

Comparative analysis of the complete mitochondrial genomes of three representatives in Ricaniidae (Hemiptera: Fulgoroidea), with phylogenetic implications

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Abstract: In this paper, the mitogenomes of three species in Ricaniidae, *Ricanoides flabellum* Noulhier, *Ricanula fujianensis* Ren, Stroiński & Qin and *Euricania clara* Kato, were sequenced and assembled. Their total lengths were 15,906, 15,809 and 16,127 bp, respectively. The whole mitogenomes showed a positive AT skew and negative GC skew. The A+T content of the rRNAs was slightly higher than that of the tRNAs. All protein-coding genes (PCGs) begin with the start codon ATN and end with TAA, TAG or an incomplete stop codon single T. The sliding window, Ka/Ks, and genetic distance analyses indicated that the *cox1* gene was the slowest evolving. The *cox1* gene was determined to be the most conserved and exhibited a slow evolution rate compared to other PCGs. It can be used as one of the genetic markers for phylogenetic analysis of the higher taxa of Ricaniidae. The phylogenetic relationships of 5 genera and 11 species in this family were also analyzed based on available mitochondrial genome data.

Key words: Auchenorrhyncha; taxonomy; phylogeny

广蜡蝉科三个代表种的线粒体基因组结构比较及其系统发育含义（半翅目：蜡蝉总科）

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摘要: 对广蜡蝉科内的类广蜡蝉属、曲广蜡蝉属和疏广蜡蝉属各 1 个代表种的线粒体基因组进行了测序和组装, 发现琼边类广蜡蝉, 福建曲广蜡蝉和透明疏广蜡蝉的完整线粒体基因组长度分别为 15,906, 15,809 和 16,127 bp, 整个线粒体基因组呈正 AT 偏斜和负 GC 偏斜, rRNAs 基因的 A+T 含量略高于 tRNAs; 所有蛋白编码基因 (PCGs) 均以起始密码 ATN 开头, 以 TAA、TAG 或 1 个不完整的终止密码子单 T 结尾。划窗分析、Ka/Ks 和遗传距离分析均表明, 与其他 PCGs 相比, 基因 *cox1* 进化最慢且相对保守, 可以用于广蜡蝉科高级阶元系统发育关系探讨。本文还基于现有线粒体基因组数据分析了该科 5 属 11 种的系统发育关系。

关键词: 头喙亚目; 分类; 系统发育

Introduction

Ricaniidae is a relatively large group in Fulgoroidea, and 443 species in approximately 70 genera of two subfamilies are known so far. They are widely distributed in the Oriental,

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Palaearctic, Afrotropical, Australian and Neotropical Regions, but primarily around the tropics (Bourgoin 2023). 43 species of 6 genera are known in the Chinese fauna, mainly distributed in the south of Yangtze River (Chou *et al.* 1985; Yang 1989; Xu *et al.* 2006; Zhang *et al.* 2014, 2021; Ren *et al.* 2015, 2016). A few species in this family are of great economic importance. For example, *Pochazia shantungensis* (Chou & Lu, 1977) is an important pest in China and has invaded France, Germany, Italy, South Korea, Turkey and Russia, which has brought huge losses in agroforestry production by laying eggs that may lead to shrubs rotting and withering in severe cases (Rahman *et al.* 2012; Yao *et al.* 2011; Hizal *et al.* 2019; Bourgoin *et al.* 2020; Stroiński *et al.* 2022; Zhuravleva *et al.* 2023).

The phylogenetic relationships within Ricaniidae has never been comprehensively studied to date. The diagnostic relationship between two genera, *Pochazia* Amyot & Audinet-Serville and *Ricania* Germar remains obscure. In order to provide more evidence for phylogenetic analysis and character re-definition between closely-related genera in this family, the mitochondrial genomes of three species in each of the three genera, *Ricanoides* Zia, *Ricanula* Melichar and *Euricania* Melichar were sequenced and assembled here. This brings the mitochondrial genome information known up to 11 species in this family (Song *et al.* 2012; Kwon *et al.* 2017; Kang *et al.* 2020; Lee *et al.* 2020; Zhang *et al.* 2022). The phylogenetic relationship based on available mitochondrial genome data in this family was also analyzed.

Material and methods

Eleven ricaniid species were selected as ingroups, of which three were newly-sequenced in this study: *Ricania flabellum* Noulhier (Fig. 1A) collected in Qiandongnan, Guizhou (GenBank accession no. OR529197); *Ricanula fujianensis* Ren, Stroiński & Qin (Fig. 1B) collected in Nanchang, Jiangxi (GenBank accession no. OR529196) and *Euricania clara* Kato (Fig. 1C) collected in Xianyang, Shaanxi (GenBank accession no. OR620182). Three species including 1 in Cixiidae and 2 in Delphacidae were selected as outgroups (GenBank accession numbers in Table 1). Adult specimens of these three ricaniid species were preserved in 100% ethanol at -20°C to allow DNA extraction. All specimens were identified by the first author before DNA extraction. The genomic DNA was extracted using the DNeasy DNA Extraction Kit (Qiagen).



Figure 1. Photos of three Ricaniidae specimens. A. *Ricania flabellum* Noulhier; B. *Ricanula fujianensis* Ren, Stroiński & Qin; C. *Euricania clara* Kato.

The whole genomic DNA for each of the three ricaniid species was sequenced once by the next-generation sequence method on the Illumina NovaSeq platform. Sanger sequencing was performed on the COI genes of the three ricaniid species and compared with the second-generation sequencing results. The quality trimming and assembly of the paired reads

were checked by Geneious 11.0.2 with default parameters (Kearse *et al.* 2012). The annotation of genomic features was conducted using Geneious 11.0.2, with *Ricania speculum* and *Pochazia shantungensis* as references. The open reading frames (ORFs) was created based on the invertebrate mitochondrial genetic codes. The mitogenomic maps of these three species were visualized using the CGview Server (Grant & Stothard 2008).

Base composition and RSCU values (relative synonymous codon usage) were analyzed using PhyloSuite 1.2.2 (Zhang *et al.* 2020). The Tandem Repeats Finder Online Server was employed to obtain the tandem repeats in the control region (Benson 1999). The sliding window analysis was performed with DnaSP 6.0 based on concatenated alignments of PCGs and rRNA genes among eleven Ricaniidae mitogenomes (Rozas *et al.* 2017). The non-synonymous (Ka)/synonymous (Ks) substitution rates and genetic distances were estimated via DnaSP 6.0 and MEGA-X based on 13 PCGs of the eleven Ricaniidae mitogenomes, respectively (Rozas *et al.* 2017; Kumar *et al.* 2018).

The phylogenetic analyses were reconstructed based on PCGR by Bayesian Inference (BI) and Maximum Likelihood (ML) methods. ML analysis was conducted in IQ-TREE 1.6.5 (Nguyen *et al.* 2015) using 1000 replicates of ultrafast bootstraps. BI analysis was performed using MrBayes 3.2.6 (Ronquist *et al.* 2012), each run for 10,000,000 generations with sampling every 100 generations. A consensus tree was calculated from the remaining samples after discarding the first 25% of the trees.

Table 1. Taxa used in this study

Taxon	Species	GenBank number
Outgroups		
Cixiidae	Cixiidae sp.	OR062441
Delphacidae	<i>Ishiharodelphax matsuyamensis</i> (Ishihara)	NC_052693
	<i>Bambusiphaga maculata</i> Chen & Li	MH293455
Ingroups		
Ricaniidae	<i>Pochazia confusa</i> Distant	NC_060807
	<i>Pochazia discreta</i> Melichar	NC_060730
	<i>Pochazia guttifera</i> Walker	NC_060806
	<i>Pochazia shantungensis</i> (Chou & Lu)	MW036196
	<i>Ricania speculum</i> (Walker)	MT834932
	<i>Ricania fumosa</i> (Walker)	NC_060809
	<i>Ricania marginalis</i> (Walker)	NC_019597
	<i>Ricania simulans</i> (Walker)	NC_060808
	<i>Ricania flabellum</i> Noulhier	OR529197
	<i>Ricanula fujianensis</i> Ren, Stroiński & Qin	OR529196
	<i>Euricania clara</i> Kato	OR620182

Results

The complete circular mitogenomes of *Ricanoides flabellum*, *Ricanula fujianensis* and *Euricania clara* were 15,906, 15,809 and 16,127 bp in length, respectively (Fig. 2). The three newly-sequenced mitogenomes were consistent with the gene arrangement of other

planthopper mitogenomes, comprised of the typical 37 genes: 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs), and an A+T-rich region (control region). The total length of the complete mitogenomes is mainly due to the variation in the length of the control region (Tables 2, 3).

Table 2. Mitogenomic organization of mitogenomes of *Ricanoides flabellum*, *Ricanula fujianensis* and *Euricania clara*

Gene	Position			Position			Position		Size	Strand
	From	To	Size	From	To	Size	From	To		
	<i>Ricanoides flabellum</i>			<i>Ricanula fujianensis</i>			<i>Euricania clara</i>			
<i>trnI</i>	1	63	63	1	64	64	1	67	67	J
<i>trnQ</i>	65	133	69	64	132	69	71	139	69	N
<i>trnM</i>	133	197	65	133	196	64	159	221	63	J
<i>nad2</i>	198	1163	966	197	1162	966	222	1187	966	J
<i>trnW</i>	1162	1226	65	1168	1230	63	1215	1278	64	J
<i>trnC</i>	1219	1282	64	1223	1285	63	1271	1333	63	N
<i>trnY</i>	1286	1347	62	1289	1350	62	1346	1407	62	N
<i>cox1</i>	1352	2887	1536	1354	2889	1536	1415	2950	1536	J
<i>trnL</i>	2888	2950	63	2891	2953	63	2951	3013	63	J
<i>cox2</i>	2951	3623	673	2954	3626	673	3014	3683	670	J
<i>trnK</i>	3624	3692	69	3627	3696	70	3684	3752	69	J
<i>trnD</i>	3693	3757	65	3697	3763	67	3755	3828	74	J
<i>atp8</i>	3758	3910	153	3764	3919	156	3829	3987	159	J
<i>atp6</i>	3907	4555	649	3916	4564	649	3984	4667	684	J
<i>cox3</i>	4556	5338	783	4565	5347	783	4633	5415	783	J
<i>trnG</i>	5343	5403	61	5354	5414	61	5416	5477	62	J
<i>nad3</i>	5404	5751	348	5415	5762	348	5478	5825	348	J
<i>trnA</i>	5751	5815	65	5763	5829	67	5834	5898	65	J
<i>trnR</i>	5825	5884	60	5834	5897	64	5905	5964	60	J
<i>trnN</i>	5884	5946	63	5901	5965	65	5968	6032	65	J
<i>trnS</i>	5946	6005	60	5966	6023	58	6032	6091	60	J
<i>trnE</i>	6006	6070	65	6025	6088	64	6095	6157	63	J
<i>trnF</i>	6069	6133	65	6093	6155	63	6165	6232	68	N
<i>nad5</i>	6133	7797	1665	6156	7818	1663	6233	7897	1665	N
<i>trnH</i>	7814	7874	61	7835	7897	63	7914	7976	63	N
<i>nad4</i>	7876	9181	1306	7899	9225	1327	7978	9301	1324	N
<i>nad4L</i>	9194	9466	273	9219	9491	273	9295	9567	273	N
<i>trnT</i>	9469	9532	64	9499	9564	66	9570	9632	63	J
<i>trnP</i>	9538	9603	66	9568	9632	65	9646	9708	63	N
<i>nad6</i>	9605	10099	495	9634	10128	495	9710	10204	495	J
<i>cytb</i>	10092	11213	1122	10121	11245	1125	10197	11330	1134	J
<i>trnS</i>	11212	11276	65	11248	11312	65	11338	11403	66	J
<i>nad1</i>	11283	12207	925	11319	12243	925	11410	12343	934	N

Continued Table 2.

Gene	Position		Size	Position		Size	Position		Size	Strand
	From	To		From	To		From	To		
	<i>Ricanoides flabellum</i>			<i>Ricanula fujianensis</i>			<i>Euricania clara</i>			
<i>trnL</i>	12209	12272	64	12245	12308	64	12345	12410	66	N
<i>rrnL</i>	12273	13479	1207	12309	13507	1199	12411	13634	1224	N
<i>trnV</i>	13480	13545	66	13508	13570	63	13635	13697	63	N
<i>rrnS</i>	13546	14268	723	13571	14295	725	13698	14422	725	N
<i>CR</i>	14269	15906	1638	14296	15809	1514	14423	16127	1705	J

Table 3. Nucleotide composition of mitogenomes of *Ricanoides flabellum*, *Ricanula fujianensis* and *Euricania clara*

Regions	Size (bp)	T(U) %	C%	A%	G%	AT (%)	GC (%)	AT skew	GC skew
<i>Ricanoides flabellum</i>									
PCGs	10890	42.4	12.7	33.4	11.5	75.8	24.2	-0.118	-0.047
1st codon position	3630	35.7	11.5	36	16.8	71.7	28.3	0.004	0.19
2nd codon position	3630	47.7	19	20	13.2	67.7	32.2	-0.41	-0.18
3rd codon position	3630	43.6	7.6	44.2	4.6	87.8	12.2	0.007	-0.247
Control Region	1638	36.8	7.3	49.3	6.7	86.1	14	0.146	-0.044
tRNAs	1410	35.7	10.1	40.9	13.3	76.6	23.4	0.069	0.133
rRNAs	1930	50.8	8.1	26.8	14.2	77.6	22.3	-0.309	0.276
Full genome	15906	28.3	13.7	48.9	9.1	77.2	22.8	0.267	-0.204
<i>Ricanula fujianensis</i>									
PCGs	10914	44.3	11.8	33.3	10.7	77.6	22.5	-0.142	-0.048
1st codon position	3638	37.1	11.4	35.7	15.8	72.8	27.2	-0.019	0.163
2nd codon position	3638	49.1	18.3	19.8	12.8	68.9	31.1	-0.425	-0.177
3rd codon position	3638	46.5	5.7	44.3	3.5	90.8	9.2	-0.025	-0.234
Control Region	1514	35.4	11.1	47.2	6.3	82.6	17.4	0.143	-0.278
tRNAs	1413	35.4	10.1	41.3	13.2	76.7	23.3	0.077	0.133
rRNAs	1924	50.3	7.6	28.8	13.3	79.1	20.9	-0.271	0.269
Full genome	15809	29.3	13.4	48.9	8.4	78.2	21.8	0.25	-0.231
<i>Euricania clara</i>									
PCGs	10968	43	12.4	33.1	11.6	76.1	24	-0.13	-0.035
1st codon position	3656	36.6	11.2	36	16.2	72.6	27.4	-0.009	0.181
2nd codon position	3656	48.3	18.7	20	13.1	68.3	31.8	-0.415	-0.175
3rd codon position	3656	44	7.3	43.3	5.4	87.3	12.7	-0.008	-0.15
Control Region	1705	32.7	9.8	49.3	8.2	82	18	0.202	-0.092
tRNAs	1421	35.8	10.1	41.1	13	76.9	23.1	0.069	0.128
rRNAs	1949	50	8	28.3	13.7	78.3	21.7	-0.278	0.262
Full genome	16127	28.5	13.7	48.7	9.2	77.2	22.9	0.262	-0.2

Table 4. Start and stop codons of mitogenomes of *Ricanoides flabellum*, *Ricanula fujianensis* and *Euricania clara*

Gene	Codon		Codon		Codon	
	Start	Stop	Start	Stop	Start	Stop
	<i>Ricanoides flabellum</i>		<i>Ricanula fujianensis</i>		<i>Euricania clara</i>	
<i>nad2</i>	ATT	TAA	ATT	TAA	ATT	TAA
<i>cox1</i>	ATG	TAG	ATG	TAA	ATG	TAA
<i>cox2</i>	ATA	T	ATA	T	ATA	T
<i>atp8</i>	ATT	TAA	ATT	TAA	ATA	TAA
<i>atp6</i>	ATA	T	ATA	T	ATA	TAA
<i>cox3</i>	ATG	TAA	ATG	TAA	ATG	TAA
<i>nad3</i>	ATA	TAG	ATG	TAA	ATA	TAA
<i>nad5</i>	ATG	TAG	ATG	T	ATG	TAA
<i>nad4</i>	ATA	T	ATG	T	ATG	T
<i>nad4L</i>	ATG	TAA	ATG	TAA	ATG	TAA
<i>nad6</i>	ATC	TAA	ATT	TAA	ATT	TAA
<i>cytb</i>	ATG	TAG	ATG	TAA	ATG	TAA
<i>nad1</i>	ATG	T	ATG	T	ATG	T

In these three ricaniid species, the nucleotide composition for *Ricanoides flabellum* was: A = 48.9%, C = 13.7%, G = 9.1%, and T = 28.3%; for *Ricanula fujianensis*: A = 48.9%, C = 13.4%, G = 8.4%, and T = 29.3%; and for *Euricania clara*: A = 48.7%, C = 13.7%, G = 9.2%, and T = 28.5%. These whole mitogenomes show a positive AT skew and negative GC skew. The lengths of the 13 PCGs of *Ricanoides flabellum*, *Ricanula fujianensis* and *Euricania clara* were 10,890, 10,914 and 10,968 bp, respectively. All PCGs represented a negative AT skew and GC skew. The A+T content of the third codon was the highest, while that of the second codon was the lowest (Table 3).

The tRNAs were for *Ricanoides flabellum* 1,410 bp; *Ricanula fujianensis* 1,413 bp and *Euricania clara* 1,421 bp in length. The tRNAs showed a positive AT skew and GC skew with a heavy AT nucleotide bias that reached 76.6% in *Ricanoides flabellum*; 76.7% in *Ricanula fujianensis* and for *Euricania clara* 76.9%. The three rRNAs show a negative AT skew and positive GC skew. The A+T content of the rRNAs is slightly higher than that of the tRNAs. And the A+T content of the rRNAs of *Ricanula fujianensis* (79.1%) is higher than that of *Ricanoides flabellum* (77.6%) and *Euricania clara* (78.3%) (Table 3). The control region shows a positive AT skew and a negative GC skew. The A+T content of *Ricanoides flabellum* (86.1%) is higher than that of *Ricanula fujianensis* (82.6%) and *Euricania clara* (82.0%) (Table 3). Comparing tandem repeat regions of these three ricaniid mitogenomes, the results show that one repeat region was detected in *Ricanula fujianensis* and *Euricania clara*, and two repeat regions were present in *Ricanoides flabellum*. A poly (T) was found in *Ricanoides flabellum* and a poly (A) was found in *Euricania clara* (Fig. 3).

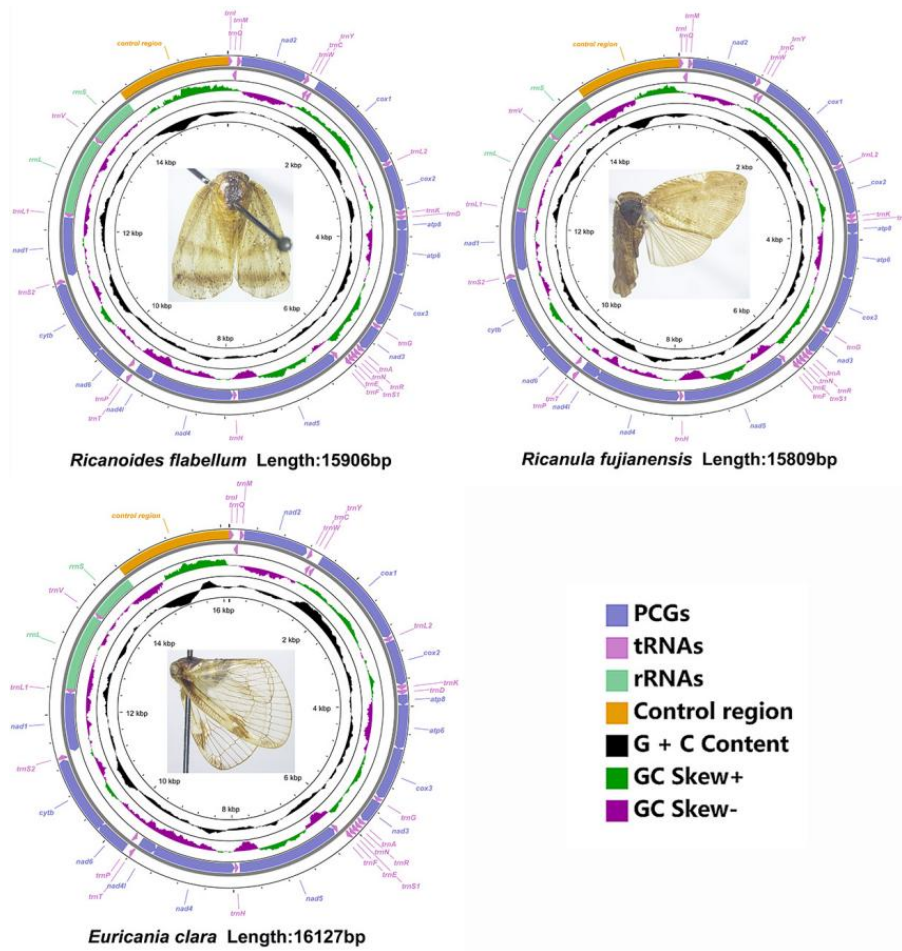


Figure 2. The mitochondrial genome of *Ricanoides flabellum*, *Ricanula fujianensis* and *Euricania clara*.

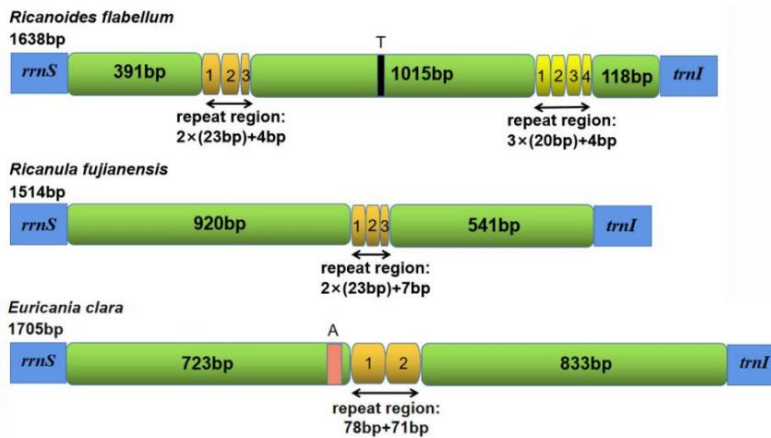


Figure 3. Organization of the control regions in the three Ricaniidae mitogenomes. The chrome yellow and yellow ochre indicate the tandem repeats. Non-repeat regions are represented by a green rounded rectangle. The red and black blocks indicate the structures of poly (A) or poly (T).

In the three new ricaniid mitogenomes, the typical start codon ATN (ATA/T/G/C) was found in all PCGs. Correspondingly, the terminated codon TAA and TAG were found in most PCGs, but the *cox2*, *nad4* and *nad1* genes ended with a single T, *atp6* in *Ricanoides flabellum* and *Ricanula fujianensis* ended with a single T and *nad5* in *Ricanula fujianensis* uses a single T as the stop codon (Table 4), which is universal in planthoppers (Wang *et al.* 2019, 2021; Ai *et al.* 2021; Zhang *et al.* 2022). The relative synonymous codon usage (RSCU) is shown in Fig. 4. Phe (UUU), Ile (AUU), Met (AUA), Ser2 (UCA), Thr (ACA) and Leu2 (UUA) were observed to be the most frequently used codons. The amino acid compositions were mostly A or U, indicating the strong AT bias in the whole mitochondrial genome. However, the codon Leu1 (CUC), Arg (CGC) and Ser1 (AGC) were missing in *Ricanula fujianensis* and Ser1 (AGC) was missing in *Euricania clara* (Fig. 4). These missing codons were observed in other planthoppers.

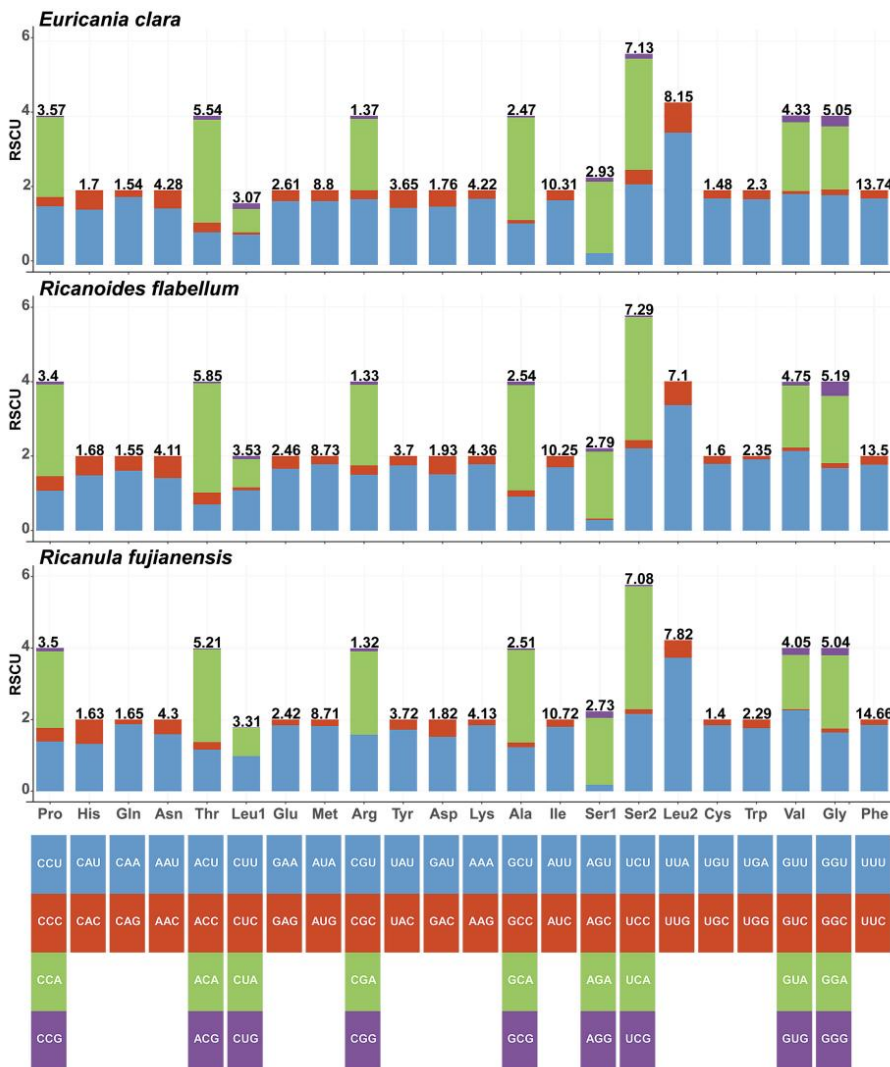


Figure 4. Relative synonymous codon usage (RSCU) of the mitogenomes of *Ricanoides flabellum*, *Ricanula fujianensis* and *Euricania clara*.

The nucleotide diversity of 13 PCGs and 2 rRNAs among the eleven Ricaniidae species is shown in Figure 5. The value of nucleotide diversity ranges from 0.148 (*rrnS*) to 0.278 (*nad2*). The results indicate that *cox1* ($Pi = 0.159$) and *nad5* ($Pi = 0.172$) show a relatively low nucleotide diversity, whereas *nad3* ($Pi = 0.229$), *atp8* ($Pi = 0.243$), *nad6* ($Pi = 0.252$) and *nad2* ($Pi = 0.278$) present a higher nucleotide diversity. Two rRNAs were highly conserved genes with lower values of 0.148 for *rrnS* and 0.152 for *rrnL* (Fig. 5).

The Ka/Ks rates of 13 PCGs of the eleven Ricaniidae species were separately calculated with *Bambusiphaga maculata* as the reference sequence. The Ka/Ks values greater than 1 represent positive selection in each gene. In *Ricania fumosa*, the Ka/Ks ratio of *atp8* was greater than 1, indicating that the *atp8* gene was under positive selection. In addition, the Ka/Ks of *atp8* in *Ricanoides flabellum* was as high as 0.912, and the Ka/Ks of *nad4* was 0.902 and 0.900 in *Pochazia confusa* and *Ricania marginalis*, respectively. The Ka/Ks of *nad4l* was 0.956, 0.938 and 0.905 in *Ricania fumosa*, *Pochazia shantungensis* and *Ricania simulans*, respectively (Fig. 6). The lowest Ka/Ks ratio was for the *cox1* genes of all species, which signifies fewer amino acid changes. Furthermore, the genetic distances of 13 PCGs of the eleven Ricaniidae species were separately calculated with *Bambusiphaga maculata* as the reference sequence, and show that *cox1* genes of all species with the lowest value were evolving relatively slower. The gene *atp8* in *Pochazia confusa* (1.080) and *Pochazia discreta* (1.060), and the gene *nad2* in *Euricania clara* (1.020), *Pochazia discreta* (1.030), *Pochazia guttifer* (1.040), *Ricania fumosa* (1.060), *Pochazia shantungensis* (1.010) and *Ricania speculum* (1.080) are evolving comparatively faster (Fig. 7).

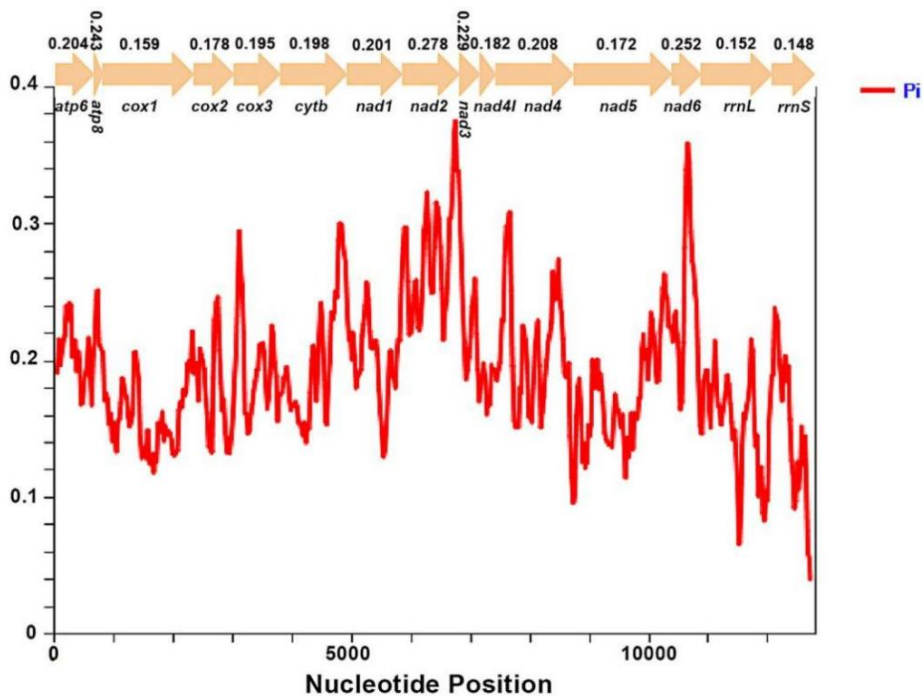


Figure 5. Sliding window analysis of 13 PCGs and 2 rRNAs based on eleven Ricaniidae species. The red line shows the value of nucleotide diversity Pi (window size = 100 bp, step size = 25 bp). The Pi value for each gene is shown in the graph.

The nucleotide diversity and evolutionary rate analysis in this study reveals that the *cox1* gene is determined to be the most conserved gene and exhibits a slow evolution rate compared to other PCGs. This result is consistent with the investigations in some other groups in Fulgoroidea (Wang *et al.* 2021, 2023; Zhang *et al.* 2022). The *cox1* gene has been widely used as a DNA barcode for species identification or as one of the genetic markers for phylogenetic analysis among taxa of planthoppers (Urban & Cryan 2009; Urban *et al.* 2010; Gnezdilov *et al.* 2015, 2020; Huang *et al.* 2017). Here we believe that the combination of the *cox1* gene with other mitochondrial genes and nuclear genes can be used to explore the phylogenetic relationships of the higher taxa of Ricaniidae.

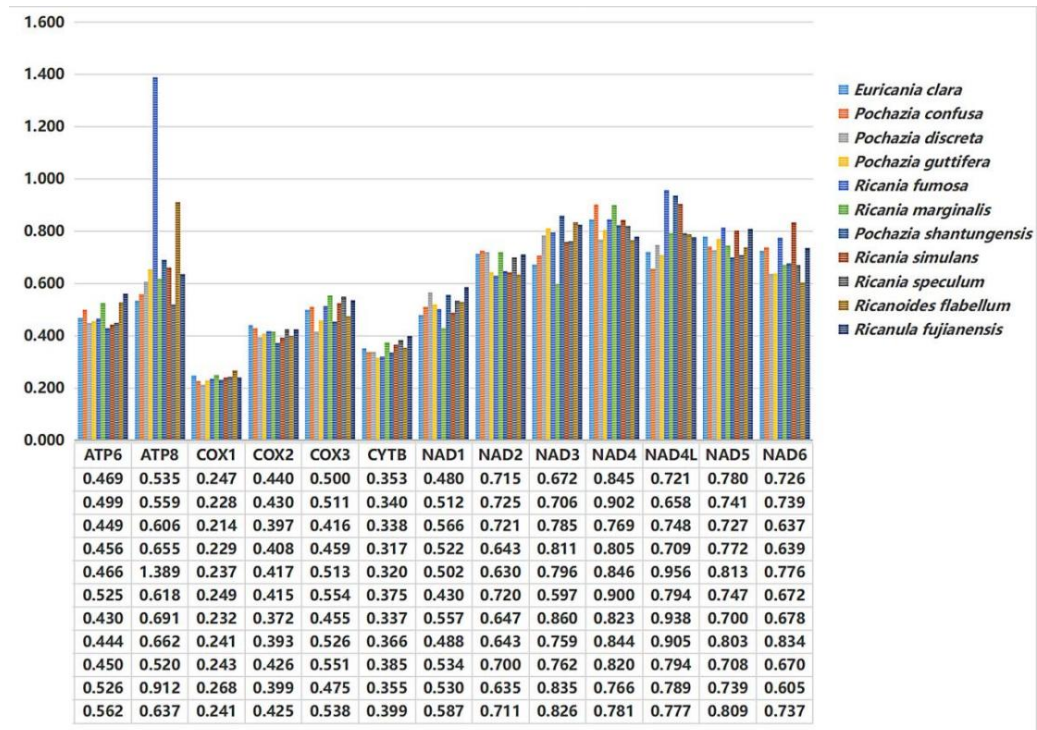


Figure 6. The ratio of Ka/Ks of 13 PCGs of the eleven Ricaniidae species, separately calculated by using *Bambusiphaga maculata* as a reference sequence.

The tree topologies of the ML and BI analyses are identical based on data sets PCGR (Fig. 8). This result based on current mitochondrial genome data shows that the genus *Ricanoides* is the most basal in the Ricaniidae, *Ricanula* is a clade sister to the other remaining Ricaniid species, and *Euricania clara* is placed as sister to *Ricania fumosa*. This result recovers *Pochazia guttifera* and *Pochazia shantungensis*, *Ricania speculum* and *Ricania marginalis* as sister taxa, respectively. This result fails to support the monophyly of both *Pochazia* and *Ricania*, which is consistent with the analysis of Zhang *et al.* (2022). The monophyletic status of these two genera has long been debated, and their morphological definition and species composition remain definitively unclear (Stroiński & Bourgoin 2022). The diagnosis between *Pochazia* and *Ricania* needs to be re-evaluated further.

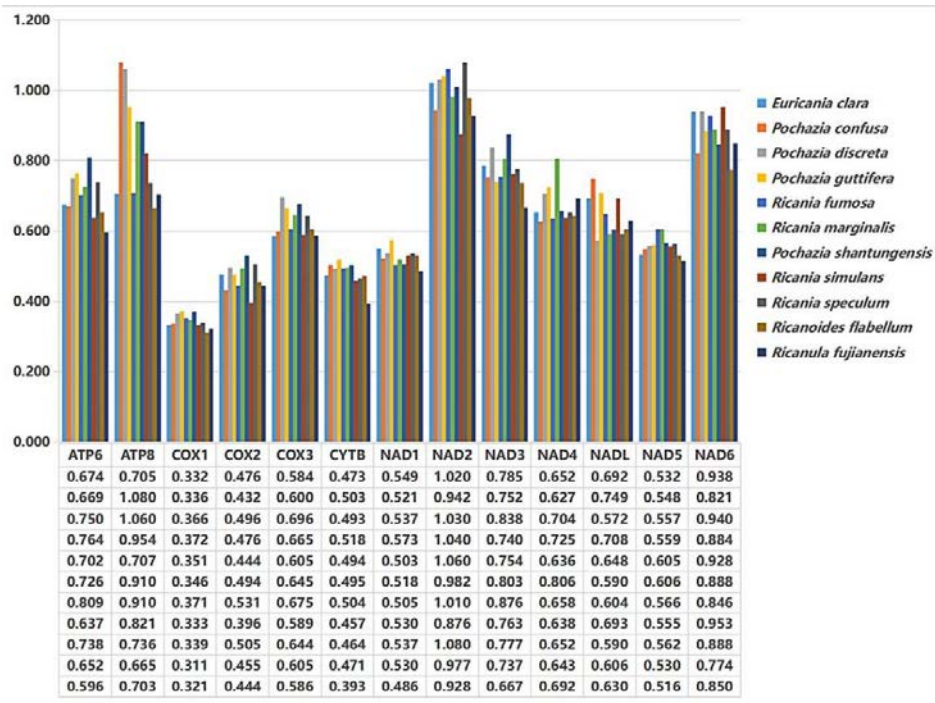


Figure 7. Genetic distances of 13 PCGs of the eleven Ricaniidae species, separately calculated by using *Bambusiphaga maculata* as a reference sequence.

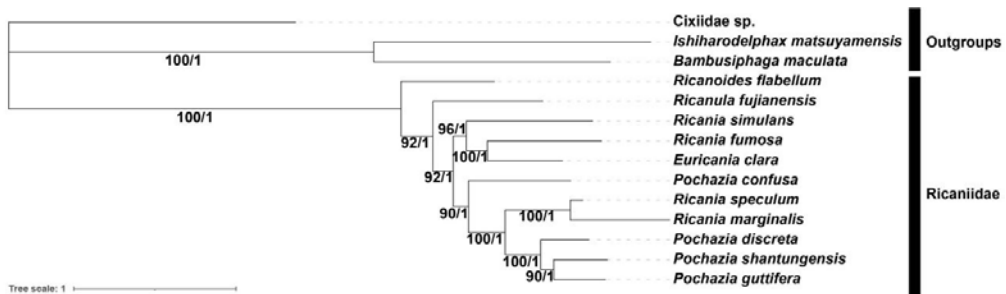


Figure 8. ML analysis and BI analysis based on PCGR. Numerals at nodes are bootstrap values (BS) and Bayesian posterior probabilities (PP).

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