

# Patterns of morphological simplification and innovation in the megadiverse Holometabola (Insecta)

Rolf Georg Beutel<sup>\*a,b</sup>, Frank Friedrich<sup>c</sup> and Evan P. Economo<sup>b</sup>

<sup>a</sup>Entomology Group, Institut für Zoologie und Evolutionsforschung, Friedrich-Schiller-Universität Jena, Erbertstrasse 1, Jena, D-07743, Germany;

<sup>b</sup>Biodiversity and Biocomplexity Unit, Okinawa Institute of Science and Technology Graduate University, 1919-1 Tancha, Onna-son, Kunigami-gun, Okinawa, 904-0495, Japan; <sup>c</sup>Institut für Zoologie, Universität Hamburg, Martin-Luther-King-Platz 3, Hamburg, D-20146, Germany

Accepted 22 June 2021

---

## Abstract

We analyzed patterns of complexity and simplicity in holometabolan insects using parsimony and maximum-likelihood. By contrast with other groups of arthropods (and most other groups of animals), insects have undergone a stepwise process of structural simplification in their evolution. The megadiverse Holometabola are characterized mainly by structurally simplified larvae, which differ strongly from the adults in their morphology and usually also in their life habits. Although smaller groups such as Neuropterida have largely maintained their structural complexity in adults and immature life stages, a series of reductions occurred with the appearance and diversification of Coleopterida, Mecopterida and especially Antliophora. Parasitic Strepsiptera or fleas display conspicuous patterns of reduction in different life stages and body regions, and high degrees of simplification also occur in groups with short-lived adults. Larvae living in moist substrates display far-reaching structural simplifications and also morphological uniformity, especially in the species-rich Diptera, but also in other groups. Liquid feeding leads to correlated simplifications and innovation of adult head structures, especially of the mouthparts. Functional or anatomical dipterism leads to an optimization of the flight apparatus in most holometabolous groups, which is correlated with reductions in one of the pterothoracic segments, and coupled (e.g. by hamuli), partly reduced or transformed wings (e.g. halteres). In flightless groups, the pterothoracic skeleto-muscular apparatus is strongly simplified. In the abdomen of adult females a stepwise reduction of the lepismatoid ovipositor occurs. By contrast, the male genital apparatus often undergoes an extreme diversification. Our evaluations revealed a highly correlated complexity between larval and adult stages.

© 2021 The Authors. Cladistics published by John Wiley & Sons Ltd on behalf of Willi Hennig Society.

---

## Introduction

Evolutionary systems are driven by the reciprocal processes of innovation and simplification, which serve to either increase or decrease complexity (Darwin, 1859; Simpson, 1949; Ramírez and Michalik, 2014; O'Malley et al., 2016). Innovation can lead to diversification when a new trait or ability allows lineages to take advantage of ecological opportunities (i.e. habitats, behaviours etc.). However, diversity also can be generated through specialization and “streamlining” of different descendant lineages, resulting in simplified

structural configurations (Darwin, 1859; Simpson, 1949, 1953; O'Malley et al., 2016). Interestingly, these trends are not only the domain of biological evolution. Languages apparently are also subject to patterns of simplification in their “evolution”, as for instance from Sanskrit to modern Hindustani, from Latin to the Romance languages, or from ancient to modern Greek (e.g. Bopp, 1842; see also, e.g., Kusters, 2000). The balance between these tendencies toward simplification or complexification during major episodes of diversification is not well understood.

Trends towards structural simplification and/or a reduced morphological diversity can be observed in different groups of animals. Within the ecdysozoan Cycloneuralia (several groups of former

---

\*Corresponding author:

E-mail address: rolf.beutel@uni-jena.de

Nemathelminthes), likely the sistergroup of Arthropoda (e.g. Giribet, 2003), the species-poor Scali-dophora (e.g. Priapulida) display a remarkable structural complexity. A retractile introvert with rings of scalids as sensorial structures is present in all three groups, the body of the small Kinorhyncha is externally segmented, and a complex apparatus of plates (lorica) characterizes the minute Loricifera (Schmidt-Rhaesa, 2013). By contrast, the closely related species-rich worm-like nematodes (*c.* 25 000 known species) are extremely uniform in their morphological structures. They lack a differentiated head region (except for the terminal mouth opening and simple ring-shaped brain), sense organs, body appendages, breathing organs, ring muscles and nephridial organs (e.g. Schmidt-Rhaesa, 2013). In Annelida, formerly considered as sister taxon of Arthropoda (“Articulata”; see, e.g., Giribet, 2003), the “basal” marine polychaetes display a high structural diversity, and contain groups with structurally highly complex species (e.g. Nereidae), including a complex head region with lens eyes, antennae, palps, jaws, and also segmental bilobed parapods with gills, tentacles, bundles of long chaetae, internal aciculae and branches of the circulatory system with capillary regions. By contrast to this, the Clitellata (*c.* 11 000 spp.), a clade characterized by the secretion producing clitellum as an apomorphy, displays a distinctly simplified body organization and apparently a low morphological diversity, without complex sense organs, tactile structures, mouth parts, breathing organs and segmental body appendages (e.g. Aguado et al., 2014). Examples in Craniota (vertebrates) are the simplified robust skull of mammals with the dentale as the only bone forming the lower jaw (and the shift of the malleus and incus into the middle ear as innovations), the constant number of only seven cervical vertebrae (with two different exceptions in sloths), and the simplified shoulder girdle with only the scapula preserved in most groups of Eutheria (=Placentalia; e.g. Mickoleit, 2004).

Patterns of simplification also are well-known in Hexapoda. Examples are the simplified thoracic muscle system in Coleoptera, especially the megadiverse Polyphaga (Beutel and Haas, 2000; Friedrich et al., 2009), or the simplified mandibular musculature in the entire Neoptera (Staniczek, 2000; Blanke et al., 2012, 2015). Whereas eight pairs of mandibular muscles are present in Archaeognatha and the zygentoman Lepidotrichidae (Bitsch, 1963; Blanke et al., 2015), only two pairs plus a very thin proprioceptor are present in the neopteran orders (groundplan; e.g. Beutel and Pohl, 2006; Beutel and Vilhelmsen, 2007; Wipfler et al., 2011; Matsumura et al., 2015; Antunes-Carvalho et al., 2017; Richter et al., 2020), where two mandibular articulations function as ball-and-socket joints, thus reducing the degrees of freedom to a single plane of movement.

This feature is highly stable in neopteran insects, without a single case of a secondary increase of the number of mandibular muscles (e.g. Beutel et al., 2011, 2014). The case of reduced tarsomeres in different groups of beetles was discussed by Brown (1965) in a study on numerical taxonomy and evolutionary reduction. Using the endoparasitic Strepsiptera and the cucujiform beetle family Rhipiphoridae as an example, he pointed out that a secondary gain of a 5th hind tarsomere in the former group is highly unlikely, and thus dismissed a close relationship between these two otherwise phenotypically similar groups (Crowson, 1955). Indeed, not a single case of secondarily acquired five-segmented tarsi is known in the very species rich tenebrionoid beetles (“Heteromera”; Lawrence et al., 2011). It also was pointed out by Brown (1965) that the concept of the meristic reduction rule holds extraordinarily well in arthropods (except for cases of increased numbers of antennomeres in some groups) and vertebrates, and that a rule of evolutionary reduction has also been explicitly recognized by plant morphologists (e.g. Eames, 1936). Directional evolution in the morphology of Hymenoptera was studied using a Bayesian approach by Klopstein et al. (2015), who discerned a distinct trend towards reduction in the wing venation and musculature.

A study specifically focused on evolutionary patterns of simplification in a large supraordinal subdivision of insects has not been carried out so far. For evaluating this phenomenon, we chose Holometabola, a group with an unparalleled diversity, comprising *c.* 830 000 described species (e.g. [https://en.wikipedia.org/wiki/Insect\\_biodiversity](https://en.wikipedia.org/wiki/Insect_biodiversity)), >50% of all organisms known on this planet. The phylogeny of this extremely successful group has been analyzed with different datasets in the last decades. Recent analyses of several single copy protein-coding nuclear genes (Wiegmann et al., 2009), extensive morphological character sets (Beutel et al., 2011), and genomic (Niehuis et al., 2012) or transcriptomic data (Misof et al., 2014; Peters et al., 2014) yielded a robust phylogeny: Hymenoptera is placed as sister to the remaining Holometabola (Aparaglossata), and a clade Neuropterida + Coleopterida (Coleoptera + Strepsiptera) as sister to Mecopterida comprising Amphiesmenoptera (Trichoptera + Lepidoptera) and Antliophora (Mecoptera, Siphonaptera, Diptera).

Since Beutel et al. (2011), numerous studies on the morphology of different life stages and body regions have been published, most of them based on innovative approaches (e.g. Wipfler et al., 2016). A comprehensive work on beetle morphology and phylogeny was published by Lawrence et al. (2011) and new *Handbook of Zoology* volumes on this megadiverse order and on Nannomecoptera and Neomecoptera are available (Beutel and Leschen, 2016; Leschen and

Beutel, 2014; Beutel and Friedrich, 2019). Studies on specific groups and body regions include, for instance, Schneeberg et al. (2011, 2012, 2013), Hünefeld et al. (2012), Schneeberg and Beutel (2014), Liu et al. (2017) and Richter et al. (2019, 2020).

This enormous increase in morphological information inspired us to screen and modify the dataset of Beutel et al. (2011) and to re-analyze it with new approaches and a large-scale perspective. Here, we perform a qualitative and quantitative analysis of the evolution of morphological simplicity and complexity. We focus on the role that structural simplification and innovation played in different groups (Figs. 1–3), especially in the megadiverse “Big Four”, Hymenoptera, Coleoptera, Lepidoptera and Diptera. Character states are categorized as reductions/simplifications (e.g. loss of appendages or muscles, fusion of sclerites by reduction of sutures or ridges) or innovations leading to increased complexity (e.g. acquisition of cleaning devices of the fore legs, formation of a sperm pump). The character evolution is reconstructed in a robust phylogenetic framework, based on transcriptomic data (Misof et al., 2014; Peters et al., 2014).

In addition to qualitatively analyzing trends in different character systems observed across the phylogeny, we reconstruct statistical trends in complexity and simplicity by analyzing aggregate patterns across over 260 characters. We assess whether complexity increases or decreases in different parts of the tree, both in general and for specific body regions (i.e. head, thorax, abdomen) and life stages (i.e. larvae, adult). The theory behind such possible correlations is not well-developed, and this analysis is exploratory. However, positive correlations could indicate that there are common genomic, ecological or behavioural pressures favouring correlated change (for example, parasitism may result in reduced complexity across all stages/parts). However, it also is possible there are no correlations, where different stages and segments go through innovations and simplifications independently, or negative

correlations, where increased complexity of one life stage/body region is associated with reduced complexity elsewhere.

## Materials and methods

### Character dataset

We modified the dataset of Beutel et al. (2011), deleting characters with high levels of homoplasy (see Beutel et al., 2011: fig. 3) or too many missing entries. We also omitted features not clearly fitting into the categories increased complexity (innovation) or simplification, for instance modifications of shape. The reduced list of 264 characters (vs. 356 in Beutel et al., 2011) and the matrix (nexus and WINCLADA format) are presented in Appendix S1 (Data S1).

Simple character states (simplification) include absence/losses (e.g. specific muscles or sclerites), fusions (e.g. borders between sclerites separated in other groups are absent), and a lower number of appendage segments (=meres; e.g. fewer antennomeres, palpomeres, tarsomeres). Complex states include novel structures or organs (e.g. additional sclerites or muscles, cleaning devices, sperm pump) and modified structures with novel functions (e.g. antennomeres bearing labellae, halteres as gyroscopic sense organs).

### Phylogenetic tree and analyses of character evolution

The generally accepted topology obtained with genomic (Niehuis et al., 2012) or transcriptomic data (Misof et al., 2014; Peters et al., 2014) with monophyletic Aparaglossata, Neuropteroidea (Neuropterida + Coleopterida) and Mecopterida (Amphiesmenoptera + Antliophora) was used for reconstructing the character evolution with parsimony. The analysis was carried out using MES-QUITE (Maddison and Maddison, 2018; stored tree, trace character history, parsimony ancestral states). The obtained presumptive apomorphies are listed in Appendix S2 (for additional references, see Appendix S3).

As the maximum-likelihood (ML) character reconstruction analyses required a tree with realistic branch lengths corresponding to divergence times, we used the tree from Misof et al. (2014; for topology, see Appendix S4) as a basis for comparative analysis, but adding a few families based on their inferred position and branching times from order-level phylogenetic studies on the Hymenoptera (Peters et al., 2017), Coleoptera (McKenna et al., 2019) and Neuroptera (Vasilikopoulos et al., 2020).

### Maximum-likelihood analysis of character evolution

We also sought to estimate the evolutionary histories of each character to map whether in the aggregate, complexity increases or decreases in different locations of the tree for groups of characters representing different life stages (larval vs. adult) and body regions (head, thorax, abdomen). Our data consisted of binary discrete characters, some with gaps (not applicable or undefined for a taxon) and missing data (where the character likely exists but the state is unknown). The states of each binary character were assigned to be either “simple” or “complex” based on the criteria described above. For each character, we estimated ancestral states using Mk models (Pagel, 1994; Lewis, 2001) of character evolution. When gaps were present in some taxa, the character was reconstructed as having three states rather than binary, with the third state reflecting the undefined condition. For taxa with missing data for a character owing to our incomplete knowledge rather than undefined state, these taxa were

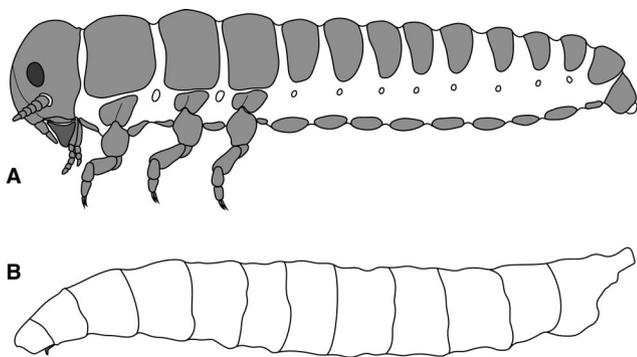


Fig. 1. (a) Hypothetical groundplan of Holometabola in the larval stage (based on Peters et al., 2014); (b) strongly simplified larva (maggot) of *Drosophila* (based on Wipfler et al., 2013).

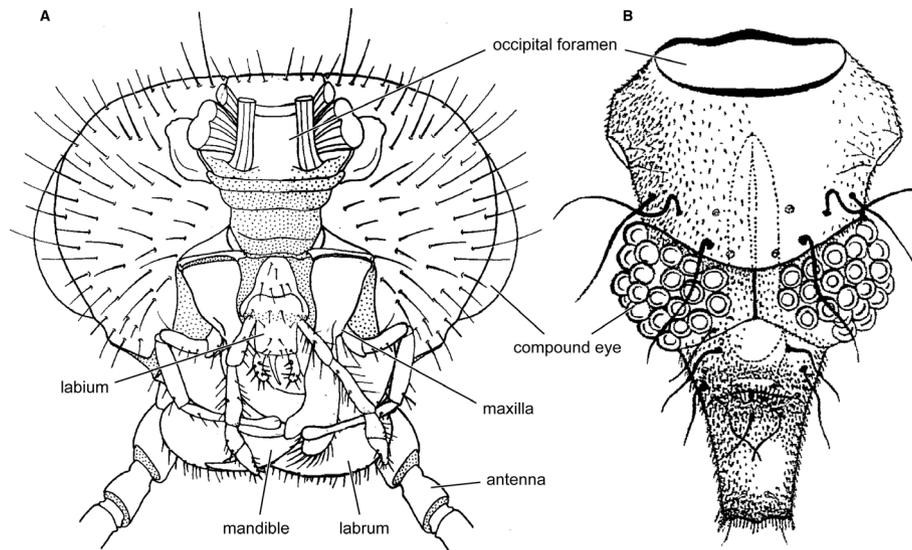


Fig. 2. Comparison of adult head features of (a) the saw fly *Macroxyela ferruginea* (Xyelidae, Hymenoptera) (based on Beutel and Vilhelmsen, 2007) and (b) the nematoceran fly *Nymphomyia alba* (Nymphomyiidae, Diptera) (based on Tokunaga, 1935). The complete set of mouthparts shown in *Xyela* (including maxillary and labial endite lobes) comes close to the groundplan condition in Holometabola. The mouthparts are almost completely reduced in *Nymphomyia*.

pruned from the tree when reconstructing that character. We first used the function *fitDiscrete* from the R package *GEIGER* (Pennell et al., 2014) to compare models where all transition rates were equivalent (equal rates) using Akaike's Information Criterion (AIC; Akaike, 1973), transitions among pairs of states were equivalent (symmetrical, equivalent to the "equal rates" in all characters with no gaps), or where all rates could differ (all rates different), with the more complex model preferred if  $\Delta\text{AIC}$  is  $>2$ . For each character, 250 stochastic character maps (Bollback, 2006) were simulated with the selected model with the function *make.simmap* in the R package *PHYTOOLS* (Revell, 2012), and the marginal probability of each state was calculated for each internal node. Although nonstationarity of character states is known to be a source of systematic bias, each character was analyzed independently on a reference tree, thus attraction caused by long branches or compositional heterogeneity is not a focal problem. Additionally, it is not currently possible to implement variable root composition probabilities in *PHYTOOLS*. Refined analyses may be the aim of future studies.

In order to summarize "complexity" for each character set for each node, we took the mean marginal probability of being in the complex state across all characters at that node. This summary was calculated for characters corresponding to different life stages and body regions, and both the observed tip means and reconstructed internal state means were visualized on the tree. To assess whether the results were robust to methodology, we also used maximum parsimony (MP) in the same analysis pipeline and compared the node-wise percentage complexity estimates. This was achieved in the same framework by setting the transition of the  $Q$  matrix to equal and extremely low values (0.000001) instead of using the ML  $Q$  matrix, and again performing stochastic character mapping. In the few cases where there were multiple configurations with equal parsimony, the node state was averaged over the different solutions.

#### Correlation between complexity across life stages and body regions

We also evaluated the correlation of complexity across non-overlapping character sets. If a taxon has a more complex head, is it

also likely to have a more complex thorax (larva vs. adult, head vs. abdomen, etc.)? To do this, we calculated the mean complexity of each taxon for each character set (as described in the previous section). To get an overall correlation between the complexities of character sets across taxa, we calculated Spearman's rho ( $\rho$ ). We used Phylogenetic Generalized Least Squares (PGLS; Grafen, 1989) analysis to assess significance of the correlation while accounting for phylogenetic non-independence using the *gls* function in the R package *NMLE*, but using a Brownian motion correlation structure determined by the *corBrownian* function in the R package *APE* (Paradis and Schliep, 2019). Directional models (e.g. Ornstein–Uhlenbeck) were not considered as they cannot be implemented for discrete data in R at present.

#### Character Evolution

(See Appendix S2 for detailed list of apomorphies).

The analysis of character evolution based on MP and on ML both support a scenario of a stepwise structural simplification within the megadiverse Holometabola (e.g. partial reduction of the lepidomatoid ovipositor in Neuropterida, and complete absence in Mecopterida; Figs 4–6). This affects immature stages and adults, and also different body regions, but with patterns distinctly varying among the major subgroups and orders (Figs 5 and 6). The presumptive apomorphies of clades presented in the following are based on parsimony reconstruction. As shown in Fig. 5d, the results obtained with MP and ML are nearly identical.

#### Holometabola

A first wave of simplification apparently took place in the holometabolan stem group (Fig. 4: 9 innovations vs. 20 simplifications in the MP-based analysis),

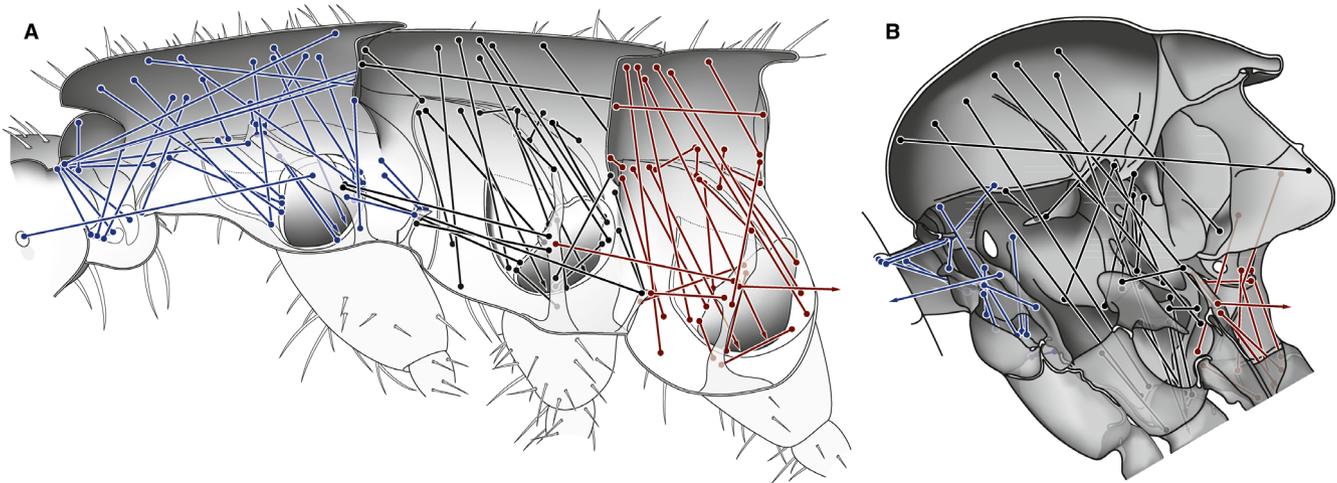


Fig. 3. Comparison of thoracic segments and muscle sets. Three similarly sized segments each with a complex set of muscles are present in (a) the polyneopteran ground louse *Zorotypus hubbardi* (Zoraptera) (based on Friedrich and Beutel, 2008), which is likely close to the groundplan condition in Neoptera and the thoracic morphology in ancestral holometabolans (e.g. Neuropterida). Within Holometabola, trends towards size reduction of single thoracic segments along with a decrease of muscle numbers in these subunits are recognizable. This is often due to a specialized flight mode (i.e. postero- or anteromotorism). The latter is typical for true flies (Diptera), for instance (b) the fruit fly *Drosophila melanogaster* (based on Fabian et al., 2016), resulting in strongly reduced metathorax, accompanied with a distinctly reduced muscle apparatus in this segment. Colouration: muscles associated with: blue, neck and prothorax; black, mesothorax; and red, metathorax. For detailed information on individual muscles, see Friedrich and Beutel (2008) and Fabian et al. (2016).

probably beginning as early as in the Devonian, >350 Ma (Misof et al., 2014). Holometabola are mainly characterized by larval stages displaying structural simplifications of different body regions (Fig. 1a). This includes the absence of ocelli (possibly a synapomorphy shared with Paraneoptera; see Appendix S1 for characters and character states), simplified or reduced larval compound eyes, antennae with a strongly reduced number of antennomeres and lacking Johnston's organ and intrinsic muscles, reduced muscles of the simplified maxillary and labial endite lobes, unsegmented tarsi, and the absence of abdominal segment XI and cerci (also in Paraneoptera, reversal in Strepsiptera). Adult structural features of Holometabola appear less affected by simplification considering the results of the MP analysis, even though the ML analysis of character evolution indicated that about 30% of the groundplan characters are in the complex state in both larvae and adults (Fig. 5a,b). Less than 30% of characters in the complex state were also obtained for the adult head in the ML analysis, vs. c. 50% for the thorax (Fig. 4b). This result appears surprising, as "basal" groups of Hymenoptera (Beutel and Vilhelmsen, 2007) and also Neuroptera (e.g. Randolph et al., 2014) are mostly characterized by plesiomorphic cephalic features, with largely unmodified biting mouthparts and a complex set of muscles. As a whole, the thorax of Holometabola (groundplan) has maintained its structural complexity to fulfil its essential functions of locomotion on the ground and flight.

A conspicuous important character transformation of each of the pterothoracic segments is the internalization of the true sternum, which now forms the internal median ridge (discrimen), and is externally replaced by pleural elements (preepisterna). The MP analysis of character evolution assigns the absence of several muscles to the groundplan of Holometabola (e.g. *M. frontolabralis*, *M. pronoto-trochanteralis*, *M. profurcamesospinalis*). However, in these cases multiple losses appear more likely than reversal in various subgroups. Cases of secondarily regained muscles (e.g. muscles of the adult head or thorax) are unknown, for instance, in the megadiverse Coleoptera (Larsén, 1966; Beutel and Haas, 2000; Friedrich et al., 2009), but also in other groups of Holometabola (Beutel et al., 2011).

Even though the ML analysis yielded <30% of the adult abdominal characters in the complex state, this tagma appears comparatively unmodified in the groundplan of Holometabola (Beutel et al., 2011). One plesiomorphic character complex preserved in the groundplan of Hymenoptera is the well-developed lepisematoid ovipositor (Vilhelmsen, 2000). A derived condition of the abdomen is the distinct reduction or absence of the cerci. Shortening and the reduced segmentation of segment XI are potential synapomorphies with the related paraneopteran orders, with independent complete loss in different holometabolan groups (e.g. Coleoptera) and all orders of Paraneoptera (=Acercaria; e.g. Beutel et al., 2011).

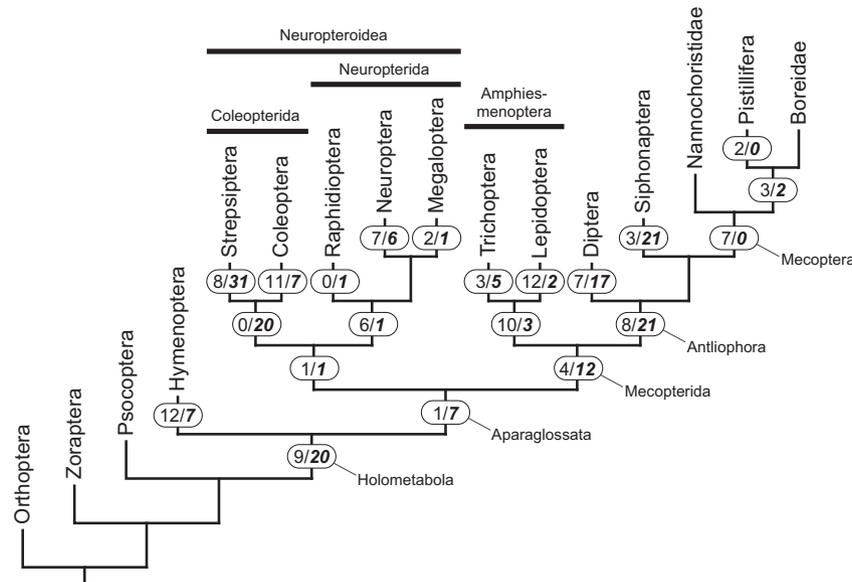


Fig. 4. Cladogram based on Misof et al. (2014) with apomorphies mapped on it. Innovations coded as (1) and simplifications or reductions as (2). Below nodes number of innovations versus simplifications (bold italics).

### Hymenoptera

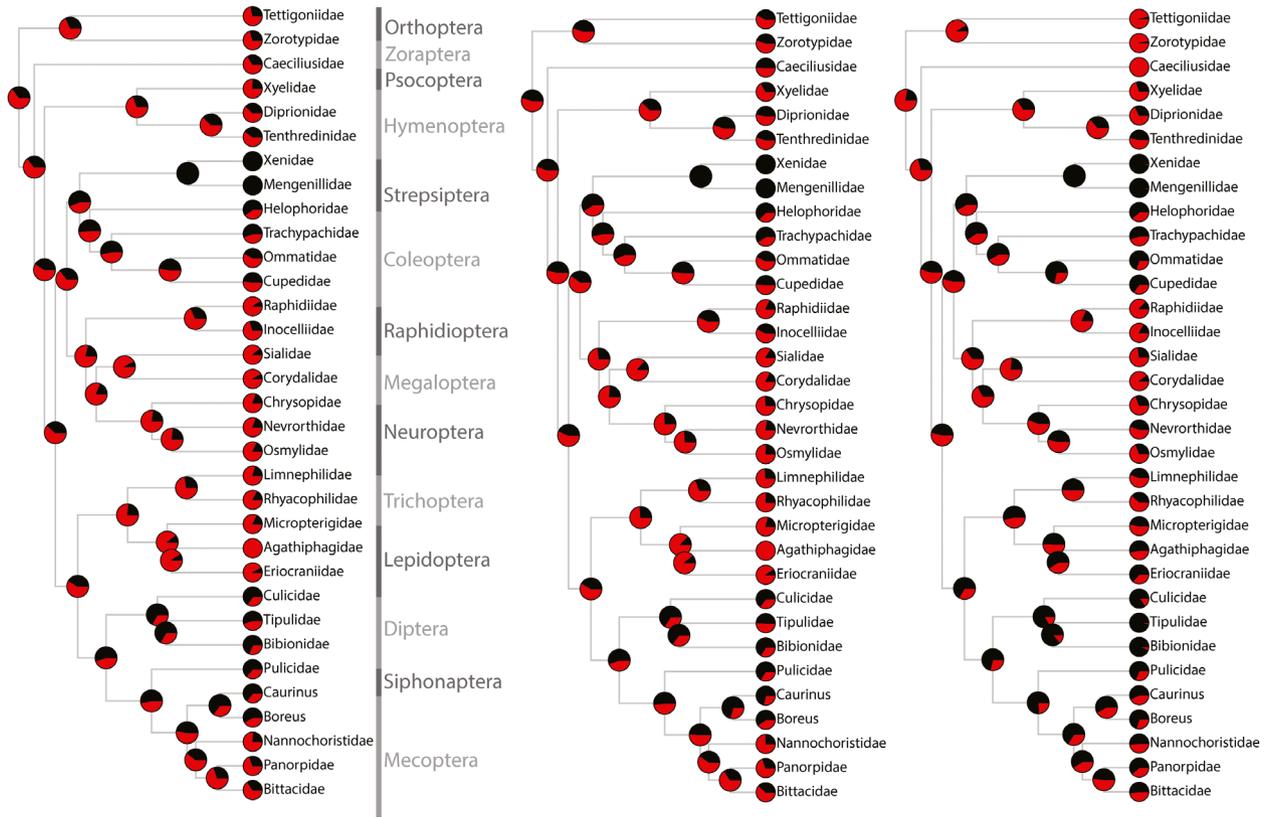
Hymenoptera (represented by “symphytan” terminals here), the sister group of the remaining holometabolous orders, also display distinct simplifications in adults (Fig. 3a: *c.* 40% in the complex state) and in immature stages (Fig. 3b: *c.* 40% in the complex state). This includes the restricted lateral movability of the larval maxillae (maxillolabial complex), the single claws of larvae, cervical sclerites of adults partly or completely fused with the propleuron, the absence of the mesotrochantin, completely fused terminal abdominal segments IX–XI, basally fused gonapophyses IX (= 2nd valvulae), and absence of the spermathecal duct. Major innovations are the haplo-diploid reproductive system, modifications of the cephalic digestive tract (e.g. very strongly developed dorsal longitudinal muscle; Beutel and Vilhelmsen, 2007: fig. 6 [lm]), a specialized cleaning organ of the foreleg, functional dipterism (fore- and hind wings linked by hamuli) with a greatly enlarged mesothorax (and strongly reduced metathorax), and a basal articulation between the 2nd valvifer and 2nd valvulae, which allows the terebra (1st and 2nd valvulae) to be extended between the ovipositor sheaths (Vilhelmsen, 2000). Functional dipterism evolved several times in pterygote insects (e.g. the megadiverse Auchenorrhyncha and Heteroptera; Beutel et al., 2014) and within Holometabola, possibly for the first time in Hymenoptera. It is conceivable that an enhanced flight performance resulting from coupled fore- and hind wings is one factor that has contributed to the very successful radiation of the megadiverse order. However, specific advantages of

functional (or anatomical) dipterism (e.g. Brodsky, 1994) remain elusive.

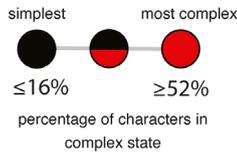
Preserved plesiomorphic features of Hymenoptera are distinctly developed labial endite lobes equipped with muscles, and a comparatively large number of Malpighian tubules (e.g. Beutel et al., 2011). The formation of a wasp waist, a key innovation of Apocrita (still indistinct in Orussidae), likely was correlated with the switch to a parasitoid lifestyle (Vilhelmsen, 2003). The increased movability of the metasoma also facilitates the use of a modified ovipositor as a stinging apparatus in Aculeata (e.g. Grimaldi and Engel, 2005). Strongly simplified immature stages of Orussidae and Apocrita, apparently also a result of parasitic habits (with external 1st and 2nd instars in orussids; Wharton et al., 2004), are likely a derived groundplan feature of this extremely diverse monophyletic lineage (e.g. Peters et al., 2017).

### Aparaglossata (*Holometabola* excl. *Hymenoptera*)

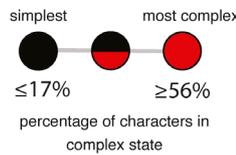
As the name suggests, Aparaglossata (Peters et al., 2014) is characterized by the reduction of the labial paraglossae and their muscles. The larval stemmata, arranged in groups of five or six in the typical case in the aparaglossatan orders (e.g. Beutel et al., 2011), are arguably an innovation. However, they are structurally simple compared to the unicorneal faceted eyes of symphytan larvae (ocularium), which still contain retinulae with isolated rhabdoms composed of eight rhabdomeres (Paulus, 1979). A major aparaglossatan apomorphy is the simplification of the lepismatoid ovipositor, with partially reduced gonocoxae and gonapophyses VIII. Another simplification is the



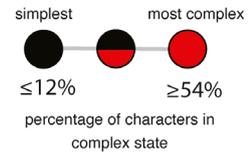
**A All Characters (264)**



**B Adult Characters (209)**



**C Larval Characters (49)**



**D Node Estimates (% complex)**

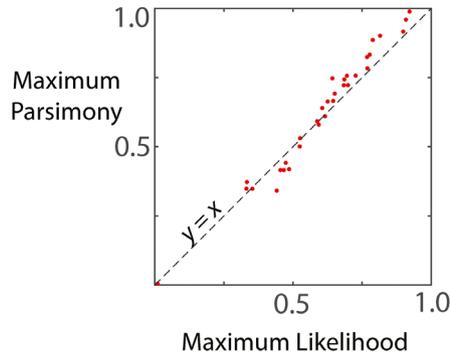


Fig. 5. Summary of node-wise character reconstructions using stochastic character mapping for the adult and larval life stages. The pie marker at the tips represent the fraction of characters that are in the “simple” (black) and “complex” (red) states, whereas the pie marker at internal nodes represent the posterior probability of each character being in the complex state, taken as a mean across all characters in the character set. The pattern for all characters (not shown) looks similar to the pattern for the adult life stages given that most characters are adult. The ranges of the pie markers are scaled to the variation in the data so that differences can be more visible. (a) All characters, (b) adult characters, (c) larval characters, and (d) reconstructions and node estimates also were performed with maximum parsimony (MP) and compared to the maximum-likelihood (ML) estimates for all characters (e.g. those depicted in (a)), and were nearly identical (Spearman’s  $\rho = 0.99$ ), indicating a lack of sensitivity to methods.

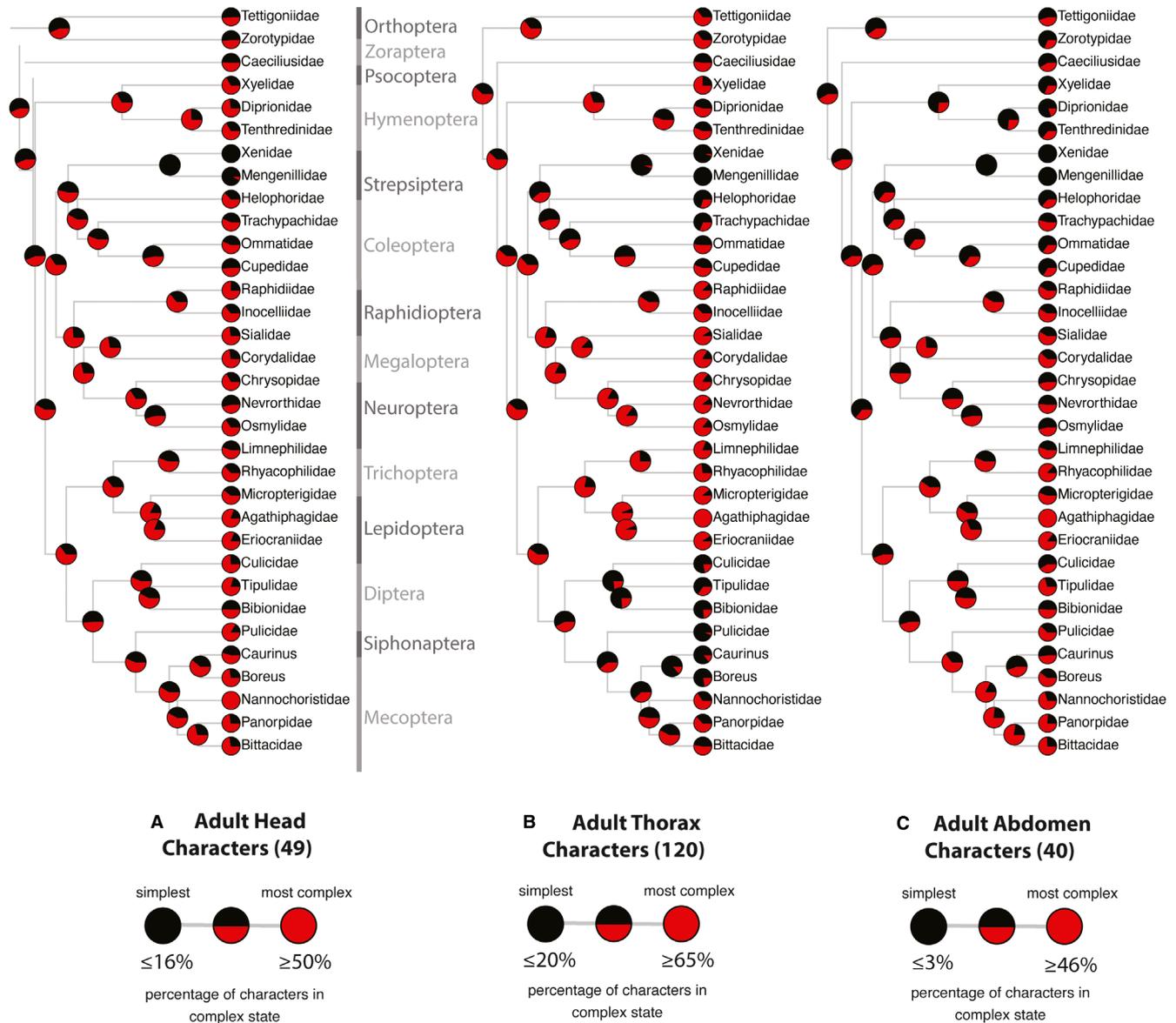


Fig. 6. Summary of node-wise character reconstructions using stochastic character mapping for the head, thorax, and abdomen of the adult life stage. The pie marker at the tips represent the fraction of characters that are in the “simple” (black) and “complex” (red) states, whereas the pie marker at internal nodes represent the posterior probability of each character being in the complex state, taken as a mean across all characters in the character set. The ranges of the pie markers are scaled to the variation in the data so that differences can be more visible. Characters of the (a) adult head, (b) adult thorax and (c) adult abdomen.

distinctly reduced number of Malpighian tubules, with a maximum number of eight in the groundplan (Neuropterida), but only six or four in most groups (Beutel et al., 2011). The adult abdomen of Aparaglossata is clearly more affected by simplification than the other two tagmata (Fig. 5a–c).

*Neuropterida* (*Raphidioptera*, *Megaloptera*, *Neuroptera* [= *Planipennia*])

With *c.* 6.300 described species (*c.* 90% in Neuroptera; Aspöck and Aspöck, 2008), the three orders

of Neuropterida belong to the smaller subunits of Holometabola. Compared with most other holometabolous orders including Hymenoptera, they have maintained a high structural complexity (Figs. 4–6). They have retained a largely unmodified configuration of head structures with biting mouthparts (e.g. Beutel et al., 2010; Randolph et al., 2013, 2014, 2017), and also approximately equally sized pterothoracic segments. With functional quadrupterism (with two uncoupled pairs of wings) the flying abilities are moderate in Neuropterida, with few exceptions (e.g. Ascalaphidae; Pfau, 2018). A long and fully functional ovipositor,

albeit with some reductions and fusions, is present in females of Raphidioptera (Achtelig, 1977). By contrast, it is greatly shortened in the other two orders (e.g. Aspöck and Aspöck, 2003). Only a short and partly membranous gonocoxite IX with a palp-like appendage is present in Megaloptera, whereas the condition is less simplified and more diverse in Neuroptera (Hünefeld et al., 2012).

Innovations of the relatively small group are the increased number of retinula cells in the larval stemmata, the presence of >5 costal cross veins in the forewing, and a trichobothria field on tergum X (e.g. Aspöck and Aspöck, 2003).

Whereas the immature stages of Raphidioptera and Megaloptera have largely preserved plesiomorphic morphological features (except for aquatic habits in the latter with adaptations like abdominal gills), larvae of the largest of the three orders, Neuroptera, display an intriguing pattern of innovations and reductions (Fig. 5b). This is mainly correlated with specialized predaceous habits and liquid feeding (e.g. Aspöck and Aspöck, 2008; Beutel et al., 2010; Jandausch et al., 2018, 2019), and possibly one reason for their increased diversity compared to Megaloptera and Raphidioptera. A unique derived feature of neuropteran larvae is the formation of combined mandibulo-maxillary sucking stylets, associated with a set of different cephalic glands. Reductions are the complete fusion of the labrum with the clypeal region, the loss of the maxillary palps and endite lobes, and the loss of different head muscles (Beutel et al., 2010, 2011). An unusual feature of the digestive tract, likely linked with liquid feeding, is the posterior closure of the midgut, a condition that evolved independently in apocritan larvae (Vilhelmsen, 2003).

### *Coleopterida*

Coleopterida, the sister group of Neuropterida (Misof et al., 2014), comprise the highly specialized Strepsiptera and the megadiverse Coleoptera (e.g. Beutel et al., 2019). This clade is almost exclusively characterized by structural simplifications (Fig. 4). In adults, this includes an undivided clypeus, loss of the salivary duct, fusion of the hypopharynx with the dorsal prelabium, antennae with eleven segments or less, a firm connection of the pronotum and propleura, absence of a mesocoxal meron, the loss of the pterothoracic katapisternal coxal joint, and considerable number of losses of thoracic muscles, mainly but not only in the distinctly smaller mesothorax (Beutel et al., 2011). A key feature of the group is posteromotorism, with the metathorax moderately or strongly (Strepsiptera) enlarged, accompanied by a distinct size reduction of the mesothorax (Beutel and Haas, 2000).

Innovations are the transformation of the forewings, either into protective elytra (autapomorphy of Coleoptera) or into gyroscopic sense organs, the halteres (autapomorphy of Strepsiptera). Similar halteres also occur in Diptera, but in their case are formed by the hind wings.

The small order Strepsiptera (*c.* 600 spp.) is a highly specialized group, in which the larvae are endoparasitic, a lifestyle found also in the females of *c.* 90% of the species (Stylopodia; Pohl and Beutel, 2008). Among Holometabola, it reaches the maximum of characters in the simplified state in the adults and larvae (Figs. 5 and 6). Even the less modified free-living males are characterized by numerous structural simplifications (Fig. 5a). This includes the loss or vestigial condition of the labrum, antennae with eight segments or less, complete reduction of the tentorium, absence of the labium and hypopharynx, extensive simplification of the maxilla, distinctly reduced prothorax, loss of the pro- and mesotrochantin, and multiple muscle losses in the thorax (Koeth et al., 2012). Skeletal and muscular features of the head of the extremely miniaturized 1st instar larvae also are distinctly simplified (e.g. absence of antenna and labrum and associated muscles; Knauthe et al., 2016), whereas the thoracic skeleto-muscular system is well-developed (Osswald et al., 2009). A noteworthy innovation is a jumping apparatus at the abdominal apex, involving well-developed muscles and bristle-like structures, that are possibly secondarily re-acquired cerci (Pohl, 2000; Beutel et al., 2011). The secondary larval stages living and feeding inside the insect host are greatly simplified structurally (Tröger et al., 2020). The free-living females of Menge-nillidae are flightless and weakly sclerotized, lack defined internal genital organs and also lack any trace of an ovipositor. The endoparasitic females of Stylopodia are extremely simplified, with a largely undivided sclerotized cephalothorax comprising head thorax and abdominal segment I, and a large sack-shaped membranous posterior body located within the host. Evolutionary innovations are the functional unit formed by the last larval exuvia and the female integument, a series of birth organs, and a birth channel releasing the tiny primary larvae (Tröger et al., 2019).

Coleoptera (beetles), by far the largest insect order in terms of described species (*c.* 380 000), are mainly characterized by a strongly armoured body, without externally exposed membranes when the elytra are closed, and invaginated terminal abdominal segments (e.g. Beutel and Haas, 2000). Probably in correlation with reduced degrees of freedom in the skeletal apparatus, especially at the coxal bases, the thoracic skeleto-muscular system is distinctly simplified, with approximately ten muscles fewer than in Neuropterida in the small suborder Archostemata (Beutel and Haas, 2000; Friedrich et al., 2009), and again approximately

ten fewer in the remaining suborders. Interestingly, the minimum number is reached in the megadiverse Polyphaga (*c.* 320 000 spp.; Larsén, 1966; Beutel and Haas, 2000), supposedly the sister group of the remaining extant beetles (McKenna et al., 2019). A separate mesokatepisternum and exposed metatrochantin are absent in all beetles except for Archostemata. Polyphaga (with very few exceptions) and Myxophaga share the fusion or rigid connection of the meso- and metaventrite as a common structural simplification, and also the fusion of the protrochantin and propleura. Finally, the propleura is completely internalized in all adult polyphagan beetles (e.g. Beutel and Haas, 2000).

Simplifications also occur in the larval stages of beetles but they are limited, especially compared to Strepsiptera (Fig. 5b). The head capsule and mouthparts are always well-developed. The larval legs are usually six-segmented in Archostemata and Adephaga, and five-segmented in Myxophaga and most groups of Polyphaga. They are more or less simplified or reduced in the extremely species-rich weevils (Curculionidae), likely related to mining in plant tissue, especially fruit. It is noteworthy that the mouthparts of beetle larvae are highly variable, by contrast with those of adults. Innovations include sucking mandibles (several times independently within Adephaga and Polyphaga), maxillolabial complexes (Elateriformia part., Cleroidea), simplified maxillae resembling ventral antennae (Adephaga excl. Gyrinidae, Hydrophiloidea, Staphylinidae partim), and highly complex filter apparatuses (Beutel and Yavorskaya, 2019).

Innovations in adult beetles are mostly linked with the elytra, for instance mesoscutellar and metanotal locking devices (e.g. Beutel and Haas, 2000). The air storage space below these sclerotized forewings is a preadaptation for an aquatic lifestyle, which was acquired in Coleoptera at least ten times independently, usually not only by the adults but also by the larvae (Beutel, 1997). Larval innovations linked with aquatic habits are mandibular sucking channels, tracheal or spiracular gills, and terminal abdominal hooks (e.g. Beutel, 1997; Beutel et al., 2019).

### *Mecoptera*

A new series of simplifications occurred with the rise of Mecoptera, which comprise the remaining five orders, together *c.* 350 000 described species (Fig. 4: four innovations vs. 12 simplifications). The immature stages have preserved distinctly fewer characters in the complex state according to the ML analyses (Fig. 7a,b). Apomorphies of the larval head are an antenna comprising three segments or less and moved by only two extrinsic muscles (implying

reversal in fleas), vestigial or absent dorsal tentorial arms, indistinctly separated or fused larval maxillary endite lobes, and a simplified musculature of the ventral mouthparts. The larval legs, if present, bear only a single claw. By contrast to Coleoptera, the mesothorax of adults is enlarged in relation to the metathorax, and few muscle reductions occur in the pterothoracic segments. The ovipositor is missing, with gonopods and gonapophyses completely reduced, and a new mode of oviposition with telescoping terminal abdominal segments (Beutel et al., 2011; Hünefeld et al., 2012).

### *Amphiesmenoptera*

Amphiesmenoptera comprise the medium-sized Trichoptera (caddisflies, *c.* 14 500 spp.) and the megadiverse Lepidoptera (moths and butterflies, *c.* 175 000 spp.). Their most obvious innovation is a dense vestiture of hairs or scales on the wings and other areas of the body surface (e.g. Kristensen and Skalsi, 1999).

Another unusual derived feature is the female heterogametic sex determination. A simplification characterizing adults and larvae is the fusion of the prelabium with the hypopharynx, associated with the innovation of an apically placed silk gland orifice in the immatures (e.g. Hasenfuss and Kristensen, 2003; Friedrich et al., 2015). Simplifications in the adult thorax (Fig. 6b) are minimal, considering the groundplan of Amphiesmenoptera. By contrast with this, several innovations occur in the female genital tract (Fig. 4c): the extrusion of the terminal unit VIII and IX by muscle force, a muscularis of the spermathecal duct formed by circular fibres, a spermathecal gland, and a bursa copulatrix connected with the genital chamber

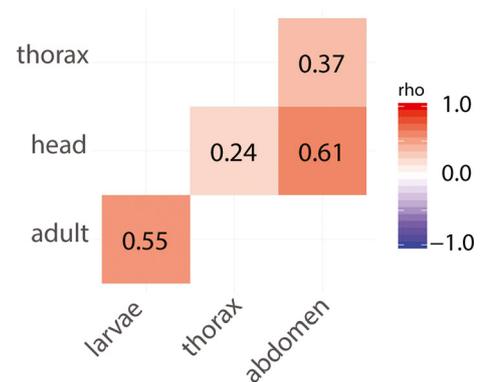


Fig. 7. Pairwise correlations between complexity of life stages and body regions. The percentage of characters in the complex state (depicted on tips of the tree in Figs 5 and 6) was positively correlated between adults and larvae, and between body regions, although correlations between the thorax and head/abdomen was weaker than between head and abdomen. Spearman rho correlations are presented, and they were all significant from Phylogenetic Generalized Least Squares regressions ( $P < 0.02$  for all).

by a distinct duct. As a whole, Amphiesmenoptera is less affected by simplification than by innovation, compared to the groundplan of Mecoptera.

Trichoptera are more affected by simplification in the adult stage than their megadiverse sister taxon. Slightly <50% of adult characters are preserved in the complex state in Lepidoptera (groundplan), vs. *c.* 40% in caddisflies according to the results of the ML analysis (Fig. 5a). Greatly reduced adult mouthparts are one conspicuous apomorphic feature, arguably correlated with the formation of a prelabial-hypopharyngeal haustellum (Klemm, 1966; Kubiak et al., 2015), which allows efficient uptake of liquid food. A major innovation of the immature stages is the aquatic lifestyle, with a wide spectrum of breathing organs and feeding habits (Malicky, 1973). Terminal abdominal hooks are a novel feature occurring in different groups with aquatic larvae (Megaloptera, Gyrinidae, Hydraenidae; e.g. Beutel et al., 2014). The larval case is a characteristic innovation, but does not belong to the groundplan of the order (Kjer et al., 2002: fig. 2).

A conspicuous innovation in Lepidoptera is the transformation of the setae of the body surface into scales, often linked with conspicuous colour patterns. Even though the shape is clearly more complex than that of hair-like setae, the loss of mechanoreceptive neurons is a simplification. Other novel features are protibial cleaning devices, pterothoracic tegular arms, and a balloon-shaped bursa copulatrix of females. Within the group, the large clade Glossata is characterized by a far-reaching reduction of the mouthparts, and the formation of a proboscis for liquid food uptake (Kristensen and Skalski, 1999). Within the order, the flight apparatus is optimized, resulting in very efficient functional dipterism, with major changes including muscle losses characterizing Heteroneura and Ditrysia, large subgroups of Glossata (Kozlov, 2012; Liu et al., 2017).

### *Antliophora*

The strongly supported clade Antliophora comprises the species-poor and relatively unspecialized Mecoptera (scorpionflies s.l., *c.* 750 spp.), the ectoparasitic flightless Siphonaptera (fleas, *c.* 2000 spp.) and the megadiverse Diptera (true flies, *c.* 154 000 spp.). It is characterized by another wave of reductions. About 30% of the adult characters and *c.* 25% of the larval features are in the complex state in the ML analysis (Fig. 5b), and the MP analysis yielded eight innovations vs. 21 simplifications. Simplifications of the adult head (Fig. 6a) are the fusion of the labrum with the head capsule, the loss of the lateral labral retractor (*M. frontoepipharyngalis*), the cranio-cardinal muscle, and all transverse muscles of the prepharynx, and a very compact mass formed by the brain and suboesophageal

complex around the pharynx, with extremely shortened circumoesophageal connectives. An entire series of muscles of the pterothorax is reduced (Fig. 3b), and several simplifications occur in the thoracic skeletal elements, for instance the absence of the propina, or the loss of the membranous zone between the mesoscutum and mesopostnotum (Beutel et al., 2011). The flight apparatus is largely unspecialized in the groundplan, and the flying abilities of mecopterans are moderate, with almost equally sized pterothoracic segments and functional quadruputerism (forewings and hind wings uncoupled; Friedrich and Beutel, 2010a,b). An innovation of the antliophoran head is the formation of a postcerebral pumping apparatus (Beutel and Baum, 2008). The MP analysis suggests that a labroepipharyngeal food channel, elongate and blade-like lacinia, and consequently liquid feeding belong to the groundplan of Antliophora. However, considering the presumably plesiomorphic condition in Mecoptera excl. Nannochoristidae (e.g. Beutel et al., 2008a), this appears unlikely. A spermathecal duct with a muscularis with longitudinal fibres is a novel feature in the female genital apparatus (Hünefeld et al., 2012).

Within Antliophora, Mecoptera has preserved the maximum number of groundplan features (e.g. Beutel et al., 2008a, 2009; Friedrich and Beutel, 2010a,b; Fabian et al., 2015). A conspicuous apomorphy is the genital capsule of males. However, this feature is not fully developed in the flightless snow fleas (Boreidae; Willmann, 1981). A major innovation that evolved within the group is the transfer of liquid sperm, with a sperm pump resembling that of fleas in the enigmatic Gondwanan relict group Nannochoristidae, and an advanced type with a pistil formed by the roof of the endophallus in Pistillifera (Mecoptera excl. Nannochoristidae and Boreidae; Mickoleit, 2009).

Like Strepsiptera, fleas reach a high degree of simplification (Fig. 5a,b), in this case with the maximum reached in the wingless thorax (Fig. 6b). The morphology is strongly affected by the parasitic habits in both groups, even though the pattern differs very distinctly. Fleas, which are ectoparasites as adults, are characterized by a strongly transformed helmet-shaped head, with largely or completely reduced compound eyes and missing ocelli, short and club-shaped 11-segmented antennae, completely reduced mandibles and an obsolete maxillary cardo (e.g. Snodgrass, 1946; Michelsen, 1996/97). Innovations are linked with blood-sucking feeding habits, such as an unpaired labral stylet and paired stylets formed by the laciniae. The thorax is characterized by the complete loss of the flight apparatus, accompanied by multiple muscle losses. A conspicuous innovation obviously linked with ectoparasitic habits are the ctenidia, regular rows of strongly developed, flattened setae. The larvae are cylindrical and completely legless like the maggots of true flies.

Another series of simplifications and reductions is linked with the rise of Diptera, one of the four megadiverse “Big Four” of Holometabola. A maximum of simplification of characters of immature stages is reached within the group, especially in Brachycera (Fig. 5b). Dipteran larvae are legless maggots (Fig. 1b) like those of fleas. The adult head of true flies is mainly characterized by piercing sucking stylets, likely formed by the labrum, mandibles, maxillae and hypopharynx in the groundplan (Hennig, 1973; Schneeberg and Beutel, 2014). However, the paired mouthparts are completely or largely reduced in the species-rich nematoceran Tipulidae, in Nymphomyiidae and Deuterophlebiidae, groups presumably very close to the root of the order, and in the vast majority of families of the megadiverse Brachycera (Schneeberg and Beutel, 2014). The thorax is mainly characterized by anatomical dipterism, with hind wings transformed into specialized gyroscopic sense organs (halteres) and a metathorax distinctly reduced in size and simplified. A series of muscles is reduced in the thoracic segments (e.g. Fabian et al., 2016) and skeletal simplifications occur, like the connection of the propleura with the anterior mesanepisternum, the fusion of the mesepimeron with the meron of the mesocoxa, and the fusion of the metepimeron with the meron of the metacoxa.

We assessed whether complexity in Holometabola is correlated among different life stages and body regions (Fig. 7), and found that complexity between larval and adult stages was highly correlated (Spearman’s  $\rho = 0.55$ ) and this correlation was significant even considering phylogenetic nonindependence (PGLS  $P$ -value  $< 0.013$ ). Likewise, the complexity of the head, thorax and abdomen were all positively correlated to some degree, but the thorax was less correlated with the head and abdomen (thorax-head:  $\rho = 0.24$ , PGLS  $P < 0.01$ , thorax-abdomen:  $\rho = 0.37$ , PGLS  $P < 0.01$ ), than the head and abdomen are with each other (head-abdomen:  $\rho = 0.61$ , PGLS  $P < 0.0004$ ).

## Discussion

Our analysis reveals both ensemble trends and individual stories in the evolution of simplicity and complexity. There is no universal pattern observed; indeed there are cases of both simplification and innovation across the tree. Overall, the head and abdomen are associated with more innovation and a rise of complexity, the thorax with trends toward simplification, although there is much biologically relevant variation among individual clades and lineages. Overall complexity is correlated positively among life stages and regions, indicating common factors driving complexity overall, although again the thorax is the least correlated. In the following sections, we interpret these

macro patterns in light of our qualitative analysis of how different character complexes, clades and ecological transitions shaped the gain and loss of characters.

### *Arthropoda and the early evolution of Hexapoda*

Among Arthropoda in the widest sense, the miniaturized Tardigrada are morphologically obviously simplified, with a five-segmented body, monocellular muscles, a completely missing circulatory system and only about 1000 cells (Gross et al., 2019). A study explicitly addressing structural complexity in spiders using the Spider Anatomy Ontology (SPD), revealed a distribution of transitions skewed towards simplification in this successful subgroup of chelicerates (Ramírez and Michalik, 2014). By contrast, no distinct trend towards structural simplification has been identified so far in Onychophora (velvet worms; e.g. de Sena Oliveira et al., 2012), non-aranean chelicerates (e.g. Dunlop and Lambdell, 2017), and Myriapoda (e.g. Edgecombe, 2004). In the crustaceans (Pancrustacea excl. Hexapoda) morphological simplifications occur in specialized parasitic forms (e.g. Castro et al., 2015), whereas increased morphological complexity in multiple parallel lineages was revealed by Adamowicz et al. (2008).

The results presented here and in earlier studies suggest that structural simplification has played an important role in Hexapoda, the extremely species-rich terrestrial subgroup of Pancrustacea (=Tetraconata; e.g. Meusemann et al., 2010). The loss of the ligamentous (mesodermal) endoskeleton in the head and thorax took place early in the evolution of the group, and also the simplification of the ventral abdominal sclerites, the reduction of the serial abdominal styli and coxal vesicles, and a distinct simplification of the cephalic muscular system (e.g. Bitsch, 1963, 1994; Matsuda, 1965; Kristensen, 1997; Beutel et al., 2014, 2017). These processes, already quite advanced in the apterygote silverfish (*Zygentoma*; e.g. ligamentous head endoskeleton missing in all groups except for *Tricholepidion*, abdominal coxosternites fused, coxal vesicles and styli reduced on most abdominal segments; e.g. Klass and Kristensen, 2001; Blanke et al., 2014), were completed in the Pterygota (e.g. Klass and Kristensen, 2001; Blanke et al., 2012). This monophyletic unit, which comprises c. 99% of all known insect species, is characterized by the complete absence of pre-genital abdominal appendages. The presence of two pairs of wings is likely to be the most important innovation in the evolutionary history of insects, greatly increasing the structural complexity (e.g. the development of the meso- and metathoracic muscle sets) and ecological versatility (e.g. Grimaldi and Engel, 2005; Beutel et al., 2017). Another major innovation of Pterygota is internal fertilization, with a postabdominal aedeagus in Ephemeroptera and Neoptera, in

many groups characterized by high variability and complexity of the involved structures (e.g. Beutel et al., 2017; Boudinot, 2018).

#### *Polyneoptera and Paraneoptera*

The structural complexity of the body is largely maintained in the hemimetabolous Polyneoptera, a head with more or less unmodified biting mouthparts (e.g. Wipfler et al., 2011, 2016), a complex thoracic skeletomuscular system (Fig. 3a: *Zorotypus hubbardi* Caudell; Friedrich and Beutel, 2008), and usually a well-developed orthopteroid ovipositor (e.g. Klass, 1998).

The monophyly of Paraneoptera, comprising Psocodea (bark lice and lice), thrips, and the megadiverse hemipteran orders, has recently been challenged in studies based on transcriptomes (Misof et al., 2014; Johnson et al., 2018). Aside from this, this successful group (c. 120 000 spp.) displays a distinct pattern of simplifications and reductions. This includes a reduced number of tarsomeres, a maximum of four Malpighian tubules (>100 in many polyneopteran groups; Beutel et al., 2014), abdominal ganglia fused in a single complex, and completely reduced cerci (e.g. Beutel et al., 2014). The transformation of the mandibles and laciniae into sucking-piercing stylets and the formation of a sheath by the labium are a major innovation, and arguably, an evolutionary breakthrough in the case of the well-founded Hemiptera, which comprise >100 000 described species (e.g. Beutel et al., 2017).

#### *Factors leading to simplification in Holometabola*

A distinct step towards further structural simplifications took place in Holometabola, partly linked with complete metamorphosis, and larval stages differing profoundly from the adults in their morphology (Fig. 1a), and often also in their microhabitats and feeding habits (e.g. Belles, 2020; Beutel et al., 2011, 2017). This profound developmental innovation, which includes a complex pattern of structural simplification in the immature stages, arguably resulted in reduced intraspecific competition. The phylogenetic pattern (Fig. 4) shows that this transformation did not trigger a major wave of diversification (e.g. Beutel et al., 2011, 2017). Nevertheless, it appears likely that this complex transformation played a role in the background, facilitating later bursts of diversification in different lineages.

Parasitism is one factor leading to far-reaching structural simplification, in the larval stages in the parasitoid groups of Hymenoptera (Orussidae [likely ectoparasitoids], Chalcidoidea etc.; e.g. Vilhelmsen, 2003), mainly in secondary larvae and females in the

endoparasitic Strepsiptera; e.g. Pohl and Beutel, 2008; Richter et al., 2017; Tröger et al., 2020; Fig. 5b), and in adults of the ectoparasitic fleas (Figs 5a and 6b). However, structural innovations also are linked with parasitism, for instance the unique jumping apparatus and attachment devices of primary larvae of Strepsiptera (Pohl, 2000; Pohl and Beutel, 2004), or the ctenidia, jumping hind legs and stylet-like mouthparts in adult fleas (e.g. Beutel et al., 2011, 2014).

Another factor linked with simplification in larval stages is burrowing in moist substrates (e.g. mud at the edges of water bodies, faeces) or plant tissues (e.g. leaves, fruit, wood). The loss of legs in Diptera is likely linked with a preference for a burrowing life-style, which also very likely led to parallel events of a far-reaching reduction of cephalic structures (e.g. Tipuloidea, major part of Brachycera; e.g. Hennig, 1973; Neugart et al., 2009; Wipfler et al., 2013). Whereas basal lineages of Diptera display a remarkable structural diversity in the larval stages (e.g. Hennig, 1968a, 1968b), Brachycera, especially Muscomorpha are characterized by a great uniformity of the maggots with a largely reduced head and a more or less worm-like body (Fig. 1b). Burrowing in parts of plants and moist substrates also has played a role in different groups of beetles. This includes for instance the wood-boring larvae of Archostemata (c. 40 spp.), jewel beetles (Buprestidae, c. 15 000 spp.) and longhorn beetles (Cerambycidae, c. 26 000 spp.), which are characterized by shortened or reduced legs and a weakly sclerotized postcephalic body, or in the highly diverse weevils (Curculionidae, c. 83 000 spp.). The fruit-mining larvae of weevils (Curculionoidea) have the legs distinctly or often completely reduced.

Other major evolutionary transformations are linked with liquid feeding of adults, leading to different patterns of linked simplifications and innovations. Biting (“orthopteroid”) mouthparts are usually preserved in Neuropterida (e.g. Beutel et al., 2010; Randolph et al., 2013, 2014) and Coleoptera (Beutel and Yavorskaya, 2019). In contrast to this, a far-reaching reduction of the paired mouthparts took place in Trichoptera and in major subgroups of Lepidoptera and Diptera. Biting mouthparts are certainly part of the groundplan of Hymenoptera (Fig. 2a; Beutel and Vilhelmsen, 2007), even though proboscises have evolved multiple times within the order (Jervis and Vilhelmsen, 2000). A very unusual feature of Hymenoptera is that many groups have biting and sucking mouthparts at the same time (L. Vilhelmsen, pers. comm.).

Reduction of the number of cephalic muscles is a major trend in Hexapoda, also occurring in different orders of Holometabola. This apparently is mainly linked with a simplification and specialization of the mouthparts (Fig. 2). Approximately 70 pairs of intrinsic head muscles are present in the silverfish

*Tricholepidion* (Blanke et al., 2014), 60 in the cockroach *Periplaneta* (Wipfler et al., 2016), 45 in the “basal” hymenopteran *Macroxyela* (Xyelidae; Beutel and Vilhelmsen, 2007), 42 in the megalopteran alderfly *Chauliodes* (Maki, 1936), 40 in the neuropteran *Osmylus* (Beutel et al., 2010), 30 or slightly less in the archostematan beetle *Tetraphalerus* (Beutel et al., 2008b) and 17 in adult males of the strepsipteran genus *Mengenilla* (Beutel and Pohl, 2006). Approximately 30 are present in the phylogenetically controversial mecopteran *Nannochorista* (Beutel and Baum, 2008), 28 in *Panorpa* (Heddergott, 1938) and 26 in the nematoceran crane fly *Tipula* (Schneeberg and Beutel, 2010). The presence of 48 pairs in the “ancestral” moth *Micropterix calthella* (Hannemann, 1956; Fig. 6a) is probably close to the groundplan of Amphiesmenoptera and Mecoptera. Forty-five are present in the trichopteran *Philopotamus* (Kubiak et al., 2015), and only *c.* 34 in Eriocraniidae (Kristensen, 1968).

Another factor leading to far-reaching reductions is a short adult lifespan. This can lead to simplified mouthparts (Fig. 2b), and to an inflated gut not suitable for food processing in the case of males of Strepsiptera (Beutel and Pohl, 2006). As a rare exception in beetles, the mouthparts of short-lived adults of some rhipiphorid beetles are completely reduced (Beutel and Yavorskaya, 2019). A similar condition is present in the “ancestral” dipteran Nymphomyiidae (Fig. 2b; Tokunaga, 1935) and Deuterophlebiidae, where mouthparts are completely missing (Schneeberg et al., 2011). The cephalic musculature reaches a peak of reduction in the latter group: only eight pairs of muscles are preserved, five of them belonging to the antenna.

The optimization of the thoracic locomotor apparatus of adults (Figs 3 and 5b) plays a major role in Holometabola, with functional dipterism in Hymenoptera, Coleoptera and “higher Lepidoptera”, and anatomical dipterism in Strepsiptera and true flies (Fig. 3b; e.g. Beutel et al., 2011, 2017; Liu et al., 2017). These evolutionary processes were accompanied by some modifications of the skeletal elements, but especially by a distinct simplification of the muscular system (Fig. 3). Whereas the complexity of the prothoracic muscle system, crucial for movements of the head and fore legs, was not distinctly affected (e.g. Friedrich and Beutel, 2008, 2010b), a trend towards reduction is clearly present in the pterothorax of Holometabola. Seventy-six pterothoracic muscles (excl. intrinsic leg muscles) were identified in the relatively unspecialized megalopteran fish fly *Chauliodes* (Maki, 1936), 59 in the archostematan beetle *Priacma serrata* (Baehr, 1975) and only 23 in the diving beetle *Dytiscus* (Dytiscidae; Larsén, 1966; Beutel and Haas, 2000). Remarkably, the functional metathoracic flight apparatus of

the whirligig beetle *Orectochilus villosus* comprises only nine muscles (Liu et al., 2017). Fifty-two pterothoracic pairs are present in *Nannochorista* (Friedrich and Beutel, 2010a), but only *c.* 36 in *Drosophila* (some of them divided into subunits; Fig. 3b; Fabian et al., 2016). Not surprisingly, the pterothoracic musculature can be strongly reduced in flightless forms, with 24 pterothoracic muscles preserved in males of the boreid *Caurinus* and only 18 in the females (Beutel and Friedrich, 2019). Only ten pairs are present in workers of the ant species *Myrmecia nigrocincta* Smith, excluding intrinsic leg muscles and muscles of the propodeum (Liu et al., 2019).

An element of the abdomen subject to simplification and finally complete reduction is the lepidematoid ovipositor. It is simplified in Neuropterida, especially in Megaloptera and Neuroptera, largely reduced in Coleoptera, and completely absent in Strepsiptera (together with the internal female genital organs) and Antliophora (Hünefeld et al., 2012). A major innovation of the male abdominal genital apparatus is transmission of liquid sperm, independently achieved in Strepsiptera, Mecoptera, fleas and within Diptera (Hünefeld and Beutel, 2005). Complex sperm pumps have evolved several times independently in Antliophora (Hünefeld and Beutel, 2005; Mickoleit, 2009). By contrast with simplifications in different body regions including the female abdomen, an accelerated evolution of the male genitalia apparently took place within Holometabola (Boudinot, 2018), leading to an unparalleled diversity between and within the orders.

#### *Simplification and innovation*

A formal assessment of simplification and innovation (or increased complexity) is impeded by the fact that an insect body is not a “construction kit” with well-defined and delimited subelements, but rather a continuum of sclerites, semimembranous areas, membranes, apodemes, complicated articulations, and muscles that often shift their origin. It is important in this context that both phenomena are often correlated, as in the case of the switch from four-winged flight (e.g. Neuropterida, Mecoptera) to functional (Hymenoptera, Coleoptera, “higher” Lepidoptera) or anatomical dipterism (Strepsiptera, Diptera). This evolutionary transformation has occurred multiple times in insects. It is always accompanied by a reduced size of either the meso- (e.g. Coleoptera) or metathorax (e.g. Diptera), and usually also simplified muscle equipment in the same segment (Friedrich and Beutel, 2010b). However, specialized structures also can be formed in the evolutionary process, for instance hamuli connecting the fore- and hind wings, halteres as gyroscopic sense organs or sclerotized

elytra protecting the upper side of the body (Friedrich and Beutel, 2010b; Beutel et al., 2011). The formation of mandibular and maxillary stylets in Diptera (and Hemiptera) can be interpreted as a simplification of these paired mouthparts, but also as an innovation, involving the formation of thin channels for transportation of liquid food and saliva. The complete reduction of paired stylets in the majority of brachyceran groups is accompanied by the formation of complex premental labellae equipped with pseudotracheae, suitable for the uptake of liquids from surfaces (e.g. Schneeberg and Beutel, 2014). Similarly, the meso- (Strepsiptera) or metathoracic (Diptera) halteres can be seen as partially reduced wings not contributing propelling forces during flight, but also as specific and complex gyroscopic sense organs and structures stabilizing flight with their rotating movements. The stepwise reduction of the primary ovipositor is compensated for by a telescoping postabdomen (Hünefeld et al., 2012), suitable for the deposition of eggs in moist substrates.

The genetic mechanisms of reductions (e.g. loss of appendages or muscles) or the formation of novel structures (e.g. sucking stylets) are not known yet. However, the presently available data strongly suggest that a secondary regain of reduced structures is extremely rare. Ridges dividing thoracic sclerites or thoracic muscles once reduced in polyphagan beetles, do not re-appear in any family of the megadiverse suborder as far as known at present (Larsén, 1966; Beutel and Haas, 2000; Friedrich et al., 2009). The lepismatoid ovipositor does not resurface in any of the orders of Mecoptera (Beutel et al., 2011; Hünefeld et al., 2012), including the megadiverse Lepidoptera and Diptera.

#### *Limitations and future directions*

We attempted a first order of analysis of simplicity and complexity, even though there are a number of points for improvement. First, the definition used here mostly in terms of presence and absence of various parts, is a reasonable starting point but not entirely sufficient to capture complexity. A more holistic approach would be to consider the functioning of different systems, and the relationships of different parts as they work together to result in relevant phenotypes. We attempted this qualitatively to some degree by discussing system-level changes, yet more could be done quantitatively by modelling interactions between parts. For example, a more narrowly focused study could build explicit understanding of how the muscles of the thorax operate to facilitate locomotion, and then how those systems have evolved over time to gain and lose complexity. Toward that end, more sophisticated definitions of complexity exist (Mitchell, 2009) and could

be explored in an evolutionary framework, and perhaps take advantage of more complicated statistical models of multivariate character evolution that continue to improve (e.g. Adams and Collyer, 2019).

#### **Conclusions**

Understanding trends in organismal complexity, and how those trends relate to diversification, are major goals of evolutionary science. Our analyses both provide a macroscale view of how innovation and simplification have shaped the evolution of the megadiverse Holometabola, and a number of specific examples for different characters and taxa.

By contrast with other groups of Arthropoda, a distinct trend towards simplification obviously has played a role in the extremely species-rich Hexapoda, especially in the largest subgroups Paraneoptera and Holometabola. A complex meshwork of structural simplifications and innovations has played a major role in the evolution of the group, with different degrees of positive correlation between structural complexity in adults and larvae, and also between different adult body regions. Several waves of simplification occurred, with a series of reductional features in the groundplan of the entire lineage (especially larvae), Aparaglossata (Holometabola excl. Hymenoptera), Coleoptera (beetles and Strepsiptera), Mecoptera and Antliophora. This trend has reached a peak in parasitic groups, in groups with a very short adult lifespan (e.g. reduction of mouthparts) and in the larvae of the megadiverse dipteran Brachycera, which are usually specialized on mining in moist substrates. In many cases, structural simplifications and innovations are linked in a specific functional context, for instance the formation of mouthparts specialized for the uptake of liquids, or the optimization of the flight apparatus, showing how both the gain and loss of complexity can facilitate the exploration of new ecological niches.

#### **Acknowledgements**

The research stay of R.G.B. at the Biodiversity and Biocomplexity unit (host E.P.E.) of the Okinawa Institute of Science and Technology (OIST) was made possible by generous financial support from this institution. This is gratefully acknowledged. E.P.E. also acknowledges subsidy support to OIST for supporting this research. We are very grateful for detailed and constructive reviews provided by Seraina Klopffstein (Naturhistorisches Museum Basel) and Lars Vilhelmsen (Zoologisk Museum, Copenhagen). Numerous comments made by an anonymous reviewer also helped to improve the manuscript. We are also

grateful for stimulating discussions with B. Boudinot and H. Pohl (Entomology Group, Institut für Zoologie und Evolutionsforschung, FSU Jena) and valuable suggestions made by both. We also thank Benjamin Fabian (MPICE, Jena) for providing original drawings of the thorax of *Drosophila*.

### Conflict of interest

None declared.

### References

- Achtelig, M., 1977. Skelet und Muskulatur des Abdomens weiblicher Raphidioptera (Insecta, Neuropteroidea). Zool. Jahrb. Abt. Anat. Ontog. Tiere 98, 137–167.
- Adamowicz, S.J., Purvis, A. and Wills, M.A., 2008. Increasing morphological complexity in multiple parallel lineages of the Crustacea. Proc. Nat. Acad. Sci. U.S.A. 105, 4786–4791.
- Adams, D.C. and Collyer, M., 2019. Phylogenetic comparative methods and the evolution of multivariate phenotypes. Ann. Rev. Ecol. Evol. Syst. 50, 1–21.
- Aguado, M.T., Capa, M., Oceguera-Figueroa, A. and Rouse, G.W., 2014. Annelids. In: Vargas, P. and Zardoya, R. (Eds.), The Tree of Life. Sinauer, Sunderland, Massachusetts, pp. 254–269.
- Akaike, H., 1973. Information theory as an extension of the maximum likelihood principle. In: Petrov, B.N. and Csaki, F. (Eds.), Second International Symposium on Information Theory. Akademiai Kiado, Budapest, pp. 267–228.
- Antunes-Carvalho, C., Yavorskaya, M., Gnaspini, P., Ribera, I., Hammel, J.U. and Beutel, R.G., 2017. Cephalic anatomy and three-dimensional reconstruction of the head of *Catops ventricosus* (Weise, 1877) (Coleoptera: Leioididae: Cholevinae). Org. Div. Evol. 17, 199–212.
- Aspöck, U. and Aspöck, H., 2003. 28. Ordnung Raphidioptera, Kamelhalsfliegen. 29. Ordnung Megaloptera, Großflügler, Schlammfliegen. 30. Ordnung Neuroptera (Planipennia), Netzflügler. In: Dathe, H.H. (Ed.), 5. Teil: Insecta. In: Lehrbuch der Speziellen Zoologie. Begründet von A. Kaestner, 2nd ed. Spektrum Akademischer Verlag, Berlin, pp. 542–584.
- Aspöck, U. and Aspöck, H., 2008. Phylogenetic relevance of the genital sclerites of Neuropterida (Insecta: Holometabola). Syst. Entomol. 33, 97–127.
- Baehr, M., 1975. Skelett und Muskulatur des Thorax von *Priacma serrata* Leconte (Coleoptera, Cupedidae). Z. Morph. Tiere 81, 55–101.
- Belles, X., 2020. Insect Metamorphosis. From Natural History to Regulation of Development and Evolution. Academic Press, London, UK.
- Beutel, R.G., 1997. Über Phylogenese und Evolution der Coleoptera (Insecta), insbesondere der Adephaga. Verhandl. Naturwiss. Ver. Hamburg NF 31, 1–164.
- Beutel, R.G. and Baum, E., 2008. A longstanding entomological problem finally solved? Head morphology of *Nanmochorista* (Mecoptera, Insecta) and possible phylogenetic implications. J. Zool. Syst. Evol. Res. 46, 346–367.
- Beutel, R.G. and Friedrich, F. (Eds.), 2019. Nannomecoptera and Neomecoptera. Handbook of Zoology. Walter de Gruyter, Berlin.
- Beutel, R.G. and Haas, F., 2000. Phylogenetic relationships of the suborders of Coleoptera (Insecta). Cladistics 16, 103–141.
- Beutel, R.G. & Leschen, R. (Eds.), 2016. Coleoptera, Beetles: Morphology and Systematics (Archostemata, Adephaga, Myxophaga, Polyphaga partim), Vol. 1 (2nd ed.). Walter de Gruyter, Berlin.
- Beutel, R.G. and Pohl, H., 2006. Head structures of males of Strepsiptera (Hexapoda) with emphasis on basal splitting events within the order. J. Morph. 267, 536–554.
- Beutel, R.G. and Vilhelmsen, L., 2007. Head anatomy of Xyelidae (Hexapoda: Hymenoptera) and phylogenetic implications. Org. Div. Evol. 7, 207–230.
- Beutel, R.G. & Yavorskaya, M. (2019). Structure and evolution of mouthparts in Coleoptera. In: Krenn, H. (Ed.), Insect Mouthparts. Zoological Monographs, Vol. 5. Springer, Cham, pp. 387–418.
- Beutel, R.G., Friedrich, F. and Aspöck, U., 2010. The larval head of Nevrothidae and the phylogeny of Neuroptera (Insecta). Zool. J. Linn. Soc. 158, 533–562.
- Beutel, R.G., Friedrich, F., Ge, S.-Q. and Yang, X.K., 2014. Insect Morphology and Phylogeny. A Textbook for Students of Entomology. De Gruyter, Berlin.
- Beutel, R.G., Friedrich, F., Hörschemeyer, T., Pohl, H., Hünefeld, F., Beckmann, F., Meier, R., Misof, B., Whiting, M.F. and Vilhelmsen, L., 2011. Morphological and molecular evidence converging upon a robust phylogeny of the megadiverse Holometabola. Cladistics 27, 341–355.
- Beutel, R.G., Friedrich, F. and Whiting, M.F., 2008a. Head morphology of *Caurinus* (Boreida, Mecoptera) and its phylogenetic implications. Arthr. Str. Dev. 37, 418–433.
- Beutel, R.G., Ge, S.-Q. and Hörschemeyer, T., 2008b. On the head morphology *Tetraphalerus*, the phylogeny of Archostemata and the basal branching events in Coleoptera. Cladistics 23, 1–29.
- Beutel, R.G., Kristensen, N.P. and Pohl, H., 2009. Resolving insect phylogeny: the significance of cephalic structures of the Nannomecoptera in understanding endopterygote relationships. Arthr. Str. Dev. 38, 427–460.
- Beutel, R.G., Pohl, H., Yan, E.V., Anton, E., Liu, S.P., Ślipiński, A., McKenna, D. and Friedrich, F., 2019. The phylogeny of Coleopterida (Hexapoda) – morphological characters and molecular phylogenies. Syst. Entomol. 44, 75–102.
- Beutel, R.G., Yavorskaya, M.I., Mashimo, Y., Fukui, M. and Meusemann, K., 2017. The phylogeny of Hexapoda (Arthropoda) and the evolution of megadiversity. Proc. Arthropod. Embryol. Soc. Jpn. 51, 1–15.
- Beutel, R.G., Zimmermann, D., Krauß, M., Randolph, S. and Wipfler, B., 2010. Head morphology of *Osmylus fulvicephalus* (Osmylidae, Neuroptera) and its phylogenetic implications. Org. Div. Evol. 10, 311–329.
- Bitsch, J., 1963. Morphologie céphalique des machilides (Insecta Thysanura). Ann. Sci. Nat. Zool. Biol. Animale 12, 585–706.
- Bitsch, J., 1994. The morphological groundplan of Hexapoda: critical review of recent concepts. Ann. Soc. Entomol. France 30, 103–129.
- Blanke, A., Koch, M., Wipfler, B., Wilde, F. and Misof, B., 2014. Head morphology of *Tricholepidion gertschi* indicates monophyletic Zygentoma. Front. Zool. 11, 16.
- Blanke, A., Machida, R., Szucsich, N.U., Wilde, F. and Misof, B., 2015. Mandibles with two joints evolved much earlier in the history of insects: dicondylly is a synapomorphy of bristletails, silverfish and winged insects. Syst. Entomol. 40, 357–364.
- Blanke, A., Wipfler, B., Letsch, H., Koch, M., Beckmann, F., Beutel, R. and Misof, B., 2012. Revival of Palaeoptera – head characters support a monophyletic origin of Odonata and Ephemeroptera (Insecta). Cladistics 28, 560–581.
- Bollback, J.P., 2006. SIMMAP: stochastic character mapping of discrete traits on phylogenies. BMC Bioinform. 7, 88.
- Bopp, F., 1842. Vergleichende Grammatik des Sanskrit, Zend, Griechischen, Lateinischen, Litthauischen, Gothischen und Deutschen, Vol. 1. F. Dümmler, Berlin.
- Boudinot, B.E., 2018. A general theory of genital homologies for the Hexapoda (Pan crustacea) derived from skeletomuscular correspondences, with emphasis on the Endopterygota. Arthr. Struct. Dev. 47, 563–613.
- Brodsky, A.K., 1994. The Evolution of Insect Flight. Oxford University Press, Oxford, UK.

- Brown, W.L., 1965. Numerical taxonomy, convergence, and evolutionary reduction. *Syst. Zool.* 14, 101–109.
- Castro, P., Davie, P., Guinot, D., Schram, F. and von Vaupel Klein, C.A. (Eds.), 2015. *Treatise on Zoology-Anatomy, Taxonomy, Biology. The Crustacea*. Brill, Leiden, The Netherlands.
- Crowson, R.A., 1955. The natural classification of the families of coleoptera. Nathaniel Lloyd and Co., LTD., London, UK.
- Darwin, C., 1859. *The Origin of Species by Means of Natural Selection, or, the Preservation of Favoured Races in the Struggle for Life*. J. Murray, London.
- Dunlop, J.A. and Lamsdell, J.C., 2017. Segmentation and tagmosis in Chelicerata. *Arthr. Struct. Dev.* 46, 395–418.
- Eames, A.J., 1936. *Morphology of Vascular Plants. Lower Groups*. McGraw-Hill, New York, NY.
- Edgecombe, G.D., 2004. Morphological data, extant Myriapoda, and the myriapod stem-group. *Contr. Zool.* 73, 207–252.
- Fabian, B., Russell, L., Friedrich, F. and Beutel, R.G., 2015. The morphology of the larval head of the enigmatic boreid *Caurinus dectes* (Mecoptera). *Arthr. Struct. Dev.* 73, 385–399.
- Fabian, B., Schneeberg, K. and Beutel, R.G., 2016. Comparative thoracic anatomy of the wild type and wingless (*wg1cn1*) mutant of *Drosophila melanogaster* (Diptera). *Arthr. Struct. Dev.* 45, 611–636.
- Friedrich, F. and Beutel, R.G., 2008. The thorax of *Zorotypus* (Hexapoda, Zoraptera) and a new nomenclature for the musculature of Neoptera. *Arthr. Struct. Dev.* 37, 29–54.
- Friedrich, F. and Beutel, R.G., 2010a. The thoracic morphology of *Nannochorista* (Nannochoristidae) and its implications for the phylogeny of Mecoptera and Antliophora. *J. Zool. Syst. Evol. Res.* 48, 50–74.
- Friedrich, F. and Beutel, R.G., 2010b. Goodbye Halteria? The thoracic morphology of Endopterygota (Insecta) and its phylogenetic implications. *Cladistics* 26, 579–612.
- Friedrich, F., Farrell, B.D. and Beutel, R.G., 2009. The thoracic morphology of Archostemata and the relationships of the extant suborders of Coleoptera (Hexapoda). *Cladistics* 24, 1–37.
- Friedrich, F., Schulz, J., Kubiak, M., Beckmann, F. and Wilde, F., 2015. The larval head anatomy of *Rhyacophila* (Rhyacophilidae) with discussion on mouthpart homology and the groundplan of Trichoptera. *J. Morph.* 276, 1505–1524.
- Giribet, G., 2003. Molecules, development and fossils in the study of metazoan evolution; Articulata versus Ecdysozoa revisited. *Zoology* 106, 303–326.
- Grafen, A., 1989. The phylogenetic regression. *Phil. Trans. R. Soc. B* 326, 119–157.
- Grimaldi, D.A. and Engel, M.S., 2005. *Evolution of the Insects*. Cambridge University Press, New York, New York.
- Gross, V., Treffkorn, S., Reichelt, J., Epple, L., Lüter, C. and Mayer, G., 2019. Miniaturization of tardigrades (water bears): morphological and genomic perspectives. *Arthr. Struct. Dev.* 48, 12–19.
- Hannemann, H.J., 1956. Die Kopfmuskulatur von *Micropteryx calthella* (L.) (Lep.). *Zool. Jb. Anat.* 75, 177–206.
- Hasenfuss, I. & Kristensen, N.P., 2003. Skeleton and muscles: immatures. In: Kristensen, N.P. (Ed.), *Lepidoptera, Moths and Butterflies*. Vol. 2: Morphology, Physiology, and Development. *Handbook of Zoology. Vol. IV Arthropoda: Insecta*. Part 36. Walter de Gruyter, Berlin, pp. 133–164.
- Heddergott, H., 1938. Kopf und Vorderdarm von *Panorpa communis* L. *Zool. Jb. Anat.* 65, 229–294.
- Hennig, W., 1968a. *Die Larvenformen der Dipteren*. 1. Teil. Akademie-Verlag, Berlin.
- Hennig, W., 1968b. *Die Larvenformen der Diptera*. 2. Teil. Akademie-Verlag, Berlin.
- Hennig, W., 1973. *Diptera (Zweiflügler)*. *Handbuch der Zoologie IV, Insecta*. Lieferung 20. Walter de Gruyter, Berlin.
- Hünefeld, F. and Beutel, R.G., 2005. The sperm pumps of Strepsiptera and Antliophora (Hexapoda). *J. Zool. Syst. Evol. Res.* 43, 297–306.
- Hünefeld, F., Mißbach, C. and Beutel, R.G., 2012. The morphology and evolution of the female postabdomen of Holometabola (Insecta). *Arthr. Str. Dev.* 41, 361–371.
- Jandausch, K., Beutel, R.G. and Bellstedt, R., 2019. The larval morphology of the spongefly *Sisyra nigra* (Retzius, 1783) (Neuroptera: Sisyridae). *J. Morph.* 280, 1742–1758.
- Jandausch, K., Pohl, H., Aspöck, U., Winterton, S.L. and Beutel, R.G., 2018. Morphology of the primary larva of *Mantispa aphavexelte* Aspöck and Aspöck, 1994 (Neuroptera: Mantispidae) and phylogenetic implications to the order of Neuroptera. *Arthr. Struct. Dev.* 76, 529–560.
- Jervis, M. and Vilhelmsen, L., 2000. Mouthpart evolution in adults of the basal, 'symphytan', hymenopteran lineages. *Biol. J. Linn. Soc.* 70, 121–146.
- Johnson, K.P., Dietrich, C.H., Friedrich, F., Beutel, R.G., Wipfler, B., Peters, R.S., Allen, J.M., Petersen, M., Donath, A., Walden, K.K.O. et al., 2018. Phylogenomics and the evolution of hemipteroid insects. *Proc. Nat. Acad. Sci. U.S.A.* 115, 12775–12780.
- Kjer, K.M., Blahnik, R.J. and Holzenthal, R.W., 2002. Phylogeny of caddisflies (Insecta, Trichoptera). *Zool. Scr.* 31, 83–91.
- Klass, K.D., 1998. The ovipositor of Dictyoptera (Insecta): homology and ground-plan of the main elements. *Zool. Anz.* 236, 69–101.
- Klass, K.D. and Kristensen, N.P., 2001. The ground plan and affinities of hexapods: recent progress and open problems. *Ann. Soc. Entomol. France* 37, 265–298.
- Klemm, N., 1966. Die Morphologie des Kopfes von *Rhyacophila* Pict. (Trichoptera). Darmtraktus von *Panorpa communis* L. und seine Anhänge bei Larve und Imago. *Zool. Jb. (Anat.)* 83, 1–53.
- Klopfstein, S., Vilhelmsen, L. and Ronquist, F., 2015. A nonstationary Markov model detects directional evolution in hymenopteran morphology. *Syst. Biol.* 64, 1089–1103.
- Knauth, P., Beutel, R.G., Hörnschemeyer, T. and Pohl, H., 2016. Serial block-face scanning electron microscopy sheds new light on the head anatomy of an extremely miniaturized insect larva (Insecta, Strepsiptera). *Arthr. Syst. Phyl.* 74, 107–126.
- Koeth, M., Friedrich, F., Pohl, H. and Beutel, R.G., 2012. The thoracic skeleto-muscular system of *Mengenilla* (Strepsiptera: Mengenillidae) and its phylogenetic implications. *Arthr. Struct. Dev.* 41, 323–335.
- Kozlov, M.V., 2012. Ground plan and evolution of pterothoracic musculature of moths and butterflies (Lepidoptera). *Entomol. Rev.* 92, 162–177.
- Kristensen, N.P., 1968. The anatomy of the head and the alimentary canal of adult Eriocraniidae (Lep., Dacnonypha). *Entomol. Medd.* 35, 239–315.
- Kristensen, N.P., 1997. 21. The groundplan and basal diversification of the hexapods. In: Fortey, R.A., Thomas, R.H. and John, D.M. (Eds.), *Arthropod Relationships*. Chapman and Hall/Springer, London, UK, pp. 281–293.
- Kristensen, N.P. & Skalsi, A.W., 1999. Phylogeny and paleontology. In: Kristensen, N.P. (Ed.), *Lepidoptera, Moths and Butterflies*. Vol. 1: Evolution, Systematics and Biogeography. *Handbook of Zoology. Vol. IV Arthropoda: Insecta*. Part 35. Walter de Gruyter, Berlin, pp. 7–25.
- Kubiak, M., Beckmann, F. and Friedrich, F., 2015. The adult head of the annulipalpan caddisfly *Philopotamus ludificatus* McLachlan, 1878 (Philopotamidae), mouthpart homologies, and implications on the ground plan of Trichoptera. *Arthr. Syst. Phyl.* 73, 351–384.
- Kusters, W., 2000. Morphological simplification: More than erosion? *St. Slav. Gen. Ling.* 28, 225–230.
- Larsén, O., 1966. On the morphology and function of locomotor organs of the Gyrinidae and other Coleoptera. *Opusc. Entomol.* 30(Suppl.), 1–241.
- Lawrence, J.F., Ślipiński, A., Seago, A.E., Thayer, M.K., Newton, A.F. and Marvaldi, A.E., 2011. Phylogeny of the Coleoptera based on morphological characters of adults and larvae. *Ann. Zool.* 61, 1–217.
- Leschen, R. & Beutel, R.G. (Eds.), 2014. *Coleoptera, Beetles: Morphology and Systematics (Phytophaga)*, Vol. 3. Walter de Gruyter, Berlin.

- Lewis, P.O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Syst. Biol.* 50, 913–925.
- Liu, S.P., Richter, A., Stoessel, A. and Beutel, R.G., 2019. The mesosomal anatomy of *Myrmecia nigrocincta* workers and evolutionary transformations in Formicidae (Hymenoptera). *Arthr. Syst. Phyl.* 77, 1–19.
- Liu, S.P., Wipfler, B., Niitsu, S. and Beutel, R.G., 2017. The thoracic anatomy of the male and female winter moth *Nyssiodes lefuarius* (Lepidoptera: Geometridae) and evolutionary changes in the thorax of moths and butterflies. *Org. Div. Evol.* 17, 565–594.
- Maddison, W.P. and Maddison, D.R., 2018. Mesquite: a modular system for evolutionary analysis. Version 3.51. <http://mesquiteproject.org>.
- Maki, T., 1936. Studies on the skeletal structure, musculature and nervous system of the Alder fly *Chauliodes formosanus* Petersen. *Mem. Fac. Sci. Agric. Taihoku Imp. Univ.* 16, 117–243 + errata, 10 pls.
- Malicky, H., 1973. Trichoptera (Köcherfliegen). *Handbuch der Zoologie IV, Insecta. Inst.* 29. Walter de Gruyter, Berlin, pp. 1–114.
- Matsuda, R., 1965. Morphology and evolution of the insect head. *Mem. Am. Entomol. Inst.* 4, 1–240.
- Matsumura, Y., Wipfler, B., Pohl, H., Dallai, R., Machida, R., Mashimo, Y., Cámera, J.T., Rafael, J. and Beutel, R.G., 2015. Cephalic anatomy of *Zorotypus weidneri* New, 1978: new evidence for a placement of Zoraptera. *Arthr. Syst. Phyl.* 73, 85–105.
- McKenna, D.D., Shin, S., Ahrens, D., Balke, M., Beza-Beza, C., Clarke, D.J., Donath, A., Escalona, H.E., Friedrich, F., Letsch, H. et al., 2019. The evolution and genomic basis of beetle diversity. *Proc. Nat. Acad. Sci. U.S.A.* 116, 24729–24737.
- Meusemann, K., von Reumont, B.M., Simon, S., Roeding, F., Strauss, S., Kuck, P., Ebersberger, I., Walz, M., Pass, G., Breuers, S. et al., 2010. A phylogenomic approach to resolve the arthropod tree of life. *Mol. Biol. Evol.* 27, 2451–2464.
- Michelsen, V., 1996/97. A revised interpretation of the mouthparts in adult fleas (Insecta, Siphonaptera). *Zool. Anz.* 235, 217–223.
- Mickleit, G., 2004. *Phylogenetische Systematik der Wirbeltiere*. Pfeil, München.
- Mickleit, G., 2009. Die Sperma-Auspreßvorrichtung der Nannocharistidae (Insecta: Mecoptera). *Entomol. Gen.* 31, 193–226.
- Misof, B., Liu, S., Meusemann, K., Peters, R.S., Donath, A., Mayer, C., Frandsen, P.B., Ware, J., Flouri, T., Beutel, R.G. et al., 2014. Phylogenomics resolves the timing and pattern of insect evolution. *Science* 346, 763–767.
- Mitchell, M., 2009. *Complexity: A Guided Tour*. Oxford University Press, New York, New York, pp. 94–111.
- Neugart, C., Schneeberg, K. and Beutel, R.G., 2009. The morphology of the larval head of Tipulidae (Diptera, Insecta) – the dipteran groundplan and evolutionary trends. *Zool. Anz.* 248, 213–235.
- Niehuis, O., Hartig, G., Grath, S., Pohl, H., Lehmann, J., Tafer, H., Donath, A., Krauss, V., Eisenhardt, C., Hertel, J. et al., 2012. Genomic and morphological evidence converge to resolve the enigma of Strepsiptera. *Curr. Biol.* 22, 1309–1313.
- O'Malley, M.A., Wideman, J.G. and Ruiz-Trillo, I., 2016. Losing complexity: the role of simplification in macroevolution. *Trends Ecol. Evol.* 31, 608–621.
- Osswald, J., Pohl, H. and Beutel, R.G., 2009. Extremely miniaturised and highly complex: the thoracic morphology of the first instar larva of *Mengenilla chobauti* (Insecta, Strepsiptera). *Arthr. Str. Dev.* 39, 287–304.
- Pagel, M., 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc. R. Soc. B* 225, 37–45.
- Paradis, E. and Schliep, K., 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinform* 35, 526–528.
- Paulus, H.F., 1979. Eye structure and the monophyly of the Arthropoda. In: Gupta, A.P. (Ed.), *Arthropod Phylogeny*. Van Nostrand Reinhold Co., New York, New York, pp. 299–384.
- Pennell, M., Eastman, J., Slater, G., Brown, J., Uyeda, J., Fitzjohn, R., Alfaro, M. and Harmon, L., 2014. geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics* 30, 2216–2218.
- Peters, R.S., Krogmann, L., Mayer, C., Donath, A., Gunkel, S., Meusemann, K., Kozlov, A., Podsiadlowski, L., Petersen, M., Lanfear, R. et al., 2017. Evolutionary history of the Hymenoptera. *Curr. Biol.* 27, 1013–1018.
- Peters, R.S., Meusemann, K., Petersen, M., Wilbrandt, J., Ziesmann, J., Donath, A., Mayer, C., Kjer, K.M., Aspöck, U., Aspöck, H. et al., 2014. The evolutionary history of holometabolous insects inferred from transcriptome-based phylogeny and comprehensive morphological data. *BMC Evol. Biol.* 14, 52.
- Pfau, H.K., 2018. Functional morphology and evolution of the flight apparatus of *Libelloides* (Neuroptera: Ascalaphidae). *Entomol. Gen.* 37, 317–352.
- Pohl, H., 2000. Die Primärlarven der Fächerflügler – evolutive Trends (Insecta, Strepsiptera). *Kaupia* 10, 1–144.
- Pohl, H. and Beutel, R.G., 2004. Fine structure of adhesive devices of Strepsiptera (Insecta). *Arthr. Str. Dev.* 33, 31–43.
- Pohl, H. and Beutel, R.G., 2008. The evolution of Strepsiptera. *Zoology* 111, 318–338.
- Ramírez, M.J. and Michalik, P., 2014. Calculating structural complexity in phylogenies using ancestral ontologies. *Cladistics* 30, 635–649.
- Randolf, S., Zimmermann, D. and Aspöck, U., 2013. Head anatomy of adult *Sisyra terminalis* (Insecta: Neuroptera: Sisyridae) – functional adaptations and phylogenetic implications. *Arthr. Str. Dev.* 42, 565–582.
- Randolf, S., Zimmermann, D. and Aspöck, U., 2014. Head anatomy of adult *Nevrorthus apatelios* and basal splitting events in Neuroptera (Neuroptera: Nevrothidae). *Arthr. Syst. Phyl.* 72, 111–136.
- Randolf, S., Zimmermann, D. and Aspöck, U., 2017. Head anatomy of adult *Coniopteryx pygmaea*: effects of miniaturization and the systematic position of Coniopterygidae (Insecta: Neuroptera). *Arthr. Struct. Dev.* 46, 304–322.
- Revell, L.J., 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Meth. Ecol. Evol.* 3, 217–223.
- Richter, A., Hita Garcia, F., Keller, R.A., Billen, J., Economo, E. and Beutel, R.G., 2020. Comparative analysis of worker head anatomy of *Formica* and *Brachyponera* (Hymenoptera: Formicidae). *Arthr. Syst. Phyl.* 78, 133–170.
- Richter, A., Keller, R.A., Rosumek, F.B., Economo, E.P., Garcia, F.H. and Beutel, R.G., 2019. The cephalic anatomy of workers of the ant species *Wasmannia affinis* (Formicidae, Hymenoptera, Insecta) and its evolutionary implications. *Arthr. Str. Dev.* 49, 26–49.
- Richter, A., Wipfler, B., Beutel, R.G. and Pohl, H., 2017. The female cephalothorax of *Xenos vesparum* Rossi, 1793 (Strepsiptera: Xenidae). *Arthr. Syst. Phyl.* 75, 327–347.
- Schmidt-Rhaesa, A. (Ed.), 2013. *Gastrotricha, Cycloneuralia, and Gnathifera: Nematomorpha, Priapulida, Kinorhyncha, Loricifera*. *Handbook of Zoology*. De Gruyter, Berlin.
- Schneeberg, K. and Beutel, R.G., 2010. The adult head structures of Tipulomorpha (Diptera, Insecta) and their phylogenetic implications. *Acta Zool.* 92, 316–343.
- Schneeberg, K. and Beutel, R.G., 2014. The evolution of head structures in lower Diptera. *Sci. Open Res.* <https://doi.org/10.14293/S2199-1006.1.SOR-LIFE.ALTCE1.v2>.
- Schneeberg, K., Courtney, G.W. and Beutel, R.G., 2011. Adult head structures of Deuterophlebiidae (Insecta), a highly derived “ancestral” dipteran lineage. *Arthr. Str. Dev.* 40, 93–104.
- Schneeberg, K., Friedrich, F., Courtney, G.W., Wipfler, B. and Beutel, R.G., 2012. The larvae of Nymphomyiidae (Diptera, Insecta) – Ancestral and highly derived? *Arthr. Struct. Dev.* 41, 293–301.

- Schneeberg, K., Polilov, A., Harris, M.O. and Beutel, R.G., 2013. The adult head morphology of the Hessian fly *Mayetiola destructor* (Diptera, Cecidomyiidae). *J. Morph.* 274, 1299–1311.
- de Sena Oliveira, I., Franke, F.A., Hering, L., Schaffer, S., Rowell, D.M., Weck-Heimann, A., Monge-Nájera, J., Morera-Brenes, B. & Mayer, G. 2012. Unexplored character diversity in Onychophora (velvet worms): a comparative study of three peripatid species. *PLoS One*, 7, e51220.
- Simpson, G.G., 1949. *The Meaning of Evolution: A Study of the History of Life and its Significance for Man*. Yale University Press, New Haven, Connecticut.
- Simpson, G.G., 1953. *The Major Features of Evolution*. Columbia University Press, New York, New York.
- Snodgrass, R.E., 1946. The skeletal anatomy of fleas (Siphonaptera). *Smiths. Misc. Coll.* 104, 1–89.
- Staniczek, A.H., 2000. The mandible of silverfish (Insecta: Zygentoma) and mayflies (Ephemeroptera): its morphology and phylogenetic significance. *Zool. Anz.* 239, 147–178.
- Tokunaga, M., 1935. A morphological study of a nymphomyid fly. *Philipp. J. Sci.* 56, 127–214.
- Tröger, D., Beutel, R.G. and Pohl, H., 2019. The abdomen of a free-living female of Strepsiptera and the evolution of the birth organs. *J. Morph.* 280, 739–755.
- Tröger, D., Grabe, V., Beutel, R.G. and Pohl, H., 2020. The endoparasitic larval stages of *Eoxenos laboulbenei*: an atypical holometabolan development (Strepsiptera, Mengenillidae). *Arthr. Str. Dev.* 56, 100932.
- Vasilikopoulos, A., Misof, B., Meusemann, K., Lieberz, D., Flouri, T., Beutel, R.G., Niehuis, O., Wappler, T., Rust, J., Peters, R.S. et al., 2020. An integrative phylogenomic approach to elucidate the evolutionary history and divergence times of Neuropterida (Insecta: Holometabola). *BMC Evol. Biol.* 20, 64.
- Vilhelmsen, L., 2000. The ovipositor apparatus of basal Hymenoptera (Insecta): phylogenetic implications and functional morphology. *Zool. Scr.* 29, 319–345.
- Vilhelmsen, L., 2003. Larval anatomy of Orussidae (Hymenoptera). *J. Hymenoptera Res.* 12, 346–354.
- Wharton, R., Vilhelmsen, L. and Gibson, G.A.P., 2004. Characterizing basal apocritans (Hymenoptera: Apocrita). *Proc. Russ. Entomol. Soc. St. Petersburg* 75, 17–23.
- Wiegmann, B.M., Trautwein, M.D., Kim, J.W., Cassel, B.K., Bertone, M.A., Winterton, S.L. and Yeates, D.K., 2009. Single-copy nuclear genes resolve the phylogeny of the holometabolous insects. *BMC Biol.* 7, 34.
- Willmann, R., 1981. Das Exoskelett der männlichen Genitalien der Mecoptera (Insecta. I. Morphologie. II). Die phylogenetischen Beziehungen der Schnabelfliegen-Familien. *Z. Zool. Syst. Evolutforsch.* 19, 153–174.
- Wipfler, B., Machida, R., Mueller, B. and Beutel, R.G., 2011. On the head morphology of Grylloblattodea (Insecta) and the systematic position of the order, with a new nomenclature for the head muscles of Dicondylia. *Syst. Entomol.* 36, 241–266.
- Wipfler, B., Schneeberg, K., Löffler, A., Hünefeld, F., Meier, R. and Beutel, R.G., 2013. The skeletomuscular system of the larva of *Drosophila melanogaster* (Drosophilidae, Diptera) – a contribution to the morphology of a model organism. *Arthr. Str. Dev.* 42, 47–68.
- Wipfler, B., Weissing, K., Lass, K.D. and Weihmann, T., 2016. The cephalic morphology of the American cockroach *Periplaneta americana* (Blattodea). *Arthr. Syst. Phyl.* 74, 267–297.

## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Additional supporting information can be found in the figshare online depository: <https://www.doi.org/10.6084/m9.figshare.14762967>.

**Appendix S1.** Characters used in the analyses.

**Appendix S2.** Apomorphies of Holometabola and its subgroups.

**Appendix S3.** References cited in Appendices S1 and S2.

**Appendix S4.** Topology used for reconstruction of character evolution (based on Misof et al., 2014).

**Data S1.** Data matrix (in nexus and WinCLADA format).

**Data S2.** Character evolution reconstructed for each character using maximum-likelihood.