# 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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Palearctic, 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℃ 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 | *Ishiharodelphax matsuyamensis* (Ishihara) | NC_052693 |
| | *Bambusiphaga maculata* Chen & Li | MH293455 |
| Ingroups | | |
| Ricaniidae | *Pochazia confusa* Distant | NC_060807 |
| | *Pochazia discreta* Melichar | NC_060730 |
| | *Pochazia guttifera* Walker | NC_060806 |
| | *Pochazia shantungensis* (Chou & Lu) | MW036196 |
| | *Ricania speculum* (Walker) | MT834932 |
| | *Ricania fumosa* (Walker) | NC_060809 |
| | *Ricania marginalis* (Walker) | NC_019597 |
| | *Ricania simulans* (Walker) | NC_060808 |
| | *Ricania flabellum* Noulhier | OR529197 |
| | *Ricanula fujianensis* Ren, Stroiński& Qin | OR529196 |
| | *Euricania clara* 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 | | Size | Position | | Size | Position | | Size Size | Strand |
|---|---|---|---|---|---|---|---|---|---|---|
| | From | To | | From | To | | From | To | | |
| | *Ricanoides flabellum* | | | *Ricanula fujianensis* | | | *Euricania clara* | | | |
| *trnI* | 1 | 63 | 63 | 1 | 64 | 64 | 1 | 67 | 67 | J |
| *trnQ* | 65 | 133 | 69 | 64 | 132 | 69 | 71 | 139 | 69 | N |
| *trnM* | 133 | 197 | 65 | 133 | 196 | 64 | 159 | 221 | 63 | J |
| *nad2* | 198 | 1163 | 966 | 197 | 1162 | 966 | 222 | 1187 | 966 | J |
| *trnW* | 1162 | 1226 | 65 | 1168 | 1230 | 63 | 1215 | 1278 | 64 | J |
| *trnC* | 1219 | 1282 | 64 | 1223 | 1285 | 63 | 1271 | 1333 | 63 | N |
| *trnY* | 1286 | 1347 | 62 | 1289 | 1350 | 62 | 1346 | 1407 | 62 | N |
| *cox1* | 1352 | 2887 | 1536 | 1354 | 2889 | 1536 | 1415 | 2950 | 1536 | J |
| *trnL* | 2888 | 2950 | 63 | 2891 | 2953 | 63 | 2951 | 3013 | 63 | J |
| *cox2* | 2951 | 3623 | 673 | 2954 | 3626 | 673 | 3014 | 3683 | 670 | J |
| *trnK* | 3624 | 3692 | 69 | 3627 | 3696 | 70 | 3684 | 3752 | 69 | J |
| *trnD* | 3693 | 3757 | 65 | 3697 | 3763 | 67 | 3755 | 3828 | 74 | J |
| *atp8* | 3758 | 3910 | 153 | 3764 | 3919 | 156 | 3829 | 3987 | 159 | J |
| *atp6* | 3907 | 4555 | 649 | 3916 | 4564 | 649 | 3984 | 4667 | 684 | J |
| *cox3* | 4556 | 5338 | 783 | 4565 | 5347 | 783 | 4633 | 5415 | 783 | J |
| *trnG* | 5343 | 5403 | 61 | 5354 | 5414 | 61 | 5416 | 5477 | 62 | J |
| *nad3* | 5404 | 5751 | 348 | 5415 | 5762 | 348 | 5478 | 5825 | 348 | J |
| *trnA* | 5751 | 5815 | 65 | 5763 | 5829 | 67 | 5834 | 5898 | 65 | J |
| *trnR* | 5825 | 5884 | 60 | 5834 | 5897 | 64 | 5905 | 5964 | 60 | J |
| *trnN* | 5884 | 5946 | 63 | 5901 | 5965 | 65 | 5968 | 6032 | 65 | J |
| *trnS* | 5946 | 6005 | 60 | 5966 | 6023 | 58 | 6032 | 6091 | 60 | J |
| *trnE* | 6006 | 6070 | 65 | 6025 | 6088 | 64 | 6095 | 6157 | 63 | J |
| *trnF* | 6069 | 6133 | 65 | 6093 | 6155 | 63 | 6165 | 6232 | 68 | N |
| *nad5* | 6133 | 7797 | 1665 | 6156 | 7818 | 1663 | 6233 | 7897 | 1665 | N |
| *trnH* | 7814 | 7874 | 61 | 7835 | 7897 | 63 | 7914 | 7976 | 63 | N |
| *nad4* | 7876 | 9181 | 1306 | 7899 | 9225 | 1327 | 7978 | 9301 | 1324 | N |
| *nad4L* | 9194 | 9466 | 273 | 9219 | 9491 | 273 | 9295 | 9567 | 273 | N |
| *trnT* | 9469 | 9532 | 64 | 9499 | 9564 | 66 | 9570 | 9632 | 63 | J |
| *trnP* | 9538 | 9603 | 66 | 9568 | 9632 | 65 | 9646 | 9708 | 63 | N |
| *nad6* | 9605 | 10099 | 495 | 9634 | 10128 | 495 | 9710 | 10204 | 495 | J |
| *cytb* | 10092 | 11213 | 1122 | 10121 | 11245 | 1125 | 10197 | 11330 | 1134 | J |
| *trnS* | 11212 | 11276 | 65 | 11248 | 11312 | 65 | 11338 | 11403 | 66 | J |
| *nad1* | 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 | Size | |
| | *Ricanoides flabellum* | | | *Ricanula fujianensis* | | | *Euricania clara* | | | |
| *trnL* | 12209 | 12272 | 64 | 12245 | 12308 | 64 | 12345 | 12410 | 66 | N |
| *rrnL* | 12273 | 13479 | 1207 | 12309 | 13507 | 1199 | 12411 | 13634 | 1224 | N |
| *trnV* | 13480 | 13545 | 66 | 13508 | 13570 | 63 | 13635 | 13697 | 63 | N |
| *rrnS* | 13546 | 14268 | 723 | 13571 | 14295 | 725 | 13698 | 14422 | 725 | N |
| *CR* | 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 |
|---|---|---|---|---|---|---|---|---|---|
| *Ricanoides flabellum* | | | | | | | | | |
| 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 |
| *Ricanula fujianensis* | | | | | | | | | |
| 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 |
| *Euricania clara* | | | | | | | | | |
| 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** |
| | *Ricanoides flabellum* | | *Ricanula fujianensis* | | *Euricania clara* | |
| *nad2* | ATT | TAA | ATT | TAA | ATT | TAA |
| *cox1* | ATG | TAG | ATG | TAA | ATG | TAA |
| *cox2* | ATA | T | ATA | T | ATA | T |
| *atp8* | ATT | TAA | ATT | TAA | ATA | TAA |
| *atp6* | ATA | T | ATA | T | ATA | TAA |
| *cox3* | ATG | TAA | ATG | TAA | ATG | TAA |
| *nad3* | ATA | TAG | ATG | TAA | ATA | TAA |
| *nad5* | ATG | TAG | ATG | T | ATG | TAA |
| *nad4* | ATA | T | ATG | T | ATG | T |
| *nad4L* | ATG | TAA | ATG | TAA | ATG | TAA |
| *nad6* | ATC | TAA | ATT | TAA | ATT | TAA |
| *cytb* | ATG | TAG | ATG | TAA | ATG | TAA |
| *nad1* | 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).

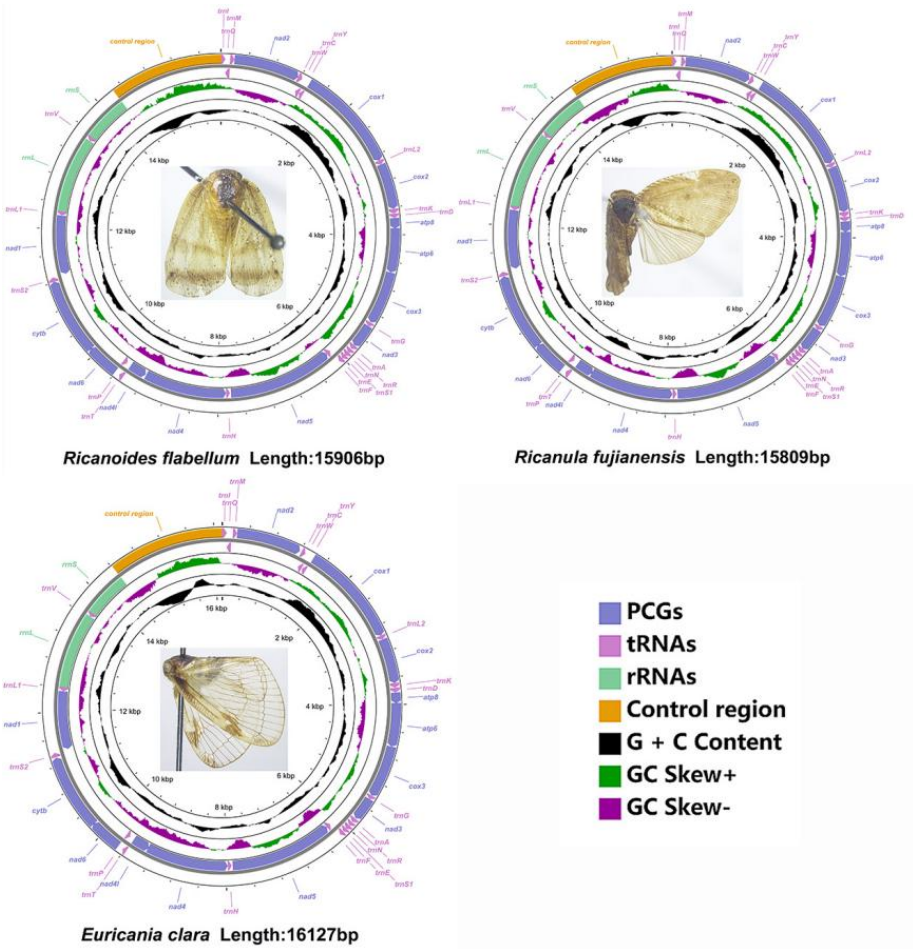



Figure 2. The mitochondrial genome of *Ricanoides flabellum*, *Ricanula fujianensis* and *Euricania clara*.

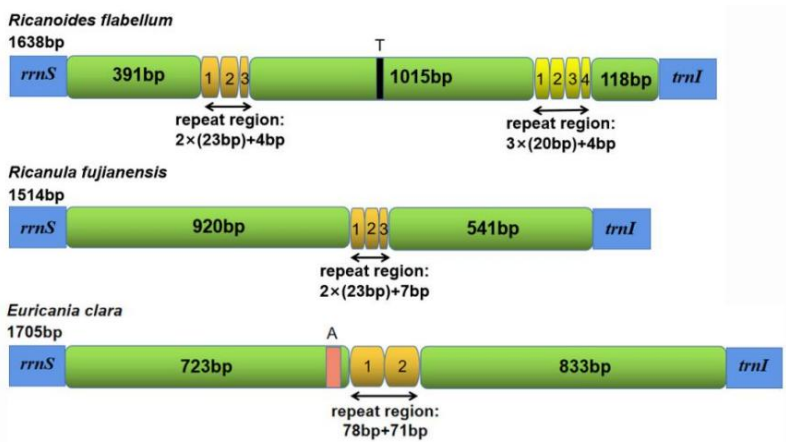



Figure 3. Organization of the control regions in the three Ricaniidae mitogenomes. The chrome yellow and yellow ochre indicate the tandem repeats. Non-repeat regions are represented by a green rounded rectangle. The red and black blocks indicate the structures of poly (A) or poly (T).

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.

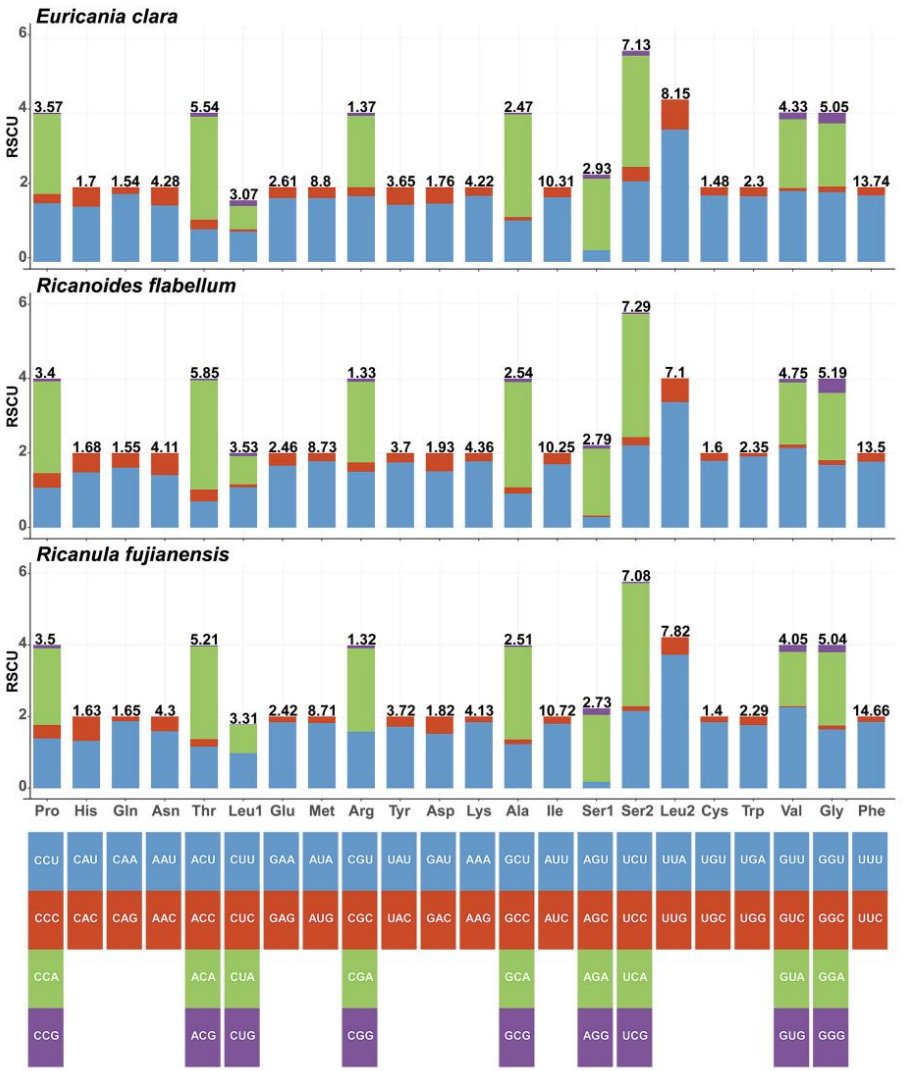



Figure 4. Relative synonymous codon usage (RSCU) of the mitogenomes of *Ricanoides flabellum*, *Ricanula fujianensis* and *Euricania clara*.

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 guttifera* (1.040), *Ricania fumosa* (1.060), *Pochazia shantungensis* (1.010) and *Ricania speculum* (1.080) are evolving comparatively faster (Fig. 7).

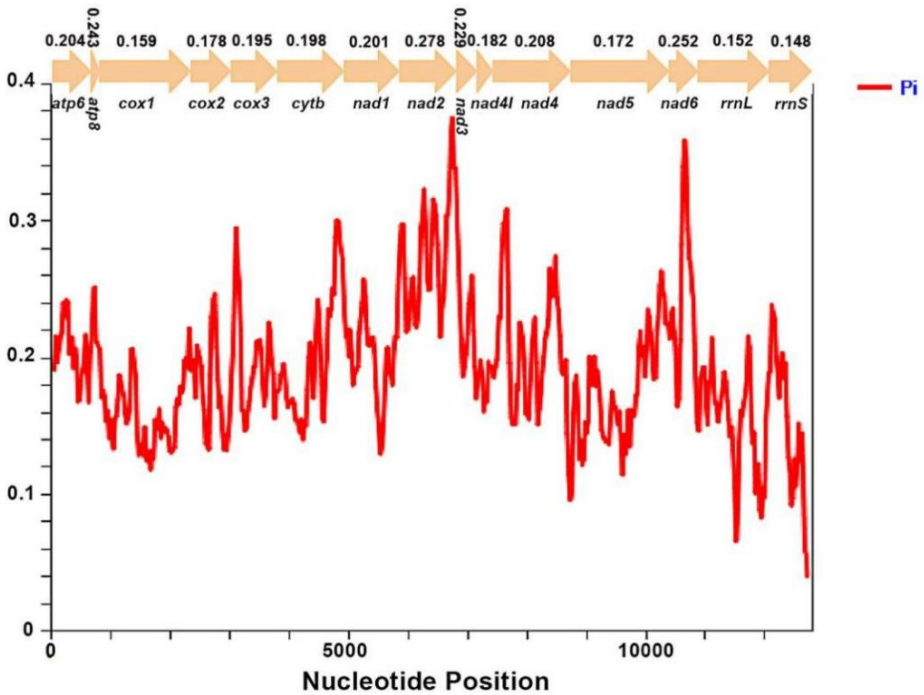
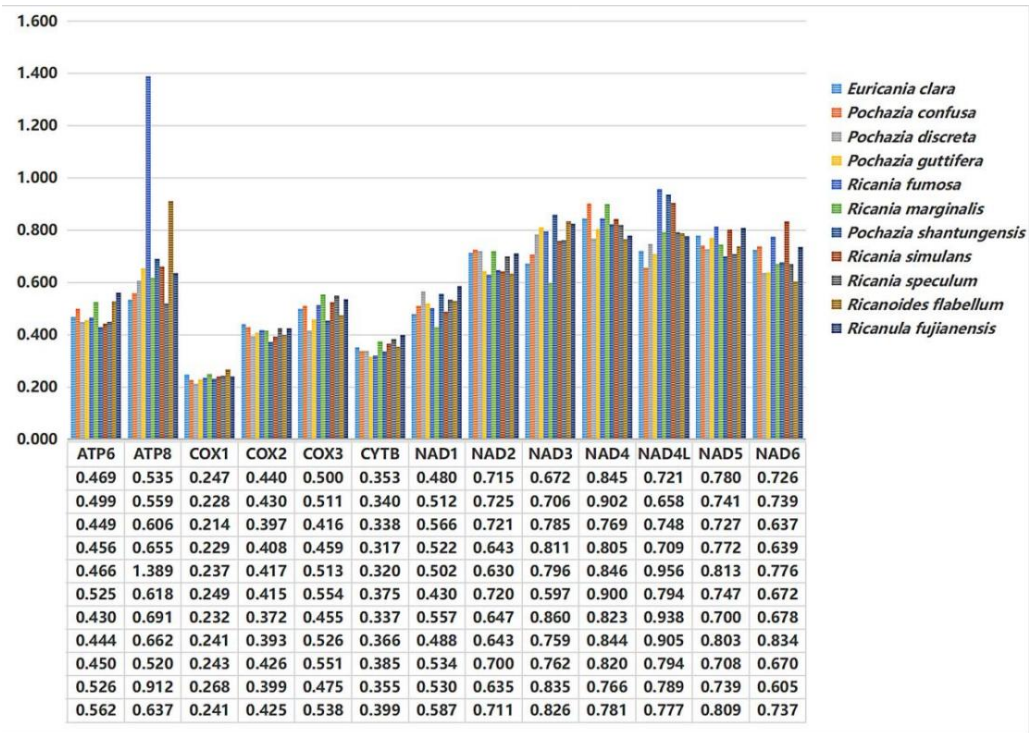



Figure 5. Sliding window analysis of 13 PCGs and 2 rRNAs based on eleven Ricaniidae species. The red line shows the value of nucleotide diversity Pi (window size = 100 bp, step size = 25 bp). The Pi value for each gene is shown in the graph.

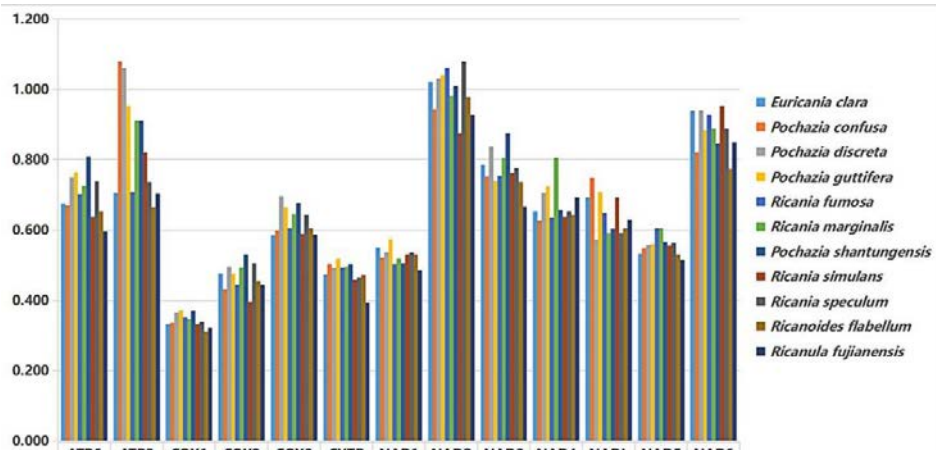
The nucleotide diversity and evolutionary rate analysis in this study reveals that the *cox1* gene is determined to be the most conserved gene and exhibits a slow evolution rate compared to other PCGs. This result is consistent with the investigations in some other groups in Fulgoroidea (Wang *et al*. 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.




| ATP6 | ATP8 | COX1 | COX2 | COX3 | CYTB | NAD1 | NAD2 | NAD3 | NAD4 | NAD4L | NAD5 | NAD6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.469 | 0.535 | 0.247 | 0.440 | 0.500 | 0.353 | 0.480 | 0.715 | 0.672 | 0.845 | 0.721 | 0.780 | 0.726 |
| 0.499 | 0.559 | 0.228 | 0.430 | 0.511 | 0.340 | 0.512 | 0.725 | 0.706 | 0.902 | 0.658 | 0.741 | 0.739 |
| 0.449 | 0.606 | 0.214 | 0.397 | 0.416 | 0.338 | 0.566 | 0.721 | 0.785 | 0.769 | 0.748 | 0.727 | 0.637 |
| 0.456 | 0.655 | 0.229 | 0.408 | 0.459 | 0.317 | 0.522 | 0.643 | 0.811 | 0.805 | 0.709 | 0.772 | 0.639 |
| 0.466 | 1.389 | 0.237 | 0.417 | 0.513 | 0.320 | 0.502 | 0.630 | 0.796 | 0.846 | 0.956 | 0.813 | 0.776 |
| 0.525 | 0.618 | 0.249 | 0.415 | 0.554 | 0.375 | 0.430 | 0.720 | 0.597 | 0.900 | 0.794 | 0.747 | 0.672 |
| 0.430 | 0.691 | 0.232 | 0.372 | 0.455 | 0.337 | 0.557 | 0.647 | 0.860 | 0.823 | 0.938 | 0.700 | 0.678 |
| 0.444 | 0.662 | 0.241 | 0.393 | 0.526 | 0.366 | 0.488 | 0.643 | 0.759 | 0.844 | 0.905 | 0.803 | 0.834 |
| 0.450 | 0.520 | 0.243 | 0.426 | 0.551 | 0.385 | 0.534 | 0.700 | 0.762 | 0.820 | 0.794 | 0.708 | 0.670 |
| 0.526 | 0.912 | 0.268 | 0.399 | 0.475 | 0.355 | 0.530 | 0.635 | 0.835 | 0.766 | 0.789 | 0.739 | 0.605 |
| 0.562 | 0.637 | 0.241 | 0.425 | 0.538 | 0.399 | 0.587 | 0.711 | 0.826 | 0.781 | 0.777 | 0.809 | 0.737 |

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.




| ATP6 | ATP8 | COX1 | COX2 | COX3 | CYTB | NAD1 | NAD2 | NAD3 | NAD4 | NADL | NAD5 | NAD6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.674 | 0.705 | 0.332 | 0.476 | 0.584 | 0.473 | 0.549 | 1.020 | 0.785 | 0.652 | 0.692 | 0.532 | 0.938 |
| 0.669 | 1.080 | 0.336 | 0.432 | 0.600 | 0.503 | 0.521 | 0.942 | 0.752 | 0.627 | 0.749 | 0.548 | 0.821 |
| 0.750 | 1.060 | 0.366 | 0.496 | 0.696 | 0.493 | 0.537 | 1.030 | 0.838 | 0.704 | 0.572 | 0.557 | 0.940 |
| 0.764 | 0.954 | 0.372 | 0.476 | 0.665 | 0.518 | 0.573 | 1.040 | 0.740 | 0.725 | 0.708 | 0.559 | 0.884 |
| 0.702 | 0.707 | 0.351 | 0.444 | 0.605 | 0.494 | 0.503 | 1.060 | 0.754 | 0.636 | 0.648 | 0.605 | 0.928 |
| 0.726 | 0.910 | 0.346 | 0.494 | 0.645 | 0.495 | 0.518 | 0.982 | 0.803 | 0.806 | 0.590 | 0.606 | 0.888 |
| 0.809 | 0.910 | 0.371 | 0.531 | 0.675 | 0.504 | 0.505 | 1.010 | 0.876 | 0.658 | 0.604 | 0.566 | 0.846 |
| 0.637 | 0.821 | 0.333 | 0.396 | 0.589 | 0.457 | 0.530 | 0.876 | 0.763 | 0.638 | 0.693 | 0.555 | 0.953 |
| 0.738 | 0.736 | 0.339 | 0.505 | 0.644 | 0.464 | 0.537 | 1.080 | 0.777 | 0.652 | 0.590 | 0.562 | 0.888 |
| 0.652 | 0.665 | 0.311 | 0.455 | 0.605 | 0.471 | 0.530 | 0.977 | 0.737 | 0.643 | 0.606 | 0.530 | 0.774 |
| 0.596 | 0.703 | 0.321 | 0.444 | 0.586 | 0.393 | 0.486 | 0.928 | 0.667 | 0.692 | 0.630 | 0.516 | 0.850 |

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

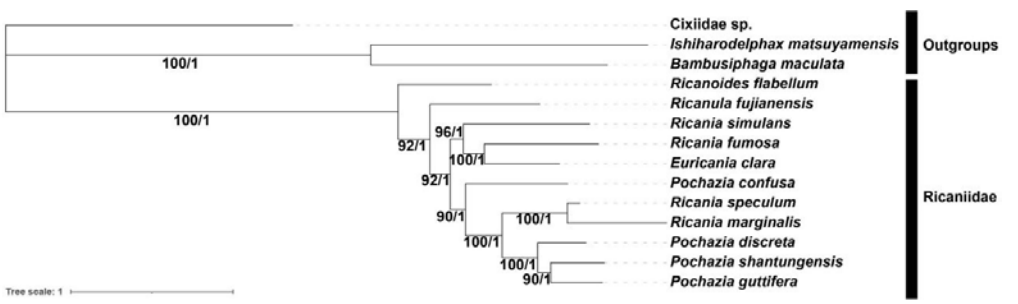



Figure 8. ML analysis and BI analysis based on PCGR. Numerals at nodes are bootstrap values (BS) and Bayesian posterior probabilities (PP).

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