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TECHNICAL NOTE

Characterization of the complete mitochondrial genome of the Asian planthopper *Ricania speculum* (Hemiptera: Fulgoroidea: Ricannidae)

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Abstract The Asian planthopper *Ricania speculum* is a major agricultural pest for a variety of crops in tropical and subtropical areas. Rapid species diagnosis and source determination of this pest would be essential to the formulation of effective management, control and prevention strategy, and mitochondrial DNA has proven powerful for such purposes. In this study, we assembled and characterized its complete mitochondrial genome from Illumina sequencing reads. The whole genome of *R. speculum* is 15,729 bp in length, and encodes the typical set of 37 mitochondrial genes (incl. 13 PCGs, 22tRNAs and 2 rRNAs) and a control region. All PCGs are initiated with the typical ATN codons, and are terminated with either the complete TAA/TAG codons or the incomplete T(aa) codon. The nucleotide composition is asymmetric (47.4%A, 14.8%C, 9.5%G and 28.3%T) with an obvious bias towards A and T. Phylogenetic analysis indicated that *R. speculum* is closely related to *Ricania marginalis*.

Keywords *Ricania speculum* · Mitochondrial genome · Phylogenetic analysis · Planthopper

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The Asian planthopper *Ricania speculum* belongs to the family Ricannidae (Hemiptera: Fulgoroidea), and is regarded as a major agricultural pest for a variety of crops (e.g. grapes, apples, pears, plums, tea trees, coffee trees, cocoa, oil palms, *Citrus* species, etc.) (Li et al. 2011; Rossi and Lucchi 2015; Xu et al. 2013). This species is widely distributed in China, India, Indonesia, Japan, Korea, Malaysia, Philippines and Vietnam, and has also recently been accidentally introduced into Italy (Mazza et al. 2014; Rossi and Lucchi 2015). Rapid species diagnosis and source determination of this pest would be essential to the formulation of effective management, control and prevention strategy, and mitochondrial DNA is a powerful tool for such purposes. Here, its complete mitochondrial genome has been assembled from Illumina sequencing data, and has been submitted to GenBank with the accession number KX371891.

Total genomic DNA was extracted from muscle tissue of a single individual using the QIAamp Tissue Kit (QIAGEN, CA, USA). The shotgun library preparation and whole-genome sequencing was conducted following the manufacturer's manual for the Illumina HiSeq 2500 Sequencing System (Illumina, CA, USA). After the quality-trimming with Trimmomatic v0.35 (Bolger et al. 2014), the sequencing data were used for the assembly of mitochondrial genome with MITObim v1.8 (Hahn et al. 2013). Mitogenomic annotation was conducted by using the MITOS Web Server (Bernt et al. 2013), and was further visually adjusted according to its alignment with the mitochondrial genomes of other Hemipteran species, e.g. *Ricania marginalis* (JN242415) (Song et al. 2012) and *Sivaloka damnosus* (FJ360694) (Song et al. 2010).

In all, 10,676 individual mitochondrial reads generated an average coverage of 104X. The resultant complete mitochondrial genome of the *R. speculum* is 15,729 bp in length. The nucleotide composition is asymmetric (47.4%A, 14.8%C,

9.5%G and 28.3%T) with an obvious bias towards A and T. As in those of most other insects, the typical panel of 37 genes (including 13 protein-coding genes/PGCs, 22 tRNAs and 2 rRNAs) and a non-coding control region are present in the mitochondrial genome of *R. speculum* (Table 1; Supplementary Fig. 1). Out of the 37 genes, 14 genes [*nad1, nad4, nad4l, nad5, tRNA-Cys, tRNA-Gln, tRNA-His, tRNA-Leu*$^{(CUN)}$, *tRNA-Phe, tRNA-Pro, tRNA-Tyr, tRNA-Val, 12S rRNA* and *16S rRNA*] are located on the heavy strand (H-strand), while all the others on the light strand (L-strand).

Table 1 Annotation of the mitochondrial genome of *Ricania speculum*

Gene	Coding strand	Location (bp)	IGS (bp)	Size (bp)	Anticodon (location in bp)	Start codon	Stop codon
tRNA-Ile	H	1–64	4	64	GAT (30–32)		
tRNA-Gln	L	69–137	14	69	TTG (105–107)		
tRNA-Met	H	152–216	0	65	CAT (183–185)		
nad2	H	217–1182	26	966		ATT	TAA
tRNA-Trp	H	1209–1272	−8	64	TCA (1239–1241)		
tRNA-Cys	L	1265–1327	4	63	GCA (1296–1298)		
tRNA-Tyr	L	1332–1393	4	62	GTA (1362–1364)		
cox1	H	1398–2933	1	1536		ATG	TAA
tRNA-Leu$^{(UUR)}$	H	2935–2997	0	63	TAA (2965–2967)		
cox2	H	2998–3679	0	682		ATT	T(aa)
tRNA-Lys	H	3680–3749	2	70	CTT (3711–3713)		
tRNA-Asp	H	3752–3815	0	64	GTC (3782–3784)		
atp8	H	3816–3977	−4	162		ATA	TAA
atp6	H	3974–4622	0	649		ATA	T(aa)
cox3	H	4623–5405	3	783		ATG	TAG
tRNA-Gly	H	5409–5469	0	61	TCC (5439–5441)		
nad3	H	5470–5817	5	348		ATA	TAA
tRNA-Ala	H	5823–5887	5	65	TGC (5851–5853)		
tRNA-Arg	H	5893–5954	2	62	TCG (5920–5922)		
tRNA-Asn	H	5957–6024	−1	68	GTT (5988–5990)		
tRNA-Ser$^{(AGY)}$	H	6024–6082	1	59	GCT (6045–6047)		
tRNA-Glu	H	6084–6148	7	65	TTC (6115–6117)		
tRNA-Phe	L	6156–6220	5	65	GAA (6185–6187)		
nad5	L	6226–7890	15	1665		ATG	TAA
tRNA-His	L	7906–7969	0	64	GTG (7935–7937)		
nad4	L	7970–9296	−7	1327		ATG	T(aa)
nad4l	L	9290–9559	2	270		ATG	TAA
tRNA-Thr	H	9562–9626	11	65	TGT (9592–9594)		
tRNA-Pro	L	9638–9702	1	65	TGG (9669–9671)		
nad6	H	9704–10,198	−8	495		ATC	TAA
cytb	H	10,191–11,321	−1	1131		ATG	TAA
tRNA-Ser$^{(UCN)}$	H	11,321–11,386	−7	66	TGA (11,349–11,351)		
nad1	L	11,380–12,318	1	939		ATG	TAA
tRNA-Leu$^{(CUN)}$	L	12,320–12,383	23	64	TAG (12,351–12,353)		
16S rRNA	L	12,407–13,604	−7	1198			
tRNA-Val	L	13,598–13,659	−3	62	TAC (13,635–13,637)		
12S rRNA	L	13,657–14,383	0	727			
Control region	N.C.	14,384–15,729	0	1346			

IGS denotes the length of the intergenic spacer region, for which negative numbers indicate nucleotide overlapping between adjacent genes. T(aa) denotes that the TAA stop codon is presumed to be completed by the addition of 3′ A residues to the mRNA. *H* heavy strand (H-strand), *L* light strand (L-strand), *N.C.* non-coding

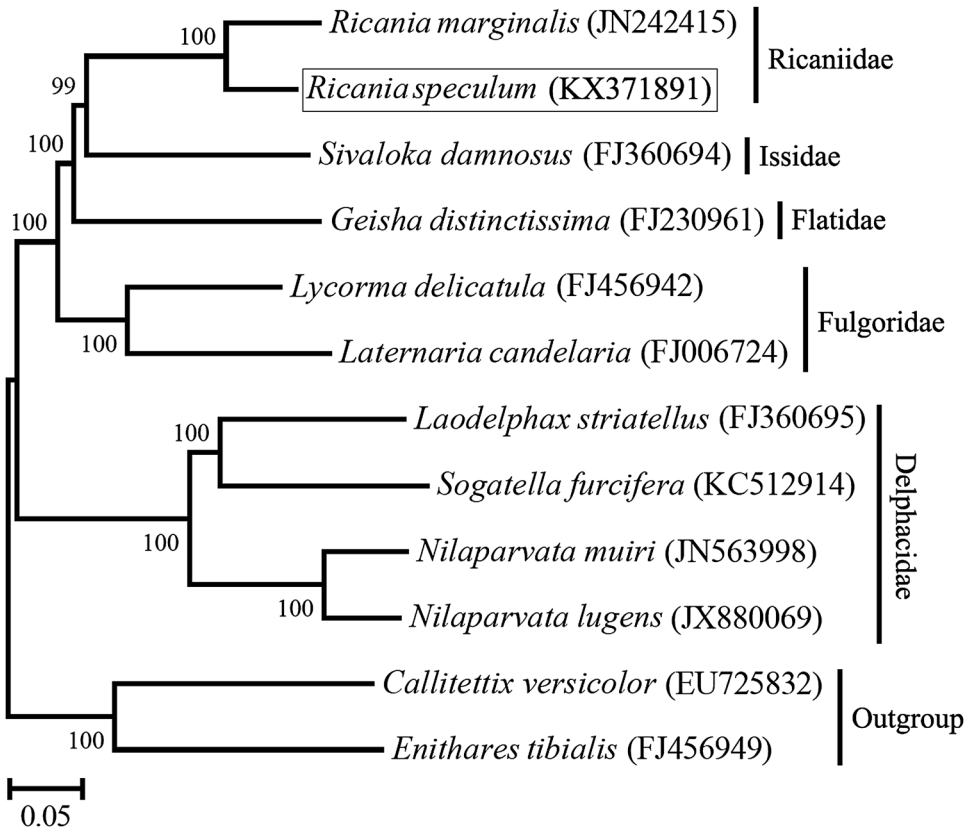

Fig. 1 Phylogenetic relationships among ten species within the superfamily Fulgoroidea based on the Neighbor-Joining (NJ) analysis of the concatenated sequences of 13 mitochondrial PCGs. The bootstrap values were based on 500 resamplings, and are indicated next to the branches. The tree was rooted with *Callitettix versicolor* (Hemiptera: Cercopidae) and *Enithares tibialis* (Hemiptera: Notonectidae).

The 13 PCGs are initiated with the typical ATA [*atp6, atp8* and *nad3*], ATC [*nad6*], ATT [*cox2* and *nad2*] or ATG [*cytb, cox1, cox3, nad1, nad4, nad4l* and *nad5*] codons. All PCGs harbor the typical TAA or TAG stop codons except for *atp6, cox2* and *nad4* with the incomplete stop codon T(aa). The 22 tRNAs vary in size between 59 [*tRNA-Ser*$^{(AGY)}$] and 70 bp [*tRNA-Lys*] with a total length of 1441 bp. All tRNAs display the typical clover-leaf structure except for *tRNA-Ser*$^{(AGY)}$, whose dihydrouridine arm forms a simple loop as in most other insects (Ohtsuki et al. 2002). The two rRNAs are 727 bp [*12S rRNA*] and 1198 bp [*16S rRNA*] in length, and are separated from each other by *tRNA-Val*. The control region is 1346 bp long with an A+T content of 80.8 %, and is located between *12S rRNA* and *tRNA-Ile*. In all, 20 instances of intergenic spacers are identified, ranging in length from 1 to 26 bp with a total length of 136 bp, and the largest spacer (26 bp) resides between *tRNA-Met* and *tRNA-Trp*. The nine intergenic overlapping regions range in size from 1 to 8 bp with a total length of 46 bp.

A phylogenetic analysis was carried out to infer the placement of *R. speculum* within the superfamily Fulgoroidea based on the 13 mitochondrial PCGs for a panel of 12 species. A Neighbor-Joining tree was obtained using MEGA6 (Tamura et al. 2013) with high bootstrap support values (Fig. 1). The phylogeny agrees with the mopho-taxonomy of the superfamily Fulgoroidea at the familial level. As expected, *R. speculum* is closely related to the congeneric planthopper *R. marginalis*.

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