

MORPHOLOGY OF ACERCARIA: INVESTIGATIONS OF THE OVIPOSITOR AND INTERNAL
ANATOMY

BY

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THESIS

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ABSTRACT

Acercaria, which includes Psocodea, Thysanoptera and Hemiptera, is a group that encompasses substantial diversity and has generated equally substantial debate about its higher-level phylogeny. The advent of molecular phylogenetics has done little to resolve arguments about the placement of various infraorders within Hemiptera, in spite of general confidence about their monophyly, which illustrates the need to take integrative approaches that include morphology as well as conduct more analyses across these higher groups as a whole. This thesis will attempt to address some of these issues in hemipteroid morphological research through projects covering two main topics.

The first chapter reviews and updates previously described morphology with a treatment focusing on the ovipositor. By comparing ovipositors among representatives of Hemiptera's infraorders and describing their character states using a common lexicon for homologous structures, it became apparent that "laciniate" (plant-piercing) ovipositors vary in such a way that implies such a phenotype was independently derived in the lineages that have them. This not only demonstrates the limited usefulness in the terms "laciniate" and "platelike" to describe hemipteran ovipositor types, but also provides support to the historically-held hypothesis that the earliest heteropterans had substantially different a life history and reproductive ecology from its relatives in Cicadomorpha and Fulgoromorpha.

The second chapter describes an effort to investigate digestive and nerve tissue morphology, which has previously been hypothesized to be phylogenetically informative

in acercarians (Goodchild 1966; Niven et al 2009). X-ray micro-computed tomography was used instead of conventional dissection for this task, which allowed for the 3-D visualization of these tissues with their natural placements and arrangements kept intact. These resulting images were found to align with previous dissections of closely related taxa where available; in addition, potential phylogenetic signal was found in the abdominal and thoracic neuromeres, the fusion of which varied among taxa. Although more taxon sampling would be needed to verify how phylogenetically informative characters of the nervous system may be, the results here demonstrate the great potential for microCT imaging in opening and exploring novel and neglected avenues of morphological investigation.

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INTRODUCTION

The research significance of Hemiptera, an order of insects including leafhoppers, planthoppers, aphids, true bugs, and related groups, can be seen directly in its higher taxonomy. Most of its infraorders respectively have a unique habitat range, with Heteroptera (true bugs) containing groups which each can be found occupying different types of aquatic or terrestrial habitat. While evolutionary hypotheses have historically assumed a general narrative of an aquatic ancestor (Nepomorpha) giving rise to semi-aquatic bugs (Gerromorpha and Leptopodomorpha), which in turn gave rise to terrestrial bugs more ecologically analogous to leafhoppers and planthoppers, verifying this narrative phylogenetically has proven difficult even in the post-genomic era. Resolving this problem is not only important for developing a more comprehensive understanding of Hemiptera's evolutionary history, but also has the potential to give entomologists a wealth of information on how insect populations are able to move through different habitats and ecological niches as they diversify.

Hemiptera is part of a superorder known as Acercaria, currently understood to also include Psocodea (Psocoptera + Phthiraptera; known respectively as bark lice and parasitic lice) and Thysanoptera (thrips). This makes it a widely diverse group with upwards of 120,000 known species, many of which are of basic and applied importance. In medicine some members take the form of nuisance parasites as well as vectors of disease, with the human louse *Pediculus humanus* Linnaeus, 1758 historically responsible for the spread of *Rickettsia* (Rault and Roux 1999) and kissing bugs (Triatominae) transmitting Chagas disease throughout Central and South America (Lent

and Wygodzinsky 1979). In agriculture they have presented a unique challenge in their involvement in the transmission of numerous plant pathogens.

Leafhoppers had the distinction of being the first insects documented as plant disease vectors when the zigzag leafhopper *Maiestas dorsalis* (Motschulsky) was implicated in the transmission of rice dwarf virus (RDV), and since then hemipterans have been recognized as important agricultural pests for their transmission abilities and the substantial economic damage caused by the diseases they spread (Nault et al. 1985). Sternorrhyncha in particular carry many pathogens: for example, whiteflies have been known to transmit tomato yellow leaf curl (Czosnek et al. 1988), and in recent years the psyllid *Bactericera cockerelli* (Sulc) has been associated with a potato disease, known as zebra chip, that emerged in the 2000s (Munyaneza et al. 2007). Vectorial potential has also been shown in Heteroptera (Mitchell 2004), but not thoroughly studied.

According to current fossil evidence available for this group, acercarians were present in small numbers during the Late Carboniferous Period (Nel et al. 2012). During the Permian they diversified into recognizable members of modern orders and infraorders (Riek 1973). After the end-Permian mass extinction wiped out around half of the known Paleozoic families, the Triassic Period gave rise to early Cicadomorpha (Martins-Neto et al. 2003), Nepomorpha and Sternorrhyncha (Evans 1957; Szwedo and Nel 2011). Superfamilies that persist in present day appeared during the Jurassic (Szwedo et al. 2010; Shcherbakov 2000), and the appearance of the first angiosperms in the early Cretaceous had enough of an impact on insect ecology at the time that it facilitated the emergence and diversification of families that are still well-represented

today (Dohlen and Moran 2008; Szwedo 2009). Acercarians endured few losses among higher taxa during the end-Cretaceous mass extinction, and saw a rise in diversity throughout the Paleogene (Shcherbakov 2002). Throughout their history, these insects experienced repeated periods of extinction and diversification which likely factor in the substantially different paths of ecological adaptation taken by the major groups.

PHYLOGENY OF ACERCARIA

Historically, Hemiptera has been grouped with Thysanoptera, Psocodea and Zoraptera under the name Paraneoptera, with the morphology originally used to justify such a grouping based mainly on wing characteristics (Martynov 1923). Wing morphology was also instrumental in Tillyard's (1918) fossil-informed hypotheses on hemipteran divergence history, which considered Heteroptera to be the earlier-diverging group. The first formal phylogeny for Hemiptera would agree on the basal placement of Heteroptera (Muir 1923), and later work with male genitalia gave further evidence for this hypothesis (Singh-Pruthi 1925). However, evidence for homology in some features of the head capsule provided a solid case for a basal Homoptera, then understood as an order including fulgoromorphans and cicadomorphans (Spooner 1938). The phylogeny drawn from these head characters placed Fulgoromorpha (then understood as Fulgoridae) and Peloridiidae at the base of Homoptera, and Geocorisae (terrestrial bugs) at the base of Heteroptera.

Recent molecular analyses of the relationships among all orders of insects argue against the monophyly of Paraneoptera, instead placing Zoraptera as sister to

Dictyoptera (Yoshizawa and Johnson 2005) and Psocodea as sister to the Holometabola (Ishiwata et al. 2011; Misof et al. 2014). However, a comprehensive treatment of zorapteran morphology by Beutel and Weide (2005) argued for a unified Paraneoptera based on characteristics of the head and mouthparts. In addition, a phylogeny involving more representatives, as well as including morphological data, placed Psocodea within Acercaria (Kjer et al. 2006), the group which also includes Thysanoptera and Hemiptera but excludes Zoraptera. It is likely that, along with taxon sampling, the small genome sizes of parasitic lice (Pittendrigh et al. 2006) influence the difficulty in not only resolving the relationships within Psocodea, but also solidifying its relationship to other insect orders. As such, more comparative morphology between lice and other insect orders will be important in constructing more accurate phylogenies, as will continued work on Zoraptera.

PSOCODEA

Early evidence for a unified Psocodea was observed in specialized structures of the antennae shared by Phthiraptera and Psocoptera (Seeger 1975). Following this was the suggestion that bark lice and book lice are paraphyletic with respect to parasitic lice, which arose as a sister taxon to Liposcelidae (Lyal 1985). Molecular data has since revealed increasing complexity in these relationships, the earliest of which provided evidence against the monophyly of Phthiraptera (Johnson et al. 2004). However, the tendency of parasitic lice to have much smaller genomes than other insects causes difficulty in not only determining Psocodea's closest relatives in Insecta, but also

resolving whether or not parasitic lice constitute a monophyletic group (Yoshizawa and Johnson 2010).

HEMIPTERA

“Homoptera”

The original phylogeny for Homoptera had a basal Fulgoroidea and grouped peloridiids with Auchenorrhyncha, which was paraphyletic with respect to Sternorrhyncha (Muir 1923). Decades later, Evans (1963) was the first to propose monophyletic Auchenorrhyncha, albeit in unresolved trichotomy with Coleorrhyncha and Sternorrhyncha. Auchenorrhyncha's monophyly, as well as Homoptera's, continued to be a topic of debate into and past the turn of the 21st century. Hamilton (1981) suggested Auchenorrhyncha to be paraphyletic with respect to Sternorrhyncha, with Fulgoromorpha as a sister group to a clade containing Sternorrhyncha and Cicadomorpha. Later, an extensive morphological treatment by Emeljanov (1987) argued for Auchenorrhyncha's monophyly, as well as grouping Cercopoidea with Cicadoidea and Cicadelloidea with Fulgoroidea. At the start of the molecular era, some concrete evidence arose in support of Auchenorrhyncha's paraphyly with respect to Heteroptera, with Sternorrhyncha as sister to Heteroptera + Auchenorrhyncha (Dohlen and Moran 1994, Campbell et al. 1994). A proposed phylogeny incorporating molecular, morphological and fossil data also supported this general scheme (Bourgoin and Campbell 2002).

In present day “Homoptera” is generally accepted as a paraphyletic group, and has been shown as such in more recent molecular analyses (Cryan and Urban 2011; Cui et al. 2013). In addition, the monophyly of Cicadomorpha, Fulgoromorpha and Sternorrhyncha have been consistently resolved by molecular phylogenetics (Cui et al. 2013) and are generally well accepted. However, there is still little agreement on the relationships between these groups and how they relate to others in Hemiptera. Heteroptera’s lineage of origin, for example, has yet to be confidently verified, and the difficulty in doing so is reflected in the recent works cited here.

Heteroptera

Early classification of Heteroptera involved its division into Geocorisae, Amphibicorisae (=Gerromorpha) and Hydrocorisae (=Nepomorpha) based on morphology and habitat (Dufour 1833). At this time, Enicocephalidae was considered part of Reduvioidae, until Reuter (1912) published a revised classification system for Heteroptera that, along with proposing seven family groups for the group based on a limited subset of morphological traits, suggested Enicocephalomorpha as an infraorder. These characters were systematically rejected by several authors, resulting in a return to Dufour’s (1833) original scheme with the added separation of Corixidae into Sandaliorrhyncha (Ekblom 1929). Ekblom also hypothesized Saldidae as a modern representative of ancestral Heteroptera. Hennig (1969) proposed Hydrocorisae as the basal group to Heteroptera and sister group to Geocorisae. This idea was followed by a

hypothesis placing Enicocephalomorpha at the base of Heteroptera (Schuh 1979) and a grouping of Nepomorpha and Leptopodomorpha (Schuh and Polhemus 1980).

Early molecular work supported Enicocephalomorpha as the basal heteropteran group. A comprehensive analysis by Wheeler et al. (1993), which included morphological data, provided a scheme in which Dipsocoromorpha is sister to the rest of Heteroptera (minus Enicocephalomorpha) and sister groups Nepomorpha + Leptopodomorpha and Cimicomorpha + Pentatomomorpha are the the most recently diverging lineages (Figure 2-A). A similar scheme by Xie et al. (2008) has Nepomorpha as sister to remaining Heteroptera (minus Enicocephalomorpha) and sister groups Cimicomorpha + Pentatomomorpha and Gerromorpha + Dispocoromorpha as the most recently diverged (Figure 2-B). However, current fossil data provide some evidence nepomorphans were the earliest modern heteropterans to appear (Shcherbakov 2010).

Cimicomorpha + Pentatomomorpha can be grouped together with confidence considering how consistently it has resolved as such both historically and with modern molecular data (Song et al. 2012; Cui et al. 2013). These works also tenuously support Nepomorpha as the earliest diverging group, a conclusion that has seen more robust evidence with increased nuclear gene sampling (Li et al. 2012). Recent molecular work has also upheld the monophyly of Gerromorpha (Damgaard et al. 2008) and Dipsocoromorpha (Weirauch and Schuh 2014). Conversely, little work has been done on the phylogenetic front for Enicocephalomorpha due to the difficulty involved in locating and collecting them, and its neglect has no doubt played a role in the continued confusion about the relationships between these major groups.

Obtaining a broader-scale understanding of Hemiptera is not as simple as assuming a division between Homoptera and Heteroptera. The complexity of these relationships can also be seen in individual groups with a history of ambiguity: for example, the unusual morphology of Peloridiidae (Coleorrhyncha) has provided evidence in taxonomic and phylogenetic analyses for its placement in both Homoptera (Myers and China 1929; Evans 1963) and Heteroptera (Emeljanov 1987). With the advent of molecular research, some evidence surfaced in favor of Coleorrhyncha as a sister group to Heteroptera (Ouvrard et al. 2000). However, phylogenetic analysis of mitochondrial genomes yielded results not consistent with this relationship (Cui et al. 2013). Molecular data also followed morphology's trend in providing varied and sometimes contradictory arrangements of the major groups in general, depending on the type of genetic or genomic information utilized in the investigation -- for example, Cui et al.'s (2013) work also presents a paraphyletic Auchenorrhyncha while that of Cryan and Urban (2012) does not. Ergo, there is still much more to be done before the relationships between superfamilies and infraorders are confidently resolved. A summary of recent competing hypotheses is shown in Figures 1 and 2.

MORPHOLOGY

A handful of synapomorphies uniting Acercaria and its orders have been suggested in a variety of sclerotized anatomical systems -- unless otherwise noted, the ones listed here are taken from Grimaldi and Engel (2005). Characters of the head include the lacinia being detached from stipes and elongated, an enlarged postclypeus

and cibarial dilator muscles, reduction or loss of labial palps, and asymmetrical mandibles. Condylgnatha (Thysanoptera + Hemiptera) share an opisthognathous head, a narrowed labrum, unicondylar stylets and expanded hypopharyngeal apodemes as well as a dorsad shift of the anterior tentorial pits, while Hemiptera have mouthparts with articulations inside the head capsule, maxillae lacking a cardo, and the familiar labial rostrum. Wing base apomorphies include fusions of the humeral plate to the subcostal base and the posterior medial plate to the anal base, respectively (Yoshizawa and Saigusa 2001). Additionally, the basal fusion of CuA with R + M in a common stem and the presence of a unique "cua-cup" crossvein (proximally concave and distally convex) which acts as a brace between CuA and CuP are wing characters proposed as synapomorphic for Acercaria (Nel et al. 2012). The subcostal base is fused to 2Ax in Condylgnatha, and Hemiptera is united by a forked anterior axillary fold-line (Yoshizawa and Saigusa 2001).

While a wealth of morphological data is available for acercarian groups, synthesizing it into a comprehensive phylogenetic analysis has proven a difficult task. One reason for this is that such an ecologically diverse group translates into a diverse set of research communities, and with diverse communities comes variation in how certain vocabulary is used to describe certain morphology. For example, this thesis will use the terms "valvifer" and "valvula" in reference to structures another entomologist may describe as the "gonacoxa" and "gonapophysis" (e.g. Scudder 1960), and similar discrepancies in terminology use between research communities exist in other anatomical systems. Overcoming the communication difficulties that result is a task that

will require detailed treatments of all morphological systems, using a common vocabulary, across all groups of Acercaria, in order to provide a more reliable foundation from which a comprehensive phylogenetic analysis can be performed.

Another more obvious issue can be seen in the gaps in data that still exist. A subtle form of misinformation takes place in the recycling of the same literature figures and references to compare and list character states, with the underlying assumption that the few species examined for a given system are morphologically representative for an entire group. In addition, the priority given to any character system also varies by group: the most extreme example of this is in Sternorrhyncha, which are often lacking in sclerotized features such as wings, legs, and common sclerites to compare to other insects, resulting in a reliance on apomorphic external characters such as wax pores and siphunculi. As such, issues lie not only in neglected taxa such as Dipsocoromorpha and Enicocephalomorpha, which still lack comprehensive morphological treatments (Weirauch and Schuh 2013), but also neglected aspects of morphology, including internal anatomy, which could potentially assist in comparisons with groups for which there are few other options.

This thesis will address some of these issues in hemipteroid morphological research through two separate projects covered in the next two chapters. The first involves reviewing and updating previously described morphology with a treatment focusing on the ovipositor, while the second utilizes CT visualizations of soft tissue morphology to investigate and propose novel targets for future research.

CHAPTER 1: Morphology of the hemipteran ovipositor

BACKGROUND

Within the realm of acercarian morphology, the ovipositor is worthy of particular attention due to its unique role in the evolution and diversification of insects. In a broad sense, it has played a role in their adaptation to living and feeding on plant substrates and the widespread ecological diversification made possible as a result -- the ability to utilize these novel oviposition sites freed insects from their ancestral terrestrial lifecycle (Emiljanov 2014). Scudder's (1959) broad treatment of the heteropteran ovipositor demonstrated its intrinsic relationship to the group's ecological diversity: while many plant-feeding heteropterans retain some form of the laciniate or saw-like valvulae used to insert eggs into plant tissues, their predaceous and aquatic relatives often possess dramatic modification or reduction of these structures. In addition, the gonangulum, which is coupled with the abdominal tergite IX in Psocodea, Thysanoptera, and Hemiptera (Scudder 1961; Yoshizawa 2005), provides another potential synapomorphy for an Acercaria that includes lice. In Homoptera, ovipositor morphology has been used to describe three distinct lineages in Fulgoroidea (Aesche 1987), a scheme that sees partial support in recent molecular work (Urban and Cryan 2006).

The sclerotized anatomy of the ovipositor in Hemiptera generally involves two complexes of characters: one consisting of the **abdominal tergite IX, gonangulum, valvifer I, and valvula I**, and the other involving **valvifer II, valvula II and valvula III** (Figure 2). In a typical bladelike or sawlike ovipositor the gonangulum acts as a fulcrum against valvifer I, and this articulates valvula I, which is coupled with valvula II. When

present, valvula III can ensheath these other components, or, as in the case of some fulgoroids, be utilized as a scooping or digging structure to facilitate oviposition into the soil (Aesche 1987). This general arrangement is shared among Thysanoptera, Auchenorrhyncha, Fulgoromorpha, some phytophagous Heteroptera, and Psylloidea with the plant-piercing or slicing function present; among homopterists and heteropterists it has historically been referred to as the “laciniate” form.

In heteropteran “plate-like” ovipositors, these parts are associated with each other in the same way, albeit with change in function and a general shift from a laterally-compressed arrangement to one that is depressed dorsoventrally. The varying character states across the suborder often appear as a result of reduction, fusion, or loss of sclerites, and there is some indication that these morphological shifts occur in higher taxonomic patterns (Scudder 1959). This would reflect current understanding of Heteroptera’s ecological and evolutionary history, which hypothesizes a basal predator with periodic returns to plant feeding in individual infraorders or superfamilies, possibly in tandem with the periodic extinction and diversification periods faced by hemipteroids as discussed earlier.

These variations in morphology allow for a wide range of oviposition strategies employed by hemipterans, with nuances beyond whether the insect cuts into plant tissues or deposits eggs on a plant surface. Laciniate valvulae can be used to pierce directly into a substrate, which has been observed in mirids (Ferran et al. 1996) and psyllids (Taylor 1992), the latter of which vary in whether eggs are embedded partially or completely into plant tissues. Sharpshooters use their valvulae to slice horizontally

along the substrate, and have the additional step of applying brochosomes to egg masses after they are deposited into the resulting crevice (Hix 2001). Those which oviposit on plant surfaces may also do so with lacinate valvulae, such as *Pelocoris femoratus* (Naucoridae), which glues eggs individually to *Ceratophyllum* leaves underwater (McPherson et al. 1987).

As has been mentioned, one of the chronic issues impeding the ability to build phylogenies with greater confidence is the lack of morphological comparisons across the orders, infraorders and superfamilies in Acercaria. Therefore, the primary goal in covering this topic is not to propose the ovipositor as a solution, but to document its variation as a beginning chapter of a thorough and comprehensive analysis of all character systems used in a larger NSF-funded Tree of Life project for this group (Johnson et al. 2012). In addition, it will present hypothetically homologous structures within the context provided by a consistent vocabulary, making it easier to explore how ecological shifts in acercarian evolution are reflected in ovipositor morphology, and use the data gathered to critique the use of the generalized terms “lacinate” and “plate-like” to describe ovipositor forms in Hemiptera.

MATERIALS AND METHODS

A collection of representative taxa was built from available insects stored with the Illinois Natural History Survey, which provided at least one species representative for each superfamily of Hemiptera with the exception of Leptopodoidea, Velocipodoidea, Microphysoidea and Joppicoidea. For ovipositor scoring, females were

cleared in a warm solution of 2% KOH and dissected where needed. Matrix-building and scoring took place in Mesquite (Maddison and Maddison 2001). The character list was largely derived from previous descriptions in literature, with a few added or modified based on personal observation, for a total of 7 binary and 11 multistate characters.

In addition to observation with a microscope, imaging and illustration were instrumental in maximizing accuracy of character state scoring. Micrographs of genitalia taken in QCapture, using a digital camera mounted on a Olympus SZX12 stereo microscope, were used as a tracing reference for the figures, which were hand-drawn in Photoshop using a Wacom drawing tablet. In addition, the actual specimen being drawn was kept available under a dissection microscope as a secondary, manipulable reference for structures not readily discernible in the static 2-D image. Individual species were chosen for illustration to represent their respective infraorders, and their selection was based on which had the most explicitly discernible morphology after being cleared.

A heuristic search in PAUP 4.0 (Swofford 2003) was used to generate trees, with the starting trees retrieved by stepwise addition using a simple addition sequence and TBR swapping algorithm. Characters were weighted equally. The best-fit trees retained were then used in the formation of strict consensus and 50% majority rule consensus trees. *Frankliniella* was selected as an outgroup for this because it was the only non-hemipteran species scored and previous analyses have established Thysanoptera as sister to Hemiptera (Misof et al. 2014). *Systelloderes*, *Ceratocombus* and non-psyllid sternorrhynchan representatives were excluded from the tree search due their general lack of distinct ovipositor morphology as defined by the matrix.

RESULTS

PHYLOGENY (Figures 4, 5 and 6)

The search yielded 1083 equally parsimonious trees, each 66 steps long, with a consistency index of 0.5152 (0.5077 excluding uninformative characters) and retention index of 0.7881. Excessive polytomy is present throughout either consensus tree provided, and the clades resolved by the majority rule tree have generally poor branch support. Heteroptera has strong support as a monophyletic group, although no heteropteran infraorders were resolved as monophyletic; however, several groupings in the strict consensus tree represent superfamilies of Heteroptera.

MORPHOLOGY

Cicadomorpha: Cicadellidae - *Agallia constricta* (Figure 7-A)

Subgenital plate (a lobe of sternum VII; not shown in figure) present and subgenital sternite reduced; tergite XIII separate from valvifer I and tergite IX separate from valvifer II. Gonangulum, valvifer I and valvula I all present and connected with a complete ramus; valvifer I internal; valvula I lacinate and externally visible. Valvifer II present internally; valvula II long and lacinate; valvula III present as a sheath covering other valvulae.

Fulgoromorpha: Achilidae - *Synecdoche impunctata* (Figure 7-B)

Subgenital plate present and subgenital sternite reduced; tergite VIII separate from valvifer I and tergite IX separate from valvifer II. Gonangulum, valvifer I and valvula I all present and connected with a complete ramus; valvifer I internal; valvula I lacinate and externally visible. Valvifer II present internally; valvula II long and lacinate; valvula III with distinct modifications -- in this case, into scooplike shapes which flank the valvulae.

Sternorrhyncha: Psyllidae - *Pachypsylla celtidis* (Figure 7-C)

Subgenital plate absent; subgenital sternite unmodified; sternites VIII and IX separate from valvifers I and II respectively. Gonangulum, valvifer I and valvula I all present and connected with a complete ramus. Valvifer I internal, shaped like a thin, tight helix; valvula I present, long and bladelike in shape. Valvifer II appears mechanically hinged against valvifer I and the gonangulum. valvula II long and bladelike; valvula III present and partially ensheathing the other valvulae posteriorly.

Psyllid ovipositors often have a **median dorsal process**, which is found dorsad of and between the valvulae. *Pachypsylla*'s median dorsal process has thin, clawlike secondary structures at the posterior end.

Coleorrhyncha: Peloridiidae - *Peloridium* sp (Figure 8-A)

Subgenital plate absent; subgenital sternite unmodified; sternites VIII and IX separate from valvifers I and II respectively. Gonangulum, valvifer I and valvula I all

present and connected with a complete ramus. Valvulae I and II, both long and lacinate in shape, appear fused together. Valvula III is present and ensheaths the other valvulae.

Leptopodomorpha: Saldidae - *Saldula pallipes* (Figure 8-B)

Subgenital plate absent, subgenital sternite unmodified; sternites VIII and IX separate from valvifers I and II respectively. Gonangulum, valvifer I and valvula I all present and connected with a complete ramus; valvula I lacinate and closely associated to enlarged, platelike valvifer I. Valvifer II platelike and widely separated; valvula II long and lacinate; valvula III present and sheathing other valvulae.

Nepomorpha: Naucoroidae - *Pelocoris femoratus* (Figure 9-A)

Subgenital plate present and subgenital sternite unmodified; sternites VIII and IX separate from valvifers I and II respectively. Gonangulum, valvifer I and valvula I all present and connected with a complete ramus; valvifer I enlarged and platelike; valvula I modified into a sclerotized scoop shape and closely associated with valvifer I. Valvifer II small and articulated within genital capsule; valvula II short, reduced, and connected to valvifer II via a membrane. Valvula III absent.

Gerromorpha: Gerridae - *Aquarius remigis* (Figure 9-B)

Subgenital plate absent, subgenital sternite large and unmodified; sternite VIII fused with valvifer I; sternite IX separate from valvifer II. Gonangulum, valvifer I and valvula I all present and connected with a complete ramus; valvifer I enlarged and

platelike, valvula I laciniate and closely associated with valvifer I. Valvula II short and reduced; valvifer II and valvula III absent.

Cimicomorpha: Tingidae - *Corythuca ciliata* (Figure 9-C)

Subgenital sternite large and unmodified; subgenital plate absent. Sternite VIII fused with valvifer I; sternite IX distinct from valvifer II. Gonangulum, valvifer I and valvula I all present and connected with a complete ramus; valvula I laciniate and closely associated with large, platelike valvifer I. Valvifer II long and externally visible along sternum; valvula II long and laciniate; valvulae III internal, reduced, fused together.

Pentatomomorpha: Coreidae - *Anasa tristis* (Figure 9-D)

Subgenital plate absent; subgenital sternite large, split and concealing the ovipositor; sternites VIII and IX separate from valvifers I and II respectively. Gonangulum present; valvifer I enlarged and platelike; valvula I laciniate and closely associated with valvifer I; ramus absent. valvifer II internal; valvula II laciniate and coupled with valvula I; valvula III absent.

Highly reduced ovipositors

Enicocephalomorpha and Sternorrhyncha (with the exception of psylloids) exhibit at least on the superficial level a near-complete reduction or fusion of the ovipositor. In addition, the dipsocoromorphan specimens examined in this study have

highly reduced ovipositors, but this is not the case for the entire group (Schuh and Slater 1995).

Gonangulum and Tergum IX

Tergum IX is internalized in most gerromorphan and nepomorphan representatives. In *Systelloderes*, abdominal segment IX is capable of telescoping into segment XIII.

Sternite VIII, valvifer I and valvula I

Sternite VIII and valvifer I are at least partially fused in all Gerromorpha as well as some Cimicomorpha and *Saldula* species (Leptopodomorpha). Representative groups of virtually all heteropteran infraorders have a broad, platelike valvifer I, which is closely associated with and sometimes appears membranously hinged to the valvula I, which is a flaplike structure in Pentatomoidea, Pyrrhocoroidea, and Coreoidea.

Valvifer II, valvula II and valvula III

Within Cimicomorpha valvifer II is externalized in Naboidea and Miroidea, but lost in Reduvioidea and Cimicoidea. In Pentatomomorpha valvifer II is reduced and often fused together to form a small plate in Pentatomoidea (as well as *Aradus* and *Dysdercus*), while in Nepomorpha they are absent in Corixoidea, but are reduced and may be fused in Nepoidea and Ochterioidea.

Valvula III is reduced in Miroidea and Naboidea, and completely absent in Pentatomomorpha and Gerromorpha. Conversely, the fulgoromorphan valvula III can bear a variety of exaggerations and modifications.

DISCUSSION

Limitations

Among the superfamilies that went unrepresented in this project were three of Cimicomorpha and one of Leptopodomorpha; in addition, several superfamilies were represented by only a single species, as was the case for the suborders Coleorrhyncha, Leptopodomorpha and Enicocephalomorpha. Furthermore, because only sclerotized characters were utilized, this character set was not able to produce much information at all for Dipsocoromorpha, Enicocephalomorpha or Sternorrhyncha (with the exception of Psylloidea). These gaps in data limit the confidence in what conclusions can be made here about the hemipteran ovipositor and the ecology and evolution of these insects with regard to the groups in which these gaps are found.

With those caveats in place, cicadomorphans generally have relatively consistent sclerotized ovipositor morphology, while heteropterans and fulgoromorphans bear more exaggerations and modifications. The modification of valvifer I into a large, often externalized plate appears to be a unifying character for Heteroptera. In many cases, it may also serve secondarily as a protective cover for the valvulae, which would provide some context to the reduction or loss of the third valvifer -- which functions as a sheath when present as in Auchenorrhyncha -- in most heteropteran taxa.

Unfortunately, in spite of the ovipositor's historical role in determining group divisions among planthoppers, the character matrix developed here was lacking in its ability to compare fulgoroids at any depth beyond acknowledging the adaptations for soil excavation exhibited by some groups. This is due to the matrix being mainly focused

on heteropteran characters as well as on higher taxa. Searching literature for morphological variation of the ovipositor among families of planthoppers would be an ideal step toward correcting this deficiency, especially given recent doubts cast on the notion that the ecological divisions among planthopper oviposition strategies represent monophyletic groups (Urban and Cryan 2007).

The oviposition behavior of psyllids involves piercing directly into plant tissue in order to partially or completely embed eggs within it, and the eggs often have a pedicel extending to the surface of their substrate to facilitate gas exchange (Taylor 1992). While this is not a wholly unique behavior, the morphology involved is distinct from that of other taxa: the presence of a median dorsal process is unique to psyllids, having no known homologies elsewhere in Hemiptera. In the past it has been suggested that it may provide musculature with extra support or leverage (Journet and Vickery 1978), and if further study were to support this hypothesis, it may indicate the median dorsal process facilitates their oviposition behavior in some way.

Phylogeny

The parsimony-based search did not resolve a monophyly for any of the heteropteran infraorders included, and in addition representatives of relatively disparate heteropteran taxa are grouped together by character states mostly defined by a loss or absence (Figure 6; Table 2). This severely limits any potential for comparison with past phylogenetic work on Acercaria given how most of Hemiptera's infraorders are well-established as monophyletic (Cui et al. 2013; Damgaard et al. 2008; Weirauch

and Schuh 2014). As such, little inference about acercarian relationships can be made with this character set without supplemental information. The fact that superfamilies are retrieved in the majority rule consensus may indicate bias in the matrix towards characters that compare groups within infraorders, given that literature on specific groups of heteropterans (e.g. Scudder 1959; Damgaard 2008) were a primary source for writing character descriptions as well as guiding preliminary rounds of manual scoring. Alternatively, it suggests ovipositor morphological diversity may be more significant phylogenetically within infraorders than it is between them.

Ecology and evolution

Aspects of reproductive ecology such as behavior and host plant preference can be convergent among hemipteran species of different infraorders or superfamilies: for example, mirids (Ferran et al. 1996) and psyllids (Taylor 1992) both use their valvulae to pierce into plant tissue directly. This investigation of ovipositor morphology across groups has shown that phytophagous heteropterans with laciniate valvulae do not have phenotypes directly comparable to those of cicadomorphans or fulgoromorphans. Homologous sclerites involved in the development of a laciniate ovipositor are instead arranged in different ways between these groups, which indicates that returning to an oviposition strategy requiring laciniate valvulae likely did not involve a return to a more ancestral state.

The most obvious difference is found in valvifer I, which is internalized in cicadomorphans and fulgoromorphans (Figure 7) but externalized and often enlarged to

a plate in Heteroptera (Figure 9). In addition, the presence and role of valvula II in heteropterans varies: in some taxa it is interlocked with valvula I in similar fashion to that of cicadomorphans (seen in *Corythuca*), and in others valvula II is decoupled from valvula I (seen in *Pelocoris*). These morphological differences likely translate to mechanical differences in muscle attachment and articulation, which in turn may play a role in the differences observed in oviposition behavior across Hemiptera.

Continued work on this system should include data on oviposition behavior and egg morphology. Because oviposition is a precise interaction between the insect, its eggs and the substrate being used, insect eggs can display as wide a suite of adaptations as the ovipositor itself. Such adaptations are involved in functions such as gas exchange, preventing desiccation and facilitating hatching, and the morphology involved in expressing them varies between groups: for example, the pseudopericolum in some pentatomorphans serves some of the same functions as the true opericolum in cimicomorphans, and other taxon-specific patterns in egg morphology are well-documented for heteropterans (Lundgren 2011). In addition to providing more context to the convergences detailed here, such information would also provide more character states for Sternorrhyncha and Enicocephalomorpha, for which there is little to no distinctive sclerotized ovipositor morphology (with the exception of psylloids).

Terminology

It has been common historically to categorize heteropteran ovipositors as either lacinate or platelike depending on whether plant-piercing structures are present. However, this is more of an ecological distinction than a morphological one: the criteria

for determining in which category a species belongs are based on what oviposition behavior is enabled by its general morphology, and not any specific character states (Matsuda 2013). In other words, these terms can refer to different combinations of character states depending on which taxa are being discussed. This is apparent not only when comparing plant-piercing ovipositors of different heteropteran infraorders, but also these heteropterans to cicadomorphans or fulgoromorphans, which have lacinate valvulae that are structured and arranged quite differently: the first valvifer being an externalized plate in Heteroptera instead of an internal sclerite changes how the first valvula can be articulated, and within Heteroptera the second valvula's presence and association with other parts of a "lacinate" ovipositor varies among superfamilies. There is also variation in the morphology of "platelike" ovipositors: for example, the fusion of valvifer II into a small plate is unique to pentatomoids.

With that in mind, one can conclude this terminology is not phylogenetically informative and has limited use when discussing this system's evolutionary biology. The pragmatic alternative to these terms would be instead to discuss the ovipositor of a given species in reference to the infraorder or superfamily in which it is currently placed and describe what character states or features, if any, make it atypical for that group: for example, according to the scoring done here, the lack of a second valvifer gives *Aquarius remigis* an atypical ovipositor among Gerromorpha. However, labeling an ovipositor as 'lacinate' or 'platelike' is still useful when discussing behavior or reproductive ecology, so long as it is accompanied by a specific account of the oviposition strategies of the species being investigated.

CONCLUSIONS

The ovipositor as a morphological system highlights not only the ecological diversity of different hemipteran groups, but also convergence of different groups to similar habitats, food sources and oviposition strategies. While plant-piercing ovipositors are present in all hemipteran infraorders, the morphology involved is distinctly different between “Homoptera” and Heteroptera as well as being varied in patterns specific to superfamilies of Heteroptera. As these morphological differences indicate that heteropterans underwent several incidences of re-adaptation to this oviposition strategy, it supports the hypothesis that the common ancestor of Heteroptera had a life history substantially divergent from its relatives in Cicadomorpha and Fulgoromorpha (e.g. Nepomorpha or Enicocephalomorpha). The complex nature of this system’s evolution also demonstrates the inadequacy of “laciniate” and “platelike” as general terms to describe them when discussing them in a phylogenetic context, and so it may be pragmatic moving forward to name ovipositor forms for the superfamilies in which they are found.

TABLES AND FIGURES

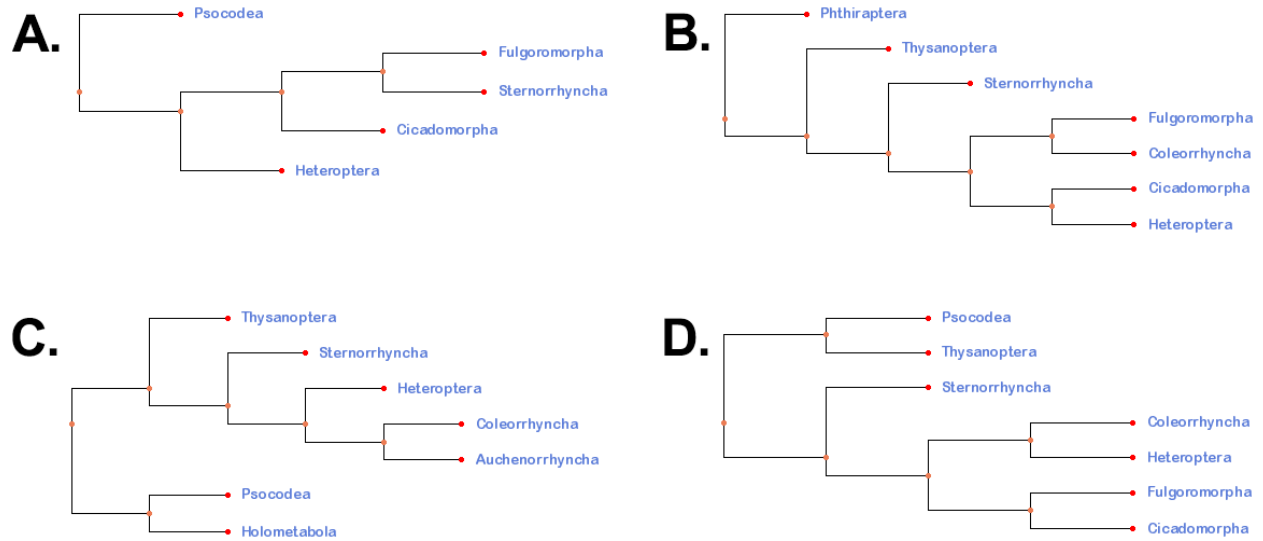


Figure 1. Recent hypotheses on the phylogeny of the major groups of Acercaria, with emphasis on hemipteran groups. **A:** Song et al. 2012 (Thysanoptera was not included in this analysis); **B:** Cui et al. 2013; **C:** Misof et al. 2014; **D:** Cryan and Urban 2011. Figure generated with Phy-fi (Fredslund 2006).

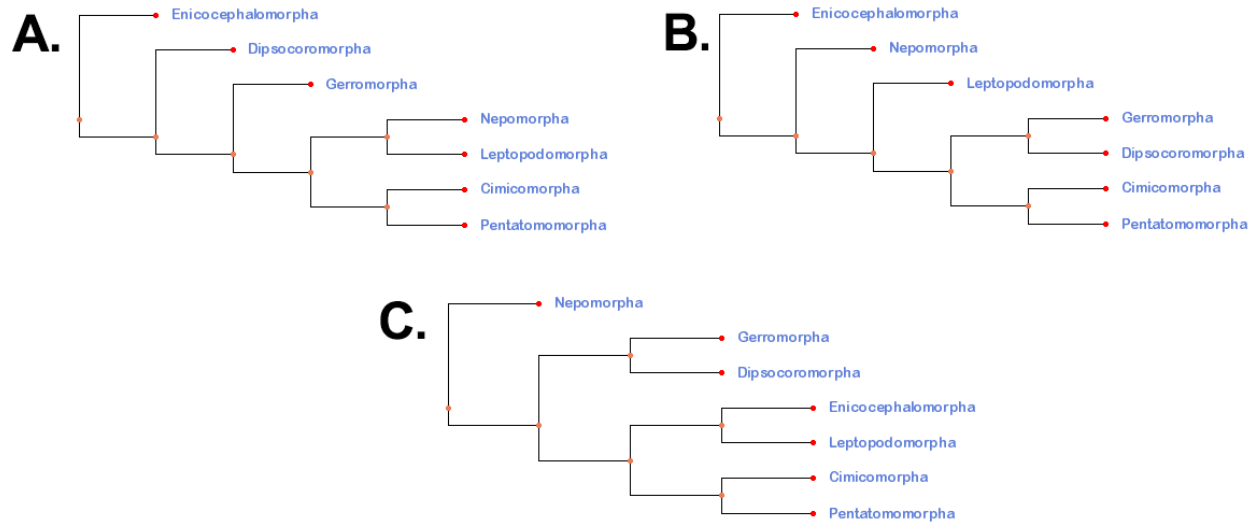


Figure 2. Recent hypotheses on the phylogeny of the major groups of Heteroptera. **A:** Wheeler et al. 1993, **B:** Xie et al. 2008; **C:** Li et al. 2012. Figure generated with Phy-fi (Fredslund 2006).

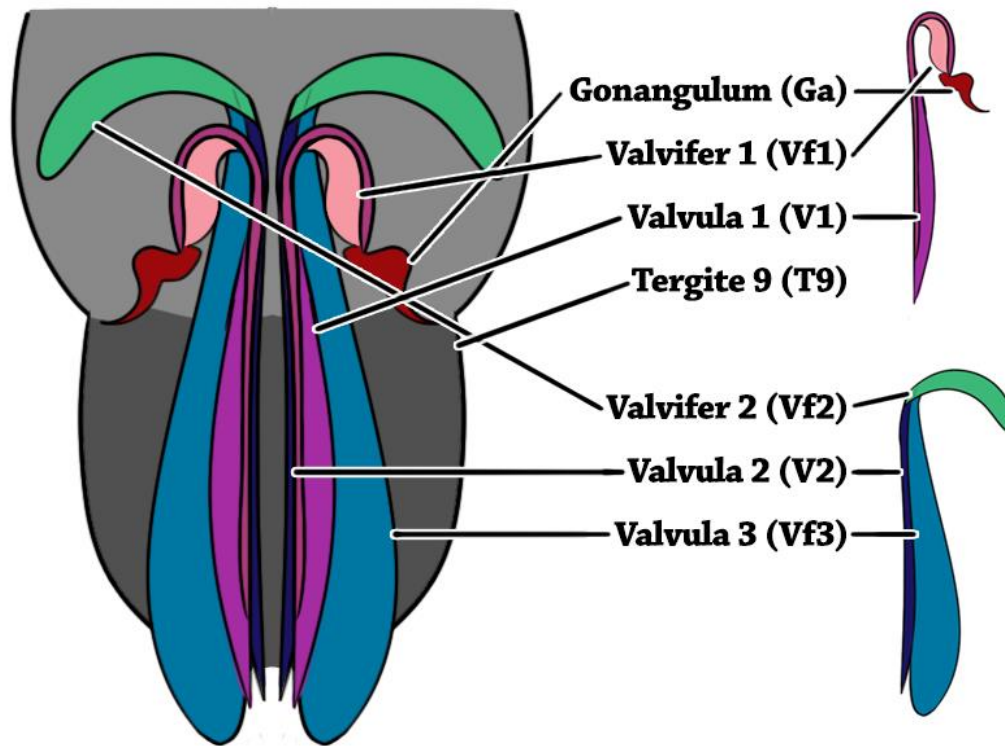


Figure 3. A generalized schematic of typical cicadomorphan ovipositor morphology. The abbreviations and color codes shown here apply to all illustrations in this section.

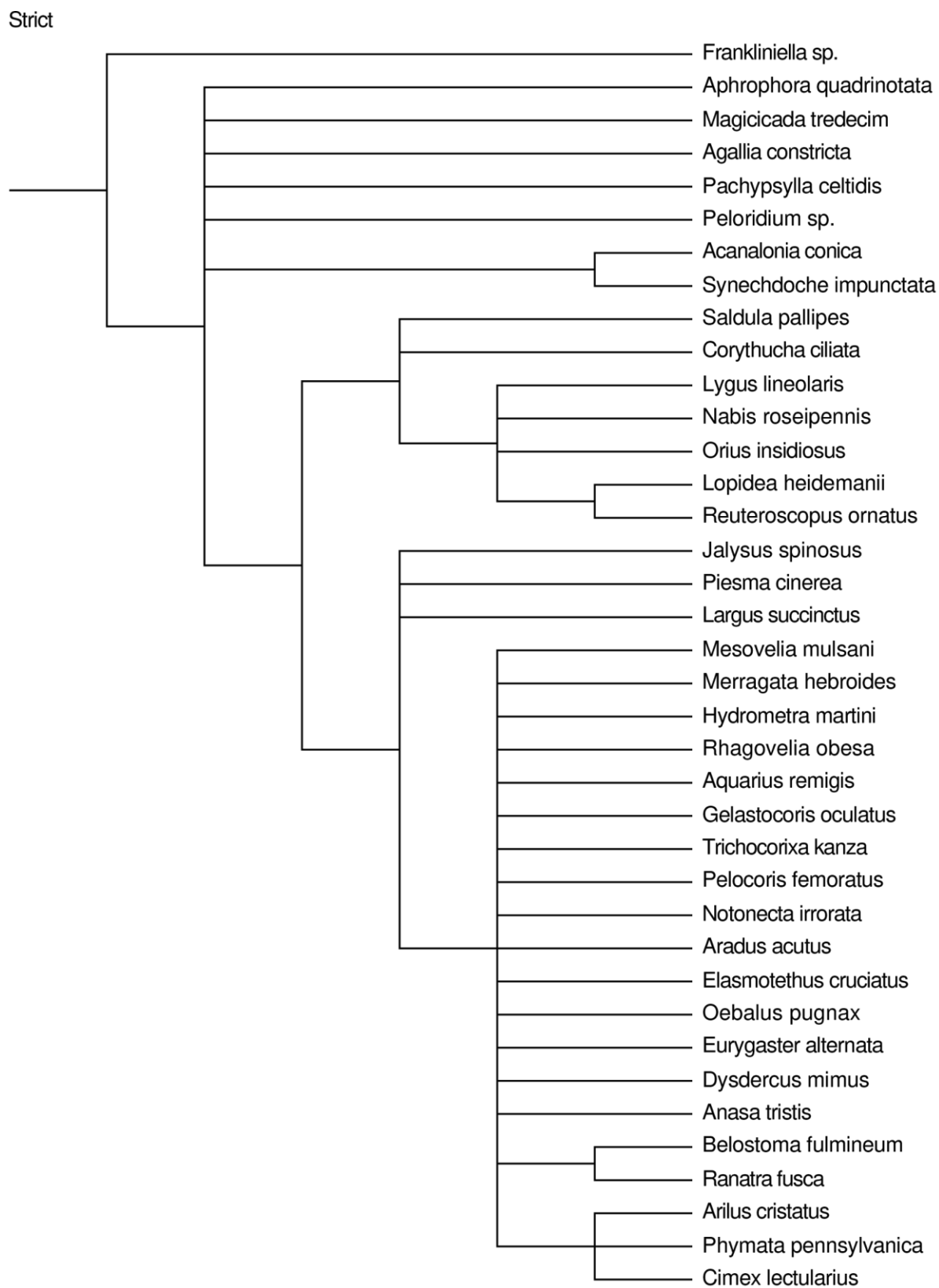


Figure 4. Strict consensus tree for representative taxa, generated in PAUP.

Majority rule

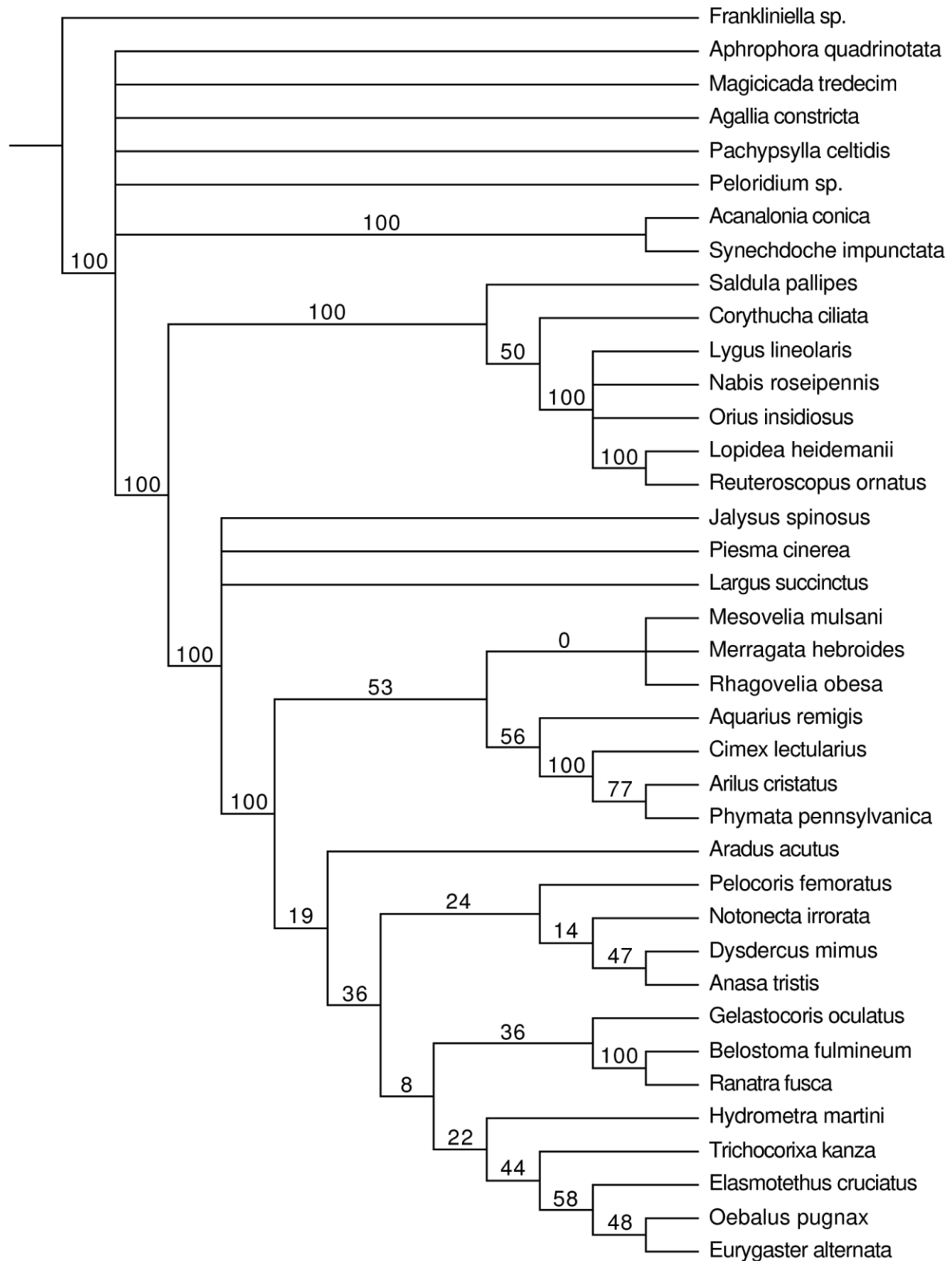


Figure 5. 50% majority rule consensus tree built with bootstrap values shown at the nodes.

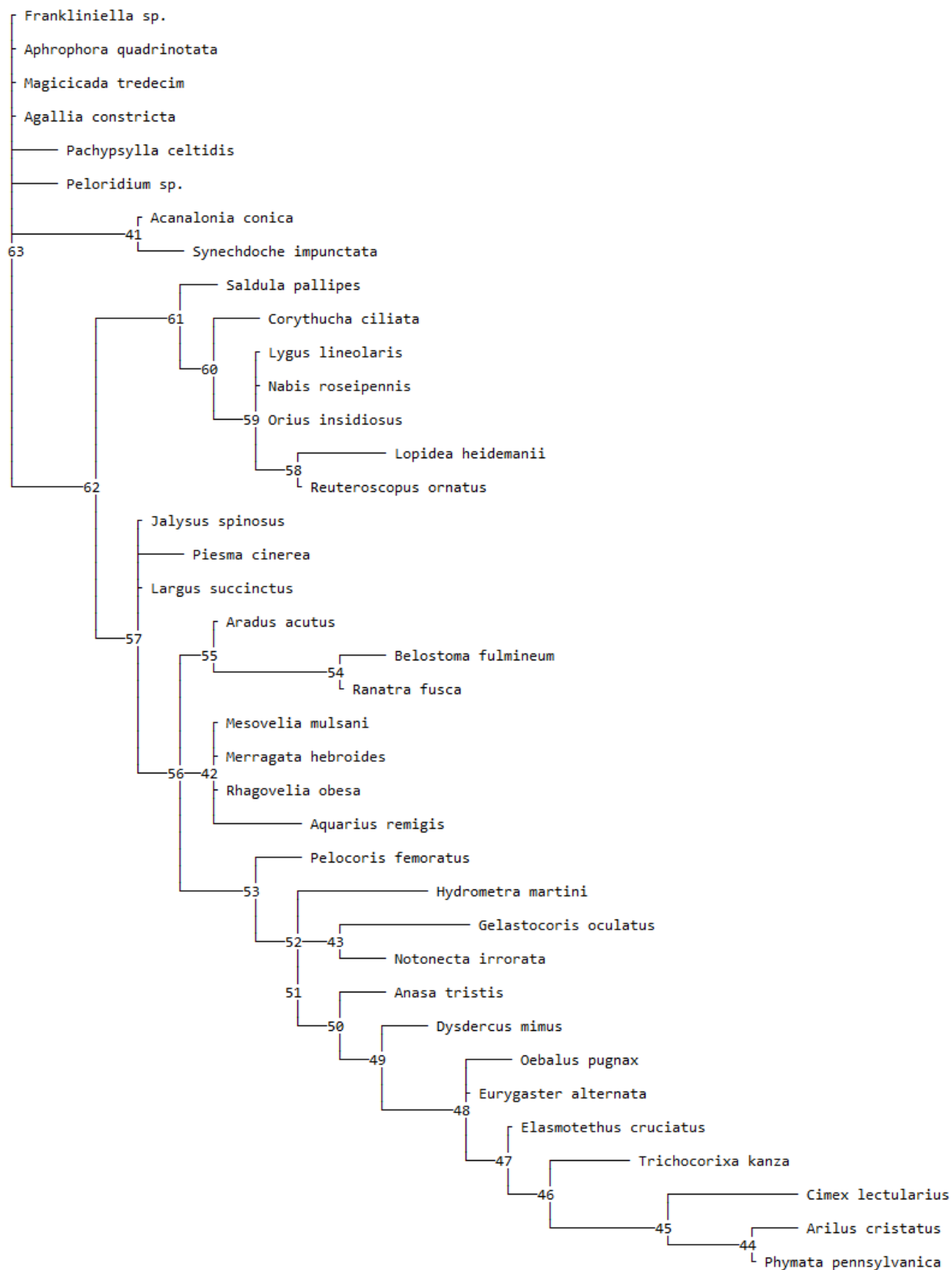


Figure 6. A select tree, with apomorphies for each node listed in Table 2.

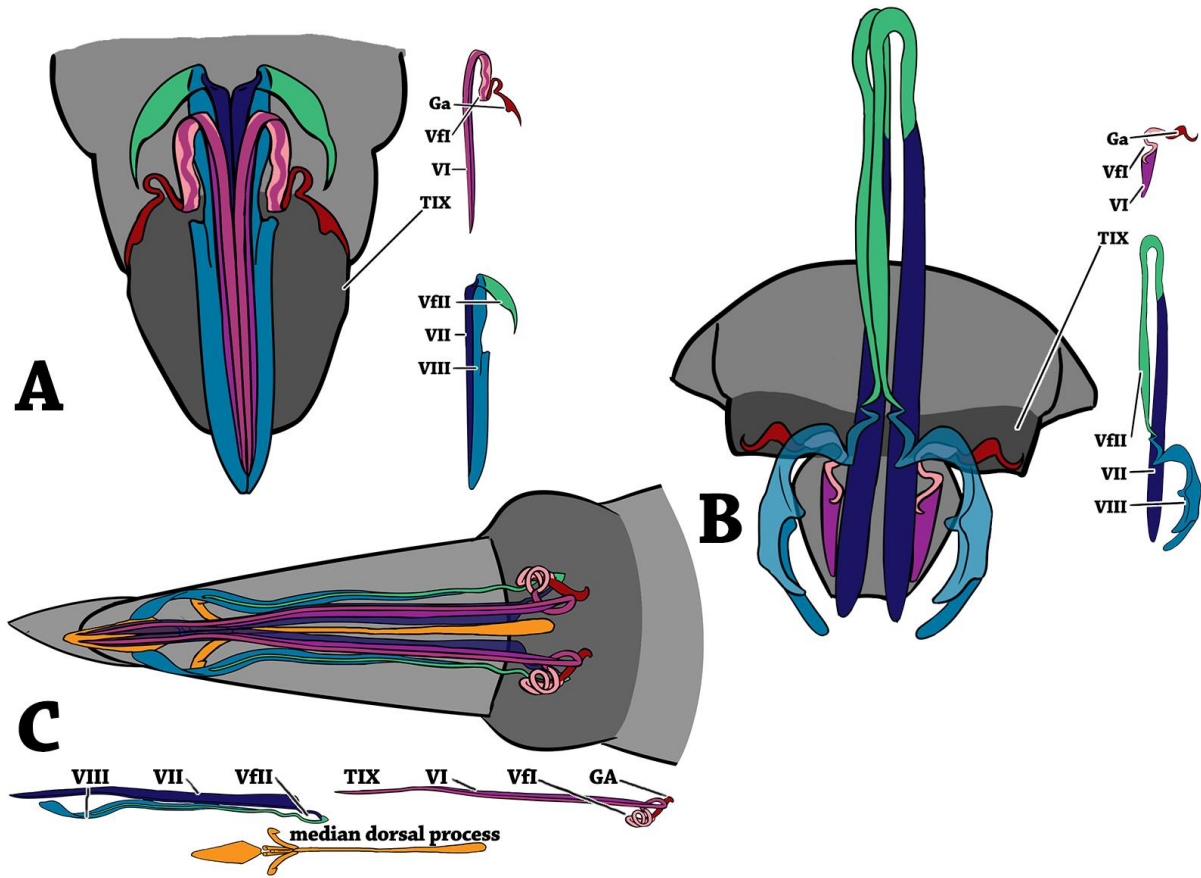


Figure 7. Ovipositor morphology of homopteran representatives. **A.** *Agallia constricta* (Cicacellidae); **B.** *Synechdoche impunctata* (Achilidae); **C.** *Pachypsylla celtidis* (Psyllidae).

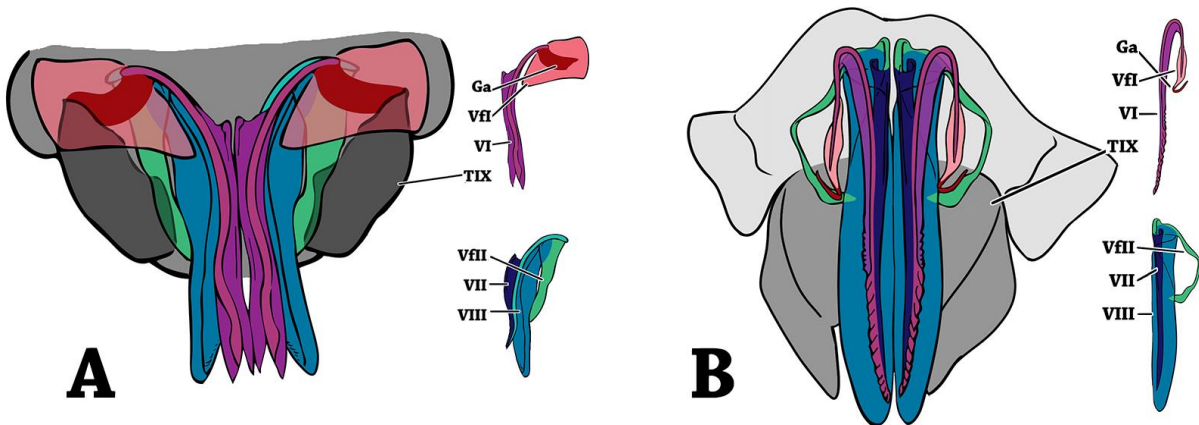


Figure 8: Ovipositor morphology of coleorrhynchan and leptopodomorphan representatives. **A.** *Saldula pallipes* (Saldidae); **B.** *Peloridium sp.* (Peloriidae).

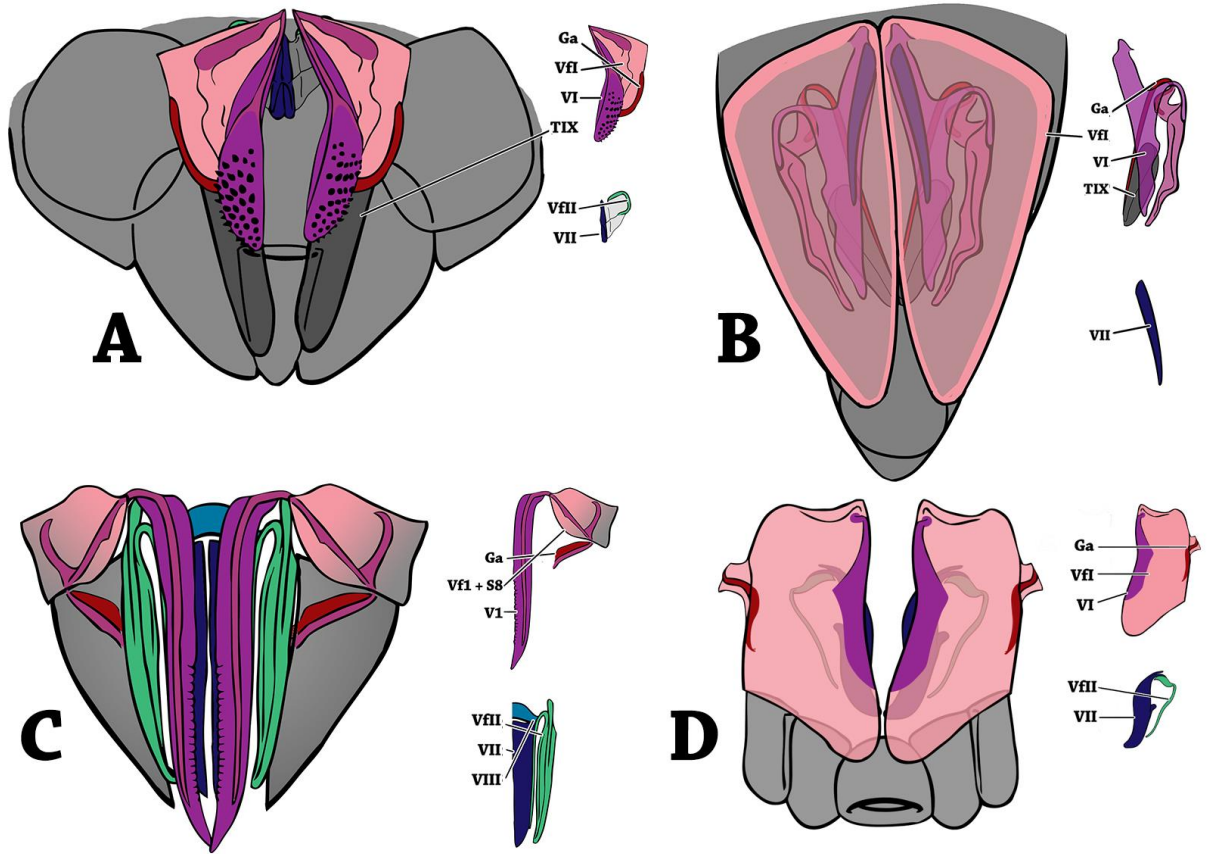


Figure 9. Ovipositor morphology of heteropteran representatives. **A.** *Pelocoris femoratus* (Naucoridae); **B.** *Aquarius remigis* (Gerridae); **C.** *Corythuca ciliata* (Tingidae); **D.** *Anasa tristis* (Coreidae).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Frankliniella sp.	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0
Aphrophora quadrinotata	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0
Magiciada tredecim	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0
Agallia constricta	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0
Acanalonia conica	0	1	1	0	1	0	0	0	1	1	0	1	0	0	0	0	0
Synechdoche impunctata	0	1	1	0	1	0	0	0	1	1	0	1	0	0	0	1	0
Pachypsylla celtidis	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1
Pelorida sp.	0	0	0	2	1	0	0	0	1	1	0	0	0	0	0	0	0
Ceratocombus sp.	3	0	0	-	0	-	-	3	0	0	4	0	3	0	-	5	0
Systelloderes biceps	3	0	0	1	0	0	1	3	0	0	4	0	3	2	-	5	0
Mesovelis mulsani	0	0	0	0	1	1	1	1	1	1	0	0	0	1	0	5	0
Merragata hebroides	0	0	0	0	1	1	1	1	1	1	0	0	0	1	0	5	0
Hydrometra martini	0	0	0	0	1	1	1	2	0	1	0	0	2	1	0	5	0
Rhagovalia obesa	0	0	0	0	1	1	1	1	1	1	0	0	0	1	0	5	0
Aquarius remigis	0	0	0	0	1	1	1	1	1	0	4	0	0	1	0	5	0
Belostoma fulmineum	0	1	0	0	1	0	1	2	1	1	2	0	1	1	0	3	0
Ranatra fusca	0	1	0	0	1	0	1	1	1	1	2	0	1	1	0	3	0
Gelastocoris oculatus	0	0	0	0	1	0	1	2	1	1	2	0	2	1	2	0	0
Trichocorixa kanza	0	1	0	0	1	0	1	2	0	0	4	0	2	1	0	5	0
Pelocoris femoratus	0	1	0	1	1	0	1	1	1	1	0	0	2	1	0	5	0
Notonecta irrorata	0	0	0	1	1	0	1	2	2	1	0	0	2	1	0	0	0
Salduia pallipes	0	0	0	0	1	1	1	1	1	1	3	0	0	0	0	0	0
Arilus cristatus	0	0	0	0	0	1	1	2	0	0	4	0	0	0	0	0	0
Phymata pennsylvanica	0	0	0	0	0	1	1	2	0	0	4	0	0	0	2	-	0
Lygus lineolaris	0	0	2	0	1	1	1	1	1	1	1	0	0	0	0	2	0
Lopidea heidemanii	0	1	2	0	1	0	1	1	1	0	1	0	0	0	0	2	0
Reuterocopus ornatus	0	1	2	0	1	1	1	1	1	1	1	0	0	0	0	2	0
Nabis roseipennis	0	0	2	0	1	1	1	1	1	1	1	0	0	0	0	2	0
Corythucha ciliata	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	4	0
Orius insidiosus	0	0	2	0	1	1	1	1	1	1	1	0	0	0	0	2	0
Cimex lectularius	0	0	2	0	0	1	1	1	1	0	4	0	2	0	2	5	0
Aradus acutus	0	0	0	0	1	0	1	1	1	1	2	0	0	1	0	5	0
Elasmotethus cruciatus	0	0	0	0	1	0	1	2	0	0	2	0	2	0	0	5	0
Oebalus pugnax	0	0	1	1	1	0	1	2	0	0	2	0	2	0	0	5	0
Eurygaster alternata	0	0	0	1	1	0	1	2	0	0	2	0	2	0	0	5	0
Jalysus spinosus	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0	5	0
Piesma cinerea	0	0	2	0	1	0	1	1	1	1	0	0	0	0	0	5	0
Largus succinctus	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0	5	0
Dysdercus mimus	0	0	0	1	1	0	1	2	1	1	2	0	2	0	1	5	0
Anasa tristis	0	0	3	1	1	0	1	2	1	1	0	0	2	0	0	5	0

Table 1. Matrix for character states, with the corresponding list of characters available in the Appendix figure.

Branch	Character	Steps	CI	Change
node_63 --> node_41	2 (Female subgenital plate)	1	0.200	0 ==> 1
	3 (Size of female subgenital sternite)	1	0.500	0 ==> 1
	12 (Valvula IIe with median basal apodeme)	1	1.000	0 ==> 1
node_41 --> Synechdoche imp.	16 (Valvula III)	1	0.714	0 ==> 1
node_63 --> Pachypsylla cel.	17 (Median dorsal process)	1	1.000	0 ==> 1
node_63 --> Peloridium sp.	4 (Ovipositor valvulae)	1	0.400	0 ==> 2
node_63 --> node_62	7 (First valvifer)	1	1.000	0 ==> 1
	8 (Valvula I)	1	0.500	0 ==> 1
node_62 --> node_57	16 (Valvula III)	1	0.714	0 ==> 5
node_57 --> node_56	14 (Shape and size of Tergite IX)	1	0.333	0 ==> 1
node_56 --> node_42	6 (Association of tergite VIII with valvifer I)	1	0.200	0 ==> 1
node_42 --> Aquarius remigis	10 (Ramus connecting valvifer II to valvula II)	1	0.333	1 ==> 0
	11 (Valvifer II)	1	0.571	0 ==> 4
node_56 --> node_53	4 (Ovipositor valvulae)	1	0.400	0 --> 1
	13 (Valvula II)	1	0.667	0 ==> 2
node_53 --> node_52	8 (Valvula I)	1	0.500	1 ==> 2
node_52 --> Hydrometra mart.	4 (Ovipositor valvulae)	1	0.400	1 --> 0
	6 (Association of tergite VIII with valvifer I)	1	0.200	0 ==> 1
	9 (Ramus connecting valvifer I to valvula I)	1	0.500	1 ==> 0
node_51 --> node_43	16 (Valvula III)	1	0.714	5 ==> 0
node_43 --> Gelastocoris oc.	4 (Ovipositor valvulae)	1	0.400	1 --> 0
	11 (Valvifer II)	1	0.571	0 ==> 2
	15 (Association of Tergite IX with valvifer II)	1	0.500	0 ==> 2
node_43 --> Notonecta irror.	9 (Ramus connecting valvifer I to valvula I)	1	0.500	1 ==> 2
node_51 --> node_50	14 (Shape and size of Tergite IX)	1	0.333	1 ==> 0
node_50 --> node_49	11 (Valvifer II)	1	0.571	0 ==> 2
node_49 --> node_48	9 (Ramus connecting valvifer I to valvula I)	1	0.500	1 ==> 0
	10 (Ramus connecting valvifer II to valvula II)	1	0.333	1 ==> 0
node_48 --> node_47	4 (Ovipositor valvulae)	1	0.400	1 ==> 0
node_47 --> node_46	11 (Valvifer II)	1	0.571	2 ==> 4
node_46 --> Trichocorixa ka.	2 (Female subgenital plate)	1	0.200	0 ==> 1
	14 (Shape and size of Tergite IX)	1	0.333	0 ==> 1
node_46 --> node_45	5 (Gonangulum (ramus extending from first valvifer))	1	1.000	1 ==> 0
	6 (Association of tergite VIII with valvifer I)	1	0.200	0 ==> 1
	15 (Association of Tergite IX with valvifer II)	1	0.500	0 --> 2
node_45 --> node_44	13 (Valvula II)	1	0.667	2 ==> 0
	16 (Valvula III)	1	0.714	5 --> 0
node_44 --> Arilus cristatus	15 (Association of Tergite IX with valvifer II)	1	0.500	2 --> 0
node_45 --> Cimex lectulari.	3 (Size of female subgenital sternite)	1	0.500	0 ==> 2
	8 (Valvula Ie)	1	0.500	2 ==> 1
	9 (Ramus connecting valvifer I to valvula I)	1	0.500	0 ==> 1
node_48 --> Oebalus pugnax	3 (Size of female subgenital sternite)	1	0.500	0 ==> 1
node_49 --> Dysdercus mimus	15 (Association of Tergite IX with valvifer II)	1	0.500	0 ==> 1
node_50 --> Anasa tristis	3 (Size of female subgenital sternite)	1	0.500	0 ==> 3
node_53 --> Pelocoris femor.	2 (Female subgenital plate)	1	0.200	0 ==> 1
node_56 --> node_55	11 (Valvifer II)	1	0.571	0 ==> 2
node_55 --> node_54	2 (Female subgenital plate)	1	0.200	0 ==> 1
	13 (Valvula II)	1	0.667	0 ==> 1
	16 (Valvula III)	1	0.714	5 ==> 3
node_54 --> Belostoma fulmi.	8 (Valvula I)	1	0.500	1 ==> 2
node_57 --> Piesma cinerea	3 (Size of female subgenital sternite)	1	0.500	0 ==> 2
node_62 --> node_61	6 (Association of tergite VIII with valvifer I)	1	0.200	0 ==> 1
	11 (Valvifer II)	1	0.571	0 --> 1
node_61 --> Saldula pallipes	11 (Valvifer II)	1	0.571	1 --> 3
node_61 --> node_60	16 (Valvula III)	1	0.714	0 --> 2
node_60 --> node_59	3 (Size of female subgenital sternite)	1	0.500	0 ==> 2
node_59 --> node_58	2 (Female subgenital plate)	1	0.200	0 ==> 1
node_58 --> Lopidea heidema.	6 (Association of tergite VIII with valvifer I)	1	0.200	1 ==> 0
	10 (Ramus connecting valvifer II to valvula II)	1	0.333	1 ==> 0
node_60 --> Corythucha cili.	16 (Valvula III)	1	0.714	2 --> 4

Table 2. List of apomorphies as according to the tree in Figure 5. Character and state numbers correspond to those described in Table 1 and the Appendix.

Appendix. List of character states.

1. **General state of ovipositor:** 0) normal; valvulae distinguishable; 1) reduced to externally visible operculum; 2) completely reduced, with only a vulva present 3) reduced, with multiple fusions between sclerites making any homologies indistinguishable
2. **Female subgenital plate:** 0) absent; 1) present
3. **Size of female subgenital sternite:** 0) relatively large, unmodified; 1) reduced or concealed by genitalia; 2) large, split by ovipositor; 3) large, split, concealing ovipositor
4. **Ovipositor valvulae:** 0) interlocked; 1) not interlocked; 2) valvulae I and II fused
5. **Gonangulum:** 0) absent or indistinguishable from associated sclerites; 1) present, fused with 9th abdominal segment internally
6. **Association of tergite VIII with valvifer I:** 0) separate; 1) at least partially fused
7. **Valvifer I:** 0) short, within genital capsule and not visible externally; 1) platelike, enlarged
8. **Valvula I:** 0) long, laciniate, distinct from valvifer and often visible externally; 1) laciniate, closely associated with valvifer I; 2) flaplike, flat, reduced; membranously associated with or attached to valvifer I; 3) absent or indistinguishable from neighboring sclerites
9. **Ramus connecting valvifer I to valvula I:** 0) absent; 1) present; 2) fragmented or nondistinct
10. **Ramus connecting valvifer II to valvula II:** 0) absent; 1) present; 2) fragmented or indistinct;
11. **Valvifer II:** 0) articulated within genital capsule and not visible externally, usually short; 1) very long, flush with sternum and visible externally; 2) reduced; often fused together 3) platelike, widely separated, may be visible externally; 4) absent or indistinguishable from neighboring sclerites
12. **Valvula II with median basal apodeme:** 0) absent; 1) present
13. **Size and shape of valvula II:** 0) long, laciniate, coupled with valvula Ie; 1) short, platelike; 2) Short, reduced, often membranously associated with valvifer II; 3) absent or indistinguishable from neighboring sclerites
14. **Shape and size of Tergite IX:** 0) normal, external, conspicuous; 1) small, reduced, internalized; 2) whole segment capable of telescoping inside segment 8
15. **Association of Tergite IX with valvifer II:** 0) separate; 1) partially fused; 2) fused, indistinguishable from one another
16. **Valvula III shape and size:** 0) normal, external, conspicuous; 1) modified for soil excavation; 2) on apex of valvifer II, reduced or vestigial; 3) stylus-shaped; 4) reduced, internalized, fused into a bridge shape; 5) absent
17. **Median dorsal process:** 0) absent; 1) present

CHAPTER 2: Exploring hemipteroid internal morphology with X-ray micro-computed tomography

BACKGROUND

Despite the potential for describing useful character states, soft tissue morphology is not often used in phylogenetic research due to the time and labor investment involved in properly preparing, dissecting and preserving organs. In addition, the act of dissection separates tissues from their original arrangement within the body cavity, resulting in loss of information. The advent of microCT technology can, however, serve as a way to address these problems. Generating an X-ray computed tomography involves taking images of a specimen at short rotational increments around a defined center point and using those data to generate a stack of virtual slices, each of which provides cross-sectional information similar to what can be visualized using conventional histological slicing techniques. These images can then be segmented, meaning that specific tissues are isolated from the rest shown so that they may be visualized independently, effectively making segmentation a means of virtual dissection. This allows for 3-D visualization of soft tissues with their natural placement within the body kept intact, and preparation methods for scanning insects are simple and inexpensive (Metscher 2009).

Hemipteroid insects have highly modified feeding and digestive structures from adaptation to a liquid diet, and older literature on their internal morphology suggests some of these adaptations are taxon-specific at higher levels (Goodchild 1966); as such, the food canal and associated tissues are good candidates for initial investigation into

soft tissue morphology using this technology. Nervous tissue is also an area of interest, given the fusion of particular thoracic and abdominal neuromeres appears in taxon-specific configurations across insect orders according to Niven et al. (2007). This work provided evidence that acercarians possess completely fused abdominal neuromeres, while hemipterans in particular also have completely fused thoracic neuromeres.

MATERIALS AND METHODS

Insects used in this project include representatives of *Saldula* sp. (Saldidae), *Vidanoana flavomaculata* (Cicadellidae), *Megathrips* sp. (Phlaeothripidae), *Phylloscelis atra* (Dictyopharidae), and *Lygus lineolaris* (Miridae) (collection locales and methods available in Table 3). Fixing, staining, and staging techniques were modified from techniques developed by Metscher (2009). Immediately following live capture, specimens were kept in alcoholic Bouin's solution for 6 hours and washed in 70% ethanol daily until all excess fixative was removed. The one exception to this was *Vidanoana*, which had been stored in 95% ethanol in a -40C freezer for a few weeks prior to being fixed. They were then stained in iodine in 95% ethanol for 2 to 6 hours and moved to pure ethanol. They were then processed in a critical point dryer and staged in a holder made from a multipurpose pipette tip -- this was to position the insects in a vertical orientation in order to maximize scan quality and create a transverse series of slices to examine initially.

All equipment and software used for microCT scanning and 3-D visualization is located at the Beckman Institute for Science and Technology in Urbana-Champaign, IL.

Scans took place in an XRadia MicroXCT-400 imaging system, for a period of 3.5 to 4 hours, at 10x magnification and a power setting of 60 kV and 8 W. As staging methods cannot ensure uniform positioning among specimens scanned, it was required to manually define the rotational axis for each scan. The long body of *Megathrips* required two scans, which were stitched together into a whole tomography before reconstruction.

Tomography files were loaded into XMReconstructor (software included with the XRadia system) in order to generate the virtual slices, which were cropped and converted to an 8-bit format in imageJ (Schneider et al. 2012). Then the slices were manually segmented and visualized in 3-D in Amira (Staling et al. 2005) at a workstation with a Wacom touch-screen tablet monitor.

RESULTS

Images of ganglia and digestive tissues can be seen for all representatives in Figures 10 and 11, respectively.

Megathrips

Individual segments of the thorax represented by its neuromeres are distinguishable. The gut is superficially simple, with a lightly coiled esophagus and three large, distinct midgut segments. Three Malphigian tubules are present, one extending anteriorly into the hemocoel and the other two posteriorly. The hindgut is very large, accounting for roughly half the volume of the entire gut, and dotted with evenly-dispersed rectal glands.

Vidanoana

Due to time constraints and scan quality, only nervous tissue was imaged from this representative. The neuromeres of the thoracic ganglion are relatively distinguishable from one another.

Phylloscelis

The thoracic neuromeres are easily distinguishable from one another, with the anterior two segments extending laterally into tapered shapes. An anterior diverticulum begins at the junction between the foregut and first section of the midgut, taking up a substantial volume of space inside the thorax and extending into the head. The midgut consists of a few long coils, part of which appear compressed by the diverticulum.

Saldula

The thoracic neuromeres appear fused to a point that makes individual segments less distinct. The food canal here is superficially simple, with an esophagus emptying into a baglike midgut flanked by a pair of large gastric caecae. Some tearing is present at the posterior half of the midgut, which is likely a result of tissue shrinkage from the ethanol-based preparation used in these scans. The four Malpighian tubules are closely associated with both the midgut and the abdominal tergum.

Lygus

The two nerves comprising the ventral nerve cord are close enough together to make them difficult to separate at the resolution used for the tomography; in addition, the thoracic neuromeres appear dramatically fused to a degree that makes the individual

segments they represent indistinguishable from one another. Only the foregut of this insect was imaged due to time constraints.

Nervous system

The brain and major neuromeres were fully segmented and visualized in all five of *Saldula*, *Vidanoana*, *Megathrips*, *Phylloscelis*, and *Lygus*, revealing substantial variety in shape and arrangement. They follow Niven et al.'s (2007) predictions of acercarians sharing fused thoracic and abdominal neuromeres, and in addition there appear to be varying degrees of fusion in the different taxa. In *Saldula* and *Lygus* the neuromeres are indistinguishable from one another, while in the others it is still possible to denote the individual segments.

Digestive system and endosymbionts

The alimentary canal was visualized in all representatives with the exception of *Vidanoana*. Salivary glands were imaged in *Lygus* and *Saldula*, and Malpighian tubes were imaged in *Saldula* and *Megathrips*. *Phylloscelis* sports an impressively large anterior diverticulum, which extends into the head and “displaces” the brain, as well as appearing to press against the coils of the midgut, which are flattened in proximity to the diverticulum. *Saldula* has large, conspicuous gastric caecae associated with the midgut, and some tearing is present at the posterior end of the midgut, which may be a result of tissue shrinkage from being prepared in ethanol. *Megathrips* has several rectal glands evenly distributed around the hindgut.

DISCUSSION

While CT visualization is indeed a powerful tool, it still has limitations that must be considered while interpreting the images produced. The first is that the whole process can be time-consuming and costly, as the technology is not widely accessible, longer scans are required for quality images, and manual segmentation is slow work requiring precision. Scanning artifacts caused by errors such as equipment miscalibration, low photon count, or movement of the specimen during a scan can obscure or distort some features, and obtaining a perfect image can be difficult (Boas and Fleischmann 2012). In addition to these problems, the fact that the generated slices consist of square pixels can lead to erasure of fine details (as one pixel can hold only one color or grayscale value) and limits the extent to which the image can be magnified without distortion, which are issues that are not encountered with conventional histology and microscopy. The upper size limit of microCT scanners (2.5cm) also biases sampling toward smaller insects, which has a possible impact on how internal morphology can be described and used in a phylogenetic context. Lastly, obtaining a scan with soft tissues intact requires fresh-caught or fixed specimens, and the ethanol-based preparation used here appears to atrophy soft tissues at least slightly. Since this effect would lead to more distortion with smaller and more soft-bodied insects, it would be necessary to develop different preparations for sternorrhynchans moving forward.

In general, the anatomy visualized in these segmentations align with previous dissections of closely related taxa where available. The tissues are distinguishable from one another in cross-section (Figure 13). Since available data on internal morphology are sparse, and much of the relevant information is found in very old literature with

illustrations of varying quality, there is definitely potential for using CT data to create an updated catalogue of these characters. The food canal and salivary glands of *Lygus* were near-identical to the same tissues in a photographed dissection of a congeneric representative (Habibi et al. 2008), and the same organs of the other hemipteran species scanned roughly match with the more idealized and exaggerated illustrations given by Goodchild (1966) for insects in the same family. For *Megathrips*, the general shape of the food canal and the presence of rectal glands in the hindgut find analogues in older dissections across a range of genera (Sharga 1933).

In Heteroptera microbial endosymbionts can be housed in gastric caecae, and some taxonomic patterns may exist in their diversity of shape and arrangement (Glasgow 1914). These were relatively easy to find and segment in *Saldula*, so continued visualization of caecal morphology may have some use in Heteroptera. Otherwise, morphology of endosymbiont-housing tissues is probably not as informative as molecular data can be: gene sequences from vertically-transmitted endosymbionts have been used to generate phylogenies for sternorrhynchan groups, for example (Andersen et al. 2010).

While the anterior diverticulum of the midgut is historically understood to be unique to planthoppers (Cheung 1983), in *Phylloscelis* it occupies a substantial amount of space in the body cavity -- so much that the supraesophageal ganglion seems to have shifted in shape and position to accommodate it. The virtual slices that include the diverticulum show a lack of X-ray attenuation in its interior, suggesting that it is filled with air. That it is air-filled has also been indirectly observed via air bubbles leaking out

during conventional dissections (Goodchild 1966). A possible explanation for this morphology is that fulgoroids use the pressure of the filled diverticulum on the midgut to mechanically mediate fluid intake, and therefore they could represent an earlier adaptation to handling a liquid diet. Naturally, more investigation would need to be conducted before any credence can be given to this hypothesis.

Nerve tissue attenuates consistently well in scans and as a result appears unambiguously in slices, making it relatively easy to segment, and the observations shown here demonstrate the potential in using neuromeres for morphological phylogenetics. Because the degree of abdominal and thoracic fusion is split among ordinal and infraordinal lines in the handful of taxa examined, it is possible this system has more character states than those provided by Niven (2007), and these could be properly described with more taxon sampling. Imaging more of the nervous system may also reveal more phylogenetic patterns or assist in verifying homologies in existing characters by tracing sclerotized traits to shared muscle and nerve connections.

CONCLUSIONS

Despite issues with precision, the anatomy revealed by visualized CT data matches that of dissections of the same or closely related taxa, affirming CT as an excellent tool for gross morphology of soft tissues in insects. This technology provides a powerful way to investigate how tissues vie for the limited three-dimensional space

inside an insect's body cavity, and examining multiple tissues at once in this fashion can reveal novel characters and new insights on adaptive physiology.

For Acercaria these data have revealed possible phylogenetic signal in degree of neuromere fusion, which warrants more taxon sampling and investigation to determine where this pattern could apply at different taxonomic levels. While similar connections could not be made for the gut morphology visualized, it was congruent enough with information in previous literature that more sampling and investigation could test the hypotheses presented by Goodchild (1966) and provide more characters for comparison among major groups such a study would be of particular use for Sternorrhyncha, for which the general lack of sclerotized characters is a hindrance in research.

TABLES AND FIGURES

Family	Genus	Collecting Locale	Date	Site, Method
Cicadellidae	Vidanoana	Los Lagos, Chile	1/23/2014	savanna, vacuum
Dictyopharidae	Phylloscelis	Rock Island Co, IL	6/24/2013	prairie, sweeping net
Saldidae	Saldula	Rock Island Co, IL	7/24/2013	silt near creek, vacuum
Miridae	Lygus	Vermilion Co, IL	8/28/2015	prairie, sweeping net
Phlaeothripidae	Megathrips	Vermilion Co, IL	8/28/2015	prairie, sweeping net

Table 3. Collection details for the insects used in microCT scans.

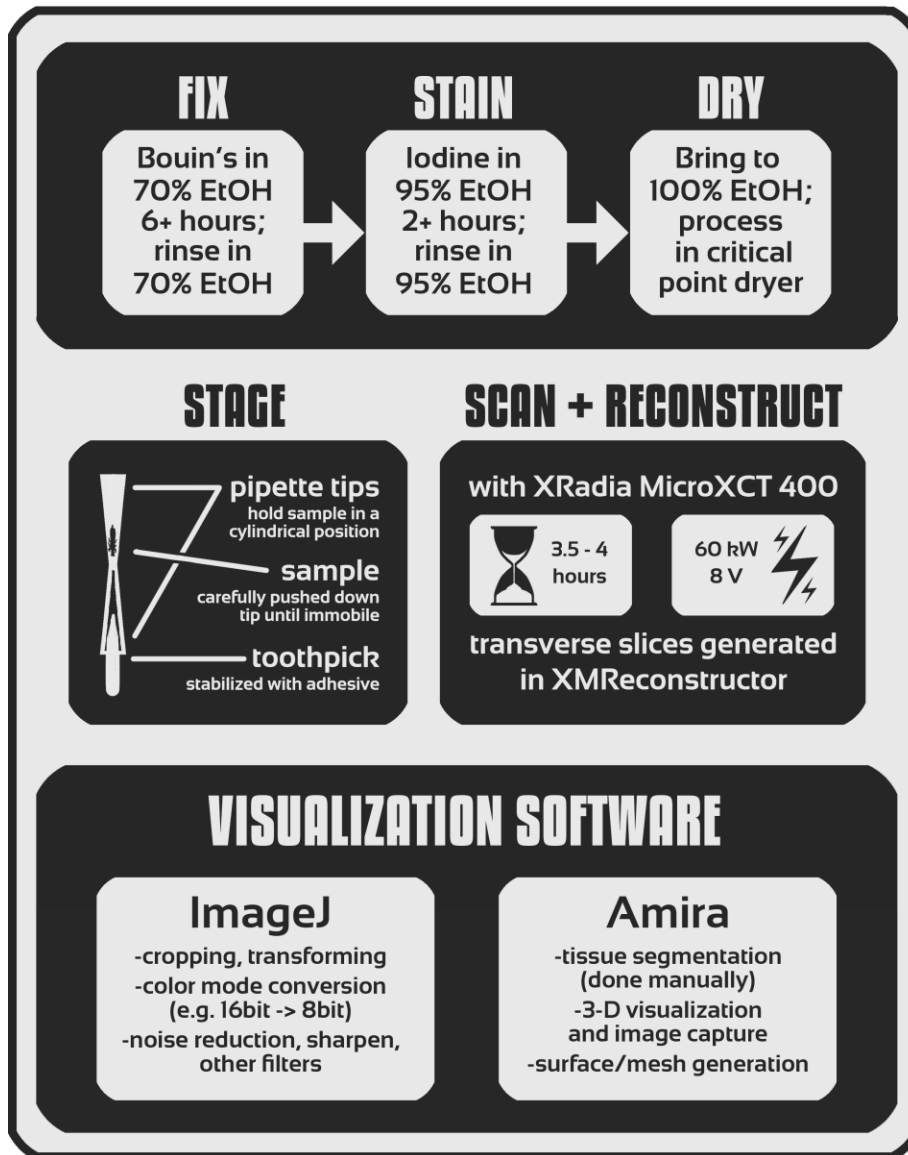


Figure 10. Graphic summary of the methods and equipment used in microCT scanning and visualization.

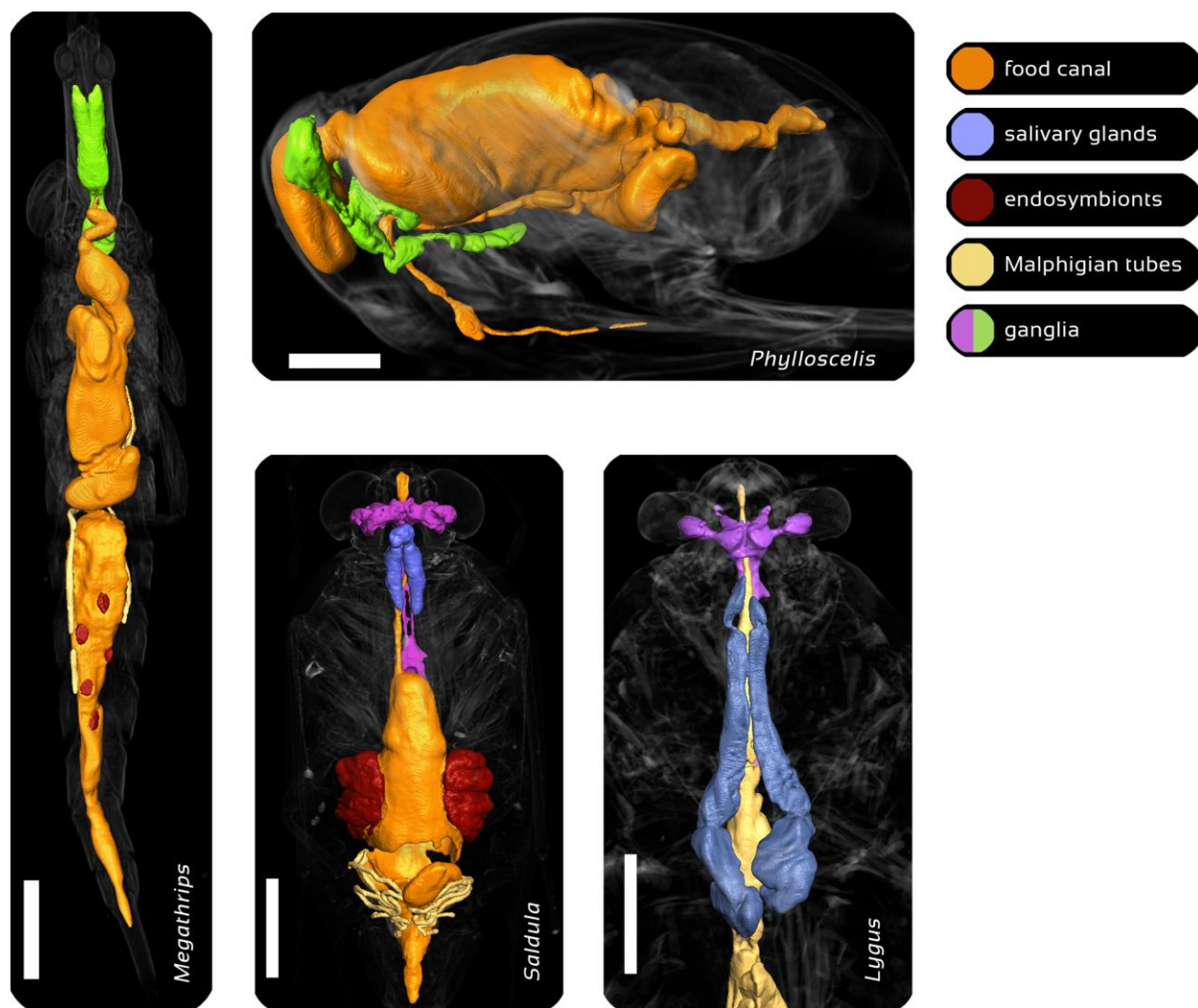


Figure 11. Lateral view of *Phylloscelis* and dorsal views of *Megathrips*, *Saldula* and *Lygus*, with select tissues imaged. The food canal is pale yellow in *Lygus*.

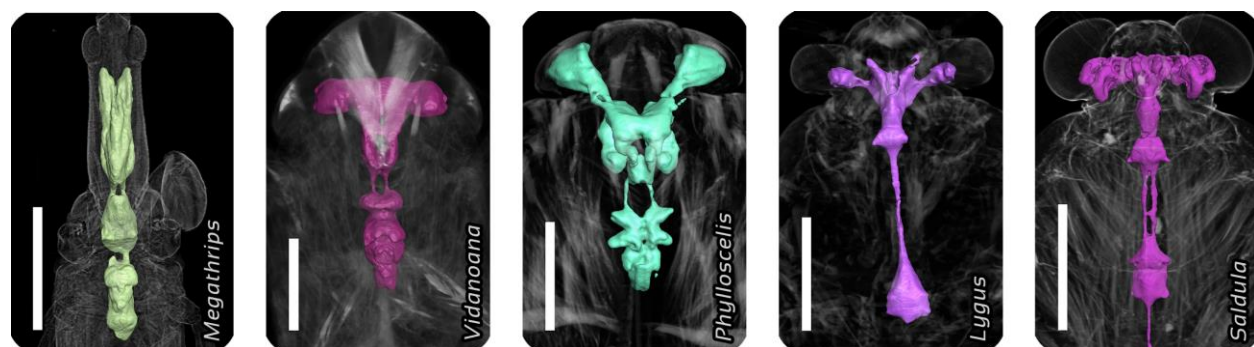


Figure 12. Ventral view of visualized ganglia from all scanned representatives. Scale bars = 254 um.

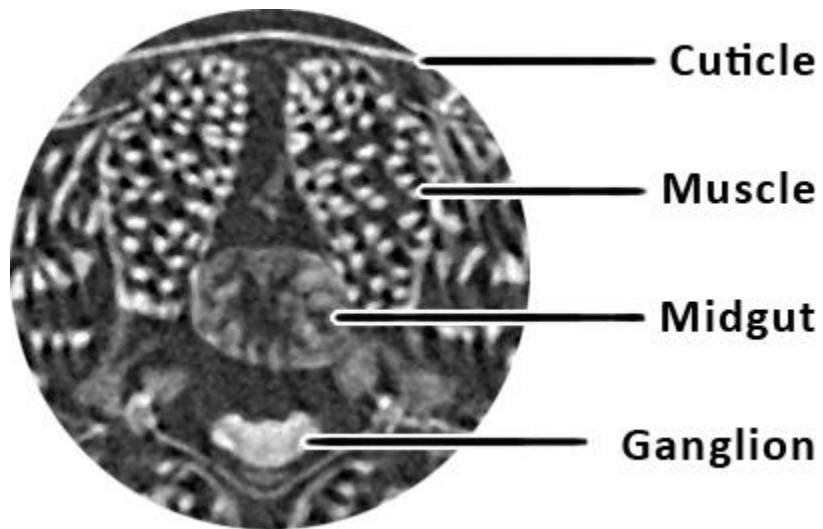


Figure 13. A select virtual slice from the raw stack for *Saldula pallipes*, demonstrating the visible tissue differentiation.

BIBLIOGRAPHY

- Andersen, J. C., Wu, J., Gruwell, M. E., Gwiazdowski, R., Santana, S. E., Feliciano, N. M., ... Normark, B. B. (2010). A phylogenetic analysis of armored scale insects (Hemiptera: Diaspididae), based upon nuclear, mitochondrial, and endosymbiont gene sequences. *Molecular Phylogenetics and Evolution*, 57(3), 992–1003.
- Asche, M. (1987). Preliminary thoughts on the phylogeny of Fulgoromorpha (Homoptera, Auchenorrhyncha). In *Proceedings of the 6th Auchenorrhyncha Meeting, Turin, Italy* (Vol. 7).
- Beutel, R. G., & Weide, D. (2005). Cephalic anatomy of *Zorotypus hubbardi* (Hexapoda: Zoraptera): new evidence for a relationship with Acercaria. *Zoomorphology*, 124(3), 121–136. <http://doi.org/10.1007/s00435-005-0117-z>
- Boas, F. E., & Fleischmann, D. (2012). CT artifacts: causes and reduction techniques. *Imaging in Medicine*, 4(2), 229–240.
- Bourgoin, T., & Campbell, B. C. (2002). *Inferring a phylogeny for Hemiptera: falling into the "autapomorphic trap."* na.
- Campbell, B. C., Steffen-Campbell, J. D., & Gill, R. J. (1994). Evolutionary origin of whiteflies (Hemiptera: Sternorrhyncha: Aleyrodidae) inferred from 18S rDNA sequences. *Insect Molecular Biology*, 3(2), 73–88.
- Cheung, W. W. K. (1983). Light and electron microscopic observations of the midgut sheath and anterior diverticulum of the lantern bug, *Pyrops candelaria* Linn.(Homoptera: Fulgoridae). *Cytologia*, 48(2), 333–342.
- Cryan, J. R., & Urban, J. M. (2012). Higher-level phylogeny of the insect order Hemiptera: is Auchenorrhyncha really paraphyletic? *Systematic Entomology*, 37(1), 7–21.
- Cui, Y., Xie, Q., Hua, J., Dang, K. A. I., Zhou, J., Liu, X., ... Bu, W. (2013). Phylogenomics of Hemiptera (Insecta: Paraneoptera) based on mitochondrial genomes. *Systematic Entomology*, 38(1), 233–245.
- Czosnek, H., Ber, R., Navot, N., Zamir, D., Antignus, Y., & Cohen, S. (1988). Detection of tomato yellow leaf curl virus in lysates of plants and insects by hybridization with a viral DNA probe. *Plant Disease*, 72(11), 949–951.
- DOHLEN, C. D. VON, & MORAN, N. A. (2000). Molecular data support a rapid radiation of aphids in the Cretaceous and multiple origins of host alternation. *Biological Journal of the Linnean Society*, 71(4), 689–717. <http://doi.org/10.1111/j.1095-8312.2000.tb01286.x>
- Dufour, L. (1833). *Recherches anatomiques et physiologiques sur les hémiptères: accompagnées de considérations relatives à l'histoire naturelle et à la classification de ces insectes* (Vol. 4). Bachelier.
- Eklom, T. (1929). New contributions to the systematic classification of the Heteroptera. *Ent.Tidskr*, 50, 169–180.
- Evans, J. W. (1963). The phylogeny of the Homoptera. *Annual Review of Entomology*, 8(1), 77–94.
- Evans, J. W. (1957). SOME ASPECTS OF THE MORPHOLOGY AND INTER-RELATIONSHIPS OF EXTINCT AND RECENT HOMOPTERA. *Transactions of the Royal Entomological Society of London*, 109(9), 275–294.
- Ferran, A., Rortais, A., Malausa, J. C., Gambier, J., & Lambin, M. (1996). Ovipositional behaviour of *Macrolophus caliginosus* (Heteroptera: Miridae) on tobacco leaves. *Bulletin of Entomological Research*, 86(02), 123–128.
- Glasgow, H. (1914). The gastric caeca and the caecal bacteria of the Heteroptera. *The Biological Bulletin*, 26(3), 101.
- Grimaldi, D. A., & Engel, M. S. (2005). The Paraneopteran Orders. *Evolution of the Insects*, 261–330.
- Habibi, J., Coudron, T. A., Backus, E. A., Brandt, S. L., Wagner, R. M., Wright, M. K., & Huesing, J. E. (2008). Morphology and histology of the alimentary canal of *Lygus hesperus* (Heteroptera: Cimicomorpha: Miridae). *Annals of the Entomological Society of America*, 101(1), 159–171.

- Hamilton, K. G. A. (1981). Morphology and evolution of the rhynchotan head (Insecta: Hemiptera, Homoptera). *The Canadian Entomologist*, 113(11), 953–974.
- Henning, W. (1969). Die Stammesgeschichte der Insekten. Frankfurt am Main: Waldemar Kramer.[English edition with supplementary notes 1981: W. Hennig: Insect Phylogeny]. J.
- Hix, R. (2001). Egg-laying and brochosome production observed in glassy-winged sharpshooter. *California Agriculture*, 55(4), 19–22.
- Ishiwata, K., Sasaki, G., Ogawa, J., Miyata, T., & Su, Z.-H. (2011). Phylogenetic relationships among insect orders based on three nuclear protein-coding gene sequences. *Molecular Phylogenetics and Evolution*, 58(2), 169–180.
- Johnson, K. P., Yoshizawa, K., & Smith, V. S. (2004). Multiple origins of parasitism in lice. *Proceedings Biological Sciences / The Royal Society*, 271(1550), 1771–1776. <http://doi.org/10.1098/rspb.2004.2798> [doi]
- Johnson, K. P., Dietrich, C., & Robertson, H. (2012, November). Phylogenomics and Morphology of the Hemipteroid Insect Orders. Retrieved from http://www.nsf.gov/awardsearch/showAward?AWD_ID=1239788
- Journet, A. R. P., & Vickery, V. R. (1978). Studies on Nearctic *Craspedolepta* Enderlein, 1921 (Homoptera: Psylloidea): external morphology. *The Canadian Entomologist*, 110(01), 13–36.
- Kjer, K. M., Carle, F. L., Litman, J., & Ware, J. (2006). A molecular phylogeny of Hexapoda. *Arthropod Syst Phylogeny*, 64(1), 35–44.
- Lent, H., & Wygodzinsky, P. (1979). Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. *Bulletin of the American Museum of Natural History*, 163(3), 123–520.
- Li, M., Tian, Y., Zhao, Y., & Bu, W. (2012). Higher level phylogeny and the first divergence time estimation of Heteroptera (Insecta: Hemiptera) based on multiple genes. *PloS One*, 7(2), e32152.
- Linnaeus, C. (1758). Systema Naturae per Regna tria Naturae, secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis Synonymis, Locis.
- Lundgren, J. G. (2011). Reproductive ecology of predaceous Heteroptera. *Biological Control*, 59(1), 37–52.
- Lyal, C. H. C. (1985). Phylogeny and classification of the Psocodea, with particular reference to the lice (Psocodea: Phthiraptera). *Systematic Entomology*, 10(2), 145–165.
- Maddison, W. P., & Maddison, D. R. (2001). Mesquite: a modular system for evolutionary analysis.
- Martins-Neto, R. G., Gallego, O. F., & Melchor, R. N. (2003). The Triassic insect fauna from South America (Argentina, Brazil and Chile): a checklist (except Blattoptera and Coleoptera) and descriptions of new taxa. *Acta Zoologica Cracoviensis*, 46(Suppl.).
- Martynov, A. V., & Deryugina, K. M. (1923). On two basic types of insect wings and their significance for the general classification of insects. *Trudy Pervogo Vserossijskogo S'ezda Zoologov, Anatomov I Gistologov*, 1, 88–89.
- Matsuda, R. (2013). *Morphology and evolution of the insect abdomen: with special reference to developmental patterns and their bearings upon systematics*. Elsevier.
- McPherson, J. E., Packauskas, R. J., & Korch III, P. P. (1987). Life history and laboratory rearing of *Pelocoris femoratus* (Hemiptera: Naucoridae), with descriptions of immature stages. *Proceedings of the Entomological Society of Washington*, 89(2), 288–295.
- Metscher, B. D. (2009). MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues. *BMC Physiology*, 9(1), 1.
- Misof, B., Liu, S., Meusemann, K., Peters, R. S., Donath, A., Mayer, C., ... Zhou, X. (2014). Phylogenomics resolves the timing and pattern of insect evolution. *Science (New York, N.Y.)*, 346(6210), 763–767. <http://doi.org/10.1126/science.1257570> [doi]
- Mitchell, P. L. (2004). Heteroptera as vectors of plant pathogens. *Neotropical Entomology*, 33(5), 519–545.

- Moran, N. A., Baumann, P., & Von Dohlen, C. (1994). Use of DNA sequences to reconstruct the history of the association between members of the Sternorrhyncha (Homoptera) and their bacterial endosymbionts. *European Journal of Entomology*, 91(1), 79–83.
- Muir, F. (1923). On the characters separating Heteroptera from Homoptera. *Entomologist's Monthly Magazine*, 59, 254.
- Munyanzeza, J. E., Crosslin, J. M., & Upton, J. E. (2007). Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with “zebra chip,” a new potato disease in southwestern United States and Mexico. *Journal of Economic Entomology*, 100(3), 656–663.
- Myers, J. G., & China, W. E. (1929). XXXVII.—The systematic position of the Peloriidiidae as elucidated by a further study of the external anatomy of *Hemiodæcus leai*, China (Hemiptera, Peloriidiidae). *Journal of Natural History*, 3(15), 282–294.
- Nault, L. R., Rodriguez, J. G., & DeLong, D. M. (1985). *The leafhoppers and planthoppers*. Wiley New York.
- Nel, A., Prokop, J., Nel, P., Grandcolas, P., Huang, D., Roques, P., ... Szwedo, J. (2012). Traits and evolution of wing venation pattern in paraneopteran insects. *Journal of Morphology*, 273(5), 480–506.
- Niven, J. E., Graham, C. M., & Burrows, M. (2008). Diversity and evolution of the insect ventral nerve cord. *Annu.Rev.Entomol.*, 53, 253–271.
- Ouvrard, D., Campbell, B. C., Bourgoin, T., & Chan, K. L. (2000). 18S rRNA secondary structure and phylogenetic position of Peloriidiidae (Insecta, Hemiptera). *Molecular Phylogenetics and Evolution*, 16(3), 403–417.
- Pittendrigh, B. R., Clark, J. M., Johnston, J. S., Lee, S. H., Romero-Severson, J., & Dasch, G. A. (2006). Sequencing of a new target genome: the *Pediculus humanus humanus* (Phthiraptera: Pediculidae) genome project. *Journal of Medical Entomology*, 43(6), 1103–1111.
- Raoult, D., & Roux, V. (1999). The body louse as a vector of reemerging human diseases. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, 29(4), 888–911. <http://doi.org/10.1086/520454> [doi]
- Reuter, O. M. (1912). Bemerkungen über mein neues Heteropterensystem.
- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675. <http://doi.org/10.1038/nmeth.2089>
- Schuh, R. T. (1979). Evolutionary trends in Heteroptera. *Part II.Mouthpart-Structures and Feeding Strategies*, 28.
- Schuh, R. T., & Polhemus, J. T. (1980). Analysis of taxonomic congruence among morphological, ecological, and biogeographic data sets for the Leptopodomorpha (Hemiptera). *Systematic Zoology*, 1–26.
- Schuh, R. T., & Slater, J. A. (1995). True bugs of the world. *Comstock, Ithaca, New York*.
- Scudder, G. G. E. (1961). The comparative morphology of the insect ovipositor. *Transactions of the Royal Entomological Society of London*, 113(2), 25–40.
- Scudder, G. G. E. (1959). THE FEMALE GENITALIA OF THE HETEROPTERA: MORPHOLOGY AND BEARING ON CLASSIFICATION1. *Transactions of the Royal Entomological Society of London*, 111(14), 405–467.
- Seeger, W. (1975). Funktionsmorphologie an Spezialbildungen der Fühlergeißel von Psocoptera und anderen Paraneoptera (Insecta); Psocodea als monophyletische gruppe. *Zoomorphology*, 81(2), 137–159.
- Sharga, U. S. (1933). On the internal anatomy of some Thysanoptera. *Transactions of the Royal Entomological Society of London*, 81(2), 185–204.
- Shcherbakov, D. (2002). *The 270 million year history of Auchenorrhyncha (Homoptera)*. na.

- Shcherbakov, D. E. (2000). The most primitive whiteflies (Hemiptera; Aleyrodidae; Bernaeinae subfam. nov.) from the Mesozoic of Asia and Burmese amber, with an overview of Burmese amber hemipterans. *BULLETIN-NATURAL HISTORY MUSEUM GEOLOGY SERIES*, 56(1), 29–38.
- Shcherbakov, D. E. (2010). The earliest true bugs and aphids from the Middle Triassic of France (Hemiptera). *Russian Entomological Journal*, 19(3), 179–182.
- Sheffield, J. (2007). ImageJ, A Useful Tool for Biological Image Processing and Analysis. *Microscopy and Microanalysis*, 13(S02), 200–201. <http://doi.org/10.1017/S1431927607076611>
- Spooner, C. S. (1938). The phylogeny of the Hemiptera based on a study of the head capsule. 16. *Illinois Biological Monographs*; v.16, no.3.
- Stalling, D., Westerhoff, M., & Hege, H. (2005). Amira: a highly interactive system for visual data analysis. *Visualization Handbook*. Retrieved from <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.382.5837&rep=rep1&type=pdf#page=768>
- Swofford, D. L. (2003). PAUP*: phylogenetic analysis using parsimony, version 4.0 b10.
- Szwedo, J. (2010). The Major Diversification of Cicadomorpha in the Jurassic (Insecta: Hemiptera). In *Marine and non-marine Jurassic--Short papers for the 8~(th) International Congress on the Jurassic System*.
- Szwedo, J. (2011). First discovery of Neazoniidae (Insecta, Hemiptera, Fulgoromorpha) in the Early Cretaceous amber of Archingeay, SW France. <http://dx.doi.org/10.5252/g2009n1a9>.
- Szwedo, J., & Nel, A. (2011). The oldest aphid insect from the Middle Triassic of the Vosges, France. *Acta Palaeontologica Polonica*, 56(4), 757–766.
- Taylor, G. S. (1992). The structure of the eggs of some Australian Psylloidea (Hemiptera). *Australian Journal of Entomology*, 31(2), 109–117.
- Urban, J. M., & Cryan, J. R. (2007). Evolution of the planthoppers (Insecta: hemiptera: Fulgoroidea). *Molecular Phylogenetics and Evolution*, 42(2), 556–572.
- Wheeler, W. C., Schuh, R. T., & Bang, R. (1993). Cladistic relationships among higher groups of Heteroptera: congruence between morphological and molecular data sets. *Insect Systematics & Evolution*, 24(2), 121–137.
- Yoshizawa, K. (2005). Morphology of Psocomorpha (Psocodea: 'Psocoptera'). *Insecta matsumurana. Series entomology. New Series*, 62, 1–44.
- Yoshizawa, K., & Johnson, K. P. (2005). Aligned 18S for Zoraptera (Insecta): phylogenetic position and molecular evolution. *Molecular Phylogenetics and Evolution*, 37(2), 572–580.
- Yoshizawa, K., & Johnson, K. P. (2010). How stable is the "Polyphyly of Lice" hypothesis (Insecta: Psocodea)? A comparison of phylogenetic signal in multiple genes. *Molecular Phylogenetics and Evolution*, 55(3), 939–951.
- Емельянов, А. Ф. (1987). Филогения цикадовых (Homoptera, Cicadina) по сравнительно морфо логическим данным. *Морфологические Основы Филогении насекомых. —Л.: На Ука*, 19–109.