Characteristics of the complete mitochondrial genome of *Suhpalacsalongialata* (Neuroptera, Ascalaphidae) and its phylogenetic implications

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The owlflies (Family Ascalaphidae) belong to the Neuroptera but are often mistaken as dragonflies because of morphological characters. To date, only three mitochondrial genomes of Ascalaphidae, namely *Libelloides macaronius*; *Ascaloptynx appendiculatus*; *Ascalohybris subjacens*, are published in GenBank, meaning that they are greatly under-represented in comparison with the 430 described species reported in this family. In this study, we sequenced and described the complete mitochondrial genome of *Suhpalacsalongialata* (Neuroptera, Ascalaphidae). The total length of the *S.longialata* mitogenome was 15,911 bp, which is the longest known to date among the available family members of Ascalaphidae. However, the size of each gene was similar to the other three Ascalaphidae species. The *S. longialata* mitogenome included a transposition of $tRNA^{Cys}$ and $tRNA^{Trp}$ genes and formed an unusual gene arrangement $tRNA^{Cys}$-$tRNA^{Trp}$-$tRNA^{Tyr}$(CWY). It is likely that the transposition occurred by a duplication of both genes followed by random loss of partial duplicated genes. The nucleotide composition of the *S.longialata* mitogenome was as follows: A=41.0%, T=33.8%, C=15.5%, G=9.7%. Both BI and ML analyse strongly supported *S. longialata* as a sister clade to (*Ascalohybris subjacens* + *L. macaronius*), and indicated that Ascalaphidae is not monophyletic.

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ABSTRACT

The owlflies (Family Ascalaphidae) belong to the Neuroptera but are often mistaken as dragonflies because of morphological characters. To date, only three mitochondrial genomes of Ascalaphidae, namely *Libelloides macaronius*; *Ascaloptynx appendiculatus*; *Ascalohybris subjacens*, are published in GenBank, meaning that they are greatly under-represented in comparison with the 430 described species reported in this family. In this study, we sequenced and described the complete mitochondrial genome of *Suhpalacsa longialata* (Neuroptera, Ascalaphidae). The total length of the *S. longialata* mitogenome was 15,911 bp, which is the longest known to date among the available family members of Ascalaphidae. However, the size of each gene was similar to the other three Ascalaphidae species. The *S. longialata* mitogenome included a transposition of $tRNA^{Cys}$ and $tRNA^{Trp}$ genes and formed an unusual gene arrangement $tRNA^{Cys}$-$tRNA^{Trp}$-$tRNA^{Tyr}$ (CWY). It is likely that the transposition occurred by a duplication of both genes followed by random loss of partial duplicated genes. The nucleotide composition of the *S. longialata* mitogenome was as follows: A=41.0%, T=33.8%, C=15.5%, G=9.7%. Both BI and ML analyses strongly supported *S. longialata* as a sister clade to (*Ascalohybris subjacens* + *L. macaronius*), and indicated that Ascalaphidae is not monophyletic.

INTRODUCTION

The study of mitochondrial genomes (mitogenomes) is of great interest to many scientific fields, including molecular evolution and evolutionary genomics (Avise et al., 1987; Salvato et al., 2008). Insect mitochondrial genomes are usually a double-stranded circular molecule with a length of 14-20 kbp, including 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), 2 ribosomal RNAs (rRNAs), and a control region (AT-rich region) (Boore, 1999). The most widespread gene arrangement in insect mtDNAs is hypothesized to be ancestral for the entire Class Insecta (Clary et al., 1985; Boore et al., 1998; Cameron et al., 2006). However, more and more researchers have found other gene rearrangements in mitogenomes, mostly related to tRNAs or non-coding regions often within a selected family or order or these may even define clades at a variety of taxonomic scales below the ordinal level. (Beard et al., 1993; Mitchell et al., 1993; Cameron et al., 2008; Salvato et al., 2008; McMahon et al., 2009; Cameron, 2014b). Consequently, the particular gene arrangement becomes a significant marker to delimit taxonomic boundaries. Furthermore, the mitogenome has been increasingly used to reconstruct phylogenetic relationships because of its simple genetic structure, maternal inheritance and high evolutionary rate properties (Boyce et al., 1989; Sheffield et al., 2008; Jia et al., 2008; Du et al., 2017).

The insect Order Neuroptera contains approximately 6,000 species worldwide (Aspöck, 2002; Haring et al., 2004). Known as net-winged insects, adults usually possess functional membranous wings with an extensive network of veins and cross-veins (Beckenbach et al., 2008). The fossil record of Neuroptera dates back to the Late Permian and indicates that they were a major group of insect fauna during the early

diversification of the Holometabola (Aspöck, 2002). Therefore, their phylogenetic position is likely to have had a key influence on the subsequent evolution of insects (Beckenbach et al., 2008). To date, only 42 mitochondrial genomes of Neuroptera are available in databases (Beckenbach et al., 2008; Cameron et al., 2009; Haruyama et al., 2011; Negrisolo et al., 2011; He et al., 2012; Zhao et al., 2013; Wang et al., 2013; Cheng et al., 2014; Yan et al., 2014; Cheng et al., 2015; Zhao et al., 2016; Zhang et al., 2016; Lan et al., 2016; Zhang et al., 2017; Song et al., 2018) and this includes 21 partial mitochondrial genomes. Hence, there is a great need to add data for more Neuroptera species in order to be able to analyze phylogenetic relationships both within this group and to further understand relationships within the Holometabola.

The owlflies (Family Ascalaphidae) belong to the Neuroptera but are often mistaken as dragonflies because of their morphological similarity. The larvae and adults of Ascalaphidae are usually predaceous and so they play an important role in maintaining ecological balance and pest control if they are well applied. At present, only three mitochondrial genomes of Ascalaphidae, namely *Libelloides macaronius* (Scopoli 1763) (Negrisolo et al., 2011); *Ascaloptynx appendiculatus* (Fabricius 1793) (Beckenbach et al., 2008); *Ascalohybris subjacens* (Walker 1853) (Cheng et al., 2014), are published in GenBank, meaning that they are greatly under-represented in comparison with the 430 described species reported in this family (Stange, 2004). These three published genomes show substantial gene rearrangements (Beckenbach et al., 2008; Negrisolo et al., 2011; Cheng et al., 2014) and it is unclear if the mitogenome of any of these species represents the common condition within the Ascalaphidae. Ascalaphidae as a sister clade of Myrmeleontidae is supported by Song et al. (2018), while Ascalaphidae within the clade of Myrmeleontidae is recovered by Wang et al. (2017). Increasing the number of sequenced species within the Neuroptera will be very helpful for phylogenetic reconstructions of Neuroptera relationships. Hence, in the present study we sequenced the complete mitogenome of *Suhpalacsa longialata* Yang 1992 (Neuroptera, Ascalaphidae) and analyzed its genomic structure and composition in comparison with the other three Ascalaphidae species including determining nucleotide composition, gene order, codon usage and secondary structure of tRNAs. Additionally, we also analyzed evolutionary relationships within Neuroptera using Megaloptera as outgroups to discuss the relationship between Ascalaphidae and Myrmeleontidae, and the relationships of inter-families of Neuroptera.

MATERIALS AND METHODS

Sample origin and DNA extraction

The sample of an adult *S. longialata* used for sequencing was collected from Hangzhou, Zhejiang province, China in July 2017 by LP Zhang. The specimen was identified by JY Zhang and preserved in 100% ethanol at -40 ℃ in the lab of JY Zhang. Total DNA was isolated from one foreleg of *S. longialata* using an Ezup Column Animal Genomic DNA Purification Kit (Sangon Biotech Company, Shanghai, China) according to the manufacturer's protocol.

PCR amplification and sequencing of *S. longialata* mtDNA

Twelve universal primers for polymerase chain reaction (PCR) amplification were modified according to Simon et al. (2006), Zhang et al. (2008) and Zhang et al. (2018) (**Table S1** and **Fig.1**) based on the mitogenome sequences of the three known species of Ascalaphidae (*L. macaronius*, *Ascaloptynx*

appendiculatus and *Ascalohybris subjacens*). Then five specific primers (**Table S1** and **Fig.1**) were designed based on the sequence information from universal primers using Primer Premier 5.0 (PREMIER Biosoft International,CA, USA). All PCR was performed with a BioRADMJMini Personal Thermal Cycler (made in Singapore) using Ta*kara Taq* DNA polymerase (TaKaRa Biotechnology Co., Ltd., Dalian, China) with the following cycling steps: denaturation at 94 ℃ for 5 min, followed by 35 cycles of 94 ℃ (50 s for denaturation), 48-60 ℃ (30-50 s for annealing), and 72 ℃ (1-3 min elongation), followed by a final elongation at 72 ℃ for 10 min. PCR reactions were carried out in a 50 μL reaction volume consisting of 32.75 μL sterile deionized water, 5.0 μL 10×PCR buffer (Mg^{2+}Free), 5.0 μL $MgCl_2$ (25 mM), 4.0 μL dNTP Mixture (2.5 mM each), 1.0 μL DNA template, 1.0 μL each primer (10 ppm), 0.25 μL Takara Taq DNA polymerase (5 U/μL). All PCR products were visualized by electrophoresis in a 1% agarose gel and sent to Sangon Biotech Company (Shanghai, China) for sequencing of both strands.

Mitogenome annotation and sequence analyses

The mtDNA sequence was assembled using DNASTAR Package v.6.0 (Burland, 2000). The transfer RNA (tRNA) genes and their cloverleaf secondary structures were determined by MITOS (http://mitos.bioinf.uni-leipzig.de/index.py) using the invertebrate mitogenome genetic code (Bernt et al., 2013). The control region and ribosomal RNA (rRNA) genes were identified by the boundary of tRNA genes (Thompson et al., 1997) as well as comparison with homologous sequences of mitogenomes from other species of Ascalaphidae (Beckenbach et al., 2008; Negrisolo et al., 2011; Cheng et al., 2014). The 13 PCGs were translated to amino acids with the invertebrate mitogenome genetic code and the open reading frames were identified using Mega 7.0 (Kumar et al., 2016; Cameron, 2014a). The nucleotide composition, codon usage and relative synonymous codon usage were calculated by Mega 7.0 (Kumar et al., 2016). The GC and AT skews were calculated using the following formulae: AT skew = (A-T)/(A+T), GC skew = (G-C)/(G+C) (Perna et al., 1995). A mitogenome map of *S. longialata* was constructed using CG View server V 1.0 (Grant et al., 2008).

Phylogenetic analyses

For the first analysis that indicated Megaloptera as a sister clade to Neuroptera, as proposed by Engel et al. (2018) and Peters et al. (2014), we used data from 43 previously sequenced species of Neuroptera (43SN) as ingroups including *S. longialata* (e.g. Beckenbach et al., 2008; Cameron et al., 2009; Haruyama et al., 2011; Negrisolo et al., 2011; He et al., 2012; Zhao et al., 2013; Wang et al., 2013; Cheng et al., 2014; Yan et al., 2014; Cheng et al., 2015; Zhao et al., 2016; Zhang et al., 2016; Lan et al., 2016; Zhang et al., 2017), with the outgroup taxa consisting of 4 species of Megaloptera (*Corydalus cornutus*; *Dysmicohermes ingens*; *Neochauliodes bowringi*; *Sialis hamata*) (Beckenbach et al., 2008; Cameron et al., 2009; Li et al., 2015; Wang et al., 2016) to discuss family-level phylogenetic relationships of Neuroptera,. Accession numbers of all mitochondrial genomes are listed in **Table S2**. Nucleotide sequences of the 13 PCGs were employed for construction of BI and ML phylogenetic trees according to Cheng et al. (2016) and Zhang et al. (2018). DNA alignment was acquired from the amino acid alignment of the 13 PCGs using Clustal W in Mega 7.0 (Kumar et al., 2016), and the conserved regions were found by Gblock 0.91b (Castresana, 2000). We estimated the best partitioning scheme and model by the program PartionFinder 1.1.1 (Lanfear et al., 2012) on the basis of Bayesian Information Criterion (BIC). The ML tree was constructed in RAxML 8.2.0 with the best model of

GTRGAMMA and the branch support inferred from 1,000 bootstrap replications (Stamatakis, 2014). BI analysis was carried out in MrBayes 3.2 with the model of GTR + I + G; the analysis was set for 10 million generations with sampling every 1,000 generations; the initial 25% of generations was discarded as burn-in (Ronquist et al., 2012). Because long branch attraction can cause a wrong relationship (Bergsten, 2005; Philippe et al., 2005), we obtained a second data set using 40 species of Neuroptera (40SN) as the ingroup by excluding *Semidalis aleyrodiformis*, *Coniopteryx* sp. and *Dilar* sp. that showed long branch attraction. The ML and the BI analyses of data 40SN were then performed as above.

RESULTS AND DISCUSSION

Mitogenome organization and structure

The complete mitogenome of *S. longialata* is a double-stranded circular DNA molecule with a length of 15,911 bp **(Fig. 1)** that has been submitted to GenBank under the accession number MH361300. It encodes the entire set of 37 mitochondrial genes including 13 PCGs, 22 tRNA genes and 2 rRNA genes that are typically present in metazoan mitogenomes (Wolstenholme, 1992). In addition, the gene arrangement of *S. longialata* is similar to the assumed commom ancestor of insects (Mueller et al., 2005; Yu et al., 2007; Erler et al., 2010; Li et al., 2011; Li et al., 2012a, 2012b), with the exception of the $tRNA^{Trp}$-$tRNA^{Cys}$-$tRNA^{Tyr}$ (WCY) triplet. *S. longialata* possessed an unusual gene order of $tRNA^{Cys}$-$tRNA^{Trp}$-$tRNA^{Tyr}$ (CWY) **(Fig. 1)**, which also occurred in the other species of Ascalaphidae available in the GenBank database (Beckenbach et al., 2008; Negrisolo et al., 2011; Cheng et al., 2014). In addition, the transposition of $tRNA^{Cys}$ and $tRNA^{Trp}$ genes has also been found in other families within the Neuroptera, including Dilaridae, Hemerobiidae, Mantispidae, Berothidae, Ithonidae, Chrysopidae, Psychopsidae, Nymphidae, Nemopteridae, and Myrmeleontidae (Wang et al., 2017; Song et al., 218), but not in the other neuropterid orders. Thus, it is widely acknowledged that it may be synapomorphic for the Neuroptera (Cameron et al., 2009; Beckenbach et al., 2008; Haruyama et al. 2011; Negrisolo et al., 2011; He et al., 2012; Zhao et al., 2013; Yan et al., 2014). The duplication-random loss model may be a possible explanation for the transposition of contiguous genes. Similar to the report by Beckenbach et al. (2008), it is likely that the $tRNA^{Trp}$-$tRNA^{Cys}$ (WC) genes were duplicated in tandem to form a tRNA cluster WCWC, which was then followed by random loss of partial duplicated genes to produce the final CW gene order**.**

The mitogenome of *S. longialata* (15,911bp) is the longest as compared with those of other Ascalaphidae species, whose mitogenomes range from 15,873 bp to 15,890 bp. The greater length of the *S. longialata* mitogenome is due largely to 16 intergenic gaps ranging from 1 bp to 54 bp and a long typical A+T-rich region (1,088 bp) as compared to 1,049 bp for *L. macaronius* (Negrisolo et al., 2011), 1,066 bp for *Ascaloptynx appendiculatus* (Beckenbach et al., 2008) and 1,051 bp for *Ascalohybris subjacens* (Cheng et al., 2014). The nucleotide composition of the *S. longialata* mitogenome is as follows: A=41.0%, T=33.8%, C=15.5%, G=9.7%. It is obvious that the *S. longialata* had a strong A+T bias of 74.8%, which is similar to other species of the Ascalaphidae: 74.5% for *L. macaronius*; 75.5% for *Ascaloptynx appendiculatus* ; 75.7% for *Ascalohybris subjacens* (Beckenbach et al., 2008; Negrisolo et al., 2011; Cheng et al., 2014) **(Table 1)**. The high A+T bias was found in PCGs, ribosomal RNA genes, transfer RNA genes and the control region.

Previous studies pointed out that the strand bias in nucleotide composition may be attributed to mutational damage primarily affecting the lagging strand during asymmetric replication (Francino et al., 1997; Hassanin et al., 2005). The skew statistics indicated that *S. longialata* had a positive AT-skew and negative GC-skew **(Table 1)**.

Protein-coding genes and codon usages

Nine PCGs (ND2, COX1, COX2, ATP8, ATP6, COX3, ND3, ND6 and CYTB) were located on the major strand (J-strand) with the remaining PCGs on the minor strand (N-strand). All PCGs genes used ATN (N represents A, G, C or T) as initiation codons, which have been accepted as the canonical mitochondrial start codons for insect mitogenomes (Wolstenholme, 1992). Termination codons for *S. longialata* were mostly complete (TAA) with some incomplete (TA or T). Such incomplete stop codons have been found in various insect species (e.g. Ma et al., 2015; Nardi et al., 2001; Fenn et al., 2007), and it has been determined that incomplete stop codons can produce functional stop codons in polycistronic transcription cleavage and polyadenylation processes (Ojala et al., 1981). The only exception was detected in ND1, where *S. longialata* exhibited TAG as the stop codon. The infrequent use of TAG may be because of the high A+T composition of the PCGs, although TAG is the conservative stop codon in most insect mitogenomes (Liu et al., 2015). However, in the other three published Ascalaphidae mitogenomes, COX1 of *L. macaronius* (Negrisolo et al., 2011), *Ascaloptynx appendiculatus* (Beckenbach et al., 2008) and *Ascalohybris subjacens* (Cheng et al., 2014) used ACG as the start codons, and ND1 of *Ascalohybris subjacens* used TTG. The other start/stop codons were identical to the *S. longialata* situation.

The total length of the 13 PCGs in the *S. longialata* mitogenome was 11,169 bp, with an average AT content of 73.0%. The PCGs displayed A-skews (A > T) and C-skews (C > G) **(Table 1)**. We calculated the relative synonymous codon usage (RSCU) of the *S. longialata* mitogenome, excluding stop codons **(Fig. 2)**. The RSCU proved that codons with A or T in the third position are always overused when compared to the other synonymous codons. The codons of amino acids being NNW (NNA/NNU) were higher than 1.0 without exception in *S. longialata*. The most frequently encoded amino acids were Leu (UUR), Phe, Ile (>300), and the least frequently used amino acid was Cys (<45) **(Table S3)**, which was similar to the other Ascalaphidae mitogenomes **(Fig. 2)**.

Ribosomal and transfer RNAs

The mtDNA of *S. longialata* contained the entire content of 2 rRNAs and 22 tRNAs genes that were also found in other neuropterid mitogenomes (Boore 1999; Song et al. 2018; Wang et al. 2017). The 16S rRNA gene with a length of 1,314 bp was located between $tRNA^{Leu}$ (CUN) and $tRNA^{Val}$ whereas the 12S rRNA gene with a size of 739 bp was located between $tRNA^{Val}$ and the control region (CR); these locations were also detected in the other ascalaphid owlfly species (Beckenbach et al., 2008; Negrisolo et al., 2011; Cheng et al., 2014). The AT content of rRNAs in the *S. longialata* mitogenome was the highest (77.8%) except for the A+T-rich region (85.1%). We found that the AT-skew was strongly positive whereas the GC-skew was highly negative, which showed that the contents of A and C were higher than those of T and G, respectively.

The size of the tRNAs was 1,476 bp with an average A+T content of 76.2%. Among the 22 tRNAs, most tRNA genes displayed the common cloverleaf secondary structure, whereas the $tRNA^{Ser(AGN)}$ had lost the

dihydrouridine (DHU) arm **(Fig. 3).** The absence of this arm in $tRNA^{Ser(AGN)}$ is a typical feature of many insect mtDNAs (Wolstenholme et al., 1992; Salvato et al., 2008; Sheffield et al., 2008; Negrisolo et al., 2011; Yan et al., 2014; Du et al., 2017; Zhang et al. 2008), and is usually demonstrated to be functional (Hanada et al., 2001; Stewart et al., 2003). We also found that the $tRNA^{Phe}$ and $tRNA^{Leu\ (CUN)}$ lack the TψC loops. Furthermore, unmatched U-U base pairs were observed in $tRNA^{Trp}$ **(Fig. 3)**.

In terms of the tRNA gene structures of the other three ascalaphid owlflies, the $tRNA^{Phe}$ in *L. macaronius* and *Ascalohybris subjacens* showed the loss of TψC loops, and the $tRNA^{Ser(AGN)}$ in *Ascalohybris subjacens* lost the DHU loop, whereas the tRNA genes of *Ascaloptynx appendiculatus* almost displayed the typical cloverleaf secondary structure.

A+T-rich region and Intergenic regions

Generally speaking, the A+T-rich region was the largest non-coding region, which was located between 12S rRNA and $tRNA^{Ile}$. The A+T-rich region of *S. longialata* mtDNA having a length of 1,088 bp was the longest when compared to the other three species of Ascalaphidae, e.g. the *L. macaronius* (1,049 bp), *Ascaloptynx appendiculatus* (1,066) and *Ascalohybris subjacens* (1,051 bp). Additionally, the composition of A+T was 85.1% in *S. longialata*, which was higher than in *L. macaronius* (84.5%) and lower than *Ascaloptynx appendiculatus* (85.7%) and *Ascalohybris subjacens* (86.2%).

The mitochondrial genomes of most insects are compact (Boore, 1999), although large intergenic regions occur in some species. In the *S. longialata* mitogenome the longest intergenic region was a 54 bp insertion between $tRNA^{Ile}$ and $tRNA^{Gln}$. This spacer was also present in *L. macaronius*, *Ascaloptynx appendiculatus* and *Ascalohybris subjacens* and spanned 55 bp, 42 bp, 54 bp, respectively (Beckenbach et al., 2008; Negrisolo et al., 2011; Cheng et al., 2014). This intergenic region of the four species also shared a 12 bp long congruent motif A(A/G)TTAA(A/C)TAAAT adjacent to $tRNA^{Gln}$. It has previously been reported that this spacer may diverge quickly among different families of the same order (Negrisolo et al., 2011). Aside from this spacer, gaps between genes ranged from 1 to 18 residues in the *S. longialata* sequence.

Phylogenetic analyses

The phylogenetic relationships including the long-branch attraction species deduced from BI analysis and ML analysis are shown in **Fig. 4**, and they present somewhat different topologies. In the ML analysis, (*Micromus* sp. + (*Neuronema laminatum* + *Drepanepteryx phalaenoides*)) is a sister clade to (*Ditaxis biseriata* + *Eumantispa harmandi*) + (*Podallea* sp. + *Stenobiella* sp.) with low support (ML 29). However, in the BI analysis (*Micromus* sp. + (*Neuronema laminatum* + *Drepanepteryx phalaenoides*)) is a sister clade to (*Apochrysa matsumurae* + (*Nothochrysa* sp. + (*Nothancyla verreauxi* + (*Abachrysa eureka* + (*Chrysopa pallens* + (*Chrysoperla nipponensis* + *Chrysoperla externa*)))))) with high support (BI 1). In the ML analysis (*Sisyra nigra* + *Climacia areolaris*) is a clade sister to (*Nevrorthus apatelios* + *Nipponeurorthus fuscinervis*), but in BI (*Sisyra nigra* + *Climacia areolaris*) is a clade sister to (*Coniopteryx* sp. + *Semidalis aleyrodiformis*). It has been demonstrated that the long branch attraction (LBA) artefact will affect both Maximum Likelihood (ML) and Bayesian Inference (BI) tree reconstruction methods (Huelsenbeck et al., 1993; Huelsenbeck, 1995; Philippe, 2000; Philippe et al., 2005). Thus, we propose that the difference between the ML and BI analyses were caused mainly by long branch attraction of *Coniopteryx* sp., *Dilar* sp. and *Semidalis aleyrodiformis*.

According to the opinion of HYPERLINK "https://xs.glgooo.top/citations?user=gjC5lywAAAAJ&hl=zh-CN&oi=sra" Bergsten (2005), a method excluding long branch taxa can avoid LBA. So we removed three species (*Semidalis aleyrodiformis*, *Coniopteryx* sp., *Dilar* sp.) and reconstructed the phylogeny of Neuroptera (**Figs. 5**). In this situation, both the ML and BI phylogenetic trees showed identical topologies and high support values for most clades, except for the internal relations within the family Chrysopidae. *Apochrysa matsumurae* is a sister clade to *Nothochrysa* sp. in ML analysis and then the clade of (*Apochrysa matsumurae* + *Nothochrysa* sp.) is the base clade of Chrysopidae, whereas the relationship of (*Nothochrysa* sp. + (*Nothancyla verreauxi* + (*Abachrysa eureka* + (*Chrysopa pallens* + (*Chrysoperla nipponensis* + *Chrysoperla externa*))))) in BI analysis is recovered. On the whole, this analysis highly supports the monophyly of Osmylidae, Sisyridae, Nevrorthidae, Berothidae, Mantispidae, Hemerobiidae, Chrysopidae, Psychopsidae, Nymphidae and Nemopteridae. But the monophyly of Ascalaphidae which was supported by Wang et al. (2017) failed to be supported in this study. Two clades of Neuroptera were supported: one clade is (Osmylidae + (Sisyridae + Nevrorthidae)) and the other clade is (Berothidae + Mantispidae) + ((Hemerobiidae + Chrysopidae) + (Ithonidae + ((Psychopsidae + (Nymphidae + ((Nemopteridae + (*Ascaloptynx appendiculatus* of Ascalaphidae + (Ascalaphidae + Myrmeleontidae). In the ML analysis long-length attraction existed with all families of Neuroptera (**Fig. 4**) and Coniopterygidae is recovered as sister clade to the remaining extant Neuroptera, which is consistent with the conclusions of Wang et al. (2017) and Winterton et al. (2010; 2018). By contrast, in the BI analysis (**Fig. 4**) Osmylidae is recovered as sister clade to (Coniopterygidae + (Sisyridae + Nevrorthidae). In the results of Haring and Aspöck (2004) and Song et al. (2018), Osmylidae as the basal position of Neuroptera was supported whereas in the results of Wang et al. (2017), the relationship of (Osmylidae + (Sisyridae + Nevrorthidae)) is supported by ML and BI analyses with the homogenous GTR+I+G model. But when Wang et al. (2017) used the heterogenous CAT-GTR model in BI analysis, (Sisyridae + Nevrorthidae) + (Osmylidae + other Neuroptera) were recovered. These difference may be caused by the model selection. In this study we also found that Nevrorthidae and Sisyridae were united with Osmylidae and sister to other extant Neuroptera, excluding Coniopterygidae (**Fig. 5**), which was also found by Wang et al. (2017) and Winterton (2010). The sister relationship of Myrmeleontidae and Ascalaphidae, Hemerobiidae and Chrysopidae, respectively, is supported as well as by Wang et al. (2017) and Song et al. (2018).

In addition, the phylogenetic trees resolved the unclear relationship between/within Myrmeleontidae and Ascalaphidae, which were previously controversial since the recent results of mitogenomic phylogeny do not support the monophyly of Myrmeleontidae or Ascalaphidae (Yan et al., 2014; Lan et al., 2016; Winterton et al. 2018; Zhao et al., 2017). In this study, the topology is as follows: ((*Myrmeleon immanis* + *Epacanthaclisis banksi*) + (*Dendroleon pantherinus* + (*Bullanga florida* + *Gatzara jezoensis*))) (ML 78, BI 1), which supports the monophyly of Myrmeleontidae. Among them, the *S. longialata* that we sequenced is a sister clade to (*Ascalohybris subjacens* + *L. macaronius*), which showed high support both in ML and BI analysis. Because of the increase in species of Neuroptera included in the present analysis, the topologies of the phylogenetic relationships were somewhat different to those of Wang et al. (2017) who reported that *Myrmeleon immanis* is a sister clade to (*Dendroleon pantherinus* + (*Ascaloptynx appendiculatus* + (*L. macaronius* + *Ascalohybris subjacens*))). However in present study showed the topology as follows: (*Ascaloptynx appendiculatus* +((*Suhpalacsa longialata* + (*Ascalohybris subjacens* + *L. macaronius*)) + the clade Myrmeleontidae). We found with the inclusion of *Suhpalacsa longialata* that the monophyly of Myrmeleontidae was recovered again,

but the monophyly of Ascalaphidae failed in our results, which was also supported by Wang et al. (2017) and Song et al. (2018). Myrmeleontidae is inside Ascalaphidae in our results. The monophyly of Ascalaphidae and Myrmeleontidae will need more species to be added before they can be discussed further. Consequently, we believe that increasing the abundance of mitochondrial genomes of Neuroptera will make a significant difference to resolving and reconstructing the phylogenetic relationships within Neuroptera.

CONCLUSION

We successfully sequenced the entire mitochondrial genome of *S. longialata*, which showed similar gene characteristics to the other three species of Ascalaphidae. Both BI and ML analyses supported *S. longialata* as a clade sister to (*Ascalohybris subjacens* + *L. macaronius*), but Ascalaphidae is not monophyletic. The different topologies of phylogenetic relationships were caused mainly by long branch attraction of *Coniopteryx* sp., *Dilar* sp. and *Semidalis aleyrodiformis*.

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316 **Figure legends**

317 **Figure 1 Mitogenome map of *S. longialata*.** The outermost circle shows the gene map of
318 *S. longialata* and the genes outside the map are coded on the major strand (J-strand), whereas the
319 genes on the inside of the map are coded on the minor strand (N-strand). The middle circle
320 (black) displays the GC content and the paracentral circle (purple & green) displays the GC skew.
321 Both GC content and GC skew are plotted as the deviation from the average value of the total
322 sequence. Seventeen arcs display the PCR amplification methods. All primers are shown in
323 Table S1.

324

325 **Figure 2 The relative synonymous codon usage (RSCU) in the *S. longialata* mitogenome.**
326 Codon families are provided on the X-axis along with the different combinations of synonymous
327 codons that code for that amino acid. RSCU are provided on the Y-axis.

328

329 **Figure 3 Secondary structures for 22 transfer RNAs in the *S. longialata* mitogenome.**

330

331 **Figure 4 Phylogenetic relationships of Neuroptera in ML and BI analyses.** The data is
332 includes 43 species of Neuroptera as the ingroup and 4 species of Megaloptera as the outgroup.
333 The red boxes on the figure mean different topology.

334

335 **Figure 5 Phylogenetic relationships of Neuroptera in ML and BI analyses after the**
336 **elimination of three species (*Semidalis aleyrodiformis*, *Coniopteryx* sp., *Dilar* sp.).** The data
337 include 40 species of Neuroptera as the ingroup and 4 species of Megaloptera as the outgroup.
338 The red boxes on the figure mean different topology.

Table Notes

Table 1 Base composition of the mitochondrial genomes of four species of Ascalaphidae.

Table S1 Universal and specific primers used to amplify the mitochondrial genome of *S. longialata*. All universal primers were modified according to Simon et al. (2006), Zhang et al. (2008) and Zhang et al. (2018) by comparing to known mayfly mitochondrial genomes. The orientation of primers is as shown in Fig. 1.

Table S2 Species used to construct the phylogenetic relationships along with GenBank accession numbers.

Table S3 The codon number and relative synonymous codon usage (RSCU) in *S. longialata* mitochondrial protein-coding genes.

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Figure 1

Mitogenome map of *S.longialata*.

The outermost circle shows the gene map of *S.longialata* and the genes outside the map are coded on the major strand (J-strand), whereas the genes on the inside of the map are coded on the minor strand (N-strand). The middle circle (black) displays the GC content and the paracentral circle (purple & green) displays the GC skew. Both GC content and GC skew are plotted as the deviation from the average value of the total sequence. Seventeen arcs display the PCR amplification methods. All primers are shown in Table S1.

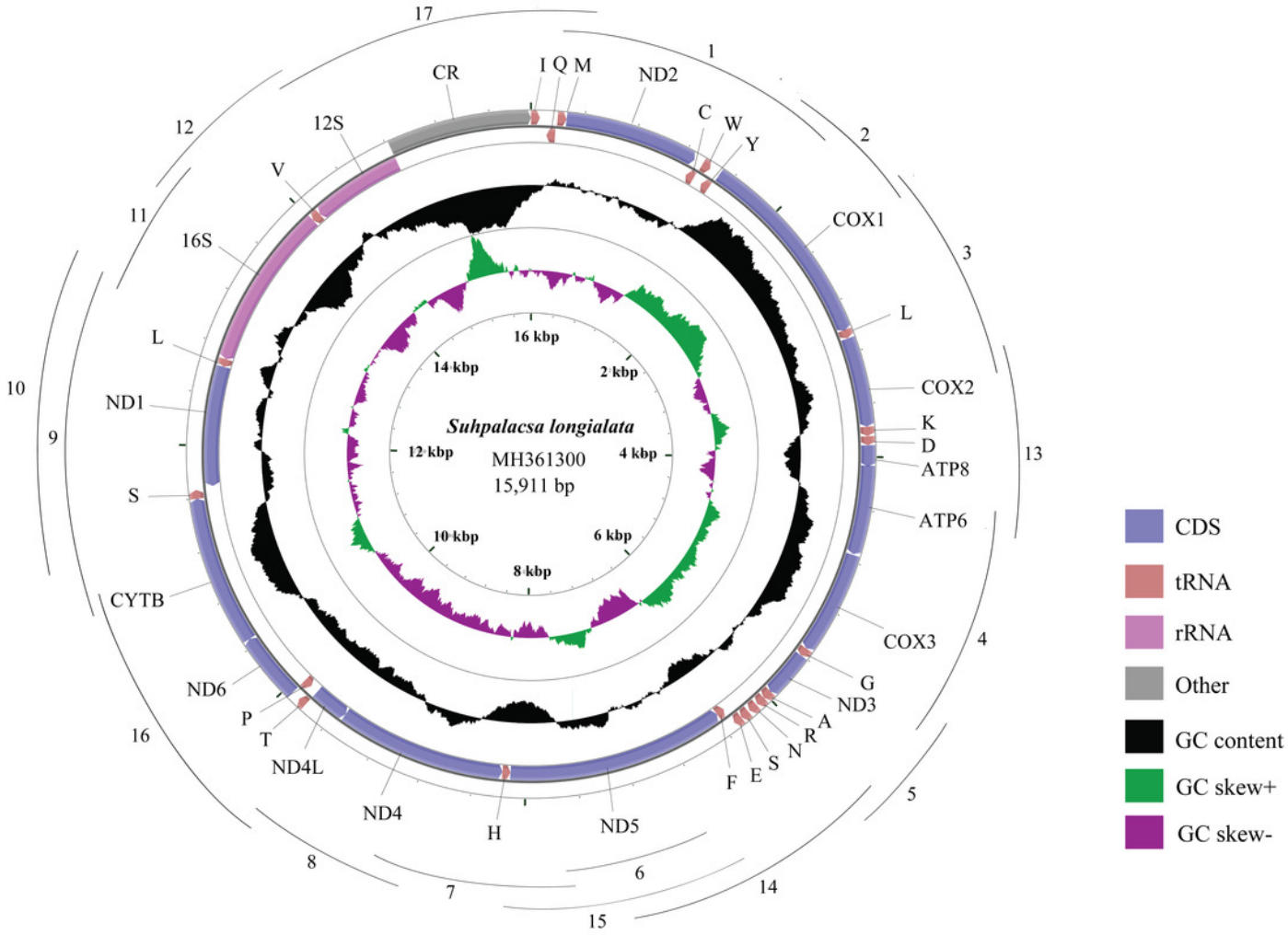

Figure 2

The relative synonymous codon usage (RSCU) in the *S.longialata* mitogenome.

Codon families are provided on the X-axis along with the different combinations of synonymous codons that code for that amino acid. RSCU are provided on the Y-axis.

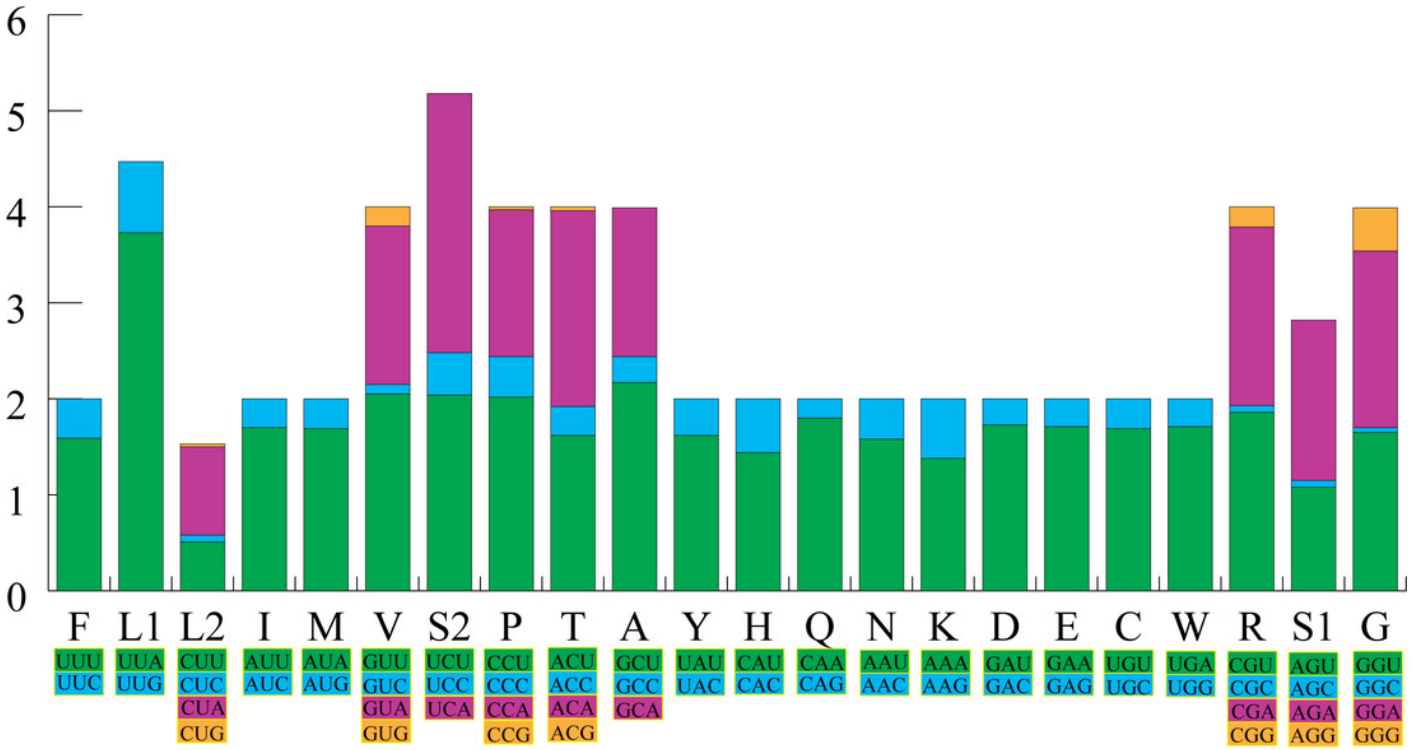

Figure 3

Secondary structures for 22 transfer RNAs in the *S.longialata* mitogenome.

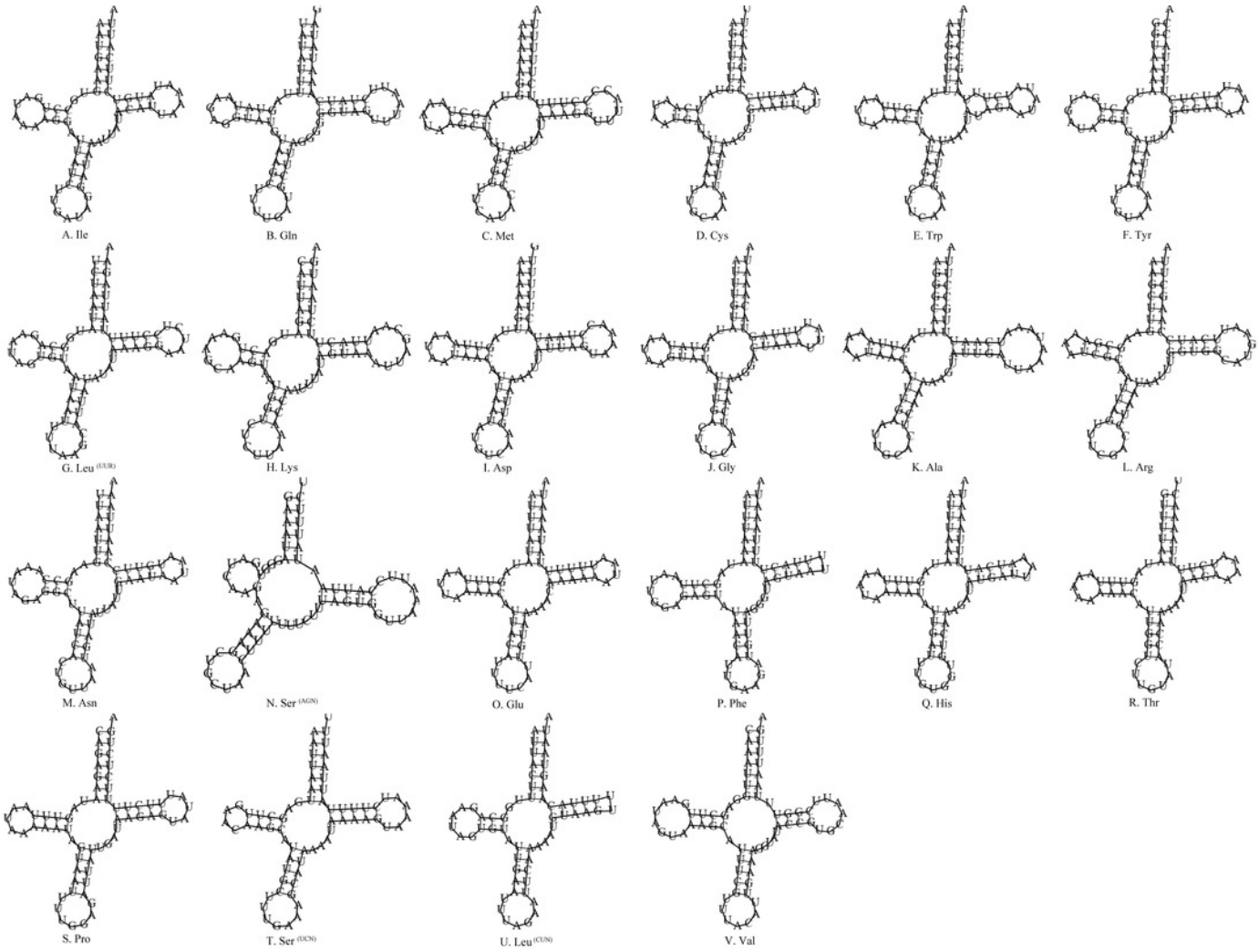

Figure 4

Phylogenetic relationships of Neuroptera in ML and BI analyses.

The data is includes 43 species of Neuroptera as the ingroup and 4 species of Megaloptera as the outgroup. The red boxes on the figure mean different topology.

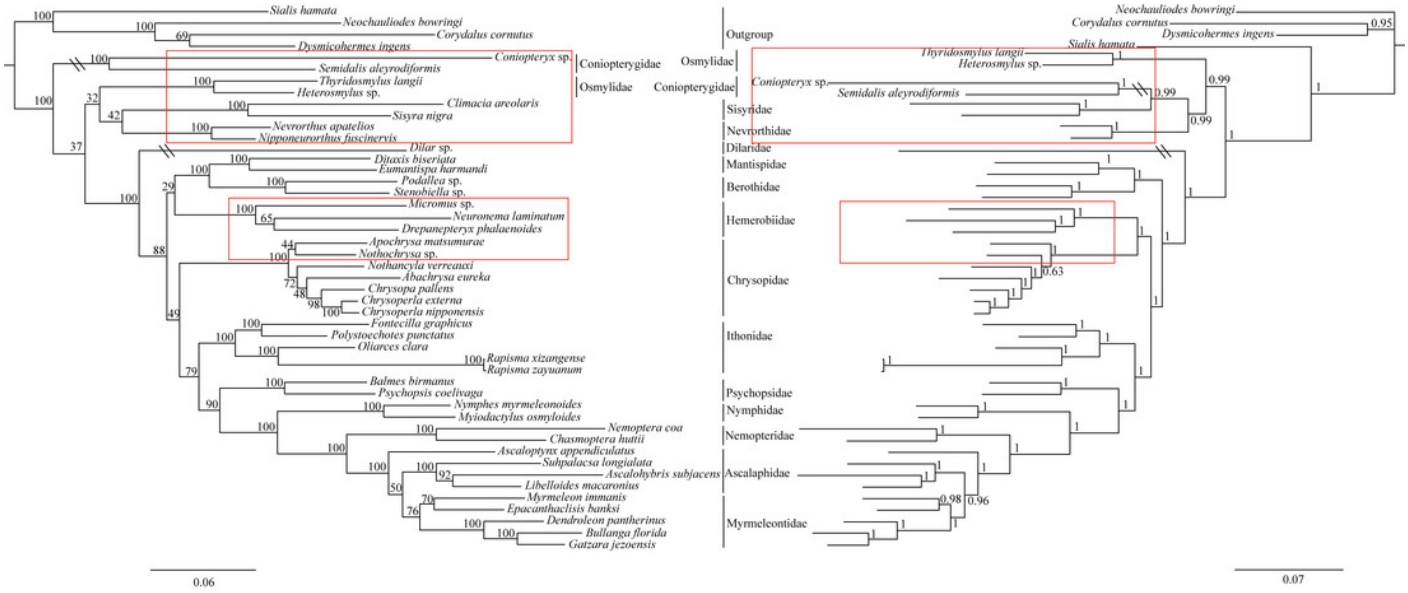

Figure 5

Phylogenetic relationships of Neuroptera in ML and BI analyses after the elimination of three species (*Semidalis aleyrodiformis*, *Coniopteryx* sp., *Dilar* sp.).

The data include 40 species of Neuroptera as the ingroup and 4 species of Megaloptera as the outgroup. The red boxes on the figure mean different topology.

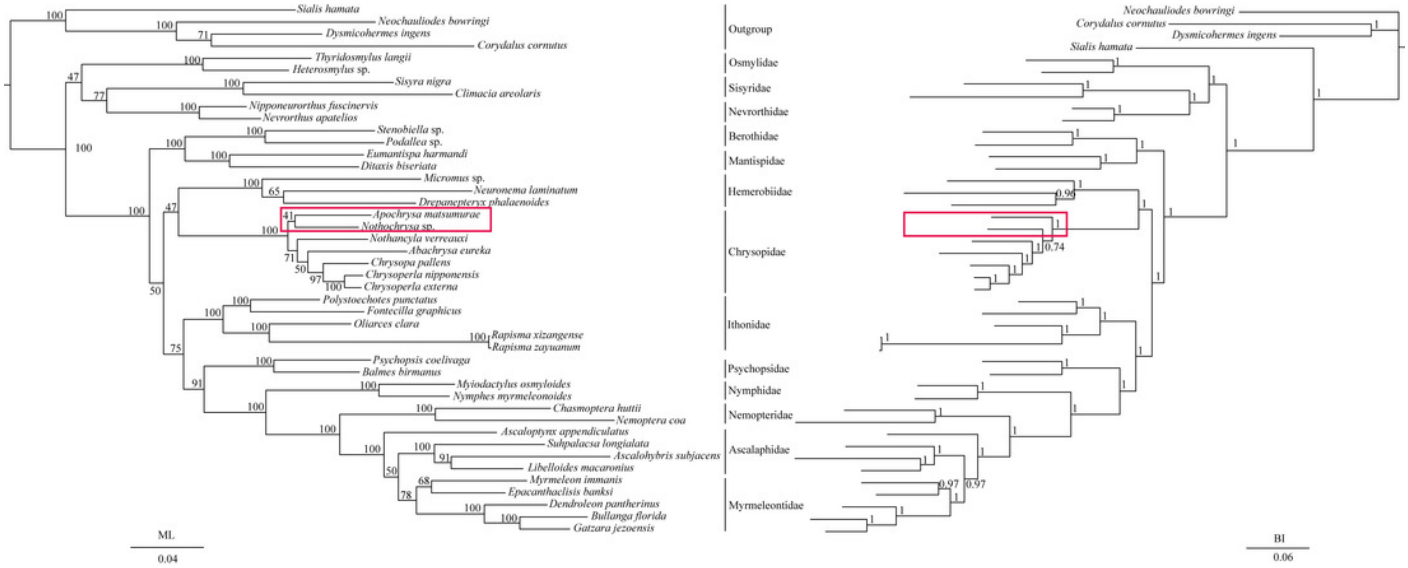

Table 1(on next page)

Base composition of the mitochondrial genomes of four species of Ascalaphidae.

1 **Table 1 Species used to construct the phylogenetic relationships along with GenBank accession numbers.**

Order	Family	Species	GenBank accession number	References
Neuroptera	Myrmeleontidae	*Myrmeleon immanis*	KM216750	Zhang et al., 2016
		Epacanthaclisis banksi	KF701327	Cheng et al., 2015
		Gatzara jezoensis	KY364372	Zhang et al., 2017
		Bullanga florida	KX369241	Lan et al., 2016
		Dendroleon pantherinus	KT425068	Wang et al., 2012
	Chrysopidae	*Apochrysa matsumurae*	AP011624	Haruyama et al., 2011
		Chrysoperla nipponensis	AP011623	Haruyama et al., 2011
		Chrysopa pallens	JX033119	He et al., 2012
		Chrysoperla externa	KU877169	Directly submitted
		Nothochrysa sp.	KP264630	Directly submitted
		Nothancyla verreauxi	KP264629	Directly submitted
		Abachrysa eureka	KY587199	Jiang et al., 2017
	Ascalaphidae	*Ascalohybris subjacens*	KC758703	Cheng et al., 2014
		Ascaloptynx appendiculatus	FJ171324	Beckenbach et al., 2008
		Libelloides macaronius	FR669150	Negrisolo et al., 2011
		Suhpalacsa longialata	MH361300	This study
	Ithonidae	*Polystoechotes punctatus*	FJ171325	Beckenbach et al., 2008
		Oliarces clara	KT425090	Wang et al., 2017
		Fontecilla graphicus	KT425072	Wang et al., 2017
	Hemerobiidae	*Neuronema laminatum*	KR078257	Zhao et al., 2016
		Drepanepteryx phalaenoides	KT425087	Wang et al., 2017
		Micromus sp.	KT425075	Wang et al., 2017
	Osmylidae	*Thyridosmylus langii*	KC515397	Zhao et al., 2013
		Heterosmylus sp.	KT425077	Wang et al., 2017
	Mantispidae	*Ditaxis biseriata*	FJ859906	Cameron et al., 2009
		Eumantispa harmandi	KT425080	Wang et al., 2017
	Rapismatidae	*Rapisma zayuanum*	KF626447	Wang et al., 2013
		Rapisma xizangense	KF626446	Wang et al., 2013
	Psychopsidae	*Balmes birmanus*	KT425083	Wang et al., 2017
		Psychopsis coelivaga	KT425082	Wang et al., 2017
	Nemopteridae	*Chasmoptera huttii*	KT425069	Wang et al., 2017
		Nemoptera coa	KT425079	Wang et al., 2017
	Berothidae	*Podallea* sp.	KT425091	Wang et al., 2017
		Stenobiella sp.	KT425081	Wang et al., 2017
	Sisyridae	*Climacia areolaris*	KT425088	Wang et al., 2017
		Sisyra nigra	KT425070	Wang et al., 2017
	Coniopterygidae	*Coniopteryx* sp.	KT425078	Wang et al., 2017
		Semidalis aleyrodiformis	KT425067	Wang et al., 2017
	Nevrorthidae	*Nipponeurorthus fuscinervis*	KT425076	Wang et al., 2017
		Nevrorthus apatelios	KT425074	Wang et al., 2017
	Nymphidae	*Nymphes myrmeleonoides*	KJ461322	Yan et al., 2014
		Myiodactylus osmyloides	KT425089	Wang et al., 2017
	Dilaridae	*Dilar* sp.	KT425073	Wang et al., 2017

Order	Family	Species	GenBank accession number	References
Megaloptera	Corydalidae	*Corydalus cornutus*	FJ171323	Beckenbach et al., 2008
		Dysmicohermes ingens	KJ806318	Wang et al., 2016
		Neochauliodes bowringi	JQ351950	Li et al., 2015
	Sialidae	*Sialis hamata*	FJ859905	Cameron et al., 2009

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