apodemes	literature record	Vondracek (1949)	-
Ulopinae				
Cephalelus brevipilus Davies, 1988	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Paracephaleus hudsoni Myers, 1923	tymbal (ribbed)	dry mounted	Microscopy	BMNH
P. marginatus (Waterhouse, 1839)	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Melizoderidae				
Melizoderes darwini (Funkhouser, 1934)	tymbal (?)	dry mounted	Microscopy	BMNH
Membracidae				
Centrotus cornutus (Linnaeus, 1758)	tymbal (ribbed)	ethanol	Dissection; Microscopy	DPC
Gargara genistae (Fabricius, 1775)	tymbal (ribbed)	ethanol	Dissection; Microscopy; Photomicrography	DPC
Membracis tectigera Olivier, 1792	tymbal (ribbed)	dry mounted	Microscopy	BMNH
Stictocephala bisonia Kopp & Yonke, 1977	tymbal (ribbed)	ethanol	Microscopy	DPC
Myerslopiidae				
Mapuchea chilensis (Nielson, 1966)	tymbal (not ribbed)	ethanol	Dissection; Microscopy; Photomicrography	PI
Myerslopia magna Evans, 1947	tymbal (not ribbed)	glycerol	Microscopy	BMNH
M. triregia Knight, 1973	tymbal (not ribbed)	glycerol	Microscopy	BMNH

Pemmation bifurca (Knight, 1973)	tymbal (not ribbed)	ethanol	Microscopy; Photomicrography	MMBC
Pemmation variabile (Knight, 1973)	tymbal (not ribbed)	ethanol	Microscopy; Photomicrography	NMPC

Table 2. List of the nomenclature for muscles operating the vibroacoustic organs of Cicadomorpha and Fulgoromorpha, as described by the studies of Davranoglou et al., (2019c), Evans (1941), Ossiannilsson (1949), Pringle (1957, 1954), Vasvary (1966), Vondráček (1949) and Young (1975, 1972), homologised with the terminology applied in the present study. No single species that we examined had all of the muscles described in the table, and muscles of doubtful homology are listed with a question mark (?). Inferences of homology and segmental identity were formulated based on our observations of the musculoskeletal system and its innervation, which were examined using SR-μCT, ethanol-preserved specimens and records from the literature. En-dash (–) denotes that the character is either absent or not reported by the study in question.

Present study	Davranoglou et al. (2019c): **Fulgoromorpha**	Evans (1941): **Tettigarctidae**	Ossiannilsson (1949): **non-cicadid Cicadomorpha (several families)**	Pringle (1957, 1954): **Cicadidae & Tettigarctidae**	Vasvary (1966): **Cicadidae**	Vondráček (1949): **Typhlocybinae (Cicadellidae)**	Young (1975, 1972): **Cicadidae**
dvmI1	Idvm	-	Iadvm3 (excluding *Neophilaenus*)	tensor muscle	95	ml	tensor muscle
dvmI2	-	-	-	-	97	-	accessory tensor muscle
dvmI3	-	-	-	-	98	-	-
dvmII1	IIedvm1	TMS	Iadvm1	tymbal muscle	94	p	tymbal muscle
dvmII2	IIidvm1	-	Iadvm2	-	-	-	-
dvmII3	IIedvm2	-	Iadvm4 (Cicadellidae); Iadvm (?)(Typhlocybinae)	tensor tympani (?)	-	pe	-

dvmII4	IIidvm2?	-	Iaism	-	-	-	-
dvmII5	-	-	IIadvm	lat. m. (posterior)	100	-	lat.m.
dvmII6	IIisdvm	-	IIaism; IIaism2 (Typhlocybinae)	lat. m. (anterior)	99	qm	-
dvmII7	-	-	IIaism1	-	-	qm	-
IIIvlm2 (metathoracic)	IIIvlm2	-	IIIvlm/IIIvlm1	vlm [upper]	91	b/m	-
vlmI1	Ivlm1	-	IIIvlm2	vlm [lower]	-	m [lower]	-
vlmII1	IIvlm1	-	Iavlm	detensor muscle	93	s/o	detensor muscle
vlmII2	-	-	-	-	-	r/nn	-
vlmII3	-	-	-	-	-	q/n	-
vlmIII1	-	-	IIavlm	vent.abd.m.	-	t	-

Table 3. Generalised areas of point of origin and insertion of muscles operating the vibroacoustic organs of Cicadomorpha and Fulgoromorpha, including their innervation, as described in previous studies. Note that muscles dvmI2 and dvmI3 have so far been found only in the Cicadidae. En-dash (–) denotes that the character is either absent or not reported by the study in question. C (Cicadomorpha) and F (Fulgoromorpha) denote that the muscle or nerve in question is found in that particular infraorder.

Muscle	Origin	Insertion
dvmI1	anterior margin of basal lobe of tergum 1 (C: in front of tymbal, when present; F: base of snapping organ)	posterior margin of st1a (C: on a small apodeme)
dvmI2	C: anterolateral margin of tergum 1; F: -	C: apodeme 2 of stn1a, next to chordotonal organ; F: -
dvmI3	C: anterolateral margin of tergum 1, beneath dvmI2; F:-	C: apodeme 2 of stn1a, next to chordotonal organ; F:-
dvmII1	C:posterior margin of tergum 1, close to spiracle 2 (behind or at level of tymbal ribs, when present); F: tergum 2 apodeme next to connector	C: median muscle attachment of stn2a; F: lateral margin of stn2a
dvmII2	C: metapostnotum (ridge?); F: membrane in front of tergum 1	C: anterior margin of median muscle attachment of stn2a, in front of dvmII1; F: median muscle attachment of stn2a
dvmII3	C: posterior margin of tergum1, between spiracle 2 and insertion of dvmII1; F: tergum 2 apodeme next to connector	extreme lateral margin of stn2a
dvmII4	C: antecosta of tergum 2, above spiracle 2; F: posterior lobe of tergum 2	C, F: posterior margin of stn2a
dvmII5	C: posterior margin of tergum 2, close to spiracle 2; F: -	C: muscle attachment on stn2b; F: -
dvmII6	C, F: anterior margin (antecosta) of tergum 3	C, F: extreme lateral margin of stn2b (or its apophysis in Typhylocibinae), often on an apodeme
dvmII7	C: anterior margin (antecosta) of tergum 3, close to origin of dvmII6; F: same, but above dvmII6	C: apex of stn2b apophysis; F: membrane immediately behind stn2b

Table 4. List of previous names for nerves supplying the muscles operating the vibroacoustic organs of Cicadomorpha, as described by the studies of Pringle (1957, 1954), Vasvary (1966), Wohlers et al. (1979), and Fulgoromorpha, as described by Davranoglou et al. (2019c), homologised with the terminology applied in the present study.

Present study	Davranoglou et al. (2019c)	Pringle (1957, 1954)	Vasvary (1966)	Wohlers et al. (1979)
n.ab. 1	n.ab. 1	tensor	IIN7/IIN7a	tensor
n.ab. 2	n.ab. 2	auditory nerve	IIN8-IIN8a	auditory nerve
n.ab. 3-9	(organized into n.ab. 4-9)	abdominal nerves	IIN9	large auditory nerve

Statement of Authorship for joint/multi-authored papers for PGR thesis

To appear at the end of each thesis chapter submitted as an article/paper

The statement shall describe the candidate's and co-authors' independent research contributions in the thesis publications. For each publication there should exist a complete statement that is to be filled out and signed by the candidate and supervisor **(only required where there isn't already a statement of contribution within the paper itself).**

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Student Confirmation

Student Name:	Leonidas-Romanos Davranoglou
Contribution to the Paper	Conceived the study, secured funding, undertook the observations, analysed and interpreted data, prepared figures, and wrote the paper.

Signature

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Supervisor Confirmation

By signing the Statement of Authorship, you are certifying that the candidate made a substantial contribution to the publication, and that the description described above is accurate.

Supervisor name and title: Professor Graham K. Taylor

Supervisor comments

Signature

Date 04/11/2019

This completed form should be included in the thesis, at the end of the relevant chapter.

Chapter 7: The pregenital abdomen of Enicocephalomorpha and morphological evidence for different modes of communication at the dawn of heteropteran evolution

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Abstract

The internal and external anatomy of the posterior metathoracic region, pregenital abdomen, and associated nervous system of the heteropteran infraorder Enicocephalomorpha are thoroughly described, using an array of state-of-the art techniques. Based on morphology, it is hypothesised which modes of communication these insects use. This study is based primarily on an undescribed species of *Cocles* Bergroth, 1905 (Enicocephalidae) and another undescribed species of *Lomagostus* Villiers, 1958 (Aenictopecheidae), but additional representatives of the infraorder are also examined. Our results are compared with the literature on other Heteroptera. The metathoracic scent gland system of Enicocephalomorpha uses the same muscles as that of more derived Heteroptera, although

the efferent system is different. The presence of a tergal plate and well-developed longitudinal musculature in the families Enicocephalidae and Aenictopecheidae, as well as a sexually dimorphic set of sclerites and membranes that allow an as yet undetermined type of motion, may indicate the presence of vibrational signaling in the infraorder, although experimental confirmation is required. Our findings raise new research questions regarding heteropteran functional morphology and communication.

1. Introduction

The pregenital abdomen of Heteroptera is a remarkably adaptable piece of evolutionary machinery. Besides its primary function of maintaining the viscera in place and protecting them externally, the pregenital abdomen has been modified multiple times to support a variety of secondary functions: it may be subdivided into several sclerites that confer flexibility when the animal is engorged with food or eggs (Sweet, 1996); it may bear mate-holding devices (Leston, 1957) or eversible glands (Schuh & Slater, 1995); and it can act as a secondary site of copulation (Tatarnic et al., 2006). The pregenital abdomen may also have a sensory function, as in some taxa it possesses trichobothria, whose number and arrangement are of phylogenetic significance (Schaefer, 1975). In addition, the pregenital abdomen and metathorax can play an important role in chemical, vibrational, and acoustic communication.

Heteroptera are known for their extensive use of chemical communication by means of metathoracic scent-glands and dorsal abdominal scent-glands (Staddon, 1979; Aldrich, 1995). Such scent-gland systems have been found in all heteropteran infraorders, and represent apomorphies of the suborder as a whole (Carayon, 1971; Staddon, 1979; Wheeler et al., 1993; Schuh & Slater, 1995). Chemical signals are not the only means of communication in Heteroptera, however, and all heteropteran infraorders besides

Dipsocoromorpha and Enicocephalomorpha have been shown to use vibrational or acoustic signalling – typically using a pregenital organ known as the tergal plate (Davranoglou et al., in preparation; Gogala, 1984, 1985b, 1985a, 2006). Whether the tergal plates producing these signals across Heteroptera are homologous with the tymbal organs of Auchenorrhyncha, has been the subject of a prolonged debate (reviewed in Wessel et al., 2014), but knowledge of the morphological basis of vibrational signalling in Heteroptera is poor, and a meaningful comparison between the vibrational organs of the two suborders has not yet been made.

Here we describe the functional morphology of the pregenital abdomen of the Enicocephalomorpha, or unique-headed bugs, which arguably represent a pivotal taxon for our understanding of the evolution of different modes of communication in Heteroptera. The Enicocephalomorpha are one of the least studied heteropteran infraorders, but are of significant interest for the higher-level systematics of Heteroptera (Štys & Kerzhner, 1975; Weirauch & Schuh, 2011). Enicocephalomorphans were originally included in the Reduviidae (Usinger, 1943, 1945), but subsequent morphological (Cobben, 1968; Štys & Kerzhner, 1975; Schuh, 1986; Wheeler et al., 1993; Yoshizawa & Saigusa, 2001) and molecular (Wheeler et al., 1993; Xie et al., 2008) studies have suggested that they form a distinct infraorder, traditionally placed as the sister group to all other heteropteran infraorders. Indeed, enicocephalomorphans share certain morphological similarities with the Coleorrhyncha (Spangenberg et al., 2013b), which are placed in many, but not all, phylogenies as the sister group to Heteroptera (Wheeler et al., 1993; Xie et al., 2008; Weirauch & Cassis, 2009; Cryan & Urban, 2012; Li et al., 2015; but see Misof et al., 2014). The alternative proposal of Nepomorpha as the sister group to all other heteropteran infraorders (Mahner, 1993; Scherbakov & Popov, 2002) should be treated with scepticism, as a recent study with extensive taxon sampling of this infraorder (Wang et al., 2016) did

not recover such a placement. Further morphological and molecular studies are needed to assess the validity of these proposed relationships.

The relationship of Enicocephalomorpha with Dipsocoromorpha has been controversial since these clades first received infraordinal status. Previous studies variously treat Dipsocoromorpha as: i) the sister-group to Enicocephalomorpha, collectively forming the sister group to all other heteropteran infraorders (Miyamoto, 1961; Wang et al., 2016), ii) another clade of Heteroptera (Yoshizawa & Saigusa, 2001; Xie et al., 2008), or iii) the sister group to Leptopodomorpha + Terheteroptera (= Geocorisae) (Weirauch & Štys, 2014). It is evident that the higher-level phylogeny of Heteroptera is still in flux, and that further insights obtained from both morphological and molecular data are required to consolidate the systematic position of certain infraorders. Nevertheless, given that Enicocephalomorpha (with or without Dipsocoromorpha) are putatively placed as the sister group to all other Heteroptera, it follows that examination of the enicocephalomorphan pregenital abdomen, metathoracic glands, and associated innervation may offer valuable insight for future systematic studies investigating the evolution of the abdominal morphology, musculature, and nervous system of Heteroptera as a whole. Furthermore, given the known phylogenetic distribution of vibrational signaling in Heteroptera, it is reasonable to expect that the pregenital morphology of Enicocephalomorpha and Dipsocoromorpha may provide the key missing information needed for future systematic studies to test the evolutionary origins of heteropteran signaling behaviours (Sulier-Perkins et al., 2007).

To this end, we provide here the first detailed description of the internal and external morphology of the pregenital abdominal segments and posterior region of the metathorax of Enicocephalomorpha. Because the segmental origin and homology of different muscles is only reliably identified after examining their innervation, we also examined the nervous system, instead of relying on topographical resemblance alone (Klug & Klass, 2006). We

use adult males of an undescribed species of the enicocephalid genus *Cocles* (females are unknown), and adults of both sexes of the aenictopecheid genus *Lomagostus*, which we compare with several other carefully selected species from both families of Enicocephalomorpha. Finally, we review the organization of the pregenital musculature of Heteroptera in detail, and compare this with that of the examined Enicocephalomorpha. It is hoped that this study will help fill some gaps in our knowledge of these enigmatic insects, and set the framework for additional morphological and functional investigations of the heteropteran pregenital abdomen.

2. Material and methods

2.1. Material

Our morphological investigations involved several undescribed taxa of Enicocephalomorpha. We consider all undescribed specimens as potential paratypes; to ensure the repeatability of our results and to facilitate future comparisons, each specimen used in this study is provided with a unique accession number accompanying the data label, allowing it to be traced. Vials containing additional specimens of the undescribed species examined by us but not directly used in the analysis below are provided with generic identification and the unique project code [MORPHOMMBC = Morphological Project on Enicocephalomorpha (MORPHO), Moravian Museum (MMBC)]. All specimens are deposited at the Moravian Museum. In the present work, a very large number of enicocephalomorphan taxa were examined from both families. The taxa described in this work were carefully selected, in order to represent the main conditions of the enicocephalomorphan pregenital abdomen, although it is certain that many more are to be found. We based our study on the following taxa:

1. *Cocles* sp.nov., 8 males (ENI001MOPRHOMMBC-ENI008MORPHOMMBC), Madagascar: Ambohitantely Special Reserve, S18°10′17.9‘‘E47°16’36.9‘‘, 1584 m; Malaise trap, 17.-25. xi. 2011, leg. P. Baňař.

Additional specimens examined. Same data label, but includes: *Cocles* sp.nov. det. P. Baňař & L.R. Davranoglou. Used in project MORPHOMMBC.

2. *Oncylocotis* sp.nov., 2 males, 2 females, Tanzania: Mt. Hanang. North East slope, S4.43058’ E35.41616’; 2275 m, 16. 12. 2012, sift sample 22, leg. V. Grebennikov. det. P. Baňař & L.R. Davranoglou. Used in project MORPHOMMBC.

3. *Phallopirates* sp.nov. (close to *Ph. borneensis-Ph. malaicus*), one male (ENI009MOPRHOMMBC), Malaysia: Sabah, Lahad Datu, Ulu Segama Forest Reserve, Danum Valley Forest Centre, 250 m alt. leg. D.J. Mann.

4. *Proboscidopirates* sp.nov. 2 females (ENI010MOPRHOMMBC-ENI011MOPRHOMMBC), Madagascar: Vohimana, Réserve Expérimentale de Vohimana, Circuit 3“; 28.viii.2012 S18°55′12.3‘‘E48°30’53.3‘‘; 807 m sifting litter; Winkler app. extr.; legs. L.S. Rahanitriniaina & E.M. Rabotoson.

Additional specimens examined. Same data label, but includes: *Proboscidopirates* sp.nov. det. P. Baňař & L.R. Davranoglou. Used in project MORPHOMMBC.

5. *Monteithostolus genitalis* Štys, 1980, one adult male specimen, 1 second instar larva, New Caledonia (South Province): Sarraméa, – 21.6267/165.8667, 480 m, 9.11.2010, trail to Dogny plateau, sifting litter, legs. M. Wanat & R. Ruta. Used in project MORPHOMMBC.

6. *Lomagostus* sp.nov. 2 males (ENI012MOPRHOMMBC-ENI013MOPRHOMMBC), 2 females (ENI014MOPRHOMMBC-ENI015MOPRHOMMBC), Madagascar: Fianarantsoa Province, 7 km W Ranomafana, 1100 m, 1–9 February 1990, leg. W. E. Steiner.

Additional specimens examined. Same data label, but includes: *Lomagostus* sp.nov. det. P. Baňař & L.R. Davranoglou. Used in project MORPHOMMBC.

The above-mentioned species were analysed using the following techniques (described in detail in Sections 2.2. –2.6.): *Cocles* sp.nov., scanning electron microscopy, synchrotron microtomography, macrophotography, microtome sections; *Lomagostus* sp.nov., synchrotron microtomography, laser confocal scanning microscopy; *Phallopirates* sp.nov., macrophotography; *Proboscidopirates* sp.nov., laser confocal scanning microscopy.

2.2. Scanning electron microscopy

Two critical point dried male specimens of *Cocles* sp.nov. were observed in a Neoscope 2000 scanning electron microscope (SEM; Nikon Instruments, UK) at 15 kV high vacuum, following coating for 150 s at 18 mA with gold/palladium (Quorum Technologies SC7620), giving a coating of 12.5 nm.

2.3. Synchrotron microtomography and 3D reconstruction

Two male specimens of *Cocles* sp.nov. (one ethanol-preserved; the other critical point dried) and four specimens of both sexes of *Lomagostus* sp.nov. (all ethanol-preserved) were used for synchrotron-radiation microcomputed-tomography (SR-μCT) at the TOMCAT beamline, Swiss Light Source (SLS), Paul Scherrer Institut, Switzerland. Cocles sp.nov. were scanned at a beam energy of 15.99 keV with final pixel size of 1.625 μm. Lomagostus sp.nov., due to their smaller size and more fragile nature were scanned with beam energy of 12 keV and pixel size of 0.325 μm. The advantage of this technique over other tomographic methods is the exceptional resolution (down to voxel sizes of 0.16–6.5 μm in fields of view of 0.4 × 0.3 mm2 and 16.6 × 14.0 mm2, respectively), which allows visualization of muscles, glands, and nerves, which is particularly suitable for minute insects such as Enicocephalomorpha. Three-dimensional reconstruction was carried out using Amira 6.1

software (Mercury Systems). Image brightness adjustment and labelling were performed in Adobe Photoshop CS6 (Adobe Systems Incorporated, San Jose, California, USA) and Adobe Illustrator CC/CS6 (Adobe Systems Incorporated, San Jose, California, USA) respectively. All drawings were generated in Adobe Illustrator CC/CS6.

2.4. Microtome sections

To ensure the quality of our SR-μCT data, two ethanol-preserved *Cocles* were stained and sectioned using a Leica Biosystems DSC1 microtome (not shown). The results of both techniques were compared and found to be congruent.

2.5. Macrophotography

Images of the thorax and abdomen of two ethanol-preserved *Cocles* and a 10% KOH-macerated *Phallopirates* sp.nov. were taken using a Leica M165c microscope with a Leica DFC490 camera, while stacked images were combined using Helicon Focus (Helicon Soft, Kharkiv, Ukraine). In Fig. 1B, the abdomen is artificially extended by means of forceps, in order to clearly show the arrangement of the different sclerites of the pregenital abdomen and components of the metathoracic scent glands.

2.6. Laser confocal scanning microscopy

Specimens of *Lomagostus* sp.nov. and *Proboscidopirates* sp.nov. were placed between two cover slips in 70% ethanol. Images were taken with an Olympus FV1000, at a laser wavelength of 488 nm.

2.7. Terminology

Our description of the thoracic musculature generally follows the terminology of Friedrich and Beutel (2008) for Neoptera, with the exception of the metathoracic scent gland musculature, for which we follow Parsons (1960a), because of its uncertain homology.

Adopting the opinion of Ferris (1940) and Weber (1952), we observe that the homologies of the ventral median sclerites of the thoracic segments in Neoptera are frequently obscured by a secondary reduction of the primary sternite. To simplify the discussion, the above mentioned sclerite of the metathorax is termed the "metasternum", noting that it is a secondary structure, probably incorporating subcoxal elements. The definition of dorsal and ventral laterotergites typically used in Enicocephalomorphan descriptive taxonomy (e.g. Štys & Baňař, 2008, 2009) is followed. We propose our own terminology for the musculature of the pregenital abdomen, but are influenced by Snodgrass (1935) in recognizing inter-and-intrasegmental groups of dorsoventral muscles, and interpret that only (internal) medial sets of longitudinal muscles exist in the examined taxa. The abbreviated name appears before the full name of each muscle. We subsequently provide an extensive review of Heteropteran pregenital musculature and suggest homologies with the terminology of other authors.

Abbreviations used in the figures: **ac:** antecosta; **ascx3:** anterior supracoxal lobe of metepisternum; **apl:** anapleural ridge; **apo:** apodeme of sternacosta; **ce:** connexival edge; **cl:** coxal cleft; **cmp:** connective membrane of movable plate of sternum 3; **cxm:** coxal membrane; **cx:** coxa; **dag:** dorsal abdominal gland; **djm:** dorsal junctional membrane of metathorax-abdomen; **dlm:** dorsal longitudinal muscle; **dltg:** dorsal laterotergite; **dm:** dorsolateral membrane of mediotergum 2; **edvm:** external dorsoventral muscle of abdomen; **efp:** efferent pouch; **eg:** epidermal gland; **el:** elevated cuticle of dorsal abdominal gland; **ep3:** metepimeron; **es3:** metepisternum; **fa:** apophysis of metafurca; **IIIdvm8:** dorsoventral muscle of metafurca; **fm:** femur; **fo:** laterotergal fold; **fp:** pit of metafurca; **fr:** metapostcoxal frame; **IIIvlm2:** ventral longitudinal muscle of metafurca; **gc:** ganglionic connective; **gl:** metathoracic scent gland; **idvm:** internal dorsoventral muscle of abdomen; **lp:** lateral plate of sternum 2, composed of laterosternite 2 + vltg2; **ltg:** laterotergite; **lw:** line of weakness

of triangular sclerite of sternum 2; **ma:** muscle attachment; mab: main abdominal nerve; **mb:** membrane; **mf:** membranous fold of sternum 1; **mg:** midgut; **mgt:** musculus glandulae thoracicae; **mp:** movable plate of sternum 3; **msg:** mesothoracic ganglion; **msu:** median sulcus of tergal plate; **mscl:** muscle-bearing sclerite of sternum 1; **mt:** mediotergite; **mtg:** metathoracic ganglion; **mtst:** metasternum; **n.ab.:** abdominal nerve; **n.ms.:** mesothoracic nerve; **n.mt.:** metathoracic nerve; **o:** ostiole; **ph:** phragma; **pl:** pleural membrane; **prcx:** precoxale; **pscx3:** posterior supracoxal lobe of metepimeron; **ptcx:** postcoxale; **pn3:** metapostnotum; **sa:** sensilla; **sc3:** scutum of metathorax; **scl1a:** median sclerite of sternum 1; **scl1b:** second sclerite of sternum 1; **scl1c:** third sclerite of sternum 1; **scl1d:** fourth, anterior-most sclerite; **sf:** sternal fold of sternum 3; **sl:** sclerotized line of scl1d; **sm3:** scutellum of metathorax; **sp:** spiracle; **stc:** sternacosta; **stn:** abdominal sternum; **sts:** sternacostal sulcus; **t:** abdominal tergum 1; **tc:** trochanter; **tcut:** tuberculate cuticle of dorsal abdominal gland; **tr:** trough of valve of dorsal abdominal gland; **ts:** triangular sclerite of sternum 2; **tscl:** tergal sclerite; **tsu:** transverse sulcus of tergal plate; **tp:** tergal plate; **xy:** metasternal xyphus; **v:** valve of dorsal abdominal gland; **va:** valvular apparatus of metathoracic scent gland; **vc:** valvular channel; **vlm:** ventral longitudinal muscle of abdomen; **vjm:** ventral junctional membrane of abdomen; **vltg:** ventral laterotergite.

3. Results

3.1. *Cocles* sp.nov. (Enicocephalidae: Enicocephalinae)

The morphology of this species is described in a sequential manner, starting from the posterior region of the metathorax, succeeded by abdominal segments 1–4. The musculature of these regions is provided in the corresponding subsections. Pregenital segments 5–7 are not described as they do not differ significantly from segment 4.

3.1.1. Posterior region of metathorax

The metathorax is greatly reduced compared to the mesothorax. In lateral view, two parts are most distinctive: a greatly enlarged metepisternum (es3), and a reduced metepimeron (ep3; Figs. 1–3). The metepisternum, metepimeron and the metacoxal cleft (cl3) have been displaced posterodorsally (Fig. 3), to the extent that the metepimeron has assumed a nearly dorsal position (Fig. 3). The metapostnotum (pn3) is transverse, its distal end being distinctly concave and heart-shaped (Figs. 1, 4A), forming an indistinct metaphragma (Fig. 10). The metasternum (mtst) is tapered basally, where it joins the mesothorax, and concave distally, at the ventral thoracic-abdominal boundary (Figs. 1, 6A). The metasternum is produced posteriorly into a short metaxyphus (xy) (Figs. 1, 2, 5–9). The ventral boundary of metepisternum is delimited by a distinct (anapleural or a secondary episternal) ridge (apl) (Figs. 1, 8A). The furcal pits (fp) are broadly separated, each situated anteriorly, at the extreme base of the coxal cleft (Figs. 1, 2, 6, 8A and B). A distinct, U-shaped sternacosta (stc) is present, its median portion located beneath the metaxyphus (Fig. 1B). The sternacosta connects the two furcal pits via its sulcus (sts) (Fig. 6A, B). Internally, at the base of the metaxyphus, the sternacosta bears a pair of equidistant rounded apodemes (apo) (Fig. 1B, asterisks; 10). The proximal portion of the rim of metacoxal cavity is formed laterally by the anterior supracoxal lobe (ascx3) of the metathorax and ventrally by the sternacosta (Figs. 1–3). The metapostcoxal frame (fr; Figs. 1, 2, 7D) probably represents the fusion of the antecosta of abdominal sternum 1 to the metathorax (Brindley, 1938), and is dorsally continuous with the metapostnotum. The narrow sternacostal sulcus, which starts from the base of the metaxyphus and ends at the furcal pit (Fig. 6A, B), probably does not aid in disseminating secretions once they have been externalized, as it does not connect internally with the valvular apparatus and lacks the distinct ultrastructure (e.g. mycoid microsculpture) which would indicate the presence of a thoracic evaporatorium (Fig. 6C).

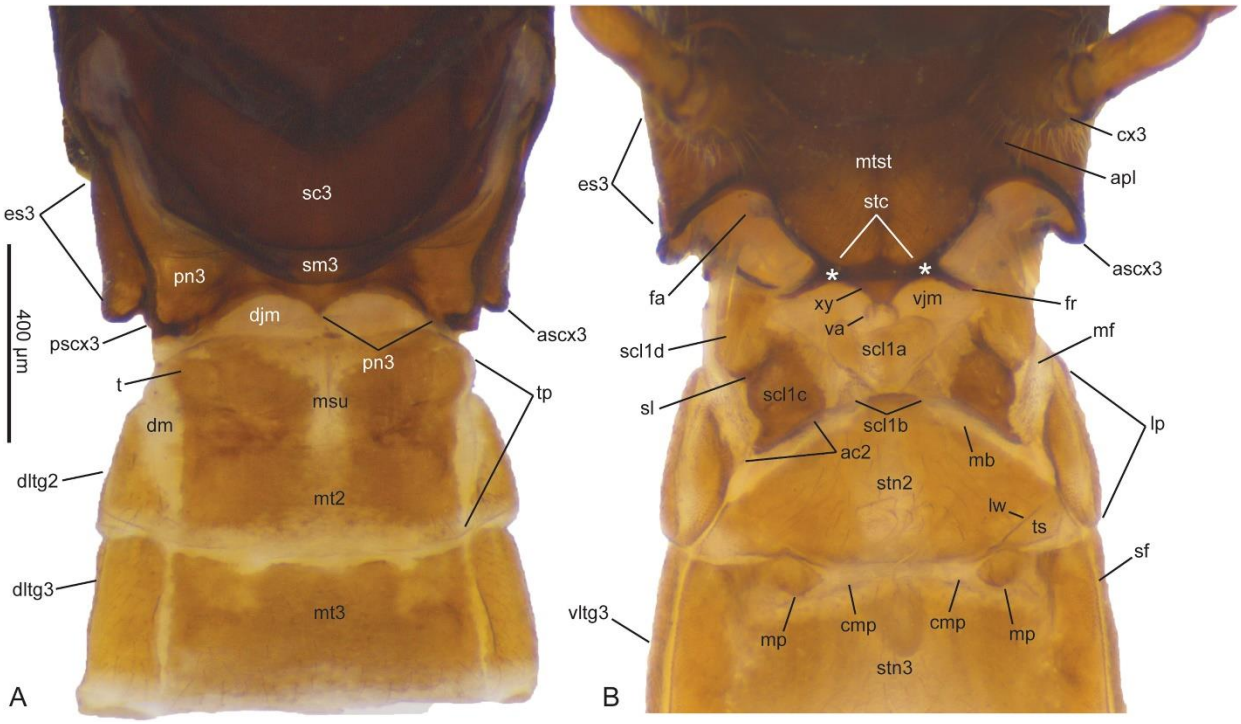


Fig. 1. External abdominal and metathoracic morphology of *Cocles* sp. nov. A: Dorsal view of abdomen and metathorax. B: Ventral view, artificially extended abdomen by using forceps, allowing full view of junctional membranes, abdominal sclerites and metathoracic scent gland valve; note that scl1a has a somewhat distorted shape due to the artificial extension. Asterisks indicate the internal position of the sternacostal apodemes; ac2, antecosta of sternum 2; apl: anapleural ridge; ascx3, anterior supracoxal lobe of metepisternum; cmp, connective membrane of movable plate of sternum 3; djm, dorsal junctional membrane of metathorax-abdomen; dltg2, dorsal laterotergite 2; dltg3, dorsal laterotergite 3; dm, dorsolateral membrane of mediotergum 2; es3, metepisternum; fa, apophysis of metafurca; fr, metapostcoxal frame; lp, lateral plate of sternum 2, composed of laterosternite 2 + vltg2; lw, line of weakness of triangular sclerite of sternum 2; mb, membrane; mf, membranous fold of sternum 1; mp, movable plate of sternum 3; msu, median sulcus of tergal plate; mt, mediotergite; mtst, metasternum; pn3: metapostnotum; pscx3, posterior supracoxal lobe of metepimeron; sc3: scutum of metathorax; scl1a, median sclerite of sternum 1; scl1b, second sclerite of sternum 1; scl1c, third sclerite of sternum 1; scl1d, fourth, anterior-most sclerite; sf, sternal fold of sternum 3; sl, sclerotized line of scl1d; sm3: scutellum of metathorax; stc, sternacosta; stn, abdominal sternum; t, abdominal tergum 1; ts, triangular sclerite of sternum 2; tp, tergal plate; xy,

metasternal xyphus; va, valvular apparatus of metathoracic scent gland; vjm, ventral junctional membrane; vltg3, ventral laterotergite 3. Image link.

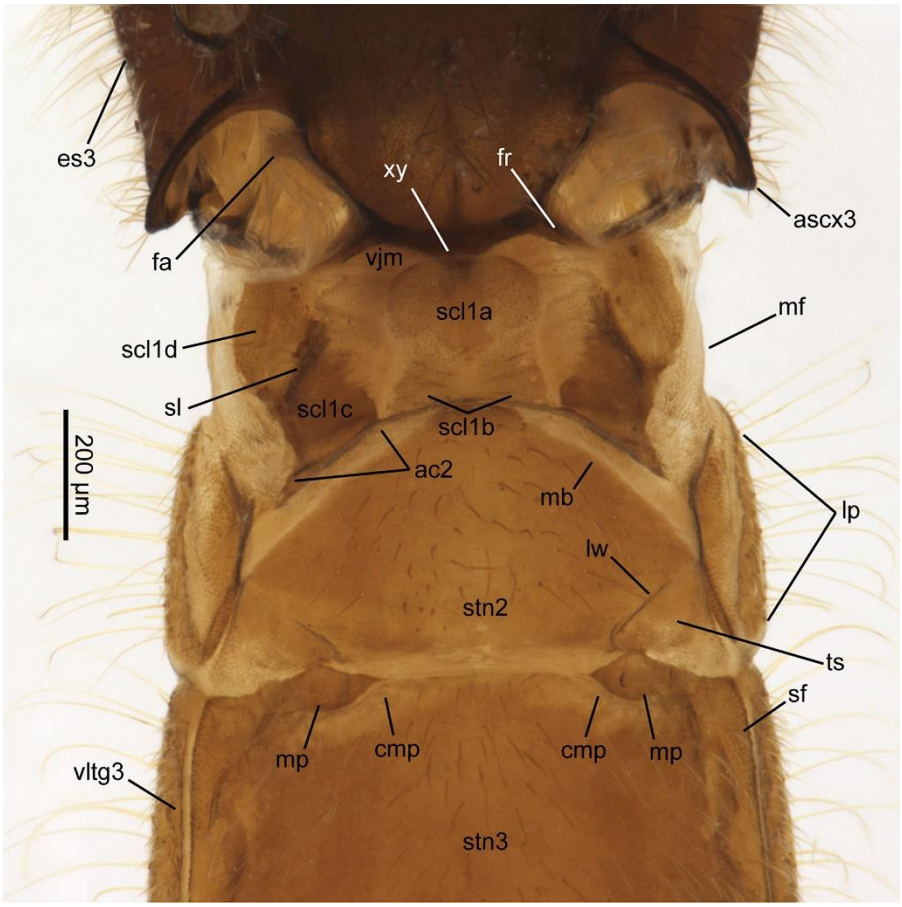


Fig. 2. Ventral view of the first three abdominal segments of *Cocles* sp. nov., in natural position. ac2, antecosta of sternum 2; ascx3, anterior supracoxal lobe of metepisternum; cmp, connective membrane of movable plate of sternum 3; es3, metepisternum; fa, apophysis of metafurca; fr, metapostcoxal frame; lp, lateral plate of sternum 2, composed of laterosternite 2 + vltg2; lw, line of weakness of triangular sclerite of sternum 2; mb, membrane; mf, membranous fold of sternum 1; mp, movable plate of sternum 3; scl1a, median sclerite of sternum 1; scl1b, second sclerite of sternum 1; scl1c, third sclerite of sternum 1; scl1d, fourth, anterior-most sclerite; sf, sternal fold of sternum 3; sl, sclerotized line of scl1d; stn, abdominal sternum; ts, triangular sclerite of sternum 2; xy, metasternal xyphus; vjm, ventral junctional membrane; vltg3, ventral laterotergite 3. Image link.

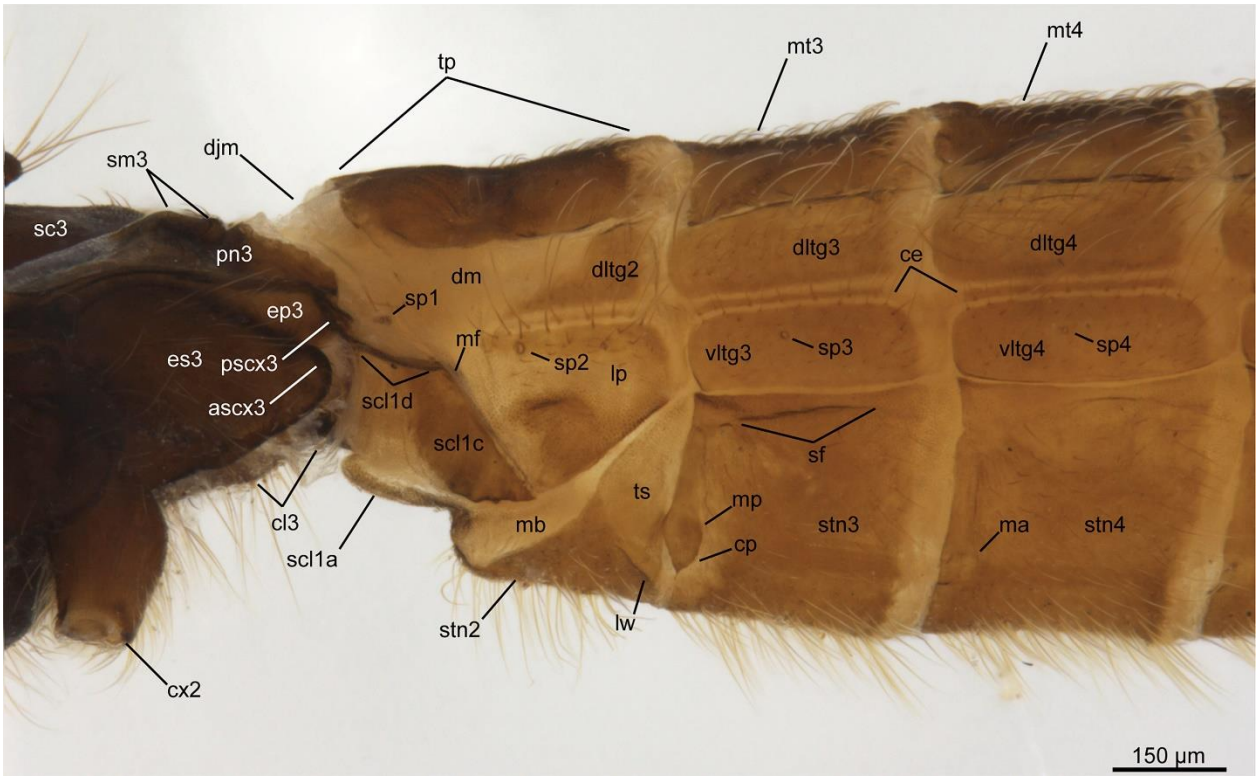


Fig. 3. Lateral view of the first three abdominal segments of *Cocles* sp. nov. ascx3, anterior supracoxal lobe of metepisternum; ce, connexival edge; cl3, coxal cleft 3; cmp, connective membrane of movable plate of sternum 3; cx2, coxa of mid leg; djm, dorsal junctional membrane of metathorax-abdomen; dltg2, dorsal laterotergite 2; dltg3, dorsal laterotergite 3; dltg4, dorsal laterotergite 4; dm, dorsolateral membrane of mediotergum 2; es3, metepisternum; lp, lateral plate of sternum 2, composed of laterosternite 2 + vltg2; lw, line of weakness of triangular sclerite of sternum 2; mb, membrane; mf, membranous fold of sternum 1; mp, movable plate of sternum 3; mt, mediotergite; pn3: metapostnotum; pscx3, posterior supracoxal lobe of metepimeron; sc3: scutum of metathorax; scl1a, median sclerite of sternum 1; scl1b, second sclerite of sternum 1; scl1c, third sclerite of sternum 1; scl1d, fourth, anterior-most sclerite; sf, sternal fold of sternum 3; sm3: scutellum of metathorax; sp, spiracle; stn, abdominal sternum; ts, triangular sclerite of sternum 2; tp, tergal plate; xy, metasternal xyphus; vltg3, ventral laterotergite 3; vltg4, ventral laterotergite 4. Image link.

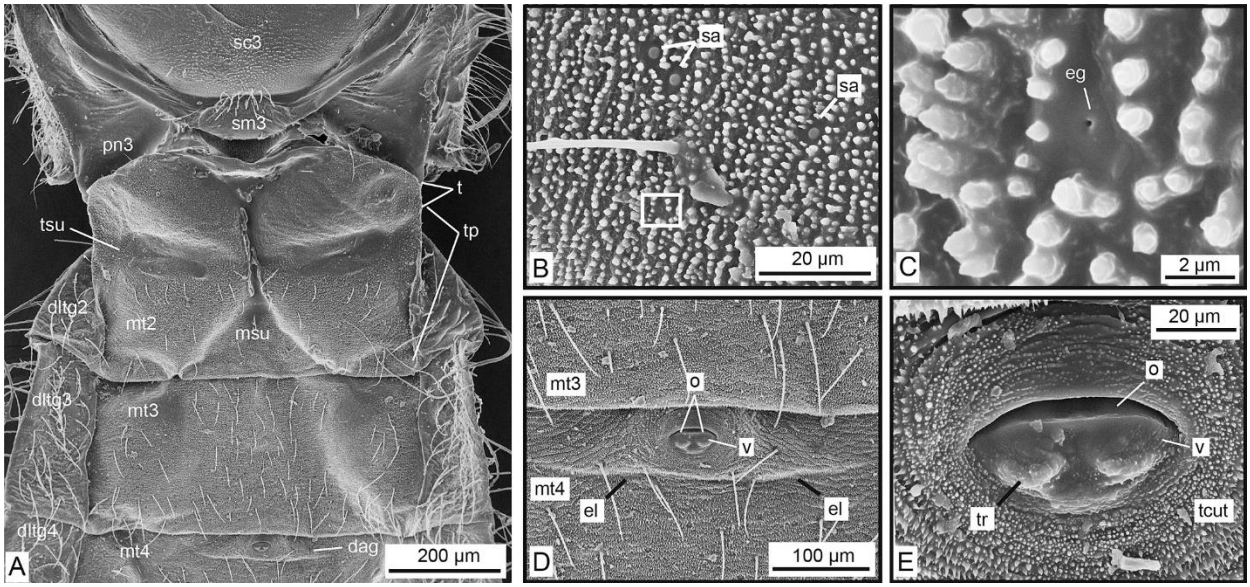
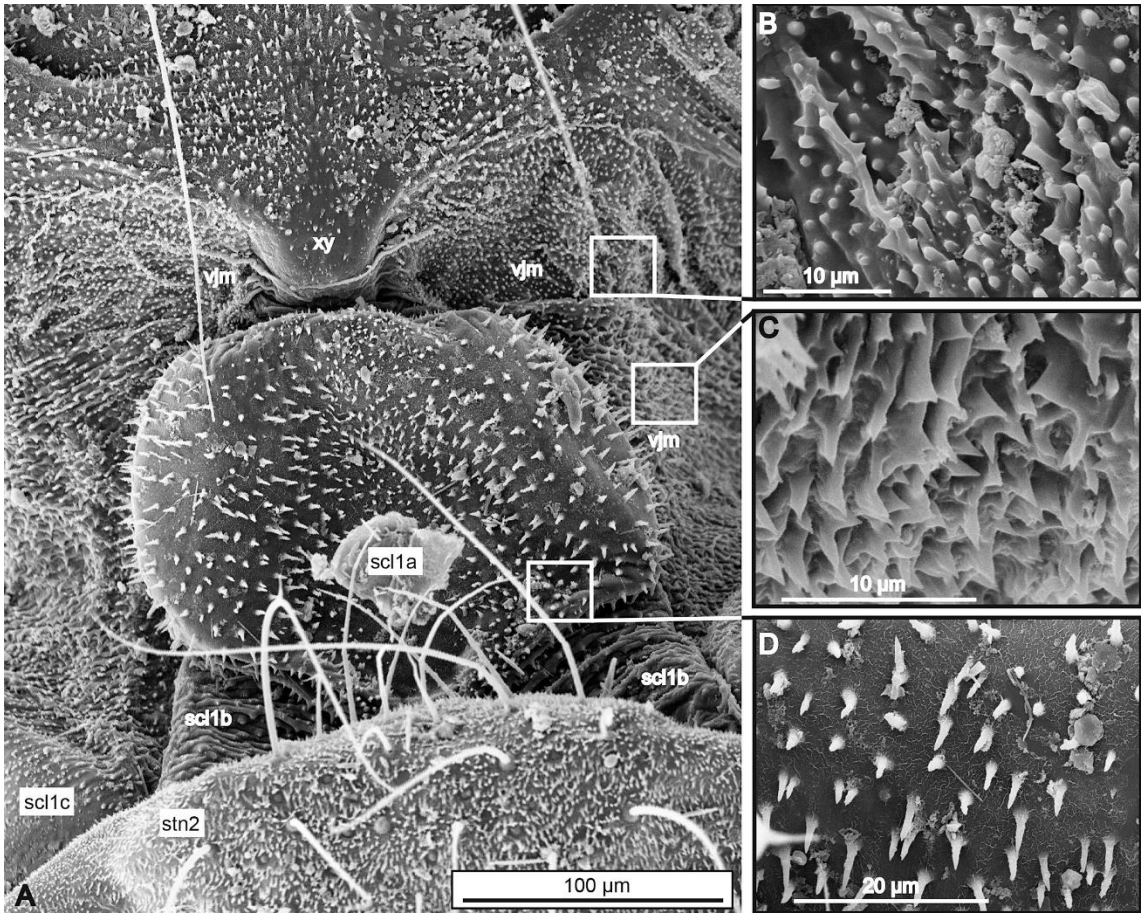


Fig. 4. SEM examination of dorsal structures in the abdomen of *Cocles* sp. nov. A: Dorsal view of metathorax and first four abdominal segments (SEM); B: Sculpture of cuticular surface of mediotergum 3, small window indicating the position of an epidermal gland. Note the presence and density of sensilla; C: Closeup of epidermal gland and structure of cuticular microsculpture in selected region of mediotergum 3; D: Proximal portion of mediotergum 4, dorsal abdominal gland; E: Enlarged view of dorsal abdominal gland and surrounding cuticle. dag, dorsal abdominal cuticle; dltg2, dorsal laterotergite 2; dltg3, dorsal laterotergite 3; dltg4, dorsal laterotergite 4; eg, epidermal gland; el, elevated cuticle of dag; msu, median sulcus of tergal plate; o, ostiole; pn3: metapostnotum; sa, sensilla; t, abdominal tergum 1; tcut, tuberculate cuticle of dorsal abdominal gland; tp, tergal plate; tr, trough of valve of dorsal abdominal gland; tsu, transverse sulcus of tergal plate; v, valve of dag. Image link.

Fig. 5. Structure of basal portion of abdomen (SEM). A: Sclerites of sternum 1 and metaxyphus, mtst, metasternum; scl1a, median sclerite of sternum 1; scl1b, second sclerite of sternum 1; scl1c, third sclerite of sternum 1; scl1d, fourth, anterior-most sclerite; stn2, sternum 2; xy, metasternal xyphus; vjm, ventral junctional membrane. B: Microsculpture of vjm; C: Same, posterior portion of vjm; D: Same, scl1a. Image link.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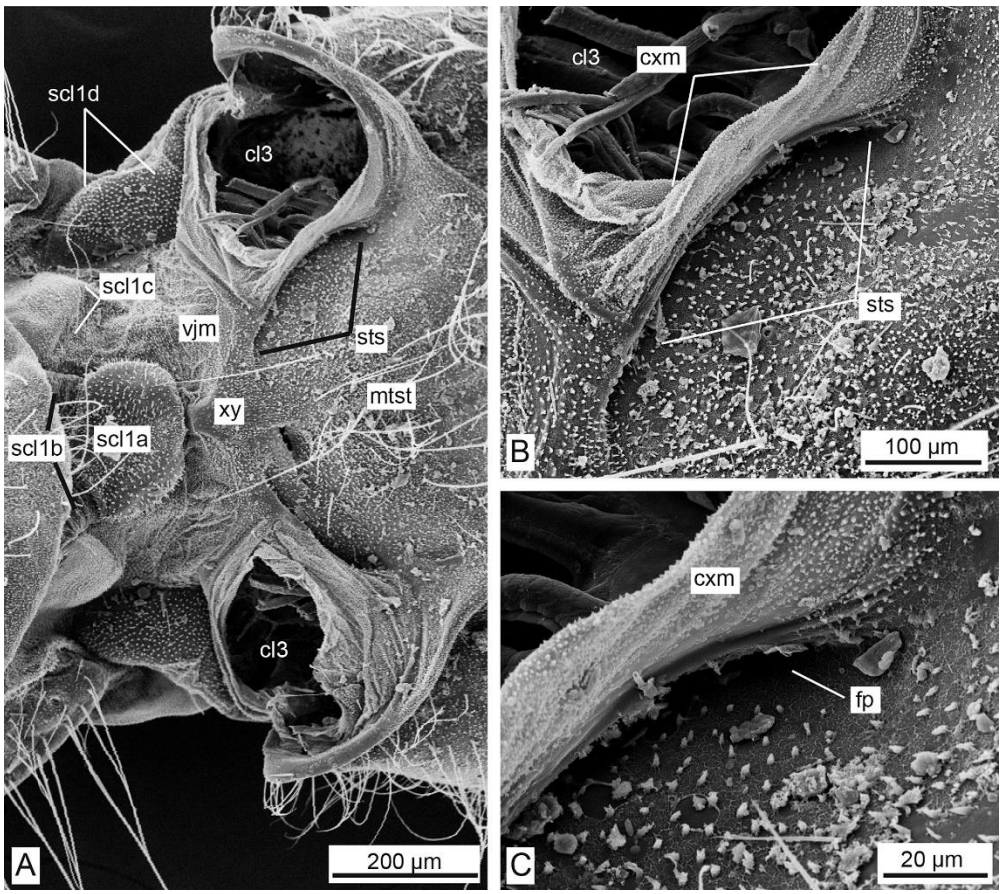
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Fig. 6. Ventral aspect of abdomen and metathorax of *Cocles* sp. nov., indicating the position and structure of the sternacostal sulcus (SEM). A: Metathorax and sclerites of sternum 2, ventral view, hind coxae omitted, cl3, coxal cleft 3; scl1a, median sclerite of sternum 1; scl1b, second sclerite of sternum 1; scl1c, third sclerite of sternum 1; scl1d, fourth, anterior-most sclerite; sts: sternacostal sulcus; stn2, sternum 2; xy, metasternal xyphus; vjm, ventral junctional membrane; B: Sternacostal sulcus, cxm, coxal membrane; C: Enlarged view of metafurcal pit (fp), marking the end of the sternacostal sulcus. Image link.

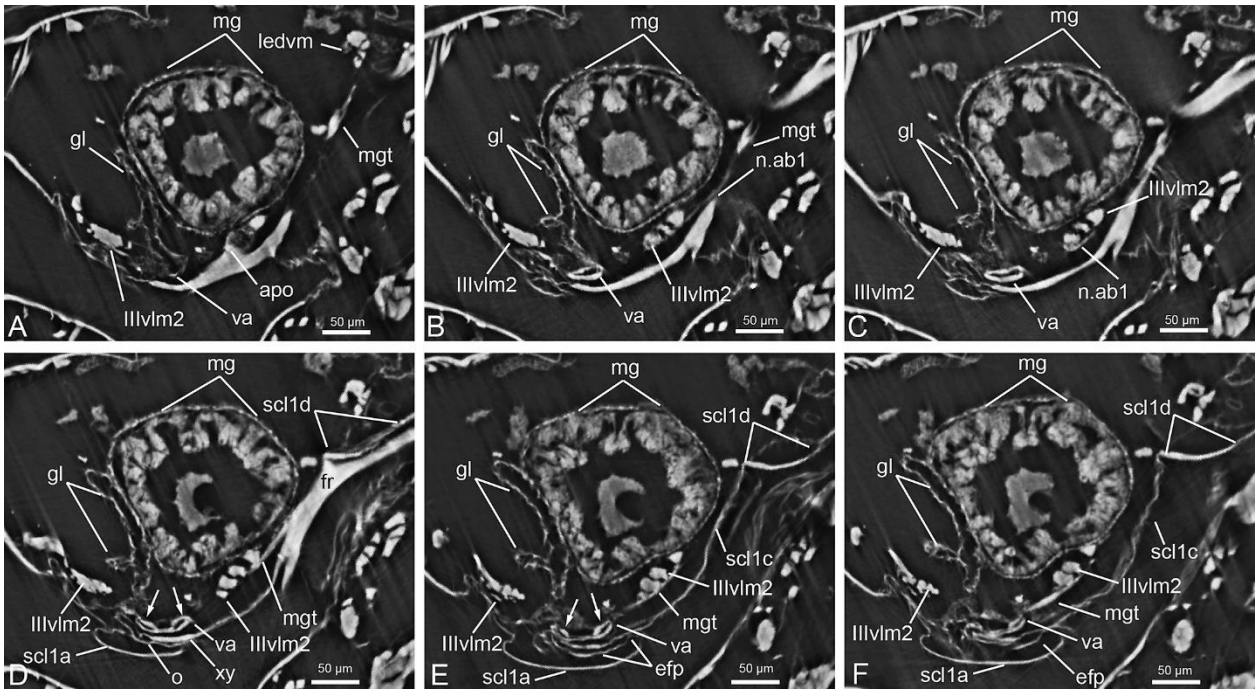


Fig. 7. Serial SR-µCT cross-sections of metathorax and abdomen (moving from anterior to posterior), illustrating the internal morphology of the metathoracic scent gland system of *Cocles* sp. nov. A: Extreme anterior region of metathoracic scent gland (gl), connecting ventrally with the base of the sclerotized valvular apparatus (va). Both structures are located between the sternacostal apodemes (apo, visible on the right), which form the origin for the furcal ventral longitudinal muscles (IIIvlm2, shown on the left). The position of gl next to the midgut (mg) represents its location when the specimen was killed; it can assume any position ventral or lateral to mg. The anterior region of musculus glandulae thoracicae (mgt) and the external dorsoventral of sternum 1 (Iedvm), as they reach the metaphragma is clearly visible; B: Immediately after the sternacostal apodemes, both

IIIvlm2 are visible. Note the first abdominal nerve (n.ab. 1) beneath IIIvlm2, innervating mgt; C: The position of n.ab.1 immediately beneath IIIvlm2 is clearly visible, while va is more flattened; D: The distal 1/3d of the va is distinctly compressed medially, the arrows showing its two lobes, which form the lower insertion point for mgt. The apex of metaxyphus is located between the va dorsally, and ventrally by the base of scl1a; they collectively form a small orifice, which might represent the metathoracic scent gland ostiole (o); Note that the base of scl1d connects with the metapostcoxal frame (fr); E: The association of scl1d and xy form a hollow efferent pouch (efp), the roof of which is formed by the latter and its base by the former; the position of mgt ventral to IIIvlm2 is particularly distinct, while the plate-like base of scl1d is visible; F: At approximately the mid portion of scl1a, efp has largely disappeared, and the insertion of mgt on the lateral lobe of the va is particularly distinct. Image link.

The metathoracic scent gland system comprises a single gland (gl), a median valvular apparatus (va), the metaxyphus, and an efferent pouch (efp) (Figs. 7–9). The gland is an elongate, tubular structure (Fig. 8), composed of very thin cuticle (Fig. 7). The anterior portion of the gland is located between the metafurcal apophyses (Fig. 8) and then extends beyond the fourth abdominal segment. The gland discharges directly into the base of the strongly sclerotized valvular apparatus (Figs. 7, 8), which lacks a distinct opening/closing device and is continuous with the gland and its contents. The valvular apparatus is located mid-ventrally above the base of the metaxyphus (Figs. 1, 8A, B), between the sternacostal apodemes. In its proximal portion, the valvular apparatus has the shape of a sac (Fig. 7B, C). However, after the first third of its length, it becomes strongly flattened medially, whilst its lateral margins retain their original thickness and appear lobe-like (Fig. 7D–F). In cross-section, the valvular apparatus consists of an internal valvular channel (vc), which ends in two orificia (Fig. 9). Each orifice connects ventrally into the efferent pouch and thus links the valvular apparatus with the latter (Figs. 7, 9). The roof of this pouch represents the distal part of the metaxyphus (Fig. 7D), while its base is formed

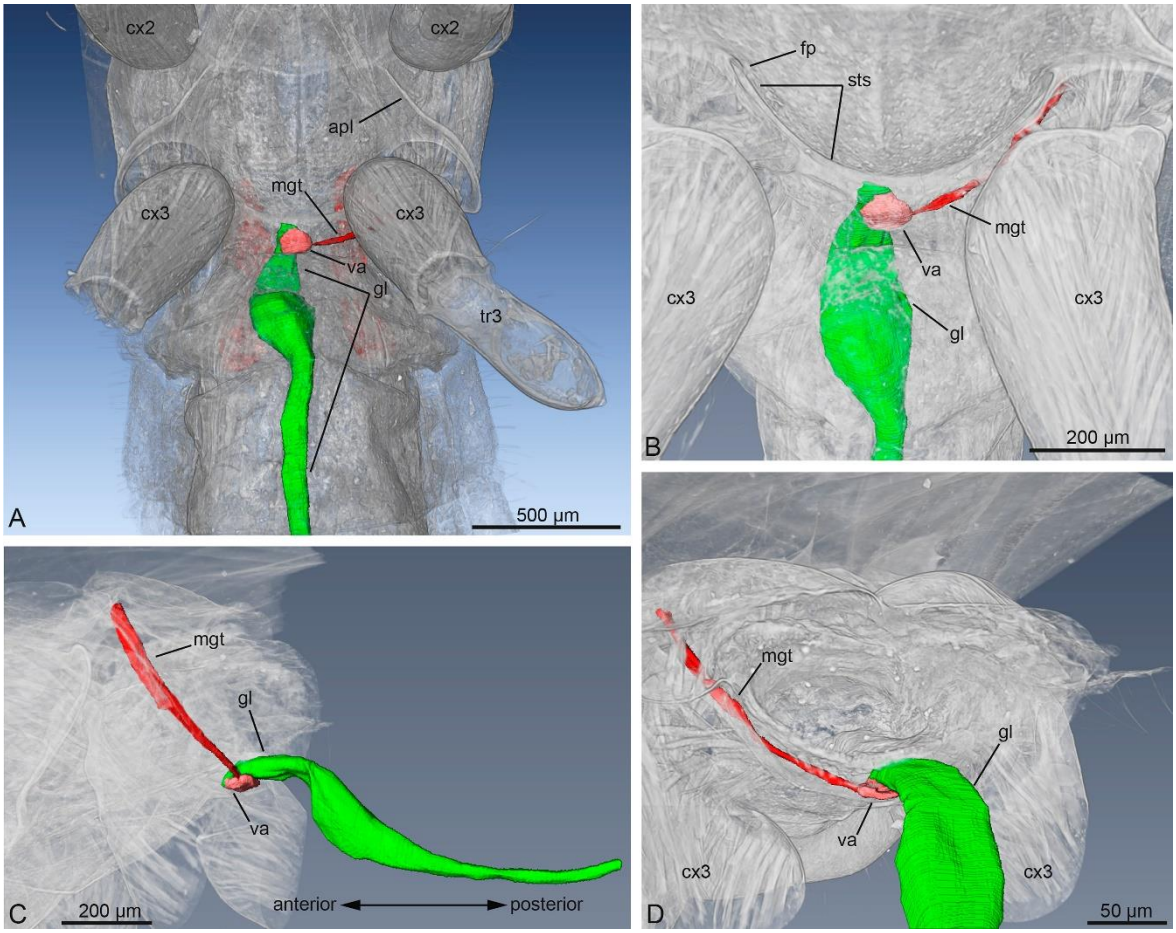
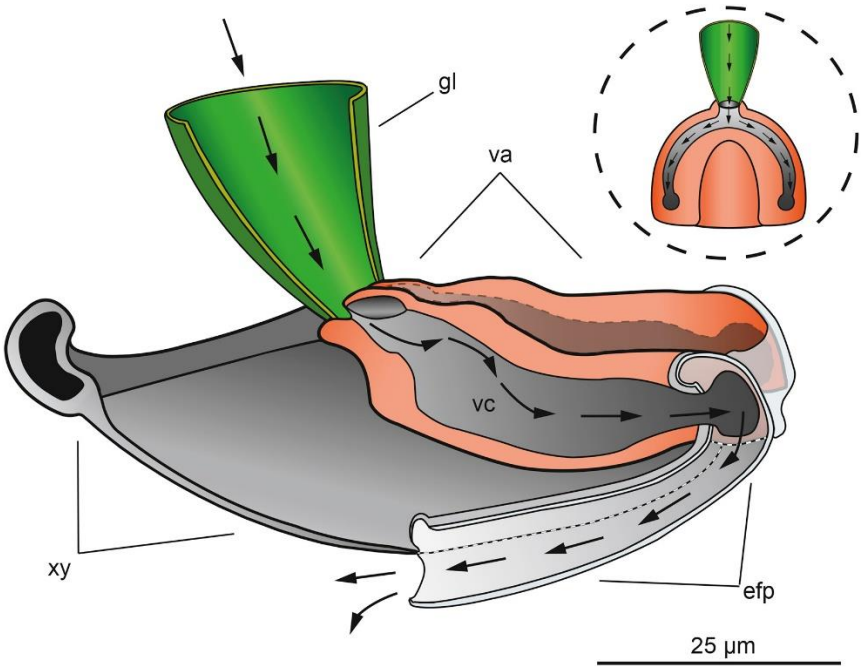


Fig. 8. SR-µCT, volume-rendered 3D reconstruction of the metathoracic scent gland system of *Cocles* sp. nov. A: Ventral view of thorax and abdomen, components of metathoracic scent gland system coloured, apl, anapleural ridge; cx2, mid coxa; cx3, metacoxa; gl, metathoracic scent gland; mgt, musculus glandulae thoracicae; tr3, hind trochanter; va, valvular apparatus of metathoracic scent gland; B: Same, enlarged view at metathoracic-abdominal junction, fp, metafurcal pit; sts, sternacostal sulcus; C: Same, lateral view; D: Same, caudal view, mgt originates on metaphragma. Image link.

Fig. 9. Schematic of metathoracic scent gland system of *Cocles* sp. nov. Arrows indicate assumed flow of secretion from the metathoracic gland (gl) to the valvular apparatus (va). Once the secretion enters the valvular channel (vc) it discharges in the efferent pouch (efp) (presumably following contraction of musculus glandulae thoracicae), where it is then externalised on the ventral junctional membrane. Top right inset shows a slice of the proximal part of the metathoracic gland and valvular apparatus in dorsal view; efp and xyphus (xy) are omitted in inset. Image link.



by the thin cuticle of the ventral junctional area of the abdomen (Fig. 7, Fig. 9). The external appearance of ostioles (o) could not be reconstructed, although the efferent pouch ends into two orificia, which are interpreted as paired, adjacent ostioles (Fig. 7D).

Musculature. Musculus mesophragma-metaphragmalis (IIIdlm1) (Fig. 10): Small and compact muscle, O (= origin) – posteromedian surface of mesothoracic phragma; I (= insertion) – anteromedian surface of metathoracic phragma; F (= function) – unclear, possibly retracts abdomen. Musculus glandulae thoracicae (mgt): very long and slender muscle. O – posterolateral surface of metathoracic phragma; I – lateral lobe of valvular apparatus (Figs. 7, 8), immediately beneath ventral longitudinal muscle of metafurca (Figs. 7, 10); F – valve-opener of metathoracic scent-gland system. Musculus metafurca-abdominosternalis (IIIvlm2): long and broad, largest muscle of abdomen, O – posterior surface of sternacostal apodeme (Figs. 7, 10); I – anterolateral surface of antecosta of abdominal sternum 2 (Figs. 10, 11); F – Retracts the abdomen inwards.

3.2. The pregenital abdomen

3.2.1. The first abdominal segment

The thorax and abdomen are joined dorsally by an extensive membranous region between the metanotum and abdominal tergum 1 (Figs. 1, 3). This junctional membrane (djm) is a desclerotised part of the latter. The abdomen is directly fused to the metathorax: dorsolaterally on the metapostnotum and metepimeron (via the dorsal junctional membrane and scl1d, respectively) and ventrolaterally on the metathoracic sternacosta and the metacoxal rim by means of the ventral junctional membrane (vjm) (Figs. 1, 2, 5).

The first and second abdominal terga are partially fused, forming a "tergal plate (tp)" (sensu Gogala, 2006) (Figs. 1, 4). The posterior boundary of the first abdominal tergum is demarcated by a faint transverse sulcus (tsu), which represents the antecosta of tergum 2 (Fig. 4A). The tergal plate is split medially by an hourglass-shaped sulcus (msu) into two reniform lobes (Figs. 1, 4). Abdominal segment 1 lacks dorsal or ventral laterotergites, its lateral region being completely membranous (Figs. 1–4A). Uniquely, abdominal sternum 1 is entire, somewhat quadrangular, subdivided into a complex of sclerites (Figs. 1–3). Immediately posterior to the metathoracic scent gland valvular apparatus, there is a median, semicircular plate which is termed sclerite 1a (scl1a) (Figs. 1–3, 5–7D–F). The region between the thoracic tagma and scl1a is composed of the ventral junctional membrane (Figs. 1, 2, 5, 6A). When the abdomen is at rest (or deflated, in dry specimens), this membrane folds inwards and scl1a covers most of the metaxyphus (protects metathoracic scent gland apparatus) (Figs. 2, 3). Immediately beneath scl1a there is a pair of minute, triangular sclerites termed scl1b (Figs. 1, 2, 5A). On either side of the median scl1a there is an elongate, trapeziform, darkly pigmented lateral sclerite (scl1c). The base of scl1c is strongly sclerotized and posteriorly fuses with the antecosta of sternum 2 (Fig. 1B). This lateral scl1c ends anteriorly into a transverse sclerotized line (sl), which forms the posterior ventral margin of the anterior-most sclerite, scl1d (Fig. 1A). The ventral portion of scl1d is plate-like (Figs. 3, 7E, F), somewhat rounded in ventral view (Figs. 1, 2, 6A). In lateral view, the

proximal portion of scl1d is fused to the posterior supracoxal lobe (Fig. 2). Due to the absence of laterotergites on abdominal segment 1, an extensive membrane (dm) forms the entire lateral and dorsal region of scl1d, acting as a bridge between tergum and sternum (Figs. 1–3). The first abdominal spiracle (sp1) is found in this membrane (Figs. 3, 10). The lateral margin of the membrane, starting from the posterodorsal region of scl1d and reaching the base of scl1c, forms a strong fold (mf), which is particularly distinct when the abdomen is viewed ventrally (Figs. 1–3).

Musculature. I musculus dorsalis medialis (Idlm) (Fig. 10): O – posteromedian surface of metaphragma; I – anteromedian surface of tergal antecosta 2; F – Raises the abdomen. I musculus dorsoventralis externus (Iedvm) (Figs. 7, 10): O – posteromedian surface of metaphragma, between Idlm and mgt; I – base of sclerotized plate formed by scl1d, immediately beneath spiracle 1, lateral to Iidvm; F – abdominal retraction/abduction. I musculus dorsoventralis internus (Iidvm) (Fig. 10): O – posterior surface of tergum 1, behind antecosta 2; I – immediately lateral to sclerotized base of scl1d; F – compressor of tergum 1 and scl1d. Musculus metafurca-abdominosternalis (IIIvlm2): details in Section 3.1.

3.2.2. The second abdominal segment

This segment is markedly different from that preceding and the ones following, in both its tergal and sternal components. Tergum 2 is split into a mediotergite (mt2) which laterally bears 1 + 1 subtriangular dorsal laterotergites 2 (dltg2) (Fig. 1A). The proximal portion of dltg2 is membranous (Fig. 3), while dorsally, it is separated from mt2 by a broad dorsolateral membrane (dm), which gradually narrows posteriorly and almost disappears at the extreme apex of dltg2, the latter almost completely fusing to mediotergum 2 (Figs. 1, 3). The dorsal laterotergite 2 is separated by its ventral counterpart by a narrow, flexible fold (connexival edge) (Fig. 3). Laterally, segment 2 is complex: a large, quadrangular, lateral plate (lp) is

composed by the complete fusion of a laterosternal subdivision of sternum 2 and vltg2, with no trace of a sternal-laterotergal boundary (Figs. 3, 10). This lateral plate bears a large abdominal spiracle 2 (sp2) (twice as large as all other abdominal spiracles; Fig. 3), while its base is separated from sternum 2 and its antecosta by an extensive membrane (mb), which further adds to the flexibility of the abdomen (Fig. 3). Immediately beneath the lateral plate, towards its posterior margin, there is a triangular sclerite (ts) of sternum 2 (Figs. 1–3, 11A, C). A line of weakness (lw) (Figs. 1–3) probably allows its flexion by means of muscular action. The main body of sternum 2 is strongly oval anteriorly, its posterior margin being somewhat straighter (Figs. 1, 2). The region bearing antecosta 2 is strongly inflected inwards to form a deep fold, which is easily observed in lateral view (Fig. 3).

Musculature. II musculus dorsalis medialis (IIdlm) (Fig. 10): O – Posteromedian surface of antecosta 2; I – anteromedian surface of antecosta 3; F – Retracts tergum 3 dorsad. II musculus dorsoventralis externus (IIedvm) (Figs. 10, 13C): O – lateral margin of tergum 2, lateral to IIdlm; I – Basal third of lateral plate; F – compressor of segment 2. II musculus ventralis medialis (IIvlm) (Figs. 10, 11B, D): O – posteromedian surface of antecosta 2; I – anterior region of movable plate of sternum 3; F – Ventrad retraction of sternum 3, contraction of movable plate of sternum 3.

3.2.3. The third abdominal segment

The morphology of this segment is much simpler compared to the preceding ones. Mediotergum 3 is transverse, quadrangular, its length being almost as long as the entire tergal plate (Fig. 4A). Mediotergum 3 is linked with the broad, quadrangular dorsal laterotergite 3 via a membranous, extensible fold (fo) (Figs. 3, 19B). The connexival

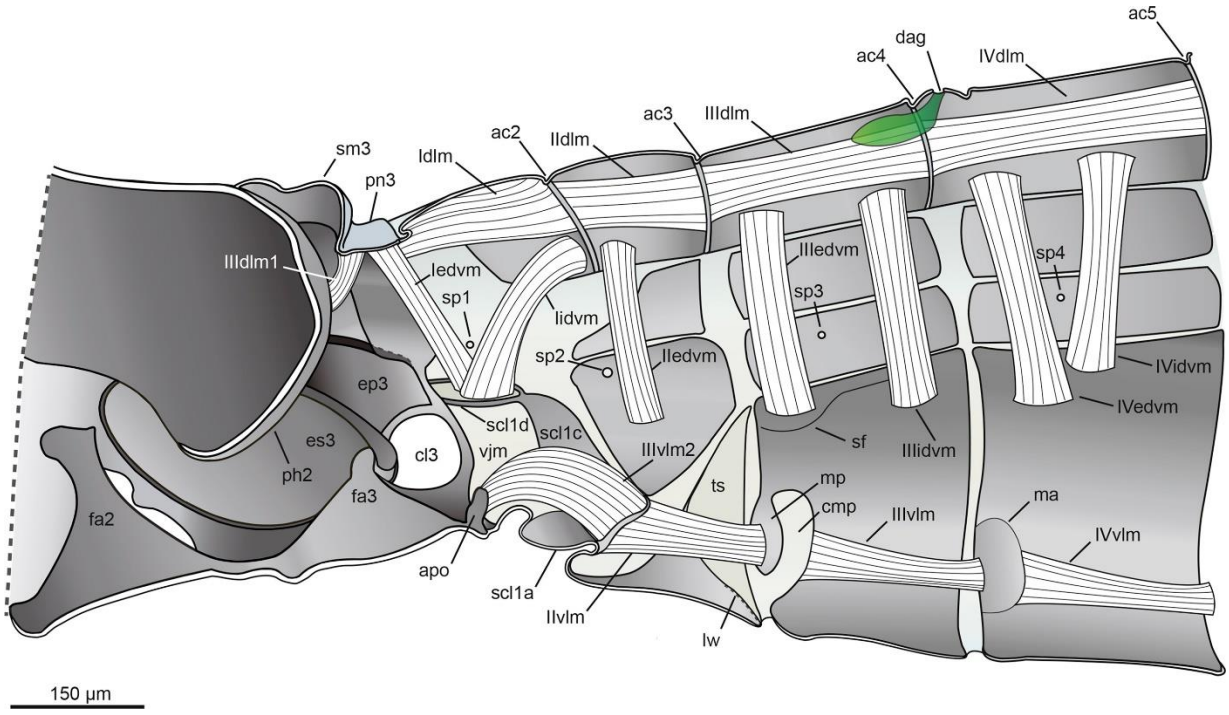


Fig. 10. Sagittal view of metathoracic and pregenital abdominal musculature (segments 1–4) of adult male *Cocles* sp. nov. Mesothoracic muscles, metathoracic scent gland system, musculus glandulae thoracicae and muscles of dorsal abdominal glands not indicated. ac, antecosta; apo, apodeme of sternacosta; cl3, coxal cleft 3; cmp, connective membrane of movable plate of sternum 3; dag, dorsal abdominal gland; ep3, metepimeron; es3, metepisternum; fa, furcal apophysis; lw, line of weakness; mtst, metasternum; ph2, mesophragma; pn3, metapostnotum; scl1a, median sclerite of sternum 1; scl1c, third sclerite of sternum 1; scl1d, fourth, anterior-most sclerite; sf, sternal fold of sternum 3; sm3, scutellum of metathorax; sp, spiracle; ts, triangular sclerite of sternum 2. Muscles: IIIvlm2, musculus metafurca-abdominosternalis; IIIdlm1, musculus mesophragma-metaphragmalis; dlm, dorsal longitudinal muscle; edvm, external dorsoventral muscle; idvm, internal dorsoventral muscle; vlm, ventral longitudinal muscle. Latin numerals on muscles indicate segmental identity, while Arabic numerals indicate muscle set. Image link.

edge is distinctly pointed in cross-section (Fig. 19B), while ventral laterotergite 3 is of similar length and structure to its dorsal counterpart. At the extreme lateral margin of sternum 3, throughout its length, there is a strong sternal fold (sf) (Figs. 1–3, 10, 11A, C),

its dorsal margin marking the laterotergal-sternal boundary. This fold is broader anteriorly, becoming strongly narrowed after its proximal half.

Sternum 3 is a broad, strongly convex quadrangular plate. The anterior margin of sternum 3 bears 1 + 1 movable sternal plates (mp), the region between them being membranous (cmp) (Figs. 1, 2, 10, 11A, C). The anterior margin of the movable plate is in close contact with the triangular sclerite of sternum 2.

Musculature. III musculus dorsalis medialis (IIIdlm): O – posteromedian surface of tergal antecosta 3; I – anteromedian surface of antecosta 4; F – raises tergum 4. III musculus dorsoventralis externus (IIIedvm) (Fig. 10): O – anterior lateral margin of mediotergum 3; I – base of sternal fold; F – unknown; presumably compresses mediotergum 3 and sternal fold. III musculus dorsoventralis internus (IIIidvm) (Fig. 10): O – posterior lateral margin of mediotergum 3; I – Posterolateral margin of sternum 3, beneath distal apex of sternal fold; F – unknown; presumably compresses tergum and sternum 3. III musculus ventralis medialis (IIIvlm) (Figs. 10, 11B, D): O – proximal 1/3d of sternum 3; I – muscle attachment of sternum 4; F – ventrad retraction of sternum 4.

3.2.4. The fourth abdominal segment

Very similar to preceding segment. The differences of this segment with the previous one are summarized below: Mediotergum 4 is slightly longer. The membranous fold linking mediotergum to connexivum is slightly larger, the dorsal laterotergite is slightly longer, the connexival edge (ce) is slightly more acute in histological sections (Fig. 19B), while the ventral laterotergite is narrower. A narrow membrane (mb) forms the laterotergal-sternal boundary in cross-sections (Fig. 19B). Sternum 4 extends slightly more anteriorly, and a distinct sternal fold is absent (Fig. 3). The structures corresponding to the movable plates of

the previous sternite are present in the form of 1 + 1 circular muscle attachments (ma), surrounded by membranous but presumably immobile cuticle (Figs. 10, 11A, C).

The proximal portion of the mediotergum is provided with a single dorsal abdominal gland (dag) (Figs. 4, 10). The gland is internally equipped with two ostioles, which are set so close to each other, that they externally appear as a single opening (Fig. 4D, E). The valve lip is broad, provided with 1 + 1 trough-shaped protuberances (Fig. 4E). The cuticle surrounding the external surface of the gland (tcut) is modified into a ring of hundreds of small granulose tubercles (Fig. 4E). Outside of this ring, the cuticle is covered by processes similar to the remainder of the mediotergum (see 3.3. Cuticular microstructures). The cuticle bearing the gland is distinctly elevated (el) (Fig. 4A, D).

Musculature. IV musculus dorsalis medialis (IVdlm): O – posteromedian surface of antecosta 4; I – anteromedian surface of antecosta 5; F – raises tergum 5. IV musculus dorsoventralis externus (IVedvm) (Fig. 10): O – mediolateral surface of mediotergum 4; I – immediately beneath infolding of ventral laterotergite, on an externally visible muscle attachment; F – unknown. IV musculus dorsoventralis internus (IVidvm) (Fig. 10): O – lateral margin of mediotergite 4, slightly more anterior to IVedvm; I – mediolaterally on sternum 4; F – unknown. IV musculus ventralis medialis (IVvlm) (Figs. 10, 11B, D): O – proximal third of sternum 4; I – muscle attachment of sternum 5; F – ventrad retraction of sternum 5.

3.3. Cuticular microstructures

The thorax and abdomen are largely covered with setae; these are short and sparse on the abdominal terga (Fig. 4A), but become very long and dense in the laterotergites, sterna and metathorax (Figs. 3, 4A). Abdominal sclerites scl1a, scl1c, scl1d and laterotergites are covered with long, conical protruberances (Fig. 5D). Those of scl1c-d are somewhat shorter

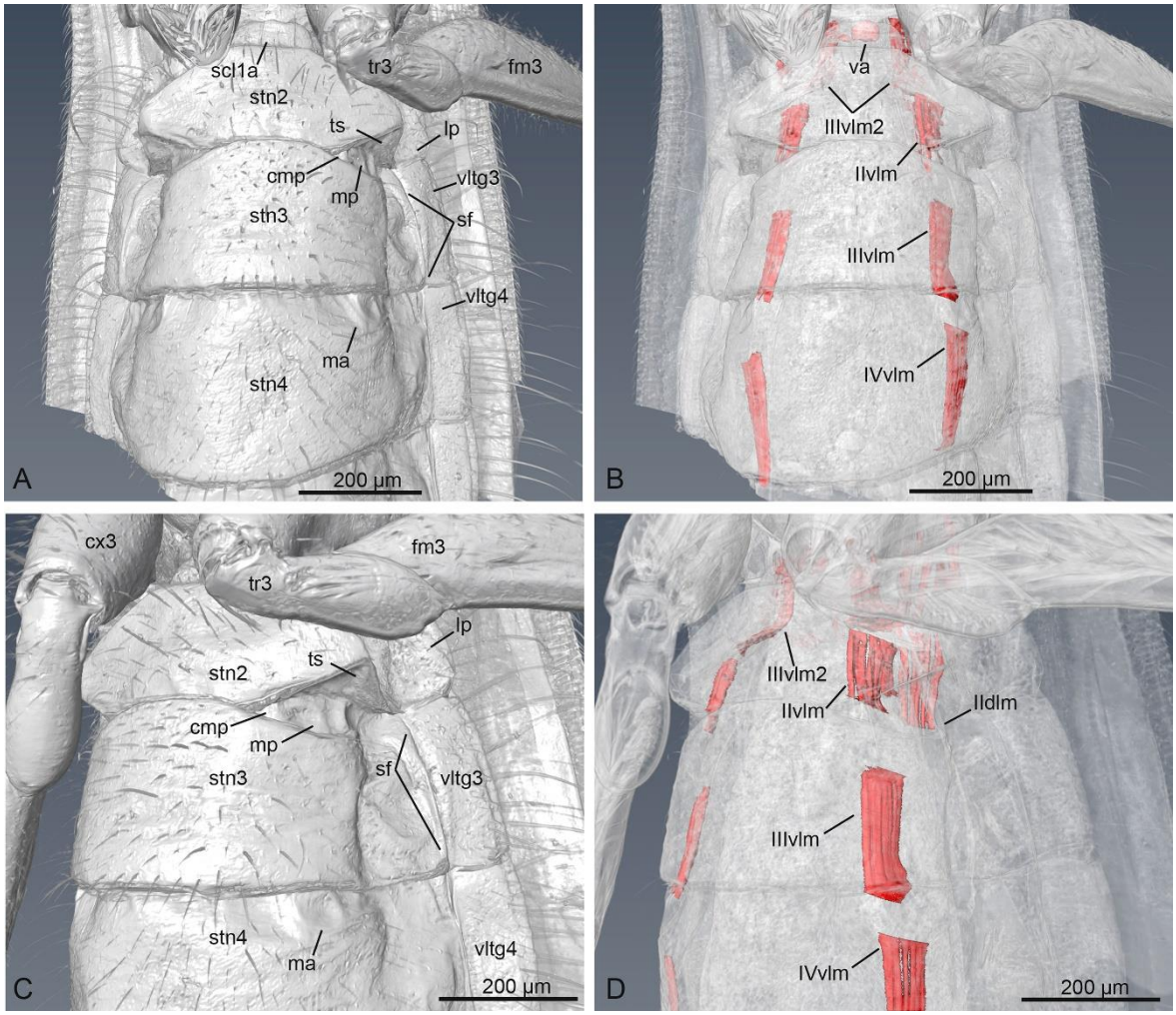


Fig. 11. SR-µCT, volume-rendered 3D reconstruction of ventral abdominal musculature of *Cocles* sp. nov. (dorsal longitudinal and dorsoventral musculature omitted). A: Ventral view of external abdominal morphology, cmp, connective membrane of movable plate of sternum 3; fm3: femur of hind leg; lp, lateral plate, composed of laterosternite 2 and ventral laterotergite 2; ma, muscle attachment of sternum 4; mp, movable plate of sternum 3; scl1a, first abdominal sclerite of sternum 1; sf, sternal fold of sternum 3; stn2, sternum 2; stn3, sternum 3; stn4, sternum 4; tr3, trochanter of hind leg; ts, triangular sclerite of sternum 2; vltg3, ventral laterotergite 3; vlg4, ventral laterotergite 4; B: Same, with cuticle faded to reveal ventral longitudinal muscles and their position in the exoskeleton, IIIvlm2, musculus metafurca-abdominosternalis; va, valve of metathoracic scent gland system; IIvlm, ventral longitudinal muscle of sternum 2; IIIvlm, ventral longitudinal muscle of sternum 3; IVvlm, ventral longitudinal muscle of sternum 4; C: Ventrolateral view of pregenital

abdomen with emphasis on the abdominal sclerites; D: Same, with exoskeleton faded to reveal the attachments of ventral longitudinal muscles on these sclerites. Image link.

and thicker compared to scl1a, while the ones present in laterotergites are of equal length but much thinner (Fig. 12B). Similar conical microsculpture coats the remaining abdominal sterna and metaxyphus, although in the former it is much shorter and extremely dense (Fig. 5A). The surface of scl1b is strongly rugose and has only a few, irregularly spaced serrate processes (Fig. 5A). The ventral junctional membrane possesses a distinct type of microsculpture, being composed of lines of serrate processes, intermixed with rounded granules of great density (Fig. 5B). The posterior portion of ventral junctional membrane, immediately lateral to scl1a, is covered with very dense, serrate microsculpture (Fig. 5C). Other membranous regions, such as the dorsal junctional membrane and the coxal membranes (cxm) are covered with even smaller, rounded granules (Fig. 6C). The entirety of the abdominal dorsum is uniformly covered by densely arranged, short granules of varying shape (irregular, rounded or conical) (Fig. 4B, C). The cuticle of both abdomen and metathorax bears sparse, irregularly arranged epidermal glands (eg) (Fig. 4C), externally similar to simple glands sensu Lawrence & Staddon (1975). A unique cuticular structure might represent a type of sensilla (sa) (same with spherical bodies of Cobben, 1968 in *Oncylocotis*; bare spot of Wygodzinsky & Schmidt, 1991 in *Systelloderes*) (Fig. 12A): it consists of a rounded portion of sunken cuticle, medially bearing an elevated, rounded area (Fig. 12A). These tentative sensilla are present at considerable density on the abdominal terga, sterna 2 onwards and dorsal-ventral laterotergites (Figs. 4, 12B). They are sparse on sternum 1 and the metathoracic sternum and could not be located on the thoracic dorsum. They are also found in the pro-and-mesoxyphus.

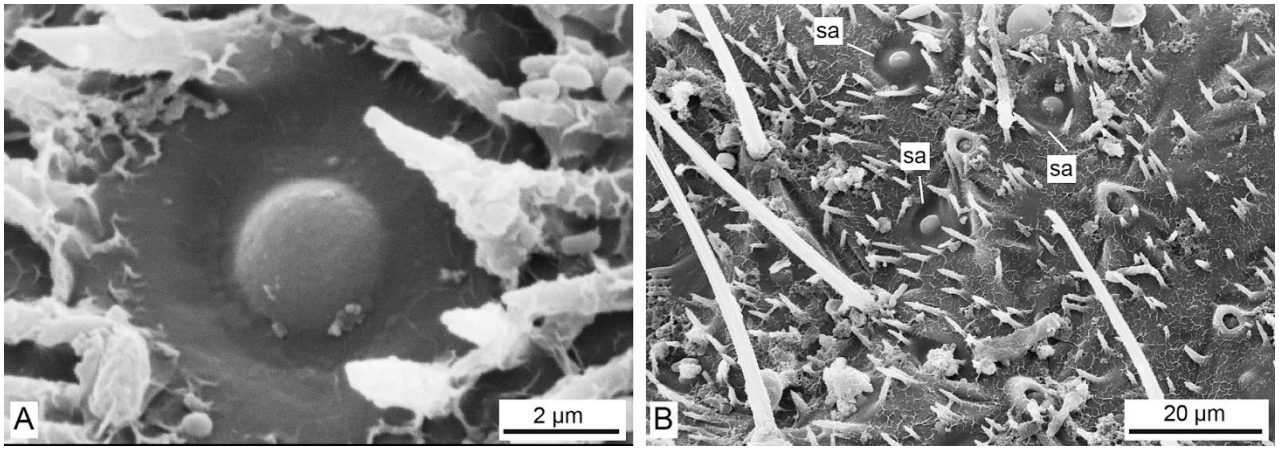


Fig. 12. Structure and distribution of sensilla in *Cocles* sp. nov. A: External morphology of sensilla; B: Distribution and density of sensilla at a given location on the lateral plate (ventral laterotergal portion) of sternum 2, sa, sensilla. Image link.

3.4. The nervous system

The structure of the nervous system of *Cocles* could only be partially reconstructed due to suboptimal preservation of the samples used. The nervous system is organized into a mesothoracic ganglion (msg) which represents the fusion of the mesothoracic, metathoracic and abdominal ganglia (Fig. 13A). The abdomen is innervated by two main pairs of nerves: a thin nervus abdominalis primus-tertius (n.ab. 1–3) and a thicker, medial nervus abdominalis quartus-quintus (n.ab. 4–9) (Fig. 13A). These nerves are termed as such, based on the segments their branches innervate. n.ab. 1–3 is located immediately posterior to a nerve which supplies the hind leg and metathoracic wing muscles and runs parallel to n.ab. 4–9 for most of its length. Before reaching the metathoracic xyphus, this nerve splits into two smaller component nerves (n.ab. 1, n.ab. 2–3; Fig. 13B). The first nerve (n.ab. 1) sends a branch ventral to IIIvlm2 (Fig. 7B, C), which innervates the latter and musculus glandulae thoracicae (Fig. 7B). Further branches from this nerve are sent anteriorly presumably to innervate the dorsal longitudinal and dorsoventral muscles, but could not be traced precisely.

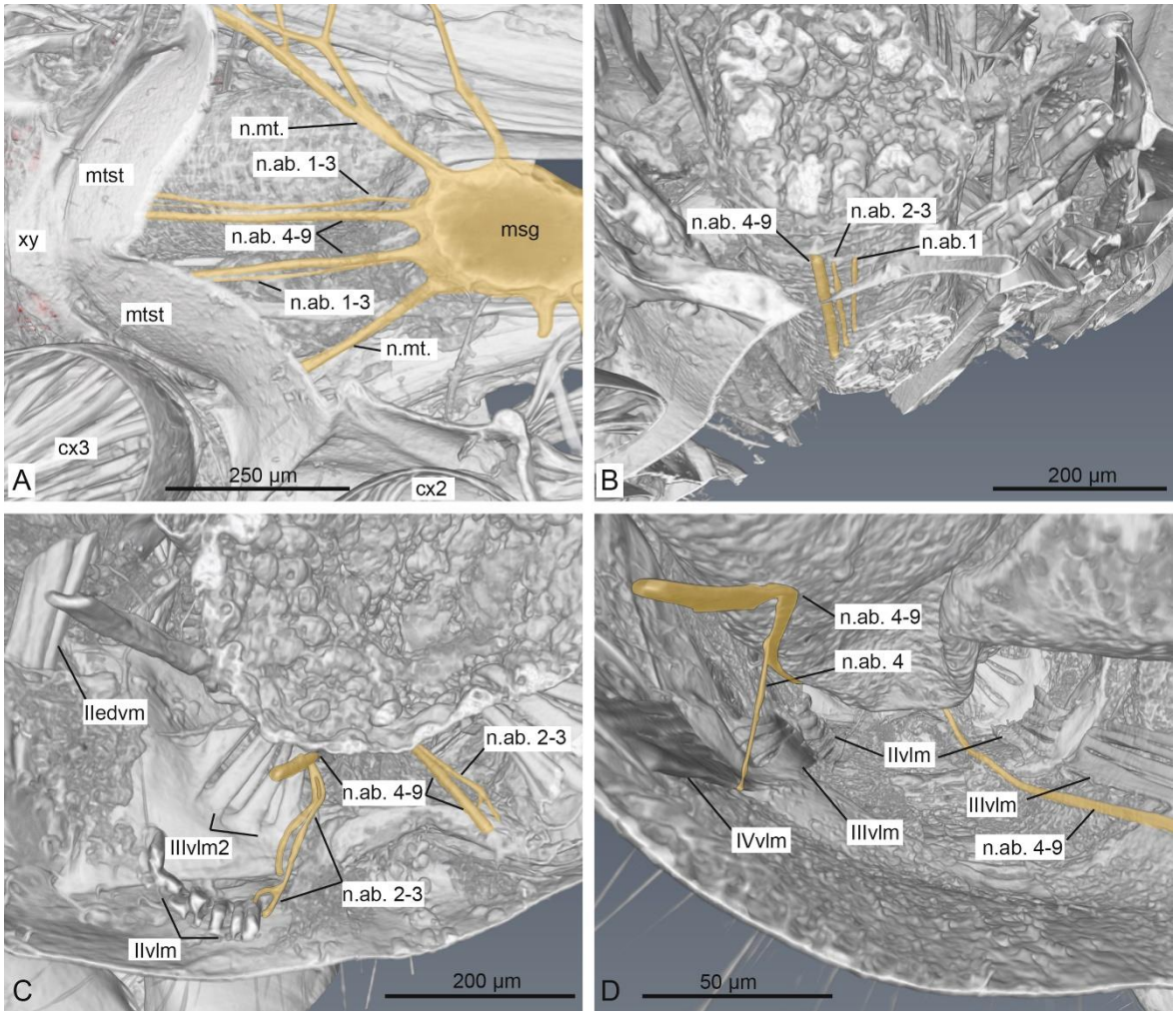


Fig. 13. Mesothoracic ganglion and abdominal innervation in *Cocles* sp. nov. (CPD-dried specimen, SR-µCT, false-coloured, volume-rendered 3D reconstruction). A: Basic organization of mesothoracic ganglion (msg) (median portion of meso and metathorax removed); cx3, coxa of hind leg; mtst, metasternum; n.ab. 1–3, nervus abdominalis primus-tertius; n.ab. 4–9, nervus abdominalis quartus-quintus; xy, metathoracic xyphus; B: Nerves at level of metathoracic xyphus, n.ab. 1–3 has split into two component nerves, the first abdominal nerve (n.ab. 1) and the fused second and third abdominal nerves (n.ab. 2–3); C: Cross-section of abdominal segment 2, n.ab. 2–3 innervates ventral longitudinal muscle of sternum 2 (IIvlm); IIIvlm2, musculus metafurca-abdominosternalis; IIedvm, external dorsoventral muscle of segment 2; D: Cross-section of abdominal segment 4, the fourth abdominal nerve splits from fused nerves 4–9 (n.ab. 4–9), IIIvlm, ventral longitudinal muscle of sternum 3; IVvlm, ventral longitudinal muscle of sternum 4. Image link.

n.ab. 2–3 passes lateral to musculus ventralis furco-abdominalis and bifurcates: one short branch supplies musculus ventralis 2 (Fig. 13C), while the other, longer branch extends posteriorly and innervates musculus ventralis 3. The two large medial nerves (n.ab. 4–9) innervate segments 4–9 (e.g. n.ab. 4; Fig. 13D).

3.5. Other Enicocephalidae

The following subsections concern additional species of Enicocephalidae, covering all subfamilies except Alienatinae and Megenicocephalinae. The description will not be repeated to the same detail as with *Cocles* sp.nov., but mainly serves to note similarities and differences within this family.

3.5.1. *Proboscidopirates* sp.nov. (Enicocephalinae)

Female (known only from tentatively thelytokous females). The general morphology of the metathorax is similar to *Cocles* sp.nov., the main differences being that the metepisternum is considerably more acute, facing more anteriad and that the metaxyphus is almost entirely absent (Fig. 14A). None of the components of the metathoracic scent gland system are present. The metafurcal apophyses are located quite anteriorly in the coxal cavity, as in *Cocles* (Fig. 14B). The abdomen is slightly physogastric and largely membranous, with dark patches of sclerotized cuticle, typically marking areas of muscle attachment (Fig. 14A). The sternum 1 is largely membranous, completely lacking scl1a/scl1b, while scl1c/scl1d are present but poorly sclerotized (Fig. 14B). The remaining abdominal subdivisions largely reflect the condition found in *Cocles*, but are modified in this desclerotized abdomen: the laterosternal plate of sternum 2 is present, largely sclerotized, the movable plate of sternum 3 is present only as a transverse sclerotized line, while sternal areas marking insertions of dorsoventral muscles are sclerotized. There are no indications of any laterotergal subdivision (other than laterosternal plate of sternum 2).

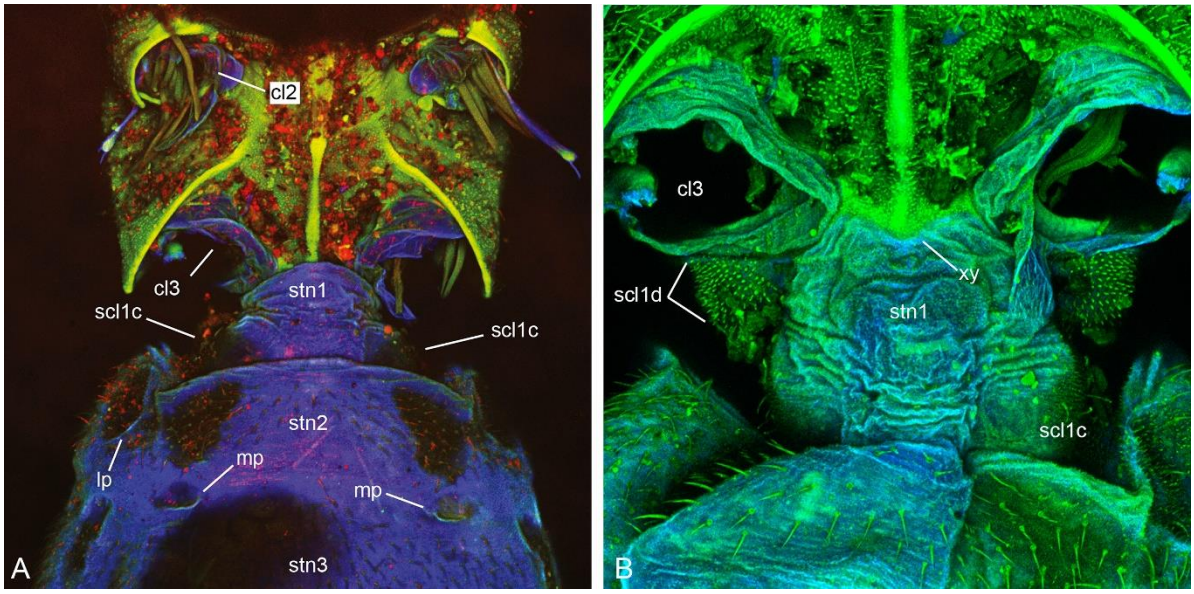


Fig. 14. CLSM images of *Proboscidopirates* sp. nov. A: Ethanol-preserved specimen, ventral view of meso-metathorax and abdominal segments 1–3, cl2, coxal cleft 2; cl3, coxal cleft 3; lp, lateral plate of sternum 2, composed of laterosternite 2 + vltg2; mp, movable plate of sternum 3; scl1c, third sclerite of sternum 1; stn, abdominal sternum; B: Ventral view of dried specimen, showing reduced metaxyphus (xy) and anterior-most sclerite (scl1d). Image link.

3.5.2. Brachypterous *Oncylocotis* sp.nov. (Enicocephalinae)

Male. The thorax is affected significantly by the loss of flight: it is broader, the metepisternum is greatly enlarged and the metaxyphus reduced. The metafurcal apophyses are located anteriorly in the coxal cavity. No metathoracic scent-gland system could be traced. The abdomen is also much broader, flattened and extensively sclerotized. The abdominal dorsum is characterized by a broad tergal plate (fusion of tergum 1 and mediotergum 2) and segments 2–4 are subdivided into mediotergites and undivided laterotergites (similar to *O. neotenicus*, Štys, 1982). Laterotergite 2 is fused to the lateral plate of sternum 2, as in *Cocles* and *Proboscidopirates*. Sternum 1 is medially membranous (no distinct scl1a/scl1b), laterally subdivided into scl1c and scl1d. Sternum 2 is provided with a pair of movable plates, separated by a connective membrane.

Female. Metathorax constructed similarly to male, also lacking metathoracic glands. The abdomen is inflated and much less sclerotized than the male. The boundaries between mediotergum, laterotergites and sternum are not visible. Dark patches frequently coincide with areas of muscle attachment. The first two abdominal terga are strongly reduced, strip-like, not forming a tergal plate. Construction of sternum 1 as in male, sternum 2 somewhat smaller, less sclerotized, experiencing a greater degree of fusion (movable plates fused and immobile). Lateral plate as in male.

Note. Examined Enicocephalinae exhibit marked sexual dimorphism in the construction of the abdomen. The illustration of the abdomen (segments 2–9) of male *O. swezeyi* Usinger & Wygodzinsky, 1960 (both sexes macropterous) is identical to that of Cocles sp.nov. The nearly physogastric, desclerotized abdomen of the female of *O. swezeyi* (Usinger & Wygodzinsky, 1960) is identical to that of *Proboscidopirates* sp.nov. (Section 3.5.1.), the female of the brachypterous *Oncylocotis* sp.nov. and a very large series of *Oncylocotis* spp. and *Systelloderes* spp. we have examined.

3.5.3. *Phallopirates* sp.nov. (Phallopiratinae)

Male. All components of thorax and abdomen essentially identical to that of *Cocles* sp.nov. The main differences are as follows: metaxyphus tapered, with two adjacent ostioles at its apex (Fig. 15B, C); valvular apparatus with 1 + 1 black spots (accessory components?) (Fig. 15B, C). The abdomen is generally less sclerotized, the segments separated from each other by broad intersegmental membranes (Fig. 15). The dorsal junctional membrane is enlarged and the median sulcus is entirely membranous (Fig. 15A). The proximal portion of dorsal laterotergites 2–4 is membranous, providing them with a triangular appearance (Fig. 15A). The connexival edges connecting the dorsal to ventral laterotergites are strongly sclerotized (Fig. 15D). Sternum 1 is broad and largely membranous, scl1b/c are fused to each other (Fig.

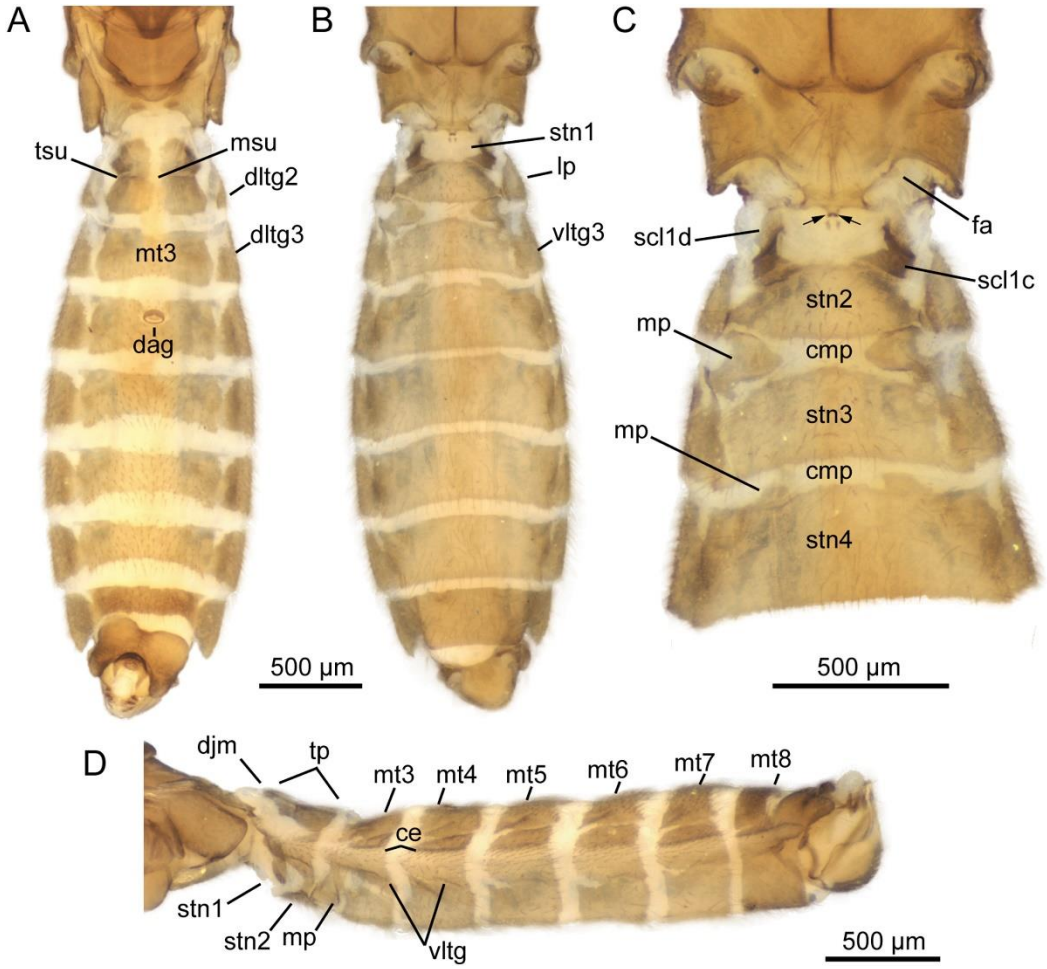


Fig. 15. Abdomen and metathorax of *Phallopirates* sp. nov. macerated in 10% KOH. A: Dorsal view; note the more membranous nature of the abdomen compared to Cocles sp. nov.; dag, dorsal abdominal gland; dltg, dorsal laterotergite; msu, median sulcus; mt3, mediotergite 3; tsu, transverse sulcus; B: Ventral view; C: Meso-and metathorax and abdominal segments 1–4, ventral view; sternum 1 (stn1) is largely membranous, but scl1c/scl1d persist. Movable plates (mp) are present in sternites 2–3, separated by a broad connective membrane (cmp). Arrows indicate the two ostioles at the edge of the metaxyphus; fa, furcal apophysis; D: Lateral view; the connexival edge (ce) is not membranous, and the ventral laterotergites (vltg) almost completely fuse to the sternites; djm, dorsal junctional membrane. Image link.

15B, C). On sternum 2, the triangular sclerite is completely membranous. The movable arms of sternum 3 are greatly enlarged, linked to each other by a very broad connective membrane

(Fig. 15C, D). The corresponding movable arms on sternum 4 are much smaller and the membrane connecting them is narrower.

Note. The sclerites of sterna 1-2 of Phallopiratinae were interpreted by Štys (1985) as abdominal evaporatoria.

3.5.4. *Monteithostolus genitalis* Štys, 1980 (Phthirocorinae)

Male. The metepisternum and coxal bases are greatly enlarged. The metasternum is small and narrow, with a short but distinctive metaxyphus. Metathoracic scent glands could not be detected, although they are probably present, covered by sternum 1. The abdomen is poorly sclerotized but not membranous. The dorsal junctional membrane of the abdomen is narrow, strip-like, while its ventral counterpart is significantly broader. Tergum 1 is short and transverse, not subdivided into laterotergites (different interpretation from Štys, 1981a) and is separated from tergum 2 by a narrow, faint line. Abdominal segments 2–4 are dorsally subdivided into medioterga and a single laterotergite. Although no connexival edge is visible (contrary to the interpretation of Štys, 1981a), the laterotergite is divided below its midline by dense pilosity into two distinct regions: a dorsal glabrous and a ventral pilose region respectively – these could represent undefined dorsal and ventral laterotergites separated by an imaginary line. Sternum 1 consists of a single broad plate, which is strongly sclerotized basally, where it meets the ventral junctional membrane. No subdivision into sclerites scl1b-scl1c could be traced; abdominal evaporatorium 1 forms the base of a spiracle-bearing sclerite (homologous to scl1d). The lateral portion of sternum 2 forms the second evaporatorium, while laterotergite 2 is entire and not fused to the latter. Movable plate of sternum 3 is present, being very large and spatulate. The connective membrane is greatly enlarged, presumably allowing for considerable flexion. Fully formed movable plates are

present on sternites 3–5, although they are much smaller, and the connective membranes are narrower.

3.6. *Lomagostus* sp.nov. (Aenictopecheidae)

3.6.1. Male. Posterior region of metathorax

The metasternum (mtst) is rounded, its posterior apex distinctly tapered, not produced into a metaxyphus (Fig. 16). The metepisternum and metepimeron are greatly enlarged, the metacoxal cleft (cl3) being almost as wide as the metasternum (Fig. 16); the precoxale (prcx) is separated from the metasternum by a narrow membrane (Fig. 16). A true postcoxale (ptxc) is present, which fuses dorsally to the metepimeron and is basally much thicker than the precoxale, almost spatulate (Fig. 16). As a result, the underlying furcasternum and sternacosta are completely obscured by the postcoxale (Fig. 18). Only the furcal pits are visible (apparently misinterpreted as ostioles by Wygodzinsky & Schimdt, 1991), located immediately above the junction between precoxale and postcoxale (Fig. 16A). A single metathoracic gland is present, not subdivided into a reservoir and lacking accessory components (Fig. 16B). The gland is short and ovoid, barely extending to sternum 2. A minute valvular apparatus could be located between the area of fusion of the two arms of the metapostcoxal frame (Fig. 16A). Two adjacent ostioles are located on the sternacosta (not shown).

Musculature. Musculus glandulae thoracicae (mgt): very long and slender muscle. O – posterolateral surface of metathoracic phragma, lateral to Idlm; I – sternacosta (Fig. 18), immediately beneath ventral longitudinal muscle of metafurca (Fig. 18); F – Retracts sternacosta, opens ostioles (?). Musculus metafurca-abdominosternalis (IIIvlm2): O – Metafurcal tip and distal portion of sternacosta (Fig. 18); I – movable plates (part of antecosta 2) posterior to sternum 1 (Figs. 16, 18); F – Raises the abdomen.

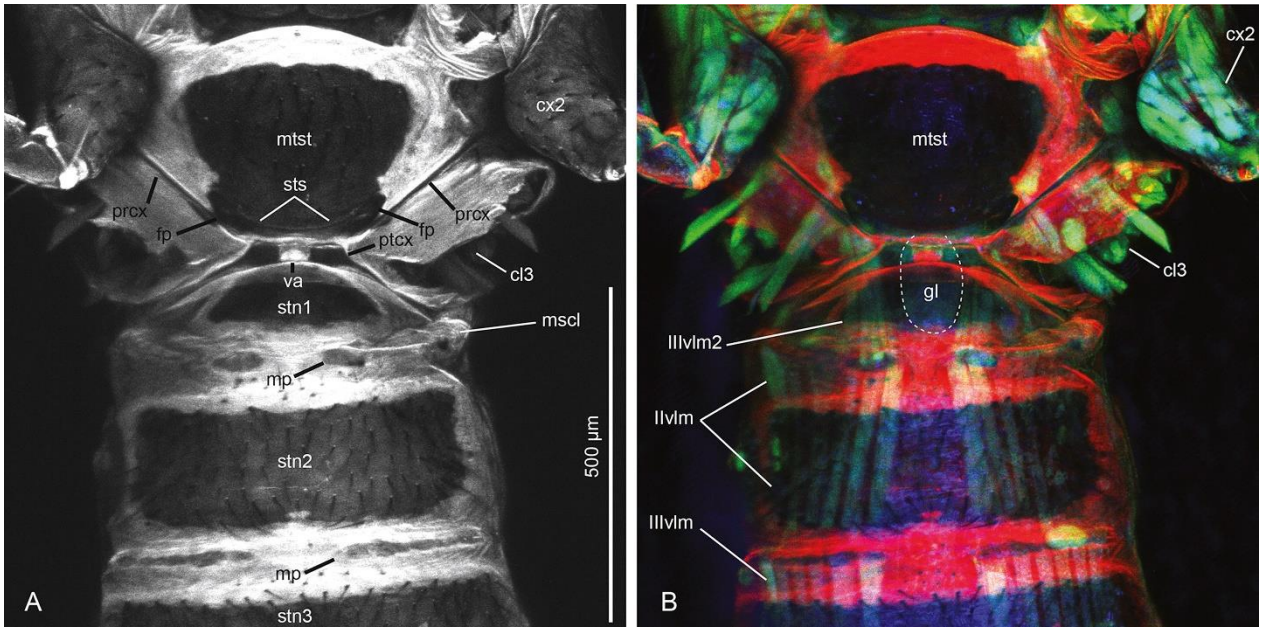


Fig. 16. Ventral view of metathorax and abdomen of male *Lomagostus* sp. nov. using CLSM. A: Greyscale image illustrating contrast between sclerotized and membranous regions of thorax and abdomen; cl3, coxal cleft 3; cx2, coxa 2; fp: pit of metafurca; mscl, muscle-bearing sclerite of sternum 1; mtst, metasternum; prcx: precoxale of metathorax; ptcx, postcoxale; stn: sternum; va: valvular apparatus; B: CLSM image showing musculature and other soft tissue. Red colour indicates membranous regions. IIIvlm2 is relatively undeveloped, while II-IIIvlm are greatly enlarged. The white dashed line indicates the outline of the metathoracic scent gland (gl). Image link.

3.6.2. First abdominal segment

The dorsal surface of abdomen is identical to *Australostolus monteithi* Štys, 1980 as illustrated in Štys (1980; Fig. 31). The dorsal junctional membrane is very broad, occupying most of tergum 1. The sclerotized portion of tergum 1 is also narrow, its posterior median surface distinctly emarginated inwards (forming an inverse U-shape), closely associated but not distinctly fused to tergum 2. A small, partly membranous muscle-bearing sclerite (mscl) at the level of metepimeron bears spiracle 1 (homologous with scl1d of Enicocephalidae; interpreted by Wygodzinsky & Schimdt, 1991 as laterotergite 1) (Figs. 16, 18). Sternum 1 is subtrapezoidal, linked laterally to postcoxale and sternum 2 via a small, triangular

membrane. The posterior surface of sternum 1 bears a pair of elongate, transverse, movable plates (mp, no homology assumed with structure in Enicocephalidae) (confluent with sternal antecosta 2).

Musculature. I musculus dorsalis medialis (Idlm) (Fig. 18): O – posteromedian surface of metaphragma; I – anteromedian surface of tergal antecosta 2; F – Raises the abdomen. I musculus dorsoventralis externus (Iedvm) (Fig. 18): O – posteromedian surface of metaphragma, between Idlm and mgt; I – base of muscle-bearing sclerite, immediately beneath spiracle 1, lateral to Iidvm; F – abdominal retraction/abduction (?). I musculus dorsoventralis internus (Iidvm) (Fig. 18): O – posterior surface of tergum 1, behind antecosta 2; I – muscle-bearing sclerite; F – compressor of tergum 1 (?). Musculus metafurca-abdominosternalis (IIIvlm2): details in Section 3.6.1.

3.6.3. Segments 2–8

The abdomen is largely membranous and segments 2–8 both dorsally and ventrally consist of medially sclerotized regions, which are surrounded by broad membranous areas (Fig. 16). The true segmental limits are well-defined only artificially (in SEM, stained or SR-μCT specimens) or after dissection. The terga are slightly convex, extending ventrad. Each tergum is provided with two pairs of 1 + 1 slit-like impressions (one pair is dorsomedian, the other is dorsolateral), corresponding to muscle attachments. Tergum 4 is provided with a dorsal abdominal gland. Spiracles 2–8 are isomorphic, located at the extreme ventral margin of their respective tergum (Figs. 18, 19). The tergal areas are connected to the sterna by a broad "pleural" membrane (sensu Štys, 1980) (Figs. 18, 19A). There is no evidence of laterotergal subdivision or the presence of a connexival edge (Fig. 19A). Sterna 2–8 are strongly convex; the anterior surface of sternum 3 with a pair of large, transverse

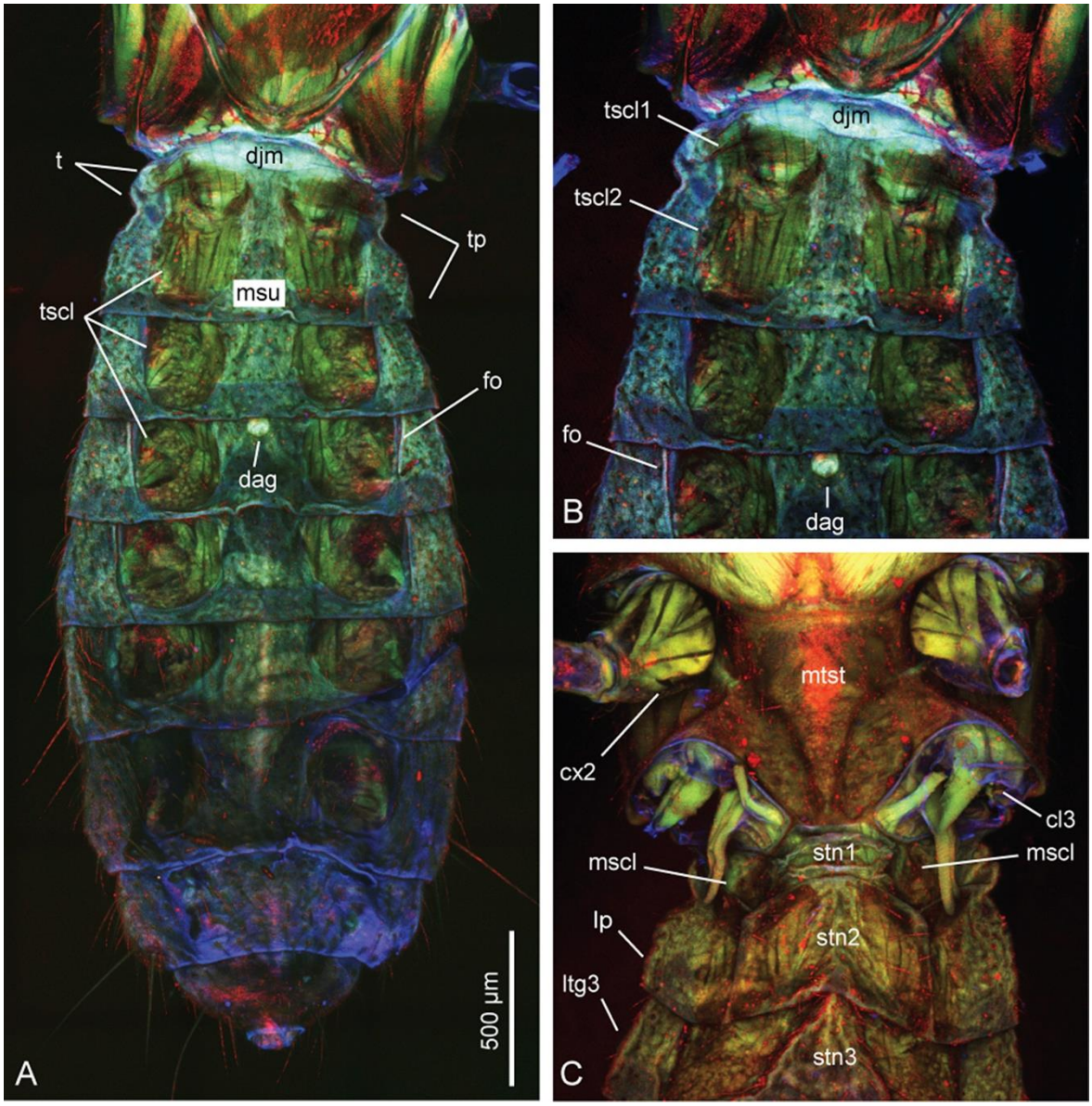


Fig. 17. CLSM images of female *Lomagostus* sp. nov. A: Metathorax and abdomen, dorsal view; note that dorsal longitudinal muscles are confined in distinct tergal sclerites (tscl), dag, dorsal abdominal gland; djm, dorsal junctional membrane; fo, laterotergal fold; msu, median sulcus of tergal plate; t, tergum 1; tp, tergal plate; B: Same, closer view of terga 1–4; C: Ventral view of meso- and metathorax and abdominal sterna 1–3, cl3, coxal cleft 3; cx2, coxa 2; lp, lateral plate of sternum 2, composed of laterosternite 2 + ltg2; ltg3, laterotergite 3; mscl, muscle-bearing sclerite of sternum 1; mtst, metasternum; stn: sternum. Image link.

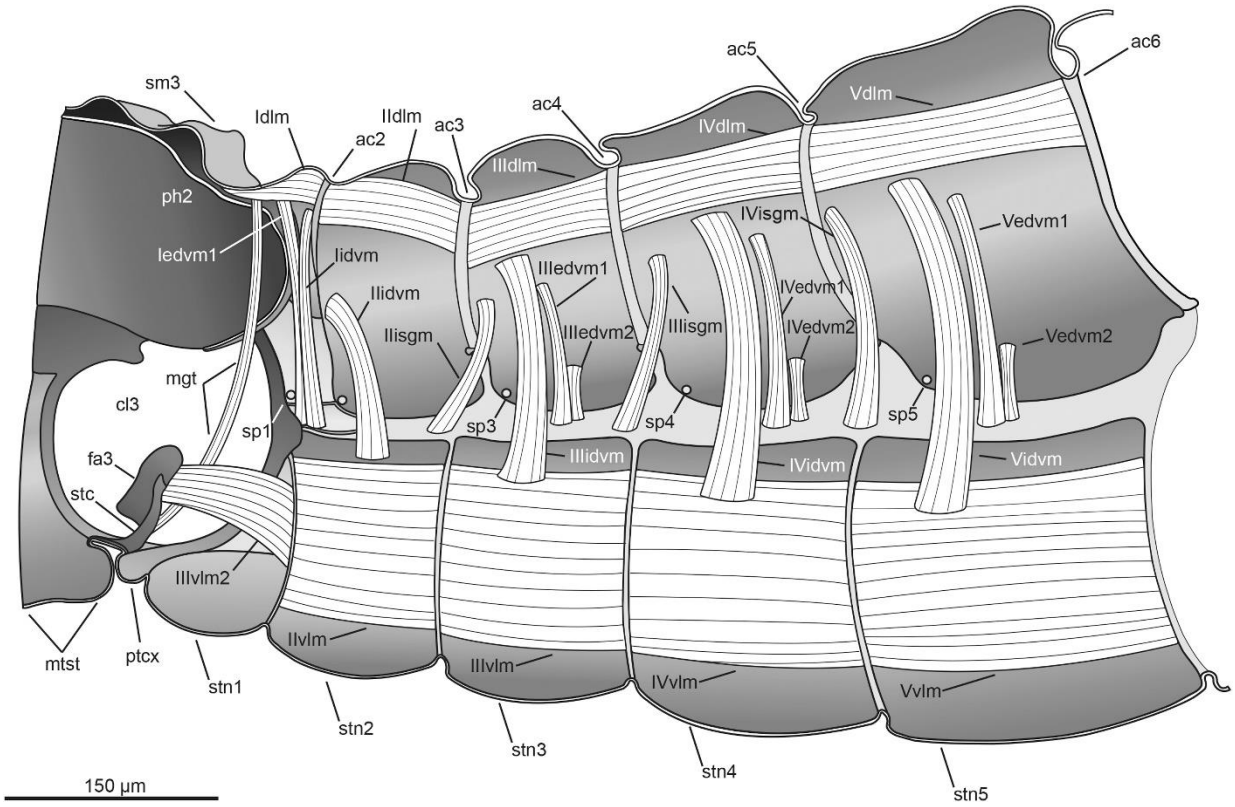


Fig. 18. Sagittal section of male *Lomagostus* sp. nov. showing metathoracic and pregenital abdominal musculature. Metathoracic scent gland and dorsal abdominal gland omitted. ac, antecosta; cl3, coxal cleft 3; fa, furcal apophysis; mtst, metasternum; ph2, mesophragma; ptcx, postcoxale; sm3, scutellum of metathorax; sp, spiracle; stc, sternacosta; stn, sternum. Muscles: IIIvlm2, musculus metafurca-abdominosternalis; dlm, dorsal longitudinal muscle; edvm1, external dorsoventral muscle 1; edvm2, external dorsoventral muscle 2; idvm, internal dorsoventral muscle; isgm, intersegmental dorsoventral muscle; mgt, musculus glandulae thoracicae; vlm, ventral longitudinal muscle. Latin numerals on muscles indicate segmental identity, while Arabic numerals indicate muscle set. Image link.

movable plates, of similar nature to those found between sterna 1-2 (Fig. 16). Sterna 4–8 without such sclerites.

Musculature (segment 2). II musculus dorsalis medialis (IIdlm) (Fig. 18): O – posteromedian surface of tergal antecosta 2; I – anteromedian surface of tergal antecosta 3; F – Raises the abdomen. II musculus dorsoventralis internus (IIidvm) (Fig. 18): O – anterior surface of

tergum 2, behind antecosta 2, on slit-like muscle attachment; I – lateral margin of sternum 2; F – compressor of tergum 2. II musculus dorsoventralis intersegmentalis (IIisgm) (Fig. 18): O – tergum 3, behind antecosta 3; I – posterior portion of pleural membrane of segment 2; F – Inward retraction of membranous antecosta 3. II musculus ventralis medialis (IIvlm) (Fig. 18): O – posterior surface of sternal antecosta 2, including movable plate of sternum 2 (Fig. 16B); I – anterior surface of antecosta 3, on movable plate of sternum 3; F – Largest muscle group in abdomen; ventrad retraction of sternum 3.

Musculature (segment 3). III musculus dorsalis medialis (IIIdlm) (Fig. 18): O – posteromedian surface of tergal antecosta 3; I – anteromedian surface of tergal antecosta 4; F – Contraction of tergum 3. III musculus dorsoventralis externus primus (IIIedvm1) (Fig. 18): O – anterior surface of tergum 3, on slit-like muscle attachment; I – pleural membrane, behind IIIedvm2; F – compressor of tergum 3. III musculus dorsoventralis externus secundus (IIIedvm2) (Fig. 18): O – lateral margin of tergum 3, slightly above level of spiracle; I – exterior margin of pleural membrane, in front of IIIedvm1; F – Unclear; similar position to dilator of abdomen of Vasvary (1966). III musculus dorsoventralis internus (IIIidvm) (Fig. 18): O – anterior surface of tergum 3, on slit-like muscle attachment; I – sternum 2, arising between muscle fibers of IIIvlm (Fig. 19A); F – compressor of tergum 3. III musculus dorsoventralis intersegmentalis (IIIisgm) (Fig. 18): O – tergum 4, behind antecosta 4; I – posterior portion of pleural membrane of segment 3; F – Inward retraction of membranous antecosta 4. III musculus ventralis medialis (IIIvlm) (Fig. 16, Fig. 18): O – posterior surface of sternal antecosta 3, on movable plate of sternum 3; I – anterior surface of antecosta 4; F – Largest muscle group in abdomen; ventrad retraction of sternum 3. The remaining pregenital segments follow the same muscle conformation as segment 3 (Fig. 18).

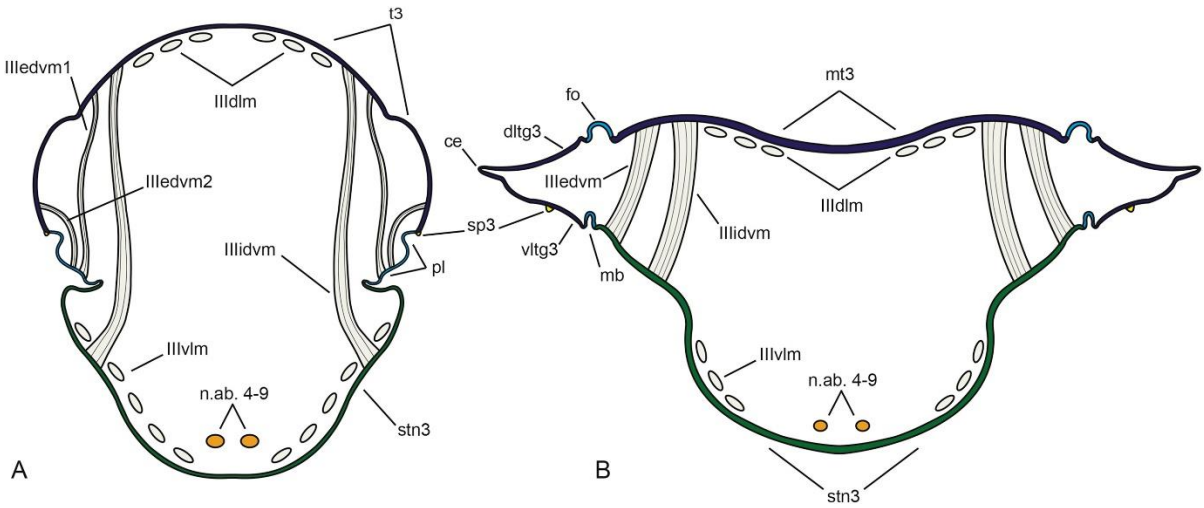


Fig. 19. Schematic drawings of cross-sections of abdominal segment 3 in *Lomagostus* sp. nov. and *Cocles* sp. nov., demonstrating the basic construction of the abdomen in the families Aenictopecheidae and Enicocephalidae. Purple, blue and green colours indicate tergal, membranous and sternal components respectively. A: Abdominal segment 3 of Lomagostus sp. nov. The abdomen consists of an undivided tergum (t3), bearing spiracle 3 (sp3) at is extreme lateral margin, a pleural membrane (pl) and sternum 3 (stn3). There are two pairs of external dorsoventral muscles (IIIedvm1-2), and single, large internal dorsoventral (IIIidvm) arising between the fibres of the sheet-like ventral longitudinal muscle (IIIvlm), n.ab. 4–9, abdominal nerves 4–9; IIIdlm, dorsal longitudinal muscle of segment 3. Intersegmental dorsoventral muscles are omitted; B: The same segment in Cocles sp. nov. is organized differently. The tergum is flat, subdivided into a mediotergite (mt3) and dorsal-ventral laterotergites (dltg3, vltg3) by a membranous, extensible fold (fo). An acute connexival edge (ce) separates dltg3 from vltg3. vltg3 is separated from the convex sternum by a narrow membrane (mb); it is possible that this is the remnant of the pleural membrane of Aenictopecheidae, the rest being lost or incorporated to the ventral laterotergites. The number of dorsoventral muscles is reduced, while the ventral longitudinal muscles are reduced in size. Image link.

3.6.4. Nervous system

Organized into prothoracic, mesothoracic (msg) and metathoracic ganglia (mtg), linked to each other by distinct interganglionic connectives (gc) (Fig. 20). Precise innervation patterns

could not be determined due to inadequate preservation. The two large main abdominal nerves innervate segments 4–9 and a single nerve sends branches to segments 1-2, as in *Cocles* (Fig. 20). Innervation for segment 3 could not be determined.

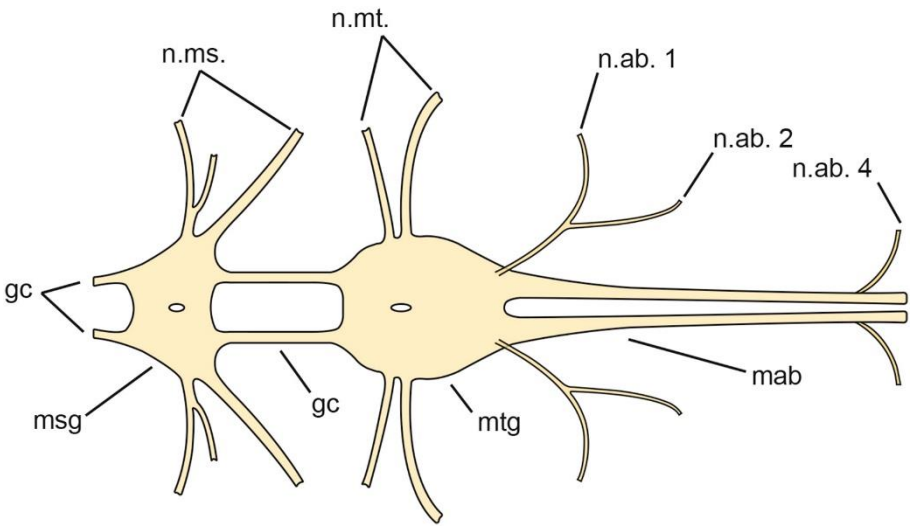


Fig. 20. Thoracic ganglia of male *Lomagostus* sp. nov. (brain and prothoracic ganglion omitted). The innervation of the third abdominal segment is uncertain. gc, ganglionic connectives; mab, main abdominal nerve; msg, mesothoracic ganglion; mtg, metathoracic ganglion; n.ab. 1, first abdominal nerve; n.ab. 2, second abdominal nerve; n.ab. 4, fourth abdominal nerve; n.ms., mesothoracic nerves; n.mt. metathoracic nerves. Image link.

3.6.5. Female

The ventral portion of the metathorax differs strikingly from the male: the precoxale is greatly enlarged, bulging outwards. The metasternum is much larger than the male, shaped like an inverted triangle (Fig. 17C). The posterior apex of metasternum is distinctly tapered, as it is not produced into a metaxyphus (Fig. 17C). The metafurcae are displaced anteriorly, located at the middle of precoxale. There is no indication of a metathoracic scent gland.

The abdomen is elongate, fusiform. Dorsally, there is a broad dorsal junctional membrane, followed by a tergal plate (terga 1-2 fused) (Fig. 17A, B). Terga 3–7 each possess a pair of sclerotized, ovoid, elevated regions (tscl) which host the dorsal longitudinal muscles (Fig.

17A); these regions are fused into a single entity in terga 1-2 (Fig. 17B). The terga are faintly subdivided into laterotergites, which fold ventrally, although they are not distinctly subdivided into a dorsal and ventral series. The laterotergite of segment 2 is fused with a laterosternal subdivision of sternum 2, forming a lateral plate (lp) (Fig. 17C), as in *Cocles*. The spiracles are isomorphic, located in a median region of the ventral part of the laterotergite. No distinct pleural membrane is present. Sternum 1 (stn1) is membranous (Fig. 17C), on its lateral sides possessing distinct muscle-bearing sclerites (mscl) (homologous to scl1d of Enicocephalidae). Sterna 2–7 are without particulars.

4. Discussion

4.1. Musculature

In the following sections, the pregenital abdominal musculature of *Cocles* and *Lomagostus* is homologized with that of other heteropterans, and in some cases with Auchenorrhyncha, Coleorrhyncha and Polyneoptera, when there is sufficient evidence to do so. The musculature of the metathoracic scent glands is compared with that of Nepomorpha (Staddon & Thorne, 1973, 1974, 1979), Gerromorpha (Moller-Andersen, 1982), Leptopodomorpha (Parsons, 1963), some families of Pentatomomorpha (Remold, 1962, 1963), and the Cimicomorphan family Reduviidae (Weirauch, 2006).

4.1.1. Metathoracic muscles

Polyneoptera have a plethora of ventral and dorsoventral muscles originating on the metafurca and the mesospina which insert on the proximal segments of the abdomen (e.g. supplementary table of Friedrich & Beutel, 2008). Most of these muscles are lost in Acercaria [assuming the latter are monophyletic, as Huang et al. (2016) show, contradicting the findings of Misof et al. (2014)], probably in relation to the reduction or loss of the spinae and the simplification of the abdominal sterna. There are only two muscles originating on

the metafurca of Acercaria which attach to the abdomen: the furcal ventral longitudinal muscle (IIIvlm2) and the furcal dorsoventral (IIIdvm8). Given that there is considerable confusion about the identity of these muscles in most works of hemipteran morphology, their systematic distribution and function are discussed in detail (refer to Table 1 for proposed homologies of these muscles in acercarian insects).

In most of the insects examined – both Acercaria and Polyneoptera – IIIvlm2 usually originates immediately beneath the posteroventral surface of the metafurcal tip and inserts on sternal antecosta 2 (i.e. the posterior boundary of sternite 1); its presumed function is to raise the abdomen (Parsons, 1960b). The origin of IIIvlm2 at the metafurcal tip was probably present in the ground plan of Hemiptera, as this is the condition found in adult Auchenorrhyncha (Pringle, 1957; Vasvary, 1966; Young, 1975; Simmons & Young, 1978), Peloridiidae (Davranoglou & Hartung, in preparation), male *Lomagostus* sp.nov. (Fig. 16, Fig. 18), some Ceratocombidae (personal observation), Nepomorpha, Leptopodomorpha and various Cimicomorpha (e.g. Brindley, 1938; Larsen, 1945; Parsons, 1963, 1969).

The condition in *Cocles* and the other examined Enicocephalidae is instead quite modified, where the origin of IIIvlm2 has shifted posteriorly to a pair of sternacostal apodemes. The reasons underlying this change in attachment are assumed to be mechanical: The metafurca of *Cocles* has been displaced anteriorly towards the base of the coxa (Figs. 1, 2, 6), while the metapostcoxal frame forms an elevated bridge (Fig. 11). The origin of IIIvlm2 at the sternacostal apodemes provides direct access to the abdomen and possibly allows the muscle to perform its function more efficiently from this location. However, the precise function of IIIvlm2 is ambiguous. It is one of the largest muscles of the abdomen, suggesting it performs a highly strenuous task in male *Cocles*. The shift in its attachment was probably accompanied by changes in function: instead of simply raising the abdomen, the origin of this muscle at the sternacostal apodemes is roughly parallel to antecosta 2, which allows for

inward retraction of the membranous sternum 1, much like an accordion. Indeed, in male *Lomagostus,* the furcal pits are located at the extreme posterior margin of the metathorax and IIIvlm2 originates from the latter (Figs. 16, 18).

Although IIIvlm2 is largely unmodified in most Heteroptera in terms of attachment and function, the condition in Pentatomomorpha is more specialized. This muscle is partially or fully resorbed soon after the final molt of *Carpocoris purpureipennis* (De Geer, 1773) (Remold, 1962), *Nezara viridula* (Linnaeus, 1758) and *Piezodorus lituratus* (Fabricius, 1794) (Malouf, 1933b; Brindley, 1938), while in some Lygaeoidea (including Berytidae) it has become a component of the metathoracic scent gland system and persists in the adult stage (our homology with ventral muscle of Remold, 1962). Although its precise function in the latter is poorly understood (Staddon, 1979), based on the illustrations of Remold (1962, 1963), in *Lygaeus saxatilis* (Scopoli, 1763), IIIvlm2 has undergone a posterior shift in its origin from the metafurcal tip to a distinct apodeme at the base of the metafurca (chitinhebel of Remold, 1962). Nevertheless, IIIvlm2 retains its origin at the metafurcal tip in Gastrodes grossipes (De Geer, 1773) (Remold, 1962), suggesting that this shift in attachment is not a universal feature of the metathoracic scent glands of Lygaeoidea. The above suggest that IIIvlm2 has shifted its origin at least twice in Heteroptera, possibly in relation to a modification of its original function.

The other muscle of the metafurca involved in the movement of the thoracic-abdominal region is IIIdvm8, which originates from the metaphragma and inserts on the dorsal surface of the metafurca (Parsons, 1969). It is thought to constrict the metaphragma in relation to movements of the abdomen (Parsons, 1960b). This muscle is generally present in adult Nepomorpha (e.g. Lauck, 1959; Parsons, 1969), but is absent in adult *Cocles, Lomagostus* (Figs. 10, 18) and many Pentatomomorpha (personal observation). IIIdvm8 is present in the nymphs of Cicadidae, but is usually lost in adults (Malouf, 1933b; Pringle, 1957). Malouf

(1933b) interpreted the metathoracic scent gland opener in *N. viridula* as IIIdvm8, which he named as "TSF3". We disagree with this interpretation and suggest that the muscle actually involved is musculus glandulae thoracicae (mgt) of Parsons (1960a), which is discussed later.

A theory proposed by Brindley (1938) and Parsons (1969), which postulates that IIIdvm8 and IIIvlm2 represent the ventral longitudinal and dorsoventral muscles of the first segment, which shifted their attachment onto the metafurca due to the reduction of sternum 1, is not supported here. Both of these muscles have serial homologues in the other thoracic segments (Friedrich & Beutel, 2008), and they also exist in insects which possess a more complete set of ventral longitudinal and dorsoventral muscles in the first abdominal sternum (Ford, 1923; Carbonell, 1947).

The final metathoracic muscle of interest is one that does not contribute to movement, but is an essential component of the metathoracic scent glands of many Heteroptera. Musculus glandulae thoracicae (mgt) is the primary opener muscle of the metathoracic glandular system of all Nepomorpha excluding Notonectidae (Parsons, 1960a; Staddon & Thorne, 1973, 1974, 1979), Gerromorpha (our interpretation of Figs. 48–51 in Moller-Andersen, 1982) and Pentatomomorpha (Remold, 1962, 1963; Gonzaga-Segura et al., 2013; Benelli et al., 2014) examined so far, and at least one species of Ceratocombidae (personal observation). Parsons (1963) stated that mdv, the valve opener of *Saldula pallipes* (Fabricius, 1794) (Leptopodomorpha) is not homologous with mgt, although she did not offer an explanation for this statement. The opener muscle of the reduced metathoracic scent glands of Reduviidae (Cimicomorpha) sits on a distinct apodeme adjacent to the metafurca (Weirauch, 2006), and is possibly homologous to mgt. Given that metathoracic scent glands are considered a Heteropteran apomorphy (Staddon, 1979; Wheeler et al., 1993; Schuh & Slater, 1995), the presence of this muscle in Enicocephalomorpha is not surprising.

A striking feature of mgt, however, is its remarkably conserved position: in taxa possessing a IIIvlm2 (including *Cocles* and *Lomagostus*), the position of the latter always covers the basal portion of mgt, with only its anterior portion being visible in dorsal view (Figs. 8, 10, 18) (Staddon & Thorne, 1973, 1974, 1979). In addition, mgt receives the first abdominal nerve, after it passes beneath IIIvlm2 (Fig. 7B, C), characters present in Nepomorpha as well (Staddon & Thorne, 1973, 1974, 1979). The developmental origin of mgt is ambiguous. Matsuda (1970) considered mgt (mentioned as s-stg) a modified sternocoxal muscle; the insertion of mgt of *Lomagostus* on the metafurca (Fig. 18) suggests otherwise.

4.1.2. Longitudinal muscles of the abdomen

The longitudinal musculature of the pregenital abdomen of *Cocles* shares many similarities with that of other Heteroptera. The ventral longitudinal muscle of sternum 1 is invariably absent in adult Heteroptera due to the great reduction of sternum 1 and its antecosta (e.g. Parsons, 1969; Moller-Andersen, 1982) and the examined Enicocephalomorpha do not depart from this condition. The presence of ventral longitudinal muscles on sterna 2–4 and dorsal longitudinal muscles on terga 1–4 is shared with *Gelastocoris oculatus* (Fabricius, 1798) (Parsons, 1960a, 1960b), *Naucoris cimicoides* (Linnaeus, 1758) and *Cimex lectularius* (Linnaeus, 1758) (Larsen, 1945); *Aphelocheirus aestivalis* (Fabricius, 1794) is an exception as Idlm is lost in the adult stage (Parsons, 1969). The enormous, sheet-like ventral longitudinal muscles of male *Lomagostus* are similar to those of some Auchenorrhyncha (Vasvary, 1966). Pentatomoidea (Pentatomomorpha) differ from other

Table 1. List of the different terminologies regarding metathoracic muscles involved in the movement of the abdomen of Acercaria and the metathoracic scent gland system of Heteroptera. – denotes that character is either absent or not reported by study in question.

Taxon	Author	IIIdvm8	IIIvlm2	mgt

Psocoptera	Badonnel (1934)	U	lv	-
Thysanoptera	Davies (1969)	l.inter	vp	-
Cicadidae	Vasvary (1966)	-	91	-
Cicadidae	Pringle, 1957	-	illustrated but not described	-
Tettigarctidae	Pringle, 1957	-	illustrated but not described	-
various Cicadomorpha	Ossiannilsson (1949)	-	IIIvlm (IIIvlm1 when ventral longitudinal of sternum 1 present)	-
Aphrophoridae	Savinov (1990)	2m.tgst.3	2m.v3	-
Cicadellidae	Vondracek (1949)	-	musculus ventralis lateralis	-
Delphacidae	Ossiannilsson (1949)	-	IIIvlm	-
Aphidae	Weber (1930)	IIIism	IIIvlm2	-
Nepomorpha	Parsons (1970; 1969)	60	VL1	mgt
N.viridula	Malouf (1933)	TSF3 (in nymph)	SL^{1}_{3} (lost in adult)	TSF3 (confused with IIIdvm8 of nymph)

Heteropteran infraorders in that all longitudinal muscles following abdominal segment 2 are lost in the adult stage (Malouf, 1933b; Maki, 1938; Kuštor, 1989; personal observation). As a result, the ventral longitudinal muscle of sternum 2 (IIvlm) serves as the sole depressor of the abdomen, attaching on a distinct apodeme (Kuštor, 1989), while I-IIdlm act as the primary levators.

4.1.3. Dorsoventral abdominal muscles

Our understanding of Hemipteran dorsoventral musculature is considerably less understood, and in some cases, as in the tymbal organs of Auchenorrhyncha, there is no consensus even in the segmental identity of the muscles involved (e.g. the interpretations of Snodgrass, 1933 and Kramer, 1950, contrasting those of Ossiannilsson, 1949 and Wessel et al., 2014). As a result, the homologies proposed here should be considered only tentative. Spiracular muscles are not discussed.

Compared to the complex dorsoventral musculature of Polyneoptera (Klug & Klass, 2006), the number of these muscles in the adult Heteropteran pregenital abdomen is strongly reduced, a typical abdominal segment usually having no more than 2 pairs of dorsoventral muscles (for exceptions, refer to Section 4.1.4.). In the adults of some Nepomorpha (Parsons, 1969, 1970, 1971) and a species of *Nabis* (Brindley, 1938), a single dorsoventral muscle originates from the anterolateral margin of tergum 1 and inserts on a distinct process of the postcoxal bridge. *Eurostus validus* Dallas, 1851 (Tessaratomidae) and *N. viridula* differ from other examined Heteroptera in that two dorsoventral muscles arise from this process: one that originates from the metaphragma (LCra in *N. viridula*, 50 in *E. validus*) and another (LCr1 and 51 in *N. viridula* and *E. validus* respectively) that originates behind the antecosta of tergum 2 (Malouf, 1933b; Maki, 1938). This process has received various names [spiracular process (Parsons, 1969); process of postcoxal bridge (Brindley, 1938); process for attachment of muscle EL1 (Parsons, 1971)] and is usually closely associated with the first abdominal spiracle, or the area it would normally occupy in taxa where the latter is lost.

In *Cocles* and *Lomagostus*, due to the greater development of sternum 1, no postcoxal process is present, but two dorsoventral muscles, Iedvm and Iidvm attach on an abdominal sclerite (scl1d) (Figs. 10, 18). Given that structures similar to a postcoxal process exist in

cicadas and other Auchenorrhyncha (lateral apodemal arm of anterior edge of sternum of Vasvary, 1966; sclerite on the metepimeron of Ossiannilsson, 1949), it would be interesting to investigate whether its absence in the examined Enicocephalomorpha is autapomorphic, or this process has evolved independently in Auchenorrhyncha and Heteroptera. Regarding musculature, Iedvm of *Cocles*, due to its association with spiracle 1 and external insertion, is probably homologous with the single external dorsoventral and muscles LCra/50 of the aforementioned Heteropteran taxa, while Iidvm is lost in adults. *E. validus* and *N. viridula* are exceptions, as 51 and LCr1 are likely homologous with Iidvm.

The dorsoventral musculature of the remaining pregenital segments of Heteroptera and Peloridiidae is quite uniform. In *Xenophyes cascus* (Bergroth, 1924) (Fig. 12 in Pendergrast, 1962), *Gelastocoris oculatus* (Fig. 17 in Parsons, 1960a, 1960b), and *Gerris lacustiris* (Linnaeus, 1758) (Guthrie, 1961), there are two pairs of dorsoventral muscles in pregenital segments 2–4: an external dorsoventral which is typically adjacent to the spiracle, and an internal dorsoventral. The conformation of dorsoventral musculature of *Cocles* is similar to the above-mentioned taxa, with some particularities: The external dorsoventral muscles of segments 3-4 insert immediately below the sternal-laterotergal boundary, while the internal dorsoventral muscles insert more ventrally (Fig. 10B). Adults of other Heteroptera such as *Aphelocheirus aestivalis* (Parsons, 1969), *Naucoris cimicoides* (Larsen, 1945), *Sigara substriata* Uhler, 1896 (Maki, 1938), *E. validus* (Maki, 1938) and *N. viridula* (Kuštor, 1989, Malouf, 1933b) usually retain only a single dorsoventral muscle, which is interpreted here as the external dorsoventral, due to its association with the spiracles.

4.1.4. Ambiguous dorsoventral muscles

The complex dorsoventral musculature of *Lomagostus* sp.nov. is impossible to homologize based on the current state of knowledge on heteropteran muscles. It should be noted

however, that the fourth abdominal segment of some cicadas (Vasvary, 1966) and the abdomen of cicadellids (Maki, 1938) possess 4 and 3 pairs of dorsoventral muscles respectively, bearing a remarkable topographic resemblance with the musculature of *Lomagostus* and may well be homologous. It is likely that the great reduction of the pleural membrane in Enicocephalidae (Fig. 19B) resulted in a loss of muscle groups edvm2-isgm which inserted onto it, leading to a simplification of the abdominal musculature in this family. Muscle idvm is tentatively homologized with the internal dorsoventral of *Cocles*, as it inserts on the main body of sternum and was retained.

In other Heteroptera, dorsoventral muscles become most complex in Aradidae, wherein both adults and nymphs possess up to 4 or 5 pairs of glabrous areas (Usinger & Matsuda, 1959), interpreted by Sweet (2006) and Vásárhelyi (1987) as dorsoventral muscle scars. It is predicted that other paedomorphic taxa might retain muscles which are typically lost in adult forms. An additional muscle is Depressor Tymbali reported from *N. viridula*, which originates on tergal antecosta 2 and inserts on the antecosta of sternum 2 (Kuštor, 1989). Documentation of changes in musculature during the transition from nymph to adult might elucidate the homologies of dorsoventral muscles of Aenictopecheidae with Auchenorrhyncha and other Heteroptera.

4.2. The metathoracic scent gland system

Although the internal structure of the metathoracic scent glands of more derived Heteroptera are relatively well studied, our knowledge of the system occurring in Enicocephalomorpha is based on only two species: the aenictopecheid *Murphyanella aliquantula* Wygodzinsky & Štys, 1982 and the enicocephalid *Oncylocotis* (formerly *Didymocephalus* Jeannel, 1942) *basalis curculio* (Karsch, 1893) (Carayon, 1948).

The single, very long gland of *Cocles* (Fig. 8), is similar to *O. basalis curculio*, while the shorter gland of *Lomagostus* is shared with *M. aliquantula*. The only Heteroptera characterized by a single, undivided glandular unit are Enicocephalomorpha, some Ceratocombidae (personal observation) and some Cryptostemmatidae (Carayon, 1949). Other Heteroptera, including Stemmocryptidae (Dipsocoromorpha) (Štys, 1983), typically possess a glandular system subdivided into a reservoir and a lateral gland (one of which or both may be paired), or in some Gerromorpha, the single, median reservoir is ensheathed by a distinct type of glandular tissue (Moller-Andersen, 1982). In other taxa, a suite of accessory glandular components is present (Carayon, 1971; Staddon, 1979). It is uncertain which part(s) of these glandular systems are homologous to the single gland of Enicocephalomorpha, although Carayon (1948) describes the basal part of the Enicocephalomorphan gland as reservoir-like. Detailed histological studies will help determine homologies between the gland of Enicocephalomorpha and the glandular systems of other Heteroptera.

In SR-μCT sections, the valvular apparatus of *Cocles* appears as a simple chitinous sac, which lacks secretory cells (Fig. 7) - this purely cuticular nature is shared with the valvular apparatus of other Heteroptera (Carayon, 1948) and the two structures are possibly homologous. It is generally thought that after leaving the valvular apparatus, the secretions are externalized via the efferent system, consisting of (at least) a vestibule and a scent canal (Kment & Vilimova, 2010). Given that these structures are absent in all Enicocephalomorpha examined, the efferent pouch described here forms the efferent system of this infraorder. Although the efferent pouch has not been described in other Heteroptera, it might have been overlooked due to its extremely small size and position beneath the valvular apparatus.

The number and position of ostioles formed the basis of Carayon's (1971) scheme on the evolution of metathoracic scent glands. This scheme postulates that the earliest diverging lineages possess an omphalian system (one ostiole or two closely associated ones) at the thoracic-abdominal boundary. More derived Heteroptera are diastomian (two broadly separated ostioles), which gradually move anteriorly to the metathorax, and become closely associated with the metafurcal apophyses (for a review, refer to Carayon, 1971, Staddon, 1979).

This scheme is partially confirmed: there are two closely associated ostioles present in *Cocles*, *Phallopirates* and *Lomagostus*, but whether the prototype metathoracic scent gland system possessed a single ostiole, as Carayon claims, is still uncertain. Male *Monteithostolus genitalis* Štys, 1981a and female *Heissaptera janaki* Štys and Baňař, 2006 are also thought to possess two broadly separated ostioles, while the sternacosta of *Gamostolus* (Bergroth, 1924) clearly possesses two adjacent ostioles (our interpretation of Fig. 35A of Wygodzinsky & Schimdt, 1991). Nevertheless, both sexes of *Boreostolus sikhotalinensis* Wygodzinsky & Štys, 1970 are reported possessing one ostiole at the thoracic-abdominal junction (Wygodzinsky & Štys, 1970). Further studies are necessary to conclusively determine the number of ostioles throughout the different lineages of Enicocephalomorpha and test Carayon's model. From a systematic perspective, an important observation is the apparent transfer of the ostioles from the sternacosta in Aenictopecheidae to the apex of the metaxyphus in Enicocephalidae (Figs. 9, 15C).

4.2.1. Mode of action

The valvular apparatus is directly continuous with the gland and is probably filled with the secretions of the latter at all times, as the absence of a closing structure indicates. Its possible function is to release the glandular contents when mgt, which attaches to it laterally (Figs.

7, 8), contracts and allows the secretion to travel from the valvular channel to the lumen of the efferent pouch. However, it is uncertain whether the contraction of mgt affects the conformation of the valvular apparatus itself, or the underlying efferent pouch, in order to enable the flow of secretion. Once the secretion accumulates in the efferent pouch, it is externalized, probably forming a droplet or a thin film on the abdomen, which then needs to disseminate in the air. Given that in *Lomagostus* mgt attaches on the sternacosta, it is possible that that the secretion mechanism is different e.g. mgt contracts, pulling the sternacosta, allowing the ostioles to open.

Tentative evaporatoria in Enicocephalomorpha are known from the abdominal sternal sclerites of *M. genitalis* (Štys, 1981a) [the ostioles of Wygodzinsky & Schimdt, 1991 in aenictopecheids (e.g. Fig. 20E, F, egress of ventral scent gland; Figs. 31A–E; 41E; 43A, scent gland orifice), in fact represent metafurcal pits]. The complex microstructures present in the ventral junctional membrane of *Cocles*, consisting of a mix of granulose, conical and dentiform protruberances (Fig. 5B–D) are remarkably similar to the evaporative cuticle surrounding the dorsal abdominal glands of some Rhopalidae (Rohanova et al., 2016) and possibly form an abdominal evaporatorium. The cuticular microstructures of scl1a-d are identical to those of the other abdominal sterna and are unlikely to play a role in the evaporation of the secretion, except if the latter spreads on the entire abdomen. In addition, the large sternacostal sulcus (Fig. 6C, sts) of *Cocles* also lacks distinct specializations that could aid in the evaporation of any substance. Interestingly, the anapleural suture forms part of the efferent system in at least a few Nepomorpha and Cimicomorpha and is characterized by evaporative structures (paracoxal groove of Matsuda, 1962, 1970; Staddon & Thorne, 1973, 1979). The absence of such structures in the anapleural suture of *Cocles* indicates that its modification as part of the efferent system arose later in heteropteran phylogeny.

4.2.2. A different interpretation of Enicocephalomorphan metathoracic scent glands

Contrary to Carayon (1948, 1971) and Wygodzinsky & Štys (1970, 1982), Wygodzinsky & Schimdt (1991) provided a different hypothesis regarding the scent gland system of Enicocephalomorpha. They described a "scent gland auricle" (i.e. the scent canal of Staddon & Thorne, 1979) on the metapostnotum (Fig. 30 D–F in Wygodzinsky & Schimdt, 1991) which in their view represented the "true metathoracic scent gland of other Heteroptera". They described this auricle from various New World Enicocephalomorpha, but mentioned it was lost in wingless forms. Regarding the large gland at the thoracic-abdominal junction which Carayon (1948) and the present study describe, they suggested it represents a different, secondary system which is diastomian (Figs. 31 and 35A in Wygodzinsky & Schimdt, 1991).

Štys (1998) briefly criticized the glandular hypothesis of the aforementioned authors and suggested that the "gland" instead represents the scutellar process which harbors the frenum, a part of the metapostonotal wing coupling device. The study of Weirauch & Cassis (2009) on Hemipteran wing coupling structures provides images of the scutellar process and frenum in a range of taxa, including some Enicocephalids (Figs. 6, 21 and 22). This structure is indeed identical with the "scent gland auricle" of Wygodzinsky & Schimdt (1991). In addition, a glandular "duct" mentioned in Wygodzinsky & Schimdt (1991) actually represents the internal surface of the hollow cuticle of the metapostnotum, as illustrated in Parsons (1963; Fig. 9, SP). This also explains why the "gland" is lost in wingless enicocephalid taxa, which do not need a wing-coupling apparatus.

4.3. The nervous system

Based on studies examining the nervous system of Heteroptera (e.g. Malouf, 1933a; Parsons, 1960a), abdominal innervation in this group can be broken down to the following components: The mesothoracic ganglion sends four small nerves to the abdomen, each

successively innervating segments 1–4 respectively (N.ABI-IV of Parsons, 1960a). There also two large medial nerves which innervate segments 5–9 (N.ABV of Parsons, 1960a).

The condition in cicadas is somewhat different: The first two abdominal segments receive two separate nerves (the "tensor" and "auditory" nerves respectively), while there are two large medial nerves ("abdominal nerves"), which innervate segments 3–9 (Pringle, 1957; Vasvary, 1966; Simmons & Young, 1978). Interestingly, the conformation of *Okanagana rimosa* (Say, 1830) is different to that of the cicada species examined by the previous authors. In this species, two additional nerves have been described (Strauss & Lakes-Harlan, 2009): a nerve between the tensor and auditory nerves and small abdominal nerve following the auditory nerve. Unfortunately, the segments the nerves innervate were not indicated.

The nervous system of *Cocles* is organized differently from that of cicadas and other Heteroptera. The main particularity is that the branches which form distinct nerves 1–3 and 1-2 in Heteroptera and cicadas respectively are fused into a n.ab.1–3 and the fourth independent nerve of Heteroptera is fused to the medial nerve n.ab. 4–9.

The ganglia of *Lomagostus* are strikingly different; they are organized into prothoracic, mesothoracic and metathoracic ganglia (Fig. 20), the latter representing a fusion of the metathoracic and abdominal ganglia. Interestingly, the nervous system of Peloridiidae and Cercopidae is organized in the same way (Pflugfelder, 1936; Pendergrast, 1962). The nervous system of the thorax in Heteroptera and cicadas is usually organized into a prothoracic and mesothoracic ganglion (although in some taxa all ganglia condense into a single entity), the latter representing a fusion of mesothoracic, metathoracic and abdominal ganglia during embryonic development (Springer, 1967). Based on the above, it is possible that fusion of the ganglia of the mesothorax and metathorax into a single mesothoracic ganglion occurred multiple times in Hemiptera.

4.4. Possible patterns in the evolution of the abdomen of Enicocephalomorpha

Due to their unique morphology, the monophyly of Enicocephalomorpha has rarely been questioned (Weirauch & Schuh, 2011). Our findings support this opinion. The largely complete sternum 1 is unique to Enicocephalomorpha (e.g. Štys, 1980, 1996; Štys & Baňař, 2008, 2009). The single, undivided metathoracic scent-gland, the absence of a metathoracic evaporatorium, the unique efferent system, the various abdominal sclerites, and the distinct lateral sclerite harboring the dorsoventral muscles and spiracle of segment 1, have not been found outside Enicocephalomorpha.

The two families of Enicocephalomorpha possess very different abdominal morphology from each other. The construction of the pregenital abdomen of Aenictopecheidae is quite uniform and appears simpler than that of Enicocephalidae: The abdomen is subdivided into a tergum (with spiracles located at its ventral margin), pleural membrane, and sternum (Fig. 19A) (Štys, 1980). We interpret the pleural membrane as part of the sternum, as indicated by the attachment of dorsoventral muscles edvm1-2 and isgm (Fig. 19A). This abdominal conformation is considered the plesiomorphic condition for the infraorder (Štys, 1980). A putative aenictopecheid apomorphy is the location of the metafurca at the extreme posterior border of the metathorax (Fig. 18). All known species of Aenictopecheidae conform to this scheme, the female of *Lomagostus* being the sole exception, resembling the system of some Enicocephalids (subdivision into laterotergites, spiracles 2–4 located medially on ventral part of laterotergite, metafurca displaced anteriorly) (Fig. 17). Due to the paucity of information regarding the relationships within this family, we cannot assert the phylogenetic significance of the condition found in the female of this genus, but it is possible that these characters are homoplastic. Finally, the presence of movable plates on sterna 2–3 (Fig. 16) in *Lomagostus* is shared with *Gamostolus* (Fig. 35A in Wygodzinsky & Schimdt, 1991). Their presence in other Aenictopecheid genera is likely but has probably been overlooked.

Within Enicocephalidae, the pregenital abdomen has undergone a series of transformations. The Pthirocorinae are considered as the earliest diverging lineage within Enicocephalidae, based on genitalic characters (Štys, 1981a, 1985). The abdomen is composed of the following: mediotergite; membrane; single spiracle-bearing laterotergite (Section 3.5.4.); reduced (pleural?) membrane; sternum; movable plates present in sterna 3–4. In Phallopiratinae, another plesiomorphic taxon (also based on genitalic characters, Štys, 1985), the abdomen shares the same complex system of dorsal-ventral laterotergites, sclerites (e.g. scl1a-d, movable plates, lateral plates; Fig. 15) and membranes with derived *Cocles* (Enicocephalinae), which indicates that these characters were acquired early in the phylogeny of Enicocephalidae. We postulate that the dorsal and ventral laterotergites of Enicocephalidae represent subdivisions of the single laterotergite of Pthirocorinae, which in turn is a detached part of the tergum of Aenictopecheidae. Incorporation of sternal components (e.g. pleural membrane) in the ventral laterotergites is also possible.

If the ancestral condition is indeed displayed by Pthirocorinae, it is possible that within Enicocephalidae, there has been a steady acquisition of characters that allow increasing flexibility and mobility of the abdomen, conferred by increasing complexity in the above-mentioned exoskeletal components, which is however marked by a simplification of the dorsoventral musculature and a reduction in the size of ventral longitudinal muscles (Fig. 19B). We postulate that these traits represent adaptations for more energetically efficient movement of the abdomen, requiring less investment in muscle mass, compared to the large and complex muscles of Aenictopecheids. Similar ganglionic condensation, reduction in dorsoventral musculature and subdivision into laterotergites has taken place in other Heteroptera and is here assumed to be the result of evolutionary parallelism.

To further understand the evolution of the Enicocephalid abdomen, comparative studies examining many genera and all subfamilies (including Alienatinae and Megenicocephalinae, omitted in this work) are necessary.

4.5. A comparison with other Hemiptera

A complete sternum 1, which lacks a distinct antecosta 1 is shared between Enicocephalomorpha and Peloridiidae, contrasting the situation in Auchenorrhyncha, where a very short Ivlm is frequently present (Pringle, 1957; Davranoglou et al., in preparation).

An additional similarity concerns the large size and insertion of IIIvlm2. One of the largest muscles of the abdomen in Peloridiidae (Davranoglou & Hartung, in preparation), Enicocephalidae, and other Heteroptera is IIIvlm2 and it is assumed to be the primary levator of the abdomen (Parsons, 1960a, 1960b). This system is different to that of most Cicadomorpha (Auchenorrhyncha), which combine abdominal tremulation, with the buckling of membranous structures. Although IIIvlm2 is frequently well-developed in many Auchenorrhyncha and certainly plays an important role in abdominal tremulation, the muscles responsible for membrane buckling are thought to be enlarged dorsoventral muscles (Ossiannilsson, 1949). Ergo, a basiabdominal vibrational organ, which relies primarily on longitudinal muscles (Sweet, 1996; Wessel et al., 2014), including IIIvlm2, is present in several hemipteran lineages.

4.6. Comparison within Heteroptera

In most Heteroptera, including Enicocephalomorpha and Dipsocoromorpha, abdominal terga 1-2 are closely associated and form a “tergal plate” (Fig. 4A; Sweet, 1996; Gogala, 1984, 2006; Štys & Baňař, 2006,). Its muscles are largely responsible for abdominal tremulation in Pentatomomorpha (Kuštor, 1989; Amon & Čokl, 1990), and they might be important for vibration production in other infraorders as well. Previous studies (Gogala,

1984; Štys & Baňař, 2006) have suggested its presence may be a synapomorphy of Heteroptera. A complete fusion of terga 1–3 into a syntergite and loss or reduction of the corresponding musculature is present in Gerromorpha (Moller-Andersen, 1982); as a result, a basiabdominal vibrational organ is presumed to have been secondarily lost in this infraorder (Sweet, 1996).

Concerning the ventral part of the abdomen, the extreme reduction of sternum 1 and its fusion to the metathorax, forming a secondary postcoxale, may represent a putative synapomorphy of Heteropera (excluding Enicocephalomorpha and possibly Dipsocoromorpha). Rhagadotarsine gerrids have a slightly more developed sternum 1 compared to other Heteroptera, but this is attributed to a reversal (Moller-Andersen, 1982; Štys, 1996). Interestingly, an extensive membranous region between the metathorax and abdominal sternum 2 in some Ceratocombidae (personal observation) and Stemmocryptidae (Štys, 1983) could represent sternum 1. If confirmed, this feature could support the sister-group relationship between Dipsocoromorpha and Enicocephalomorpha (Wang et al., 2016). However, the metathoracic scent apparatus of some examined Dipsocoromorpha is composed of multiple glands (Štys, 1983) and many species of this infraorder have well-developed metathoracic evaporatoria (Carayon, 1971), contrasting the condition found in Enicocephalomorpha (Fig. 6), although the latter character has evolved multiple times (Schuh et al., 2009). Morphological studies examining the head of the two infraorders yielded inconclusive results about their relationships, probably due to the fact that head morphology is strongly conserved in even derived Heteropteran taxa (Spangenberg et al., 2013a). Detailed investigations on the abdominal morphology of Dipsocoromorpha may shed light on their phylogenetic position within Heteroptera.

The loss of IIIvlm2, the action of I-IIdlm and IIvlm as the sole levators and depressors of the abdomen respectively (all other longitudinal muscles are lost) and the presence of a

distinct apodeme for IIvlm may also represent putative synapomorphies for Pentatomoidea, as we have observed this condition in the families Acanthosomatidae, Cydnidae, Pentatomidae, Plataspidae, (some, but not all) Scutelleridae, Tessaratomidae and Urostylididae (Davranoglou, in preparation; Redei, personal communication). The incorporation of IIIvlm2 into the metathoracic scent gland system of some Lygaeoidea and Berytidae (Remold, 1962), may be useful in determining the relationships between the two groups.

Regarding capacity for chemical communication, our findings are fully congruent with earlier studies (Wheeler et al., 1993) which indicated that metathoracic scent glands are an autapomorphy of Heteroptera. We also find that the principal opener muscle of the scent glands of most Heteropteran infraorders is also present in Enicocephalomorpha. It is thus possible that metathoracic scent glands of the common ancestor of Heteroptera used this muscle as well.

4.7. Possible modes of communication in Enicocephalomorpha

Heteroptera make extensive use of both chemical and acoustic-vibrational signals (Schuh & Slater, 1995; Gogala, 2006). This is in contrast to Auchenorrhyncha and Peloridiidae (Coleorrhyncha), which are known to communicate only via acoustic and/or vibrational signals (Drosopoulos & Claridge, 2006; Hoch et al., 2006). As a result, the chemical ecology (Aldrich, 1995), bioacoustics and vibrational communication (e.g. Gogala, 2006) of Heteroptera have been studied extensively, while *N. viridula* is used as a model organism in the emerging field of biotremology (Čokl, 2008). Nevertheless, the vexing question is whether the two signaling behaviours evolved in conjunction, or whether vibrational and acoustic signals appeared later in the evolution of Heteroptera. The description of

Enicocephalomorphan morphology in this paper provides key missing evidence needed to allow us to hypothesize how bimodal signaling evolved in Heteroptera.

Although there are no reports of chemical communication in Enicocephalomorpha, the use of such signals has been taken for granted by all studies on the group (e.g. Carayon, 1971; Štys, 1981b) due to the presence of metathoracic glands. The metathoracic scent gland system of Enicocephalomorpha is unique in several respects. The structure of both gland and valve mechanism is so aberrant that they currently cannot be homologized with structures occurring in other Heteroptera. In addition, the way these secretions disseminate in the air differs from all other Heteroptera. However, the musculature used is the same as that of other Heteroptera, implying a single origin of metathoracic scent glands in the common ancestor of Heteroptera, in agreement with previous studies (Wheeler et al., 1993).

Turning to vibrations, such signals have never been recorded from Enicocephalomorpha, although there are reports of tentative stridulatory organs in the legs of at least two taxa (Štys & Baňař, 2006, 2007). Novel morphological features presented in this paper may hint the presence of vibrational communication in Enicocephalomorpha: The pregenital segments are unusually flexible, due to their subdivision into several sclerites and a multitude of membranes (Figs. 1, 2, 3), which allow these segments to be extended and retracted considerably, by means of muscular action. This type of movement is not possible with the strongly sclerotized cuticle of other Heteroptera (excluding Dipsocoromorpha). Parallels can be drawn with the subdivided sterna 1-2 of Auchenorrhyncha (Snodgrass, 1933; Kramer, 1950), which presumably allow them to move their abdomen inwards and outwards in order to modulate their songs. In addition, the presence of a tergal plate in Enicocephalomorpha, a structure responsible for vibration generation in other Heteroptera (Gogala, 1984, 2006), is suggestive of the existence of this behaviour in this infraorder (Štys & Baňař, 2006). Importantly, these morphological features exhibit strong sexual dimorphism in both fully

winged and brachypterous/micropterous taxa, indicating a sex-specific behaviour not obligately associated with nuptial swarming behaviours (Schuh, 1970).

Although the heteropteran pregenital abdomen is frequently sexually dimorphic, in most taxa, the differences are subtle (e.g. the size of dorsal abdominal glands, the proportions and shape of tergal structures, pilosity etc.), in the examined Enicocephalomorpha, sexual dimorphism is apparent in the structures that presumably allow some type of motion of the abdomen. The observation that the structures involved in this movement are reduced in females indicates that this movement has some sex-specific function, in the same way that metathoracic scent glands exhibit sexual dimorphism as well (Carayon, 1971). In addition, the fact that Enicocephalomorpha possess a (sexually dimorphic) tergal plate, which is a structure crucial or important in vibration generation in other taxa (e.g. Barber, 1971; Gogala, 1984, 2006; Kuštor, 1989), indicates that its sexual dimorphism is related to differences in function, possibly vibration. The presence of a tergal plate in female *Lomagostus* (Fig. 17A, B) and its reduction in males might indicate that sexual behaviours in this species may have reversed.

Although we cannot exclude the possibility that the unusual motions generated by the Enicocephalomorphan abdomen have a function other than vibration, we consider it unlikely for several reasons. Optical signals can be excluded, as this morphology is present in brachypterous taxa with reduced eyes (e.g. *Oncylocotis* sp.nov. in Section 3.5.2.). Chemical signals probably disperse on basiabdominal evaporatoria (4.2.1.). The use of the abdomen in controlling flight manoeuvers is possible, although the presence of the same abdominal morphology in brachypterous taxa (3.5.2.) indicates that this is not its original purpose. In addition, one of us (P. Baňař) has observed that live Enicocephalomorpha are capable of producing left-right (abduction) and peristaltic movements of the abdomen. Finally, given the small size of Enicocephalomorpha, and their generally ground and leaf litter-dwelling

habits (reviewed in Wygodzinsky & Schimdt, 1991), substrate-borne vibrations would represent an ideal communication channel, as predicted both by current theories and experimental evidence (Bennet-Clark, 1998).

More data will be required on the behavior of a variety of Enicocephalomorpha (swarming and non-swarming) to confirm the presence of vibrational communication and in what contexts. Regardless of whether this group does engage in vibrational communication or not, the abdominal morphology of these Heteroptera may represent a body plan that is pre-disposed for vibrational communication. It is hoped that the morphological description provided herein will act as a primer and facilitate future morphological and behavioural investigations in these relict insects.

Author contributions

L.R. Davranoglou conceived the study, secured funding, undertook the experiments, analysed and interpreted data, prepared figures, and wrote the paper. P. Baňař collected, sorted and identified specimens and contributed to interpretation of data. C.M. Schlepütz undertook experiments and analysis of data at the Swiss Light Source. B. Mortimer and G.K. Taylor supervised the study, secured funding, and undertook experiments at the Swiss Light Source. All authors commented on and approved the final version of the manuscript.

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Chapter 8: Conclusions

At the beginning of this dissertation, our knowledge on the morphology and biomechanics of hemipteran basi-abdominal vibroacoustic organs was confined to the tymbals and tergal plates of some Cicadomorpha and Heteroptera, respectively, leaving the condition in the majority of euhemipteran lineages unstudied (Chapter 1). Due to the biological significance of vibroacoustic communication to most Hemiptera, it was natural that hypotheses would arise in order to explain the evolution, systematic distribution and biomechanics of the basi-abdominal organs producing them. The problem, however, was, that these hypotheses were based on a series of assumptions – from Hennig (1981) to Wessel et al (2014) – and not actual morphological evidence, which was invariably insufficient or entirely absent (Chapter 1).

The results presented in this dissertation have revolutionised our understanding of the morphology of the pregenital abdomen of Hemiptera, which is now one of the best known among insects, a previously neglected topic (e.g. Snodgrass, 1931; Klug and Bradler, 2006; Klug and Klass, 2006). This in turn has led to developments in several fields, which are briefly summarised here. My findings on planthopper biomechanics provide a novel way of understanding how small animals solve the physical challenges imposed by their small size in order to produce energetically efficient vibrational signals (Chapter 3). The biomechanical and morphological insights gained from this study also have broader biological and evolutionary implications, especially in the fields of bioacoustics, biotremology and sensory biology. The vibrational songs of most planthoppers, for example, were noted by Tishechkin (2003, 2008) to be remarkably uniform in their temporal structure, while those of delphacid planthoppers and the Cicadomorpha where more complex, comprising pulses of variable amplitude and temporal pattern. At that time, an explanation was not possible, as both infraorders were assumed to possess tymbal organs. We now know that most planthoppers

communicate using a morphologically conservative snapping organ, which is the likely cause of the uniformity in their signals (Chapter 3). Delphacids use a modified snapping organ, which combines enlarged dorsoventral and dorsal longitudinal muscles (S1 text, Appendix), and likely underlies the complexity of their songs. The Cicadomorpha exhibit considerable morphological plasticity in their tymbal organs, and also use tymbal-like mechanisms and sternal apodemes (Chapter 6) all of which are likely implicated in the structural diversity of their vibrational calls.

The morphological characters documented here may also prove useful in systematic and taxonomic studies. In fact, a morphological phylogeny of Hemiptera based entirely on characters of the basi-abdominal organs is currently under way (Davranoglou and Hartung, in preparation). Furthermore, the morphology of vibroacoustic organs can also be used to reconstruct the taxonomic affinities and behavioural ecology of both extant and extinct taxa, as has been done for a Jurassic katydid (Gu et al, 2012). This is applicable to Hemiptera as well, and a snapping organ from a planthopper fossilised in Burmese amber (S1 Table, Appendix) has been preliminary assessed, and I am also using basi-abdominal morphology to reconstruct the relationships of extinct taxa of Coleorrhyncha (Davranoglou and Hartung, in preparation).

Most importantly, the results presented here have provided new insights on the origins of hemipteran vibroacoustic communication, which I will briefly discuss. In Chapters 5, 6, I have provided evidence which suggests that the morphological criteria used in the formulation of the Tymbalia hypothesis were based on misinterpretations. I argue that the limited evidence presented by the authors of Wessel et al (2014), could have been used to support either hypothesis i.e. that basi-abdominal vibroacoustic mechanisms evolved once or multiple times. Regarding terminology, I have suggested that the use of “tymbalian tymbal organs” to describe all the vibroacoustic organs of Euhemiptera is both

biomechanically and morphologically imprecise and impractical, regardless of the homology or non-homology between the different mechanisms.

Expanding on the issue of homology, the nearly ubiquitous distribution of vibrational signalling in Euhemiptera makes it parsimonious to suggest that this behaviour likely evolved once. This thesis, however, has shown that from a morphological perspective, the situation is far from clear, being confounded by deep anatomical divergences and the possibility of parallel evolution. I have found that although both the snapping organ and tymbals differ biomechanically, they do share exoskeletal features of uncertain homology (e.g. ridge, connector), as well as a presumably ancestral set of homologous muscles which has been repurposed in strikingly different ways. The homology of these muscles to those of the simpler tergal plates of Heteroptera is currently challenging to ascertain.

Overall, the results presented in this dissertation have advanced our understanding of the biomechanics, morphology and systematic distribution of hemipteran vibroacoustic organs to unprecedented levels. There is little doubt, however, that the debate on the origins of hemipteran vibroacoustic behaviour is far from settled. As a student of hemipteran abdominal morphology, I identify several understudied areas which beg for improvement, and are summarised below:

1) Homologies of dorsoventral muscles and their innervation. This group of muscles is one of the hardest to study, as in different taxa they may switch attachments, get lost in the adult stage, or are simply hard to observe (Chapters 6, 7). Yet the function of these muscles as the primary operators of several types of basi-abdominal vibroacoustic organs (e.g. in most Cicadomorpha, Chapter 6), makes their homologies a potentially important topic. I have presented some tentative homologies for these muscles in the Auchenorrhyncha (Chapter 6), although their affinities to the smaller number of dorsoventral muscles of most

Heteroptera are ambiguous (Chapter 7). Mapping their innervation may be hepful in establishing homologies, as has been done for the Polyneoptera (e.g. Klug and Klass, 2006), although comparative developmental studies at different life stages may be equally important (Chapter 7).

2) Developmental biology. Expanding on the last point mentioned above, nothing is known regarding the developmental origins of hemipteran vibroacoustic organs, both from a molecular and a morphological perspective. However, this type of information will be essential in elucidating the homologies between certain morphological features of the vibroacoustic mechanisms of the Auchenorrhyncha (e.g. ridge, connector, subdivided abdominal sterna), and indicate the developmental changes required to generate a complex organ such as a tymbal from simpler precursors, presumably an organ similar to the tergal plate of Heteroptera.

3) Biomechanics. Our entire knowledge on the functional morphology of hemipteran basi-abdominal organs stems from only three examples – the cicadid tymbals (Bennet-Clark and Young, 1992), the tymbals of treehoppers (Miles et al, 2017), and the snapping organs of planthoppers (Chapter 3). However, Hemiptera display staggering morphological diversity in the vibroacoustic mechanisms used, which range from tymbal-like organs, deltocephaline organs, sternal apodemes, delphacid organs, tergal plates and the currently undescribed coleorrhynchan organs. All of these organs are expected to provide interesting examples of biomechanical innovation, which may inform us regarding the biological factors and physical constraints that have shaped vibrational communication in these insects. Furthermore, many larval Cicadomorpha, Fulgoromorpha and Heteroptera generate abdominal vibrations using currently undescribed mechanisms (Cocroft, 1999; Tishechkin, 2003; Benediktov, 2007), which should be investigated further and compared with the vibroacoustic organs of the adults.

4) Examination of Sternorrhyncha. The abdominal morphology of Sternorrhyncha is understudied, and the homology of their musculature to that of Euhemiptera is poorly understood (Chapter 4). The sternorrhynchan abdomen may indeed prove particularly important in determining the origins and evolutionary transformations of euhemipteran vibrational organs, especially since certain sternorrynchan taxa are known to generate substrate borne vibrational signals with abdominal oscillations (Kanmiya and Sonobe, 2002).

From the above description, it is apparent that elucidating the origins of hemipteran vibrational communication will undoubtedly be a lengthy process, which will require considerable multidisciplinary effort. It is my hope that this thesis will be a valuable aid in tackling this long-standing evolutionary question.

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