Characteristics of the complete mitochondrial genome of *Suhpalacsa longialata* and its phylogeny

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The total length of the mitogenome of the owlfly, Suhpalacsa *longialata*, was 15,911 bp, which is the longest known to date among the available family members of Ascalaphidae. The size of each gene was similar to the other three known species of Ascalaphidae. Transposition of $tRNA^{Cys}$ and $tRNA^{Trp}$ genes in *S. longialata* occurred and formed an unusual gene order $tRNA^{Cys}$-$tRNA^{Trp}$-$tRNA^{Tyr}$ (CWY). 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* + *Libelloides macaronius*).

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Author Cover Page

Dear editor,

We would like to submit the interesting research article entitled “Characteristics of the complete mitochondrial genome of Suhpalacsa longialata and its phylogeny”, which we wish to be considered for publication in PeerJ. It is only submitted to PeerJ and never published elsewhere.

The total length of the mitogenome of the owlfly, *Suhpalacsa longialata*, was 15,911 bp, which is the longest known to date among the available family members of Ascalaphidae. The size of each gene was similar to the other three known species of Ascalaphidae. Transposition of $tRNA^{Cys}$ and $tRNA^{Trp}$ genes in *S. longialata* occurred and formed an unusual gene order $tRNA^{Cys}$-$tRNA^{Trp}$-$tRNA^{Tyr}$ (CWY). Both BI and ML analyses strongly supported *S. longialata* as a sister clade to (*Ascalohybris subjacens* + *Libelloides macaronius*).

The work described has not been submitted elsewhere for publication, in whole or in part, and all the authors listed have approved the manuscript that is enclosed.

Sincerely,

Yours

Jia-Yong Zhang

ABSTRACT

The total length of the mitogenome of the owlfly, *Suhpalacsa longialata*, was 15,911 bp, which is the longest known to date among the available family members of Ascalaphidae. The size of each gene was similar to the other three known species of Ascalaphidae. Transposition of $tRNA^{Cys}$ and $tRNA^{Trp}$ genes in *S. longialata* occurred and formed an unusual gene order $tRNA^{Cys}$-$tRNA^{Trp}$-$tRNA^{Tyr}$ (CWY). 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* + *Libelloides macaronius*).

Keywords: Neuroptera, Ascalaphidae, Mitochondrial genome, Phylogenetic relationship

INTRODUCTION

The study of mitochondrial genomes (mtDNAs) is of great interest to many scientific fields, such as molecular evolution, evolutionary genomics and so on (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). However, more and more researchers have found special mitochondrial genomic structures. According to Boyce et al. (1989), the sizes of the mitogenomes of bark weevils were greater than 20 kbp, perhaps related to an expansion of the main non-coding region, i.e. the control region. The most widespread gene order in insect mtDNAs is present in fruit flies (*Drosophila yakuba*), and is thought to be ancestral for the entire class Insecta (Clary et al., 1985; Boore et al., 1998; Cameron et al., 2006). However, gene rearrangements often occur within a selected family or order (Beard et al., 1993; Mitchell et al., 1993; Cameron et al., 2008; Salvato et al., 2008; McMahon et al., 2009). Consequently, the particular gene order becomes a significant marker to delimit taxonomic boundaries. Indeed, mtDNAs are likely to be superior to nuclear DNAs to reconstruct phylogenetic relationships as demonstrated by several studies (Boyce et al., 1989; Sheffield et al., 2008; Jia et al., 2008).

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) 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* (Negrisolo et al., 2011); *Ascaloptynx appendiculatus* (Beckenbach et al., 2008); *Ascalohybris subjacens* (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 basal condition within the Ascalaphidae. Increasing the number of sequenced species within the Neuroptera will be very helpful for phylogenetic reconstructions of Neuroptera relationships. Hence, the present study has taken a first step to by sequencing the complete mitogenome of *Suhpalacsa longialata* (Neuroptera, Ascalaphidae) and analyzing 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.

MATERIALS AND METHODS

Sample origin and DNA extraction

The *S. longialata* sample used for sequencing was collected from Hangzhou, Zhejiang province, China in July 2017 by LP Zhang. Total DNA was isolated from one leg of *S. longialata* using Ezup Column Animal Genomic DNA Purification Kit (Sangon Biotech Company, Shanghai, China).

PCR amplification and sequencing of *S. longialata* mtDNA

The conserved primers for polymerase chain reactions (PCR) amplification were designed according to Zhang et al. (2008), and the specific primers were designed based on the sequence information from universal primers using Primer Premier 5.0. A complete list of universal primers used for PCR amplification is given in **Table 1.** PCR was performed using *Takara Taq* DNA polymerase under normal PCR conditions (product length < 3,000 bp) with the following cycling steps: denaturation at 94 ℃ for 5 min, followed by 35 cycles of 94 ℃ (50 s), 40-60 ℃ (30-50 s), and 72 ℃ (1-3 min), followed by a final elongation at 72 ℃ for 10 min. All PCR products were visualized by electrophoresis in a 1% agarose gel and sent to Sangon Biotech Company 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. 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 (Cameron, 2014). 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

In order to discuss the phylogenetic relationships of Neuroptera, we used 43 species of Neuroptera previously sequenced as ingroups (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). Accession numbers of all mitochondrial genomes are listed in the **Table 2**. The nucleotide sequences of the 13 PCGs were employed for construction of BI and ML phylogenetic trees according to Zhang et al. (2018). DNA alignment was acquired from the amino acid alignment of the 13 protein-coding genes 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). Consequently, we discovered 9 subsets. 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). The 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 of the long branch attraction, we eliminated three species that were at unstable positions in the phylogenetic relationships and reconstructed the phylogenetic tree (Bergsten, 2005; Philippe et al., 2005). Likewise, the ML and the BI analyses were performed as the previous method.

RESULTS AND DISCUSSION

Mitogenome organization and structure

The complete mitogenome of *S. longialata* is a double-stranded circular DNA molecule with the length of 15,911 bp **(Fig. 1)**, which has been submitted to GenBank under the accession number (MH361300). The mitogenome of *S. longialata* is the longest when compared to those of other Ascalaphidae species, whose mitogenomes range from 15,873 bp to 15,890 bp. It encodes the entire set of 37 mitochondrial genes including 13 protein-coding genes, 22 tRNA genes and 2 rRNA genes that were typically present in metazoan mitogenomes (Wolstenholme, 1992). In addition, the gene order of *S. longialata* was similar to the assumed basal 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), 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 account for the transposition of contiguous genes. Similar to the report by Beckenbach et al. (2008), we also inferred that the $tRNA^{Trp}$ - $tRNA^{Cys}$ (WC) genes were duplicated in tandem to form a tRNA cluster WCWC, which was followed by random loss of partial duplicated genes to produce the final CW gene order **(Fig. 2).**

Of all four sequenced Ascalaphidae mitogenomes, the length of the mitogenome of *S. longialata* (15,911bp) was the longest, largely due to 16 intergenic gaps ranging from 1 bp to 54 bp and a long typical A+T-rich region (1,088 bp) 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 3)**. The high bias towards A and T nucleotides was also found in protein-coding genes, 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 3)**.

Protein-coding genes and codon usages

All 13 protein-coding genes were annotated by Mega 7.0 using the invertebrate mitogenome genetic code (Cameron, 2014). Nine PCGs including ND2, COX1, COX2, ATP8, ATP6, COX3, ND3, ND6 and CYTB were located on the majority strand (J-strand) with the rest on the minority strand (N-strand). All protein-coding genes used ATN (N represents A, G, C, 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. 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. This is not used frequently probably because of the high composition of AT nucleotides used by the PCGs, though TAG is the conservative stop codon in insect mitochondrial genomes (Liu et al., 2015). In the other three published Ascalaphidae mitogenomes, however, COX1 of *L. macaronius* (Negrisolo et al., 2011), *Ascaloptynx appendiculatus* (Beckenbach et al., 2008) and *Ascalohybris subjacens* (Cheng et al., 2014) used ACG as start codons, and ND1 of *Ascalohybris subjacens* used TTG. The other start/stop codons are 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 protein-coding genes displayed A-skews (A > T) and C-skews (C > G) **(Table 3)**. We

189 calculated the relative synonymous codon usage (RSCU) of the *S. longialata* mitogenome, excluding stop
190 codons **(Fig. 3)**. The RSCU proved that codons with A or T in the third position are always overused when
191 compared to the other synonymous codons. The codons of amino acids being NNW (NNA/NNU) were higher
192 than 1.0 without exception in *S. longialata*. The most frequently encoded amino acids were Leu (UUR), Phe,
193 Ile (>300), and the least frequently used amino acid was Cys (<45) **(Table 4)**, which was similar to the other
194 Ascalaphidae mitogenomes.
195
196 **Ribosomal and transfer RNAs**
197 The mtDNA of *S. longialata* contained the entire content of 2 rRNAs and 22 tRNAs genes that were also
198 found in other neuropterid mitogenomes (Boore, 1999). The 16S rRNA gene with a length of 1,314 bp was
199 located between $tRNA^{Leu}$ (CUN) and $tRNA^{Val}$ whereas the 12S rRNA gene with a size of 739 bp was located
200 between $tRNA^{Val}$ and the control region (CR); these locations were also detected in the other ascalaphid owlfly
201 species. The AT content of rRNAs in the *S. longialata* mitogenome was the highest (77.8%) except for the
202 A+T-rich region (85.1%). We found that the AT-skew was strongly positive whereas the GC-skew was highly
203 negative, which showed that the contents of A and C were higher than those of T and G, respectively.
204 The size of the tRNAs was 1,476 bp with an average A+T content of 76.2%. Among the 22 tRNAs, most
205 tRNA genes displayed the common cloverleaf secondary structure, whereas the $tRNA^{Ser(AGN)}$ had lost the
206 dihydrouridine (DHU) arm **(Fig. 4).** The absence of this arm in $tRNA^{Ser(AGN)}$ is a typical feature of many insect
207 mtDNAs (Wolstenholme et al., 1992; Salvato et al., 2008; Sheffield et al., 2008; Negrisolo et al., 2011; Yan et
208 al., 2014; Du et al., 2017), and is usually demonstrated to be functional (Hanada et al., 2001; Stewart et al.,
209 2003). We also found that the $tRNA^{Phe}$ and $tRNA^{Leu\ (CUN)}$ lack the TψC loops. Furthermore, unmatched U-U
210 base pairs were observed in $tRNA^{Trp}$ **(Fig. 4)**.
211 In terms of the tRNA gene structures of the other three ascalaphid owlflies, the $tRNA^{Phe}$ in *L. macaronius*
212 and *Ascalohybris subjacens* showed the loss of TψC loops, and the $tRNA^{Ser(AGN)}$ in *Ascalohybris subjacens* lost
213 the DHU loop, whereas the tRNA genes of *Ascaloptynx appendiculatus* almost displayed the typical cloverleaf
214 secondary structure.
215
216 **A+T-rich region and Intergenic regions**
217 Generally speaking, A+T-rich region was the largest non-coding region, which was located between 12S
218 rRNA and $tRNA^{Ile}$. The A+T-rich region of *S. longialata* mtDNA having a length of 1,088 bp was the longest
219 when compared to the other three species of Ascalaphidae, e.g. the *L. macaronius* (1,049 bp), *Ascaloptynx*
220 *appendiculatus* (1,066) and *Ascalohybris subjacens* (1,051 bp). Additionally, the composition of A+T was
221 85.1% in the *S. longialata*, which was higher than *L. macaronius* (84.5%) and lower than *Ascaloptynx*
222 *appendiculatus* (85.7%) and *Ascalohybris subjacens* (86.2%).
223 The mitochondrial genomes of most insects are compact (Boore, 1999), although large intergenic regions
224 occur in some species. In the *S. longialata* mitogenome the longest intergenic region was a 54 bp insertion
225 between $tRNA^{Ile}$ and $tRNA^{Gln}$. This spacer was also present in *L. macaronius*, *Ascaloptynx appendiculatus* and
226 *Ascalohybris subjacens* and spanned 55 bp, 42 bp, 54 bp, respectively. This intergenic region of the four
227 species also shared a 12 bp long congruent motif A(A/G)TTAA(A/C)TAAAT adjacent to $tRNA^{Gln}$. It has
228 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 deduced from BI analysis and ML analysis are shown in **Fig. 5 and 6**, respectively, 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 a low support (ML 29), whereas 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 a 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*). We inferred that these difference were caused mainly by long branch attraction which possessed unstable position in phylogenetic relationships (Philippe et al., 2005). Thus, we removed three unstable species (*Semidalis aleyrodiformis*, *Coniopteryx* sp., *Dilar* sp.) and reconstructed the phylogeny of Neuroptera (**Figs. 7 and 8**) (Bergsten, 2005). 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 in the family Chrysopidae. *Apochrysa matsumurae* is a sister clade to *Nothochrysa* sp. in ML analysis, whereas the relationship was (*Nothochrysa* sp. + (*Nothancyla verreauxi* + (*Abachrysa eureka* + (*Chrysopa pallens* + (*Chrysoperla nipponensis* + *Chrysoperla externa*))))) in BI analysis, although the monophyly of Chrysopidae was well supported (ML 100, BI 1). More Chrysopidae samples need to be sequenced in the future to confirm this. In additionally, the phylogenetic trees resolved the unclear relationship between/within Myrmeleontidae, which were previously controversial (Yan et al., 2014; Lan et al., 2014; Zhao et al., 2017). The topology was as follows: (*Ascaloptynx appendiculatus* + ((*S. longialata* + (*Ascalohybris subjacens* + *L. macaronius*)) + ((*Myrmeleon immanis* + *Epacanthaclisis banksi*) + (*Dendroleon pantherinus* + (*Bullanga florida* + *Gatzara jezoensis*))))) (ML 100, BI 1). 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 that of Wang et al. (2017). The major difference is the position of Osmylidae that was more primitive than Sisyridae and Nevrorthidae in our studies. Furthermore, the Ascalaphidae branched earlier than the Myrmeleontidae. 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 analysis strongly supported *S. longialata* as a clade sister to (*Ascalohybris subjacens* + *Libelloides macaronius*), but the monophyly of Ascalaphidae is still undetermined.

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Statement of Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Figure legends

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 majority strand (J-strand), whereas the genes on the inside of the map are coded on the minority 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.

Figure 2 Putative mechanism of rearrangement in mitogenomes of *S. longialata* under the duplication-random loss model.

Figure 3 The relative synonymous codon usage (RSCU) in *S. longialata* mitogenome.

Figure 4 Secondary structures for 22 transfer RNAs in *S. longialata* mitogenome.

Figure 5 Phylogenetic relationships of Neuroptera in ML analysis.

Figure 6 Phylogenetic relationships of Neuroptera in BI analysis.

Figure 7 Phylogenetic relationships of Neuroptera in ML analysis based on the elimination of three species (*Semidalis aleyrodiformis*, *Coniopteryx* sp., *Dilar* sp.).

Figure 8 Phylogenetic relationships of Neuroptera in BI analysis based on the elimination of three species (*Semidalis aleyrodiformis*, *Coniopteryx* sp., *Dilar* sp.).

297 **Table Notes**

298 **Table 1 Universal primers used to amplify the mitochondrial genome of *S. longialata.***

299 **Table 2 Species used to construct the phylogenetic relationships along with GenBank**
300 **accession numbers.**

301 **Table 3 Base composition of Ascalaphidae mitochondrial genomes.**

302 **Table 4 The codon number and relative synonymous codon usage in *S. longialata***
303 **mitochondrial protein coding genes.**

304

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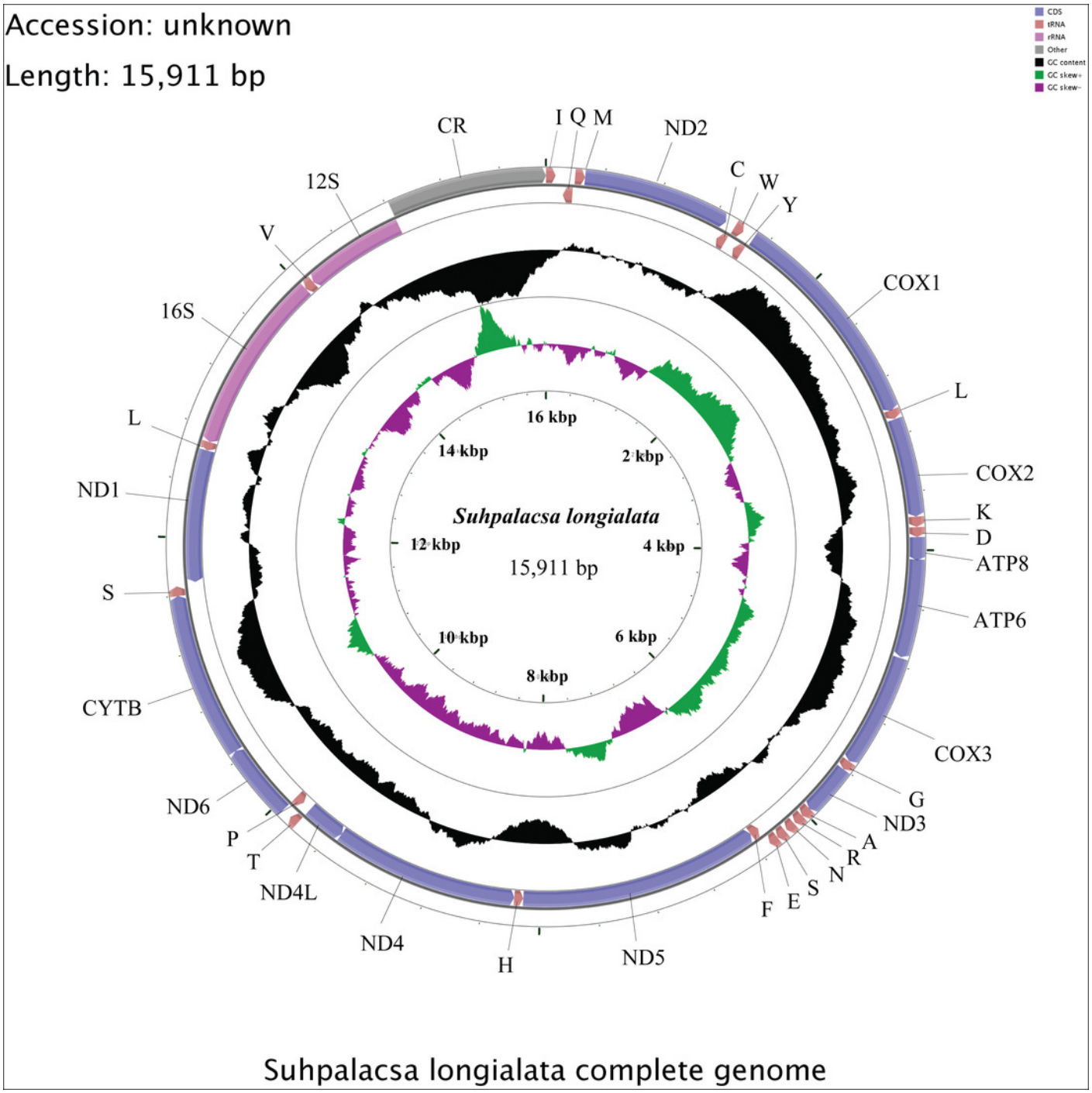
Figure 1

Figure 1 Mitogenome map of *S.longialata*.

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 majority strand (J-strand), whereas the genes on the inside of the map are coded on the minority 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.



Suhpalacsa longialata complete genome

Figure 2

Figure 2 Putative mechanism of rearrangement in mitogenomes of *S.longialata* under the duplication-random loss model.

Figure 2 Putative mechanism of rearrangement in mitogenomes of *S.longialata* under the duplication-random loss model.

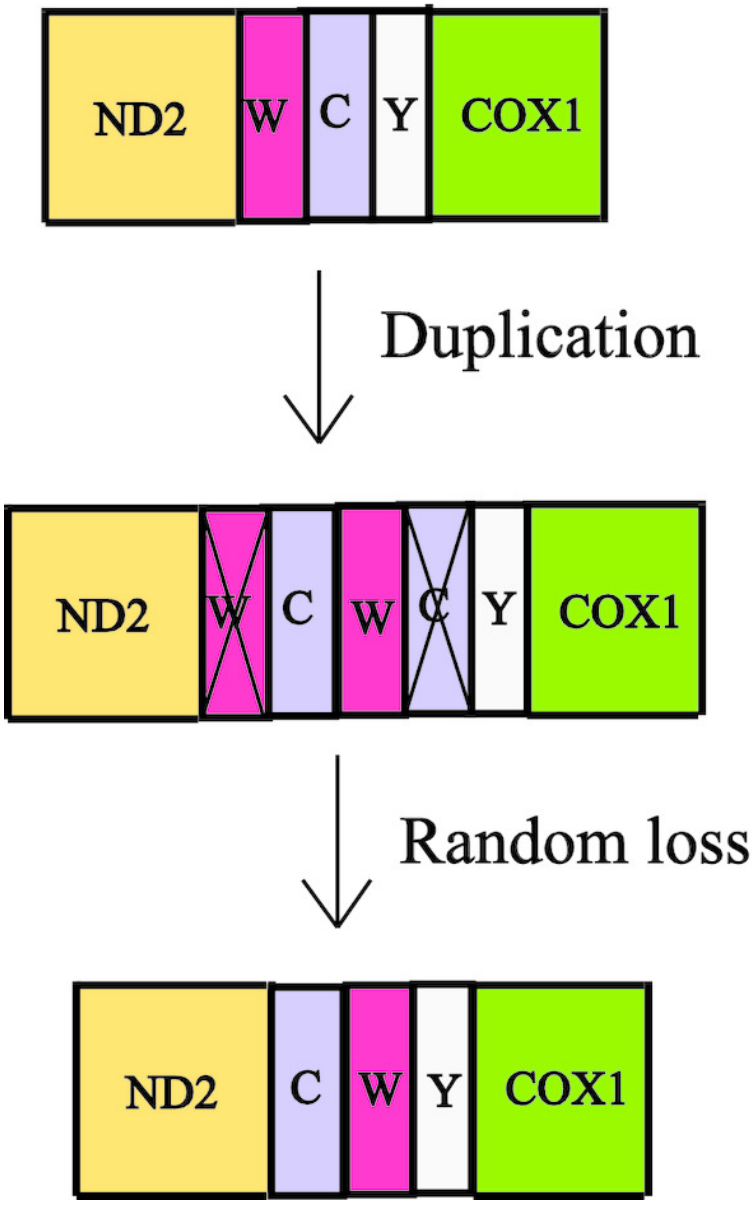


Figure 3

Figure 3 The relative synonymous codon usage (RSCU) in *S.longialata* mitogenome.

Figure 3 The relative synonymous codon usage (RSCU) in *S.longialata* mitogenome.

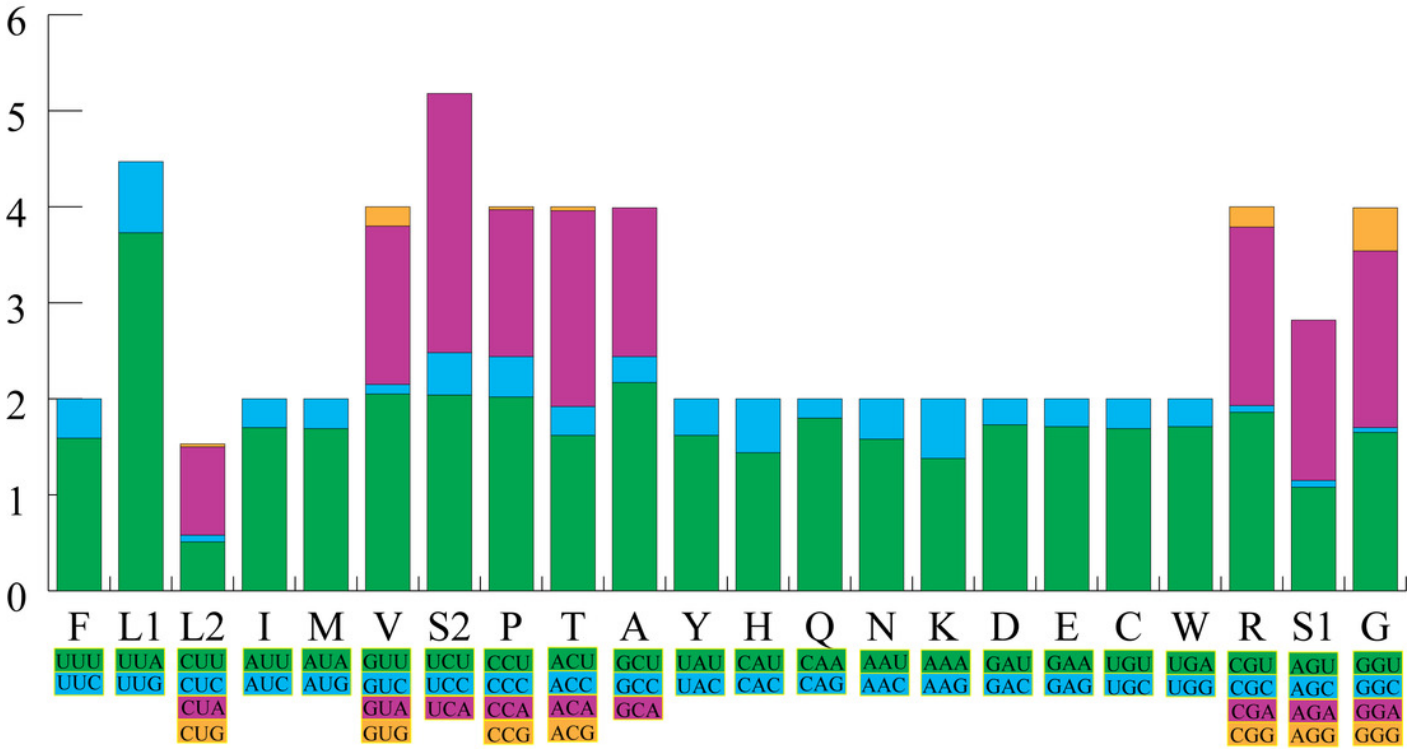


Figure 4(on next page)

Figure 4 Secondary structures for 22 transfer RNAs in *S.longialata* mitogenome.

Figure 4 Secondary structures for 22 transfer RNAs in *S.longialata* mitogenome.

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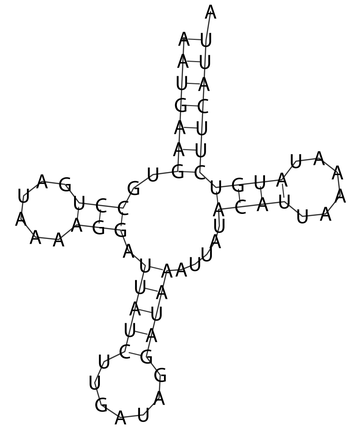
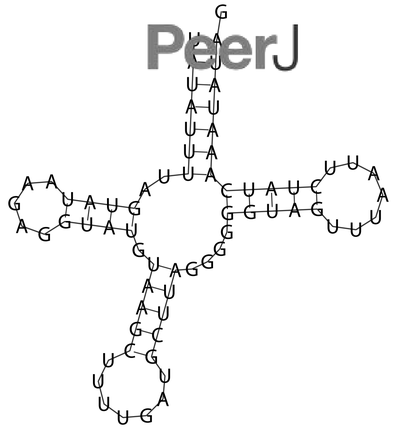
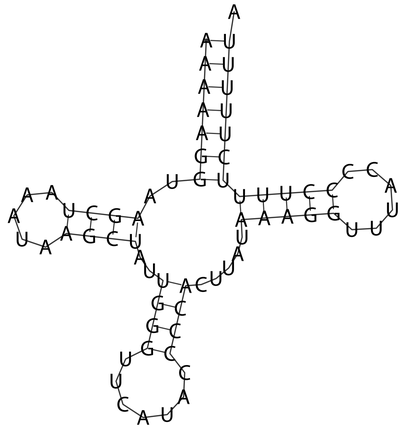


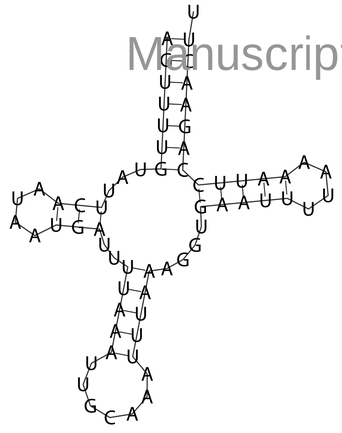
A. Ile



B. Gln



C. Met



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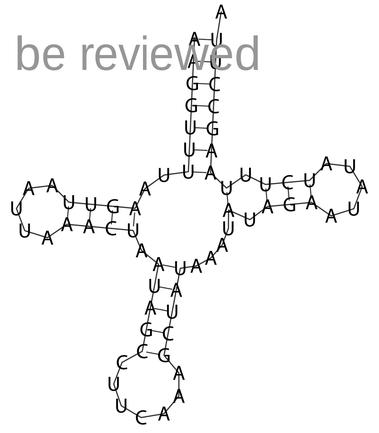

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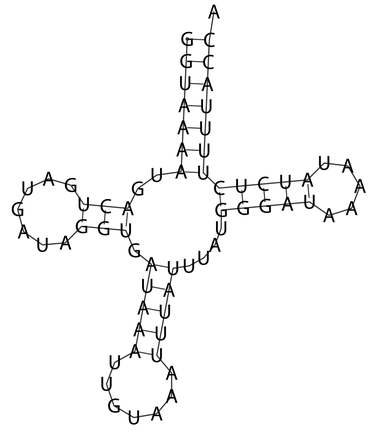
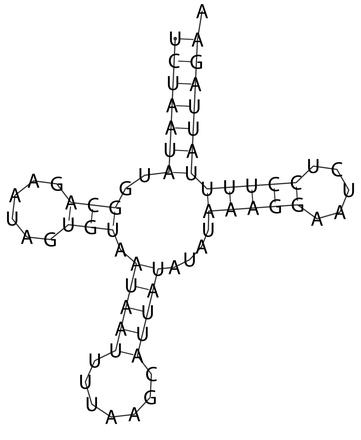


F. Tyr



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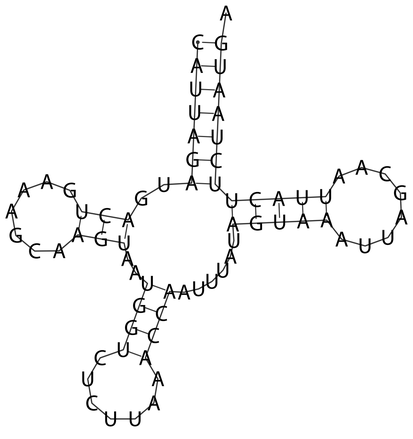
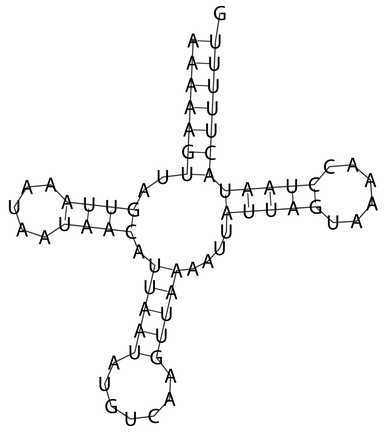


H. Lys



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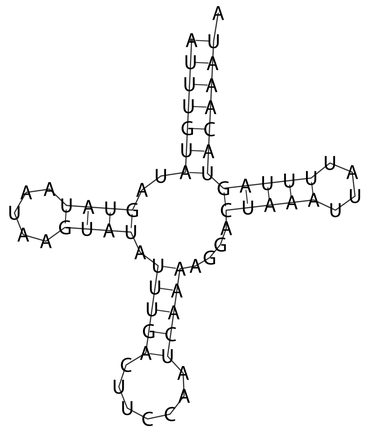

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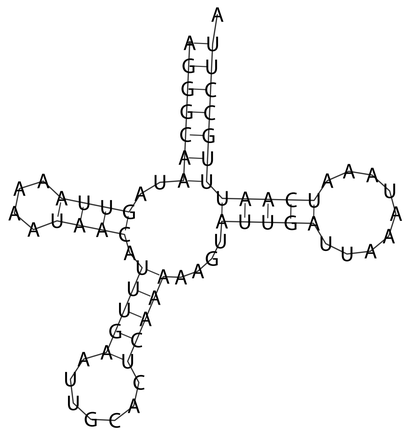

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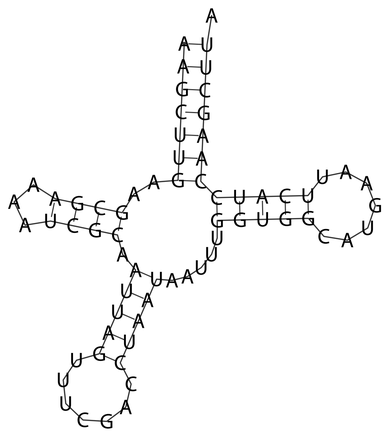
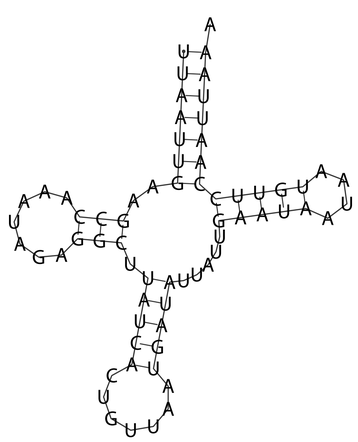


L. Arg



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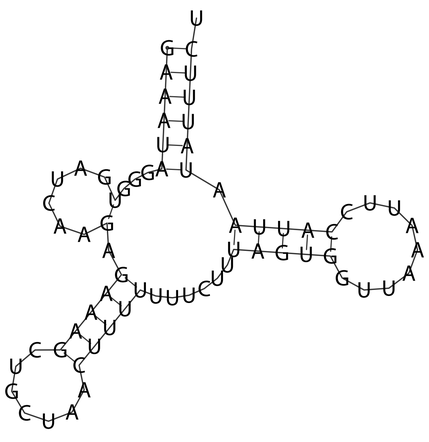

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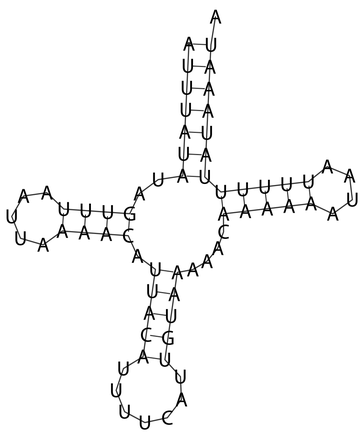
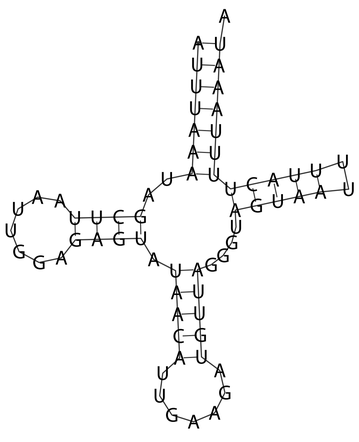
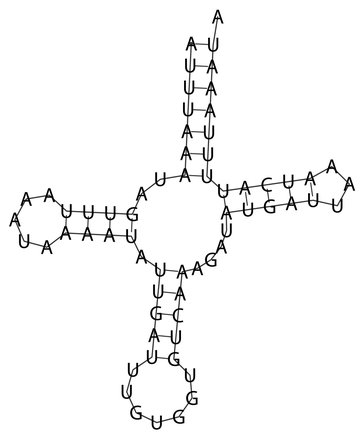


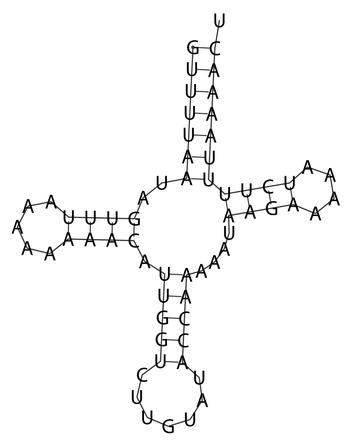
O. Glu



P. Phe



Q. His



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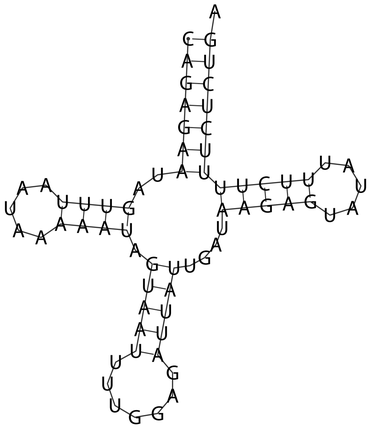
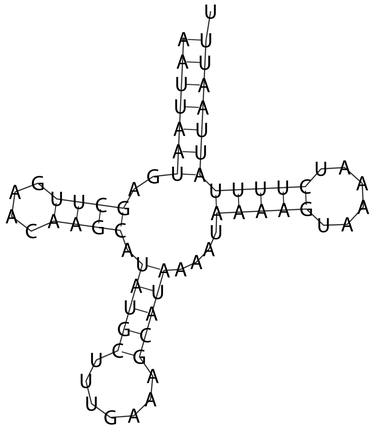


S. Pro



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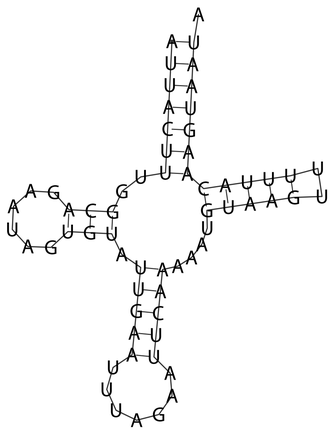
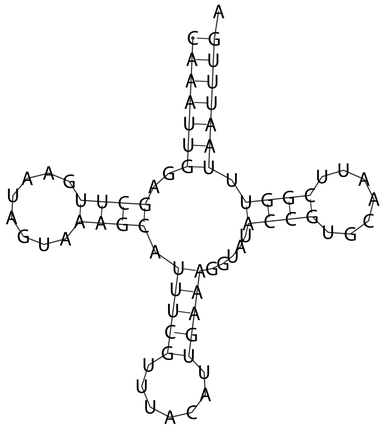


U. Leu$^{(CUN)}$



V. Val

Figure 5

Figure 5 Phylogenetic relationships of Neuroptera in ML analysis.

Figure 5 Phylogenetic relationships of Neuroptera in ML analysis.

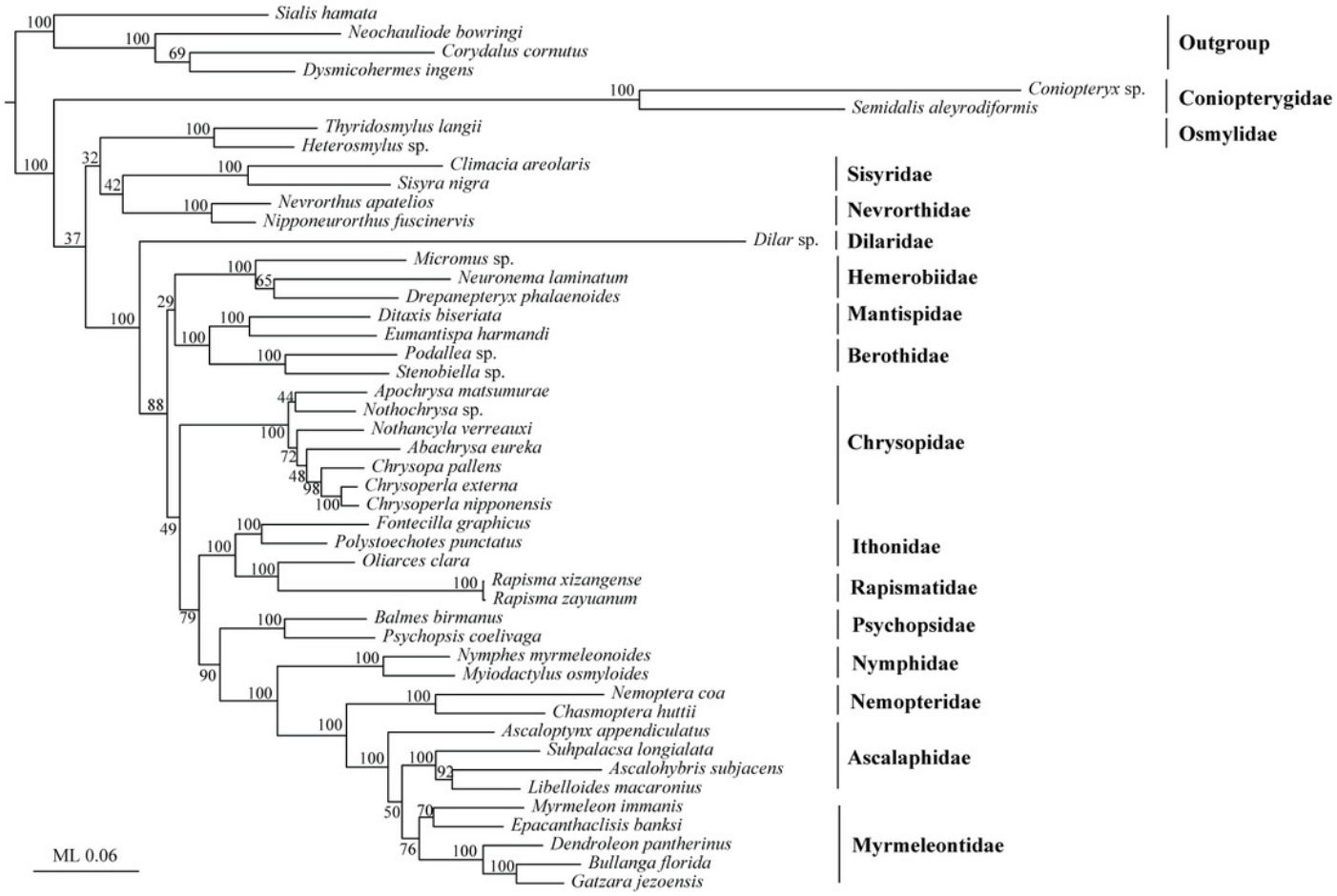


Figure 6

Figure 6 Phylogenetic relationships of Neuroptera in BI analysis.

Figure 6 Phylogenetic relationships of Neuroptera in BI analysis.

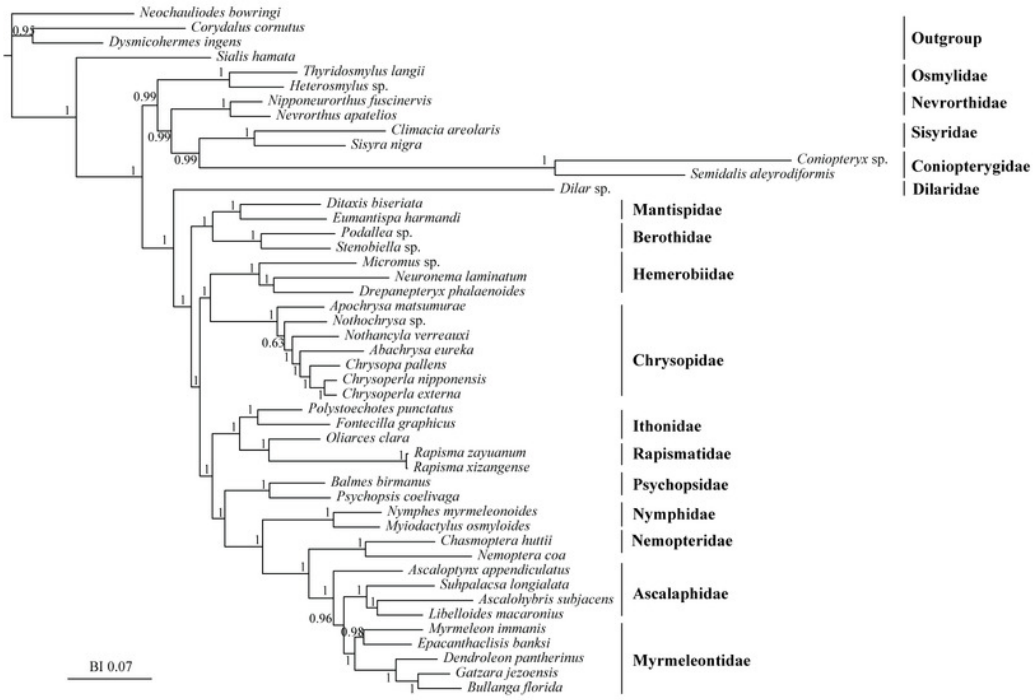


Figure 7

Figure 7 Phylogenetic relationships of Neuroptera in ML analysis based on the elimination of three species (*Semidalis aleyrodiformis*, *Coniopteryx* sp., *Dilar* sp.).

Figure 7 Phylogenetic relationships of Neuroptera in ML analysis based on the elimination of three species (*Semidalis aleyrodiformis*, *Coniopteryx* sp., *Dilar* sp.).

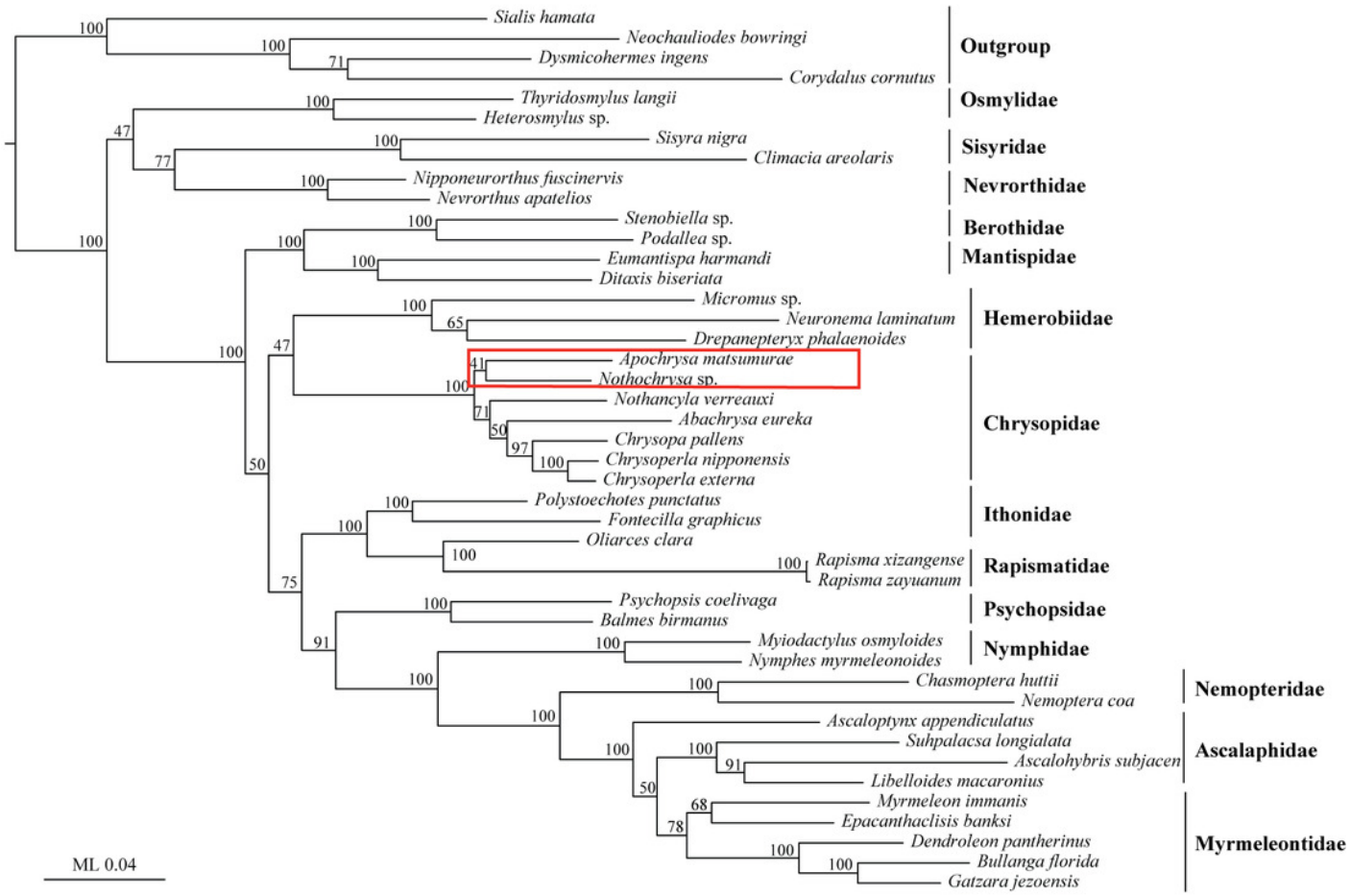


Figure 8

Figure 8 Phylogenetic relationships of Neuroptera in BI analysis based on the elimination of three species (*Semidalis aleyrodiformis*, *Coniopteryx* sp., *Dilar* sp.).

Figure 8 Phylogenetic relationships of Neuroptera in BI analysis based on the elimination of three species (*Semidalis aleyrodiformis*, *Coniopteryx* sp., *Dilar* sp.).

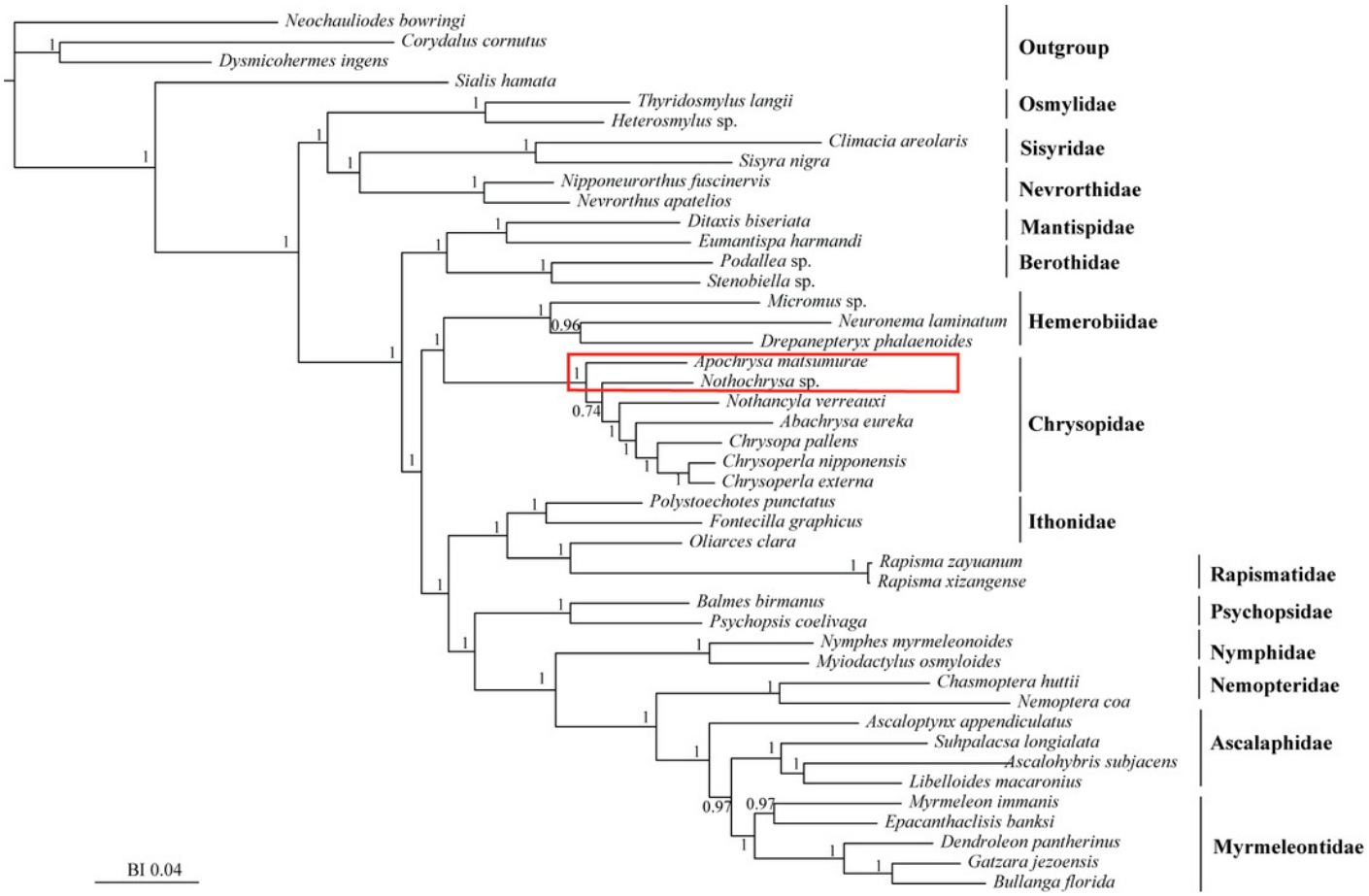


Table 1(on next page)

Table 1 Universal primers used to amplify the mitochondrial genome of *S.longialata.*

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1 **Table 1 Universal primers used to amplify the mitochondrial genome of *S. longialata***

Number	Primer name	Sequence (5'-3')
1	FY-J-210	AAGCTMNTRGGYTCATACCC
	FY-N-1284	RCTTTGAAGGYTAWTWGTTT
2	FY-J-1423	ACDAAYCAYAARGAYATYGG
	FY-N-2329	ACDGTRAAYATRTGRTGNGCYCA
3	FY-J-2198	TATTHTGATTYTTYGGNCAYCCHGAAGT
	FY-N-3705	GCYCCRCARATTTCNGAACATTG
4	FY-J-4463	TTYGCHCAYYTDGTNCCNCARGG
	FY-N-5748	GGRTCRAANCCRCAYTCRAANGG
5	FY-J-5747	CCATTYGAATGTGGATTTGAYCC
	FY-N-6160	YCAATTMTATCATTAACAGTGA
6	FY-J-7077	AARTCCTTWGARTAAAAKCC
	FY-N-7793	TTRGGTWGRGATGGDTTRGG
7	FY-J-7572	AAANGGRATYTGNGCDCTYTTHGT
	FY-N-8741	AYTTCRATNGYTTGHCCHT
8	FY-J-8641	CNGAHGAACAHARNCCRTG
	FY-N-9629	GTHTGYGARGGAGCWYTKGG
9	FY-J-10885	AYGTYCTRCCYTGRGGWCARATRTC
	FY-N-12964	TTACCTTARGGATAACAGCRTAW
10	FY-J-11335	CAYATYCARCCHGARTGRTA
	FY-N-12965	TTACCTTAGGGATAACAGCRTWA
11	FY-J-12831	CGGTYTGAACTCAGATCATGTA
	FY-N-13889	KTACCTTKTGTATCAGGGTT

12	FY-J-13286	CTTTGCACRGTCAVWATACYGC
	FY-14722	GTGCCAGCVDCCGCGGTTANA

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Table 2(on next page)

Table 2 Species used to construct the phylogenetic relationships along with GenBank accession numbers.

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1 **Table 2 Species used to construct the phylogenetic relationships along with GenBank accession numbers.**

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

Order	Family	Species	GenBank accession number
Megaloptera	Corydalidae	*Corydalus cornutus*	FJ171323
		Dysmicohermes ingens	KJ806318
		Neochauliodes bowringi	JQ351950
	Sialidae	*Sialis hamata*	FJ859905

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Table 3(on next page)

Table 3 Base composition of Ascalaphidae mitochondrial genomes.

Table 3 Base composition of Ascalaphidae mitochondrial genomes.

1 **Table 3 Base composition of Ascalaphidae mitochondrial genomes.**

Region	*S. longialata*				*L. macaronius*				*A. appendiculatus*				*A. subjacens*			
	Length (bp)	AT%	AT-skew	GC-skew	Length (bp)	AT%	AT-skew	GC-skew	Length (bp)	AT%	AT-skew	GC-skew	Length (bp)	AT%	AT-skew	GC-skew
Whole genome	15911	74.8	0.096	-0.230	15890	74.5	0.071	-0.176	15877	75.5	0.068	-0.205	15873	75.7	0.054	-0.177
Protein-coding genes	11169	73.0	0.090	-0.234	11181	73.1	0.078	-0.182	11169	74.0	0.059	-0.338	11183	74.1	0.050	-0.169
Ribosomal RNA genes	2053	77.8	0.159	-0.297	2095	76.4	0.094	-0.241	2078	78.6	0.125	-0.280	2094	77.8	0.108	-0.270
Transfer RNA genes	1476	76.2	0.055	-0.122	1471	75.6	0.037	-0.115	1464	75.5	0.057	-0.135	1466	77.7	0.037	-0.135
A+T-rich region	1088	85.1	0.086	-0.168	1049	84.5	0.030	0.006	1066	85.7	0.048	-0.077	1051	86.2	0.035	-0.014

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Table 4(on next page)

Table 4 The codon number and relative synonymous codon usage in *S.longialata* mitochondrial protein coding genes.

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1 **Table 4 The codon number and relative synonymous codon usage in *S. longialata* mitochondrial protein coding genes.**

Codon	Count				RSCU				Codon	Count				RSCU			
	SL	LM	AA	AS	SL	LM	AA	AS		SL	LM	AA	AS	SL	LM	AA	AS
UUU(F)	264	261	279	271	1.59	1.58	1.67	1.62	UAU(Y)	150	141	151	167	1.62	1.58	1.59	1.72
UUC(F)	69	69	55	64	0.41	0.42	0.33	0.38	UAC(Y)	35	38	39	27	0.38	0.42	0.41	0.28
UUA(L1)	358	358	344	403	3.73	3.76	3.66	4.18	CAU(H)	56	55	58	61	1.44	1.45	1.55	1.56
UUG(L1)	71	42	62	39	0.74	0.44	0.66	0.4	CAC(H)	22	21	17	17	0.56	0.55	0.45	0.44
CUU(L2)	49	66	71	66	0.51	0.69	0.76	0.68	CAA(Q)	62	66	66	58	1.8	1.76	1.86	1.68
CUC(L2)	7	9	6	3	0.07	0.09	0.06	0.03	CAG(Q)	7	9	5	11	0.2	0.24	0.14	0.32
CUA(L2)	88	90	76	65	0.92	0.95	0.81	0.67	AAU(N)	147	153	155	156	1.58	1.69	1.68	1.71
CUG(L2)	3	6	5	3	0.03	0.06	0.05	0.03	AAC(N)	39	28	29	26	0.42	0.31	0.32	0.29
AUU(I)	296	309	339	307	1.7	1.73	1.81	1.69	AAA(K)	56	65	69	68	1.38	1.44	1.66	1.68
AUC(I)	52	49	36	56	0.3	0.27	0.19	0.31	AAG(K)	25	25	14	13	0.62	0.56	0.34	0.32
AUA(M)	212	225	208	233	1.69	1.76	1.79	1.81	GAU(D)	63	60	57	64	1.73	1.67	1.68	1.75
AUG(M)	39	31	24	25	0.31	0.24	0.21	0.19	GAC(D)	10	12	11	9	0.27	0.33	0.32	0.25
GUU(V)	102	89	105	83	2.05	1.77	2.14	1.78	GAA(E)	66	72	71	73	1.71	1.76	1.73	1.83
GUC(V)	5	6	2	4	0.1	0.12	0.04	0.09	GAG(E)	11	10	11	7	0.29	0.24	0.27	0.18
GUA(V)	82	95	85	90	1.65	1.89	1.73	1.94	UGU(C)	33	36	32	37	1.69	1.67	1.78	1.76
GUG(V)	10	11	4	9	0.2	0.22	0.08	0.19	UGC(C)	6	7	4	5	0.31	0.33	0.22	0.24
UCU(S2)	83	67	81	77	2.04	1.64	1.92	1.82	UGA(W)	82	83	89	87	1.71	1.66	1.76	1.78
UCC(S2)	18	13	8	17	0.44	0.32	0.19	0.4	UGG(W)	14	17	12	11	0.29	0.34	0.24	0.22
UCA(S2)	110	122	129	113	2.7	2.98	3.06	2.67	CGU(R)	27	20	28	21	1.86	1.48	1.96	1.5
UCG(S2)	0	4	1	3	0	0.10	0.02	0.07	CGC(R)	1	0	2	0	0.07	0	0.14	0
CCU(P)	67	52	63	59	2.02	1.59	1.94	1.79	CGA(R)	27	30	27	28	1.86	2.22	1.89	2
CCC(P)	14	13	11	16	0.42	0.40	0.34	0.48	CGG(R)	3	4	0	7	0.21	0.30	0	0.5
CCA(P)	51	63	55	55	1.53	1.92	1.69	1.67	AGU(S1)	44	32	40	42	1.08	0.78	0.95	0.99
CCG(P)	1	3	1	2	0.03	0.09	0.03	0.06	AGC(S1)	3	4	4	5	0.07	0.10	0.09	0.12
ACU(T)	85	73	91	70	1.62	1.47	1.75	1.45	AGA(S1)	68	85	73	80	1.67	2.08	1.73	1.89
ACC(T)	16	21	10	21	0.3	0.42	0.19	0.44	AGG(S1)	0	0	1	2	0	0	0.02	0.05
ACA(T)	107	101	104	96	2.04	2.04	2.00	1.99	GGU(G)	91	79	86	62	1.65	1.50	1.62	1.2
ACG(T)	2	3	3	6	0.04	0.06	0.06	0.12	GGC(G)	3	4	1	3	0.05	0.08	0.02	0.06
GCU(A)	95	78	78	78	2.17	1.72	1.77	1.82	GGA(G)	101	102	109	106	1.84	1.93	2.06	2.05
GCC(A)	12	21	21	22	0.27	0.46	0.48	0.51	GGG(G)	25	26	16	36	0.45	0.49	0.3	0.7
GCA(A)	68	79	72	69	1.55	1.75	1.64	1.61									

GCG(A)	0	3	5	2	0	0.07	0.11	0.05

Notes. SL, *S. longialata* (MH361300); LM, *L. macaronius*; AA, *A. appendiculatus*; AS, *A. subjacens*