Report

Current Biology

Evolutionary History of the Hymenoptera

Highlights

- The most comprehensive dataset ever compiled for inferring the phylogeny of Hymenoptera
- A major radiation of primarily ectophytic sawflies (Eusymphyta) is hypothesized
- A major radiation of parasitoid wasps (Parasitoida) is identified
- The phylogenetic origins of wasp-waisted wasps, stinging wasps, and bees are resolved

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In Brief

Peters et al. infer a time-calibrated and statistically solid phylogenetic tree of the mega-diverse insect order Hymenoptera (sawflies, wasps, ants, and bees) from the analysis of phylogenomic data. This sheds new light on the early history of this intriguing group, as well as on the origins and radiation of parasitoids, stinging wasps, and bees.

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Evolutionary History of the Hymenoptera

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SUMMARY

Hymenoptera (sawflies, wasps, ants, and bees) are one of four mega-diverse insect orders, comprising more than 153,000 described and possibly up to one million undescribed extant species [1, 2]. As parasitoids, predators, and pollinators, Hymenoptera play a fundamental role in virtually all terrestrial ecosystems and are of substantial economic importance [1, 3]. To understand the diversification and key evolutionary transitions of Hymenoptera, most notably from phytophagy to parasitoidism and predation (and vice versa) and from solitary to eusocial life, we inferred the phylogeny and divergence times of all major lineages of Hymenoptera by analyzing 3,256 protein-coding genes in 173 insect species. Our analyses suggest that extant Hymenoptera started to diversify around 281 million years ago (mya). The primarily ectophytophagous sawflies are found to be monophyletic. The species-rich lineages of parasitoid wasps constitute a monophyletic group as well. The little-known, species-poor Trigonaloidea are identified as the sister group of the stinging wasps (Aculeata). Finally, we located the evolutionary root of bees within the apoid wasp family "Crabronidae." Our results reveal that the extant sawfly diversity is largely the result of a previously unrecognized major radiation of phytophagous Hymenoptera that did not lead to wood-dwelling and parasitoidism. They also confirm that all primarily parasitoid wasps are descendants of a single endophytic parasitoid ancestor that lived around 247 mya. Our findings provide the basis for a natural classification of Hymenoptera and allow for future comparative analyses of Hymenoptera, including their genomes, morphology, venoms, and parasitoid and eusocial life styles.

RESULTS AND DISCUSSION

We sequenced whole-body transcriptomes of 167 species of Hymenoptera and selected outgroups and supplemented our

dataset with sequenced and annotated genomes of five hymenopterans and a beetle (for details, see Supplemental Experimental Procedures and Data S1A–S1D). Our study includes 54 families of Hymenoptera, representing all major superfamilies. The phylogenetic inferences are based on the analysis of 1.5 million amino acid and 3.0 million nucleotide positions, respectively, derived from 3,256 single-copy protein-coding genes (Data S1E) and inferred by using a combination of domain-, gene-, and codon position-based data partition schemes to improve the fitting of the applied substitution models. Considering the taxonomic and molecular sampling, this is the most comprehensive dataset ever generated for investigating phylogenetic relationships within Hymenoptera or any other insect group. The dataset was furthermore used to estimate divergence times with an independent-rates as well as with a correlated-rates molecular clock approach (Data S1H) and a validated set of 14 fossils (Data S1F).

The inferred phylogenetic relationships and divergence time estimates were used to assess where in the phylogeny of Hymenoptera, when in their geological history, and how often major evolutionary transitions took place. Specifically, we studied the switch from feeding on plants to feeding on an insect host (parasitoidism), the formation of a wasp waist, the evolution of a venomous stinger to subdue mobile hosts, the evolution of eusociality, and the switch from hunting prey to collecting pollen. These evolutionary transitions are partially reflected by the historic classification of Hymenoptera: sawflies (''Symphyta'') are those Hymenoptera that lack the wasp waist that characterizes all remaining Hymenoptera (Apocrita), ''Parasitica'' encompasses the primarily parasitoid Apocrita that lack a stinger, and Aculeata comprises the stinging wasps, ants, and bees (Anthophila) [1]. Yet, how many major lineages each of these groups encompasses has been controversial for decades [4–11].

The results of our phylogenomic study received strong support in all analyses, unless stated otherwise, and alter previous ideas regarding the evolutionary history of Hymenoptera (Figure 1B; for full results and detailed experimental procedures, see Figure S1, Supplemental Experimental Procedures, and additional figures deposited at Mendeley Data, http://dx.doi.org/10.17632/s5j2f62z3d.2). According to our analyses, extant Hymenoptera started to diversify between the Carboniferous and the Triassic (95% confidence interval [CI]: 329–239 million years ago [mya]; mean: 281 mya; node 1 [n.1] in Figure 1B), with the oldest currently known Hymenoptera fossils being from the Triassic, ~224 million years old [8]. Previous studies suggested this divergence to have occurred between the sawfly lineage Xyeloidea and the remaining Hymenoptera [5, 7–11], whereas our analysis identified a much more inclusive clade of sawflies (Eusymphyta; n.2) that also contains Pamphilioidea and Tenthredinoidea as closest relatives of all remaining Hymenoptera (Unicalcarida). These superfamilies had been thought to form a paraphyletic grade [5, 7, 9, 11]. Instead, they represent an unexpected and previously unrecognized major radiation of primarily ectophytophagous insects that comprises more than 7,000 described species [1]. We estimate the first diversification of the extant eusymphytan lineages to have occurred 276–157 mya (mean 212 mya). Note that Eusymphyta were corroborated as the sister group of all remaining Hymenoptera when additionally scrutinizing the analyzed molecular data for conflicting phylogenetic signal (Supplemental Experimental Procedures). Given the novelty and importance of our finding, we anticipate that it will significantly influence future research on Hymenoptera relationships, and we encourage researchers to further assess this particular phylogenetic hypothesis in future studies, for example by extending the taxon sampling within Eusymphyta and the outgroup.

A clade Eusymphyta representing the extant sister lineage of all remaining Hymenoptera (Unicalcarida) has profound consequences for inferring ground-plan characters of Hymenoptera. For example, Hymenoptera were previously thought to have been ancestrally ectophytophagous, based on the assumption that eusymphytans form a paraphyletic assemblage. Considering that the sister group of Hymenoptera (Aparaglossata) was ancestrally likely predacious [12], the inferred relationship between Eusymphyta and Unicalcarida implies that the most recent common ancestor of Hymenoptera could have been ecto- or endophytophagous. A sister group relationship between Eusymphyta and Unicalcarida furthermore implies that the remarkable ability of male Hymenoptera to restore diploidy in their muscle cells was already present in the last common ancestor of all Hymenoptera (with a secondary loss in Xyelidae), or that this feature evolved at least twice (in Unicalcarida and Tenthredinoidea) [13]. Finally, the unexpected finding that the turnip sawfly, *Athalia rosae* (Tenthredinoidea), whose genome has recently been sequenced by the i5K initiative [14], is a representative of the sister lineage of all remaining Hymenoptera will improve our understanding of the genetic composition of the most recent common ancestor of Hymenoptera: genomic features shared between the turnip sawfly and species of Unicalcarida with sequenced genomes (e.g., *Nasonia* parasitoid wasps, ants, bees) were likely inherited from their common ancestor.

In agreement with earlier studies [9, 10], we found a single origin of the endophytic sawfly lineages (i.e., Cephoidea, Orussoidea, Siricoidea, and Xiphydrioidea; n.3), which form a paraphyletic grade, in which Orussoidea (parasitoid woodwasps) represent the closest relatives of Apocrita (n.4). Morphological data have suggested a sister group relationship of Orussoidea and Apocrita (Vespina) [6, 15], but results from analyzing molecular data have been inconsistent [7, 9]. Our analyses provide strong support for the monophyly of Vespina and of Apocrita (n.5) and imply that the bulk of primarily parasitoid wasps are descendants of a single endophytic parasitoid ancestor that lived in the Permian or in the Triassic (CI: 289–211 mya; mean: 247 mya). Contrary to earlier hypotheses of sawfly relationships (see [10]), we identified Cephoidea, and not Siricoidea and/or Xiphydrioidea, as the closest extant relatives of Vespina (n.6), a result only recently suggested [7].

The evolution of the wasp waist, a constriction between the first and the second abdominal segment greatly improving the maneuverability of the abdomen's rear section, including the ovipositor, was a major innovation in the evolution of Hymenoptera that undoubtedly contributed to the rapid diversification of Apocrita (n.5) [6]. Our analysis is the first to persuasively demonstrate that the most diverse parasitoid wasp lineages (i.e., Ceraphronoidea, Ichneumonoidea, and Proctotrupomorpha) constitute a natural group (Parasitoida; n.7) whose astonishing radiation was likely triggered by further optimization of the parasitoid lifestyle and related traits (e.g., endoparasitoidism,

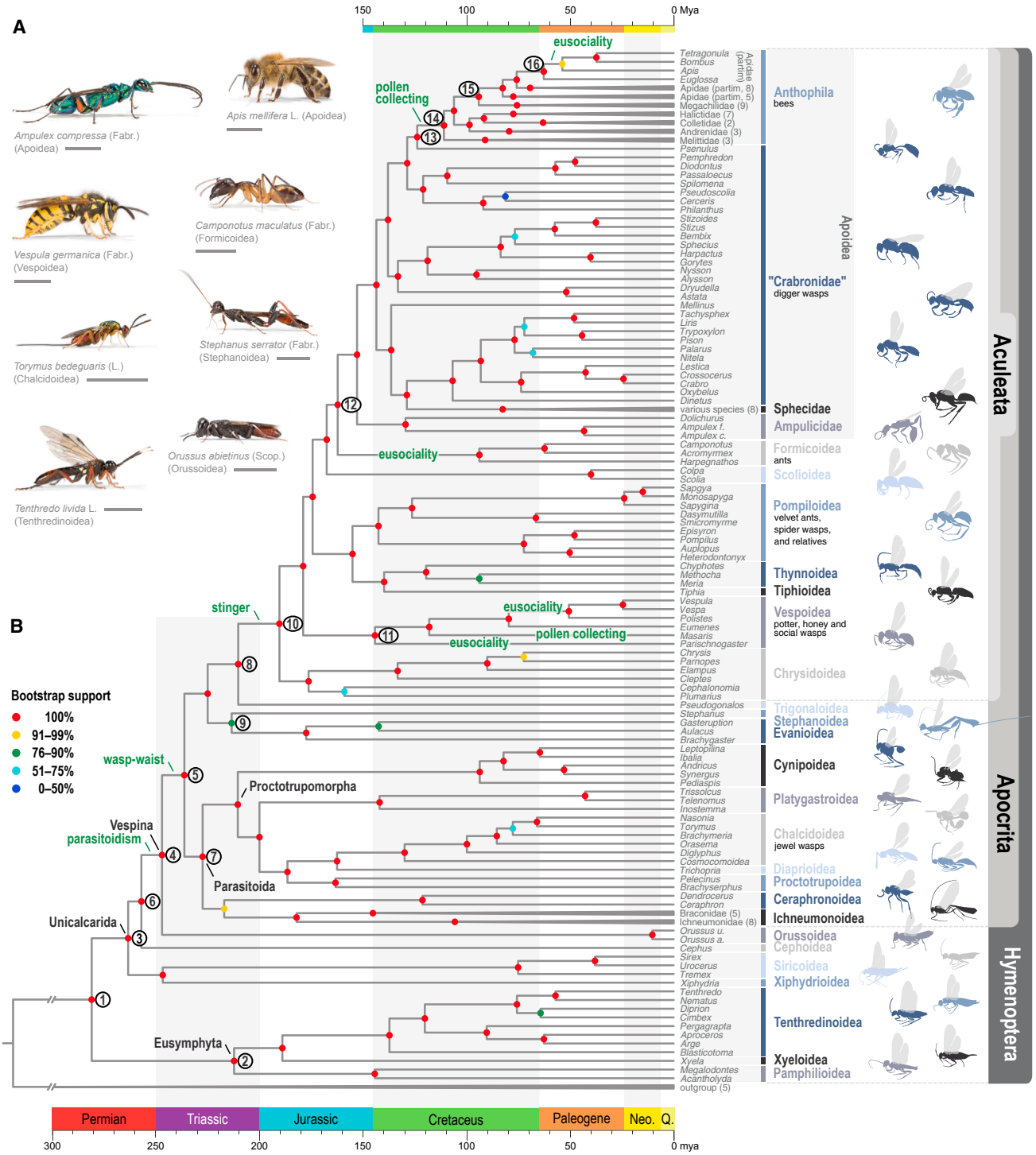

Figure 1. Evolutionary History of the Hymenoptera

(A) Representatives of sawflies, wasps, ants, and bees. Scale bars represent 5 mm.

(B) Phylogenetic relationships and divergence time estimates of Hymenoptera. Key evolutionary events are indicated at the respective clades (note that only the major eusocial lineages are considered). The tree was inferred under the maximum-likelihood optimality criterion, analyzing 1,505,514 amino acid sites and applying a combination of protein domain- and gene-specific substitution models. Divergence times were estimated with an independent-rates molecular clock approach and considering 14 validated fossils. Triangular branches cover multiple species (number of species in parentheses) whose relationships are shown in detail in Figure S1. Nodes with circled numbers are referred to in the main text.

miniaturization), which allowed for successfully attacking a variety of new hosts. We estimate the beginning of the group's radiation at 266–195 mya (mean: 228 mya), only a few million years after Parasitoida separated from the remaining Apocrita (CI: 276–203 mya; mean: 236 mya). The early radiation of Parasitoida thus falls within a time period when the parasitoids' major host lineages (e.g., Hemiptera, Holometabola) also started to diversify [16].

We identified the enigmatic Trigonaloidea as the closest extant relatives of Aculeata with strong node support (n.8), a hypothesis only recently put forth [7, 9]. Evanioidea, which had also been discussed as a possible sister group of Aculeata [5, 10, 17, 18], cluster with Stephanoidea (n.9). Node support for this relationship is low, however, and it needs to be investigated further in future studies that include additional types of characters and samples of Megalyroidea, a lineage that we were unable to sequence. Note that in contrast to Aculeata, the Evanioidea, Stephanoidea, and Trigonaloidea have all remained species-poor. The identification of the closest relatives of Aculeata will be important for better understanding which traits (e.g., venoms) fostered the diversification of the stinging wasps.

Our analysis sheds new light on the phylogeny of Aculeata (n.10), whose early diversification occurred 224–160 mya (mean: 190 mya). Chrysidoids are confirmed as the sister group of all remaining Aculeata [19]. We corroborate the artificial nature of the former superfamily "Vespoidea" (i.e., all Aculeata except Apoidea and Chrysidoidea) [5], which comprises four major lineages that are paraphyletic with respect to Apoidea [20]. The potter, honey, and social wasps (Vespoidea sensu Pilgrim et al. [20]: Vespidae; n.11) were identified as the sister lineage of all remaining non-chrysidoid Aculeata. However, the phylogenetic position of the species-poor Rhopalosomatidae (Vespoidea sensu Pilgrim et al. [20]), an aculeate wasp family that we were unable to sequence and possible sister lineage of Vespidae, remains controversial [9, 10, 20]. The inferred phylogenetic relationships within Vespidae suggest two independent origins of eusociality, a previously fiercely contested hypothesis [21, 22]. In agreement with an earlier phylogenomic study [23], we inferred ants (Formicoidea) as being the closest extant relatives of Apoidea (n.12) in all of our analyses, except when applying a Bayesian approach, which suggested ants plus scoliid wasps (Scolioidea, possibly including also the family Bradynobaenidae [20], which we were unable to sequence) as being sister to Apoidea (figure deposited at Mendeley Data, http://dx.doi.org/10.17632/s5j2f62z3d.2). We estimate the last common ancestor of ants and Apoidea to have lived in the Jurassic or the Cretaceous (CI: 192–136 mya; mean: 162 mya).

We located the phylogenetic origin of bees (Anthophila) within the apoid wasp family "Crabronidae" (n.13), which our study shows to be an artificial construct comprising five major lineages. The crabronid wasp lineage in our study most closely related to bees is the species-poor tribe Psenini. This result substantiates the idea that the switch from a predatory to a herbivorous lifestyle was a key to the tremendous diversification of bees [24]. We estimate the origin of bees to have been in the Cretaceous (CIs: 147–93 mya; means: 124 and 111 mya), a result that is consistent with a close temporal link between the diversifications of bees and angiosperms [24]. Melittid bees were identified as the sister lineage of all remaining Anthophila (n.14), which implies that short-tongued bees do not represent a natural group. In contrast, we confirmed long-tongued bees (i.e., Apidae and Megachilidae) to constitute a natural entity (n.15) [24]. We also found the eusocial apid bee lineages to be monophyletic, corroborating the hypothesis that eusociality has evolved once, not twice, in corbiculate (pollen basket) bees (n.16) [25].

Our study confirms the power of phylogenomic approaches for deciphering difficult-to-resolve arthropod phylogenetic relationships [12, 16, 26, 27] by yielding well-supported answers to some of the most pressing questions regarding the evolutionary history of the sawflies, wasps, ants, and bees. We provide strong evidence for understanding the phylogenetic relationships among all major lineages of Hymenoptera, and we were able to date the individual divergence events, both paramount for deciphering the tempo and mode of diversification of ecologically, economically, sociobiologically, and/or pharmaceutically relevant traits of interest (e.g., gene repertoires, haplodiploidy and sex determination, eusociality, chemosensation, and venoms). Finally, our study offers the basis for establishing a natural classification of the insect order Hymenoptera.

EXPERIMENTAL PROCEDURES

We sequenced the transcriptomes of 134 species of Hymenoptera using Illumina HiSeq 2000 sequencing technology (Data S1A–S1C). We complemented our dataset by including previously published transcriptomes of 29 Hymenoptera and four Neuropteroidea [16, 28]. Finally, we considered the official gene sets of five Hymenoptera and the flour beetle *Tribolium castaneum* (Data S1D). All paired-end reads were assembled with SOAPdenovo-Trans-31kmer (version 1.01) [29], the assembled transcripts were filtered for possible contaminants, and the raw reads and filtered assemblies were submitted to the NCBI SRA and TSA archives. We searched the assemblies with the software Orthograph (version beta4) [28] for transcripts of 3,260 protein-coding genes that the OrthoDB v7 database [30] suggested to be single-copy in Hymenoptera and Neuropteroidea (outgroup) by applying the best reciprocal hit criterion. Orthologous transcripts were aligned with MAFFT (version 7.017) [31] at the translational (amino acid) level. All multiple sequence alignments (MSAs) were quality assessed and, if necessary, improved and masked using the procedure outlined by Misof et al. [16]. The resulting MSAs were concatenated to a supermatrix that we simultaneously partitioned based on a combination of Pfam protein domains and genes [16]. The phylogenetic information content of each partition was assessed with MARE (version 0.1.2-rc) [32], and all uninformative partitions were removed. We subsequently used PartitionFinder (developer versions 2.0.0-pre2, 2.0.0-pre9, and 2.0.0-pre10) [33] to simultaneously infer a partition scheme and proper amino acid substitution models for analyzing each partition with the rcluster algorithm. We applied the same partition scheme when analyzing the corresponding supermatrix at the transcriptional (nucleotide) level, except that we modeled the first and second codon position of each partition separately (note that we excluded the hypervariable third codon position from our analyses). Phylogenetic trees were reconstructed with ExaML (versions 3.0.15 and 3.0.17) [34], conducting 50 independent tree searches per supermatrix. Node support was inferred with the bootstrap method [35]. Decisive datasets were used for testing the possible impact of missing data at the partition level on the inferred phylogenetic tree [36], and four-cluster likelihood mapping was used for assessing the phylogenetic signal for alternative phylogenetic relationships [37]. Permutation tests allowed assessing the impact of heterogeneous amino acid sequence composition, non-stationarity of substitution processes, and non-random distribution of missing data on the inferred phylogenetic tree [16]. We additionally conducted phylogenetic inferences in a Bayesian framework, using ExaBayes [38] with its default settings, enabling automatic substitution model detection and applying the same data partitioning scheme that we used in analyses under the maximum-likelihood optimality criterion. We analyzed three independent runs with four coupled Markov chain Monte Carlo

chains and 200,000 generations each. The consense tool (part of the ExaBayes software package) was used to obtain a consensus tree based on the extended majority rule method (MRE), discarding the first 25% of the sampled topologies as burn-in. Divergence times were calibrated using 14 fossils (Data S1F), selected following best-practice recommendations [39] and representing extant lineages distributed across the entire Hymenoptera Tree of Life. Divergence times were estimated with mcmctree in conjunction with codeml (both part of the PAML software package, version 4.9) [40]. We analyzed a subset of the amino acid and of the nucleotide supermatrix, both comprising only sites that had amino acids or nucleotides present in at least 95% of the species, both with an independent-rates model and with a correlated-rates model (Figure 1B; Data S1H) and sampling parameters previously assessed for convergence of results.

Data Resources

Data reported in this paper have been published in Mendeley Data and are available at http://dx.doi.org/10.17632/trbj94zm2n.2 (inferred matrices and statistics) and http://dx.doi.org/10.17632/s5j2f62z3d.2 (figures). All sequencing data are available at NCBI via the Umbrella BioProject accession number NCBI: PRJNA183205 ("The 1KITE project: evolution of insects").

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure, Supplemental Experimental Procedures, and one dataset and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2017.01.027.

AUTHOR CONTRIBUTIONS

B.M., L.K., O.N., and R.S.P. conceived the study. C.P., J.H., K.M., K.M.K., L.K., O.N., P.D., R.M., R.S.P., S.K., and T.S. collected or provided samples. A.D., K.M., L.P., O.N., R.S.P., S.L., and X.Z. sequenced, assembled, and processed the transcriptomes. A.K., C.M., K.M., M.P., O.N., R.L., and R.S.P. phylogenetically analyzed the transcriptomes. J.R., L.K., O.N., R.S.P., S.G., and T.W. are responsible for the dating of the inferred phylogeny. All authors contributed to the writing of the manuscript, with L.K., O.N., and R.S.P. taking the lead.

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