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MITOGENOME REPORT

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The complete mitochondrial genome of *Parotis chlorochroalis* (Hampson, 1912) (Lepidoptera: Crambidae)

Mei Xiong[a,b], Chun-Sheng Wu[a], Chao-Dong Zhu[a,b] and Qing-Song Zhou[a]

[a]Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing, China; [b]University of Chinese Academy of Sciences, Beijing, China

ABSTRACT

The complete mitochondrial genome of the *Parotis chlorochroalis* was sequenced, revaeling a length of 15239 bp with 37 genes and an A + T-rich region. All c13 PCGs begin with typical ATN codons, except COI gene, which starts with CGA. Eleven genes terminate with TAA, two with T–. All 22 tRNA genes exhibit typical cloverleaf structure except for trnS1 *P. chlorochroalis* has two relatively conserved intergenic regions and two relatively conserved overlapping regions. Phylogenetic analysis support *P. chlorochroalis* belongs to subfamily Spilomelinae, the topologies of Crambidae are highly congruent with previous studies. This newly sequences mitochondrial genome provides valuable resources for taxonomic inference and evolutionary studies of genus *Parotis*.

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1. Introduction

Crambidae is the largest family of superfamily Pyraloideae (Insecta: Lepidoptera), with over 10487 currently recognized species in 1021 genera (Nuss et al. 2003–2024). They not only include various important agricultural and forestry economic pests, but are also an ideal group for studying biodiversity (Léger et al. 2021). The genus *Parotis* Hübner, 1831 is an easily distinguishable taxon where the whole body is uniformly colored green or yellow-green, belongs to subfamily Spilomelinae. Genus *Parotis* currently has 43 species worldwide, distributed in the Palearctic, Oriental, and Australian regions. Among them, 15 species recorded in China, mainly distributed in the southern region (Nuss et al. 2003–2024; Yang 2021). Compare with other genus in Spilomelinae, genus *Parotis* has received less attention, previous studies have primarily focused on taxonomic identification and revisions (Du 2008; Yang 2021). Among these, *P. chlorochroalis* was first described by George Hampson (1912) based on the specimens from Cameroon and Nigeria. Subsequent records of this species have expanded its distribution to include Congo (Janse 1924; Meyrick 1933; Ghesquière 1942) and China (Yang 2021). In terms of molecular data, only a few mitochondrial Cytochrome c oxidase I (cox1) gene data are available and some species were unidentified or misidentified, which hinders our understanding of the taxonomy and the phylogenetic relationships within the genus *Parotis*. To address this gap, we present here the complete mitochondrial genome of *P. chlorochroalis*, offering new insights into its molecular characteristics and contributing to the broader understanding of the genus *Parotis*.

2. Materials and methods

2.1. Sample collection and DNA extraction

Specimen of *Parotis chlorochroalis* (Figure 1) was collected by light-trap in Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences in Yunnan Province (101.322832E, 21.873654 N) on 15 July 2022. Species was identified by Mei Xiong using morphological characters (including male external genitalia) followed by Hampson (1912) and Yang (2021). The dried voucher specimens and DNA sample under voucher number P2021-453 were stored in Institute of Zoology, Chinese Academy of Sciences (Zhou QS, zhouqingsong@ioz.ac.cn). Total genomic DNA was extracted from the muscle tissue of single individual by DNeasy Blood and Tissue Kit (QIAGEN), and then stored at −20 °C.

2.2. Genome sequencing, assembly and annotation

The library preparation and genome sequencing were conducted by Shanghai Majorbio Pharm Technology Co. Ltd.

CONTACT Qing-Song Zhou zhouqingsong@ioz.ac.cn Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, 1 Beichen West Road, Chaoyang District, Beijing 100101, PR China.

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Figure 1. Species reference image of *Parotis chlorochroalis*. (the photo was taken by Yong Wang in Yunnan Province, China (101.322832E, 21.873654 N) on 15 July 2022.)

(Shanghai, China). The extracted DNA mixtures were prepared for library construction using the Illumina TruSeq@ DNA PCR-Free HT Kit (Illumina, San Diego, CA), and sequenced on the platform of lumina HiSeq sequencer (150 bp paired-end). The minimum and maximum reads mapping depths for assembled mitochondrial genome were 5919× and 23510×, respectively (Supplementary Figure S1). After removing unpaired, short, and/or low-quality reads with fastp (Chen et al. 2018), clean reads were used for de novo mitogenome assembly with MitoZ v2.3 (Meng et al. 2019) under the all module by default.

The whole mitochondrial genomes were initially annotated by MitoZ v2.3 then refined using the Mitos WebServer (http://mitos2.bioinf.uni-leipzig.de/index.py) under the invertebrate mitochondrial code (Bernt et al. 2013). Transfer RNA (tRNA) genes were confirmed by online ARWEN (http://130.235.46.10/ARWEN/) (Laslett and Canbäck 2008) and tRNAscan-SE 2.0 (Chan et al. 2021) with the extended option for invertebrate mitochondrial genetic code. The remaining undetected tRNAs were compared with homologous sequences from other Pyraloidea species available in GenBank. The tandem repeats in the A + T-rich region were predicted using the Tandem Repeats Finder (http://tandem.bu.edu/trf/trf.html) (Benson 1999). The mitochondrial gene maps of the *Parotis* species were generated using the Proksee online services (https://proksee.ca) (Grant et al. 2023).

2.3. Phylogenetic analysis

Phylogenetic analyses were performed under Bayesian inference (BI) criteria using 13 protein-coding genes (PCGs) from *Parotis chlorochroalis* and 33 other Crambidae mitochondrial genomes, with three pyralid species were selected as outgroups due to the close relationship between Pyralidae and Crambidae (Supplementary Table S1). The protein coding genes (PCGs) were extracted using PhyloSuite (Zhang et al. 2020). MACSE (Ranwez et al. 2011) and MAFFT v7.453 (Katoh and Standley 2013) were used for PCGs sequences alignment. Alignment was performed, followed by the removal of non-homologous sites using trimAl (Capella Gutiérrez et al. 2009) and then the sequences were concatenated. Optimal meta-partition schemes and substitution models were determined using through PartitionFinder 2 (Lanfear et al. 2016). MrBayes version 3.2 (Ronquist et al. 2012) was employed, conducting two simultaneous runs of 10,000,000 generations, with a burn-in of 2,500,000 generations and sampling every 1000 generations.

Table 1. Annotation table of *Parotis chlorochroalis* mitogenome.

Gene	Strand	Position	Length (bp)	INC	Start codon	Stop codon	Anticodon
trnM	J	1–68	68	0			CAT
trnI	J	69–133	65	1			GAT
trnQ	N	135–203	69	53			TTG
nad2	J	257–1270	1014	4	ATT	TAA	
trnW	J	1275–1342	68	−8			TCA
trnC	N	1335–1401	67	6			GCA
trnY	N	1408–1474	67	8			GTA
cox1	J	1483–3013	1531	0	CGA	T	
trnL2	J	3014–3080	67	0			TAA
cox2	J	3081–3762	682	0	ATG	T	
trnK	J	3763–3833	71	4			CTT
trnD	J	3838–3906	69	0			GTC
atp8	J	3907–4068	162	−7	ATT	TAA	
atp6	J	4062–4736	675	−1	ATG	TAA	
cox3	J	4736–5524	789	2	ATG	TAA	
trnG	J	5527–5592	66	0			TCC
nad3	J	5593–5946	354	7	ATC	TAA	
trnA	J	5954–6016	63	−1			TGC
trnR	J	6016–6080	65	0			TCG
trnN	J	6081–6146	66	4			GTT
trnS1	J	6151–6216	66	1			GCT
trnE	J	6218–6285	68	−2			TTC
trnF	N	6284–6349	66	0			GAA
nad5	N	6350–8084	1735	0	ATT	T–	
trnH	N	8085–8151	67	−1			GTG
nad4	N	8151–9491	1341	−1	ATG	TAA	
nad4l	N	9491–9781	291	2	ATG	TAA	
trnT	J	9784–9850	67	0			TGT
trnP	N	9851–9918	68	2			TGG
nad6	J	9921–10457	537	4	ATT	TAA	
cob	J	10462–11610	1149	−2	ATG	TAA	
trnS2	J	11609–11675	67	14			TGA
nad1	N	11690–12628	939	1	ATG	TAA	
trnL1	N	12630–12697	68	24			TAG
rrnL	N	12722–14048	1327	0			
trnV	N	14049–14116	68	0			TAC
rrnS	N	14117–14902	786	0			
CR		14903–15239	337				

3. Results

The complete sequence of *Parotis chlorochroalis* was 15239 bp in length with A + T content of 81.09% contain 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNA), 2 ribosomal RNAs (rRNA), and a putative control region (CR) (Table 1, Figure 2). Analysis of the *Parotis chlorochroalis* complete mitochondrial genome revealed the same gene rearrangement as other lepidopteran species and only one tRNA rearrangement compared to the ancestral insect mitogenome (from trnI-trnQ-trnM to trnM-trnI-trnQ). Additionally, 16 intergenic spacers (137 bp in total) and 8 overlapping regions (46 bp in total) are dispersed throughout the genome. All 13 PCGs were initiated by typical ATN codons

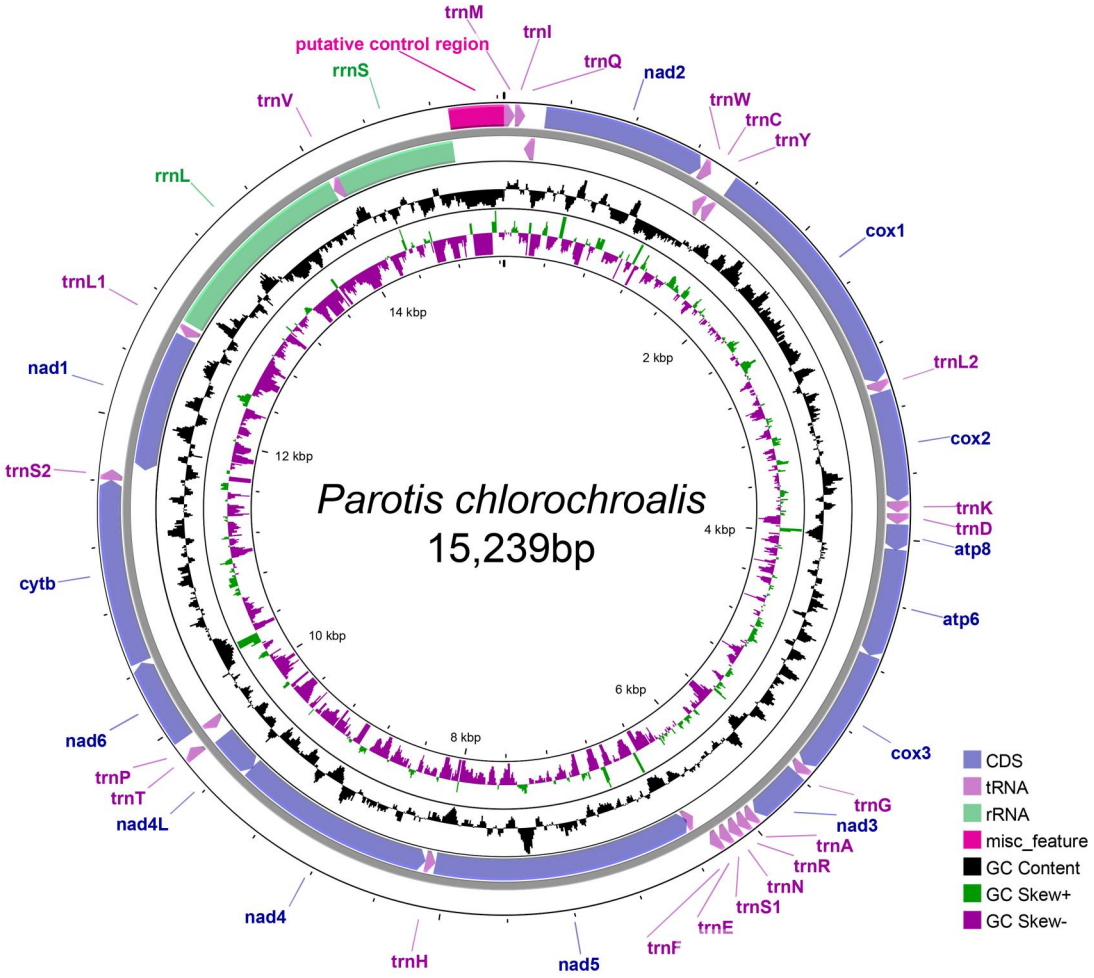

Figure 2. Mitochondrial genome map of *Parotis chlorochroalis.*

except cox1, which was start with CGA. This phenomenon was found in most Lepidoptera species (Wang et al. 2013). Eleven genes terminate with TAA, two genes (cox1 and cox2) have incomplete stop codons. All the 22 tRNA genes, ranging from 63 to 71 bp, have a typical cloverleaf structure except for trnS1, whose dihydrouridine (DHU) arm forms a simple loop. The control region was 337 bp long and 95.6% A + T content. The rrnL and rrnS genes are 1327 and 786 bp in length, with an average A + T content of 83.8% and 85%, respectively. Additional, two conserved gene-overlapping regions (located in trnQ-NAD2 and trnS2-NAD1) and two conserved intergenic regions (located in atp8-atp6 and trnW-trnC) were detected, which was also found in other lepidoptera species (Wang et al. 2013).

The phylogenetic results showed that *Parotis chlorochroalis* placed, and Crambidae form two major clades (Figure 3) which correspond to the 'PS clade' and 'non-PS clade' defined by Regier et al. (2012) and well supported by previous studies (Liu et al. 2016; 2021). Within Crambidae, the monophyly of all subfamilies were well supported, as well as the division of two major clades. The subfamily Spilomelinae and Pyraustinae were placed as the sister groups (Mally et al. 2019) with strong support (BPP = 1.00), and formed the first clade. Another clade contains the remaining subfamilies. The phylogeny also confirmed the placement *Parotis chlorochroalis* within subfamily Spilomelinae was highly supported, the *Parotis chlorochroalis* was closely related to *Botyodes principalis.*

4. Discussion and conclusion

In this study, we reported the complete mitogenome sequence of *P. chlorochroalis* by high-throughput sequencing and assembly. We described the structural features of the mitochondrial genome and its phylogenetic position within the subfamily Spilomelinae and the family Crambidae. The completed mitogenome of *P. chlorochroalis* was 15,239 bp in length with a high AT content of 81.09%, which are similar with other Crambidae species (14838 bp ~ 15689 bp in length, AT content between 77.17% and 82.27%), the orientation and gene order of all 37 genes were conserved across family Crambidae. The presence of atypical initiation codons and incomplete stop codons in PCGs has also been reported in other Lepidoptera species. The lack of the DHU arm in trnS1 is common in Lepidoptera (Lee et al. 2006; Zhao et al. 2013) and other insect mitogenome (Cameron 2014). Phylogenetic analysis supported the placement of *P. chlorochroali* in the subfamily Spilomelinae, and the phylogenetic relationships within Crambidae were mainly consistent with the results of previous studies. These results will contribute to

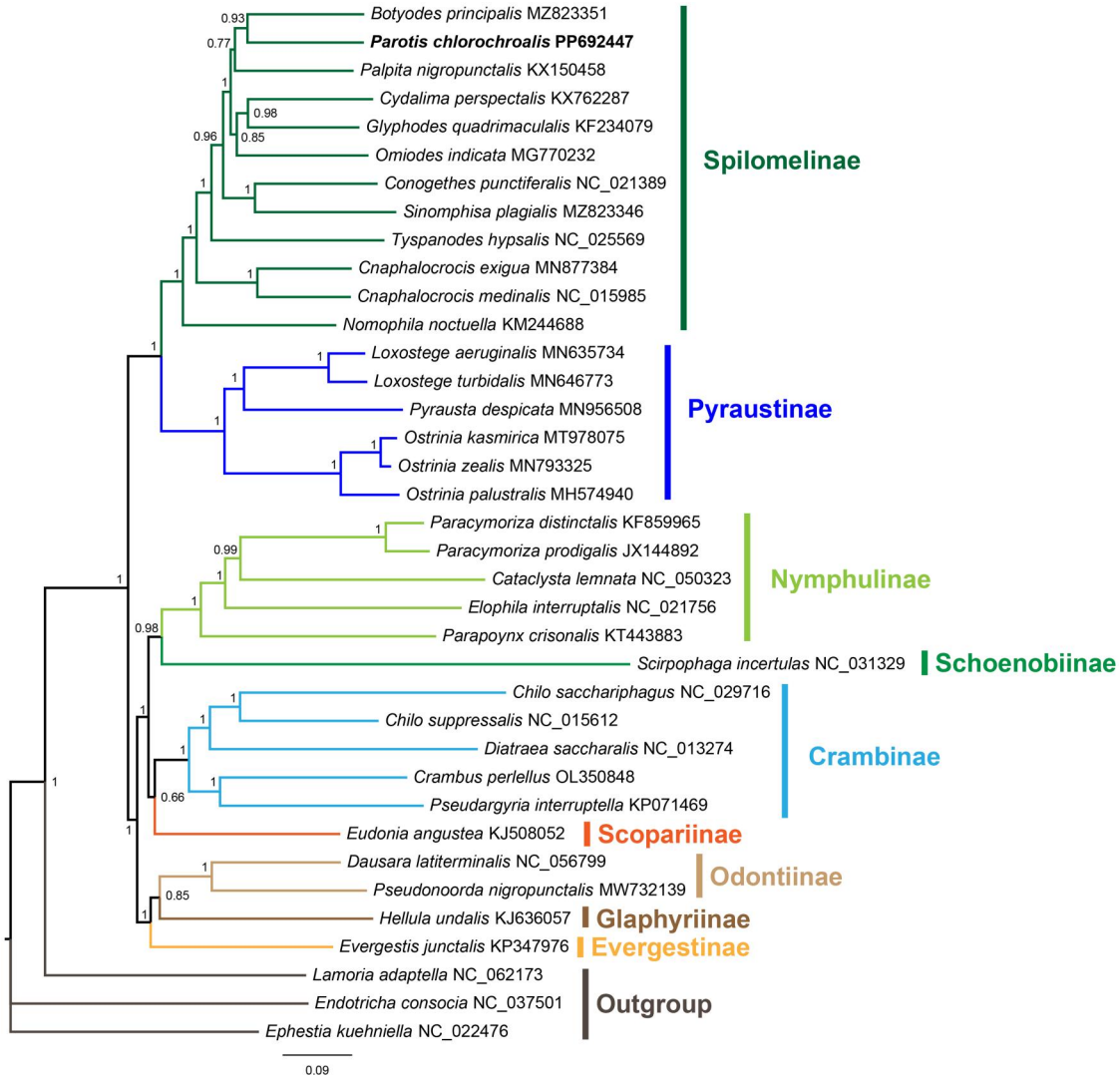

Figure 3. The Bayesian inference (BI) phylogenetic tree of Crambidae based on concatenated 13 mitochondrial protein coding genes. All species involved in the tree have scientific names with accession number on right side. The following sequences were used: MZ823351 *Botyodes principalis* (Liu et al. 2021); NC_050323 *Cataclysta lemnata* (Direct submission); NC_029716 *Chilo sacchariphagus* (Direct submission); NC_015612 *Chilo suppressalis* (Chai et al. 2012); MN877384 *Cnaphalocrocis exigua* (Zhang et al. 2020); NC_015985 *Cnaphalocrocis medinalis* (Chai et al. 2012); NC_021389 *Conogethes punctiferalis* (Wu et al. 2013); OL350848 *Crambus perlellus* (Wu et al. 2023); KX762287 *Cydalima perspectalis* (Direct submission); NC_056799 *Dausara latiterminalis* (Qi et al. 2021); NC_013274 *Diatraea saccharalis* (Li et al. 2011); NC_021756 *Elophila interruptalis* (Park et al. 2014); KJ508052 *Eudonia angustea* (Timmermans et al. 2014); KP347976 *Evergestis junctalis* (Direct submission); KF234079 *Glyphodes quadrimaculalis* (Park et al. 2015); KJ636057 *Hellula undalis* (Dong et al. 2016); MN635734 *Loxostege aeruginalis* (Wu et al. 2023); MN646773 *Loxostege turbidalis* (Wu et al. 2023); KM244688 *Nomophila noctuella* (Tang et al. 2014); MG770232 *Omiodes indicata* (Yang et al. 2018); MT978075 *Ostrinia kasmirica* (Luo et al. 2021); MH574940 *Ostrinia palustralis* (Ju Hwang et al. 2019); MN793325 *Ostrinia zealis* (Zhou et al. 2020); KX150458 *Palpita nigropunctalis* (Direct submission); KF859965 *Paracymoriza distinctalis* (Ye and You 2016); JX144892 *Paracymoriza prodigalis* (Ye et al. 2013); KT443883 *Parapoynx crisonalis* (Direct submission); KP071469 *Pseudargyria interruptella* (Direct submission); MW732139 *Pseudonoorda nigropunctalis* (Qi et al. 2021); MN956508 *Pyrausta despicata* (Wu et al. 2023); NC_031329 *Scirpophaga incertulas* (Unpublished); MZ823346 *Sinomphisa plagialis* (Liu et al. 2021); NC_025569 *Tyspanodes hypsalis* (Wang et al. 2016); NC_062173 *Lamoria adaptella* (Unpublished); NC_037501 *Endotricha consocia* (Zhu et al. 2018); NC_022476 *Ephestia kuehniella* (Traut et al. 2013).

enhancing the mitochondrial genomic data for the genus *Paortis*.

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Ethical approval

This study does not need ethical approval or permissions to collect, handling, and transport of the samples.

Author contributions

CSW and CDZ conceived the original idea. MX and QSZ carried out the experiment. MX, CSW and QSZ analyzed the data. MX, CDZ and QSZ wrote and proofread the manuscript. All authors agree to be accountable for all aspects of the work.

Disclosure statement

No potential competing interest was reported by the authors.

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Data availability statement

The genome sequence data annotation results of this study are publicly available in GenBank under accession number PP692447. The associated BioProject, SRA and Bio-Sample numbers are PRJNA1108866, SRR28963170 and SAMN41256338, respectively.

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