The complete mitochondrial genomes of two moths in the tribe Trichaeini (Lepidoptera: Crambidae) and the phylogenetic implications for Pyraloidea

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Abstract

The complete mitochondrial genomes of two Prophantis Warren, 1896 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 Pyraloidea were reconstructed based on mitogenomic data using Maximum likelihood (ML) and Bayesian inference (BI) analysis methods. The results showed that Trichaeini formed a monophyletic group with high branch support in Spilomelinae, sister to Nomophilini. In addition, the phylogenetic relationships among subfamilies of Pyraloidea were generally stable: (Galleriinae + ((Epipaschiinae + Pyralinae) + Phycitinae)) + ((Pyraustinae + Spilomelinae) + ((Odontiinae + Glaphyrinae) + CAMMSS clade)), although the affinities of some subfamilies in the "CAMMSS clade" were still unresolved.

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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 Pyraloidea were reconstructed based on mitogenomic data using Maximum likelihood (ML) and Bayesian inference (BI) analysis methods. The results showed that Trichaeini formed a monophyletic group with high branch support in Spilomelinae, sister to Nomophilini. In addition, the phylogenetic relationships among subfamilies of Pyraloidea were generally stable: (Galleriinae + ((Epipaschiinae + Pyralinae) + Phycitinae)) + ((Pyraustinae + Spilomelinae) + ((Odontiinae + Glaphyrinae) + CAMMSS clade)), although the affinities of some subfamilies in the "CAMMSS clade" were still unresolved.

Keywords

mitogenome; phylogeny; *Prophantis* ; Spilomelinae

1 Introduction

The Pyraloidea, with more than 16,000 described species worldwide, is one of the largest groups in Lepidoptera, and is composed of two families, Pyralidae and Crambidae (Munroe & Solis 1999, Nuss et al., 2003–2022). There are many important agricultural pests in this group, such as *Cnaphalocrocis medinalis* (Guenée, 1854), *Ostrinia nubilalis* (Hübner, 1796),*Maruca vitrata* (Fabricius, 1787), *Spoladea recurvalis*(Fabricius, 1775), etc. In the present systematics of Pyraloidea, Crambidae, comprising about 60% of Pyraloidea, includes 15 subfamilies. Phylogenetic relationships within Crambidae were revealed based on ten genes totalling 11,247 bp by Léger et al. (2021): (Pyraustinae + Spilomelinae) + ((Odontiinae + (Linostinae + Glaphyrinae)) + ((Lathrotelinae + Musotiminae) + ((Midilinae + (Schoenobiinae + Acentropinae)) + (Hoploscopinae + ((Erupinae + (Heliothelinae + Scopariinae)) + Crambinae))))), where the sister group relationship of Pyraustinae and Spilomelinae has also been demonstrated in previous studies using molecular and morphological data (Regier et al., 2012; Ma et al., 2016; Yang et al., 2018 a and b; Mally et al., 2019; Zhang et al., 2020; Liu et al., 2021). Pyralidae comprises five subfamilies: Chrysauginae, Epipaschiinae, Galleriinae, Phycitinae, and Pyralinae (Regier et al., 2012; Léger et al., 2021).

Spilomelinae is the most species-rich subfamily in Crambidae, with 4,132 described species in 340 genera (Léger et al., 2021). A total of 13 tribes have been defined for Spilomelinae (Mally et al., 2019): Hydririni + ((Udeini + Lineodini) + (Wurthiini + (Agroterini + (Margaroniini + (Spilomelini + (Herpetogrammatini + ((Hymeniini + Asciodini) + (Trichaeini + (Steniini + Nomophilini)))))))))). Among them, Trichaeini is a tribe with less species richness, with only four genera and 22 species. This clade was established by Mally et al. (2019) in a phylogenetic analysis of Spilomelinae based on six molecular markers and 114 adult morphological characters. This tribe includes the genus*Prophantis* Warren, 1896, which consists of eight species that have all been poorly studied besides their original descriptions.*Prophantis octoguttalis*Felder & Rogenhofer, 1875 and *P. adusta* Inoue, 1986 have been recorded from China. *Prophantis octoguttalis* , the type species of the genus, is widespread, and is mainly distributed in southern China, Australia, India, and the Afrotropical region. Its larvae feed on*Coffea arabica* Linneaus, 1757, and a single larva can harm several berries in succession, which can seriously impact coffee production. The adults of *P. adusta* are very similar in appearance to those of *P. octoguttalis* , which can make identifying these moths very challenging.

The mitochondrial genome (mtDNA) is mostly 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 noncoding region of variable length (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 research on the origin and evolution of species, molecular phylogeny, lineage geography, and relationship between mitochondrial genes and diseases (Taanman, 1999; Johns, 1995; Suzuki et al., 2013).

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. Combined with the 72 sequences of Pyraloidea obtained from NCBI, we reconstructed the phylogenetic tree of Pyraloidea by using Maximum likelihood and Bayesian inference methods. The aim was to provide genomic data and genomic characteristics, and to provide a scientific basis for phylogenetic research of Trichaeini.

2 Materials and methods

2.1 Specimen collection and DNA sequencing

Prophantis octoguttalis was collected from Wuzhi Mountain in Hainan Province, China, in March 2021; *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. tRNA genes were predicted using the MITOS Web Server (Donath et al., 2019), and their 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 74 mitogenome sequences were used as ingroups, including 25 Pyralidae and 49 Crambidae species. One Bombycidae and one Noctuidae species were selected as outgroups (Table 1). We used four datasets: 1) PCG123: all three codon positions of 13 protein-coding genes; 2) PCG123R: all three codon positions of 13 protein-coding genes and two rRNA genes; 3) PCG123RT: 37 genes of the mitogenome; 4) PCG12: first and second codon positions of 13 protein-coding genes. Maximum likelihood (ML) and Bayesian inference (BI) were used to construct phylogenetic trees.

PartitionFinder 2 (Lanfear et al., 2016) was used to find the best partitioning scheme and base substitution models for ML and BI, with the following parameters: the model parameters were set to "mrbayes," the branch length was selected to "linked," and the "greedy" strategy was used to search; Bayesian Information Criterion (BIC) was used to partition the data; and the three codon sites of the protein-coding genes were represented by pos1, pos2, and pos3, respectively. Maximum likelihood was analyzed using IQ-TREE v1.6.8 (Minh et al., 2013; Nguyen et al., 2015), with Ultrafast 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 by the minority strands. The remaining 23 genes were encoded by 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. 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 (*nad1* , *nad4* , *nad5* , and *nad4l*) were encoded by the minority strand. 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 3732 and 3739 amino acids, respectively. Statistics on the relative synonymous codon usage (RSCU) of *P. octoguttalis*showed that the codons AUU (I), UAA (*) and AAU (N) were used most frequently; these codons, AUU (I), UUU (F) and AAA (K), were most frequently used in the mitogenome of *P. adusta.* 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 (*trnQ* ,*trnC* , *trnY* , *trnF* , *trnH* , *trnP* ,*trnL1* , and *trnV*) were encoded by the minority chain, with the length of each gene ranging from 64–71 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 76 Lepidoptera species were used in this study, including four subfamilies of Pyralidae and eight subfamilies of Crambidae, including Trichaeini, with *Helicoverpa armigera*(Hubner, 1805) (Noctuidae) and *Bombyx mori* (Bombycidae) as outgroups. Phylogenetic trees of Pyraloidea were reconstructed using ML and BI analyses based on four datasets: PCG123, PCG123R, PCG123RT, and PCG12. The phylogenetic trees obtained showed almost identical topological structures, and the monophyly of both Pyralidae and Crambidae was strongly supported (BS=100, PP=1). The topological structure of the phylogenetic tree with PCG123RT was different from that of the other three datasets; for brevity, only two phylogenetic hypotheses (PCG123 and PCG123RT) were presented here (Figures 4, 5).

In Pyralidae, 25 species were divided into four major branches, and the relationships of the four subfamilies could be expressed as Galleriinae + ((Epipaschiinae+ Pyralinae) + Phycitinae), which was consistent with previous results based on mitochondrial genomic data analysis (Yang et al., 2018a; Zhang et al., 2020; Liu et al., 2021; Qi et al., 2021). Morphologically, *Orybina* Snellen, 1895 is considered to be a genus of Pyralinae. However, all of our phylogenetic trees showed that*O. plangonalis* (Walker, 1859) and *O. regalis* Leech, 1889 were consistently located in the basal branches of the Pyralidae clade, rendering Pyralinae paraphyletic. The monophyly of *Orybina* was very strongly supported (BS=100, PP=1), which was consistent with the results of Liu et al. (2021).

Interestingly, *Orthaga* Walker, 1859 is recognized morphologically as a genus of Epipaschiinae, but *O. olivacea* (Warren, 1891) grouped with species of Pyralinae in both phylogenetic trees in this study, and *O. euadrusalis* Walker, 1859 was placed with*Lista haraldusalis* (Walker, 1858) to form the Epipaschiinae branch. The phylogenetic analysis of Yang et al. (2020) inferred that*O. olivacea* is more closely related to *Hypsopygia regina* (Butler, 1879) (Pyralinae), which is consistent with the affinities shown in our phylogenetic trees. Therefore, the monophyly of*Orthaga* has not been well established. Additional samples of this genus, with 49 species worldwide, are necessary for further investigations to explore its monophyly and higher relationships.

Figure 4. Phylogenetic tree constructed by BI analyses based on the PCG123 dataset. Posterior probability (PP) values was shown on the nodes

The Crambidae was divided into two major sister lineages, one consisting of Pyraustinae and Spilomelinae, forming the "PS clade," and the other consisting of the remaining subfamilies that form the "ono-PS clade," which was first defined by Regier et al. (2012). The "ono-PS clade" consists of the "OG clade" and "CAMMSS clade," and our phylogenetic trees showed that (Glaphyriinae + Odontiinae) aggregate into a branch with high support to form the "OG clade," and that (Schoenobiinae + Acentropinae) + (Scopariinae + Crambinae) aggregate into a "CAMMSS clade" with high support, which was compatible with previous studies that combined mitochondrial and nuclear genes for analysis. Notably, the ML and BI trees constructed based on PCG123RT (Figure 5) showed differences in the topological structures of the "CAMMSS clade": Scopariinae + (Crambinae + (Schoenobiinae + Acentropinae)), with strong support. This difference was also evident in the topology of the ML tree constructed by Liu et al. (2021) based on PCG123RT. In the present taxonomic system, Crambidae consists of 15 subfamilies, containing more than 10,000 species, and the mitochondrial genome data and nuclear genes of more species should be used to infer the phylogenetic relationships within this family.

Figure 5. Phylogenetic tree constructed by ML and BI analyses based on the PCG123RT dataset. Bootstrap support (BS) and posterior probability (PP) values were shown on the nodes

In Spilomelinae, *P. octoguttalis* and *P. adusta* aggregated into a branch with high support (BS=100, PP=1) to form the Trichaeini, which formed a sister-group relationship with Nomophilini. The phylogenetic analysis by Mally et al. (2019) identified Trichaeini + (Steniini + Nomophilini) in a tree constructed using COI, CAD, EF-1a, GAPDH, IDH, and RpS5. Our results do not conflict with theirs. Trichaeini and Nomophilini constituted a sister-group relationship in the phylogenetic analysis of Japanese Pyraustinae and Spilomelinae by Matsui et al. (2022), which was consistent with the results of the present study. All phylogenetic trees based on the different datasets showed satisfactorily high support values, confirming the monophyly and position of Trichaeini.

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 Pyraloidea, the results of the phylogenetic analyses reconstructed on different datasets indicated that Trichaeini was a monophyletic group, sister to Nomophilini. The phylogenetic relationships within Pyraloidea were in general agreement with previous studies, whereas the affinities in the "CAMMSS clade" were still unclear and require further study. Therefore, it is necessary to improve sample coverage and combine different molecular markers, such as nuclear genes with morphological characters, to further explore the phylogenetic relationships among the pyraloid subfamilies.

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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 by BI analyses based on the PCG123 dataset. Posterior probability (PP) values was shown on the nodes

Figure 5. Phylogenetic tree constructed by ML and BI analyses based on the PCG123RT dataset. Bootstrap support (BS) and posterior probability (PP) values were shown on the nodes

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

Family	Subfamily	Species	GenBank ID	References
		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
		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	*Orthaga euadrusalis*	MZ823349	Liu et al., 2021
		Orthaga olivacea	MN078362	Yang et al., 2020
		Lista haraldusalis	KF709449	Ye et al., 2015
	Galleriinae	*Cathayia obliquella*	MK550620	Roh et al., 2020
		Corcyra cephalonica	HQ897685	Wu et al., 2012
		Galleria mellonella	KT750964	Park et al., 2017

Family	Subfamily	Species	GenBank ID	References
		Paralipsa gularis	MW135332	Direct Submission
	Phycitinae	*Acrobasis inouei*	MZ823347	Liu et al., 2021
		Amyelois transitella	KT692987	Chang and Shen, 2016
		Dioryctria rubella	MZ823345	Liu et al., 2021
		Dioryctria yiai	MN658208	Wu et al., 2020
		Dusungwua basinigra	MZ902334	Liu et al., 2021
		Ephestia elutella	MG748858	Liu et al., 2018
		Ephestia kuehniella	KU877167	Direct Submission
		Euzophera pyriella	KY825744	Yang et al., 2017
		Meroptera pravella	MF073207	Living Prairie Mitogenomics Consortium, 201
		Plodia interpunctella	MN619781	Liu et al., 2016
	Pyralinae	*Aglossa dimidiata*	MW542312	Hu et al., 2021
		Endotricha consocia	MF568544	Zhu et al., 2018
		Endotricha olivacealis	MZ823344	Liu et al., 2021
		Hypsopygia regina	KP327714	Direct Submission
		Orthopygia glaucinalis	MN461479	Mao et al., 2019
		Orybina plangonalis	MF568543	Zhu et al., 2018
		Orybina regalis	MZ823350	Liu et al., 2021
		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		

Gene	Strand	Position	Position	Size	Size	Intergenic nucleotides	Intergenic nucleotides	Start / Stop Codon	Start / Stop Codon
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-
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		

Gene	Strand	Position	Position	Size	Size	Intergenic nucleotides	Intergenic nucleotides	Start / Stop Codon	Start / Stop Codon
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 ske
	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	-0.196
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	-0.181
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	-0.071
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	-0.333
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	-0.183
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	-0.32
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	-0.144
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	-0.242
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	-0.333