

# The complete mitochondrial genome of *Aleuropteryx sinica* (Neuroptera: Coniopterygidae) and its phylogenetic analysis

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**Abstract:** *Aleuropteryx sinica* Liu & Yang, 2003 is the only species of *Aleuropteryx* in the family Coniopterygidae found in China. In this study, we used high-throughput sequencing methods to assemble the complete mitochondrial genome of *A. sinica*. The results showed that the complete mitogenome is 15,600 bp in length and had a high AT content (76.30%), with a typical set of 37 genes, including 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), 2 ribosomal RNA genes (rRNAs), and 1 control region (CR). Based on the sequences of 13PCGs+2rRNAs, the phylogenetic analysis revealed that *A. sinica*, *Coniopteryx* sp., *Conwentzia sinica* Yang, 1974, *Semidalis macleodi* Meinander, 1972 and *Semidalis aleyrodiformis* Stephens, 1836 form a clade, which is a sister group to other families of Neuroptera.

**Key words:** Aleuropteryinae; molecular data; taxonomy

中国囊粉蛉 *Aleuropteryx sinica* 线粒体全基因组及系统发育分析（脉翅目:粉蛉科）

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**摘要:** 中国囊粉蛉 *Aleuropteryx sinica* Liu et Yang, 2003 是在中国发现的唯一一例粉蛉科 Coniopterygidae 囊粉蛉亚科 Aleuropteryinae 物种。本研究利用高通量测序技术测定中国囊粉蛉线粒体全基因组。结果显示中国囊粉蛉线粒体基因组全长 15,600bp, AT 含量为 76.30%, 呈现明显的 AT 偏斜; 包含 37 个基因 (蛋白质编码基因 (PCGs) 13 个, tRNA 基因 22 个, rRNA 基因 2 个) 和一段非编码控制区 (CR)。基于 13 个蛋白质编码基因和 2 个 rRNA 基因的线粒体基因组序列分析粉蛉科系统发育关系, 显示中国囊粉蛉 *Aleuropteryx sinica*、*Coniopteryx* sp.、中华啮粉蛉 *Conwentzia sinica* Yang, 1974、马氏重粉蛉 *Semidalis macleodi* Meinander, 1972 和广重粉蛉 *Semidalis aleyrodiformis* Stephens, 1836 形成了一个支系, 是脉翅目其它科的姐妹群。

**关键词:** 囊粉蛉亚科; 分子数据; 分类

## Introduction

*Aleuropteryx sinica* Liu & Yang, 2003 belongs to Coniopterygidae, or dusty wings, with

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571 extant species worldwide (Oswald & Machado 2018). Coniopterygidae is the smallest group in Neuroptera, with a unique morphology that makes it easy to distinguish from other families (Yang 1951; Wang & Liu 2007). The larvae often appear in habitats with trees and shrubs, feeding on scale insects, aphids, and whiteflies, so that it has great potential for application in biological pest control (Meinander 1975; Henry 1976; Oswald & Machado 2018; Chen *et al.* 2022). *Aleuropteryx* is the type of genus of Aleuropteryginae, established by Löw in 1885, and widely distributed in the Holarctic region, Africa, and the northern part of the neotropical region (Meinander 1972). However, currently only one species, *A. sinica*, has been discovered in China (Liu *et al.* 2003).

The mitogenome has the advantages of a stable gene composition, relatively conservative gene arrangement, widespread maternal inheritance, and minimal recombination (Wolstenholme 1992). However, there is limited research on the mitogenome of Coniopterygidae. In GenBank, 48 species have DNA molecular data, of which only 4 species have relatively complete mitogenome data (Song *et al.* 2019; Sayers *et al.* 2021). In this study, we report the complete mitogenome of *A. sinica* and analyze its phylogenetic relationships for the first time, providing data to support future research.

## Material and methods

### Sampling and DNA extraction

A specimen of *Aleuropteryx sinica* was collected from Panzhihua, Sichuan, China (101°43'4.88" E, 26°34'30.84" N) on April 3, 2019, and was preserved in the Insect Specimen Museum of China Agricultural University. Total DNA was extracted from the thoracic muscle tissue using the TIANamp Genomic DNA Kit (Beijing).

### Mitochondrial genome assembly and annotation

The mitogenome was sequenced using the Illumina HiSeq 2500 sequencing platform with 6× sequencing depth and 150 bp paired-end sequencing reads at Berry Genomics (Beijing, China). After removing unpaired, short and low-quality reads, high-quality reads were assembled from scratch using MEANGS v1.0 (Song *et al.* 2022). Then the assembled sequences were uploaded to NCBI for blastn (<http://blast.ncbi.nlm.nih.gov/>) to determine the sequence orientation.

The acquired mitogenome sequences were preliminarily annotated using MITOS v2.1.7 (Donath *et al.* 2019) in Galaxy online server (<https://usegalaxy.org/>) (Jalili *et al.* 2020), and the results were then compared with close relatives using MEGA v7.0.26 (Kumar *et al.* 2016) for manual correction. The obtained genome sequence data has been deposited in the NCBI database under accession number PQ230793. The tRNA secondary structure was predicted to use tRNAscan-SE v2.0 online server (<http://lowelab.ucsc.edu/tRNAscan-SE>) (Lowe & Eddy 1997). Mitochondrial genome mapping was visualized using the Proksee online server (<https://proksee.ca>) (Grant *et al.* 2023). Nucleotide bias was calculated as follows: AT skew =  $(A-T)/(A+T)$ ; GC skew =  $(G-C)/(G+C)$  (Perna & Kocher 1995).

### Phylogenetic analysis

A phylogenetic tree was constructed based on the 13PCGs+2rRNAs dataset. 15 species

of Neuroptera were selected from GenBank as the in-group, with 3 species of Megaloptera as the out-group (Table 1). Sequences of PCGs and rRNAs were aligned in MAFFT (Katoh & Standley 2013), followed by MACSE v2.03 (Ranwez *et al.* 2018) in PhyloSuite v1.2.2 (Zhang *et al.* 2020) software for optimization. Then, poorly aligned sites were eliminated using Gblocks (Talavera & Castresana 2007). Processed sequences were concatenated using PhyloSuite v1.2.2. Model analysis was performed using PartitionFinder v2.0 (Lanfear *et al.* 2017). Finally, the evolutionary tree was constructed using Bayesian inference (BI) with MrBayes v3.2.6 (Ronquist *et al.* 2012).

**Table 1. Species involved in the phylogenetic analysis**

Order	Family	Species	GenBank Accession Number	References
Megaloptera	Sialidae	<i>Sialis hamata</i>	FJ859905	Cameron <i>et al.</i> (2010)
	Corydalidae	<i>Orohermes crepusculus</i>	MW642298	Jiang <i>et al.</i> (2022)
Neuroptera	Coniopterygidae	<i>Neochauliodes bowringi</i>	JQ351950	Unpublished
		<i>Aleuropteryx sinica</i>	PQ230793	This study
	Sisyridae	<i>Coniopteryx</i> sp.	KT425078	Unpublished
		<i>Conwentzia sinica</i>	MN200022	Song <i>et al.</i> (2019)
		<i>Semidalis macleodi</i>	MN506226	Unpublished
		<i>Semidalis aleyrodiformis</i>	KT425067	Unpublished
		<i>Sisyra aurorae</i>	MZ159968	Unpublished
	Nevrorthidae	<i>Climacia areolaris</i>	KT425088	Unpublished
		<i>Nipponeurorthus fuscinervis</i>	KT425076	Unpublished
	Osmylidae	<i>Nevrorthus apatelios</i>	KT425074	Unpublished
		<i>Osmylus fulvicephalus</i>	MN818867	Xu <i>et al.</i> (2020)
		<i>Thyridosmylus langii</i>	KC515397	Zhao <i>et al.</i> (2013)
		<i>Thaumatomylus hainanus</i>	MK408756	Xu <i>et al.</i> (2019)
	Hemerobiidae	<i>Hemerobius spodipennis</i>	MT268963	Zhao <i>et al.</i> (2020)
		<i>Hemerobius japonicus</i>	MN852445	Zhao <i>et al.</i> (2020)
Chrysopidae	<i>Chrysoperla externa</i>	KU877169	Unpublished	
	<i>Chrysopa pallens</i>	JX033119	He <i>et al.</i> (2012)	

## Results

The complete mitochondrial genome of *A. sinica* was 15,600 bp in length (Fig. 1), including 13 PCGs, 22 tRNAs, and 2 rRNAs. 23 genes (9 PCGs and 14 tRNAs) were encoded on the majority strand (J-strand) while the remaining 4 PCGs, 8 tRNAs, and 2 rRNAs were on the minority strand (N-strand). The overall composition of the mitogenome was 38.8% A, 37.5% T, 14.9% C, and 8.8% G, with a strong bias towards AT at 76.3%. It shows a positive AT-skew (0.017) and a negative GC-skew (-0.257). In contrast to *Drosophila yakuba*, *tRNA<sup>Cys</sup>* has rearranged from the rear of *tRNA<sup>Trp</sup>* to its front (trnC-trnW-trnY). The total length of the

13 PCGs was 11,131 bp, and all PCGs used ATN as the initiation codon, of which seven genes (*ND1*, *ND2*, *ND3*, *ND5*, *ND6*, *ATP8* and *COI*) started with ATT, five genes (*ND4*, *ND4L*, *ATP6*, *COIII* and *CytB*) started with ATG and *COI* started with ATC. Most PCGs terminated with TAA as their stop codon, except *ND5* ended with a single T. The lengths of the 22 tRNA genes ranged from 59 to 69 bp, with 14 located on the J-strand and the rest on the N-strand. *lrRNA* and *srRNA* were 1297 bp and 750 bp long, respectively, separated by *tRNA<sup>Val</sup>* (Table 2).

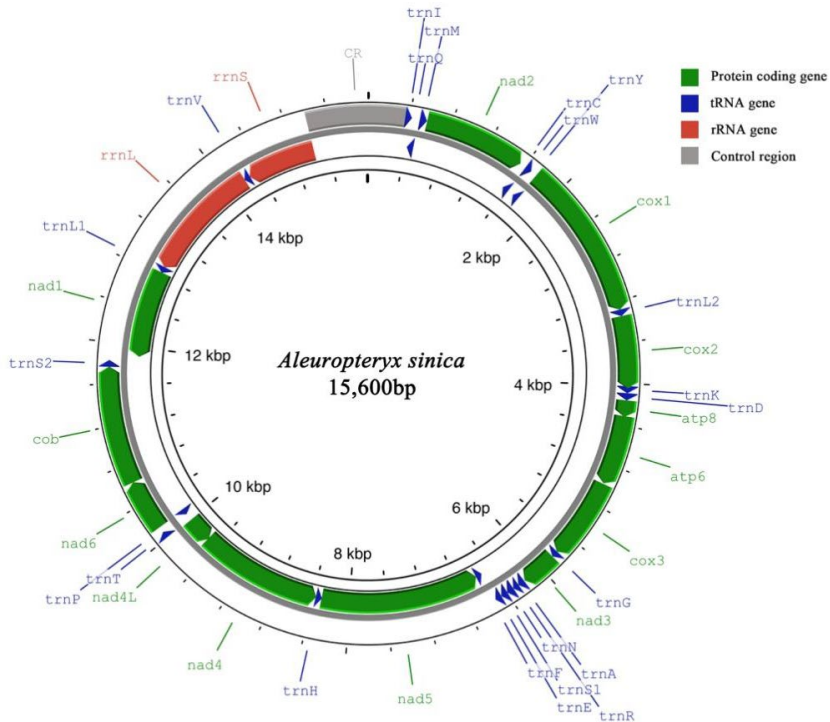


Figure 1. The mitogenome map of *Aleuropteryx sinica*, drawn by Proksee (<https://proksee.ca/>) (Grant *et al.* 2023).

**Table 2. The annotation of the mitochondrial genome of *Aleuropteryx sinica***

Genes	Strand	Location	Size	Anticodon	Start codon	Stop codon	Intergenic nucleotides
<i>tRNA<sup>Ile</sup></i>	J	355-418	64	383-385 GAT			
<i>tRNA<sup>Gln</sup></i>	N	416-484	69	452-454 TTG			-3
<i>tRNA<sup>Met</sup></i>	J	496-564	69	526-528 CAT			11
<i>ND2</i>	J	568-1554	987		ATT	TAA	3
<i>tRNA<sup>Cys</sup></i>	N	1553-1616	64	1582-1584 GCA			-2
<i>tRNA<sup>Trp</sup></i>	J	1617-1682	66	1647-1649 TCA			0
<i>tRNA<sup>Tyr</sup></i>	N	1689-1751	63	1719-1721 GTA			6
<i>COI</i>	J	1744-3282	1539		ATC	TAA	-8

Table 2 (continued )

Genes	Strand	Location	Size	Anticodon	Start codon	Stop codon	Intergenic nucleotides
<i>tRNA<sup>Leu(UUR)</sup></i>	J	3283-3346	64	3312-3314 TAA			0
<i>COII</i>	J	3347-4042	696		ATT	TAA	0
<i>tRNA<sup>Lys</sup></i>	J	4023-4090	68	4053-4055 CTT			-20
<i>tRNA<sup>Asp</sup></i>	J	4091-4159	69	4124-4126 GTC			0
<i>ATP8</i>	J	4160-4318	159		ATT	TAA	0
<i>ATP6</i>	J	4312-4986	675		ATG	TAA	-7
<i>COIII</i>	J	4994-5782	789		ATG	TAA	7
<i>tRNA<sup>Gly</sup></i>	J	5783-5845	63	5812-5814 TCC			0
<i>ND3</i>	J	5847-6197	351		ATT	TAA	1
<i>tRNA<sup>Ala</sup></i>	J	6206-6271	66	6235-6237 TGC			8
<i>tRNA<sup>Arg</sup></i>	J	6272-6330	59	6297-6299 TCG			0
<i>tRNA<sup>Asn</sup></i>	J	6332-6400	69	6363-6365 GTT			1
<i>tRNA<sup>Ser(AGN)</sup></i>	J	6401-6466	66	6426-6428 GCT			0
<i>tRNA<sup>Glu</sup></i>	J	6468-6531	64	6498-6500 TTC			1
<i>tRNA<sup>Phe</sup></i>	N	6529-6594	66	6557-6559 GAA			-3
<i>ND5</i>	N	6595-8311	1717		ATT	T-	0
<i>tRNA<sup>His</sup></i>	N	8312-8372	61	8341-8343 GTG			0
<i>ND4</i>	N	8372-9712	1341		ATG	TAA	-1
<i>ND4L</i>	N	9706-9987	282		ATG	TAA	-7
<i>tRNA<sup>Thr</sup></i>	J	10005-10068	64	10036-10038 TGT			17
<i>tRNA<sup>Pro</sup></i>	N	10069-10131	63	10100-10102 TGG			0
<i>ND6</i>	J	10125-10625	501		ATT	TAA	-7
<i>CytB</i>	J	10625-11767	1143		ATG	TAA	-1
<i>tRNA<sup>Ser(UCN)</sup></i>	J	11766-11830	65	11797-11799 TGA			-2
<i>ND1</i>	N	11883-12830	948		ATT	TAA	52
<i>tRNA<sup>Leu(CUN)</sup></i>	N	12831-12893	63	12862-12864 TAG			0
<i>lrRNA</i>	N	12894-14190	1297				0
<i>tRNA<sup>Val</sup></i>	N	14191-14254	64	14221-14223 TAC			0
<i>srRNA</i>	N	14255-15004	750				0

J: majority strand; N: minority strand

Negative intergenic nucleotides indicate overlapping of adjacent genes.

The relative synonymous codon usage (RSCU) values for the mitochondrial genome of *A. sinica* are presented in Table 3. There are 64 different codons, of which codon TTA (Leu2) has the highest RSCU value of 3.98, while CUG (Leu1) and CCG (Pro) have the lowest RSCU values, both of which are 0.07. The most commonly used codons in descending order are ATT (Ile), TTA (Leu2), TTT (Phe), AAT (Asn), ATA (Met) and TAT (Tyr).

**Table 3. The relative synonymous codon usage (RSCU) in the mitochondrial genome of *Aleuropteryx sinica***

Amino acid	Codon	Count	RSCU	Amino acid	Codon	Count	RSCU
Phe	UUU (F)	321	1.65	Tyr	UAU (Y)	154	1.66
	UUC (F)	69	0.35		UAC (Y)	31	0.34
Leu2	UUA (L)	332	<u>3.98</u>		UAA (*)	36	1.31
	UUG (L)	42	0.5		UAG (*)	19	0.69
Leu1	CUU (L)	52	0.62	His	CAU (H)	56	1.6
	CUC (L)	14	0.17		CAC (H)	14	0.4
	CUA (L)	54	0.65	Gln	CAA (Q)	53	1.83
	CUG (L)	6	<u>0.07</u>		CAG (Q)	5	0.17
Ile	AUU (I)	339	1.65	Asn	AAU (N)	189	1.63
	AUC (I)	71	0.35		AAC (N)	43	0.37
Met	AUA (M)	186	1.71	Lys	AAA (K)	90	1.49
	AUG (M)	31	0.29		AAG (K)	31	0.51
Val	GUU (V)	73	1.7	Asp	GAU (D)	50	1.56
	GUC (V)	14	0.33		GAC (D)	14	0.44
	GUA (V)	71	1.65	Glu	GAA (E)	57	1.75
	GUG (V)	14	0.33		GAG (E)	8	0.25
Ser2	UCU (S)	93	1.99	Cys	UGU (C)	28	1.27
	UCC (S)	29	0.62		UGC (C)	16	0.73
	UCA (S)	65	1.39	Trp	UGA (W)	88	1.61
	UCG (S)	5	0.11		UGG (W)	21	0.39
Pro	CCU (P)	62	2.21	Arg	CGU (R)	12	1.09
	CCC (P)	24	0.86		CGC (R)	5	0.45
	CCA (P)	24	0.86		CGA (R)	23	2.09
	CCG (P)	2	<u>0.07</u>		CGG (R)	4	0.36
Thr	ACU (T)	93	1.93	Ser1	AGU (S)	57	1.22
	ACC (T)	28	0.58		AGC (S)	25	0.54
	ACA (T)	59	1.22		AGA (S)	75	1.61
	ACG (T)	13	0.27		AGG (S)	24	0.51
Ala	GCU (A)	56	1.98	Gly	GGU (G)	38	0.83
	GCC (A)	21	0.74		GGC (G)	13	0.28
	GCA (A)	32	1.13		GGA (G)	86	1.88
	GCG (A)	4	0.14		GGG (G)	46	1.01

Twenty-two tRNA genes were measured with lengths ranging from 59 to 69 bp. The tRNA secondary structures are predicted in Fig. 2. Isoacceptors are found on *tRNA<sup>Ser</sup>* and *tRNA<sup>Leu</sup>*. Except for *tRNA<sup>SI</sup>*, which lacked the Dihydrouridine (DHU) arm, the remaining 21 tRNA genes have typical cloverleaf structures.

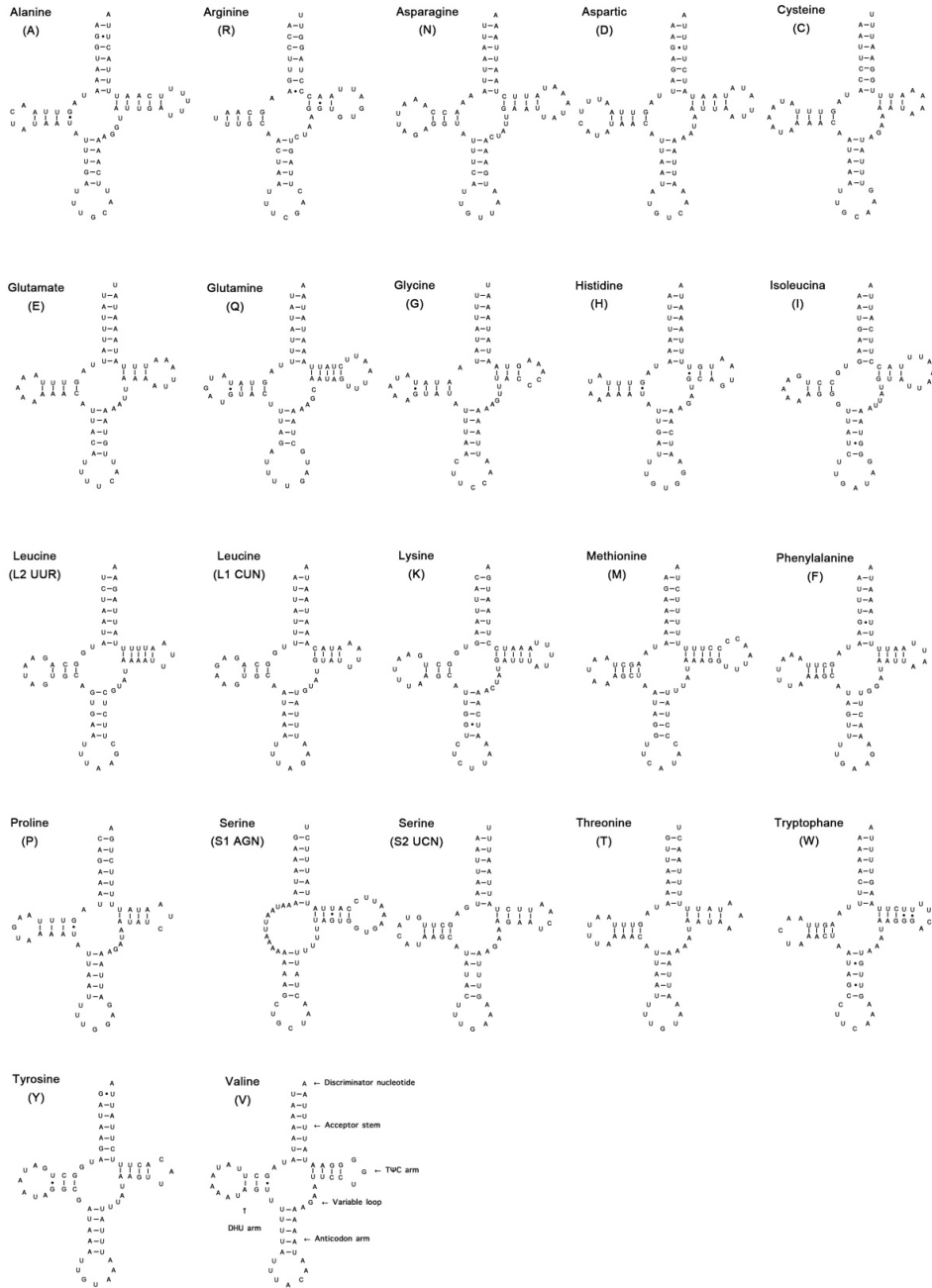


Figure 2. Predicted secondary structure of tRNA genes in the mitochondrial genome of *Aleuropteryx sinica*.

Phylogenetic analysis reveals that the six families of Neuroptera, including Coniopterygidae, Sisyridae, Nevrorthidae, Osmylidae, Hemerobiidae, and Chrysopidae, are monophyletic groups, suggesting that Coniopterygidae is a sister group of other families of Neuroptera. Within Coniopterygidae, *A. sinica*, belonging to Aleuropteryginae, is located at the root of the tree, while *Coniopteryx* sp., *Conwentzia sinica*, *Semidalis macleodi*, and *Semidalis aleyroformis*, belonging to Coniopteryginae, are grouped together (Fig. 3).

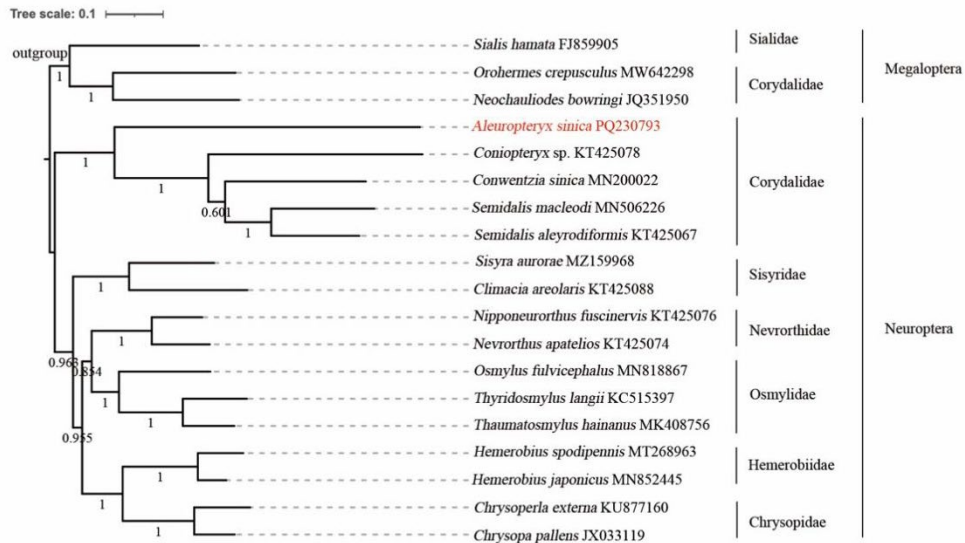


Figure 3. Phylogenetic tree of *Aleuropteryx sinica* and 18 related insect species constructed by using the Bayesian inference (BI) method, based on the 13PCGs+2rRNAs dataset.

## Discussion and conclusion

We sequenced and reported the first complete mitochondrial genome of *A. sinica*. Wang (2012) and Zhao *et al.* (2013) found a gene rearrangement in some taxa in Neuroptera. Wang *et al.* (2017) concluded that the rearrangement did not occur in the primitive taxa, including Coniopterygidae, Nevrorthidae, Sisyridae, and Osmylidae. However, in our study, a gene rearrangement occurred in *A. sinica*, suggesting that gene rearrangements are not systematically significant in Neuroptera.

The monophyly of Coniopteryginae of Coniopterygidae is further confirmed. Based on 16S rRNA, Wang and Liu (2007) showed that *Semialis* and *Coniopteryx* were sister groups, and *Conwentzia* was more primitive than the former two. However, based on the morphological characteristics of the male external genitalia, *Semialis* and *Conwentzia* are sister groups (Meinander 1972). Our results are consistent with the morphology-based conclusions.

At present, the molecular data of Coniopterygidae is limited, so it is difficult to clearly present the systematic relationships within it. In this study, we determined the first complete mitochondrial genome of Aleuropteryginae, which lays the foundation for subsequent research on the phylogeny and evolution of Neuroptera.

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The genome sequence data supporting this study's findings are available in GenBank of NCBI at (<https://www.ncbi.nlm.nih.gov>) under assessment PQ230793.

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