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Complete mitochondrial genome of *Plecia longiforceps* Duda, 1933 (Diptera, Bibionidae) and its implications for the evolution of mass outbreaks in flies

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Title: Complete mitochondrial genome of *Plecia longiforceps* Duda, 1933 (Diptera, Bibionidae) and its implications for the evolution of mass outbreaks in flies

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Abstract

Over the past decade, the prevalence of mass outbreaks involving non-native insects has sparked concerns about their potential negative impact on human inhabited areas and local environments. *Plecia longiforceps* Duda, 1933 (Diptera, Bibionidae) was recently recognized as an invasive pest in South Korea, causing public nuisance through mass outbreaks in the Seoul Metropolitan Area during early summer. In this study, we present the first complete mitochondrial genome of *Plecia longiforceps*, generated from the PacBio HiFi long-read sequencing data. Notably, the length of the circular genome is found to be larger than any annotated reference sequences of mitochondrial genomes for the infraorder Bibionomorpha, which is attributable to an unusually long A+T rich control region. We conducted a phylogenetic analysis of Bibionomorpha, leveraging all mitochondrial genome data sequenced to date, to elucidate relationships among genera within Bibionidae. Our resulting phylogeny of Bibionomorpha recovered a strong monophyly of the family Bibionidae and its three subfamilies: Bibioninae (*Bibio* + *Dilophus*), Hesperininae (*Hesperinus* + *Penthetria*), and Pleciinae (*Plecia*), corroborating the recently proposed taxonomic classification system of Bibionidae. Furthermore, we discuss evolutionary trends within Bibionidae based on our well-supported higher relationships of the superfamily Bibionoidea.

Keywords

Bibionomorpha, Bibionidae, long-read sequencing, mitochondrial genome, phylogeny, *Plecia longiforceps*

Introduction

The infraorder Bibionomorpha is a species-rich group of the insect order Diptera, comprising approximately 15,000 species worldwide (Evenhuis and Pape 2023). Members of these nematoceran flies are renowned for their remarkable morphological and ecological adaptations to diverse habitats, reflecting their biodiversity. While most species of Bibionomorpha show little association with human activities, certain groups hold economic significance as pests. For instance, the larvae of gall midges (Diptera, Cecidomyiidae) and dark-winged fungus gnats (Diptera, Sciaridae) are widely recognized for occasionally damaging crops and mushroom cultivation. Synchronized mass emergence of the saprophagous family Bibionidae has been historically reported usually during the spring season. Particularly, this swarming behavior of adult bibionids is considered a public nuisance and is increasing in both frequency and geographical range in recent years, indicating their potential introduction to anthropogenic environments and influence from climate changes (Qvenild and Rognerud 2017). Therefore, understanding the nature of insect outbreaks became important for potential control or even prevention by regulating the factors related to population dynamics of the interested species.

Plecia longiforceps Duda, 1933 (Diptera, Bibionidae) is an invasive pest in East Asia, characterized by its seasonal mass outbreak of adult mating pairs near suburban areas. The recent introduction of this species to the Korean Peninsula caused public disturbance since the summer of 2022, and previous range expansion analyses suggest that such occurrences will become increasingly prevalent, favoring the trend of climate changes within the north temperate zones (Kim et al. 2022). This case parallels an exemplar of the so-called “lovebug” or “march flies”, namely the high-profile species *Plecia nearctica* Hardy, 1940 in southeastern states of USA, with its documented history of Central American origin and gradual expansion to North American regions through the 20th century (Hardy 1940,

Buschman 1976). Despite this, the evolutionary history and biology of species in the genus *Plecia* Wiedemann, 1828, along with the other bibionids, remain understudied, hindering the assessment of their ecological impacts and prediction of future outbreaks. Traditionally, the genus *Plecia* has been considered closely related to the genus *Penthetria* Meigen, 1803 and treated as a genus within the subfamily Pleciinae (Hardy and Takahashi 1960). Later cladistic methods, focusing on extant species, found Pleciinae concept of *Plecia* + *Penthetria* to be paraphyletic, and treated *Plecia* as the sole member of Pleciinae and placed it as the sister group to the subfamily Bibioninae (Fitzgerald 2004), following the four-subfamily system proposed by Pinto and Amorim (2000). The recent phylogenies of Bibionidae based on molecular markers (Ševčík et al. 2016) and fossil taxa incorporating morphology (Skartveit and Ansoerge 2020) support the three-subfamily system of Bibionidae, including the monophyly of the subfamily Hesperininae Schiner, 1868, consisting of *Hesperinus* Walker, 1848 and *Penthetria*. However, the synapomorphies supporting this relationship have not been firmly established, leaving the placement of *Plecia* in the phylogeny of Bibionidae and its sister relationship uncertain.

In recent years, with increased accessibility to next-generation sequencing technologies, genomic data for various insects have been generated rapidly, primarily emphasizing mitochondrial sequences. This preference is due to the practicality of using DNA markers derived from the mitochondrial genome (mitogenome) for molecular identification and phylogenetic analysis (Cameron 2014). Moreover, the widespread adoption of long-read sequencing technology has simplified the assembly and annotation of complete mitogenomes, providing opportunities for comparative studies of previously neglected features, such as tandem repeats in the control region. This advancement overcomes technological constraints associated with short-read sequencing (Morgan et al. 2022). The application and utility of resolving deep relationships within Diptera using mitogenomes have

been extensively tested over a decade (Cameron et al. 2007), including Bibionomorpha. Previous studies have predominantly focused on resolving relationships within nematoceran lineages based on mitogenomes (Beckenbach 2012, Zhang et al. 2023, Xiao et al. 2023). Despite the significance of this group in addressing the phylogenetic gap between “lower flies” (Nematocera) and more derived “higher flies” (Brachycera), a comprehensive analysis of all extant subfamilies of Bibionidae based on mitochondrial genomic data is yet to be conducted.

In this study, we present the first complete and annotated mitogenome of *Plecia longiforceps* to provide insights into the phylogeny and evolutionary trends of Bibionidae from a mitochondrial genomic perspective. Furthermore, we manually annotated mitochondrial genes from previously available mitogenome data or whole-genome assemblies of Bibionomorpha and one species of Axymiidae (Diptera, Axymyiomorpha). We then incorporated these genes into the phylogenetic analysis to reconstruct the evolutionary history at the infraorder level.

Materials and methods

Sample collection, DNA extraction and sequencing

We collected a larval specimen of *Plecia longiforceps* from Incheon (37°23.34'N; 126°43.24'E), South Korea, in April 2023. After the adult emerged from its pupa, we performed identification based on the morphology of adult male terminalia. Subsequently, we snap-froze the specimen in liquid nitrogen and stored it at -80 °C until genomic DNA extraction.

The high molecular weight (HMW) genomic DNA (gDNA) was extracted from a single adult specimen using a modified version of the cetrimonium bromide (CTAB) precipitation method. To ensure the high molecular weight of the extracted gDNA, we assessed the gDNA extract through gel electrophoresis on a 1% agarose gel with the lambda DNA marker, and we quantified it with the Quantus Fluorometer (Promega, USA) and Nanodrop Spectrophotometer (Thermo Fisher Scientific, USA). Additionally, we treated the purified HMW gDNA with the Short Read Eliminator (SRE) XL kit (Pacific Biosciences, USA) to remove short DNA fragments below 40 kb and then sheared it into 20 kb fragments using the Megaruptor 2 (Diagenode, Belgium). We constructed the PacBio SMRT library using the SMRTbell Prep Kit 3.0 and sequenced it on a single SMRT HiFi cell of the PacBio Sequel IIe system (Pacific Biosciences, USA) at the National Instrumentation Center for Environmental Management (NICEM), Seoul National University (Seoul, Republic of Korea). Finally, we processed the raw base-called data through the Circular Consensus Sequence analysis application of SMRT® Link (ver. 12.0.0.177059) to identify high fidelity (HiFi) reads.

Mitochondrial genome assembly and annotation

From the newly produced PacBio HiFi reads, we isolated the mitochondrial reads and assembled them using the MitoHiFi pipeline v3.2 (Uliano-Silva et al. 2023), with *Bradysia odoriphaga* Yang & Zhang, 1985 (NCBI: NC_061662.1) as the reference sequence. Among the candidate contigs, we chose a single representative mitochondrial contig based on the annotation result obtained from MitoFinder (Allio et al. 2020). Manual curation and annotation of non-coding regions were performed in Geneious Prime® 2023.2.1. The online version of Proksee (Grant et al. 2023) was used for the calculation of GC content and GC skew, and for preparing a structure map in a JSON file. The circular map of mitogenome with gene elements was drawn using the CGview server (Grant and Stothard 2008). Furthermore, unannotated mitogenomes of four bibionomorphs (*Bibio marci*, *Bibio rufiventris*, *Dilophus febrilis*, and *Plecia* sp.), one species of the family Axymyiidae (*Protaxymyia* sp.), and four whole genome assemblies of bibionomorphs (*Bolitophila hybrida*, *Diadocidia ferruginosa*, *Penthetria funebris*, and *Symmerus nobilis*) were retrieved from NCBI GenBank (as of October 2023) and underwent processing using the same annotation methodology that was applied to our newly assembled mitogenome (Table 1).

Phylogenetic analysis

We employed both maximum-likelihood (ML) and Bayesian inference (BI) methods to infer phylogenetic trees. Our final dataset included a total of 27 terminal taxa, encompassing mitogenomes from 25 representative dipteran species and partial sequences of the 12S, 16S ribosomal RNA genes and cytochrome oxidase *c* subunit I (*COI*) from two *Hesperinus* species (*Hesperinus brevifrons* and *Hesperinus ninae*). As outgroup taxa, three non-bibionomorphan species of the suborder Nematocera (*Coboldia fuscipes*, *Tipula aestiva* and *Protaxymyia* sp.) and three species of the suborder Brachycera (*Dolichopus galeatus*, *Parastratiosphecomyia szechuanensis* and *Tabanus chrysurus*) were incorporated (Tables 1,

2). We aligned the sequences of 13 protein-coding and two ribosomal RNA genes of mitogenomes using the command-line version of MAFFT v7.475 with default parameters (Kato and Standley 2013). The concatenated matrix of gene alignments was generated using the 'create_concatenation_matrix' function implemented in PhyKIT (Steenwyk et al. 2021). Best-fitting nucleotide substitution models were determined for each gene alignment using PartitionFinder2 (Lanfear et al. 2017) under the corrected Akaike Information Criterion (AICc). Data blocks for the protein-coding genes were pre-defined to reflect all three codon positions. The ML analysis was conducted in IQ-TREE v2.2.2.6 (Minh et al., 2020) using the partition mode, and branch support values assessed via ultra-bootstrap approximation method with 5,000 bootstrap replicates. The BI analysis was carried out in MrBayes v3.2.7a (Ronquist et al. 2012), running four chains for 10 million generations with trees sampled every 10,000 generations. Convergence of the runs was diagnosed in Tracer v1.7.2 (Rambaut et al. 2018) with the first 2.5 million generations discarded as burn-in.

Results and discussion

General mitochondrial genomic characteristics

Using the PacBio HiFi long-read sequencing technology, we successfully sequenced and assembled the complete mitochondrial genome (mitogenome) of *Plecia longiforceps*. Our initial PacBio HiFi data yielded a total of 27,997,014,611 base pairs (bp) in 1,891,452 reads, of which MitoHiFi identified only 20 reads as mitochondrial. The assembled mitogenome of *P. longiforceps* is circular, measuring 17,739 bp in length, with a base composition of 42.0% A, 39.6% T, 7.5% C and 10.8% G. The annotation result comprised of 37 genes—13 protein-coding genes, 2 ribosomal RNA genes and 22 transfer RNA genes—along with one A+T rich control region (Figure 1), consistent with the putative ancestral insect mitogenome in gene contents and arrangements (Cameron 2014). These highly conserved aspects of the *P. longiforceps* mitogenome are readily observed in other bibionomorph lineages, with a few exceptions reported in families such as Cecidomyiidae and Keroplatidae (Xiao et al. 2023). Notably, our mitogenome assembly of *P. longiforceps* is significantly longer than that of *Plecia* sp. (GenBank accession: MZ562680.1; 15,763 bp), despite the overall similarity between the two mitogenomes in terms of gene contents and respective nucleotide sequences, with the only difference found in the control region. We found similar patterns other genera of the family Bibionidae—for instance, between *Bibio marci* (OU343120.2; 16,014 bp) and *Bibio rufiventris* (MZ562678.1; 14,717 bp); and between the reference mitogenome of *Dilophus febrilis* (OY284474.1; 19,009 bp) and its partial genome assembly (MT872668.1; 15,236 bp)—in which the differences in length are specifically due to shorter control regions. In all three cases, the larger genome assemblies were based on long-read sequence data, suggesting the superiority of long-read data in capturing control regions rich in tandem repeats compared to the Illumina short-read data, consistent with the previous observation in rhinoceros beetles (Morgan et al. 2022).

Phylogenetic accounts

Our final concatenated supermatrix consisted of 14,192 bp nucleotide positions for 27 taxa, with the objectives of elucidating the relationships among the three subfamilies of Bibionidae. Within Bibionidae, the genera *Biblio*, *Dilophus*, *Penthetria* and *Plecia* are represented by at least one complete mitogenome sequence (Table 1, 2); however, due to the lack of mitogenomic sequence for the genus *Hesperinus*, we incorporated partial sequence data of three mitochondrial genes (12S and 16S rRNAs and COI). For outgroup taxa, we included *Tipula aestiva* (Diptera, Tipulomorpha, Tipulidae) as the most distant outgroup, and three brachyceran species for testing the monophyly of Bibionomorpha *sensu lato* (i.e., Anisopodoidea, Bibionoidea, Scatopsoidea and Sciaroidea), as well as for addressing the persisting debate about the phylogenetic placements of Anisopodidae and Scatopsidae.

Despite the limited data for *Hesperinus*, both our ML and BI analyses produced congruent phylogenies across all nodes (Figure 2). Along with the monotypy of the subfamily Pleciinae, the relationships within Bibionoidea—the family Pachyneuridae as a sister to Bibionidae, and Bibioninae as a sister group to Hesperininae and Pleciinae at the family- and subfamily-levels, respectively—were consistent with the previously reported phylogeny of Bibionomorpha (Ševčík et al. 2016). However, the monophyly of the subfamily Hesperininae (*Hesperinus* + *Penthetria*) were only marginally supported in the ML analysis, possibly due to the lack of complete mitogenomic sequences for the two *Hesperinus* species analyzed. Nonetheless, the overall relationship as the sister group remains unaffected. Notably, the tip branch lengths of *Plecia longiforceps* and *Plecia* sp. (MZ562680.1) were found to be zero in both resulting trees, suggesting that these two species are potentially identical. However, further information about the specimen of *Plecia* sp. (MZ562680.1) is required to confirm this observation.

While the relationships within the family Bibionidae were the primary focus of our study, here we provide brief accounts on several strongly supported higher-level relationships within Bibionomorpha. The Bibionomorpha *sensu stricto* was recovered to be composed of two distinct superfamilies, Bibionoidea and Sciaroidea, with monophylies of most of the currently recognized families within Bibionomorpha showing strong supports in all analyses. Within the superfamily Bibionoidea, the family Pachyneuridae, currently recognized by five extant species worldwide, was recovered as the earliest-branching lineage [BI posterior probability (BPP) = 1.0; ML bootstrap (MLB) = 87%], which was followed by the members of the monophyletic family Bibionidae (BPP = 1.0; MLB = 100%). Within the superfamily Sciaroidea, the family Bolitophilidae was recovered as the earliest-branching lineage, sister to the remaining Sciaroidea sampled (BPP= 1.0; MLB = 94%). This result is consistent with the previous study that was based on morphological traits (Fitzgerald 2004), as well as the Bayesian molecular phylogeny (Ševčík et al. 2014), despite with only minimal support.

Nevertheless, the monophyly of the Bibionomorpha *sensu lato* was not supported in our analyses, with the bibionomorphan families Anisopodidae and Scatopsidae being recovered to be more closely related to the suborder Brachycera with relatively strong supports (BPP= 0.99; MLB = 87%) (Figure 2). Hennig (1973) originally hypothesized that Bibionomorpha *sensu lato* is a monophyletic group and sister to Brachycera, a relationship supported by multiple subsequent molecular studies (Bertone et al. 2008, Wiegmann et al. 2011, Ševčík et al. 2016). However, Oosterbroek et al. (1995) considered Anisopodidae to be separated from Bibionomorpha and placed as the only sister to Brachycera, a relationship congruent with the recent mitochondrial phylogeny by Zhang et al. (2023). Given the ongoing debate on the placement of Anisopodidae and Scatopsidae in Bibionomorpha *sensu lato*, as well as our finding of their potential affinity with Brachycera, a more comprehensive

mitogenomic sampling of the two families and Brachycera is warranted to resolve their systematic positions within Diptera.

The origin of mass outbreaks in Bibionidae

Swarming behavior, which is commonly observed in many bibionomorph and other nematoceran species, occurs in adult flies under various environmental conditions. For instance, the species of the family Trichoceridae (Diptera, Tipulomorpha), commonly referred to as "winter crane flies," prefer cold temperatures and are known to form swarms on snowy surfaces. A recent notable case from this family involves *Trichocera maculipennis* Meigen, 1818, which was reported to have been introduced anthropogenically from the Northern Hemisphere into ice-free areas of Antarctica (Potocka and Krzemińska 2018). One of the common and most important traits found across the dipteran species that are known to cause mass outbreaks is found in their larval stage, in which the majority show detritivorous feeding habit in humid soil. Likewise, most bibionid species, except for the genus *Hesperinus*, exhibit similar habits and biology, exhibiting mass aggregation in humid soil environments to resist cold and desiccation stress. In these species, ecological stressors induce phenotypic plasticity within their life cycles, modulating the timing of adult emergence. This adaptive response synchronizes their swarming outbreak, which aligns with optimal environmental conditions (Krivosheina et al. 2019). With the recent climate change impacting global ecosystems, the range expansion of species like *Plecia longiforceps* has been reported in East Asia (Kim et al. 2022). Originating from southeastern China, this subtropical species has recently introduced to the Ryukyu Islands and the Korean Peninsula, likely due to its pre-adaptation to subtropical condition. However, this pattern of expansion raises a question about the evolutionary history of mass outbreak in Bibionidae—specifically, whether the

outbreak behavior is ancestral to Bibionidae and if so, why some groups, such as *Hesperinus*, has lost this remarkable phenomenon.

Based on our newly inferred phylogeny of Bibionomorpha, in which *Hesperinus* is recovered as one of the most derived lineages within Bibionidae, we can postulate that the swarming behavior was lost in this particular lineage. In fact, adult flies of *Hesperinus* differ markedly from other bibionids, for having broadly separated compound eyes, and elongated, filiform antennal flagellomeres in both sexes, which make resemble the species of other primitive bibionomorphan families, such as Bolitophilidae and Pachyneuridae. The inclusion of the genus *Hesperinus* in the family Bibionidae was supported by previous morphological and molecular studies (Fitzgerald 2004, Ševčík et al. 2016), though synapomorphies supporting this treatment have not yet been proposed. The larvae of *Hesperinus* develop inside decaying woods and possess soft body segments with sclerotized spiracles, but lacking flesh protuberance, a synapomorphy of Bibionidae. These simplified traits might represent adaptations to a secure habitat with a limited need for locomotion, as observed in many wood-boring beetles (Chiappini and Aldini 2011). In contrast to many bibionid species, *Hesperinus* larvae do not engage in mass aggregation and migration, potentially leading to lower larval density and the absence of adult mating swarms. This necessitates that adult *Hesperinus* flies require a broader range to search for appropriate mates, relying more on chemical signals over visual cues. The antennae of adult *Hesperinus* exhibit sexual dimorphism, in which males present significantly longer antennae than females, a characteristic also present in Bolitophilidae and Pachyneuridae. The elongated antennae in males may be associated with detecting pheromones, although further evidence through ultrastructure studies or behavioral experiments is required (Vockeroth 1974). Interestingly, in two of the five species of extant *Hesperinus*, adult females present reduced wings and have lost their flight abilities (Kurina 2013). Given the biology of Bibionidae, fecundity likely plays a crucial role in reproductive success (Krivosheina et al. 2019), prompting females to conserve energy for egg production and release pheromones to attract males. Fossil species of this genus, such as †*Hesperinus electrus* Skartveit, 2009, exhibit a significantly shortened antennae, albeit still longer than other bibionids (Skartveit 2009). This observation provides further speculation regarding the derived characteristics of *Hesperinus*, particularly in response to habitat changes and its secondary adaptation to deciduous forest environments. A comprehensive study on the evolutionary history of mass outbreaks in Bibionidae with dense taxon sampling is required to unveil the biological response of these flies' adaptation to

different habitats and environmental stress, which is especially important in the face of global climate change.

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Figure 1. Circular map of the newly sequenced and assembled mitochondrial genome of *Plecia longiforceps* with a habitus of adult male specimen in lateral view at center. All protein coding genes, ribosomal RNA genes, transfer RNA genes and a control region are shown with the feature table definition from the International Nucleotide Sequence Database Collaboration (INSDC). The direction of gene transcription is indicated with an arrow. The length of each gene is proportional to its nucleotide length. The innermost and middle circles indicate the GC skew and GC content, respectively. The outermost circle displays gene arrangement. Abbreviations: CDS, coding sequences; rRNA, ribosomal RNA; tRNA, transfer RNA; CR, control region.

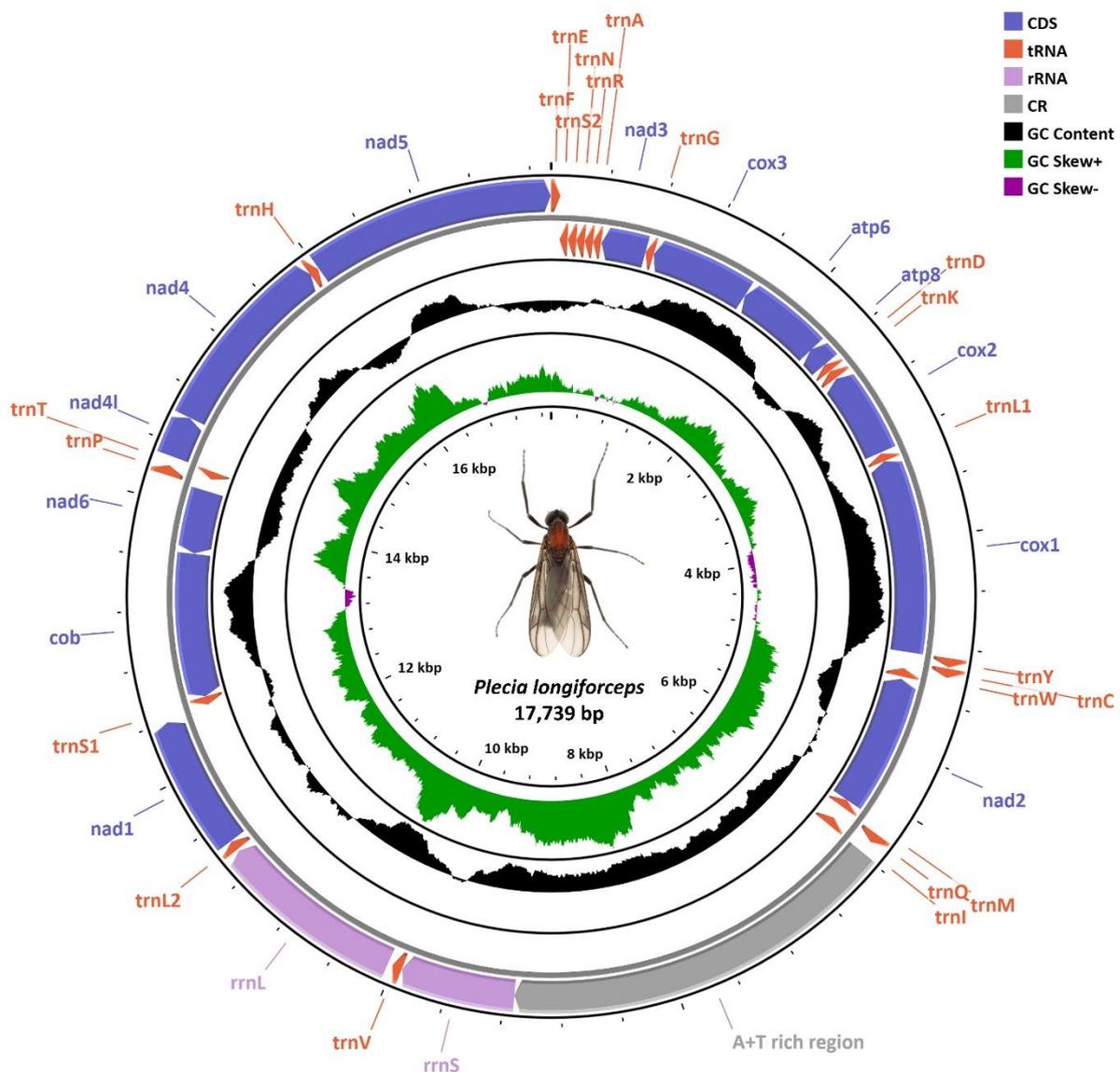


Figure 2. Bibionomorpha phylogeny inferred from the Bayesian analysis. Inferred phylogeny utilizing Bayesian analysis based on a matrix comprising 13 protein-coding genes and two ribosomal RNA genes originated from mitochondrial genomes, in total 14,192 base pairs. The analysis involved 25 taxa and included partial sequences of 12S, 16S ribosomal RNA genes, and mitochondrial cytochrome *c* oxidase subunit I (*COXI*) from two *Hesperinus* species (*H. brevifrons* and *H. ninae*). Nodal values refer to posterior probability/ Ultrafast bootstrap support, respectively, are provided only for nodes lacking full support. The species highlighted in red is the newly sequenced mitochondrial genome in this study.

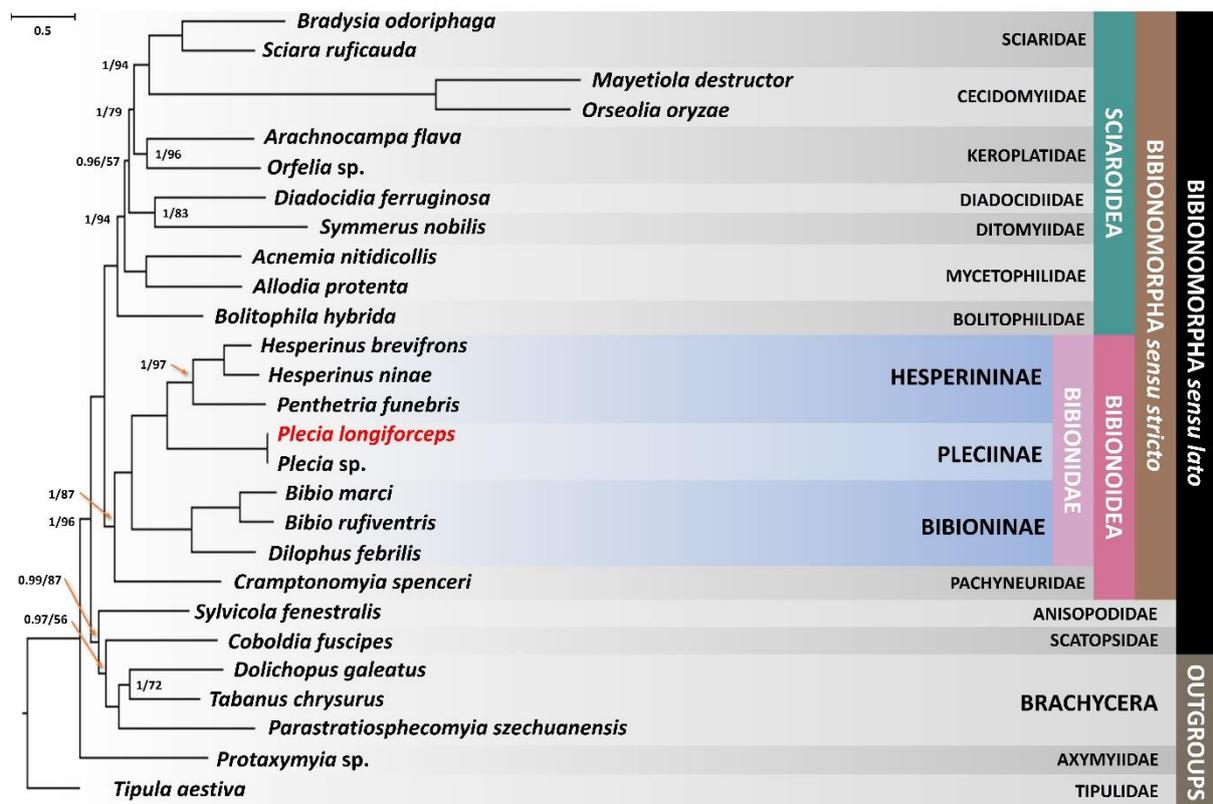


Table 1. Information on the mitochondrial genome sources used in the present phylogenetic analysis. NCBI GenBank accession number of manually annotated taxa are highlighted in bold, and those assembled and annotated have an asterisk.

Suborder/ Infraorder	Family	Species	GenBank accession numbers
Axymyiomorpha	Axymyiidae	<i>Protaxymyia</i> sp.	MZ562679.1
Bibionomorpha	Anisopodidae	<i>Sylvicola fenestralis</i> (Scopoli, 1763)	NC_016176.1
Bibionomorpha	Bibionidae	<i>Bibio marci</i> (Linnaeus, 1758)	OU343120.2
Bibionomorpha	Bibionidae	<i>Bibio rufiventris</i> Duda, 1930	MZ562678.1
Bibionomorpha	Bibionidae	<i>Dilophus febrilis</i> (Linnaeus, 1758)	OY284474.1
Bibionomorpha	Bibionidae	<i>Hesperinus brevifrons</i> Walker, 1848	See table 2
Bibionomorpha	Bibionidae	<i>Hesperinus ninae</i> Papp & Krivosheina, 2009	See table 2
Bibionomorpha	Bibionidae	<i>Penthetria funebris</i> Meigen, 1804	GCA_027564355.1*
Bibionomorpha	Bibionidae	<i>Plecia longiforceps</i> Duda, 1933	This study*
Bibionomorpha	Bibionidae	<i>Plecia</i> sp.	MZ562680.1
Bibionomorpha	Bolitophilidae	<i>Bolitophila hybrida</i> (Meigen, 1804)	GCA_027564075.1*
Bibionomorpha	Cecidomyiidae	<i>Mayetiola destructor</i> (Say, 1817)	GQ387648.1
Bibionomorpha	Cecidomyiidae	<i>Orseolia oryzae</i> (Wood-Mason, 1889)	KM888183.1
Bibionomorpha	Diadocidiidae	<i>Diadocidia ferruginosa</i> (Meigen, 1830)	GCA_027564275.1*
Bibionomorpha	Ditomyiidae	<i>Symmerus nobilis</i> Lackschewitz, 1937	GCA_027564815.1*
Bibionomorpha	Keroplastidae	<i>Arachnocampa flava</i> Harrison, 1966	NC_016204.1
Bibionomorpha	Keroplastidae	<i>Orfelia</i> sp.	MW394227.1

Bibionomorpha	Mycetophilidae	<i>Acnemia nitidicollis</i> (Meigen, 1818)	NC_050318.1
Bibionomorpha	Mycetophilidae	<i>Allodia protenta</i> Laštovka & Matile, 1974	NC_060624.1
Bibionomorpha	Pachyneuridae	<i>Cramptonomyia spenceri</i> Alexander, 1931	NC_016203.1
Bibionomorpha	Sciaridae	<i>Bradysia odoriphaga</i> Yang & Zhang, 1985	NC_061662.1
Bibionomorpha	Sciaridae	<i>Sciara ruficauda</i> Meigen, 1818	NC_046767.1
Brachycera	Dolichopodidae	<i>Dolichopus galeatus</i> Loew, 1871	NC_070101.1
Brachycera	Stratiomyidae	<i>Parastratiosphecomyia szechuanensis</i> Lindner, 1954	NC_053880.1
Brachycera	Tabanidae	<i>Tabanus chrysurus</i> Loew, 1858	NC_062705.1
Psychodomorpha	Scatopsidae	<i>Coboldia fuscipes</i> (Meigen, 1830)	MZ567016.1
Tipulomorpha	Tipulidae	<i>Tipula aestiva</i> Savchenko, 1960	NC_063751.1

Table 2. Information on the three mitochondrial genes of two *Hesperinus* species used in this study.

Species	Genbank accession numbers		
	12S	16S	COXI
<i>Hesperinus brevifrons</i> Walker, 1848	KP288705.1	KP288737.1	JN294723.1
<i>Hesperinus ninae</i> Papp & Krivosheina, 2009	KP288687.1	KP288719.1	KT316856.1