

# Evolutionary history of Polyneoptera and its implications for our understanding of early winged insects

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**Polyneoptera represents one of the major lineages of winged insects, comprising around 40,000 extant species in 10 traditional orders, including grasshoppers, roaches, and stoneflies. Many important aspects of polyneopteran evolution, such as their phylogenetic relationships, changes in their external appearance, their habitat preferences, and social behavior, are unresolved and are a major enigma in entomology. These ambiguities also have direct consequences for our understanding of the evolution of winged insects in general; for example, with respect to the ancestral habitats of adults and juveniles. We addressed these issues with a large-scale phylogenomic analysis and used the reconstructed phylogenetic relationships to trace the evolution of 112 characters associated with the external appearance and the lifestyle of winged insects. Our inferences suggest that the last common ancestors of Polyneoptera and of the winged insects were terrestrial throughout their lives, implying that wings did not evolve in an aquatic environment. The appearance of the first polyneopteran insect was mainly characterized by ancestral traits such as long segmented abdominal appendages and biting mouthparts held below the head capsule. This ancestor lived in association with the ground, which led to various specializations including hardened forewings and unique tarsal attachment structures. However, within Polyneoptera, several groups switched separately to a life on plants. In contrast to a previous hypothesis, we found that social behavior was not part of the polyneopteran ground plan. In other traits, such as the biting mouthparts, Polyneoptera shows a high degree of evolutionary conservatism unique among the major lineages of winged insects.**

lower winged insects | Polyneoptera | Pterygota | Neoptera | phylogenomics

The evolution of insect wings, which happened ~400 Mya, led to a unique radiation and gave rise to the most species-rich group of organisms relative to their phylogenetic age (1, 2). One of the major lineages of winged insects is Polyneoptera, which comprises ~40,000 described species in a total of 10 taxonomic orders. These include the well-known grasshoppers, crickets and allies (Orthoptera), stoneflies (Plecoptera), earwigs (Dermaptera), roaches and termites (Blattodea), mantids (Mantodea), stick and leaf insects (Phasmatodea), and also some of the least known and species-poor insect groups, including heelwalkers (Mantophasmatodea), ice

crawlers (Grylloblattodea), webspinners (Embioptera), and ground lice (Zoraptera). Polyneoptera feature a wide spectrum of different lifestyles and body shapes. Some groups (e.g., roaches) exhibit extreme adaptations toward a ground-dwelling lifestyle, with hardened forewings and a dorsoventrally flattened body. Other groups, such

## Significance

**Polyneoptera is the only major lineage of winged insects (Pterygota) with an unresolved evolutionary history concerning important phenotypic traits like external shape, social behavior, and lifestyle. These ambiguities have far-reaching consequences for our understanding of the early evolution of winged insects. We closed this knowledge gap through large-scale phylogenomic analyses tracing traits concerning lifestyle and habitus within Polyneoptera and Pterygota. Both groups were ancestrally terrestrial in all developmental stages, implying that wings did not evolve in species living in water. All polyneopteran insects derive from a ground-dwelling insect with a largely unmodified body relative to the last common ancestor of winged insects. Intriguingly, different forms of social behavior, changes in lifestyle, and associated morphological specializations evolved multiple times within Polyneoptera.**

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as stick and leaf insects and some mantids, live in the foliage and mimic leaves or twigs. Polyneoptera also feature a wide range of diets: some species, including most roaches and earwigs, are omnivorous, while others, such as stick and leaf insects and some grasshoppers, are strictly herbivorous. Additionally, the group includes carnivorous taxa (e.g., ambush predators like mantids and heelwalkers). Polyneoptera have also evolved a wide spectrum of insect social behaviors, ranging from maternal and biparental brood care to eusociality with a complex caste system.

The evolution of the above-mentioned traits is poorly understood, largely due to the lack of studies on character evolution and unresolved phylogenetic relationships among Polyneoptera. Previously published phylogenetic hypotheses were incongruent, including disagreement concerning the common ancestry of the group (*SI Appendix, Fig. S9*). As a result, Polyneoptera is the only major lineage of winged insects with a largely unresolved evolutionary history and thus strongly differs in this respect from Holometabola (insects with a complete metamorphosis) and from Acercaria (mostly sucking insects such as lice or true bugs). For the latter two lineages, detailed evolutionary scenarios, including ground-plan reconstructions for various character systems such as habitus (3), the holometabolan larvae (4), or social patterns (5), are available, although the common ancestry of Acercaria was recently challenged by a transcriptomic study (6). The evolutionary history of Polyneoptera is considered one of the major unresolved subjects in insect evolution (7) and not only affects our knowledge on Polyneoptera itself, but also has broad implications for our understanding of the early evolution of the winged insects. A major unresolved question related to this is whether winged insects evolved in an aquatic or terrestrial environment. The immatures of the two early diverging groups of winged insects—mayflies (Ephemeroptera) and damsel- and dragonflies (Odonata)—have an aquatic lifestyle; the same is observed in the polyneopteran stoneflies (Plecoptera), which have been hypothesized to represent the sister group of all remaining Polyneoptera or of all insects that can flex their wings above the abdomen (Neoptera) (8, 9). Thus, various authors assumed that early winged insect evolution occurred in an aquatic environment (e.g., refs. 9–12). Another unanswered question in the early evolution of winged insects concerns the ancestral lifestyle of adults. Many Polyneoptera inhabit narrow spaces such as litter, soil, and small cracks, and a similar lifestyle is found in the closest relatives of the winged insects: wingless bristletails (Archaeognatha) and silverfish (Zygentoma). However, this lifestyle is not found in mayflies, in damselflies or dragonflies, or in most groups of the other major lineages of winged insects (Acercaria and Holometabola). The question thus remains whether a ground-dwelling lifestyle represents an ancestral condition of the winged insects or whether some Polyneoptera returned secondarily to a life on ground. The current fossil record does not provide answers to these questions due to the lack of transitional fossils (13). Thus, a sound understanding of the phylogenetic relationships among the extant lineages of Polyneoptera is essential to trace the currently unresolved evolutionary trends within the in group, with possibly major implications for our knowledge of the evolution of winged insects.

We aim to close the above-outlined knowledge gaps in insect evolution by combining phylogenetic analyses of the largest transcriptomic dataset ever used for this purpose, comprising 3,014 protein-coding genes sampled from a total of 106 extant insect species, with a critical reevaluation of morphological and embryological arguments for all recovered interordinal nodes. We use the obtained phylogeny to reconstruct the evolution of 112 characters associated with habitus, habitat of larvae and adults, diet, and social behavior. Our study provides a formal reconstruction of the evolutionary history of both Polyneoptera and early winged insects (Pterygota).

## Results

**Phylogenomic Analyses.** Our dataset comprised, in total, 106 insect species, representing all currently recognized polyneopteran orders and a representative sampling of outgroup taxa (*Dataset S1*). Phylogenetic analyses are based on five different datasets derived from 3,014 protein-coding genes: (i)  $D_{AA,all}$ , the complete dataset comprising 1,246,506 aligned amino acid sites; (ii)  $D_{AA,decisive}$ , a protein domain-based decisive dataset (i.e., a dataset which included only data blocks with representatives of selected taxonomic groups, see *Materials and Methods*) comprising 909,873 aligned amino acid sites; (iii)  $D_{nuc,decisive}$ , a corresponding decisive dataset comprising 909,873 aligned sites of second codon positions only; (iv)  $D_{AA,genes}$ , a gene-based decisive amino acid dataset of 2,061 genes comprising 832,237 amino acid sites; and (v)  $D_{nuc,genes}$ , a corresponding dataset comprising 832,237 aligned sites of second codon positions only. In addition to a maximum likelihood (ML) tree reconstruction based on a supermatrix approach and a multispecies coalescent (MSC) tree reconstruction, we applied Four-cluster Likelihood Mapping (FcLM) (14) to evaluate alternative signal for the major phylogenetic splits within Polyneoptera and to assess potential incongruent signal in our datasets that might not be revealed by a multispecies tree. To assess plausibility of our phylogenomic results, we compiled and assessed arguments from morphological and embryological data that support the inferred phylogenetic relationships (*SI Appendix*).

Our various phylogenomic analyses consistently revealed the monophyly of Pterygota, Neoptera, Eumetabola (Holometabola + Acercaria), and Holometabola. The only notable difference we observed was that the phylogenetic inferences from the analysis of amino acids (Fig. 1 and *SI Appendix, Figs. S3 and S9*) support monophyletic Acercaria, while the phylogenetic inferences from the analysis of the second codon positions (*SI Appendix, Figs. S4 and S10*) support lice (Psocodea) as the sister group to Holometabola. All our phylogenomic analyses found strong support for a monophyletic origin of Polyneoptera (Fig. 1 and *SI Appendix, Figs. S3–S10*), a result that is also corroborated by morphological and embryological evidence (*SI Appendix, section 5*) and that is not contradicted by the FcLM analyses (*SI Appendix, section 4.4*). Within Polyneoptera, all five supermatrix phylogenomic analyses place earwigs (Dermaptera) and ground lice (Zoraptera) as a sister group to the remaining Polyneoptera (Fig. 1 and *SI Appendix, Figs. S3, S4, S9, and S10*). Although this phylogenetic relationship is challenged by the MSC analyses, additional in-depth analyses of confounding signal and heterogeneity revealed further support for a sister group relationship of Dermaptera and Zoraptera (see discussion in *SI Appendix, section 4.5*). Consistent with earlier studies (6, 15), a polyneopteran clade (“core Polyneoptera”) comprising grasshoppers, crickets and allies (Orthoptera), roaches and termites (Blattodea), mantids (Mantodea), stick and leaf insects (Phasmatodea), webspinners (Embioptera), heelwalkers (Mantophasmatodea), and ice crawlers (Grylloblattodea) is well supported by all our analyses. Stoneflies (Plecoptera) are placed as sister group to these core Polyneoptera. Furthermore, our analyses provide strong support for Dictyoptera, which is a close relationship of mantids, termites, and roaches. Stick and leaf insects (Phasmatodea) are inferred as the sister group of the webspinners (Embioptera) [Phasmatodea + Embioptera = Eukinolabia *sensu* (15)]. Eukinolabia form the sister group of Xenonomia *sensu* (15) [i.e., ice crawlers (Grylloblattodea) and heelwalkers (Mantophasmatodea)] (Fig. 1).

**Character Evolution.** To understand major evolutionary transitions, we applied the maximum parsimony and ML optimality criteria to trace a total of 112 behavioral, ecological, and morphological characters that played a major role during the evolution of Pterygota and of Polyneoptera (*SI Appendix, section 6*). Specifically, we studied characters associated with (i) the habitus of adults, (ii) social behavior, (iii) the habitat of larvae and of

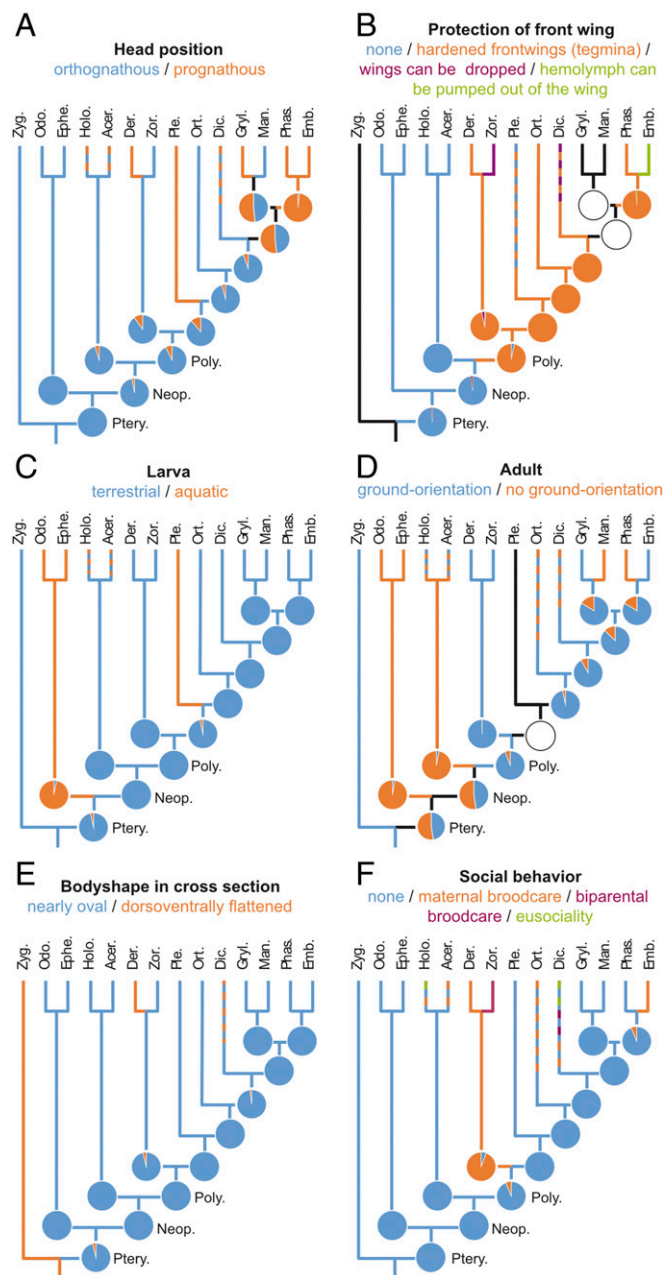




evolved at least four times separately within Polyneoptera (Fig. 24). The actual number might even be higher, because our morphological reconstruction for a clade containing Eukinolabia (= Embioptera + Phasmatodea) and Xenonomia (= Grylloblattodea + Mantophasmatodea) remained ambiguous. Prognathy is usually associated with a raptorial lifestyle (17). Intriguingly, however, the



**Fig. 3.** Virtual model of the last common ancestor of Polyneoptera, inferred from analyzing 112 morphological characters. *SI Appendix, Fig. S17* illustrates which parts of the model are based on results of the present analyses.



**Fig. 2.** (A–F) Reconstructed evolution of selected characters in Polyneoptera and related lineages (full list in *Dataset S11*). Pie charts indicate the ML results for the respective hypothesis at that node. Black lines and white pie charts imply ambiguous results or characters that are not applicable. Dotted lines indicate changes within the terminal taxa. Acer., Acercaria; Der., Dermaptera (earwigs); Dic., Dictyoptera (mantids, roaches, and termites); Emb., Embioptera (web-spinners); Ephe., Ephemeroptera (mayflies); Gryl., Grylloblattodea (ice crawlers); Holo., Holometabola; Man., Mantophasmatodea (heelwalkers); Neop., Neoptera; Odo., Odonata (damself- and dragonflies); Orth., Orthoptera (crickets, katydids, and grasshoppers); Phas., Phasmatodea (stick and leaf insects); Ple., Plecoptera (stoneflies); Poly., Polyneoptera; Ptery., Pterygota (winged insects); Zor., Zoraptera (ground lice); Zyg., Zygentoma (silverfish).

only exclusively predatory polyneopteran groups—the mantids (Mantodea) and the heelwalkers (Mantophasmatodea)—are not prognathous but orthognathous. A possible explanation for this evolutionary conservation in these two predatory groups is the fact that species of these two lineages use their forelegs rather than their mouthparts to catch prey (18). Prognathous polyneopteran insects show a wide spectrum of different diets, which include pure or partial herbivory (stick and leaf insects, stoneflies, and web-spinners), omnivory (most earwigs), wood feeding (termites), or feeding on dead animals (ice crawlers). Prognathy also evolved in various subgroups of Holometabola and Acercaria (19), the other two major clades of Neoptera. Our data thus imply that a change of mouthpart orientation, typically resulting in far-reaching modifications of the head capsule, is a comparatively frequent evolutionary transition. Based on our reconstruction, the first polyneopteran insects also exhibited other ancestral pterygote traits such as long-segmented abdominal appendages (cerci), thoracic segments with approximately equal dimensions, and pentamerous tarsi. Although extant representatives of many orders of Polyneoptera, like stoneflies (Plecoptera) and earwigs (Dermaptera), reduced at least some of these features, the fossil record shows that their stem group representatives still had these ancestral characteristics (13). This implies that such reductions occurred several times separately (13) (*SI Appendix, section 6.3*).

Given the wide distribution of social behaviors among extant polyneopterans, it has been hypothesized that the last common polyneopteran ancestor exhibited social behavior in the form of maternal care (20). Although different forms of social behavior, such as maternal or biparental care, are indeed found in almost all polyneopteran insect groups, including ground lice (Zoraptera), earwigs (Dermaptera), crickets and grasshoppers (Orthoptera), roaches and termites (Blattodea), mantids (Mantodea), and web-spinners (Embioptera) (20), our study contradicts this hypothesis. Instead, our results strongly suggest that maternal care evolved at least five times independently within Polyneoptera (Fig. 2F). The actual number might even be higher, since the social behavior of several lineages of mantids (Mantodea) is not documented and the evolution of maternal care in the roaches (Blattodea) (21) and crickets and grasshoppers (Orthoptera) (22) is only poorly understood. In accordance with Gilbert and Manica (20), we find that biparental care likely evolved separately in ground lice (Zoraptera) (23) and multiple lineages of roaches (21). Additionally, we confirm that the eusocial termites are the sister group of one of the subsocial groups of roaches, the Cryptocercidae (Fig. 1) (24).

It has been assumed that the first winged insects had aquatic larvae and that wings also evolved as an adaptation to an aquatic environment (e.g., refs. 9–12). This hypothesis is based on the presence of aquatic nymphs in mayflies (Ephemeroptera), in damself- and dragonflies (Odonata), as well as in stoneflies (Plecoptera). In this respect, the morphology of stoneflies was considered to

reflect the ancestral condition of both Polyneoptera and Neoptera (8, 9). However, our results confirm the contradicting hypothesis, which states that the last common ancestors of Polyneoptera, Neoptera, and Pterygota were terrestrial throughout their entire lives (Fig. 2C) (13, 25). Specifically, this hypothesis is based on the derived position of the stoneflies within the polyneopteran insects and the increasing evidence for monophyletic Paleoptera (26) that is also supported by our analyses (Fig. 1). In addition, there is a third group of paleopteran insects, the extinct Paleodictyoptera: these large insects with beaks and, in many of its species, strongly widened prothoracic plates that contained a venation similar to those of wings (“six-winged insects”) were also terrestrial throughout their life stages (13). The question concerning the habitat of the first winged insects also has direct implications for the ancestral function of wings. Although extant winged insects use their wings primarily for flight, this was likely not their original purpose, since early winglets were likely much shorter, immovable, and therefore incapable of supporting powered flight. Three competing hypotheses have been proposed concerning the early function of these winglets: (i) winglets were used to control the descent while falling or jumping from a raised stand (e.g., a plant) (27), (ii) winglets evolved as organs of steering and propulsion in aquatic nymphs (10), and (iii) winglets were used as sails to achieve a quick distribution after the adults hatched from aquatic larvae (11, 12). Recent developmental studies have shown that the anatomical origin of wings does not provide any insight into this question because wings most likely evolved from a combination of the dorsal plates of the thoracic segments and branches of the legs (2, 28). However, because our results suggest that an aquatic larva was not part of the ground plan of winged insects, they consequently also reject the latter two hypotheses, which postulate the most recent common ancestor of winged insects having lived in an aquatic habitat. Our results thus favor the theory that wings originally developed as organs used for directed aerial descent when gliding from a raised stand (27). This concept is further supported by the fact that this behavior is also observed in some primarily wingless bristletails (Archaeognatha) (29).

Our comprehensive datasets allowed us to shed light on the ancestral habitat of adult early winged insects. Many adult extant Polyneoptera live on the ground or inhabit narrow spaces, such as leaf litter, cracks, crevices, or the spaces under bark. Most representatives of silverfish and bristletails, the closest relatives of winged insects, prefer a similar habitat. However, Paleoptera and most representatives of the other two major groups of neopteran insects, Holometabola and Acercaria, do not live in this kind of habitat. It thus remains unclear whether a preference for the ground represents an ancestral condition in the winged insects or whether Polyneoptera returned secondarily to this habitat. Our analyses suggest that the last common ancestor of Polyneoptera had a ground-dwelling lifestyle (Fig. 2D), although it remains ambiguous whether this is an ancestral or a derived feature (Fig. 2D). The shape of the body provides some hints on the evolutionary origin of the ground preference: specialized ground-dwelling insects, such as silverfish (*Zygentoma*), earwigs (*Dermaptera*), and roaches (*Blattodea*), usually have dorsoventrally flattened bodies. Based on our data, this was not the case for the ancestral polyneopteran insects (Fig. 2E). Instead, dorsoventrally flattened bodies evolved secondarily and separately in several polyneopteran lineages. Additionally, Paleodictyoptera apparently exhibited a non-ground-dwelling lifestyle (13). Thus, it appears likely to us that the ancestral polyneopteran returned secondarily to the ground and that the ancestral terrestrial Pterygota and Neoptera lived on plants or trees, which is congruent with the idea of early wings being used for directed aerial descent. According to our analyses, this change of habitat led to many adaptations in the body of the first polyneopteran insects (Fig. 3): Its antennae were comparatively long, it evolved unique attachment structures on the tarsi (euplantulae) that provided

additional grip, and it had hardened forewings with a complete wing venation (tegmina) that protected the delicate hind wings when entering the substrate (Fig. 2B). The sclerotization of the forewings resulted in a reduced lift during flight that had to be counterbalanced by the hind wings that became triangular in shape (30). In contrast to other insects with sclerotized forewings, such as beetles and true bugs, Polyneoptera achieved this counterbalancing by a distinctly enlarged hind wing vannus with additional anal veins, which is also found in the last common ancestor (Fig. 2B). However, our data show that some polyneopteran insects secondarily and separately from each other reduced the sclerotized forewings—that is, most stoneflies (Plecoptera), ground lice (Zoraptera), webspinners (Embiopoda), and termites (subgroup of Blattodea). Interestingly, species of the latter three groups live in habitats that provide little space to move and are closely associated to the substrate. In these groups, completely different mechanisms for wing protection evolved: Ground lice and termites evolved separately from each other the ability to drop their wings when entering a life in the ground. Male webspinners (females are wingless) can pump the hemolymph out of the wings, which then become extremely flexible and can be folded or crumpled over the thorax without damage (31). As effective as sclerotized forewings are as a protective measure, there is a tendency within exclusively ground-dwelling polyneopteran insects to either replace them with another mechanism (webspinners) or to use the flight capability only as a one-time method of dispersal and then to dispose the wings (ground lice and termites). Although the first polyneopteran lived most likely on the ground or was associated with the substrate, we can show that, within Polyneoptera, several groups adapted secondarily to a life in bushes and trees. Our analyses suggest at least four independent transitions to this habitat (Fig. 2D), either related to a plant diet (stick and leaf insects and some crickets, katydids, and grasshoppers) or to a predatory life on plants (most mantids and heelwalkers). However, different groups of grasshoppers and katydids (Orthoptera) might have colonized an arboreal habitat separately from each other (22), which would increase the number of transitions within Polyneoptera. To disguise themselves, all these lineages developed camouflage patterns, including extreme forms such as morphological and behavioral leaf and twig mimicry (e.g., ref. 32).

In summary, our study reveals that the highly specialized polyneopteran groups we observe today, such as the herbivorous stick and leaf insects, the eusocial termites, and the predatory mantids, are derived from an insect with many ancestral traits in both morphology and behavior. We demonstrate that some transitions, such as the evolution of social behavior or a life in the foliage, occurred several times separately. However, some polyneopteran features, such as the retention of the biting mouthparts, are uniquely conserved compared with the other major groups of winged insects. With our approach to combine a robust phylogeny with a formal reconstruction of character evaluation, we provide a comprehensive evolutionary picture of the Polyneoptera, thus closing a major gap in our understanding of insect evolution.

## Materials and Methods

**Phylogenomic Analyses.** Our taxon sampling comprises a total of 106 extant insect species, including 72 polyneopteran representatives. [Dataset S1](#) provides a detailed list of all species, including their collection data and National Center for Biotechnology Information (NCBI) accession numbers. Detailed information on the orthology prediction, the matrix assembly, the phylogenetic analyses, the FcLM strategy, and analyses to detect confounding signal are available in [SI Appendix, sections 2–4](#). RNA extraction, cDNA library preparation, transcriptome sequencing, de novo assembly, and transcriptome quality assessment, as well as the submission procedure to the NCBI Transcriptome Shotgun Assembly database were performed as described by Peters et al. (5). Final assemblies were searched for transcripts of 3,014 protein-coding single-copy genes. Orthologous amino acid sequences were aligned, and resulting multiple sequence alignments (MSA) were assessed for quality and, if necessary, improved (or removed). We performed



ML phylogenetic analyses using a supermatrix approach with a partitioning scheme based on protein domains. According to ref. 6, we assume that protein domains are better evolutionary units to model sequence evolution than genes. In addition, we performed phylogenetic analyses with an MSC method and gene-based partitioned supermatrices applying the ML optimality criterion. All analyses have been carried out (i) on amino acid datasets and (ii) on corresponding nucleotide datasets using second codon positions only. For the gene-based ML and MSC analyses as well as for the protein domain-based ML analyses, MSA segments with putative alignment ambiguities or randomized data, identified with Aliscore (v.1.2) (33), were removed from the genes and the protein domain-based data blocks. The information content of each gene and protein domain-based data block was characterized with Mare (34), and uninformative genes and data blocks were removed. The resulting datasets were further optimized by including only those data blocks or genes that contained sequence information of selected taxa [decisive dataset, *sensu* (35)]. For the selection of optimal partitions and appropriate substitution models for the protein domain-based partitioning scheme, we applied PartitionFinder 2.0.0 (prerelease 10) using the rcluster algorithm (36). For the nucleotide supermatrix of the decisive dataset, we used PartitionFinder v.2.0.0 (prerelease 5) to select the partitions and the best-fitting substitution model using the iterative k-means search (37). Fifty independent phylogenetic tree inferences were performed using ExaML (v.3.0.16) (38), starting from different starting trees (40 random starting trees and 10 random stepwise addition parsimony starting trees). Phylogenetic analyses of the supermatrices with a gene-based partitioning scheme were inferred under the ML optimality criterion as implemented in IQ-TREE (v.1.5.5) (39) using the best-scoring substitution matrix for each gene partition as selected with ModelFinder implemented in IQ-TREE (40). In addition to the nonparametric bootstrap analysis, support for specific phylogenetic relationships was further assessed by FcLM (14) implemented in the software IQ-TREE v.1.4.1 (39). Gene trees used in the two MSC analyses

(decisive amino acid datasets and corresponding nucleotide datasets using second codon positions only) were computed with IQ-TREE (v.1.6.3) with a model selection described in *SI Appendix, section 4.1* and performing 1,000 nonparametric bootstrap replicates. The MSC analyses were carried out in ASTRAL III (v.5.5.6 and v.5.6.1) (41) on each dataset separately, with and without bootstrapping.

**Character Evolution.** To reconstruct the major transitions in morphology and lifestyle, we coded a total of 112 behavioral, ecological, and morphological characters for 106 selected species. We traced evolutionary transformations by maximum parsimony (all characters unordered) and ML-based mapping using Mesquite (42). *Dataset S10* contains the character matrix. *Dataset S11* contains the reconstructed character states for Polyneoptera. We discuss our results with respect to plausibility and paleontological findings in detail in *SI Appendix, section 6.3*. Based on these results, we created a virtual model that illustrates the retrieved characters of the last common ancestor of Polyneoptera (Fig. 3).

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