



neopteran (or polyneopteran) tree and interpreted the aquatic life history stage to be an ancestral feature in the life history of the winged insects (12–14). This inference further informed the historically contentious interpretation that the insect wing represented a derivation of ancestral tracheal gills—like those seen in mayfly, dragonfly, and stonefly aquatic nymphs—placing the aquatic larva squarely in the transitional series that gave rise to the first volant arthropod (12–15). This controversial homology schema has since been tested using developmental genetic comparisons of insect wings, nymphal gills, and crustacean gills (16–19). Available data now strongly support the idea that the insect wing represents a fusion of a paranotal extension of the body wall and secondary limb branches of the proximal leg (a fusion of both historical, competing hypotheses). These data nevertheless do not address the evolutionary significance of the aquatic larva of basally branching pterygotes (*sensu* 15)—the answer to which hinges upon a robust insect phylogeny.

Enter the efforts of the 1K Insect Transcriptome Evolution (1KITE) team, which produced in 2014 a modern and comprehensive phylogeny of insect orders based on just shy of 1,500 protein-coding genes (20). While this effort established a robust phylogeny of major insect groups and resolved nearly all higher-level relationships, neither the monophyly nor the placement of Paleoptera were supported in that study (nodes 20 and 134 in figure 1 of ref. 20). Two higher-level nodes within Polyneoptera were also not supported. These regions of topological uncertainty hindered the utility of that tree topology for understanding the evolution of flight, to which the diversification of insects is most certainly linked.

In PNAS, the 1KITE team, led by Benjamin Wipfler and Sabrina Simon, emphasized three strategies to achieve phylogenetic robustness: First, Wipfler et al. (5) increased the taxonomic sampling of Polyneoptera from 28 exemplars in 2014 to 72 in the present dataset. Second, they leveraged advances in the computational efficiency of tree inference algorithms, increasing the dataset size from 1,478 genes to 3,014. Third, they deployed established single-copy orthologs for the analysis, with model fitting based on the architecture of protein domains (for supermatrix approaches) together with multispecies coalescent approaches for reconciling gene trees. The datasets were also algorithmically filtered for informativeness and completeness. The resulting supermatrix tree topology (based on a subset of decisive data partitions) recovered a monophyletic Paleoptera sister group to Neoptera, with maximal nodal support for both relationships. As also found by Misof et al. (20), Plecoptera was found nested within Polyneoptera, forming a grade with the clade (Zoraptera + Dermaptera) at the base of Polyneoptera. All higher-level relationships in this tree were supported.

Onto this tree, Wipfler et al. (5) mapped 112 morphological and life history characters, aiming to reconstruct the last common ancestor of Polyneoptera. Given the distant placements of Paleoptera and stoneflies in their tree, they infer that the polyneopteran ancestor was most likely terrestrial throughout its life, controverting the older notion that an aquatic nymph represents a transitional step in the evolution of flight. Aquatic larvae then, as well as their gills, must represent derived steps in insect evolution, like the aquatic larvae of mosquitos and midges (members of Holometabola). Wipfler et al. infer that wings could have originated from unspecialized trunk winglets used to stabilize aerial descent in terrestrial groups, which subsequently diversified. This interpretation is consistent with the incidence of groups in the fossil record with additional wings or

winglike structures, as well as developmental genetic manipulations that can incur ectopic winglets in modern milkweed bugs (21, 22). Beyond upending the traditional evolutionary reconstruction of winged flight, Wipfler et al. (5) also infer, based on their phylogeny, a dorsoventrally compressed, ground-dwelling polyneopteran common ancestor with hardened anterior wings, and multiple origins of such social behaviors as maternal care.

### Together, the analyses by Wipfler et al. establish a robust internal phylogeny of Polyneoptera and highlight the effects of increased gene and taxonomic sampling in improving phylogenetic resolution of old clades.

Ancestral state reconstructions must always be interpreted critically, particularly when based only on extant terminals and when the fossil record for a group suggests historical extinctions of higher-level clades. As it relates to the present case, entire orders of insects are known from the Paleozoic fossil record (21, 23, 24), but the character states they exhibit (e.g., additional wing pairs; gigantism, as exemplified by Carboniferous fossils) cannot, by definition, be recovered as the ancestral states in an analysis based only on extant species that exhibit none of those conditions. Integrating paleontological and neontological data into the phylogeny would certainly clarify the effect of extinct groups on the reconstruction of insect evolution, but doing so comes with its own procedural hurdles. Few researchers have attempted to integrate morphological data (on the order of hundreds of characters) with genome-scale phylogenomic matrices (millions of characters) in phylogenetic reconstruction, owing to controversies in modeling morphological evolution and ambiguity in how these two data classes should be weighted in a single analysis (25, 26). Nevertheless, the establishment of such a dataset enables, in principle, future integration of paleontological and neontological datasets with foreknowledge of a robustly resolved polyneopteran phylogenomic backbone.

Phylogenomic datasets bring ambiguities and challenges of their own, and larger datasets are not a panacea for phylogenetic problematica (27, 28). While the relationships between polyneopteran orders recovered by Misof et al. (20) and Wipfler et al. (5) were identical (with higher nodal support in the latter for key aforementioned nodes), topologies inferred by multispecies coalescent approaches consistently failed to recover the monophyly of Paleoptera in their study, regardless of how the datasets were partitioned or filtered. This result recapitulates the marked gene tree incongruence incident in this part of the insect Tree of Life (11). Similarly, Wipfler et al. (5) recovered the monophyly of Acercaria (a group of insects with sucking mouthparts, such as true bugs)—the putative sister group of Holometabola—only in their supermatrix trees, not in their multispecies coalescent analyses. This second result is all the more curious because the 2014 analysis of Misof et al. (20) recovered the nonmonophyly of Acercaria with support using a supermatrix approach; Psocodea (true lice) were recovered in that work as the sister group of Holometabola. The two datasets differ significantly with respect to gene and taxonomic sampling; Acercaria and Holometabola, which constitute outgroups for Wipfler et al. (5), were far better sampled by Misof et al. (20), but the former study features more sequence

data. These results suggest that the identity of the sister group of Holometabola is ambiguous, prone to gene tree incongruence, sensitive to dataset composition, or some combination thereof.

Together, the analyses by Wipfler et al. (5) establish a robust internal phylogeny of Polyneoptera and highlight the effects of

increased gene and taxonomic sampling in improving phylogenetic resolution of old clades. They also showcase how the integration of morphological datasets with genome-scale molecular datasets can redress disputes over the evolutionary history of some of nature's most charismatic phenomena.

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