Complete mitochondrial genomes of two moths in the tribe Trichaeini (Lepidoptera: Crambidae) and their phylogenetic implications

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Abstract

The complete mitochondrial genomes of two Prophantis species in the tribe Trichaeini (Lepidoptera: Crambidae) were sequenced using high-throughput sequencing technology. They were assembled and annotated: the complete mitogenomes of P. octoguttalis and P. adusta were 15,197 bp and 15,714 bp, respectively, and contain 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes, and an A + T-rich region. Their arrangement was consistent with the first sequenced mitogenome of Lepidoptera, from Bombyx mori (Bombycidae). The nucleotide composition was obviously AT-biased, and all protein-coding genes, except for the cox1 gene (CGA), used ATN as the start codon. Except for trnS1, which lacked the DHU arm, all tRNA genes could fold into the clover-leaf structure. Phylogenetic trees of Crambidae were reconstructed based on mitogenomic data using Maximum likelihood (ML) and Bayesian inference (BI) analysis methods. Results showed that Trichaeini in this study robustly constitute a monophyletic group in Spilomelinae, with the relationships (Trichaeini + Nomophilini) + ((Spilomelini + (Hymeniini + Agroterini)) + Margaroniini). However, the affinities of the six subfamilies Acentropinae, Crambinae, Glaphyrinae, Odontiinae, Schoenobiinae and Scopariinae within the "non-PS Clade" in Crambidae remained doubtful with unstable topologies or low supports.

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Abstract

The complete mitochondrial genomes of two *Prophantis* species in the tribe Trichaeini (Lepidoptera: Crambidae) were sequenced using high-throughput sequencing technology. They were assembled and annotated: the complete mitogenomes of *P. octoguttalis* and*P. adusta* were 15,197 bp and 15,714 bp, respectively, and contain 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes, and an A + T-rich region. Their arrangement was consistent with the first sequenced mitogenome of Lepidoptera, from *Bombyx*

mori(Bombycidae). The nucleotide composition was obviously AT-biased, and all protein-coding genes, except for the *cox1* gene (CGA), used ATN as the start codon. Except for *trnS1* , which lacked the DHU arm, all tRNA genes could fold into the clover-leaf structure. Phylogenetic trees of Crambidae were reconstructed based on mitogenomic data using Maximum likelihood (ML) and Bayesian inference (BI) analysis methods. Results showed that Trichaeini in this study robustly constitute a monophyletic group in Spilomelinae, with the relationships (Trichaeini + Nomophilini) + ((Spilomelini + (Hymeniini + Agroterini)) + Margaroniini). However, the affinities of the six subfamilies Acentropinae, Crambinae, Glaphyriinae, Odontiinae, Schoenobiinae and Scopariinae within the "non-PS Clade" in Crambidae remained doubtful with unstable topologies or low supports.

Keywords

mitogenome; phylogeny; Trichaeini; Spilomelinae; Crambidae

1 Introduction

The Pyraloidea, with more than 16,000 described species worldwide, is one of the largest groups in Lepidoptera, and it is composed of two families: Pyralidae and Crambidae, with Crambidae species accounting for 60% (Munroe & Solis 1999, Nuss et al., 2023). Regier et al. (2012) present a most detailed molecular estimate of relationships to date across the subfamilies of Pyraloidea based on five nuclear genes, in which the Crambidae was divided into three major lineages based on phylogenetic relationships: the "PS clade" (Pyraustinae, Spilomelinae, and Wurthiinae), the "OG clade" (Evergestinae, Glaphyriinae, Noordinae and Odontiinae), and the "CAMMSS clade" (Acentropinae, Crambinae, Musotiminae, Midilinae, Scopariinae and Schoenobiinae), forming a system of PS clade + (OG clade + CAMMSS clade). However, combined with the phylogenetic tree topology of the Pyraloidea based on mitogenic data, the phylogenetic relationship within "non-PS Clade" is not completely resolved in previous study (Yang et al., 2018b; Zhang et al., 2020; Qi et al., 2021; Liu et al., 2021). More molecular data, such as the mitogenomes, are in demand to reveal the phylogenetic relationships of the subfamilies in Crambidae.

Spilomelinae is the most species-rich subfamily in Crambidae, with 4,135 described species in 344 genera (Nuss et al., 2023). Currently, a total of 13 tribes in Spilomelinae have been defined by Mally et al. (2019) based on six molecular markers (COI, CAD, EF-1α, GAPDH, IDH and RpS5) and 114 adult morphological characters, including: Hydririni, Udeini, Lineodini, Wurthiini, Agroterini, Margaroniini, Spilomelini, Herpetogrammatini, Hymeniini, Asciodini, Trichaeini, Steniini and Nomophilini. Among them, Trichaeini is a tribe with the lowest species richness, with only four genera and 22 species (Nuss et al., 2023). This tribe includes the genus *Prophantis* Warren, 1896, which consists of eight species that have all been poorly studied besides their original descriptions (Warren, 1896). Only *Prophantis octoguttalis* Felder & Rogenhofer, 1875 and *P. adusta* Inoue, 1986 have been recorded from China. *P. octoguttalis* , the type species of the genus, is widespread, and is mainly distributed in southern China, Australia, India, and the Afrotropical region (Wang, 1980; Ratnasingham & Hebert, 2007). Its larvae feed on *Coffea arabica* Linnaeus, 1757, and a single larva can harm several berries in succession, which can seriously impact coffee production (Wang, 1980). The adults of*P. adusta* are very similar in appearance to those of *P. octoguttalis* , which makes species identification in these moths very challenging.

The mitochondrial genome (mtDNA) is a closed-loop DNA double helix molecule that varies significantly in length among taxa. The mtDNA of lepidopteran insects is generally 15–16 kb in size and consists of 37 genes, including 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs), and a control region of variable length also known as A+T-rich region and D-loop region (Boore, 1999). Because of its conserved genetic components, compact arrangement, fast evolutionary rate, and maternal inheritance, it contains relevant genetic and developmental information that can be used in phylogenetic studies for different research purposes (Wesley et al., 1979; Cameron, 2014). The mtDNA has been widely used in molecular phylogeny, phylogeography and genetic differentiation (Heise et al., 1995; Suzuki et al., 2013; Wang et al., 2019).

To date, only 23 mitogenomes of Spilomelinae have been published in GenBank, and no mitogenomes of

Trichaeini have been reported. In this study, we sequenced the mitogenomes of *P. octoguttalis* and*P. adusta* of the Trichaeini for the first time, and performed preliminary bioinformatics analysis, which can help us to understand the features of mitogenomes of Trichaeini. Meanwhile, to understand the phylogenetic relationship, indicated by mitochondrial genome, of Trichaeini in Spilomelinae, we reconstructed the phylogenetic tree based on the mitogenomes data of these two species with other available mitogenomes data of Crambidae in GenBank by using maximum likelihood and Bayesian inference methods. It will provides new perspectives and genomics data for the phylogenetic research in Trichaeini and Spilomelinae.

2 Materials and methods

2.1 Specimen collection and DNA sequencing

The specimen of *Prophantis octoguttalis* investigated was collected from Wuzhi Mountain in Hainan Province, China, in March 2021; the specimen of *P. adusta* was collected from Fanjing Mountain in Guizhou Province, China, in September 2020. Fresh specimens obtained by light trapping were soaked in anhydrous alcohol and stored at -80 °C in the Insect Collection of Southwest University, Chongqing, China. DNA was extracted from the thoracic muscle of each specimen. The mitogenome was entrusted to BGI Genomics for next-generation sequencing.

2.2 Sequence assembly, annotation and analysis

The high-quality data (clean data) of the samples, which were trimmed by BGI Genomics, were saved as fastq. format and imported into Geneious Prime v2022.1.1. The mitogenome with the closest affinity to the sample as a reference sequence was downloaded from GenBank, and sequence extension was performed using the "Map to reference" function until repetitive base alignments appeared, indicating that the mitochondrial genome was assembled into a loop.

MAFFT (Multiple Alignment using Fast Fourier Transform) alignment was used to align the reference sequence with the sample sequence, and protein-coding genes (PCGs) were determined based on the similarity between genes. With the help of EditSeq v7.1.0, PCGs were translated into amino acids to further verify the correctness of the start codon, stop codon, and amino acid sequence, to ensure the accuracy of PCGs. The location and secondary structure of tRNA genes were predicted using the MITOS Web Server (Donath et al., 2019), and the chart of secondary structure was mapped using Adobe Illustrator v26.0. rRNA genes are relatively conserved, and can be determined by the position between the two genes (Boore, 2006). The A+T-rich region was generally located behind the *rrnL* gene. Mitogenome maps were generated using Proksee (https://proksee.ca/). Sequence length, base composition, gene spacing, and overlap were viewed directly using Geneious Prime v2022.1.1. The base skew was calculated using the formula: AT skew = (A - T) / (A + T) and GC skew = (G - C) / (G + C) (Perna and Kocher, 1995). Relative synonymous codon usage (RSCU) was analyzed using MEGA v10.2.5.

2.3 Phylogenetic analysis

A total of 55 mitogenome sequences (2 newly determined in this study, 53 available from GenBank) were used to construct the phylogenetic tree. The ingroups included 5 species of Acentropinae, five species of Crambinae, one species of Glaphyriinae, three species of Odontiinae, eight species of Pyraustinae, one species of Schoenobiinae, one species of Scopariinae and 25 species of Spilomelinae. The four species (*Lista haraldusalis* , *Galleria mellonella* ,*Dioryctria yiai* and *Pyralis farinalis*) of Pyralidae,*Bombyx mori* of Bombycidae and *Helicoverpa armigera* of Noctuidae were selected as outgroups (Table 1).

We used two datasets: 1) PCG123: all three codon positions of 13 protein-coding genes; 2) PCG123RT: all three codon positions of 13 protein-coding genes, two rRNA genes and 22 tRNA genes. Maximum likelihood (ML) and Bayesian inference (BI) were used to construct phylogenetic trees.

ModelFinder (Kalyaanamoorthy et al., 2017) was used to partition the data based on Bayesian Information Criterion BIC, and find the best partitioning scheme and base substitution models for ML and BI. Maximum likelihood was analyzed using IQ-TREE v1.6.8 (Minh et al., 2013; Nguyen et al., 2015), with the standard

bootstrap of 1000 replications; bootstrap values (BS) > 70% were considered to represent high confidence. Bayesian inference was analyzed using MrBayes v3.2.6, with the following parameters: two independent runs, each with four independent Markov Chain Monte Carlo runs, including three heated chains and one cold chain, were set to run for 1 x 10^7generations, with simultaneous sampling every 1,000 generations. The initial 25% of the sampled trees were discarded as burn-ins. Chain convergence was assumed when the mean standard deviation of the split frequencies fell below 0.01. Bayesian posterior probability, in which the support of each node of the BI tree was greater than or equal to 0.95, was considered high confidence. The phylogenetic tree was constructed using Figtree v.1.4.4.

3 Results and discussion

3.1 Basic structure

The full length of the mitochondrial genomes of *Prophantis octoguttalis* and *P. adusta* were 15,197 bp and 15,714 bp, respectively, including 37 genes and non-coding regions (Figure 1). Four protein-coding genes (*nad1* , *nad4* , *nad5* , and*nad4l*), two rRNA genes (*rrnL* and *rrnS*), and eight tRNA genes (*trnQ* , *trnC* , *trnY* , *trnF* ,*trnH* , *trnP* , *trnL1* , and *trnV*) were encoded from the minority strands. The remaining 23 genes were encoded from the majority of the strands (Table 2). The mitogenomes of both species were arranged in the same order as that of *Bombyx mori* (Linnaeus, 1758), which is the model organism in Lepidoptera (Dai et al., 2013). There were eight gene overlaps and 15 gene gaps in the mitogenome of*P. octoguttalis* , while five genes overlapped and 18 gene gaps were found in the mitogenome of *P. adusta* .

Figure 1. Visualization of the mitochondrial genomes of*Prophantis octoguttalis* and *P. adusta*

The mitogenome sequences of both species showed obvious AT biases. The nucleotide content of the *P. octoguttalis* mitogenome was A: 41.0%, T: 40.5%, C: 11.0%, and G: 7.5%, and for the *P. adusta*mitogenome was A: 40.8%, T: 40.7%, C: 11.0%, and G: 7.4%. The AT contents were 81.5% and 81.6%, respectively, which were much higher than the GC content. The AT skew was 0.006 and 0.001, and the GC skew was -0.189 and -0.196, respectively, showing a slight A skew and a significant C skew (Table 3).

3.2 Protein-coding genes and codon usage

Thirteen protein-coding genes were identified in the mitogenomes of *P. octoguttalis* and*P. adusta* . Among them, *atp8* , *atp6* , *cox1* ,*cox2* , *cox3* , *nad2* , *nad3* , *nad6* , and*cytb* were encoded by the majority strand, and the remaining four genes were encoded by the minority strand. In *P. octoguttalis* , there was a 7 bp overlap between *atp8* and *atp6* and 1 bp overlap between *atp6* and *cox3* . In *P. adusta* , there was only a 7 bp overlap between *atp8* and *atp6* . The start codons of all genes were typical ATN (ATT, ATA, ATG), except for*cox1* , whose start codon was CGA. The stop codons of *cox1*and *cox2* in *P. octoguttalis* were terminated by an incomplete stop codon T, and the remaining genes were terminated by TAA, which was the most frequent stop codon. Among the protein-coding genes, the AT content was 80.3% and 79.6%, respectively. The AT bias of these two species was more significant in the third codon, and the AT content of the third codon (83.2%, 85.8%) was higher than that of the first (73.1%, 82.7%) and second codons (74.9%, 79.8%). The AT skew of these two species was 0.01 and 0.003, and their GC skew was -0.173 and -0.181, respectively, showing a slight A skew and an obvious C skew.

Figure 2. Relative synonymous codon usage (RSCU) of*Prophantis octoguttalis* and *P. adusta*

The concatenated lengths of the 13 PCGs of *P. octoguttalis* and *P. adusta* were 11,196 bp and 11,219 bp, encoding 3721 and 3728 amino acids, respectively. Statistics on the relative synonymous codon usage (RSCU) of *P. octoguttalis*and *P. adusta* showed that the codons UUA(L), AUU(I), UUU(F), AUA(M) and AAU(N) were used most frequently. In *P. octoguttalis* , CUG, GUC, CCG, CGG, AGC and AGG do not participate in amino acid synthesis, while in *P. adusta* , CUG and AGG do not participate. The codons of amino acids with RSCU > 1 all contained A or U (Figure 2), and the preference of these codons indirectly reflected the AT preference of the base.

3.3 rRNA genes and tRNA genes

In the mitogenomes of *P. octoguttalis* and *P. adusta* , two rRNA genes were encoded by the minority strand, with concatenated lengths of 2092 bp and 2077 bp, respectively. The *rrnL* gene was located between the *trnL1* and *trnV* genes, which were 1355 bp and 1341 bp long, respectively; the *rrnS* gene was located between the*trnV* gene and the A+T-enriched regions, which were 737 bp and 736 bp long, respectively.

In the mitogenomes of these two species, there were 22 tRNA genes with concatenated lengths of 1468 bp and 1481 bp, respectively. A total of 14 genes (*trnM* , *trnI* , *trnW* , *trnL2* , *trnK* ,*trnD* , *trnG* , *trnA* , *trnR* , *trnN* ,*trnS1* , *trnE* , *trnT* , and *trnS2*) were encoded by the majority chain, and the remaining eight genes were encoded by the minority chain, with the length of each gene ranging from 64 bp (*P. octoguttalis*) – 71 bp. Except for *trnS1* (AGN), which lacked the DHU arm, the secondary structures of the remaining 21 tRNAs folded into a typical clover-leaf structure (Figure 3). There were G-U and U-U base mismatches in the tRNA genes, which mostly occurred in the DHU, AA acceptor, and anticodon arms.

The AT content of the RNA gene of these two species was more than 80%, showing an obvious AT bias. As for base skew, both species showed a slight A skew and an obvious C skew.

Figure 3. Secondary structure of tRNA of *Prophantis octoguttalis* and *P. adusta*

3.4 Non-coding regions

The mitogenome of *P. octoguttalis* had eight gene overlaps totaling 24 bp, with a maximum overlap length of 8 bp between the *trnW* and*trnC* genes, and 15 gene spacings totaling 172 bp, with a maximum spacing length of 45 bp between the *trnQ* and *nad2* genes. The mitogenome of *P. adusta* had five gene overlaps totaling 21 bp, with a maximum overlap length of 8 bp between the *trnW* and*trnC* genes, and 18 gene spacings totaling 240 bp, with a maximum spacing length of 54 bp between the *trnS1* and *trnE* genes.

The control regions of the mitogenomes of these two species were located between the *rrnS* and *trnM* genes, with full lengths of 327 bp and 735 bp, respectively. Both sequences showed a clear AT bias, with an AT content of 96.0% and 96.7%, respectively, which was significantly higher than that of GC. The AT skew and GC skew of both sequences were negative, showing a slight T skew and an obvious C skew.

3.5 Phylogenetic relationships

The mitogenomes of 55 Lepidoptera species were used in this study, including eight subfamilies of Crambidae as ingroups, with four Pyralidae species, *Helicoverpa armigera* (Noctuidae) and*Bombyx mori* (Bombycidae) as outgroups. Four phylogenetic trees of Crambidae were reconstructed using ML and BI analyses based on two datasets: PCG123 and PCG123RT (Figure 4). All phylogenetic trees showed the monophyly of Crambidae and was strongly supported (PP=1/BS=100).

Figure 4. Phylogenetic tree constructed with BI and ML analyses based on two datasets show the similar topology except for the "non-PS clade": (A) the BI tree of dataset PCG123RT and the ML tree of dataset PCG123. (B) the "non-PS clade" of ML tree of dataset PCG123RT. (C) the "non-PS clade" of BI tree of dataset PCG123. The values around the nodes are posterior probability (PP) and bootstrap support (BS)

The eight subfamilies of Crambidae in all phylogenetic tree was divided into two major sister lineages, the "PS clade" and the "non-PS clade", which was first defined by Regier et al. (2012). Spilomelinae and Pyraustinae were sister groups to each other (PP=1/BS=100), forming the "PS clade", which was consistent with previous studies based on molecular data (Regier et al., 2012; Leger et al., 2020) or mitogenomic data (Yang et al., 2018b; Zhang et al., 2020; Jeong et al., 2021; Liu et al.,2021; Qi et al., 2021).

In Spilomelinae, all phylogenetic results showed that the monophyly of Trichaeini was well supported (PP=1/BS=100), and the relationships within Spilomelinae were (Trichaeini + Nomophilini) + ((Spilomelini + (Hymeniini + Agroterini)) + Margaroniini). With the exception of the newly sequenced species of Trichaeini, the phylogenetic relationship among the tribes was roughly consistent with Liu et al. (2021) that Agroterini, Hymeniini, Margaroniini and Spilomelini are grouped into one branch, sister to Nomophilini. Our results showed that Trichaeini and Nomophilini were related to each other as a sister group (PP=1/BS=86) and were first separated from the base of the subfamily Spilomelinae. This confirmed the results of Matsui

et al. (2022) based gene fragments. However, in the phylogenetic tree in Mally et al. (2019) based on gene fragments, Trichaeini and (Steniini + Nomophilini) formed a sister group relationship, which was inconsistent with (Trichaeini + Nomophilini) + Steniini in Matsui et al. (2022). Therefore, more samples, especially those of the closely related species of Steniini and Nomophilini, are expected to sequenced for the complete mitochondrial genomes in the future research, in order to clarify the phylogenetic relationships among these three tribes.

The differences among the four phylogenetic trees constructed in this study were mainly concentrated in the "non-PS clade", which consisted of the remaining six subfamilies (Acentropinae, Crambinae, Glaphyriinae, Odontiinae, Schoenobiinae, Scopariinae). The "non-PS clade" was divided into the "OG clade" and the "CAMMSS clade" (PP=1/BS=98) in the BI tree of dataset PCG123RT and the ML tree of dataset PCG123. The "OG clade" consisted of Glaphyriinae and Odontiinae, which were related to each other as sister groups, with a high to low support (PP= 1/BS=55) and the monophyly of Odontiinae was highly supported (PP=1/BS=100). Acentropinae, Crambinae, Schoenobiinae and Scopariinae formed the "clade CAMMSS" which presented two close relationships, Acentropinae and Schoenobiinae as sister group (PP=1/BS=81), Scopariinae and Crambinae as sister group with a high to low support (PP=0.93/ BS=57). This was consistent with the results of Regier et al. (2012) and Leger et al. (2020) based on molecular data. Meanwhile, this result also confirmed the mitogenome-based results of Qi et al. (2020); Jeong et al. (2021) and Liu et al. (2021), which were based on the ML and BI trees of dataset PCG123R, PCG12 and PCG12RT, the ML phylogenetic trees of dataset AA and the BI trees of dataset PCG123 and PCG123RT. The affinities of the subfamilies in the "CAMMSS clade", which based on the ML tree of dataset PCG123RT in this study, were exhibited different topologies: Scopariinae + (Crambinae + (Acentropinae + Schoenobiinae)), which was consistent with the ML tree of dataset PCG123 and PCG123RT in Liu et al. (2021). In the BI tree of dataset PCG123, the phylogenetic relationship of the "non-PS clade" was: Odontiinae + ((Scopariinae + Glaphyriinae) + (Acentropinae + ((Schoenobiinae + Crambinae))),with low support, which was completely different from the above situation. The phylogenetic topology varies among the subfamilies within the "non-PS clade" in different datasets, probably due to with only one sample each in Schoenobiinae, Scopariinae and Glaphyriinae, thus causing a long branch attraction.

On the basis of the above analyses, our analyses confirmed the sister relationship of Pyraustinae and Spilomelinae with strong support. Trichaeini in this study robustly constitute a monophyletic group in Spilomelinae, with the relationships (Trichaeini + Nomophilini) + ((Spilomelini + (Hymeniini + Agroterini)) + Margaroniini). Within the "non-PS clade", the monophyly of Acentropinae, Crambinae, and Odontiinae were well supported. The close relationship between Odontiinae and Glaphyriinae, between Schoenobiinae and Acentropinae, and between Scopariinae and Crambinae seemed to be more realistic.

4 Conclusions

In this study, we reported the complete mitogenomes of two*Prophantis* species, *P. octoguttalis* and *P. adusta* , belonging to the tribe Trichaeini, for the first time, and analyzed their gene size and arrangement, base composition, codon usage, and tRNA secondary structure, etc., which were highly consistent with those of other previously studied species of Spilomelinae. The two mitogenomes were typical of lepidopteran insects. Combined with the published mitogenome sequences of Crambidae, all phylogenetic trees based on the different datasets confirmed the monophyly and position of Trichaeini and showed satisfactorily high support values. However, its sister group was not completely resolved, combined with previous multisite studies. In addition, the phylogenetic relationships within Crambidae in phylogenetic tree in our present study were in general agreement with previous studies, whereas the affinities in the "non-PS clade" were still unstable and require further investigation. Therefore, improving sample coverage and combining different molecular markers, such as mitochondrial genome and nuclear genes, should be considered in the future research on these taxa.

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Conflicts of Interest

All authors declare no conflicts of interest.

Author contributions

Ci Tang : Conceptualization (equal); Software (lead); Formal Analysis (lead); Methodology (lead); Data Curation (equal); Writing-original draft (lead); Writing-review & editing (equal).

Xicui Du : Conceptualization (equal); Data Curation (equal); Funding acquisition (lead); Project administration (lead); Resource (lead); Supervision (lead); Writing-review & editing (equal).

Data Availability Statement

GenBank accession number: *Prophantis octoguttalis* (OP559507) and*P. adusta* (OP559508).

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Figures legends.

Figure 1. Visualization of the mitochondrial genomes of*Prophantis octoguttalis* and *P. adusta*

Figure 2. Relative synonymous codon usage (RSCU) of*Prophantis octoguttalis* and *P. adusta*

Figure 3. Secondary structure of tRNA of *Prophantis octoguttalis* and *P. adusta*

Figure 4. Phylogenetic tree constructed with BI and ML analyses based on two datasets show the similar topology except for the "non-PS clade": (A) the BI tree of dataset PCG123RT and the ML tree of dataset PCG123. (B) the "non-PS clade" of ML tree of dataset PCG123RT. (C) the "non-PS clade" of BI tree of dataset PCG123. The values around the nodes are posterior probability (PP) and bootstrap support (BS)

Tables.

Table 1. The mitochondrial genome sequences used in the phylogenetic analyses

Table 1. should go to 2.3 Phylogenetic analysis

Family	Subfamily	Species	GenBank ID	References
Bombycidae	Bombycinae	*Bombyx mori*	NC002355	Direct Submission
Crambidae	Acentropinae	*Cataclysta lemnata*	MT410858	Direct Submission
		Elophila interruptalis	KC894961	Park et al., 2014
		Parapoynx crisonalis	KT443883	Direct Submission
		Paracymoriza distinctalis	KF859965	Ye and You, 2016
		Paracymoriza prodigalis	JX144892	Ye et al., 2013
	Crambinae	*Chilo auricilius*	KJ174087	Cao and Du et al., 2014
		Chilo sacchariphagus	KU188518	Direct Submission
		Chilo suppressalis	JF339041	Chai et al., 2012
		Diatraea saccharalis	FJ240227	Li et al., 2011
		Pseudargyria interruptella	KP071469	Direct Submission
	Glaphyriinae	*Evergestis junctalis*	KP347976	Direct Submission
	Odontiinae	*Dausara latiterminalis*	MW732137	Qi et al., 2021

Family	Subfamily	Species	GenBank ID	References
		Heortia vitessoides	NC056800	Qi et al., 2021
		Pseudonoorda nigropunctalis	MW732139	Qi et al., 2021
	Pyraustinae	*Loxostege aeruginalis*	MN635734	Wu et al., 2022
		Loxostege sticticalis	KR080490	Ma et al., 2016
		Loxostege turbidalis	MN646773	Wu et al., 2022
		Ostrinia furnacalis	NC056248	Li et al., 2020
		Ostrinia nubilalis	NC054270	Fisher et al., 2020
		Ostrinia scapulalis	MT801073	Gschloessl et al., 2020
		Ostrinia zealis	NC048888	Zhou et al., 2020
		Pyrausta despicata	MN956508	Wu et al., 2022
	Schoenobiinae	*Scirpophaga incertulas*	NC031329	Cao et al., 2014
	Scopariinae	*Eudonia angustea*	KJ508052	Timmermans et al., 2014
	Spilomelinae	*Botyodes principalis*	MZ823351	Liu et al., 2021
		Cnaphalocrocis medinalis	JQ305693	Yin et al., 2014
		Conogethes pinicolalis	MT674993	Jeong et al., 2021
		Conogethes punctiferalis	NC021389	Wu et al., 2013
		Cydalima perspectalis	MH602288	Que et al., 2019
		Glyphodes pyloalis	NC025933	Kong and Yang, 2016
		Glyphodes quadrimaculalis	KF234079	Park et al., 2015
		Haritalodes derogata	KR233479	Zhao et al., 2016
		Marasmia exigua	MN877384	Zhang et al., 2020
		Maruca testulalis	KJ623250	Zou et al., 2016
		Maruca vitrata	NC024099	Direct Submission
		Nagiella inferior	MF373813	Direct Submission
		Nomophila noctuella	KM244688	Tang et al., 2014
		Omiodes indicata	MG770232	Yang et al., 2018a
		Palpita hypohomalia	MG869628	Yang et al., 2018b
		Palpita nigropunctalis	KX150458	Direct Submission
		Prophantis adusta		This study
		Prophantis octoguttalis		This study
		Pycnarmon lactiferalis	KX426346	Chen et al., 2016
		Pycnarmon pantherata	KX150459	Direct Submission
		Sinomphisa plagialis	MZ823346	Liu et al., 2021
		Spoladea recurvalis	KJ739310	He et al., 2015
		Syllepte taiwanalis	MZ823348	Liu et al., 2021
		Tyspanodes hypsalis	KM453724	Wang et al., 2016
		Tyspanodes striata	KP347977	Direct Submission
Noctuidae	Heliothinae	*Helicoverpa armigera*	NC014668	Yin et al., 2010
Pyralidae	Epipaschiinae	*Lista haraldusalis*	KF709449	Ye et al., 2015
	Galleriinae	*Galleria mellonella*	KT750964	Park et al., 2017
	Phycitinae	*Dioryctria yiai*	MN658208	Wu et al., 2020
	Pyralinae	*Pyralis farinalis*	MN442120	Mao et al., 2019

Table 2. Mitogenomic organization of *Prophantis octoguttalis* and *P. adusta*

Table 2. should go to 3.1 Basic structure

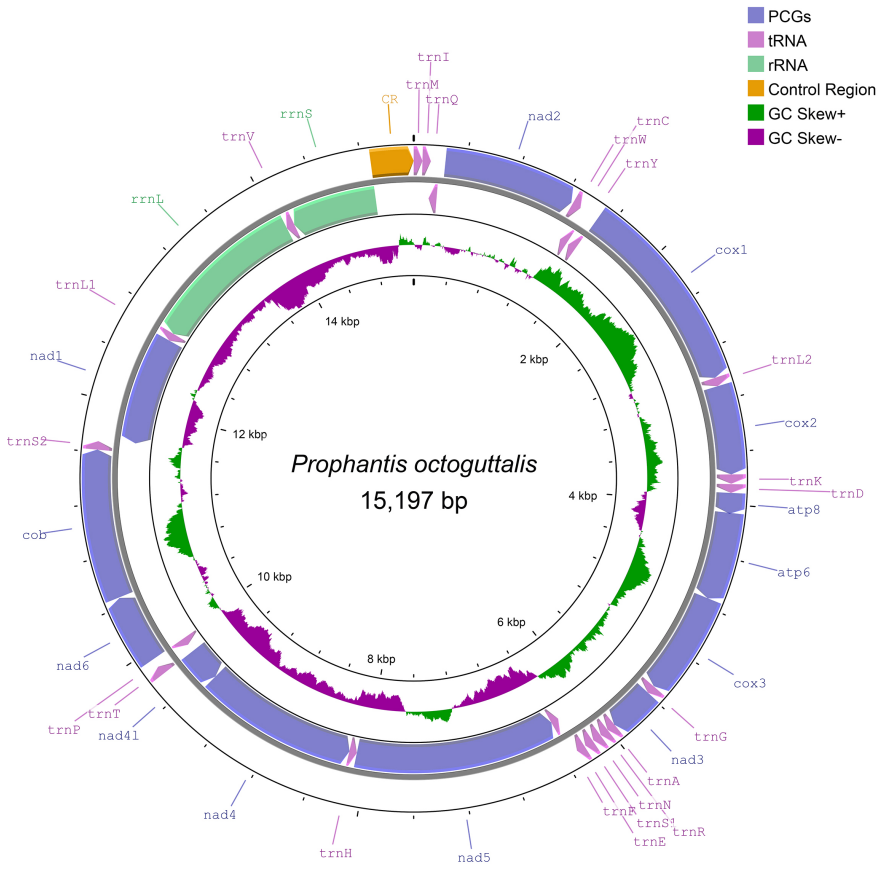
Gene	Strand	Position	Position	Size	Size	Intergenic nucleotides	Intergenic nucleotides	Start / Stop Codon	Start / Stop Codon
		Po	Pa	Po	Pa	Po	Pa	Po	Pa
trnM	J	1-67	1-68	67	68	0	0		
trnI	J	68-131	69-133	64	65	-3	-3		
trnQ	N	129-197	131-199	69	69	45	46		
nad2	J	243-1256	246-1259	1014	1014	13	11	ATT/TAA	ATT/TAA
trnW	J	1270-1337	1271-1338	68	68	-8	-8		
trnC	N	1330-1394	1331-1400	65	70	19	20		
trnY	N	1414-1482	1421-1487	69	67	8	15		
cox1	J	1491-3021	1503-3033	1531	1531	0	0	CGA/T-	CGA/T-
trnL2	J	3022-3088	3034-3100	67	67	0	0		
cox2	J	3089-3770	3101-3782	682	682	0	0	ATG/T-	ATG/T-
trnK	J	3771-3841	3783-3853	71	71	3	3		
trnD	J	3845-3911	3857-3924	67	68	0	0		
atp8	J	3912-4070	3925-4089	159	165	-7	-7	ATA/TAA	ATA/TAA
atp6	J	4064-4738	4083-4757	675	675	-1	8	ATG/TAA	ATG/TAA
cox3	J	4738-5526	4766-5554	789	789	2	2	ATG/TAA	ATG/TAA
trnG	J	5529-5593	5557-5621	65	65	0	0		
nad3	J	5594-5947	5622-5975	354	354	-1	12	ATA/TAA	ATT/TAA
trnA	J	5947-6011	5988-6053	65	66	1	-1		
trnR	J	6013-6076	6053-6118	64	66	4	14		
trnN	J	6081-6145	6113-6198	65	66	7	9		
trnS1	J	6153-6218	6208-6273	66	66	9	54		
trnE	J	6228-6293	6328-6394	66	67	-2	-2		
trnF	N	6292-6358	6393-6462	67	70	0	0		
nad5	N	6359-8093	6463-8197	1735	1735	0	0	ATT/T-	ATT/T-

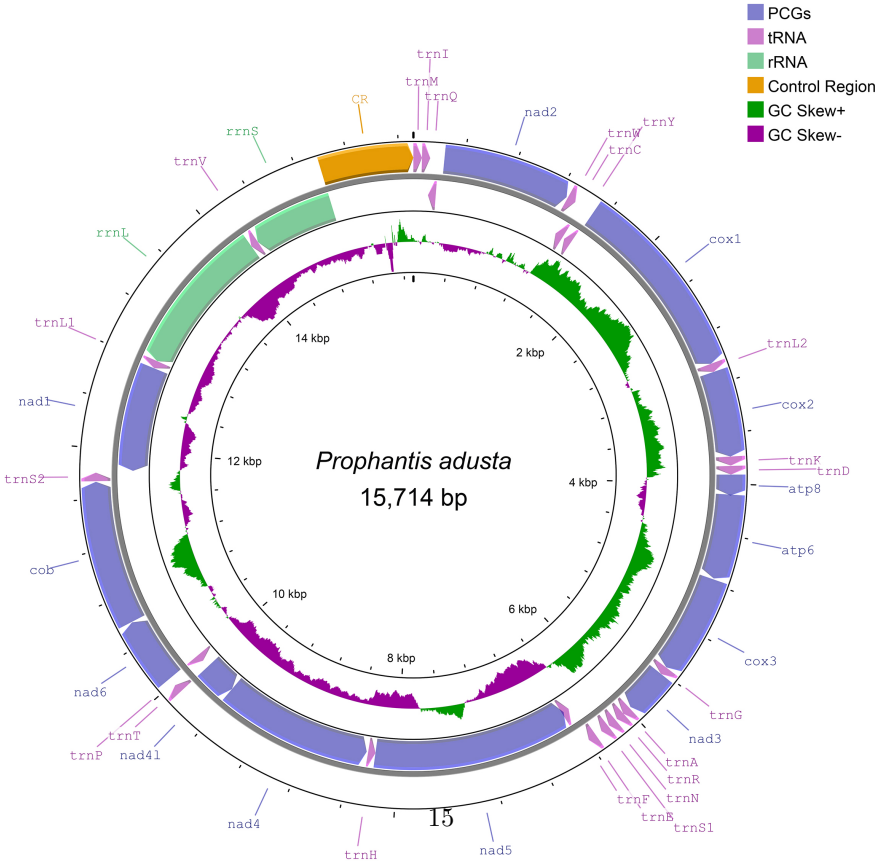
Gene	Strand	Position	Position	Size	Size	Intergenic nucleotides	Intergenic nucleotides	Start / Stop Codon	Start / Stop Codon
trnH	N	8094-8159	8198-8263	66	66	-1	13		
nad4	N	8159-9499	8277-9617	1341	1341	0	0	ATG/TAA	ATG/TAA
nad4l	N	9500-9793	9618-9911	294	294	2	2	ATG/TAA	ATG/TAA
trnT	J	9796-9862	9914-9979	67	66	0	0		
trnP	N	9863-9928	9980-10045	66	66	2	2		
nad6	J	9931-10464	10048-10581	534	534	5	4	ATT/TAA	ATT/TAA
cob	J	10470-11618	10586-11734	1149	1149	-1	5	ATG/TAA	ATG/TAA
trnS2	J	11618-11682	11740-11806	65	67	18	19		
nad1	N	11701-12639	11826-12764	939	939	0	1	ATG/TAA	ATG/TAA
trnL1	N	12640-12707	12766-12833	68	68	29	0		
rrnL	N	12708-14062	12834-14174	1355	1341	0	0		
trnV	N	14063-14133	14175-14243	71	69	0	0		
rrnS	N	14134-14870	14244-14979	737	736	0	0		
CR		14871-15197	14980-15714	327	735				

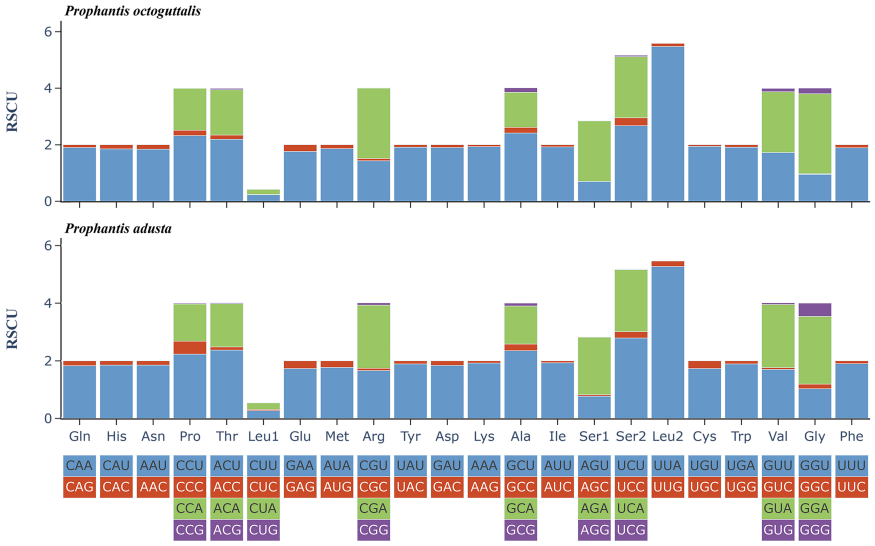
Table 3. Nucleotide composition of *Prophantis octoguttalis* and *P. adusta*

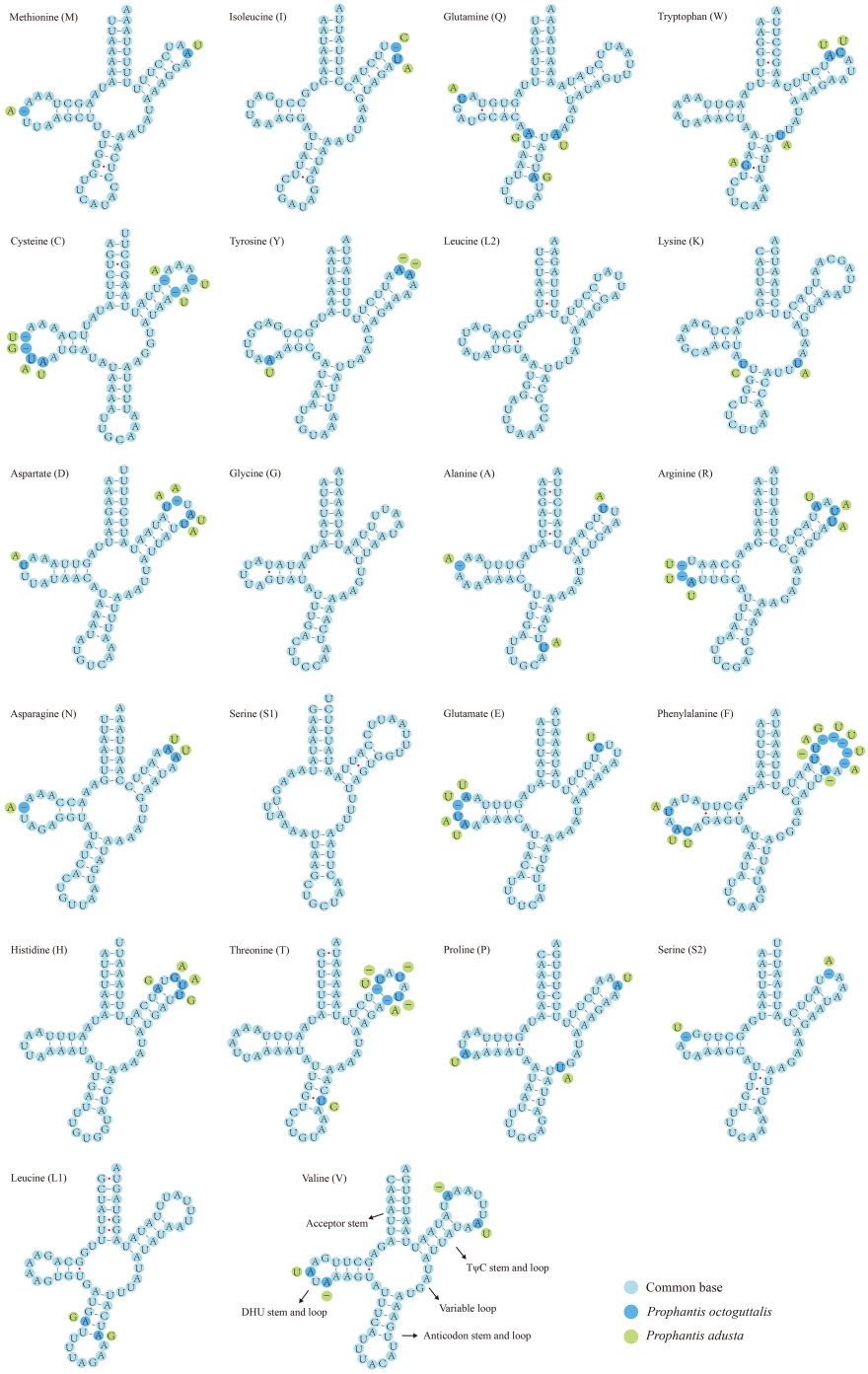
Table 3. should go to 3.1 Basic structure

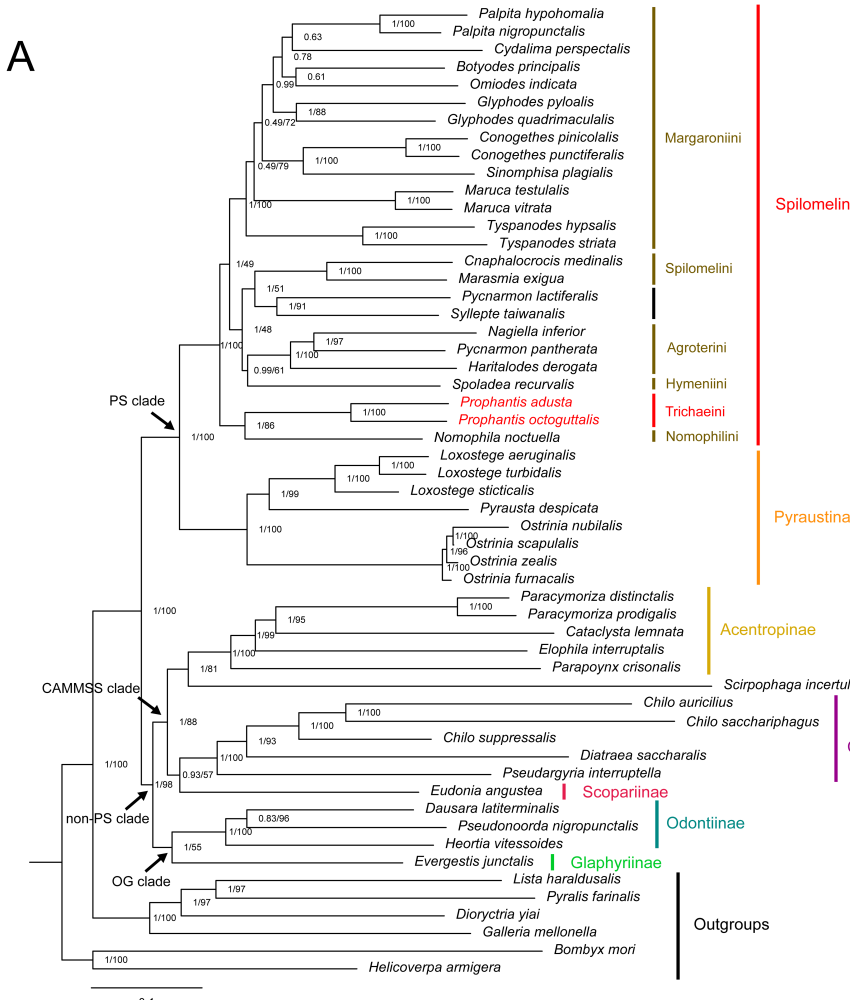
Regions	T%	T%	C%	C%	A%	A%	G%	G%	A+T%	A+T%	AT skew	AT skew	GC skew	GC
	Po	Pa	Po	Pa	Po	Pa	Po	Pa	Po	Pa	Po	Pa	Po	Pa
Whole	40.5	40.7	11.0	11.0	41.0	40.8	7.5	7.4	81.5	81.6	0.006	0.001	-0.189	-0.1
PGCs	39.8	39.7	11.6	12.1	40.5	39.9	8.2	8.4	80.3	79.6	0.01	0.003	-0.173	-0.1
1st codon	41.6	35.2	9.0	14.4	41.1	37.9	8.4	12.5	82.7	73.1	-0.006	0.037	-0.034	-0.0
2st codon	36.8	42.5	14.6	13.4	38.1	37.3	10.5	6.7	74.9	79.8	0.017	-0.065	-0.163	-0.3
3st codon	41.0	41.4	11.1	8.4	42.2	44.4	5.7	5.8	83.2	85.8	0.014	0.035	-0.321	-0.1
rRNA	42.4	43.3	10.1	9.7	42.5	42.0	5.0	5.0	84.9	85.3	0.001	-0.015	-0.338	-0.3
tRNA	40.7	39.8	9.9	10.3	41.4	42.1	7.9	7.7	82.2	82.0	0.009	0.028	-0.112	-0.1
RNAs	41.7	41.9	10.0	10.0	42.1	42.0	6.2	6.1	83.8	83.9	0.005	0.001	-0.235	-0.2
CR	49.8	49.7	3.1	2.2	46.2	47.1	0.9	1.1	96.0	96.7	-0.038	-0.027	-0.55	-0.3











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Tables 1-3.docx available at https://authorea.com/users/513468/articles/630769-complete-mitochondrial-genomes-of-two-moths-in-the-tribe-trichaeini-lepidoptera-crambidae-and-their-phylogenetic-implications