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Evolutionary timescale of chalcidoid wasps inferred from over one hundred mitochondrial genomes

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ABSTRACT

Chalcidoidea is one of the most biologically diverse groups among Hymenoptera. Members are characterized by extraordinary parasitic lifestyles and extensive host ranges, among which several species attack plants or serve as pollinators. However, higher-level chalcidoid relationships remain controversial. Here, we performed mitochondrial phylogenomic analyses for major clades (18 out of 25 families) of Chalcidoidea based on 139 mitochondrial genomes. The compositional heterogeneity and conflicting backbone relationships in Chalcidoidea were assessed using various datasets and tree inferences. Our phylogenetic results supported the monophyly of 16 families and polyphyly of Aphelinidae and Pteromalidae. Our preferred topology recovered the relationship (Mymaridae+(Signiphoridae+Leucospidae)+(Chalcididae+((Perilampidae+Eucharitidae)+ remaining Chalcidoidea))). The monophyly of Agaonidae and Sycophaginae was rejected, while the gall-associated ((Megastigmidae+Ormyridae)+(Ormocerinae+Eurytomidae)) relationship was supported in most results. A six-gene inversion may be a synapomorphy for most families, whereas other derived gene orders may introduce confusion in phylogenetic signals at deeper nodes. Dating estimates suggested that Chalcidoidea arose near the Jurassic/Cretaceous boundary and that two dynamic shifts in diversification occurred during the evolution of Chalcidoidea. We hypothesized that the potential codiversification between chalcidoids and their hosts may be crucial for accelerating the diversification of Chalcidoidea. Ancestral state reconstruction analyses supported the hypothesis that gall-inducers were mainly derived from parasitoids of gall-inducers, while other gall-inducers were derived from phytophagous groups. Taken together, these findings advance our understanding of mitochondrial genome evolution in the major interfamilial phylogeny of Chalcidoidea.

Keywords: Mitochondrial genome; Chalcidoidea; Compositional heterogeneity; Divergence time; Evolution of host and gall associations

INTRODUCTION

Chalcidoidea is a hyper-diverse group of wasps within Hymenoptera, estimated to contain 500 000 species. The group comprises 25 extant families, one extinct family (Diversinitidae, identified from Cretaceous Burmese amber) (Haas et al., 2018), 81 subfamilies (Heraty et al., 2013; Janšta et al., 2018; Zhang et al., 2022), and more than 22 500 described species (Heraty & Darling, 2009; Janšta et al., 2018; Noyes, 2019). Chalcidoid wasps vary considerably in body size, exhibiting tremendous lifestyle diversity and complex parasitoid strategies, which makes them important in agricultural ecosystems and biological control (Debach & Rosen, 1991; Greathead, 1986; Heraty, 2017). They can attack a wide range of insects within about 340 families in 13 orders, such as Lepidoptera, Hemiptera, and Orthoptera, and

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even spiders, mites, and Nematoda, at all host life stages from egg to adult (Austin et al., 1998; Gibson, 1999; Grissell & Schauff, 1997). Recent research has suggested that egg parasitism is ancestral, arising in various lineages within Chalcidoidea (Heraty et al., 2013). Although most species are parasitoids, phytophagous lifestyles exist in nine families, i.e., Agaonidae, Eulophidae, Eurytomidae, Megastigmidae, Ormyridae, Pteromalidae, Tanaostigmatidae, Tetracampidae, and Torymidae (Böhmová et al., 2022). Moreover, most species are gall-inducers, inquilines, or parasitoids of gall-inducing insects (Gómez et al., 2011; Grissell & Schauff, 1997; La Salle, 2005; Zerova, 1993). Gall-inducing species have arisen many times in Chalcidoidea, mostly from progenitor parasitoids of gall inducers, while the remaining gall-inducers most likely originated from phytophagous wasps (La Salle, 2005).

Although Chalcidoidea families are phylogenetically well studied, including Trichogrammatidae (Owen et al., 2007), Eulophidae (Burks et al., 2011), Eucharitidae (Baker et al., 2020; Murray & Heraty, 2020; Zhang et al., 2022), Chalcididae (Cruaud et al., 2021), Agaonidae (Cruaud et al., 2011, 2012), and Torymidae (Janšta et al., 2018), only a few comprehensive phylogenetic studies on families within the superfamily have been conducted, utilizing ribosomal genes (18S rDNA and 28S rDNA) (Munro et al., 2011), morphological characters and molecular data (233 characters and 18S rDNA and 28S rDNA) (Heraty et al., 2013), and transcriptomic data (Peters et al., 2018; Zhang et al., 2020b). In addition, the complexity of rapid radiations within Chalcidoidea poses a considerable challenge in generating a robust phylogenetic topology. Peters et al. (2018) provided the first phylogenomic analysis of 17 Chalcidoidea families based on 3 239 single-copy genes from 37 taxa, shedding light on the rapid diversification and evolutionary success of Chalcidoidea by elucidating the evolution of hind femoral enlargement, jump ability, and fig association. However, their study provided poor support for backbone relationships. Zhang et al. (2020b) attempted to improve the poorly resolved relationships of the same 17 Chalcidoidea families using increased taxa (a total of 55) and gene sampling (a total of 5 591), which resulted in different topologies. Despite extensive sampling and analysis in the two previous studies, most interfamilial relationships remain controversial. Notably, both studies excluded Signiphoridae and Sycophaginae, and had conflicting phylogenetic positions for Epichrysomallinae.

Mitochondrial genomes (mitogenomes) have been widely used to resolve relationships among deep Hymenoptera lineages (Song et al., 2016; Tang et al., 2019; Wei et al., 2010b, 2014). The mitogenomes of animals are usually AT-rich and show positive AT skewness and negative GC skewness on their majority strand (Reyes et al., 1998; Wei et al., 2010a). As heterogeneity in evolutionary rates can generate artefactual phylogenetic clustering (Liu et al., 2018; Rota-Stabelli et al., 2010), biased base composition may also lead to phylogenetic conflicts (Hassanin, 2006; Xu et al., 2021; Zhang et al., 2019). Nevertheless, the large-scale mitochondrial characteristics of Chalcidoidea have yet to be thoroughly investigated. In addition, mitochondrial gene rearrangement has provided additional phylogenetic evidence for different levels of controversial topologies in Coleoptera (Nie et al., 2021; Timmermans & Vogler, 2012) and mites (Ban et al., 2022; Li et al., 2019), but primarily at lower taxonomic levels in Hymenoptera (Li et al., 2016). Unique rearrangements involving five or six protein-coding gene (PCG) inversions, (*nad2*–) *cox3–atp6–atp8–cox2–cox1*, have been detected in a few families of Chalcidoidea (Wu et al., 2020; Xiao et al., 2011; Yi et al., 2022; Zhu et al., 2018), suggesting they may be synapomorphies for Chalcidoidea (Cameron, 2014). However, whether mitochondrial gene rearrangements have phylogenetic signals to help resolve the relationships of Chalcidoidea remains to be discovered.

In the present study, we inferred the phylogenetic evolution of the superfamily Chalcidoidea using mitogenomes. Based on broad taxon sampling combined with mitogenomic data, we aimed to (i) infer major relationships within Chalcidoidea and their early branches, (ii) compare some alternative phylogenetic hypotheses using several topological and heterogeneity tests, especially the monophyly of Agaonidae, (iii) assess the base composition skewness of Chalcidoidea and discuss the phylogenetic implications of gene rearrangements, (iv) estimate the divergence time of major Chalcidoidea lineages, and (v) discuss the evolution of host/gall-associations and parasitic lifestyles.

MATERIALS AND METHODS

Taxon sampling and mitogenome sequencing

Mitogenomic data were newly obtained from 104 species using individual samples, representing 18 families in Chalcidoidea. Among them, 99 sequenced mitogenomes were obtained from genomic DNA. The remaining five mitogenomes were retrieved from genomic data in NCBI (four from whole genomic data and one from transcriptome data) (Supplementary Table S1). Seven families were not included (Azotidae, Cynipencyrtidae, Eriaporidae, Rotoitidae, Tanaostigmatidae, Eutrichosomatidae, and Chrysolampidae) because they were rarely collected, or we failed to obtain their specimens or DNA sequences. Species identifications were mainly based on external morphological characters and verified by Dr. Yan-Zhou Zhang, Dr. Ling-Fei Peng, and coauthors Dr. Hui Xiao, Dr. Da-Wei Huang, and Dr. Chao-Dong Zhu. Whole genomes were extracted from the mesosoma and legs of each specimen using a DNeasy Blood and Tissue Kit (Qiagen, Germany). The DNA concentration in every sample was determined using a Qubit 4.0 fluorometer (Invitrogen, Life Technologies, USA). Several distantly related species were mixed roughly in equimolar concentrations, sonicated to 300 bp using a Covaris S220 focused-ultrasonicator, and pooled into different libraries. The remaining species were individually pooled in a single library with an insert size of 200 bp. The libraries were constructed using a VAHTS® Universal DNA Library Prep Kit for Illumina V3 and sequenced on the Illumina HiSeq sequencer platform.

Mitogenome assembly and annotation were conducted using bioinformatics pipelines (Timmermans et al., 2016) and mitoZ (Meng et al., 2019), respectively. After trimming adapter contamination with fastp v0.20.1 (Chen et al., 2018), the mitochondrial sequences were filtered from the clean data using FastqExtract script, with individual DNA library assembly using IDBA_tran v1.1.3 (Peng et al., 2012), Ray v2.3.1 (Boisvert et al., 2012), and SPAdes v3.13.0 (Bankevich et al., 2012). Geneious v2020.2 (Biomatters Ltd., USA) was used to obtain larger contigs from the above assembly software and transferred to mitoZ (Meng et al., 2019) for gene annotation. Transfer RNA (tRNA) genes were confirmed using the

tRNAscan-SE search server (Lowe & Eddy, 1997) and Mitos web server (Bernt et al., 2013). For each assembled sequence mixture, mitogenomes were assigned to corresponding individual samples using the *cox1* gene searched against sequences in the BOLD system, placing each mitogenome to at least the family level. Voucher samples and DNA were deposited at the Institute of Insect Sciences, Zhejiang University, Hangzhou, China.

Our initial dataset included 139 chalcidoid terminal taxa. The mitogenomes of 104 chalcidoid taxa were newly generated, while the remaining 35 mitogenomes were obtained from NCBI (as of July 2021).

Nucleotide (NT) feature analysis

Base composition was calculated using AMAS (Borowiec, 2016). AT skew and GC skew were calculated using AT skew=(A–T)/(A+T) and GC skew=(G–C)/(G+C). DnaSP v6.0 (Rozas et al., 2017) was used to calculate the mean synonymous (*K*s) and nonsynonymous substitution rates (*K*a) for each taxon. Gene rearrangement scenarios were assessed using CREx based on common intervals and orientations for PCGs, tRNA genes, ribosomal RNA (rRNA) genes, and AT regions of chalcidoid mitogenomes with pairwise comparisons to ancestral insect genes (Bernt et al., 2007). Scaled GC content values and mean *K*s and *K*a ratios for each taxon were determined using the R package PHEATMAP. DAMBE7 was used for saturation analyses and cumulative skews were plotted for all mitochondrial PCGs (PCG123), PCGs excluding the third codon position (PCG12), and only the third codon (P3) (Xia, 2018; Xia et al., 2003). The heterogeneity of NT variation among sequences was analyzed for different datasets with AliGROOVE v1.05 (Kück & Longo, 2014). The BLOSUM62 matrix was used as a substitution model to assess heterogeneity in the translated amino acid (AA) sequences.

Phylogenetic analysis

Phylogenetic data matrices of 145 taxa (139 ingroups and six outgroups from Proctotrupoidea and Diaprioidea) and reduced datasets of 143 taxa, 129 taxa, and 114 taxa were inferred from two combination schemes: (i) 13 mitochondrial PCGs with the first and second codon position (PCG12) partitions only (*n*=26) and (ii) AA of 13 mitochondrial PCG (13AA) partitions (*n*=13). Bayesian inference (BI) analyses were carried out using ExaBayes v1.5.1 (Aberer et al., 2014) for 145 and 143 taxa, while maximum-likelihood (ML) analyses were performed using IQ-TREE v1.6.8 (Nguyen et al., 2015) for all datasets (145, 143, 129, and 114 taxa).

Summarized information for each constructed data matrix is shown in Table 1. Full matrices containing all 145 taxa (139 ingroups and six outgroups) were reconstructed for initial phylogenetic analyses (referred to as ML/BI-AA-145 and ML/BI-PCG12-145, respectively). Within Agaonidae, *Ceratosolen solmsi* and *Dolichoris vasculosae* were excluded due to potential long-branch attraction (referred to as ML/BI-AA-143 and ML/BI-PCG12-143, respectively). To reduce the impact of NT synonymous changes, a Perl script (Degen_v1_4.pl) was used to degenerate the first and third codon positions of NTs to International Union of Pure and Applied Chemistry (IUPAC) ambiguity codes (referred to as ML-Degen-NT-143). Moreover, considering the overall compositional heterogeneous taxa within Encyrtidae, Chalcididae, and Pteromalidae, only ML trees were constructed for the reduced datasets of 129 species with lower GC content and shorter branch length. Signiphoridae was also excluded in datasets to detect the position of Epichrysomallinae (referred to as ML-AA-129 and ML-PCG12-129, respectively). In addition, more balanced sampling within each family was considered, and ML phylogenetic trees for 114 species were reconstructed to save computing time (referred to as ML-AA-114 and ML-PCG12-114, respectively).

NT and AA sequences of PCGs were aligned using MAFFT v7.205 (Katoh & Standley, 2013) under default parameters. The Perl script TranslatorX v1.1 (Abascal et al., 2010) was used for NT sequence alignment based on translated AA sequences. Concatenated matrices were then constructed from the aligned data using the Perl script FASconCAT-G v1.0 (Kück & Longo, 2014). PartitionFinder v2.1.1 (Lanfear et al., 2017) was applied to generate the best partition schemes and substitution models for each matrix. Each codon position for NT sequences of PCGs was chosen as the input for PartitionFinder to determine possible partition schemes. Protein domain-based data blocks were directly applied to PartitionFinder for analysis. The applied NT substitution model was set to “MrBayes” and Bayesian information criterion (BIC) was used to select the best partitioning scheme. Branch lengths were set as linked for AA and unlinked for PCG12.

For BI analyses, four independent ExaBayes runs, each with four coupled Markov Chain Monte Carlo (MCMC) chains,

Table 1 Summary information of each matrix used in phylogenetic analyses

Number of taxa	Matrix	Sequence length	Analysis method	Type	Usage of matrices
	BI-AA-145	3 321	BI	AA	Phylogenetic analyses
145	ML-AA-145	3 321	ML	AA	
	BI-PCG12-145	8 772	BI	PCG12	
	ML-PCG12-145	8 772	ML	PCG12	
	BI-AA-143	3 323	BI	AA	Long-branch attraction tests
143	ML-AA-143	3 323	ML	AA	
	ML-PCG12-143	8 586	BI	PCG12	
	BI-PCG12-143	8 586	ML	PCG12	
	ML_Degen-NT-143	12 879	ML	NTDegen	Synonymous change elimination test
129	ML-AA-129	3 323	ML	AA	Compositional heterogeneity tests
	ML-PCG12-129	8 528	ML	PCG12	
114	ML-AA-114	3 336	ML	AA	Balanced sampling tests
	ML-PCG12-114	8 302	ML	PCG12	

BI: Bayesian inference; ML: Maximum-likelihood; AA: Amino acid; NT: Nucleotide; PCG: Protein-coding genes.

were completed. After 10 000 000 generations, the convergence of results was determined through the average deviation of split frequencies (ASDSF criterion) and an ASDSF value below 2% was usually obtained. A majority-rule extended (MRE) consensus tree was then obtained using the consensus tool in the ExaBayes package, and the first 25% of sampled topologies were discarded. The automatic model selection strategy implemented in ExaBayes was chosen for the PartitionFinder-determined partitions. Different partition schemes were used in the BI-PCG12-143 tree because using partition settings with unlinked branch lengths led to topological convergence in the BI-PCG12-145 tree but not in the BI-PCG12-143 tree.

The best-fitting substitution model for each PartitionFinder-determined partition was identified using ModelFinder implemented in IQ-TREE (MFP). All available substitution models were tested for the AA and NT supermatrices. Branch supports were assessed with ultrafast bootstrap (Minh et al., 2013) and SH-aLRT tests (Guindon et al., 2010) using 10 000 replicates for 145 and 143 datasets. For the 129 and 114 datasets, only the ultrafast bootstrap approach (Minh et al., 2013) was used to assess branch supports.

Node support tests

Critical nodes defining basal relationships in Chalcidoidea were analyzed by four-cluster likelihood mapping (FcLM) implemented in IQ-TREE v1.6.8 (Nguyen et al., 2015). The possible supports for three alternative quartet topologies were assessed. The likelihood of each quartet was exhibited as a triangle, with every corner representing one of the three *a priori*-specified monophyletic groups. The points inside the triangle represented support for the *a priori* topology determined by the quartets of terminals drawn. Both the concatenated NT and AA sequence matrices were used for the tests. ModelFinder was used to determine the models for PCG12 and AA alignments. IQ-TREE was used to assess the significance of differences in tree topologies constructed under different methods and datasets of tree searches, and the likelihood of data was determined using Shimodaira-Hasegawa (SH) and approximately unbiased (AU) tests.

Gene rearrangement analyses

PhyloSuite v1.2.2 (Zhang et al., 2020a) was used to generate an iTOL dataset file of gene orders, with *cox1* as the starting gene after reordering. The dataset file was further utilized to visualize gene orders in the iTOL website (https://itol.embl.de). In addition, we categorized the order of PCGs of 139 species into 18 complete types and 13 incomplete types depending on PCG completeness of each species. We used the MLGO (Hu et al., 2014) web server (http://www.geneorder.org/server.php) to reconstruct topologies with non-tRNA gene orders using the Phylogenetic Tree Reconstruction inference setting.

Divergence time estimation

Divergence time for the initial matrix of 144 taxa (excluding *D. vasculosae*) was calculated from the concatenated NT and AA matrices using BEAST v2.6.2 (Bouckaert et al., 2019) under a lognormal relaxed clock model (Drummond et al., 2006). Data partitions and model selections were chosen as previously identified by PartitionFinder. The optimal topologies inferred by ExaBayes analyses (BI-AA-144 and BI-PCG12-144, excluding *D. vasculosae*) were used as a fixed input topology for analyses. Nine fossil calibrations (Supplementary Table S2) were used with soft bounds (Yang & Rannala, 2006), in which two fossils were from outgroups. Furthermore, one calibration point for the divergence of (Eucharitidae+Perilampidae) was set to 88.6 million years ago (Ma) as the median age based on the estimation of Zhang et al. (2022). We performed two independent MCMC runs for 300 million generations, with sampling every 5 000 generations. Tracer v1.7.1 was used to examine the burn-in sample sizes and convergence stationarity. We used LogCombiner v2.6.7 to resample the tree files from two runs with a frequency of 10 000 and removed the first 25% generations as burn-in, with the remaining trees combined for annotation. The summarized tree was then generated using TreeAnnotator v1.8.3 with median heights as node heights and visualized with FigTree v1.4 (Rambaut, 2012).

Ancestral state reconstruction

Members of Chalcidoidea are extremely diverse in biological lifestyle and host associations, even at the genus level. Here, we generalized ecological traits based on genus-level characteristics obtained from the Chalcidoidea database (Noyes, 2019) and previous literature (Supplementary Table S3). The associated host records are shown with different colored squares mapped beyond terminal taxa on BI-AA-144 (excluding *D. vasculosae*). As taxa have broad host ranges at the genus level, we refined most family levels of host records to their higher taxonomic level to represent host associations. We only selected orders in the five most abundant insect orders if host records were excessive. In all, the chalcidoid hosts were classified into nine groups, including Hymenoptera, Lepidoptera, Diptera, Coleoptera, Hemiptera, Mantodea, Orthoptera, Thysanoptera, and plants (phytophagous).

Ancestral state reconstruction (ASR) was performed with four characters on the BI-AA-144 and BI-PCG12-144 topologies using stochastic character mapping (SCM), parsimony mapping (PM), and likelihood mapping (LM). Polymorphic states were used in the parsimony method and coded as mix states in LM. All three methods were used for the ASR of host associations and SCM was used for the ASR of gall associations, whereas ASR of the other two parasitism strategies were inferred by PM and LM. Parsimony and likelihood reconstructions were performed in Mesquite v3.61 (Maddison & Maddison, 2019) using the "trace character history" option and unordered character state transformations. We chose the equal-rates (ER) model and used the "make.simmap" function in the R package phytools (Revell, 2012) for stochastic character mapping analyses.

(1) Host orders: we used full host records and predominant host records for ASR, respectively, due to their wide host ranges. 0, Hemiptera; 1, Hymenoptera; 2, Lepidoptera; 3, Diptera; 4, Plant; 5, Orthoptera; 6, Coleoptera; 7, Mantodea; 8, Thysanoptera (and 9, mix state, in LM).

(2) Gall-associations: Gall-inducing insects mostly exist in Hymenoptera, Lepidoptera, Diptera, and Coleoptera, so the above nine host types were classified into three gall-associated states, i.e., phytophagy, parasitoids of gall-associated insects, and other strategies.

(3) Primary parasitism or hyperparasitism: 0, Primary; 1, Hyperparasitoid; 2, Primary or Hyperparasitoid (or coded as mix state in LM).

(4) Solitary or gregarious parasitism: 0, Solitary; 1, Gregarious; 2, Solitary or Gregarious (or coded as mix state in LM).

RESULTS

Mitogenomic characteristics and heterogeneity in Chalcidoidea

Our study generated 104 newly sequenced or assembled mitogenomes in 18 Chalcidoidea families, with the mitogenomes of seven families (Eucharitidae, Leucospidae, Megastigmidae, Ormyridae, Perilampidae, Signiphoridae, and Tetracampidae) reported for the first time. The GC content in the PCGs with inclusion and exclusion of third positions differed substantially among taxa, as did the mean *Ka*/*Ks* rates (Supplementary Figure S1). At the family level, Agaonidae had a relatively higher GC content and *Ka*/*Ks* rate than most other families. Chalcididae had the highest *Ka*/*Ks* rates, whereas Tetracampidae had the lowest. Two non-pollinating fig wasps, Sycophaginae and Epichrysomallinae, exhibited mid-range GC content but relatively high *Ka*/*Ks* (Supplementary Figure S1B). At the species level, *Copidosoma chalconotum*, *Encyrtus infelix*, and *Syrphophagus nigricornis* (Encyrtidae) exhibited high GC content correlated with comparatively low *Ka*/*Ks* rates. *Ceratosolen solmsi* (Agaonidae) showed high GC content and high *Ka*/*Ks* rates, indicating NT heterogeneity (Supplementary Figure S1A).

All chalcidoid species exhibited negative AT skewness (lower A than T content), and almost all species exhibited positive GC skewness (higher G than C content) (Supplementary Figure S2A). However, Tetracampidae and *Eupelmus* sp. 1 (Eupelmidae) showed negative GC skewness, the same as the outgroup (Supplementary Figure S2A). The GC skewness of most mitochondrial genes was positive. Tetracampidae was unique in that most genes showed reverse GC skewness patterns. More than half of the mitochondrial genes with negative GC skewness in Tetracampidae exhibited positive skewness in other families (Supplementary Figure S2B).

Analysis of PCG123, PCG12, and P3 indicated that P3 experienced substitution saturation (Supplementary Figure S3). Sequence heterogeneity was assessed in AliGROOVE for different datasets separately (Supplementary Figure S4). In general, the PCG12 dataset with the third codon position excluded yielded lower sequence composition heterogeneity than the PCG123 dataset. No obvious heterogeneous outliers were detected.

Phylogenetic analyses and incongruence hypothesis

The initial phylogenetic matrix included 139 chalcidoid mitogenomes, representing 18 families and 33 subfamilies. Six mitogenomes from Proctotrupoidea and Diaprioidea were used as the outgroup taxa (Supplementary Table S1).

We conducted BI and ML analyses using two different datasets (PCG12 and 13AA) for the initial 145 taxa due to the high saturation rates of the third codon. Overall, all trees strongly supported the sister-group relationship between Diaprioidea and Chalcidoidea, monophyly of Chalcidoidea, and Mymaridae as sister to the remaining Chalcidoidea (UFboot=100, BPP=1) (Figure 1; Supplementary Figures S5–S8). The monophyly of 16 families and polyphyly of Pteromalidae and Aphelinidae were consistently recovered in almost all analyses. The monophyly of Agaonidae (Kradibiinae+Agaoninae) and Sycophaginae was rejected in most results except in the ML-AA-145 tree (Figure 1; Supplementary Figures S5–S8).

All analyses recovered Chalcididae and (Signiphoridae+Leucospidae) as early branching lineages (with or without Epichrysomallinae), followed by Perilampidae+Eucharitidae, which formed a clade with the remaining families (Figure 1; Supplementary Figures S5–S8). However, their inner relationships were not consistently resolved or well supported, except for the sister-group relationship between Perilampidae and Eucharitidae, which was robustly recovered in all analyses (UFboot=100, BPP=1). Epichrysomallinae was weakly clustered with the (Signiphoridae+Leucospidae) clade in the ML-PCG12-145 and ML-AA-145 trees (UFboot=26/41) but strongly clustered in the BI-PCG12-145 tree (BPP=0.97).

Several clades were moderately recovered in all or most trees (three out of four). In the ML/BI-AA-145 and BI-PCG12-145 trees, the monophyletic clade of ((Megastigmidae+Ormyridae)+(Ormocerinae+Eurytomidae)) (clade B) was moderately supported (UFboot=67 and BPP>0.87) (Figure 1; Supplementary Figures S5–S8). The monophyly of (Megastigmidae+Ormyridae) was moderately supported in all four trees (UFboot>58, BPP>0.87). The monophyly of clade C was also moderately recovered (UFboot=60, BPP>0.77), although several inner relationships within the clade were inconsistent across trees. Within this clade, the monophyly of Tetracampidae+Diparinae was strongly supported (UFboot=87 and BPP>0.98) in three trees; the monophyletic clade of (Miscogastrinae+((Sycoryctinae+Otitesellinae)+Pteromalinae)) was robustly recovered in all analyses (UFboot=100, BPP=1); and the close relationships between Trichogrammatidae, Cleonyminae, and Aphe_part2 (*Aphelinus certus*+*Eretmocerus* sp.) were recovered in all analyses (Figure 1; Supplementary Figures S5–S8).

The phylogenetic positions of Epichrysomallinae, Sycophaginae, and Agaonidae were discrepant among the tree construction methods. In the ML/BI-PCG12-145 datasets, the Agaonidae clade was recovered as sister to Torymidae (Figure 1; Supplementary Figures S6, S8). In the AA trees, they either formed a monophyletic clade with Sycophaginae (ML) or with the clade of (Sycophaginae+Epichrysomallinae) (BI) (Figure 1; Supplementary Figures S5–S8). Other incongruent topologies among the trees were mainly due to the polyphyletic families Aphelinidae and Pteromalidae. In the ML/BI-AA-145 tree, Coccophaginae was always the sister group to clade C, but not in the PCG12 tree (Figure 1; Supplementary Figures S5–S8).

MLGO analyses using non-tRNA genes showed that Chalcidoidea contained some of the hypothetical ancestral gene arrangements of arthropods (i.e., Mymaridae, Signiphoridae, and Leucospidae) located at the early branches (Supplementary Figure S9).

Effects of compositional heterogeneity and long branches

Dolichoris vasculosae (Agaoninae) was erroneously clustered into Kradibiinae. Despite finding no compositionally heterogeneous taxa in the chalcidoid samples, *Dolichoris vasculosae* (Agaoninae) and *Ceratosolen solmsi* (Kradibiinae) exhibited relatively high GC content and the longest branch lengths. However, after removing these two taxa, the deep nodes in the ML/BI-AA-143 and ML-PCG12-143 trees were not affected (Figure 2; Supplementary Figures S10–S13) compared to those based on the original dataset of 145 taxa. In addition to the different placements of Eupelmidae, Sycophaginae, and Tetracampidae, the topologies of BI-PCG12-143 and BI-PCG12-145 were largely congruent with

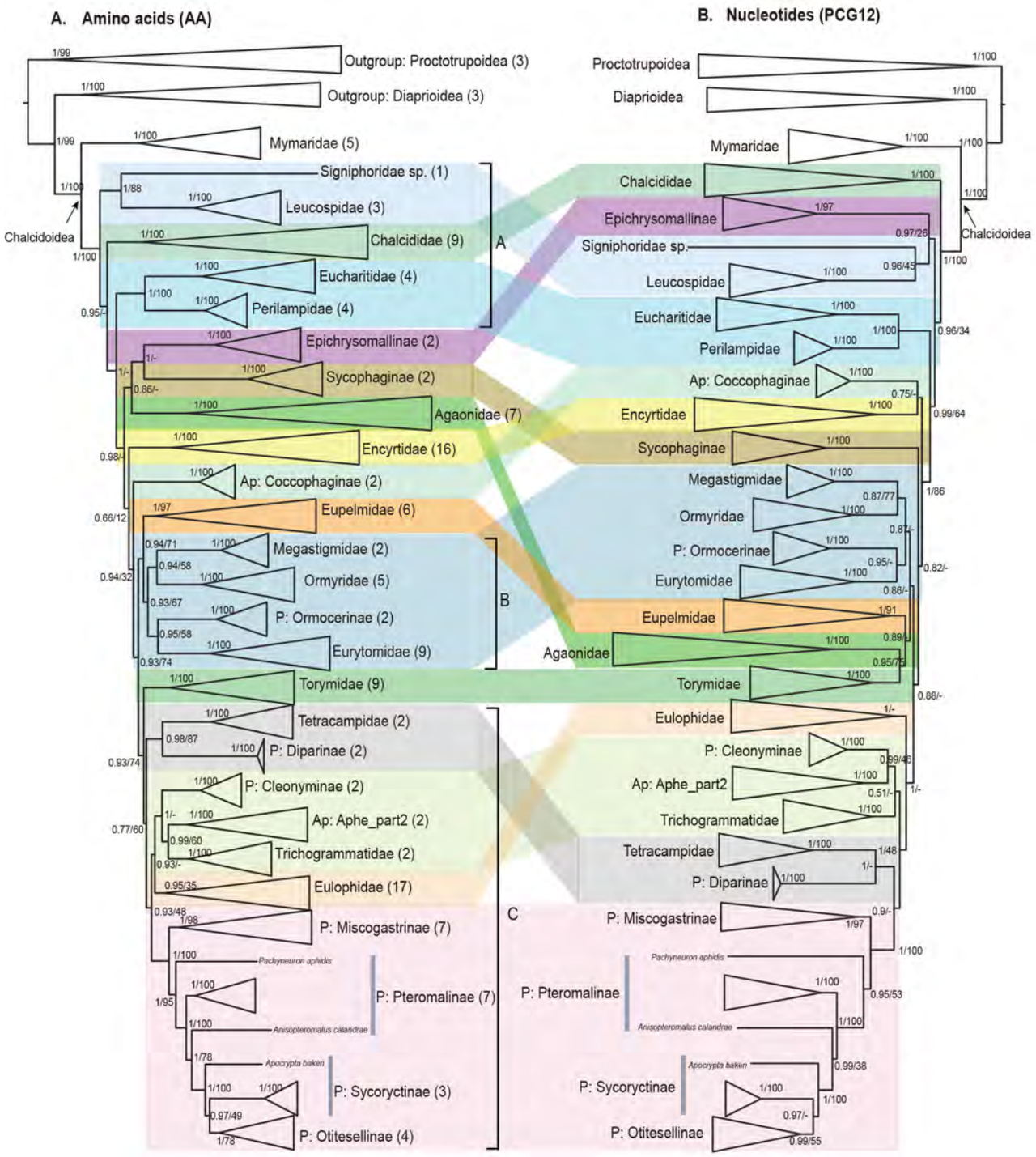

Figure 1 Phylogenetic incongruences between AA-145 (A) and PCG12-145 datasets (B)

Topology generated from Bayesian analyses with optimal partitioning scheme selected with PartitionFinder2 based on genes (A) and codons (B). Numbers around nodes from left to right are Bayesian posterior probabilities (BPP) and UFboot values. Dash means nodes are not recovered. P: Pteromalidae; Ap: Aphelinidae; Aphe_part2 means another clade of Aphelinidae. Different colors represent different monophyletic clades in phylogenetic trees. Two phylogenies are compressed to family or subfamily levels and detailed relationships are shown in Supplementary Figures. Number of sampled species for each family or subfamily is indicated in brackets behind it.

each other (Figure 2; Supplementary Figures S6, S11). *Wiebesia pumilae* (Agaoninae) was wrongly placed as sister to Kradibiinae in the ML-AA-143 tree only. Moreover, the phylogenetic tree generated by the degenerated NT dataset was more similar to the AA trees than the PCG12 trees (Supplementary Figure S14). The ML trees from the reduced datasets of 129 and 114 species using the PCG12 matrices showed almost identical topologies to the ML-PCG12-145 and ML-PCG12-143 trees with improved support values. Nevertheless, the AA dataset had reasonably congruent topologies to the ML-AA-145 and ML-AA-143 trees (Figure 2; Supplementary Figures S15–S18).

Conflicting topology tests

For the position of Epichrysomallinae, various FcLM analyses

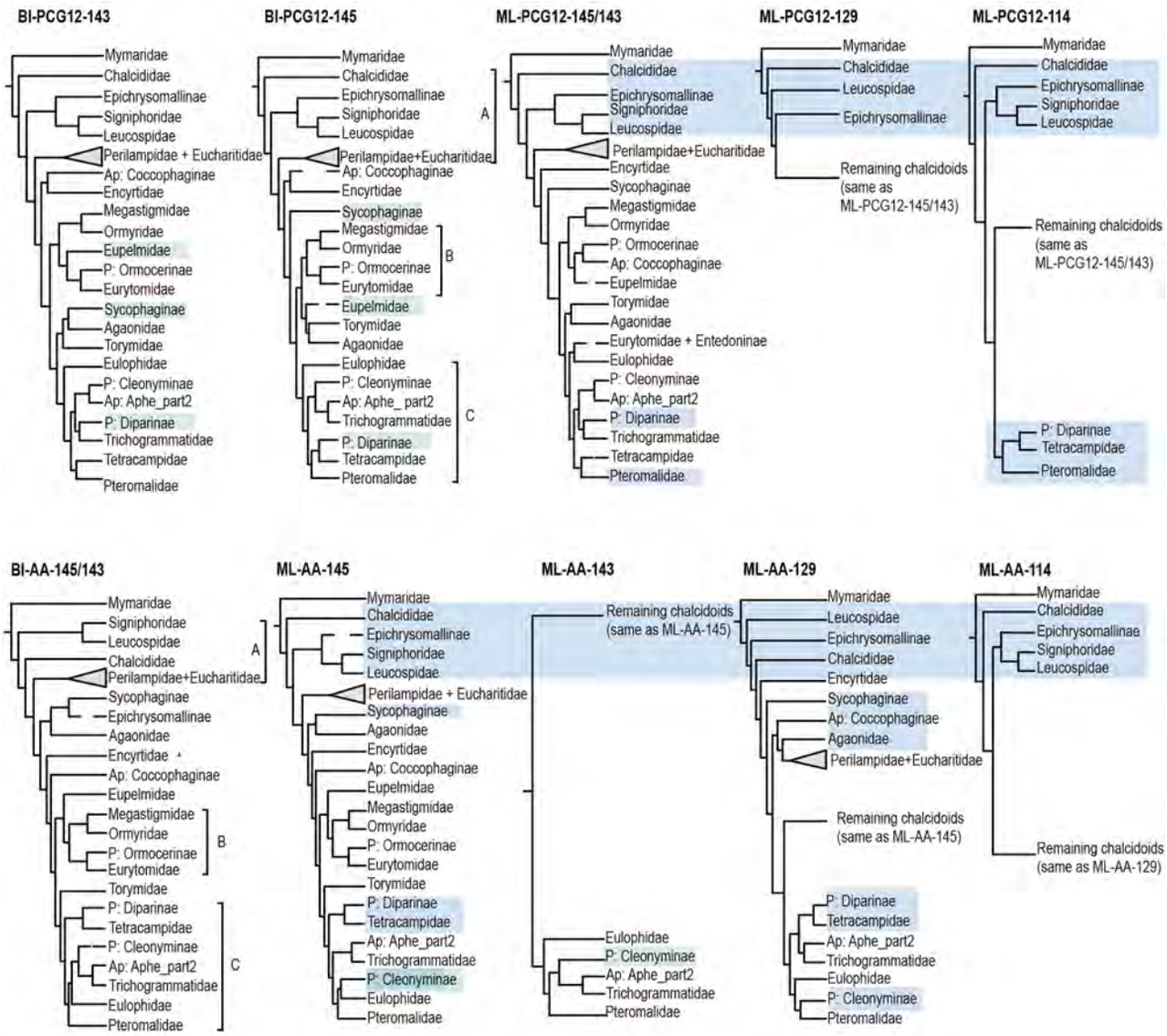

Figure 2 Alternative hypotheses of higher-level relationships within Chalcidoidea

Different phylogenetic trees of Chalcidoidea from various tree-building methods and datasets. Green background indicates discrepancies between BI-PCG12-145 and BI-PCG12-143 trees and between ML-AA-145 and ML-AA-143 trees. ML-PCG12-145 tree was identical to ML-PCG12-143 tree and BI-AA-145 tree was identical to BI-AA-143 tree. Blue backgrounds imply differences among ML trees from 145, 143, 129, and 114 datasets. Dashed lines indicate differences in ML and BI trees from 145 datasets. P: Pteromalidae; Ap: Aphelinidae; Aphe_part2 means another clade of Aphelinidae.

preferred the sister-group relationship between Epichrysomallinae and Sycophaginae (Figure 3). However, the conflicting placement of Agaonidae was not resolved based on our FcLM analyses; it was either closer to Sycophaginae and Epichrysomallinae, as favored by the AA dataset, or formed a sister relationship with Torymidae, as supported by the PCG12 dataset (Figure 3).

Based on pairwise analysis, the topologies of ML/BI-AA-145 were not rejected when using the AA dataset and the topologies of ML/BI-PCG12-145 were not rejected when using the PCG12 dataset (Table 2).

Diversified gene arrangements in Chalcidoidea

Rearrangements in the Chalcidoidea mitogenomes involved all three gene types (PCGs, rRNAs, and tRNAs), with gene inversion and translocation frequently observed. The tRNA genes were more susceptible to rearrangement than the PCGs and rRNAs. The gene cluster *cox1–trnL2–cox2–trnK–trnD–atp6–atp8–cox3–trnG–nad3* was largely inversed (except for *trnK* occasionally) and was detected at least once in 12 families (Supplementary Figure S19, pink background).

When considering only PCGs and rRNAs, the 111 species with complete PCGs showed 18 types (represented by types 1–18), whereas the 28 species with incomplete PCGs showed 13 types (represented by IM1–IM13). Mymaridae (Ancestral type 1, types 2 and 3), Signiphoridae (IM1), and Leucospidae (type 4) shared the primitive PCG order *cox1–cox2–atp8–atp6–cox3–nad3* (except *Polynema* sp., Figure 4; Supplementary Figure S20). In contrast, the remaining families of Chalcidoidea in our study shared a unique inversion rearrangement (*nad3–cox3–atp6–atp8–cox2–cox1*). Some exceptions to the above gene inversion cluster showed additional rearrangements (types 8, 9, 11, 15, 16, and 18 and IM12; Figure 4; Supplementary Figure S20). Types 5 and 6 were the most shared PCG orders within/among chalcidoid

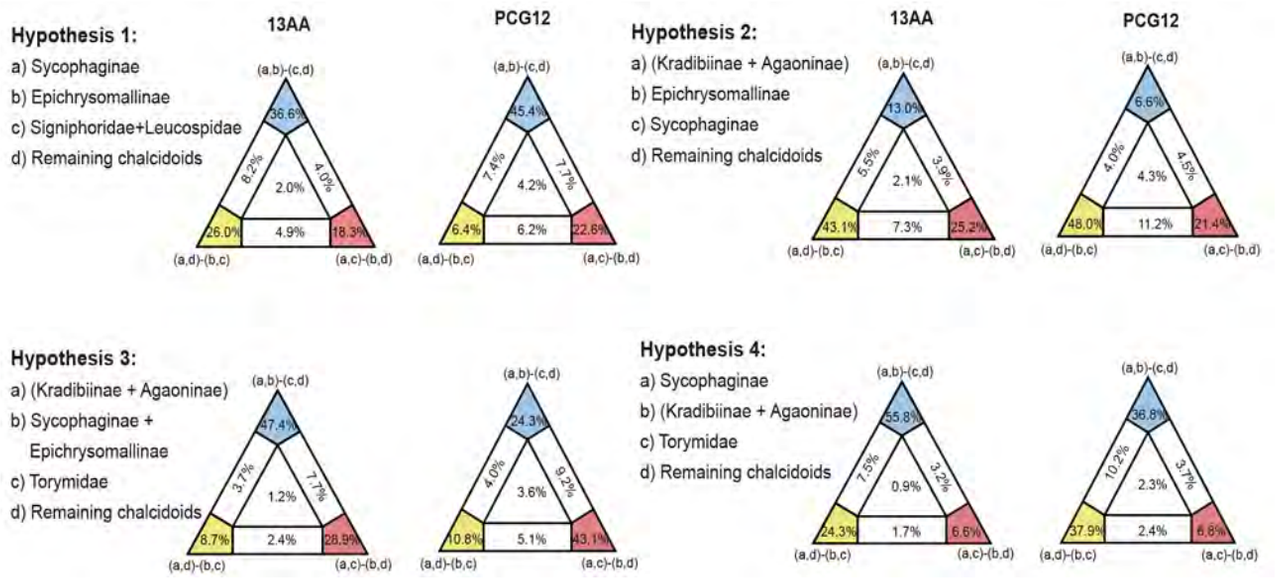

Figure 3 Four-cluster likelihood mapping for Chalcidoidea clade showing major conflicting hypotheses

Analyses are based on matrices of PCG123, PCG12, and 13AA sequences. Four *a priori* groups in analysis were: (i): Possible relationships of Sycophaginae (a), Epichrysomallinae (b), Signiphoridae+Leucospidae (c), and remaining chalcidoid taxa (d); (ii): Possible relationships of Kradibiinae+Agaoninae (a), Epichrysomallinae (b), Sycophaginae (c), and remaining chalcidoids (d); (iii): Possible relationships of Kradibiinae+Agaoninae (a), Sycophaginae+Epichrysomallinae (b), Torymidae (c), and remaining chalcidoids; (iv): Possible relationships of Sycophaginae (a), Kradibiinae+Agaoninae (b), Torymidae (c), and remaining chalcidoids.

Table 2 Assessment of conflicting tree topologies using Shimodaira-Hasegawa (SH) and approximately unbiased (AU) tests

Tree	logL	deltaL	bp-RELL	*P*-SH	*P*-WSH	c-ELW	*P*-AU
1	–330680.19	9.8645	0.38+	0.674+	0.647+	0.381+	0.417+
2	–330670.33	0	0.608+	1+	0.874+	0.607+	0.672+
3	–392937.23	27.944	0.253+	0.473+	0.497+	0.252+	0.3+
4	–392937.23	0	0.737+	1+	0.88+	0.738+	0.776+

BI and ML topologies from 13AA (Tree1, Tree2) and PCG12 (Tree3, Tree4) based on AA and PCG12 datasets, respectively, were subjected to significance tests using IQ-tree. BI: Bayesian inference; ML: Maximum-likelihood; AA: Amino acid; PCG: Protein-coding genes. deltaL: LogL difference from maximal logL in set. bp-RELL: Bootstrap proportion using RELL method (Kishino et al., 1990). *P*-SH: *P*-value of SH test. *P*-WSH: *P*-value of weighted SH test. c-ELW: Expected likelihood weight (Strimmer & Rambaut 2002). *P*-AU: *P*-value of AU test (Shimodaira, 2002). +: Values within 95% confidence sets. −: Significant exclusion. All tests included 1 000 resamplings using RELL method.

families except in Mymaridae, Leucospidae, and Signiphoridae, consistent with the patterns of the derived inversion of *nad2* (Figure 4; Supplementary Figure S20).

Seventeen genera from 14 families, with more than one species sampled from one genus, exhibited various intro-generic gene rearrangements, ranging from one tRNA gene translocation (*trnQ*: *Halticoptera* (Pteromalidae)) to several gene inversions and translocations (six PCGs and six tRNAs: *Philotrypesis* (Pteromalidae)). Nevertheless, tRNA rearrangement patterns were conserved within/among several groups (Supplementary Figure S19).

Divergence time estimations

The estimated divergence times of Chalcidoidea from the partitioned AA and PCG12 matrices exhibited similar node ages, differing by <10 Ma (Figure 5; Supplementary Figure S21). Therefore, we only summarized the results based on the AA matrix. Divergence time analysis indicated that extant Chalcidoidea originated during the very early Cretaceous (142.76 Ma, 95% confidence interval (CI): 129.68–157.62 Ma, node 1) (Figure 5). Subsequently, the crown clade of the remaining Chalcidoidea originated at 130.99 Ma (CI: 118.52–144.39 Ma, node 2). The family Chalcididae diverged from the major lineages of Chalcidoidea at 126.87 Ma (CI: 114.9–140.25 Ma, node 3). Divergence between (Perilampidae+Eucharitidae) and the main group of chalcidoids occurred at 119.76 Ma (CI: 108.34–132.42 Ma, node 4). The most recent common ancestor (MRCA) of clade B originated at 89.37 Ma (CI: 80.13–100.25 Ma, node 5) and diverged from the remaining chalcidoids at 98.74 Ma (CI: 88.71–109.13 Ma, node 6). The origin of Agaonidae occurred at 80.25 Ma (CI: 68.09–92.52 Ma, node 7). The MRCA of clade C originated at 89.56 Ma (CI: 80.08–99.53 Ma, node 8), with the (Sycoryctinae+Otitesellinae) group dated at 45.01 Ma (CI: 37.97–53.09 Ma, node 9).

Ancestral state reconstruction

The ASR analyses based on the AA and PCG12 trees exhibited similar results using different methods. Notably, ASR predicted that the ancestor of Chalcidoidea was the primary and solitary parasitoid of Hemiptera. Detailed results regarding host associations and other parasitism/parasitoid strategies are presented in Supplementary Nexus Files S1, S2 and Supplementary Figures S22–S32.

Phytophagy independently evolved at least eight times: once in (Agaonidae+(Sycophaginae+Epichrysomallinae)), Ormocerinae, Eurytomidae, Torymidae, (Sycoryctinae+Otitesellinae), and Pteromalinae, and twice in Tetrastichinae.

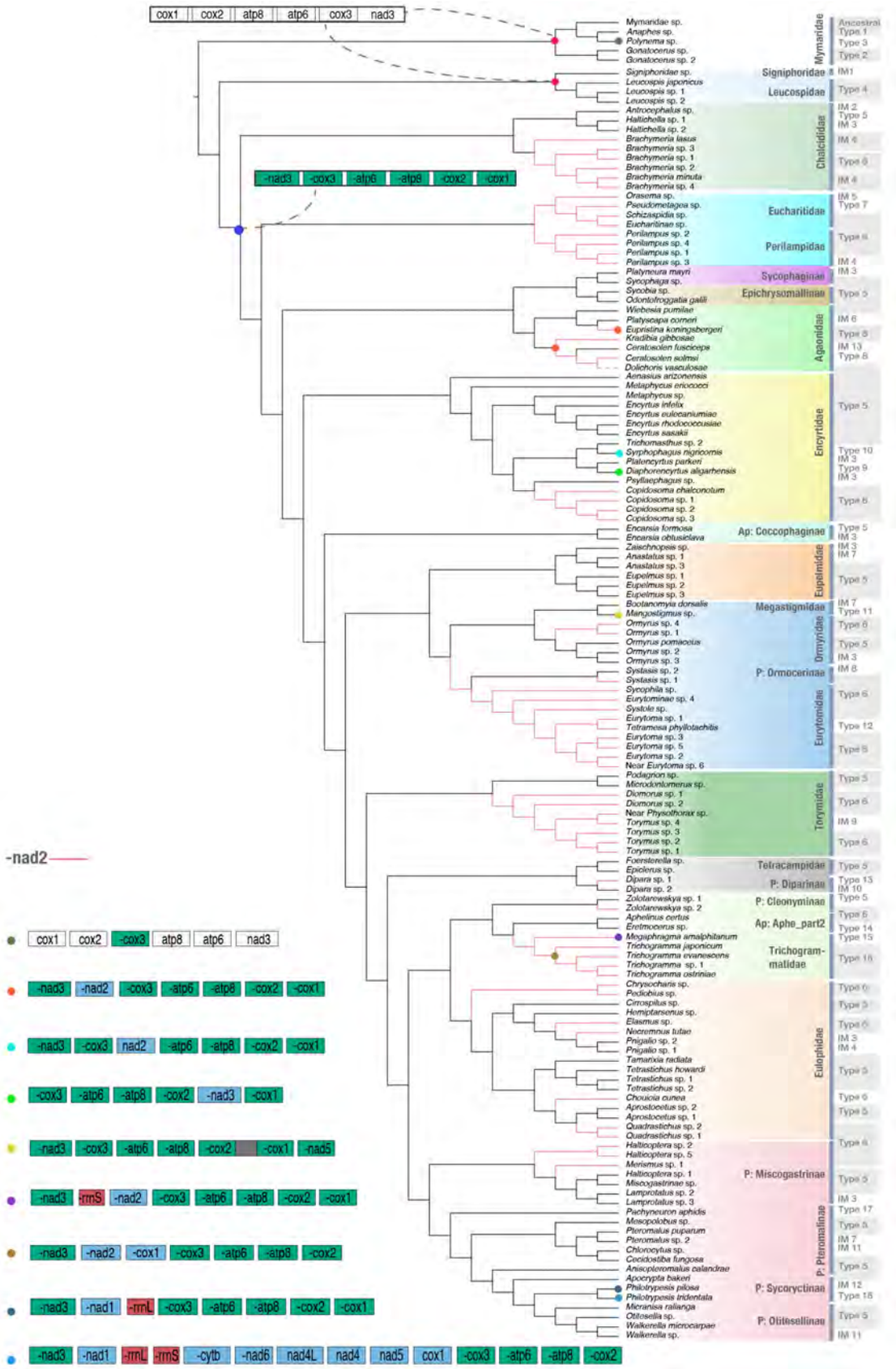

Figure 4 Phylogeny of Chalcidoidea and non-tRNA gene rearrangement patterns

Topology was generated by ExaBayes based on AA-145 dataset (BI-AA-145). Circles in front of taxon are 11 pairs of rearrangement states (color legend for circles is left of tree). Types 1 to 18 indicate rearranged gene patterns from complete mitogenomes. IM1–IM13 represent rearranged gene patterns from incomplete mitogenomes. Red branch indicates derived gene order patterns of *nad2*. Different colors represent different monophyletic clades in phylogenetic tree.

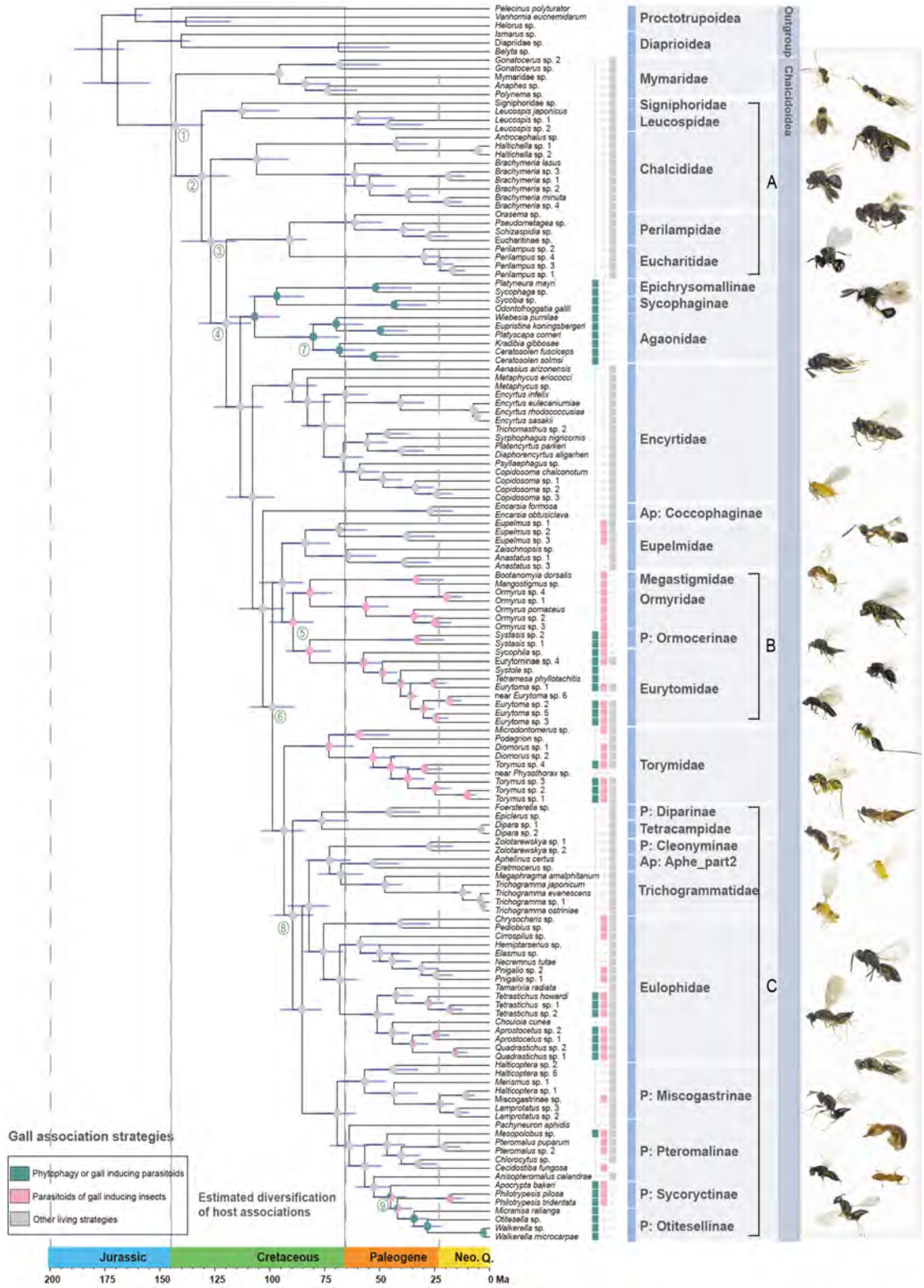

Figure 5 Chronogram showing phylogeny and divergence time estimation and ancestral state reconstruction of gall-association in Chalcidoidea

Consensus tree presenting divergence times produced by BEAST analysis of BI-AA-144 tree using nine fossils and one calibration point. Blue bars indicate 95% mean confidence intervals of each node. Geological timescale is shown at bottom. Nodes with circled numbers refer to: MRCA of Chalcidoidea (node 1); MRCA of remaining chalcidoids excluding Mymaridae (node 2); MRCA of Chalcididae and remaining chalcidoids (node 3); MRCA of (Perilampidae+Eucharitidae) and remaining chalcidoids (node 4); MRCA of clade B (node 5) and MRCA of remaining chalcidoids (node 6); origin of Agaonidae (node 7); MRCA of clade C (node 8); MRCA of (Sycoryctinae+Otitesellinae) (node 9). Squares with different colors beyond terminal taxon indicate three states of gall associations (color legends for squares and circles is left of tree). P, Pteromalidae; Ap, Aphelinidae; Aphe_part2 means another clade of Aphelinidae.

The parasitoids of gall-forming insects exhibited at least 12 independent origins: once in Eupelmidae, (Megastigmidae+Ormyridae)+(Ormocerinae+Eurytomidae), Torymidae, Entedoninae, Miscogastrinae, and Sycoryctinae, twice in Tetrastichinae and Eulophinae, and three times in Pteromalinae (Figure 5).

Based on ASR analysis, parasitism of gall-associated insects was recovered as the ancestral strategy of ((Megastigmidae+Ormyridae)+(P: Ormocerinae+Eurytomidae)) (clade B), Torymidae, (*Aprostocetus*+*Quadrastichus*), and (Sycoryctinae+Otitesellinae), and originated in the mid-Cretaceous (94.36 Ma) (Figure 5).

DISCUSSION

We annotated 104 new Chalcidoidea mitogenomes from 18 out of 25 families and 33 out of 81 subfamilies, representing the most comprehensive phylogenetic and comparative genomics study on Chalcidoidea. We observed some incongruences between the AA and NT trees and implemented various dataset treatments and tree inference methods to improve the resolution of the backbone relationships. In addition, we found that the reversal of strand asymmetry may be generally associated with the diverse mitochondrial gene rearrangements in major Chalcidoidea lineages.

Heterogeneity and conflicting topology in Chalcidoidea

Mitochondrial genes are influenced by biased NT composition, rapid evolutionary rates, and different substitution levels (Cameron, 2014; Nie et al., 2021; Tang et al., 2019). These features may lead to artificial phylogenetic clustering and incongruences between NT and AA datasets. Based on the AliGROOVE results, no obvious heterogeneity in sequence composition was observed, except for outgroup taxon *Belyta* sp., which should have limited influence on the backbone topologies. Moreover, phylogenetic trees inferred from the same datasets (PCG12 or AA) were more consistent regarding major clades than those inferred using the same tree-building methods (BI or ML), indicating that different datasets may have a significant impact on phylogenetic inferences (Figure 2; Supplementary Figures S5–S8, S10–S18).

We also assessed whether tree topology was improved by applying different treatments, such as the complex model, smaller datasets with suitable GC content and branch length, or balanced sampling. The long-branch clade Agaonidae (excluding Sycophaginae) was in an unstable position in all phylogenetic analyses. Two taxa (*Ceratosolen solms*i and *Dolichoris vasculosae*) with the longest branch lengths were falsely clustered, leading to paraphyly of Agaoninae (Supplementary Figures S5–S8). Removing these two taxa had no effect on topology, except for some minor differences among BI-PCG12 trees, possibly due to different partition schemes (Figure 2; Supplementary Figures S5–S8, S10–S13). Moreover, when excluding heterogeneous taxa or using a balanced dataset, the backbone nodes were largely congruent with the initial trees inferred from 145 taxa (Figures 1, 2; Supplementary Figures S5–S8, S10–S18). Overall, the above treatments did not significantly improve backbone topologies.

Nearly all chalcidoid species displayed a high level of reversed composition skewness (TA and CG bias) (Supplementary Figure S2), which may lead to incongruences between the AA and NT topologies (Che et al., 2021). The phylogenies generated from the AA datasets and BI analyses had more consistent backbone topologies than the PCG12 datasets and ML analyses. The degenerated NT tree yielded similar topologies to the AA trees than the NT trees (Supplementary Figure S14), indicating that compositional heterogeneity may influence NT analyses. Furthermore, AA datasets have been suggested as more suitable for deep-level analysis (Li et al., 2003; Rota-Stabelli et al., 2010). Therefore, we used the AA tree as the preferred tree for further analyses (Figures 1A, 4).

The resolution of some clades remained inadequate despite extensive investigation of the lineages, such as the unstable positions of Agaonidae and Epichrysomallinae, polyphyly of Aphelinidae, and different early branching lineages compared to Peters et al. (2018) and Zhang et al. (2020b). Discrepancies in transcriptomic research may result from rapid mitochondrial evolution, which strongly interacts with nuclear-encoded mitochondrial-associated components (Yan et al., 2019). Our results suggested that chalcidoid species have a strong bias in base composition and should be carefully examined in future higher-level phylogenetic studies.

Phylogenetic relationships within Chalcidoidea

All phylogenetic trees recovered the monophyly of Chalcidoidea and supported Mymaridae as sister to the remaining Chalcidoidea, consistent with previous studies (Heraty et al., 2013; Munro et al., 2011; Peters et al., 2018; Zhang et al., 2020b). Our phylogenetic trees recovered the monophyly of 16 families and polyphyly of Pteromalidae and Aphelinidae (Campbell et al., 2000; Gibson et al., 1999; Heraty et al., 1997, 2013; Munro et al., 2011). The polyphyly of Pteromalidae and Aphelinidae has been widely suggested (Campbell et al., 2000; Heraty et al., 1997, 2013; Mohammad, 1998; Munro et al., 2011). However, the monophyly of Aphelinidae was robustly supported in recent research and recovered as a sister group of Encyrtidae (Zhang et al., 2020b). In our analyses, only Coccophaginae (a clade in Aphelinidae) was recovered as a sister group to Encyrtidae in the NT BI trees.

The monophyly of Perilampidae+Eucharitidae and sister relationship between Miscogastrinae and ((Sycoryctinae+Otitesellinae)+Pteromalinae) were strongly supported, similar to previous studies (Heraty et al., 2013; Heraty & Darling, 2009; Peters et al., 2018; Zhang et al., 2020b). The monophyly of Tetracampidae+Diparinae was also strongly supported. Eulophidae was recovered as a monophyletic group and closely related to Trichogrammatidae and Pteromalidae, different from its previous placement as an early branching lineage of Chalcidoidea (Peters et al., 2018; Zhang et al., 2020b). Within Eulophidae, the sister-group relationship between Eulophinae and Tetrastichinae and their close relationship with Entedoninae were recovered in most analyses, congruent with the hypotheses of Burks et al. (2011) and Heraty et al. (2013).

Several recent studies have proposed that egg parasitism may be ancestral in Chalcidoidea due to the position of Trichogrammatidae (Peters et al., 2018; Zhang et al., 2020b). However, we never recovered Trichogrammatidae (egg parasitoids of Lepidoptera) as an early branching lineage. On the contrary, all resulting topologies supported Chalcididae and (Signiphoridae+Leucospidae) (or with Epichrysomallinae) as the early branching lineages after Mymaridae. Moreover, when excluding Signiphoridae (Figure 2; Supplementary

Figures S15, S16), Chalcididae, Leucospidae, and Epichrysomallinae were still recovered as one of the early branching lineages. Such discrepancies may be partially explained by the rapid evolutionary rates and compositional heterogeneity in chalcidoid mitogenomes (Che et al., 2021; Xu et al., 2021) as well as the phylogenetic implications of PCG rearrangements (and MLGO analyses in our results). Recent mitochondrial research also supports Chalcididae as an early branching lineage when implementing the heterogeneity evolution model (Zhao et al., 2021; Zhou et al., 2021).

Signiphoridae was previously recovered as a sister of Azotidae with low support (Cruaud et al., 2019; Heraty et al., 2013). Our aberrant clustering of (Signiphoridae+Leucospidae) may be due to two reasons: firstly, MLGO analyses confirmed this relationship as both contained the most primitive gene order of *cox1–cox2–atp8–atp6–cox3–nad3*, as shared by Mymaridae; secondly, our phylogenetic analysis included only one species of Signiphoridae assembled from trace concentration DNA, which may not be sufficient for robust inference. Additionally, the absence of Azotidae in our study may also have influenced the observed clustering. More genomic data and extended taxon sampling are required to further clarify the evolutionary history of these highly diverse wasps.

Limited research has recovered the lineages containing most gall inducers, inquilines, and parasitoids of gall-inducer wasps, with only a few studies proposing close relationships among some (Cruaud et al., 2011; Janšta et al., 2018; Noyes, 1990). Our study suggested a moderately but consistently supported relationship of ((Megastigmidae+Ormyridae)+(Ormocerinae+Eurytomidae), which may indicate the putative gall-associated ancestral strategy. Megastigmidae was always recovered as sister to Ormyridae in our analyses, showing a closer relationship than to Torymidae, consistent with previous conclusions (Gómez et al., 2008; Janšta et al., 2018).

In all analyses, two non-pollinating fig associates (Otitesellinae and Sycoryctinae) well clustered with Pteromalinae, in accordance with earlier studies (Heraty et al., 2013; Peters et al., 2018; Zhang et al., 2020b). Gall-makers Sycophaginae and Epichrysomallinae were originally classified as subfamilies of Torymidae (Hill, 1967; Joseph, 1964; Wiebes, 1976), but later included in Agaonidae and Pteromalidae, respectively (Bouček, 1988). However, their taxonomic status and phylogenetic position with other pollinating fig wasps (Agaonidae) remain controversial. Our inferred phylogenetic trees and FcLM results suggest that Sycophaginae may not belong to Agaonidae, as most analyses recovered a non-monophyletic relationship.

Species of *Ormyrus*, Megastigmidae, Eurytomidae, Ormocerinae, *Microdontomerus*, and *Torymus* can attack gall-inducing insects at various life stages. Agaonidae, some Eurytomidae genera, and several *Torymus* species are also phytophagous (Janšta et al., 2018). Their similar ecological traits may increase the possibility of grouping among some of them. However, more genomic data and taxonomic sampling should be included in future research to test this hypothesis. We observed that the extraordinarily diverse chalcidoid groups in terms of host, body shape, color, and biology exhibited significant differences in mitogenome and transcriptome data.

Diversified gene rearrangements across lineages of Chalcidoidea

Our study provided overall mitochondrial compositional characterization and heterogeneity for 18 families in Chalcidoidea (including seven newly reported families) for the first time. Almost all families in Chalcidoidea presented complete reversal strand asymmetry, in contrast to that observed in typical insect mitogenomes, i.e., positive AT skew and negative GC skew (Wei et al., 2010a). The reversed skewness of the Chalcidoidea mitogenomes is likely accompanied by remarkable gene rearrangements (Jakovlić et al., 2021; Wei et al., 2014; Zheng et al., 2021), as all Chalcidoidea species exhibited diversified gene rearrangements involving at least five mitochondrial genes. These results suggest that compositional heterogeneity may promote diverse gene rearrangements in Chalcidoidea.

The gene rearrangements in Chalcidoidea were highly diversified at the intrafamilial or even intrageneric levels (Supplementary Figures S19, S20) compared with other insects (Saenz Manchola et al., 2021; Wang et al., 2017). Notably, two rRNA genes in the mitogenome of *Dipara* sp. 1 were encoded by the major strand, which is the first such report in Chalcidoidea.

Mitochondrial gene orders are thought to contain phylogenetic signals for resolving higher-level phylogeny of arthropods (Boore, 2006; Mao et al., 2014; Zheng et al., 2021). In our analyses, the families containing the most hypothetical ancestral gene blocks of arthropods (i.e., Mymaridae, Signiphoridae, and Leucospidae) were recovered as early branching lineages, sharing the primitive PCG order *cox1–cox2–atp8–atp6–cox3–nad3*. While the remaining families with inversion of the above gene cluster or more intense rearrangements were located at the terminal branches, as confirmed by topological evidence (Figure 4). Therefore, the inversion of *nad3–cox3–atp6–atp8–cox2–cox1* may be a synapomorphy for the other 14 families, except for Mymaridae, Leucospidae, Signiphoridae, and Trichogrammatidae. All species in Trichogrammatidae showed more derived PCG rearrangements regarding the above PCG cluster inversion, the same as in *Philotrypesis* (Figure 4).

Divergence time of Chalcidoidea

The inferred divergence time indicated that the MRCA of extant Chalcidoidea originated at the Jurassic/Cretaceous boundary (142.76 Ma, 95% CI: 110–135 Ma, node 1) (Figure 5), older than that reported in Peters et al. (2018).

Our results suggested that the major early branching lineages of Chalcidoidea may have diverged during the early-Cretaceous from 130.99 Ma to 107.84 Ma. This period coincided with the rapid divergence of major host associations among the major lineages of Chalcidoidea, generally dated from the early-Jurassic to Cretaceous (Johnson et al., 2018; Kawahara et al., 2019; Lidgard & Crane, 1990; Magallón et al., 2015; Peters et al., 2017; Zhang et al., 2018). The early-Cretaceous radiations of major lineages of Chalcidoidea are correlated with the expansion of a wide variety of insect hosts and associated angiosperms (Crane et al., 1995, 2004).

We also found that most modern families of Chalcidoidea diverged at the mid- to late-Cretaceous, between 98.74 and 82.15 Ma (Figure 5), consistent with the estimation of Heraty et al. (2013), but older than the period suggested by Peters et al. (2018) (early-Paleogene after the Cretaceous: 75–53 Ma). The MRCA of major fig and gall-associated wasps, including gall inducers, inquilines, parasitoids of gall-inducers, and other parasitoids, diverged around 73–106 Ma. Furthermore, the origin of pollinating Agaonidae fig wasps

dated to 80.25 Ma, revealing co-divergence with the crown group of *Ficus* diverging 102–60 Ma (Cruaud et al., 2012). Sycophaginae and other non-pollinating fig wasps placed in Pteromalidae originated at 43–45 Ma, distinctly younger than the pollinating fig wasps. The overall origin of fig-associated wasps was earlier than the estimation by Peters et al. (2018), but similar to the predictions of Rønsted et al. (2005) and Cruaud et al. (2012). Our results revealed a contemporary post-Cretaceous diversification of modern chalcidoids, in line with the rapid diversification of their hosts. The potential codiversification between chalcidoids and their hosts is a key factor in the evolutionary history of Chalcidoidea.

Evolution of hosts and gall associations

The utilization of hosts by chalcidoids revealed highly diverse host switches during their evolutionary history (Supplementary Figure S22), e.g., from gall-inducing to other feeding modes in Eulophidae. Furthermore, broad shifts in living strategies were also observed in Chalcididae and Eupelmidae. We performed preliminary ASR analyses based on the preferred topology and only used predominant host records to calculate host associations. Of note, several highly diverse clades were inadequately sampled in our study and using predominant host records may be too generalized to obtain accurate ASR results. Therefore, further evidence is required to strengthen some inferences and obtain more accurate ASR results.

Our results suggested that Hemiptera may be the ancestral host for Chalcidoidea (AA datasets, SCM method: P=0.61; LM method: P=0.53) (Supplementary Nexus Files S1, S2 and Supplementary Figures S22, S25), with some subsequent backbone nodes in Chalcidoidea shifting to Hymenoptera hosts (Supplementary Nexus Files S1, S2 and Supplementary Figures S22, S23, S25, S26, S29, S30). Hymenoptera was also predicted as the ancestral host for Leucospidae and (Perilampidae+Eucharitidae), with the latter supported by Zhang et al. (2022). Our ASR results for parasitism indicated that Chalcidoidea wasps may be primitively primary and solitary parasitoids, with radiations into other parasitism strategies, such as hyperparasitism and gregarious parasitism (see Supplementary Nexus Files S1, S2, and Supplementary Figure S27, S28, S31, S32).

Our results also indicated that phytophagy and parasitism of gall-associated insects originated independently among different groups and at different times in Chalcidoidea, corroborating the hypothesis that phytophagy may not be a plesiomorphic trait but a derived one in parasitoid groups (Heraty et al., 2013; Narendran et al., 2007). Furthermore, ASR analysis of gall-associated strategies supported the hypothesis that most gall-inducers were derived from parasitoids of gall-inducers, while other gall-inducers may be derived from phytophagous groups (Figure 5). The ancestor of clade B was predicted to be a parasitoid of gall-associated insects. The larvae of *Ormyrus* and Megastigmidae are parasitoids of gall wasps (Cynipidae) and gall midges (Cecidomyiidae) (Janšta et al., 2018). *Eurytoma* exhibits a wide range of strategies, such as seed eating and gall formation, but most are parasitoids of gall-inducing hosts (Gómez et al., 2011; Lotfalizadeh et al., 2007; Zerova & Fursov, 1991). Our ASR analyses suggested that the ancestor of Torymidae may be a parasitoid of gall-associated insects (Janšta et al., 2018). All species in Agaonidae are phytophagous, as are Sycophaginae and Epichrysomallinae. Our results indicate that most fig and gall-associated wasps are generally closely clustered based on similar living and feeding biology.

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

No specific ethics permits were required for this study. The chalcidoids species used in this study are not endangered and are not included in the "List of Protected Animals in China". No specific permissions were required for sampling activities.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

J.C.Z. designed the study, collected the samples, analyzed the data, prepared the figures, and wrote the original draft. H.X. identified the species and revised the manuscript. P.T. revised the manuscript and submitted the manuscript. X.F.L. conducted experiments. X.K.L. revised the manuscript. C.D.Z., Q.W., J.H.X., and D.W.H. identified the species. C.V.A. revised the manuscript. X.X.C. applied for the funding, revised the manuscript, and supervised the study. All authors read and approved the final version of the manuscript.

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REFERENCES

Abascal F, Zardoya R, Telford MJ. 2010. TranslatorX: multiple alignment of nucleotide sequences guided by amino acid translations. *Nucleic Acids Research*, **38**(S2): W7−W13.

Aberer AJ, Kobert K, Stamatakis A. 2014. ExaBayes: massively parallel Bayesian tree inference for the whole-genome era. *Molecular Biology and Evolution*, **31**(10): 2553−2556.

Austin AD, Gibson GAP, Harvey MS. 1998. Synopsis of Australian *Calymmochilus* Masi (Hymenoptera: Eupelmidae), description of a new western Australian species associated with a pseudoscorpion, and review of pseudoscorpion parasites. *Journal of Natural History*, **32**(3): 329−350.

Baker AJ, Heraty JM, Mottern J, et al. 2020. Inverse dispersal patterns in a group of ant parasitoids (Hymenoptera: Eucharitidae: Oraseminae) and their ant hosts. *Systematic Entomology*, **45**(1): 1−19.

Ban XC, Shao ZK, Wu LJ, et al. 2022. Highly diversified mitochondrial genomes provide new evidence for interordinal relationships in the Arachnida. *Cladistics*, **38**(4): 452−464.

Bankevich A, Nurk S, Antipov D, et al. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*, **19**(5): 455−477.

Bernt M, Donath A, Jühling F, et al. 2013. MITOS: improved *de novo* metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution*, **69**(2): 313−319.

Bernt M, Merkle D, Ramsch K, et al. 2007. CREx: inferring genomic rearrangements based on common intervals. *Bioinformatics*, **23**(21): 2957−2958.

Böhmová J, Rasplus JY, Taylor GS, et al. 2022. Description of two new Australian genera of Megastigmidae (Hymenoptera, Chalcidoidea) with notes on the biology of the genus *Bortesia*. *Journal of Hymenoptera Research*, **90**: 75–99.

Boisvert S, Raymond F, Godzaridis É, et al. 2012. Ray Meta: scalable *de novo* metagenome assembly and profiling. *Genome Biology*, **13**(12): R122.
Boore JL. 2006. The use of genome-level characters for phylogenetic reconstruction. *Trends in Ecology & Evolution*, **21**(8): 439−446.
Borowiec ML. 2016. AMAS: a fast tool for alignment manipulation and computing of summary statistics. *PeerJ*, **4**: e1660.
Bouček Z. 1988. Australasian Chalcidoidea (Hymenoptera): A Biosystematic Revision of Genera of Fourteen Families, with a Reclassification of Species. Wallingford, UK: CAB International. 458.
Bouckaert R, Vaughan TG, Barido-Sottani J, et al. 2019. BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, **15**(4): e1006650.
Burks RA, Heraty JM, Gebiola M, et al. 2011. Combined molecular and morphological phylogeny of Eulophidae (Hymenoptera: Chalcidoidea), with focus on the subfamily Entedoninae. *Cladistics*, **27**(6): 581−605.
Cameron SL. 2014. Insect mitochondrial genomics: implications for evolution and phylogeny. *Annual Review of Entomology*, **59**: 95−117.
Campbell B, Heraty JM, Rasplus JY, et al. 2000. Molecular systematics of the Chalcidoidea using 28S–D2 RDNA. *In*: Austin AD, Dowton M. The Hymenoptera: Evolution, Biodiversity and Biological Control. Melbourne: CSIRO Publishing, 59–73.
Che LH, Zhang P, Deng SH, et al. 2021. New insights into the phylogeny and evolution of lady beetles (Coleoptera: Coccinellidae) by extensive sampling of genes and species. *Molecular Phylogenetics and Evolution*, **156**: 107045.
Chen SF, Zhou YQ, Chen YR, et al. 2018. Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, **34**(17): i884−i890.
Crane PR, Friis EM, Pedersen KR. 1995. The origin and early diversification of angiosperms. *Nature*, **374**(6517): 27−33.
Crane PR, Herendeen P, Friis EM. 2004. Fossils and plant phylogeny. *American Journal of Botany*, **91**(10): 1683−1699.
Cruaud A, Delvare G, Nidelet S, et al. 2021. Ultra-conserved elements and morphology reciprocally illuminate conflicting phylogenetic hypotheses in Chalcididae (Hymenoptera, Chalcidoidea). *Cladistics*, **37**(1): 1−35.
Cruaud A, Jabbour-Zahab R, Genson G, et al. 2011. Phylogeny and evolution of life-history strategies in the Sycophaginae non-pollinating fig wasps (Hymenoptera, Chalcidoidea). *BMC Evolutionary Biology*, **11**(1): 178.
Cruaud A, Nidelet S, Arnal P, et al. 2019. Optimized DNA extraction and library preparation for minute arthropods: application to target enrichment in chalcid wasps used for biocontrol. *Molecular Ecology Resources*, **19**(3): 702−710.
Cruaud A, Rønsted N, Chantarasuwan B, et al. 2012. An extreme case of plant-insect codiversification: figs and fig-pollinating wasps. *Systematic Biology*, **61**(6): 1029−1047.
Debach P, Rosen D. 1991. Biological Control by Natural Enemies. 2nd ed. Cambridge, UK: Cambridge University Press.
Drummond AJ, Ho SYW, Phillips MJ, et al. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology*, **4**(5): e88.
Gibson GAP. 1999. Sister-group relationships of the Platygastroidea and Chalcidoidea (Hymenoptera) — an alternate hypothesis to Rasnitsyn (1988). *Zoologica Scripta*, **28**(1–2): 125–138.
Gibson GAP, Heraty JM, Woolley JB. 1999. Phylogenetics and classification of Chalcidoidea and Mymarommatoidea — a review of current concepts (Hymenoptera, Apocrita). *Zoologica Scripta*, **28**(1–2): 87–124.
Gómez JF, Nieves-Aldrey JL, Nieves MH. 2008. Comparative morphology, biology and phylogeny of terminal-instar larvae of the European species of Toryminae (Hym., Chalcidoidea, Torymidae) parasitoids of gall wasps (Hym. Cynipidae). *Zoological Journal of the Linnean Society*, **154**(4): 676−721.
Gómez JF, Nieves-Aldrey JL, Nieves MH, et al. 2011. Comparative morphology and biology of terminalinstar larvae of some *Eurytoma* (Hymenoptera, Eurytomidae) species parasitoids of gall wasps (Hymenoptera, Cynipidae) in western Europe. *Zoosystema*, **33**(3): 287−323.
Greathead DJ. 1986. Parasitoids in classical biological control. *In*: Waage J, Greathead DJ. Insect Parasitoids. London: Academic Press, 289–318.
Grissell EE & Schauff ME. 1997. Superfamily chalcidoidea. *In*: Gibson GAP, Huber JT, Woolley JB. Annotated Keys to the Genera of Nearctic Chalcidoidea (Hymenoptera). Ottawa, Canada: National Research Council of Canada Research Press, 45–116.
Guindon S, Dufayard JF, Lefort V, et al. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, **59**(3): 307−321.
Haas M, Burks RA, Krogmann L. 2018. A new lineage of Cretaceous jewel wasps (Chalcidoidea: Diversinitidae). *PeerJ*, **6**: e4633.
Hassanin A. 2006. Phylogeny of Arthropoda inferred from mitochondrial sequences: strategies for limiting the misleading effects of multiple changes in pattern and rates of substitution. *Molecular Phylogenetics and Evolution*, **38**(1): 100−116.
Heraty JM. 2017. Parasitoid biodiversity and insect pest management. *In*: Foottit RG, Adler PH. Insect Biodiversity: Science and Society. Hoboken: John Wiley & Sons, 603–625.
Heraty JM, Burks RA, Cruaud A, et al. 2013. A phylogenetic analysis of the megadiverse Chalcidoidea (Hymenoptera). *Cladistics*, **29**(5): 466−542.
Heraty JM, Darling DC. 2009. Fossil Eucharitidae and Perilampidae (Hymenoptera: Chalcidoidea) from Baltic amber. *Zootaxa*, **2306**(1): 1−16.
Heraty JM, Woolley JB, Darling DC. 1997. Phylogenetic implications of the mesofurca in Chalcidoidea (Hymenoptera), with emphasis on Aphelinidae. *Systematic Entomology*, **22**(1): 45−65.
Hill DS. 1967. Figs (*Ficus* spp.) and fig-wasps (Chalcidoidea). *Journal of Natural History*, **1**(3): 413−434.
Hu F, Lin Y, Tang JJ. 2014. MLGO: phylogeny reconstruction and ancestral inference from gene-order data. *BMC Bioinformatics*, **15**(1): 354.
Jakovlić I, Zou H, Zhao XM, et al. 2021. Evolutionary history of inversions in directional mutational pressures in crustacean mitochondrial genomes: implications for evolutionary studies. *Molecular Phylogenetics and Evolution*, **164**: 107288.
Janšta P, Cruaud A, Delvare G, et al. 2018. Torymidae (Hymenoptera, Chalcidoidea) revised: molecular phylogeny, circumscription and reclassification of the family with discussion of its biogeography and evolution of life-history traits. *Cladistics*, **34**(6): 627−651.
Johnson KP, Dietrich CH, Friedrich F, et al. 2018. Phylogenomics and the evolution of hemipteroid insects. *Proceedings of the National Academy of Sciences of the United States of America*, **115**(50): 12775−12780.
Joseph KJ. 1964. A proposed revision of the classification of the fig insects of the families Agaonidae and Torymidae (Hymenoptera). *Proceedings of the Royal Entomological Society of London, Series B, Taxonomy*, **33**(3–4): 63–66.
Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, **30**(4): 772−780.
Kawahara AY, Plotkin D, Espeland M, et al. 2019. Phylogenomics reveals the evolutionary timing and pattern of butterflies and moths. *Proceedings of the National Academy of Sciences of the United States of America*, **116**(45): 22657−22663.
Kück P, Longo GC. 2014. FASconCAT-G: extensive functions for multiple sequence alignment preparations concerning phylogenetic studies. *Frontiers in Zoology*, **11**(1): 81.
La Salle J. 2005. Biology of gall inducers and evolution of gall induction in Chalcidoidea (Hymenoptera: Eulophidae, Eurytomidae, Pteromalidae, Tanaostigmatidae, Torymidae). *In*: Ramana A, Schaeffer CW, Withers TM. Biology, Ecology, and Evolution of Gall-Inducing Arthropods. Enfield, New

Hampshire: Science Publishers, 507–537.
Lanfear R, Frandsen PB, Wright AM, et al. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, **34**(3): 772−773.
Li FF, Chen XX, Piao MH, et al. 2003. Phylogenetic relationships of the Euphorinae (Hymenoptera: Braconidae) based on the D2 variable region of 28S ribosomal RNA. *Entomotaxonomia*, **25**(3): 217−226.
Li Q, Wei SJ, Tang P, et al. 2016. Multiple lines of evidence from mitochondrial genomes resolve phylogenetic relationships of parasitic wasps in Braconidae. *Genome Biology and Evolution*, **8**(9): 2651−2662.
Li WN, Shao RF, Zhang Q, et al. 2019. Mitochondrial genome reorganization characterizes various lineages of mesostigmatid mites (Acari: Parasitiformes). *Zoologica Scripta*, **48**(5): 679−689.
Lidgard S, Crane PR. 1990. Angiosperm diversification and Cretaceous floristic trends: a comparison of palynofloras and leaf macrofloras. *Paleobiology*, **16**(1): 77−93.
Liu YQ, Song F, Jiang P, et al. 2018. Compositional heterogeneity in true bug mitochondrial phylogenomics. *Molecular Phylogenetics and Evolution*, **118**: 135−144.
Lotfalizadeh H, Delvare G, Rasplus JY. 2007. Phylogenetic analysis of Eurytominae (Chalcidoidea: Eurytomidae) based on morphological characters. *Zoological Journal of the Linnean Society*, **151**(3): 441−510.
Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Research*, **25**(5): 955−964.
Maddison W, Maddison DR. 2019. Mesquite: a modular system for evolutionary analysis. Version 3.61. https://www.scirp.org/reference/referencespapers.aspx?referenceid=3344710.
Magallón S, Gómez-Acevedo S, Sánchez-Reyes LL, et al. 2015. A metacalibrated time‐tree documents the early rise of flowering plant phylogenetic diversity. *New Phytologist*, **207**(2): 437−453.
Mao M, Gibson T, Dowton M. 2014. Evolutionary dynamics of the mitochondrial genome in the Evaniomorpha (Hymenoptera)—a group with an intermediate rate of gene rearrangement. *Genome Biology and Evolution*, **6**(7): 1862−1874.
Meng GL, Li YY, Yang CT, et al. 2019. MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization. *Nucleic Acids Research*, **47**(11): e63.
Minh BQ, Nguyen MAT, Von Haeseler A. 2013. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution*, **30**(5): 1188−1195.
Mohammad H. 1998. Aphelinidae of India (Hymenoptera: Chalcidoidea): A Taxonomic Revision. Gainesville, USA: Associated Publishers.
Munro JB, Heraty JM, Burks RA, et al. 2011. A molecular phylogeny of the Chalcidoidea (Hymenoptera). *PLoS One*, **6**(11): e27023.
Murray EA, Heraty JM. 2020. Neotropical ant parasitoids (Hymenoptera: Eucharitidae): interpreting taxonomy, phylogeny and divergent morphologies. *Systematic Entomology*, **45**(2): 464−480.
Narendran TC, Santhosh S, Sudheer K. 2007. Biosystematics and biogeography of oriental Chalcidoidea (Hymenoptera) associated with plant galls. *Oriental Insects*, **41**(1): 141−167.
Nguyen LT, Schmidt HA, Von Haeseler A, et al. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, **32**(1): 268−274.
Nie RE, Vogler AP, Yang XK, et al. 2021. Higher-level phylogeny of longhorn beetles (Coleoptera: Chrysomeloidea) inferred from mitochondrial genomes. *Systematic Entomology*, **46**(1): 56−70.
Noyes J. 2019. Universal Chalcidoidea Database.https://www.nhm.ac.uk/our-science/data/chalcidoids/database/.
Noyes JS. 1990. A word on chalcidoid classification. Pages 6–7 in Chalcid Forum. Vol. 13.
Owen AK, George J, Pinto JD, et al. 2007. A molecular phylogeny of the Trichogrammatidae (Hymenoptera: Chalcidoidea), with an evaluation of the utility of their male genitalia for higher level classification. *Systematic Entomology*, **32**(2): 227−251.
Peng Y, Leung HCM, Yiu SM, et al. 2012. IDBA-UD: a *de novo* assembler for single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics*, **28**(11): 1420−1428.
Peters RS, Krogmann L, Mayer C, et al. 2017. Evolutionary history of the Hymenoptera. *Current Biology*, **27**(7): 1013−1018.
Peters RS, Niehuis O, Gunkel S, et al. 2018. Transcriptome sequence-based phylogeny of chalcidoid wasps (Hymenoptera: Chalcidoidea) reveals a history of rapid radiations, convergence, and evolutionary success. *Molecular Phylogenetics and Evolution*, **120**: 286−296.
Rambaut A. 2012. FigTree v. 1.4. 2: Tree Figure Drawing Tool.
Revell LJ. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, **3**(2): 217−223.
Reyes A, Gissi C, Pesole G, et al. 1998. Asymmetrical directional mutation pressure in the mitochondrial genome of mammals. *Molecular Biology and Evolution*, **15**(8): 957−966.
Rønsted N, Weiblen GD, Cook JM, et al. 2005. 60 million years of co-divergence in the fig–wasp symbiosis. *Proceedings of the Royal Society B:Biological Sciences*, **272**(1581): 2593−2599.
Rota-Stabelli O, Kayal E, Gleeson D, et al. 2010. Ecdysozoan mitogenomics: evidence for a common origin of the legged invertebrates, the Panarthropoda. *Genome Biology and Evolution*, **2**: 425−440.
Rozas J, Ferrer-Mata A, Sánchez-Delbarrio JC, et al. 2017. DnaSP 6: DNA sequence polymorphism analysis of large datasets. *Molecular Biology and Evolution*, **34**(12): 3299−3302.
Saenz Manchola OF, Virrueta Herrera S, D'alessio LM, et al. 2021. Mitochondrial genomes within bark lice (Insecta: Psocodea: Psocomorpha) reveal novel gene rearrangements containing phylogenetic signal. *Systematic Entomology*, **46**(4): 938−951.
Song SN, Tang P, Wei SJ, et al. 2016. Comparative and phylogenetic analysis of the mitochondrial genomes in basal hymenopterans. *Scientific Reports*, **6**(1): 20972.
Tang P, Zhu JC, Zheng BY, et al. 2019. Mitochondrial phylogenomics of the Hymenoptera. *Molecular Phylogenetics and Evolution*, **131**: 8−18.
Timmermans MJTN, Barton C, Haran J, et al. 2016. Family-level sampling of mitochondrial genomes in Coleoptera: compositional heterogeneity and phylogenetics. *Genome Biology and Evolution*, **8**(1): 161−175.
Timmermans MJTN, Vogler AP. 2012. Phylogenetically informative rearrangements in mitochondrial genomes of Coleoptera, and monophyly of aquatic elateriform beetles (Dryopoidea). *Molecular Phylogenetics and Evolution*, **63**(2): 299−304.
Wang YY, Liu XY, Garzón-Orduña IJ, et al. 2017. Mitochondrial phylogenomics illuminates the evolutionary history of Neuropterida. *Cladistics*, **33**(6): 617−636.
Wei SJ, Li Q, Van Achterberg K, et al. 2014. Two mitochondrial genomes from the families Bethylidae and Mutillidae: independent rearrangement of protein-coding genes and higher-level phylogeny of the Hymenoptera. *Molecular Phylogenetics and Evolution*, **77**: 1−10.
Wei SJ, Shi M, Chen XX, et al. 2010a. New views on strand asymmetry in insect mitochondrial genomes. *PLoS One*, **5**(9): e12708.
Wei SJ, Shi M, Sharkey MJ, et al. 2010b. Comparative mitogenomics of Braconidae (Insecta: Hymenoptera) and the phylogenetic utility of mitochondrial genomes with special reference to Holometabolous insects. *BMC Genomics*, **11**(1): 371.
Wiebes JT. 1976. A short history of fig wasp research. *Garden's Bulletin of Straits Settlement*, **29**: 207−232.
Wu YF, Yang HL, Feng ZB, et al. 2020. Novel gene rearrangement in the

mitochondrial genome of *Pachyneuron aphidis* (Hymenoptera: Pteromalidae). *International Journal of Biological Macromolecules*, **149**: 1207−1212.

Xia XH. 2018. DAMBE7: new and improved tools for data analysis in molecular biology and evolution. *Molecular Biology and Evolution*, **35**(6): 1550−1552.

Xia XH, Xie Z, Salemi M, et al. 2003. An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution*, **26**(1): 1−7.

Xiao JH, Jia JG, Murphy RW, et al. 2011. Rapid evolution of the mitochondrial genome in chalcidoid wasps (Hymenoptera: Chalcidoidea) driven by parasitic lifestyles. *PLoS One*, **6**(11): e26645.

Xu SL, Han BP, Martínez A, et al. 2021. Mitogenomics of Cladocera (Branchiopoda): marked gene order rearrangements and independent predation roots. *Molecular Phylogenetics and Evolution*, **164**: 107275.

Yan ZC, Ye GY, Werren JH. 2019. Evolutionary rate correlation between mitochondrial-encoded and mitochondria-associated nuclear-encoded proteins in insects. *Molecular Biology and Evolution*, **36**(5): 1022−1036.

Yang ZH, Rannala B. 2006. Bayesian estimation of species divergence times under a molecular clock using multiple fossil calibrations with soft bounds. *Molecular Biology and Evolution*, **23**(1): 212−226.

Yi JQ, Wu H, Liu JB, et al. 2022. Novel gene rearrangement in the mitochondrial genome of *Anastatus fulloi* (Hymenoptera Chalcidoidea) and phylogenetic implications for Chalcidoidea. *Scientific Reports*, **12**(1): 1351.

Zerova M. 1993. The new group of phytophagous chalcids of the family Eurytomidae (Hymenoptera, Chalcidoidea). *Zoologičhesky Žhurnal*, **72**(10): 68−74.

Zerova MD, Fursov VN. 1991. The Palaearctic species of *Eurytoma* (Hymenoptera: Eurytomidae) developing in stone fruits (Rosaceae: Prunoideae). *Bulletin of Entomological Research*, **81**(2): 209−219.

Zhang D, Gao FL, Jakovlić I, et al. 2020a. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources*, **20**(1): 348−355.

Zhang D, Zou H, Hua CJ, et al. 2019. Mitochondrial architecture rearrangements produce asymmetrical nonadaptive mutational pressures that subvert the phylogenetic reconstruction in Isopoda. *Genome Biology and Evolution*, **11**(7): 1797−1812.

Zhang JX, Heraty JM, Darling C, et al. 2022. Anchored phylogenomics and a revised classification of the planidial larva clade of jewel wasps (Hymenoptera: Chalcidoidea). *Systematic Entomology*, **47**(2): 329−353.

Zhang JX, Lindsey ARI, Peters RS, et al. 2020b. Conflicting signal in transcriptomic markers leads to a poorly resolved backbone phylogeny of chalcidoid wasps. *Systematic Entomology*, **45**(4): 783−802.

Zhang SQ, Che LH, Li Y, et al. 2018. Evolutionary history of Coleoptera revealed by extensive sampling of genes and species. *Nature Communications*, **9**(1): 205.

Zhao HF, Chen Y, Wang ZT, et al. 2021. Two complete mitogenomes of Chalcididae (Hymenoptera: Chalcidoidea): genome description and phylogenetic implications. *Insects*, **12**(12): 1049.

Zheng XY, Cao LJ, Chen PY, et al. 2021. Comparative mitogenomics and phylogenetics of the stinging wasps (Hymenoptera: Aculeata). *Molecular Phylogenetics and Evolution*, **159**: 107119.

Zhou QS, Xiong M, Luo AR, et al. 2021. The complete mitochondrial genome of *Metaphycus eriococci* (Timberlake) (Hymenoptera: Encyrtidae). *Mitochondrial DNA Part B:Resources*, **6**(2): 550−552.

Zhu JC, Tang P, Zheng BY, et al. 2018. The first two mitochondrial genomes of the family Aphelinidae with novel gene orders and phylogenetic implications. *International Journal of Biological Macromolecules*, **118**: 386−396.