WILEY

Cladistics

Cladistics (2025) 1–22

doi: 10.1111/cla.70006

Mitogenomics provide insights into the tribe-level systematics and historical phylogeography of band-winged grasshoppers (Orthoptera: Acrididae: Oedipodinae)

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Received 11 June 2024; Revised 17 July 2025; Accepted 19 July 2025

Abstract

Oedipodinae (Acrididae) is a species-rich and globally distributed subfamily of grasshoppers, currently comprising 807 valid species assigned to 138 genera in 16 tribes. Resolving the phylogeny of Oedipodinae has proven difficult, owing to their extensive species diversity, disjunct geographic distribution and the scarcity of informative molecular markers. To establish a more robust phylogenetic framework, we conducted a mitochondrial phylogenomic analysis of 143 mitogenomes. This dataset includes 103 Oedipodinae species representing all 16 currently recognized tribes, of which 86 mitogenomes from nine tribes were newly sequenced in this study. Divergence times and ancestral areas were also inferred to investigate evolutionary trends within this subfamily. The phylogenetic analysis supports the monophyly of nine tribes within Oedipodinae: Acrotylini, Anconiini, Bryodemini, Chortophagini, Machaerocerini, Psinidiini, Trilophidiini, Trimerotropini and Tropidolophini. Based on these results, we propose taxonomic revisions. The tribe Tropidolophini Otte, 1995 is removed from Oedipodinae and provisionally placed in Acrididae, *incertae sedis*. In addition, the genus *Ceracris* Walker, 1870 is removed from Parapleurini and placed in Acridinae, *incerta sedis*. Divergence time estimation suggests that Oedipodinae originated during the Eocene, approximately 49 Mya. The biogeographic reconstruction supports a Holarctic origin of Oedipodinae, with the Palaearctic region as the principal center of diversification, followed by subsequent dispersal into North America, the Oriental region and Africa. These patterns highlight the role of dispersal in shaping the global distribution of the subfamily.

Introduction

Acrididae, the most species-rich family within the orthopteran suborder Caelifera, comprises over 6700 valid species (Jago, 1971; Song and Wenzel, 2008; Song et al., 2015, 2018; Cigliano et al., 2025). The family is currently classified into 28 subfamilies (Cigliano et al., 2025). Among these, the subfamily Oedipodinae stands out for its high species diversity and ecological adaptability (Fig. 1), characterized by vividly coloured hind wings, often adorned with distinct dark bands (Hochkirch et al., 2023). They can be readily distinguished from other grasshoppers by the combination of the following characters: the absence of a prosternal tubercle or process, the presence of an intercalary vein in the medial area of the fore wing, and a vertical face (Otte, 1984). This subfamily comprises 16 tribes, 138 genera and 807 valid species (Table 1; Cigliano et al., 2025).

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Fig. 1. Representatives of the subfamily Oedipodinae. (a) *Locusta migratoria* (Locustini); (b) *Sphingonotus* (*Sphingonotus*) *theodori* (Sphingonotini); (c) *Psophus stridulus* (Locustini); (d) *Celes skalozubovi* (Oedipodini); (e) *Acrotylus* sp. (Acrotylini); (f) *Mioscirtus wagneri* (Oedipodini); (g) *Stethophyma grossum* (Parapleurini); (h) *Chimarocephala pacifica* (Chortophagini); (i) *Trimerotropis saxatilis* (Trimerotropini); (j) *Aiolopus tamulus* (Epacromiini); (k) *Bryodemella* (*Marikovskiella*) *zaisanica* (Bryodemini).

Table 1
The 16 currently recognized tribes within Oedipodinae, number of genera and species and distribution

Tribe	Number of genera	Number of species	Distribution
Acrotylini	2	47	Asia, Europe and Africa
Anconiini	1	2	North American
Arphiini	4	34	North, Central and South America
Bryodemini	7	46	Asia, Europe
Chortophagini	5	15	North and Central America
Epacromiini	9	59	Asia, Europe and Africa
Hippiscini	10	34	North, Central and South America
Locustini	13	88	Asia, Europe, Australia and Africa
Machaerocerini	1	1	North and Central America
Oedipodini	5	42	Asia, Europe and North Africa
Parapleurini	7	30	North American, Asia and Europe
Psinidiini	7	23	North and Central America
Sphingonotini	16	209	Cosmopolitan
Trilophidiini	1	8	Asia and Africa
Trimerotropini	5	80	North, Central and South America
Tropidolophini	1	1	North and Central America
Incerta sedis	44	88	–
Total	138	807	Cosmopolitan

Since the 19th century, taxonomists have been studying the taxonomy of band-winged grasshoppers (Saussure, 1884, 1888; Scudder, 1897; Kirby, 1910; Bei-Bienko and Mishchenko, 1951). These studies focused on the fauna of Europe, Africa, Eurasia and North America, which led to the establishment of early taxonomic schemes based on specimens collected from these regions. Oedipodinae have variously been grouped as a tribe or as a subfamily (Jago, 1996; Guliaeva et al., 2005). For instance, in 1910, Kirby established the subfamily Locustinae for species that are now placed in Oedipodinae. These species were later transferred to the tribe Oedipodini within the subfamily Acridinae by Dirsh (1965). Key and Colless (1993) and Rentz (2003) adopted a similar perspective, contending that the substantial anatomical similarities between Acridinae and Oedipodinae did not justify treating them as separate subfamilies. In the 1980s, Chinese taxonomists conducted extensive research on Oedipodinae, documenting numerous species in the region. Notably, they classified Oedipodinae as a family, belonging to the superfamily Acridoidea (Yin, 1984a, b; Yin et al., 1996; Zheng and Xia, 1998). At present, most taxonomists accept the subfamily designation (Otte, 1984). In the most recent iteration of the Orthoptera Species File (OSF) (Cigliano et al., 2025), Oedipodinae is subdivided into 16 distinct tribes.

Molecular phylogenetic studies have further complicated our understanding of Oedipodinae's position within Acrididae. Flook and Rowell (1997) conducted a systematic investigation of Caelifera using mitochondrial ribosomal RNA genes from 32 taxonomic units within the order, aiming to elucidate its phylogeny. Their results suggest that Oedipodinae may represent an early-diverging lineage within Acrididae, possibly originating in the early Tertiary or even the Cretaceous. Fries et al. (2007) investigated the intercontinental relationships and geographic origins of Oedipodinae using four mitochondrial genes from 22 species collected across the Americas, Eurasia, Africa and Australia, and found that Asia and America were the centres of the initial oedipodine radiation about 94 Mya. Chapco and Contreras (2011) analysed five genes (CO1, CO2, cytb, nd5, 16S) from 117 taxa, including 35 Oedipodinae species, to investigate the phylogeny and origins of Oedipodinae, Acridinae and Gomphocerinae. Their findings indicated that Oedipodinae is not monophyletic. Similarly, Song et al. (2018) conducted a comprehensive molecular phylogeny of Acrididae, examining five genes (18S rRNA, 28S rRNA, histone 3, CO1, CO2) across 134 Acridoidea taxa, including 15 Oedipodinae species, to assess the monophyly of Acrididae and its subfamilies. The study further confirmed that Oedipodinae is not monophyletic, in that the current concept of Oedipodinae includes species that are genetically classifiable as Acridinae and Gomphocerinae.

Likewise, the phylogenetic relationships within Oedipodinae remain largely unresolved. There are currently 16 recognized tribes within Oedipodinae (Table 1), of which only Sphingonotini have a cosmopolitan distribution, while eight tribes (Anconiini, Arphiini, Chortophagini, Hippiscini, Machaerocerini, Psinidiini, Trimerotropini and Tropidolophini) are endemic to the Americas. The species of the remaining seven tribes are distributed in Africa, Australia or the Eurasian

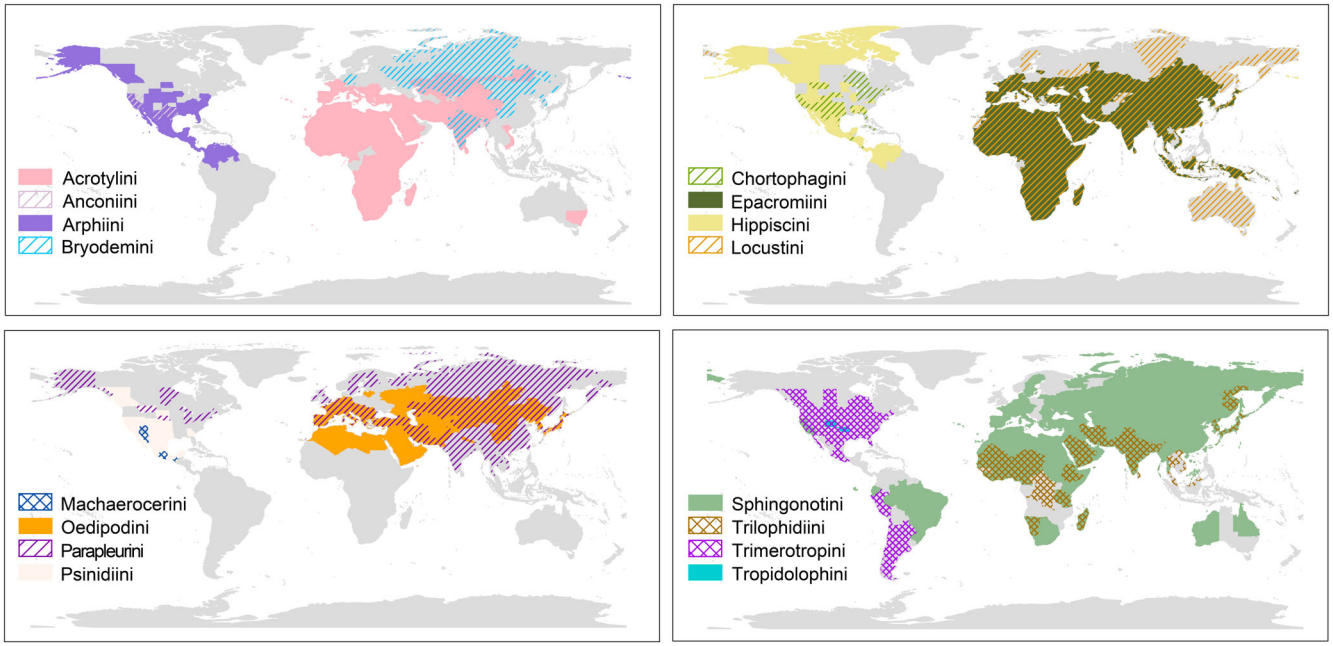


Fig. 2. Global distributions of Oedipodinae showing the disjunct pattern. Each tribe is represented by a distinct colour, showing its geographic range.

continent (Fig. 2) (Cigliano et al., 2025). In recent years, Dey, Hochkirch, Husemann and colleagues conducted multiple studies on the morphology and phylogeny of the genera *Thalpomena*, *Oedipoda*, *Sphingonotus* and the tribe Bryodemini within the subfamily Oedipodinae (Husemann et al., 2012, 2013, 2015; Dey et al., 2018, 2021a, b, c; Hochkirch et al., 2023). However, it is important to note that these studies have primarily focused on specific geographic regions or single genera and are largely biased towards the Palaearctic. A phylogenetic study with high species coverage is still needed for this subfamily, especially for resolving the relationships among tribes.

In this context, mitochondrial genomes (mitogenomes) offer a promising tool for resolving higher-level relationships. Insect mitogenomes generally exist as circular double-stranded molecules. In most insect species, the mitogenome is highly conserved in both gene content and genome size, typically forming a ~15 kilobase circular molecule that encodes a standard set of 37 genes: 13 protein-coding genes (PCGs), 2 ribosomal RNA (rRNA) genes and 22 transfer RNA (tRNA) genes (Burger et al., 2003; Cameron, 2014; Li et al., 2022; Zhu et al., 2023). Even in the age of phylogenomics, which utilizes extensive nuclear genomic information to deduce evolutionary lineages, the mitogenome continues to provide valuable information (Wu et al., 2020). Mitogenomes from many Oedipodinae lineages have been published (Chang et al., 2020; Sun et al., 2023). So far, a total of 77 complete mitogenomes from 24 genera belonging to seven tribes of Oedipodinae have been deposited in GenBank (as of May 2025). However, the bulk of this data is predominantly centred around the Bryodemini, Locustini and Sphingonotini. Therefore, a comprehensive investigation into the extensive mitochondrial characteristics of Oedipodinae is still lacking.

In conclusion, an accurate phylogeny of oedipodine grasshoppers has not been established yet. Reconstructing the evolutionary and biogeographic history of Oedipodinae has the potential to shed light on the processes driving its biodiversity and to clarify the tribal relationships within this highly diverse taxon, a task that remains a significant challenge in systematics. Previous attempts on reaching a phylogeny of Acrididae and Oedipodinae have so far been limited in either geographic scope (Song et al., 2018) or the number of molecular markers (Fries et al., 2007; Husemann et al., 2012). Using full mitogenomes of all tribes of Oedipodinae to resolve their phylogeny would be a first step towards stabilizing their systematics and taxonomy. In this study, we sequenced the mitogenomes of 86 species and subspecies of Oedipodinae. Combined with previously published mitogenomes from Oedipodinae, Acridinae and Gomphocerinae, we assembled a dataset comprising 143 mitogenomes, from which we generated the first genetic matrix including representatives from all 16 tribes of Oedipodinae. We further utilized fossils to estimate a timeline of the evolution of the subfamily and we inferred the biogeographic processes underlying its diversification.

Materials and methods

Sampling

Mitogenomic data was newly obtained for 86 Oedipodinae species or subspecies using adult grasshopper samples from Asia, Europe, Africa and America, representing all 16 tribes of Oedipodinae. The mitogenomes of 58 species from nine tribes were sequenced for the first time (Table S1). All Chinese specimens were stored in 95% ethanol and deposited in the Laboratory for Systematics and Evolution, College of Life Sciences, Shaanxi Normal University (SNNU), Shaanxi, China. The other material was pinned and dried and deposited in the Museum of Nature, Leibniz Institute for the Analysis of Biodiversity Change (LIB), Hamburg, Germany and the Staatliches Museum für Naturkunde Karlsruhe (SMNK), Germany. Prof. Zhemin Zheng and Prof. Martin Husemann identified sequenced specimens. The species names were standardized according to the OSF.

DNA extraction, sequencing and assembly

Genomic DNA was extracted from leg muscle tissues using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). DNA library construction and sequencing were conducted by the Novogene Bioinformatics Technology Co., Ltd (Beijing, China) using the Illumina HiSeq X Ten platform with a paired-end 150 bp protocol. DNA libraries were prepared using the NEB Next Ultra DNA Library Prep Kit. Low-quality and short reads were removed with Fastp (Chen et al., 2018). We assembled and annotated the draft mitogenome sequences using MitoZ v2.3 (Meng et al., 2019), with the annotation information manually examined in Geneious v11.1 (Kearse et al., 2012). Transfer RNA (tRNA) genes were confirmed using the Mitos WebServer (Bernt et al., 2013). The mitogenomes of the 86 Oedipodinae grasshopper species newly sequenced in this study were submitted to GenBank (accession numbers are listed in Table S1). The Bioproject number of this study is PRJNA1097340.

Nucleotide feature analysis

We report the length, number of genes, AT content and GC content of all sequences. AT skew and GC skew were calculated as follows: AT skew = (A–T)/(A+T) and GC skew = (G–C)/(G+C). Gene rearrangement scenarios were assessed using CREx (Bernt et al., 2007) based on PCGs, tRNA genes and ribosomal RNA (rRNA) genes comparisons to ancestral insect genes. DAMBE7 was used for substitution saturation tests, and cumulative skews were plotted for all mitochondrial PCGs, PCGs excluding the third codon position and only the third codon position (Xia, 2013, 2018). The heterogeneity of nucleotide (nt) variation among sequences was analysed for different datasets with AliGROOVE v1.05 (Kück et al., 2014).

Phylogenetic analysis

Previous studies indicated that Oedipodinae is not monophyletic, in that the current concept of the subfamily includes species that are genetically classifiable as Acridinae and Gomphocerinae (Chapco and Contreras, 2011; Song et al., 2018); hence, we conducted analyses with a comprehensive taxon sampling. In this study, mitogenome sequences of species from Acridinae and Gomphocerinae were collected from the National Center for Biotechnology Information (NCBI). To ensure the reliability of publicly available mitogenomic data, we implemented a two-step quality control procedure based on cytochrome c oxidase subunit I (CO1) sequences extracted from the mitogenomes of Acridinae and Gomphocerinae retrieved from NCBI. First, we conducted Basic Local Alignment Search Tool (BLAST) searches against the NCBI nucleotide database. Sequences were retained if the top BLAST hits (all with greater than 97% similarity) belonged to the same genus. Sequences that failed to meet this criterion—either because no match exceeded 97% similarity or because top matches belonged to a different genus—were flagged as designated for further validation and subjected to further validation. In the second step, these questionable sequences were queried against the Barcode of Life Data System (BOLD). Sequences were retained if the closest non-self match in BOLD belonged to the same species or genus. If the closest match belonged to a different genus and exceeded 97% similarity, the sequence was excluded. In cases where no match was found in BOLD, we removed sequences showing greater than 97% similarity to a different genus in BLAST. Conversely, sequences lacking high-similarity BLAST matches were retained. We initially retrieved mitogenomes for 47 species of Gomphocerinae and Acridinae, of which 40 were retained after quality control (Table S1). In addition, three outgroup species from NCBI were used (Table S1). A total of 146 mitochondrial sequences were used to construct phylogenetic data matrices, including 103 Oedipodinae, 33 Gomphocerinae, seven Acridinae and three outgroup species.

From mitogenomes, the sequences of 13 PCGs, rrnL and rrnS were extracted with Phylosuite v1.2.2 (Zhang et al., 2020). The nucleotide sequences of each mitochondrial gene were aligned individually using Mafft (Katoh and Standley, 2013), with each protein-coding gene translated and aligned as amino acid sequences using the '5 Invertebrate' option in the Phylosuite. In Phylosuite, poorly aligned regions were removed, and the concatenated alignment was partitioned by gene and codon position. Four datasets were generated for phylogenetic analyses: (1) PCG123, nucleotide sequences for all three codon positions of 13 PCGs with 11 094 nucleotides (nt); (2) AA, inferred amino acid sequences of 13 PCGs with 3698 amino acids; (3) PCG12, nucleotide sequences for the first and second codon position with 7396 nt; and (4) PCG2RNA, including PCG123 and nucleotide sequences of the two rRNA genes with 13 057 nt. Table 2 provides an overview of the details of each assembled matrix.

The optimal partitioning schemes and corresponding nucleotide substitution models for each dataset were determined using PartitionFinder 2 (Lanfear et al., 2017). Each codon position for nucleotide sequences of PCGs was chosen as the input for PartitionFinder to determine possible partition schemes. The best-fit partitioning schemes and corresponding evolutionary models (Table S4) were utilized in the following phylogenetic analyses.

Bayesian Inference (BI) analyses and Maximum-Likelihood (ML) analyses were performed on all four datasets respectively (Table 2). BI analyses were performed using MrBayes v3.2.6 (Ronquist et al., 2012), each with four coupled Markov Chain Monte Carlo (MCMC) chains, 10 million generations and sampling every 100 generations. Convergence was assessed based on an average standard deviation of split frequencies below 1%. After removing the first 25% of samples as burn-in, posterior probability values were calculated for a consensus tree. Tracer v1.5 (Rambaut et al., 2018) was employed to assess the effective sample size (ESS), with the ESS values greater than 200 being considered good evidence of convergence.

ML analyses were performed using IQ-TREE v1.6.8 (Nguyen et al., 2015). In IQ-TREE, the concatenated alignment was partitioned according to genes and codon positions. The best-fitting substitution model was determined by ModelFinder implemented in IQ-TREE (MFP + MERGE). Branch support values were calculated on the best maximum likelihood tree using 1000 ultrafast bootstrap replicates and 1000 SH-like approximate likelihood ratio test (SH-aLRT) replicates. We set the parameters to --runs 100 -bnni -allnni, with --runs 100 specifying the number of independent bootstrap runs, -bnni enabling the Bootstrap Near Neighbor Interchange (BNNI) optimization to improve accuracy and computational

Table 2
Summary information of each of the matrices used in phylogenetic analyses

Dataset	Matrix	Sequence length (nt)	Gene number	Analysis method	Type	Software
PCG12	BI-PCG12	7396	13	BI	Nucleotide	Mrbayes
	ML-PCG12	7396	13	ML	Nucleotide	IQTree
PCG123	BI-PCG123	11 094	13	BI	Nucleotide	Mrbayes
	ML-PCG123	11 094	13	ML	Nucleotide	IQTree
	MP-PCG123	11 094	13	MP	Nucleotide	TNT
PCG2RNA	BI-PCG2RNA	13 057	15	BI	Nucleotide	Mrbayes
	ML-PCG2RNA	13 057	15	ML	Nucleotide	IQTree
AA	BI-AA	3698	13	BI	Amino acid	Mrbayes
	ML-AA	3698	13	ML	Amino acid	IQTree

efficiency in ultrafast bootstrapping, and -allnni applying nearest-neighbour interchange (NNI) optimizations to all nodes, thereby enhancing the robustness and precision of the branch support estimates.

We also analysed the PCG123 dataset using Maximum Parsimony (MP) in the program TNT v1.5 (Goloboff and Catalano, 2016). It involved the execution of a New Technology search employing the following search options: tree fuse, ratchet, random drift and sectorial search, with 1000 bootstrap replicates.

Further, a topology test was conducted using the IQ-TREE to compare alternative topologies inferred from four mitogenomic matrices (PCG123, AA, PCG12 and PCG2RNA). For each matrix, four statistical tests with 1000 replicates were performed: the Shimodaira–Hasegawa (Shimodaira and Hasegawa, 1999), Kishino–Hasegawa (Kishino and Hasegawa, 1989), expected-likelihood weight (Strimmer and Rambaut, 2002) and approximately unbiased tests (Shimodaira, 2002). We also evaluated the monophyly of Arphiini and Locustini using four-cluster likelihood mapping (FcLM) (Strimmer and Von Haeseler, 1997) implemented in IQ-TREE.

Molecular dating

Divergence times were estimated using BEAST v2.6.0 (Bouckaert et al., 2014). For this analysis, we focused on species within Oedipodinae, retaining 109 taxa from the original dataset of 146 species. The PCG12 dataset, which produced the optimal topology (Table S5), was used along with the partitioning scheme and nucleotide substitution models recommended by PartitionFinder. The time-tree and clock models were linked across partitions. The Yule process of speciation was implemented as the tree prior, with exponential and lognormal prior distributions applied to the calibration points associated with fossil constraint priors.

Generally, the fossil record for Orthoptera is relatively scarce, and their taxonomic assignment, specifically due to morphological conservatism, can be difficult. Most fossils come from extinct families (e.g. Locustopsidae and Elcanidae) which cannot clearly be positioned in the phylogeny. Hence, few fossil calibration points are available. We here used four fossils for calibration of the molecular clock (Table S6). Most of these have already been used in previous studies (Husemann et al., 2012; Song et al., 2018). We use the taxonomic assignments of the fossils as currently accepted, yet are aware that this may change in the future when more research on fossil Orthoptera becomes available.

The first fossil, *Tyrbula russelli* Scudder, 1885, known from the Florissant Formation of the Eocene of the United States (37.2–33.9 Mya), is the most completely preserved specimen known from the clade comprising the subfamilies Gomphocerinae, Acridinae and Oedipodinae. Thus, we used *T. russelli* to calibrate the crown age of this clade, assigning a conservative minimum age of 33.9 Mya (Scudder, 1885). With the exponential prior distribution, we set an offset of 33.9, in which the parameter settings followed Heath (2012). We also used a lognormal prior distribution ($\mu = 1.25$, $\sigma = 1$) and node heights within the bounds of the prior ages of the fossil-calibrated nodes (Table S6). Second, we used the fossil of *Bryodema croatica* (Zeuner, 1942) from the lower Miocene (23.03–15.97 Mya) of Radoboj (Croatia), which is the oldest known fossil of Bryodemini. As previous studies have shown that the genera within the tribe Bryodemini are not monophyletic, we use this fossil as the minimum age for the split between Bryodemini and *Celes*. We set an exponential prior distribution with an offset of 15.97 and a lognormal prior distribution ($\mu = 1.96$, $\sigma = 1$). Third, we used the fossil of *Oedipoda nigrofasciolata* (Heer, 1849) from the middle Miocene (12.7–11.608 Mya) of Radoboj (Croatia), and we used this fossil as a calibration for the split between *Oedipoda* and *Pseudoceles* because it represents the oldest known fossil for the genus *Oedipoda*. We set an offset of 11.608 with the exponential prior distribution and used a lognormal prior distribution (mu 0.09, sigma 1). Finally, we used a fossil assigned to the genus *Locusta* (Théobald, 1937) without species designation from the early Oligocene (28.1–23.03 Mya) of Aix-en-Provence (France) to calibrate the split between *Locusta* and *Gastrimargus*, because it represents the oldest known fossil of the *Locusta* lineage. We set an exponential prior distribution with an offset of 23.03 and a lognormal prior distribution ($\mu = 1.62$, $\sigma = 1$).

In BEAST, the MCMC run was conducted with 300 million generations, and trees were sampled every 1000 generations. The first 25% of samples were discarded as burn-in. We further checked whether the effective sample size (ESS) was more than 200 for all parameters. The remaining subsampled trees were used to generate a maximum clade credibility tree using TreeAnnotator v2.5.0, with the annotation of mean height and 95% highest posterior density (HPD) intervals. Trees were visualized in Figtree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Biogeographic analysis

We estimated the ancestral range evolution with the R package BioGeoBEARS (Matzke, 2014) to infer biogeographical scenarios during the diversification of different tribes of Oedipodinae. A dated phylogeny inferred from the BEAST analysis with the outgroups trimmed and a file of geographical ranges indicating the presence/absence of each taxon in each discrete area was imported to BioGeoBEARS (Appendix S1). We defined six areas: Nearctic (North America), Neotropical (South America, including Central America), Ethiopian (Africa south of the Sahara and Madagascar), Palaearctic (North Africa, Arabia, Western and North Asia, Europe, North China, Greenland and Arctic islands), Oriental (South China, South Asia, including the islands up to the Sunda Islands) and Australia (Australia with associated islands and Pacific islands) following Song et al. (2018). We identified the distribution range for each

species (Table S1) based on the maps available from the OSF (Cigliano et al., 2025) and GBIF database (https://www.gbif.org/). For *Bryodema* specimens identified only to genus, we coded them as Palaearctic, as both the genus *Bryodema* and the tribe Bryodemini are restricted to the Palaearctic region. The maximum number of occupied areas for each ancestral node was set as four.

We constructed a time-stratified palaeographic model based on the changes in continental plate distribution for the four time intervals defined within geological epochs (Pleistocene, Pliocene, Miocene, Oligocene and Eocene): 0–5.33, 5.33–23.03, 23.03–33.9, 33.9–56 Mya (geological boundaries *sensu* Cohen et al., 2013). A connectivity matrix for each time interval was built to explain whether the aforementioned areas were connected to each other over time (Appendix S2). We compared the fitness of six available models based on the Akaike Information Criterion (AIC): DEC (dispersal-extinction-cladogenesis) (Ree et al., 2005); DEC + J (including founder-event speciation); DIVALIKE, a likelihood version of DIVA (dispersal-vicariance) (Ronquist, 1997); DIVALIKE+J (including founder-event speciation); BAYAREALIKE, a likelihood version of the Bayesian inference of historical biogeography for discrete areas (BayArea) (Landis et al., 2013); and BAYAREALIKE+J (including founder-event speciation). These six models included five parameters (Table S7): LnL (log-likelihood), numparams (number of parameters), d (dispersal parameter), e (extinction parameter), and Akaike information criterion (AIC).

Results

We generated 86 new mitogenomes from 16 tribes of Oedipodinae, including the first reported mitogenomes for nine tribes (Acrotylini, Anconiini, Arphiini, Chortophagini, Hippiscini, Machaerocerini, Psinidiini, Trimerotropini and Tropidolophini). The mitogenome sizes ranged from 15 150 bp to 16 825 bp.

Characteristics of Oedipodinae mitogenomes

As detected in previous studies (Gaugel et al., 2023), the Oedipodinae mitogenomes consisted of 13 protein-coding genes, two rRNA genes, 22 tRNA genes and one A + T rich region. We detected a change in gene order of *trnK* (*Lys*)-*trnD* (*Asp*) to *trnD* (*Asp*)-*trnK* (*Lys*) in all 103 Oedipodinae species compared to the ancestral insect gene order shown in Fig. 4a (Boore et al., 1998).

Total GC content, the GC content of the PCGs with inclusion and exclusion of third positions differed substantially among taxa (Fig. S1). At the tribe level, Sphingonotini had higher GC content in mitogenomes and PCGs than most other tribes (Fig. S1). The GC content of the tribe Tropidolophini in the PCGs with exclusion of third positions is the highest. All Oedipodinae species exhibited positive AT skew (higher A than T content), and almost all species exhibited negative GC skew (lower G than C content) (Table S2).

Base substitution saturation analysis revealed that Iss for both PCG12 and PCG123 was smaller than Iss.c (Table S3), suggesting that none of the datasets exhibited substitution saturation. Analysis of PCG123 and PCG12 indicated that for PCG12 both transitions and transversions fall on a straight line with transitions consistently outnumbering transversions (Fig. S2), suggesting that the sequences have not experienced substitution saturation and are appropriate for phylogenetic analysis. Sequence heterogeneity was assessed in AliGROOVE for different datasets separately (Fig. S3). No obvious heterogeneous outliers were detected.

Mitogenomic phylogeny

The phylogenetic relationships of band-winged grasshoppers were reconstructed using BI, ML and MP analyses (Figs 3, 4, Figs S4–S11). With the exception of Tropidolophini and *Ceracris*, all species of Oedipodinae formed a monophyletic clade with strong nodal support in all phylogenetic analyses (Figs 3, 4, Figs S4–S11). The only member of Tropidolophini was the sister taxon of Gomphocerinae. Further, the genus *Ceracris* of Parapleurini was sister to the genus *Phlaeoba* of Acridinae. All other species of Oedipodinae grouped together in a backbone clade, forming a sister clade with the Acridinae genus *Acrida*.

At the tribe level, the monophyly of nine tribes (i.e. Acrotylini, Anconiini, Bryodemini, Chortophagini, Machaerocerini, Psinidiini, Trilophidiini, Trimerotropini and Tropidolophini) was supported, whereas the Parapleurini, Oedipodini, Epacromiini, Hippiscini and Sphingonotini were consistently recovered as paraphyletic in almost all analyses. The tribe Locustini was recovered as monophyletic in the BI and ML nucleotide trees, but appeared paraphyletic in the BI and ML amino acid trees, primarily due to the uncertain phylogenetic placement of the genus *Heteropternis*. There are three *incerta sedis* species in our study: *Humbe tenuicornis*, *Morphacris fasciata* and *Leptopternis gracilis*. The phylogenetic positions of two species *H. tenuicornis* and *M. fasciata* suggested a relationship to Locustini. The species *L. gracilis* grouped within the Sphingonotini (Fig. 3).

In most tribes, the monophyly of genera was supported by the phylogenetic results (Fig. 3), except for Bryodemini, Sphingonotini and Trimerotropini. Although the monophyly of Bryodemini and Trimerotropini was generally recovered, several inner-group relationships within the clade were inconsistent across trees (Fig. 3, Figs. S4–S11).

Topology inconsistency assessments

We simplified the results of the ML tree inferred from the PCG12 matrix, showing only the topological relationships among tribes (Fig. 5a). Controversial topological structures exist concerning the monophyly at the tribe level, specifically: the monophyly of the Locustini tribe (Clade a: Locustini + Epacromiini,

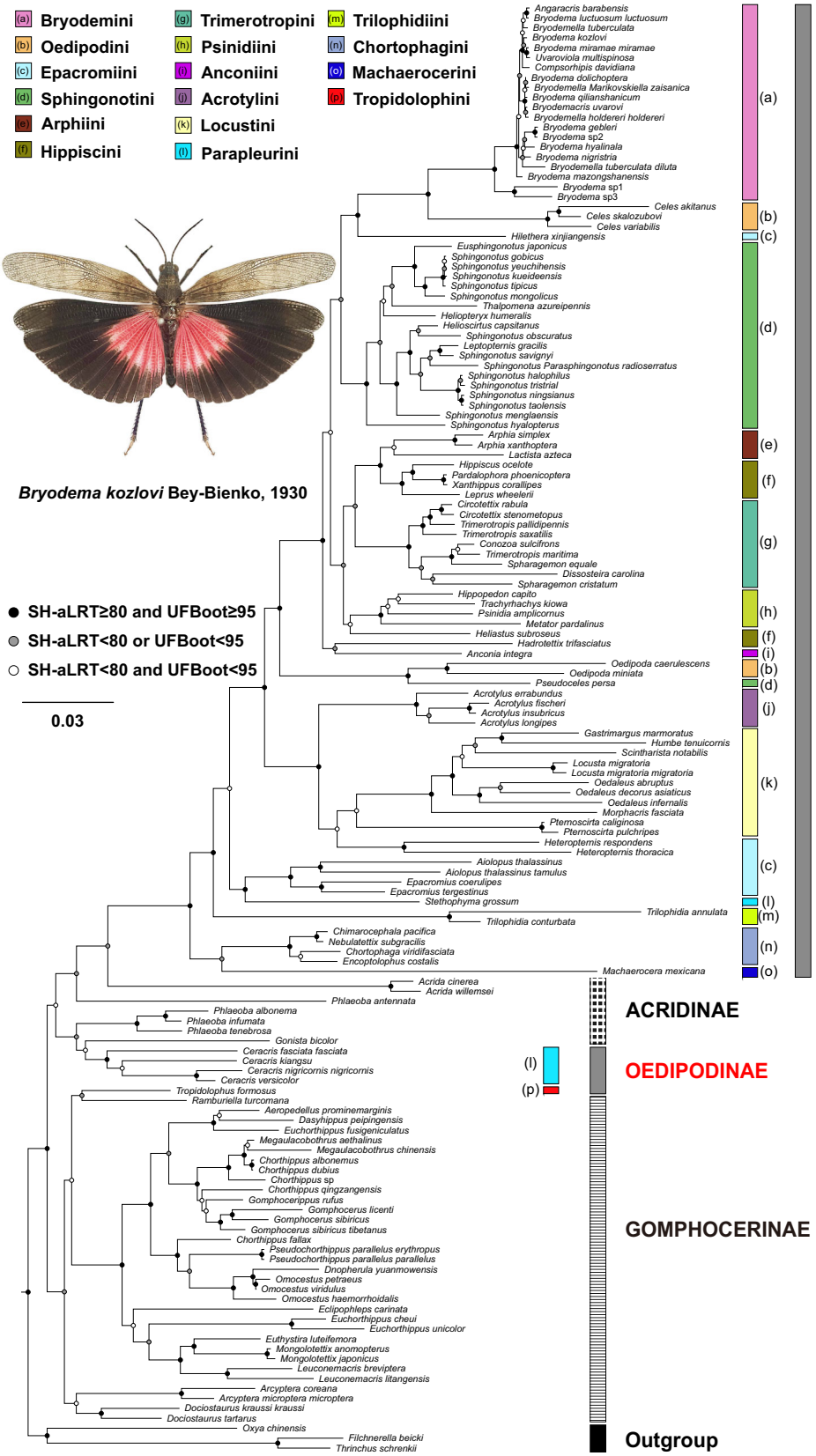


Fig. 3. Phylogeny of Oedipodinae, Acridinae and Gomphocerinae species inferred from maximum likelihood analysis based on PCG12 dataset.

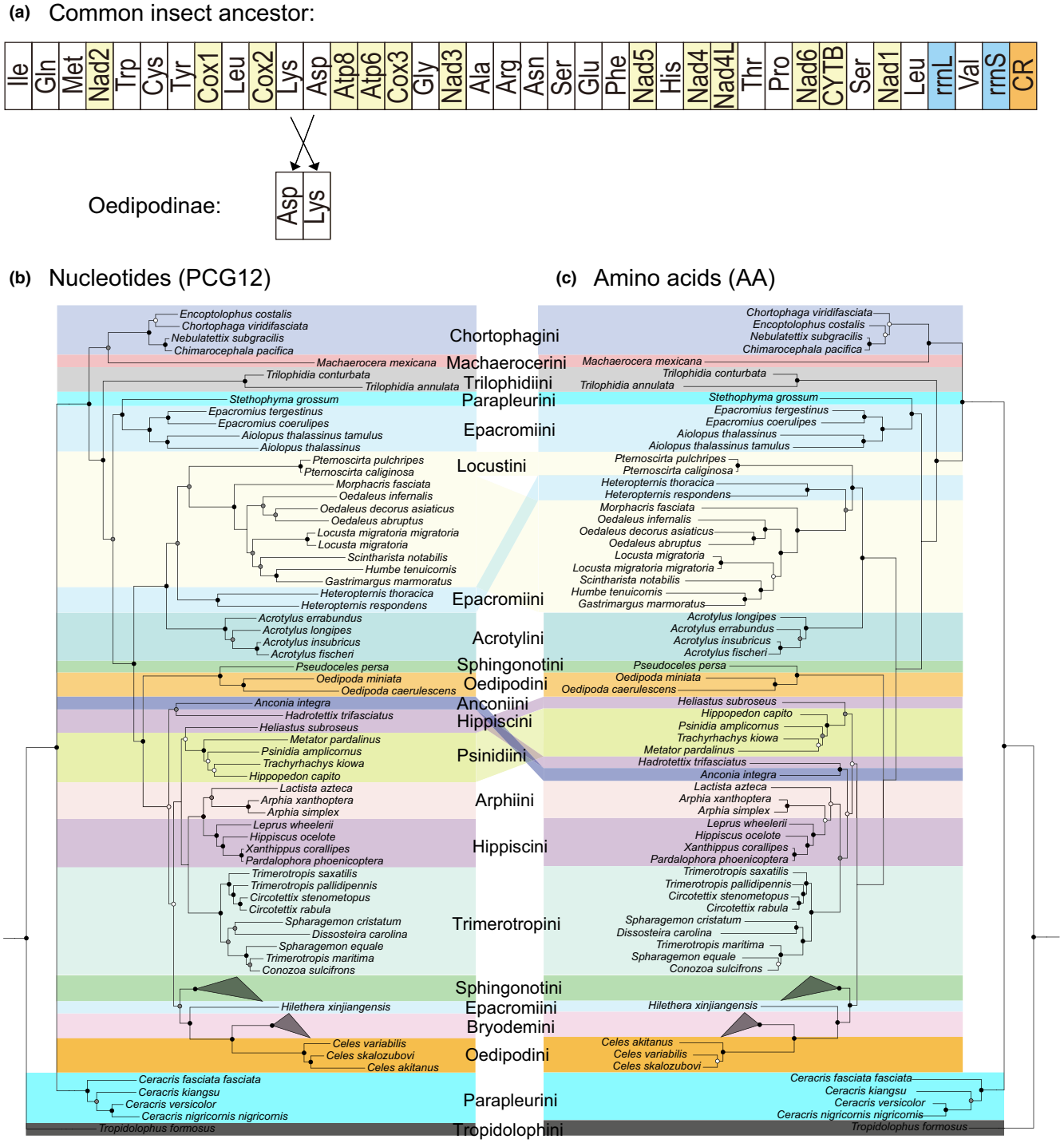


Fig. 4. (a) Mitochondrial gene order comparison between the ancestral insect and the Oedipodinae arrangement. (b and c): Phylogenetic incongruences in Oedipodinae based on the PCG12 (b) and AA (c) datasets. Black circle: clades supported in both maximum likelihood and Bayesian analyses with SH-aLRT >80%, ultrafast bootstrap (UFBoot) > 95% and posterior probability (PP) > 0.95; grey circle: clades recovered, but at least one support value is below the threshold (SH-aLRT <80%, UFBoot <95% or PP < 0.95); white circle: clades unsupported (all support values below thresholds) or not recovered in one or both analyses. Tribes are colour-coded and labelled accordingly.

Heteropternis), and the monophyly of the tribe Arphiini (Clade b: Arphiini + Hippiscini) (Fig. 5a). The monophyly of the tribe Locustini is rejected by all AA matrices (Clade a1 in Fig. 5b). However, the monophyly of Locustini received robust support in all FcLM analyses (Fig. 5c). Regarding the monophyly of

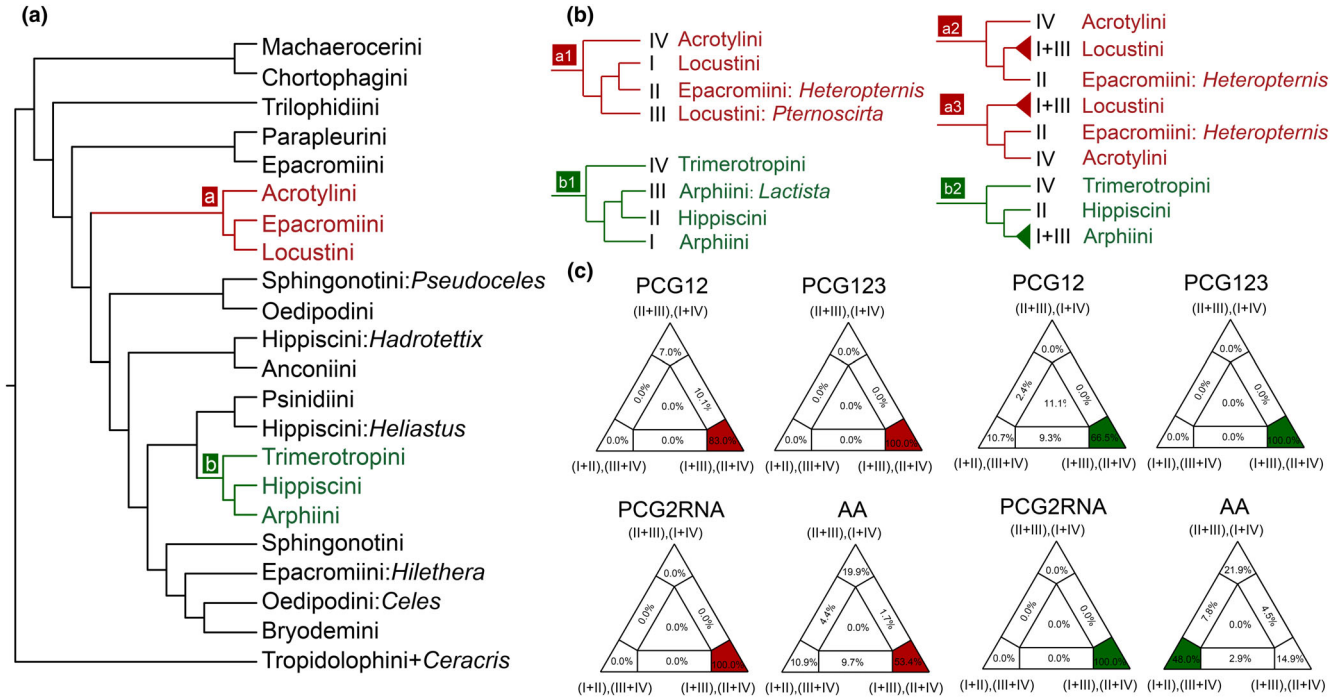


Fig. 5. (a) Phylogeny of Oedipodinae inferred from PCG12 dataset using maximum likelihood (IQTREE), summarized from Fig. 1. (b) Phylogenetic relationships within Clade a and Clade b are summarized in the following: (a1) Figs S9 and S10, (a2) Figs S4–S6 and S8, (a3) Fig. S7; (b1) Figs S5–S9, (b2) Figs S4 and S10. (c) Four-cluster likelihood mapping showing Clade a and Clade b hypotheses. Analyses are based on matrices of PCG12, PCG123, PCG2RNA and AA sequences.

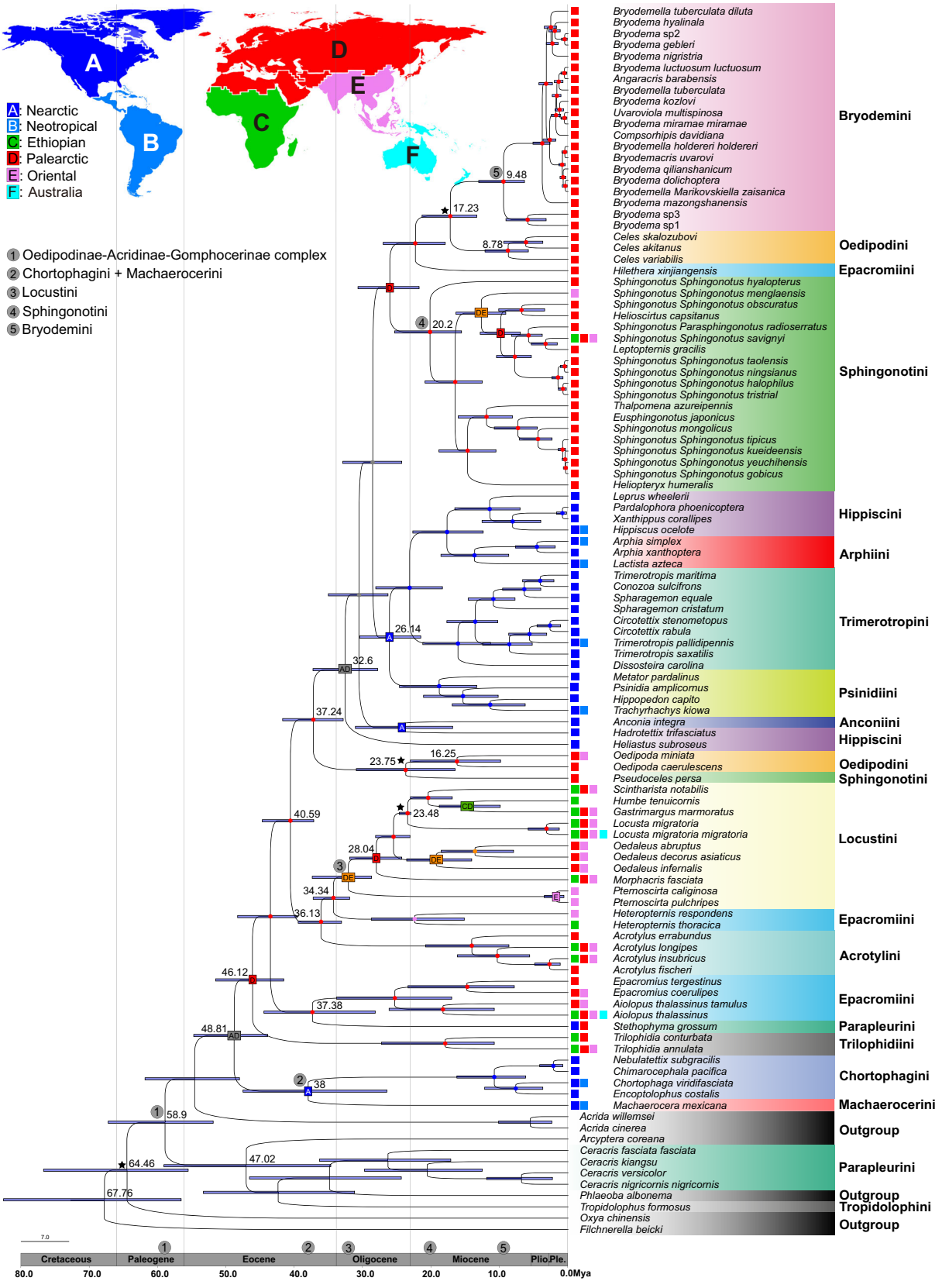
the tribe Arphiini, despite unanimous support in results presented in the PCG12 matrix (Clade b2 in Fig. 5b), the monophyly of the tribe Arphiini in matrices PCG123 and PCG2RNA is rejected (Clade b1 in Fig. 5b). Therefore, we conducted additional verification through FcLM analyses. The monophyly of Arphiini remained unresolved in the FcLM analyses, as it was supported by most matrices (PCG12, PCG123 and PCG2RNA) but rejected in the AA matrix (Fig. 5b).

Divergence time estimation and biogeography

The divergence times estimated based on the exponential distribution prior were generally younger than the lognormal distribution prior. For instance, the root of the Acridinae–Gomphocerinae–Oedipodinae complex was estimated to be 64.46 Mya (95% HPD: 55.53–76.65 Mya) based on the exponential distribution (Fig. 6 and Fig. S12), and was estimated to be 67.61 Mya (95% HPD: 56.44–80.87 Mya) based on the lognormal distribution (Fig. S13). Considering previous estimates of the root age of these three subfamilies (~60 Mya also see Discussion), we used the results of molecular dating under the exponential distribution prior for further biogeographical analysis. In BioGeoBEARS, the DEC model was the best according to the constrained test, with an AIC value of 365.260 (Table S7).

The result indicated that the most recent common ancestor of the Oedipodinae originated in the Holarctic during the Eocene period, approximately 49 Mya (95% HPD: 43.93–54.71 Mya; refer to Fig. 6). The separation between the tribes Chortophagini and Machaerocerini likely occurred around 38 Mya (95% HPD: 26.51–47.53 Mya; refer to Fig. 6), and its common ancestor was probably located in the Nearctic. Other clades began diversifying around 46 Mya (95% HPD: 41.56–51.51 Mya; refer to Fig. 6). Each of these clades independently dispersed from the Palaearctic region to the Oriental, Nearctic and Ethiopian regions (as shown in Fig. 6). The Locustini clade diversified

Fig. 6. Chronogram with estimated divergence times based on the PCG12 dataset using fossils among Oedipodinae, with ancestral areas reconstructed under DEC in BioGeoBEARS. Squares between tips and tip labels indicate distributions assigned to each single species (A–F), with colour codes corresponding to the areas indicated on the map and the legend. Numbers at nodes indicate ages in million years ago (Mya). Blue bars at nodes are 95% HPD. Coloured circles at nodes represent the most likely ancestral ranges immediately before and after each speciation event. Tribes are highlighted in distinct colours.



around 32 Mya (95% HPD: 28.75–37.39 Mya; refer to Fig. 6), with the DEC model suggesting that its common ancestor likely originated in the Oriental and Palaearctic region and later spread into the Ethiopian region (Fig. 6). The diversification of the Sphingonotini clade occurred approximately 20 Mya (95% HPD: 15.6–25.37 Mya; refer to Fig. 6), with all models indicating that its common ancestor probably originated in the Palaearctic, subsequently expanding into the Oriental region (as shown in Fig. 6, Figs. S14–S19). The Bryodemini clade began diversifying around 9.48 Mya (95% HPD: 6.41–13.08 Mya; refer to Fig. 6), with all models indicating that the Palaearctic region represents the ancestral range of Bryodemini.

Discussion

In this study, we reconstructed the most comprehensive phylogeny to date of Gomphocerinae, Acridinae and Oedipodinae using the largest mitogenomic dataset available (Fig. 3). We analysed 103 mitogenomic sequences of Oedipodinae spanning 16 tribes and 54 genera. With the exception of Tropidolophini and *Ceracris*, all species of the subfamily Oedipodinae form a monophyletic clade in our phylogenetic analysis. Most of the tribes in Oedipodinae were monophyletic; yet, the analyses also showed some inconsistencies with the current systematics suggesting that some tribes require revision. Molecular clock analysis predicted the origin of the Oedipodinae in the Eocene at about 49 Mya (with a range from 44 to 55 Mya) generally slightly predating most previous estimates for the Oedipodinae (e.g. Song et al., 2018) and matching the dating estimates for Acrididae (Song et al., 2020). Finally, ancestral area reconstruction indicates that band-winged grasshoppers primarily originated in the Palaearctic, corroborating Song et al. (2018), which suggested a Palaearctic origin for the common ancestor of the Acridinae, Gomphocerinae and Oedipodinae complexes. In the following, we discuss these major findings in more detail and emphasize some caution to be taken in interpreting some of the data.

The monophyly of Oedipodinae

Several recent studies have suggested that Oedipodinae are paraphyletic with respect to Acridinae and Gomphocerinae (Chapco and Contreras, 2011; Song et al., 2018). In our phylogenetic analyses, we used 103 Oedipodinae sequences, 33 Gomphocerinae sequences, seven Acridinae sequences and three species of Pamphagidae and Acrididae as outgroups. This is the most extensive and balanced dataset of Oedipodinae so far. Our phylogenetic analysis supports the paraphyly of Oedipodinae in the current composition, as Acridinae and Gomphocerinae are nested in this clade, consistent with previous studies (Fig. 3, Figs S4–S11) (Chapco and Contreras, 2011; Song et al., 2018).

Furthermore, Tropidolophini and *Ceracris* consistently group outside the main clade across all our analyses. The tribe Tropidolophini consists of only one species, *Tropidolophus formosus* and was established by Otte in 1995 based on morphological evidence (Otte, 1984). Our study reported the mitogenome of *T. formosus* for the first time, providing molecular data for the taxonomic position and phylogenetic relationships of both this species and the tribe. Our phylogenetic analysis indicates that Tropidolophini are more closely related to species within the subfamily Gomphocerinae than to other Oedipodinae. Specifically, it forms a sister clade with *Ramburiella turcomana*, an early-diverging member of Gomphocerinae (Fig. 3). Morphologically, *T. formosus* has an intercalary vein which would support a position within Oedipodinae. However, this vein is only weakly displayed and has no stridulation pegs on it (Fig. S20c). Notably, previous studies have reported that certain genera within Gomphocerinae also exhibit an intercalary stridulatory vein in the medial region of the tegmen (Jago, 1971), indicating that this trait is not exclusive to Oedipodinae, and some Oedipodinae do not have the intercalary vein. Another piece of supporting evidence is the presence of small pegs on the inner femur of *T. formosus*, resembling a key diagnostic character of Gomphocerinae. Previous studies have shown that the stridulatory mechanism in Gomphocerinae is relatively uniform, with the structure located on a higher femoral keel (Jago, 1971). North American genera *Coniana* and *Cibolacris*, despite their striking external resemblance to Oedipodinae, have been classified within Gomphocerinae based solely on the presence of femoral stridulatory structures (Rehn and Grant, 1960). These findings may further support the placement of *T. formosus* within Gomphocerinae. We noted that the pegs on *T. formosus* are morphologically distinct from those in known Gomphocerinae species (Fig. S20), suggesting it being a unique evolutionary lineage. Further assessments, including broader taxon sampling and genomic analysis, are necessary to determine its appropriate taxonomic status. Based on both morphological characteristics and phylogenetic analyses, we propose removing this tribe from the subfamily Oedipodinae and treat it as an unplaced taxon (*incertae sedis*) within Acrididae pending further comprehensive phylogenetic assessment.

The taxonomic position of the genus *Ceracris* also remains uncertain. Originally, *Ceracris* was considered to be a member of Acridinae or Gomphocerinae (= Arcypteridae sensu Cao et al., 2019; Jiang and Zheng, 1998; Zheng and Xia, 1998; Jago, 1971). Storozhenko et al. (2015) repositioned the genus into the Parapleurini within the subfamily Oedipodinae based on morphological evidence, which includes a distinctively

low median tubercle on the prosternum and stridulatory pegs located on the RS and M veins, as well as on the intercalary vein of the medial area. This combination of characters is typically restricted to Oedipodinae but is also present in most species of *Ceracris*. While, the stridulatory apparatus may appear to be a good trait for assigning the genus to the Oedipodinae, it has been shown to be evolutionarily labile (Hochkirch and Husemann, 2008) and may therefore have evolved multiple times. Both the current and previous findings cast doubt on the accuracy of assigning the genus *Ceracris* to Parapleurini, or even to the subfamily Oedipodinae. Song et al. (2018) found a closer relationship between *C. kiangsu* and both Acridinae and Gomphocerinae, consistent with our findings. Consequently, a more extensive investigation of the phylogenetic relationships of this genus is warranted. In our phylogenetic trees (Fig. 3), *Ceracris* is recovered as the sister taxon to *Gonista bicolor*. The genus *Gonista* Bolívar, 1898 is currently listed under Gomphocerinae in the OSF. However, according to Popov et al. (2019), *Gonista* has been transferred from Ochrilidiini (Gomphocerinae) to Phlaeobini (Acridinae) based on epiphallic characters with large articulated ancorae and broad lobiform lophi. Therefore, in the present study, *Gonista* is treated as a member of Acridinae (Fig. 3). A close relationship between *Ceracris* and the genera *Phlaeoba* and *Gonista* would suggest moving *Ceracris* to Acridinae. From a morphological point of view as well, such a relationship appears likely: the face of *Ceracris* is strongly slanted and the forewings are obliquely truncated at the apex. Slifer (1939) and Popov et al. (2019) proposed that the morphology of the proximal loop of the female spermathecal duct serves as a key diagnostic character for distinguishing Oedipodinae from Acridinae. In most Acridinae, the spermathecal duct features a distinct proximal loop and is typically coiled like a watch-spring within the body cavity (Slifer, 1939; Popov et al., 2019). In contrast, most Oedipodinae lack this loop, with the duct forming a tangled structure (Hollis, 1968). Previous studies have shown that the female genitalia of *Ceracris* possess a spermathecal duct with a watch-spring shape (Wang et al., 2023). Hence, we suggest that this genus be removed from the subfamily Oedipodinae and transferred to Acridinae.

Therefore, all species that we included in our dataset and that are currently assigned to Oedipodinae, with the exception of the tribe Tropidolophini and the genus *Ceracris*, were consistently retrieved as a monophyletic group in all our analyses (Figs 3, 4, Figs S4–S11).

Relationships of Oedipodinae at the tribe level

All our results furthermore strongly support the monophyly of nine of the 16 tribes of Oedipodinae (Acrotylini, Anconiini, Bryodemini, Chortophagini, Machaerocerini, Psinidiini, Trilophidiini, Trimerotropini and Tropidolophini). Chortophagini was previously confirmed as monophyletic (Edelman et al., 2010). The tribe was recovered as a sister group of Machaerocerini, as an early branching lineage in line with previous findings (Fries et al., 2007).

The sister relationship between *Oedipoda* (tribe Oedipodini) and *Pseudoceles* (tribe Sphingonotini) was strongly supported in all phylogenetic analyses. This relationship was also suggested by Dey et al. (2022). *Pseudoceles* is morphologically similar to *Oedipoda*, particularly *P. persa*, which shares with *Oedipoda* species a high degree of wing colour variability and the occurrence of parallel colour morphs (Dirsh, 1949). Several species of both genera also occupy ecologically analogous habitats. Further morphological studies will reveal if the genus *Pseudoceles* should be moved to the Oedipodini or if a new tribe needs to be described.

The phylogenetic placement of the genera *Hilethera* and *Celes*, currently assigned to Epacromiini and Oedipodini respectively, remains uncertain. Dong et al. (2019) recovered *Hilethera* as sister to the Bryodemini, whereas Sun et al. (2023) inferred a comparable relationship between *Celes* and the Bryodemini. This is the first phylogenetic analysis to simultaneously include *Celes*, *Hilethera* and the Bryodemini. Additional molecular data, particularly from a broader taxon sampling, alongside comparative morphological evidence, will be necessary to determine the tribal placement of *Celes* and *Hilethera* with confidence. The current data suggest including *Celes* in Oedipodini and potentially making *Hilethera* a distinct tribe. However, until morphological evidence becomes available, for the time being, we recommend that both genera be treated incertae sedis within the subfamily until further data are available.

In our study, different datasets and methods yielded distinct topologies but overall raised doubt on the monophyly of some tribes. We simplified the results of PCG12 dataset, retaining only the topological relationships of the tribes shown in Fig. 5a. At the level of tribes, Locustini and Arphiini exhibit different topological structures (Fig. 5). The monophyly of Locustini was supported in previous studies (Song et al., 2018; Sun et al., 2023) and in most of our nucleotide phylogenetic trees, but it was rejected in our amino acid trees with high support (Fig. 5). The non-monophyly of Locustini in the amino acid trees was attributed to the unstable position of the genus *Heteropternis* (Clade a in Fig. 5). FcLM results consistently supported the monophyly of Locustini, which was recovered as the sister group to *Heteropternis* (Fig. 5b). The tribe Arphiini is considered monophyletic (Fig. 4). However, the phylogenetic results of the datasets PCG123 and PCG2RNA, both for Bayesian inference and

Maximum Likelihood, reject its monophyly with high support (Clade b1 in Fig. 5b). We conducted FcLM analyses, and the results showed that only one dataset (AA) rejected the monophyly of Arphiini (Fig. 5c). Clarifying the status of Arphiini will require additional data and access to relevant specimens. To date, these genera have not been included in any phylogenetic analysis, precluding direct comparison with other taxa.

Relationships within tribes

Our results reveal incongruences with the current tribal-level systematics. Similar inconsistencies are also evident in intratribal relationships, particularly at the genus level. Notably, the tribes Bryodemini and Sphingonotini exhibit the highest degree of phylogenetic ambiguity. Both tribes have been the focus of extensive taxonomic and phylogenetic research over previous decades (Husemann et al., 2011, 2012, 2013; Benediktov, 2016; Storozhenko et al., 2017; Dey et al., 2021a). Our data provide strong support for the monophyly of Bryodemini, consistent with previous findings (Song et al., 2018; Chang et al., 2020; Sun et al., 2023). However, the inner tribe relationships so far remain unresolved, and this study also suggests that none of the genera within the tribe (*Bryodemella*, *Compsorhipis* and *Bryodema*) represent monophyletic groups. There are seven genera with 53 species or subspecies distributed in Asia and Europe of Bryodemini (Cigliano et al., 2025). The key distinguishing characteristics among genera are primarily found in the structures of the base of the male hind tibia and apex of hind femur, the size of the apex of the hind tarsus and the width of the male mesosternum and female sternal plate (Storozhenko et al., 2017). This study provides the most comprehensive phylogenetic analysis of the tribe to date, including six of the seven currently recognized genera (excluding *Andrea* Mistshenko, 1989). Among these, four genera are monotypic: *Andrea*; *Angaracris* Bey-Bienko, 1930; *Bryodemacris* Benediktov, 1998; and *Uvaroviola* Bey-Bienko, 1930. Our phylogenetic results reveal that three of the four monotypic genera (*Angaracris*, *Bryodemacris*, and *Uvaroviola*) are nested within other genera, suggesting that their current taxonomic status may require reevaluation.

The genus *Angaracris* is widely distributed across Eurasia and can be separated from other genera by the presence of shallow transverse dorsal furrows at the base of hind tibia. In the 19th and 20th centuries, more than 10 species were described within this genus, primarily differentiated by body coloration and hind wing colour. However, based on a comprehensive morphological revision by Storozhenko et al. (2017), who examined more than 1000 specimens from Kazakhstan, Russia, Mongolia and China, all previously described species were synonymized under *Angaracris barabensis* (Pallas, 1773). The authors suggested that a camouflaged body coloration, which may depend on the habitat, particularly in lichen-covered areas, represents a form of intraspecific variation, similar to the variation observed in hindwing coloration. The genus *Uvaroviola*, established by Bey-Bienko in 1930, is restricted to the Qinghai–Tibet Plateau. This genus is characterized by the base of the hind tibia bearing strong, irregular rugulae and deeply impressed punctures (Storozhenko et al., 2017). Our phylogenetic tree indicates that *Uvaroviola multispinosa* is sister to *Bryodema miramae miramae*, which was also collected from the Qinghai-Tibet Plateau. In contrast, the species *Bryodemacris uvarovi* is sister to *Bryodemella holdereri holdereri*. However, this species was reported by Bey-Bienko (1930) as '*Bryodema uvarovi*'. Benediktov (1998) established the new genus *Bryodemacris*, designating its type species based on distinctive morphological characteristics, including an arched median carina of the pronotum in the prozona, and the presence of two prominent bulges at the apex of the hind femur in dorsal view. The monotypic genus *Andrea* is endemic to Mongolia and can be readily distinguished from the other genera by the presence of vestigial hind wings in males. However, due to the limited availability of both morphological descriptions and molecular data for *Andrea gorochovi*, its phylogenetic placement remains unresolved.

The genus *Compsorhipis* Saussure, 1889 comprises seven species and is endemic to Asia. Morphologically, it can be distinguished from other genera by the presence of slender anal veins in the hind wings and a distinctly S-shaped intercalary vein in the medial field of the tegmen. In the present study, only one representative species of *Compsorhipis* was included. However, a previous phylogenetic analysis of Bryodemini based on CO1 sequences, which included three *Compsorhipis* species, revealed that the genus may be not monophyletic (Kock et al., 2024). *Bryodemella* and *Bryodema* are the most species-rich lineage within this tribe. Previous morphological studies have suggested substantial morphological differentiation in the hind femur and hind tibia structures among the genera *Bryodemella* and *Bryodema* within this tribe (Storozhenko et al., 2017); however, these distinctions lack corroborative support from molecular evidence (Dey et al., 2021a). Our result showed that the representatives of *Bryodemella* and *Bryodema* were intermingled within the whole Bryodemini clade.

In general, our data further support this lack of resolution at the genus level using whole mitogenomes. The perplexing internal phylogenetic relationships within Bryodemini in this study might be attributed to the limitations of mitochondrial genomic data in analysing the phylogenetic relationships among genera or

species, suggesting that further nuclear genetic data are required for a comprehensive analysis. However, the data so far suggest that the tribe shows only limited genetic resolution at the genus level, which may be in part the result of the large genome sizes (Hawlitschek et al., 2023) leading to high amounts of conflicting genetic signal. Further, a very recent divergence and an associated lack of lineage sorting and common hybridization may have a part in explaining this lack of signal. Bryodemini species occur in montane grasslands, forest edges and desert areas. Their similar habitats and overlapping distribution ranges make hybridization possible, which could further contribute to the observed phylogenetic uncertainty. Hence, we currently believe that the lack of clear phylogenetic resolution in this tribe is likely due not only to taxonomic issues, but also to limited genetic resolution. We noticed that the genera *Compsorhipis, Bryodemella* and *Bryodema* were paraphyletic, but due to the still limited taxon sampling of this tribe, we advocate postponing these taxonomic changes until more data become available. We suggest that this will have to wait for a more exhaustive species sampling and a careful evaluation of the morphological traits. Given that all currently recognized genera appear to be non-monophyletic, we recommend a comprehensive sampling across the tribe, including representatives from at least seven genera. Considering the potentially high morphological variability within species of Bryodemini, as observed in the recently revised the genus *Angaracris*, we further suggest a morphological revision of the species of the whole tribe using a large number of samples. In terms of molecular markers, both previous studies and our current results indicate that individual genes or mitogenomes offer limited resolution. Therefore, we recommend the use of more nuclear genes (e.g. ultraconserved elements or transcriptomes) for future phylogenetic studies of the tribe.

Similarly, the resolution in the tribe Sphingonotini is limited. Within the Sphingonotini, the largest genus is *Sphingonotus*, which, however, is not monophyletic due to the grouping of related genera like *Leptopternis*. Again, the genus has been suggested to be of young age and hence incomplete lineage sorting may play a pivotal role (e.g. Husemann et al., 2012). However, in addition to incomplete lineage sorting, the lack of a consistent taxonomy presents a major challenge in this group. *Leptopternis* is a genus with a strong adaptation to sandy habitats, which is also found in some species of *Sphingonotus* and additional related genera. Related selection pressure may have led to common adaptations and may obscure relationships derived based on morphological traits. Hence, for the Sphingonotini, we suggest that the lack of monophyly may be more a taxonomic than a resolution problem, at least at the genus level. Sphingonotini is a highly diverse tribe, comprising more than 200 species. To address the taxonomic status and monophyly of the genera within this tribe, a large number of specimens must be collected and both morphological and molecular evidence is required to resolve these issues in the future.

Similar issues are also observed within the tribe Trimerotropini. Species of Trimerotropini are endemic to the Americas and exhibit high species diversity. Our study provides the first phylogenetic analysis, encompassing all five currently recognized genera. Consistent with previous studies (Chapco et al., 1997; Husemann et al., 2012), our results indicate that the genera *Trimerotropis* and *Spharagemon* within this tribe are not monophyletic. Among the genera, *Trimerotropis* and *Spharagemon* are the most species-rich, with 52 species in *Trimerotropis* and nine species in *Spharagemon*. A key morphological feature distinguishing *Trimerotropis* from other genera is the structure of the pronotal crest. In *Trimerotropis*, the crest is typically divided by two sulci, while in other genera, the crest is usually divided by a single sulcus, although some species, such as *Spharagemon bunites* and *S. campestris*, may also exhibit two sulci (Otte, 1984), indicating that this trait may not be suitable for taxonomic purposes. Additional distinguishing features among these genera include the coloration and patterning of the forewings and hindwings, along with the coloration of the hind tibiae (Otte, 1984). Therefore, distinguishing these genera based solely on morphology can be challenging in certain cases. The phylogeny has a very clear signal in our study, but the taxonomic history is confusing. It is therefore a purely taxonomic issue. We recommend conducting extensive sampling across the Americas, combining comprehensive molecular evidence with morphological data to revise their classification and thoroughly resolve the taxonomic issues among the genera within this tribe.

In addition, although Acridinae and Gomphocerinae were included primarily as outgroups, our analyses recovered three genera—*Phlaeoba*, *Chorthippus* and *Omocestus*—as non-monophyletic (Fig. 3). These results are consistent with previous studies. The non-monophyly of *Chorthippus* has been repeatedly reported, with related genera such as *Gomphocerippus* and *Megaulacobothrus* found to be nested within it (Xu et al., 2005; Nolen et al., 2020; Gaugel et al., 2023; Schmidt et al., 2024). Similarly, earlier work has highlighted the paraphyly or polyphyly of *Omocestus* (Bugrov et al., 2006). In contrast, phylogenetic data for *Phlaeoba* remain sparse, with most studies including only a limited number of species (Gaugel et al., 2023). Comprehensive taxon sampling and targeted revisionary work will be required to clarify the phylogenetic placement and taxonomic limits of these genera. Given that the primary focus of this study is on Oedipodinae, we do not further elaborate on the

phylogenetic relationships within Acridinae and Gomphocerinae.

Historical biogeography

The subfamily Oedipodinae displays a worldwide distribution, with notable radiations in both the Palaearctic and Nearctic regions (Husemann et al., 2012). Previous studies already suggested a complex biogeographic history of the Acrididae (Song et al., 2018). Chapco and Contreras (2011) suggested an African origin for the Acridinae, Gomphocerinae and Oedipodinae. Approximately 99 Mya, one pre-acridine group migrated from Africa to South America through connected landmasses, diversifying to give rise to the Gomphocerinae. Around 96 Mya, another group entered Eurasia from Africa, facilitated by intermittent land connections across the narrow Tethys Sea, and underwent a radiation event, leading to the formation of the Oedipodinae (Chapco and Contreras, 2011). Song et al. (2018) proposed that the origin of Acrididae was in South America, subsequently spreading to North America and Eurasia. Additionally, Song et al. (2018) proposed that Oedipodinae originated in the Palaearctic region (comprising Eurasia and North Africa) and subsequently underwent recolonization events from the Old World to the New World. The migration from Eurasia to North America may have taken place through either the Thulean route (or aerial dispersal across Greenland) or via the Beringian Land Bridge. Previous molecular analyses of the group suggested a Beringian dispersal (Husemann et al., 2012). However, all the past studies were strongly biased in sampling to few geographic regions or tribes.

In this study, we have systematically addressed these challenges by amassing a diverse set of specimens from band-winged grasshoppers across Africa, Eurasia (including Europe), and North America covering all currently recognized tribes. This expanded and comprehensive dataset has enabled us to conduct a thorough analysis of biogeographical patterns and divergence time estimations. Consequently, our research yields a more intricate and nuanced understanding of the evolutionary history of Oedipodinae. However, although our sampling for Oedipodinae is relatively comprehensive, we have only included few data on the closely related Acridinae and Gomphocerinae, limiting our understanding of the extended group. According to the estimations in this study, the common ancestor of Acridinae, Gomphocerinae and Oedipodinae is estimated to be around 60.02 Mya, in line with Song et al. (2015, 2018).

Our study suggests an origin in the Holarctic realm for the Oedipodinae, with the Palaearctic as the primary centre of diversification, followed by an immigration to North America, the Oriental region, Africa and Australia, with different lineages expanding their ranges and colonizing new regions. This result corroborates the conclusion in Song et al. (2018) that the common ancestor of the Acridinae, Gomphocerinae, and Oedipodinae complexes had its origin in the Palaearctic.

Similar to other studies, our data suggest several large-scale colonization events at different time horizons. At the end of the Eocene, approximately 38 Mya, one lineage may have colonized North America from the Palaearctic (clades of Chortophagini and Machaerocerini). Though the North Atlantic was opening during the Eocene, a land connection appears to have remained between North America and Europe (Denk et al., 2011; Torsvik and Cocks, 2016), enabling the exchange between the Eurasian continent and North America. Since the early Oligocene, at around 26 Mya, the ancestors of Trimerotropini, Arphiini and Psinidiini also expanded their ranges, colonizing regions in North America again. At that time, the Turgai Strait, which separated Europe and Asia, retreated, establishing a persistent land connection between the continents and providing the main route for this colonization. Sphingonotini and Bryodemini, the two most diverse tribes within the subfamily Oedipodinae, are primarily distributed across the Eurasian continent. According to our analysis, the common ancestor of Sphingonotini and Bryodemini dispersed across the Eurasian continent in the early Oligocene, around 26 Mya, giving rise to the highest species diversity of Oedipodinae in this region.

The major diversification into tribes and genera coincided with the expansion of the grasslands between 20 and 30 Mya in the Oligocene, which explains the evolution of a variety of new forms of band-winged grasshoppers. The evolution of species within genera is difficult to reconstruct from our data as we only included some of the most diverse tribes of Bryodemini and Sphingonotini. The Sphingonotini began their differentiation approximately 20.2 Mya, dispersing from Eurasia to Africa during the Miocene period. This dispersal was facilitated by the collision between the African-Arabian Peninsula and the Eurasian landmass, leading to colonization and subsequent rapid radiation. Bryodemini rapidly diversified on the Eurasian continent approximately 9.48 Mya, possibly due to the uplift of the Qinghai–Tibet Plateau, blocking the entry of westerly winds into the Tarim Basin and causing it to become dry. The emergence of this arid environment facilitated the rapid differentiation of Bryodemini species. Here, we observe recent radiations in both groups, likely dating to the Pleistocene, when repeated climatic cycles promoted speciation through isolation and reconnection, a pattern observed in many other lineages (Hewitt, 1996, 2001).

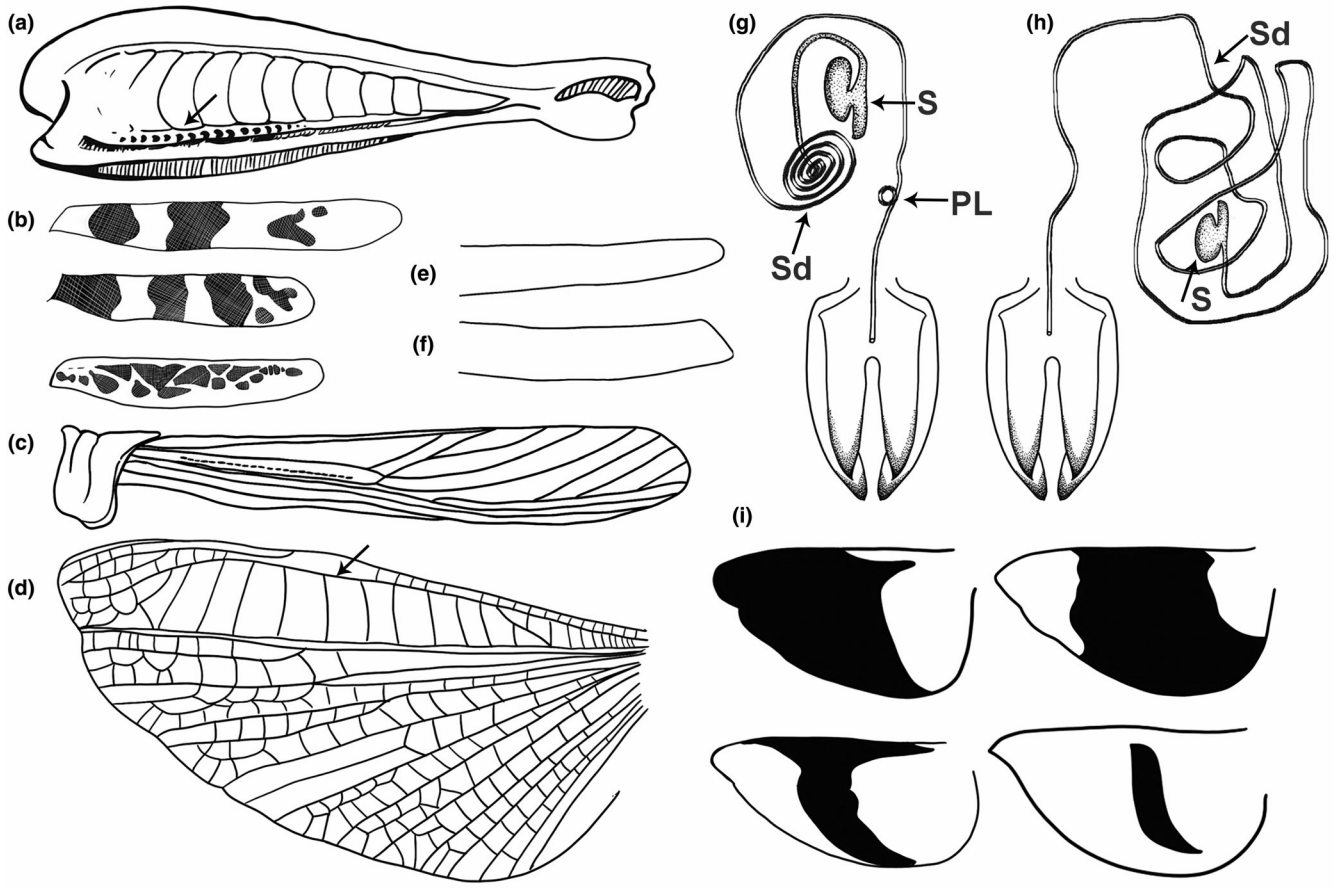


Fig. 7. Features of the Oedipodinae (b, c, e, h and i), Acridinae (d, f and g) and Gomphocerinae (a and d). (a) Inner face of male hind femora (arrow points at stridulatory pegs). (b) Forewings of Oedipodinae. (c) Forewings with raised intercalary vein. (d) Male hindwings (arrow points at enlarged cells). (e) Forewings apex of Gomphocerinae and Oedipodinae. (f) Forewings apex of Acridinae. (g) Female spermathecal ducts of Acridinae (S: spermatheca, Sd: spermathecal duct, PL: proximal loop). (h) Female spermathecal ducts of Oedipodinae. (i) Hindwings of Oedipodinae. Modified from Otte (1984) and Popov et al. (2019).

Systematics

Family Acrididae MacLeay, 1821

Subfamily Gomphocerinae Fieber, 1853 (slant-faced grasshoppers)

Type genus: *Gomphocerus* Thunberg, 1815

Diagnosis. Inner face of male hind femora with a row of stridulatory pegs (arrow in Fig. 7a). Forewings rounded at the apex (Fig. 7e), without a raised intercalary vein as in Fig. 7c (dotted line) and without prominent crossbands (not like Fig. 7b). Hindwings usually transparent and male hindwings without enlarged cells near the leading edge (arrow in Fig. 7d). Face (in side view) strongly slanted to vertical and antennae filiform or ensiform (Otte, 1984).

Subfamily Acridinae MacLeay, 1821 (slant-faced grasshoppers)

Type genus: *Acrida* Linnaeus, 1758

Diagnosis. Inner face of male hind femora without stridulatory pegs. Forewings obliquely truncated at apex, and truncation more pronounced in males (Fig. 7f), without raised intercalary vein and without distinct crossbands. Hindwings usually transparent or only faintly tinged with colour and without dark or smoky band and male hindwings always with enlarged cells near leading edge (arrow in Fig. 7d). Face moderately to strongly slanted and antennae slightly to strongly ensiform (Otte, 1984). In females, the spermathecal duct features a distinct proximal loop and is typically coiled like a watch-spring (arrow in Fig. 7g) within the body cavity (Slifer, 1939; Popov et al., 2019).

Subfamily Oedipodinae Walker, 1871 (banded-wing grasshoppers)

Type genus: *Oedipoda* Latreille, 1829

Diagnosis. Inner face of male hind femora without stridulatory pegs. Forewings rounded at apex

(Fig. 7e), usually with raised intercalary vein, as in Fig. 7c (dotted line), and with striking crossbands or large dark patches (Fig. 7b). Hindwings usually banded or brightly marked with yellows, oranges, reds or blues and often with black or smoky cross-band (Fig. 7i), male hindwings always without enlarged cells near leading edge. Face usually more vertical than slanted (Otte, 1984). In females, the spermathecal duct without a distinct proximal loop and forming a tangled structure (arrow in Fig. 7h) within the body cavity (Hollis, 1968).

Taxonomic transfers

Genus *Ceracris* Walker, 1870

Type species: *Ceracris nigricornis* Walker, 1870

Description. Head short. Face strongly slanted. Eyes situated in the middle part of the head. Vertex short. Foveolae indistinct, very small, triangular. Antennae very long. Tarsus with a large empodium between the claws, equal to the claws or extending beyond their apices. Forewings median vein area mostly without intercalary vein. Body yellowish green. Forewings brown without crossbands. Hindwings pellucid, with a pale brown tinge on the apical portion. For figures and detailed information, see Bey-Bienko and Mishchenko (1951) and Cao et al. (2019).

Transfer: The genus *Ceracris* is transferred from Oedipodinae to Acridinae based on the face strongly slanted, the inner face of male hind femora lacking stridulatory pegs (Fig. 7a) and females with watch-spring shaped spermathecal duct (Fig. 7g). Forewings obliquely truncated at the apex (Fig. 7e), and the lack of raised intercalary vein in some species (Fig. 7c).

Tribe Tropidolophini Otte, 1995

Genus *Tropidolophus* Thomas, 1873

Species *Tropidolophus formosus* (Say, 1825)

Type locality: North America, Northwestern USA, Colorado, Baxter

Description. Medium size, slender. Head short, sides parallel; front straight, nearly vertical; occiput rounded; eyes elliptical; antenna robust, passing the thorax, about 22-jointed (Fig. S20a). Pronotum compressed, slightly converging in front; the dorsum elevated into a strong arcuate crest; the front advanced at an angle upon the occiput, the posterior extremity extending at an acute angle upon the base of the elytra; no lateral carina; no transverse sutures crossing the crest; the posterior lateral margins regularly rounded inwardly from the lower angle to the apex. Forewings rounded at apex and with a weakly intercalary vein (Fig. S20a–c). Posterior femora slender, but slightly enlarged at the base; posterior tibia spined almost their entire length (Fig. S20d).

Body pale green. Antenna yellowish. Head ashy yellow; the lower sides of the pronotum pale green or cinereous; the crest green, with two yellow radii on each side, and the anterior and posterior margins yellow. Forewings pale green, with about six large brown spots on each, which are paler in the centre than near the circumference, and rounded at the apex. Hindwings pellucid, with a pale orange tinge on the basal portion and with a smoky crossband (Fig. S20b). For figures and detailed information, see Fig. S20.

Transfer: The tribe Tropidolophini is moved from Oedipodinae to Acrididae *incertae sedis* based on the specific stridulatory pegs on the hind femora. The intercalary vein is weakly expressed and without stridulation pegs, the face is straight from the side view. The morphology of this species does not conform to the morphological characteristics of any of the three subfamilies.

Conclusion

We present the most complete phylogenetic framework to date for band-winged grasshoppers, incorporating mitogenomic data from all recognized tribes within the subfamily. We find that with the exception of Tropidolophini and *Ceracris*, all species of Oedipodinae form a monophyletic clade in our phylogenetic analysis. Although most tribes are well supported by molecular data, some exhibit notable discordance. The most important factors appear to be the need for taxonomic revision and molecular processes and patterns that are likely associated with the large genomes found in many species of the family Acrididae. We make some suggestions for direct changes in the current systematics, but we also point out where additional data is needed. We have also reconstructed the biogeographic history of Oedipodinae and confirm previous findings of several waves of colonization between continents. The major diversification of Oedipodinae is likely to have been a response to the expansion of grasslands and the glacial cycles of the Pleistocene. While we present the most comprehensive dataset of the group to date, many questions remain that can only be addressed by incorporating additional taxa into the phylogeny and expanding genetic sampling to include nuclear genomic data. This will also aid in understanding the evolution of the group's large genomes.

Acknowledgements

We would like to thank all colleagues for providing samples (especially Dave Ferguson for many samples of North American species). We are deeply grateful to the late Professor Zhemin Zheng for his invaluable

guidance in the identification of specimens. We extend our gratitude to the editors and two anonymous reviewers, whose insightful comments greatly enhanced previous versions of the manuscript and significantly improved its quality. This work was financially supported by the National Natural Science Foundation of China (No. 31872273) and the Fundamental Research Funds for the Central Universities (GK202105003). This work was supported by the China Scholarship Council (CSC) (grant # 202206870020). Funding for this study was provided to JAYF by the Orthopterists' Society and the Humbolt Foundation.

Conflict of interest

The authors declare no conflict of interest.

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Supporting Information

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

Appendix S1. Presence/absence of each taxon in discrete biogeographic areas for analysis.

Appendix S2. Connectivity maxtrix used for constrained analysis with a time-stratified palaeographic model.

Appendix S3. Phylip file of ML tree based on dataset PCG12 (nucleotide data of the 13 PCGs without the third position of PCGs).

Appendix S4. Phylip file of ML tree based on dataset PCG123 (nucleotide data of the 13 PCGs).

Appendix S5. Phylip file of ML tree based on dataset PCG2RNA (nucleotide data of the 13 PCGs and two RNA genes).

Appendix S6. Phylip file of ML tree based on dataset AA (amino acids data of the 13 PCGs).

Appendix S7. Bayesian inference tree based on PCG12 dataset.

Appendix S8. Bayesian inference tree based on PCG123 dataset.

Appendix S9. Bayesian inference tree based on PCG2RNA dataset.

Appendix S10. Bayesian inference tree based on AA dataset.

Appendix S11. Maximum-likelihood tree based on PCG12 dataset.

Appendix S12. Maximum-likelihood tree based on PCG123 dataset.

Appendix S13. Maximum-likelihood tree based on PCG2RNA dataset.

Appendix S14. Maximum-likelihood tree based on AA dataset.

Figure S1. GC content for each tribe of Oedipodinae are shown as a boxplot.

Figure S2. Saturation analysis for concatenated PCG123, PCG12, Position 3, and PCG RNA.

Figure S3. AliGROOVE heterogeneity analysis of mitochondrial sequence composition for different datasets.

Figure S4. BI tree based on PCG12 matrix reconstructed using MyBayes.

Figure S5. ML tree based on PCG123 matrix reconstructed using IQTree.

Figure S6. BI tree based on PCG123 matrix reconstructed using MyBayes.

Figure S7. ML tree based on PCG2RNA matrix reconstructed using IQTree.

Figure S8. BI tree based on PCG2RNA matrix reconstructed using MyBayes.

Figure S9. ML tree based on AA matrix reconstructed using IQTree.

Figure S10. BI tree based on AA matrix reconstructed using MyBayes.

Figure S11. MP tree based on PCG12 matrix reconstructed using TNT.

Figure S12. Chronogram with estimated divergence times using exponential distribution prior.

Figure S13. Chronogram with estimated divergence times using lognormal distribution prior.

Figure S14. Ancestral area reconstruction of Oedipodinae under DEC model in BioGeoBEARS with constrained.

Figure S15. Ancestral area reconstruction of Oedipodinae under DEC + J model in BioGeoBEARS with constrained.

Figure S16. Ancestral area reconstruction of Oedipodinae under DIVALIKE model in BioGeoBEARS with constrained.

Figure S17. Ancestral area reconstruction of Oedipodinae under DIVALIKE + J model in BioGeoBEARS with constrained.

Figure S18. Ancestral area reconstruction of Oedipodinae under BAYAREALIKE model in BioGeoBEARS with constrained.

Figure S19. Ancestral area reconstruction of Oedipodinae under BAYAREALIKE + J model in BioGeoBEARS with constrained.

Figure S20. *Tropidolophus formosus*, male. (a) Body. (b) Wings. (c) Forewing. (d) Hind leg (inner face).

Table S1. Taxonomic information, collection location, distribution range and GenBank accession numbers for taxa used in mitogenome analysis.

Table S2. Characteristics of newly mitogenomes of 86 samples used in this study.

Table S3. Saturation test for 13 PCGs sequences, concentrations of PCG12, PCG123, two rRNAs, and three positions of 13 PCGs, as implemented in DAMBE.

Table S4. Best partitioning schemes and substitution models selected by PartitionFinder for four datasets.

Table S5. Topology test results of eight phylogenies based on four datasets and two methods.

Table S6. Four nodes used for calibrating the divergence time.

Table S7. The summary table of all models used in BioGeoBEARS.