

Complete mitochondrial genomes of two leafroller moths (Tortricidae: *Cydia*) and their phylogenetic positions within the tribe Grapholitini

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ABSTRACT

The leafroller moths *Cydia glandicolana* and *Cydia kurokoi* are economically important pests. Here, we report the first complete mitochondrial genomes (mitogenomes) of these two species, with length of 15,395 bp and 15,246 bp, respectively. Each mitogenome contains 13 protein-coding genes (PCGs), 22 tRNA genes, two rRNA genes, and a single A + T-rich (D-loop) region. Their GC contents are 20.4% and 21.1%, respectively. Phylogenetic analysis, based on currently available sampling, supports the monophyly of the genus *Cydia* and indicates it is sister to the genus *Leguminivora*. Our study provides valuable genomic resources for taxonomic identification, biomonitoring, phylogenomics, and studies of genome evolution.

ARTICLE HISTORY

Received 7 October 2025
Accepted 18 February 2026

KEYWORDS

Mitochondrial genome;
phylogeny; pests; leafroller
moths; evolution

Introduction

The worldwide genus *Cydia* Hübner, [1825] 1816—placed in the tribe Grapholitini of the subfamily Olethreutinae (Lepidoptera: Tortricidae)—comprises around 250 described species (Jia et al. 2025). Many *Cydia* species are important fruit- and seed-feeding pests; notably, *Cydia glandicolana* Danilevsky, 1968 and *Cydia kurokoi* Amsel, 1960 are economically significant pests in China, the Korean Peninsula, and Japan (Komai and Ishikawa 1987; Brown and Komai 2008; Gilligan and Epstein 2014). In addition to these regions, *C. glandicolana* has also been recorded from southeastern Russia (Gilligan and Epstein 2014). *C. glandicolana* is characterized by a reddish body, inconspicuous pinacula concolorous with the integument, and 19–26 crochets on the abdominal prolegs (Brown and Komai 2008). In contrast, *C. kurokoi* has a whitish body, conspicuous pinacula darker than the surrounding integument, and 25–35 crochets on the abdominal prolegs (Brown and Komai 2008) (Figure 1). Mitochondrial genomes (mitogenomes), characterized by high copy numbers in cells, maternal inheritance, and a generally conserved structure with variable regions, have become increasingly valuable for insect species identification, phylogenetics, and evolutionary studies, especially with the declining cost of sequencing (as reviewed by Cameron 2014, 2025). In this study, we report the complete mitogenomes of *C. glandicolana* and *C. kurokoi*.

Materials and methods

Sample collection and sequencing

Samples of *C. glandicolana* and *C. kurokoi* were collected during the oak fruit fall period, as both species are fruit-boring insects. Insect-infested fallen fruits exhibiting clear insect tunnels were carefully collected and reared in laboratory conditions until the larvae emerged as adults. Specimens were deposited at the Institute of Zoology, Chinese Academy of Sciences (IOZ-CAS; <http://english.ioz.cas.cn/>; Guanliang Meng, guanliang.meng@ioz.ac.cn) under the voucher numbers IOZ-CAS 47_D12 and IOZ-CAS 48_G1, respectively. *C. glandicolana* was collected from the Kunming Botanical Garden, China (25.140634° N, 102.742879° E), with *Quercus chenii* (Fagaceae) serving as the host plant. *C. kurokoi* was collected from the Baotianman National Nature Reserve in Henan Province, China (33.495223° N, 111.923999° E), where the host plant is *Quercus variabilis*. The specimens were preserved in 100% ethanol and stored at –20 °C until DNA extraction. Total DNA was extracted from the muscle using the CTAB method. DNBSEQ short-read libraries (300–400 bp insert size) were constructed and approximately 8 Gbp of raw sequence data per specimen were generated on a DNBSEQ-T7 platform using the paired-end 150 bp strategy.

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 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/23802359.2026.2636405>.

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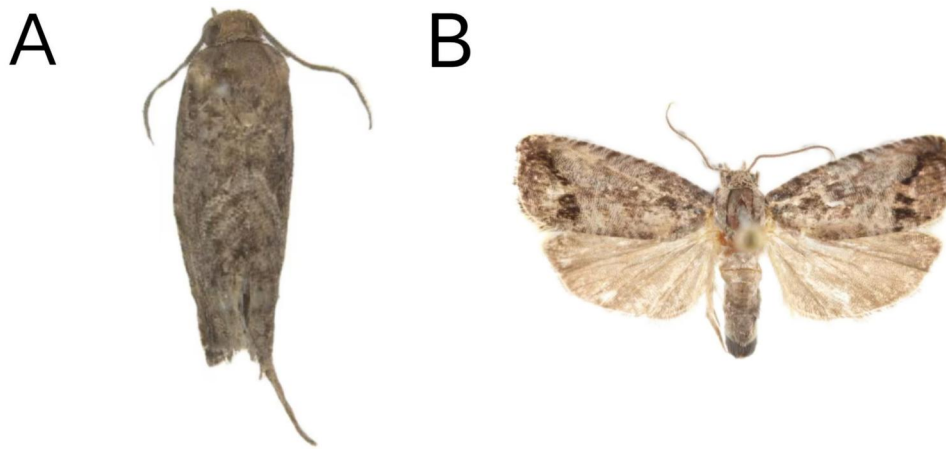


Figure 1. Photographs of the two *Cydia* species. (A) *Cydia glandicolana*. Photo credit: Tengting Liu, Shandong Normal University. License: Creative Commons—Attribution Non-Commercial Share-Alike (2020) (<https://creativecommons.org/licenses/by-nc-sa/3.0/>). Source: https://v3.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=248995. (B) *Cydia kurokoi*. Photo credit: Andrew J. Frewin, Centre for Biodiversity Genomics. License: Creative Commons—Attribution (2013) (<https://creativecommons.org/licenses/by/3.0/>). Source: https://v3.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=558580. Changes to the two photos: cropped. No warranties are given.

Genome assembly and annotation

Adapters and low-quality sequences were filtered with fastp (v0.24.0) (Chen et al. 2018) using the default parameters. Mitogenomes were assembled with MEGAHIT (v1.2.9) (Li et al. 2015) in MitoZ (v3.6) (Meng et al. 2019), and then verified by mapping the clean reads back to the mitogenomes using the BWA-MEM algorithm (0.7.18-r1243-dirty) (Li 2013). Per-site coverage was calculated with SAMtools (v1.21) (depth -a -a) (Danecek et al. 2021) and visualized with Seaborn (v0.13.2) (Waskom 2021).

Genome annotations were performed with MitoZ and MITOS2 (Donath et al. 2019). The gene boundaries predicted by the two methods were then compared, and the sequences were manually inspected in AliView (v1.27) (Larsson 2014). In cases of conflict, the gene (or protein) sequences were compared against those of closely related species (see section ‘Phylogenetic analysis’). Gene boundaries that were consistent with conserved patterns observed in closely related species were adopted. Taxonomic assignment of these two specimens was further confirmed by blasting the COX1 gene to the NT database and BOLD system (Ratnasingham et al. 2024), respectively, yielding similarities of 99.41–100%. The visualization of the two mitogenomes was conducted with OGDRAW (v1.3.1) (Greiner et al. 2019).

Phylogenetic analysis

To determine the phylogenetic placements of these two *Cydia* species in the tribe Grapholitini, we downloaded the mitogenomes of five other Grapholitini species, which include the codling moth *C. pomonella* (JX407107; Shi et al. 2013), *Grapholita dimorpha* (KJ671625; Niu et al. 2016), *Leguminivora glycinivorella* (MZ506761), *Matsumuraesia phaseoli* (OP575915), and *Thaumatotibia leucotreta* (MW697088). For rooting the tree, we selected *Adoxophyes honmai* (DQ073916; Lee et al. 2006) from another subfamily,

Tortricinae, of Tortricidae. The protein-coding genes (PCGs) of these downloaded genomes and our sequenced *Cydia* species were aligned with the mafft-linsi algorithm of MAFFT (v7.525) (Katoh and Standley 2013) and PAL2NAL (Suyama et al. 2006), where protein-level alignments were used to assist the construction of CDS-level alignments to avoid biologically meaningless frameshifts (Suyama et al. 2006).

Partitioned maximum-likelihood (ML) inference was conducted with IQ-TREE (v3.0.1) (Minh et al. 2020), with the optimal model determined by ModelFinder (Kalyaanamoorthy et al. 2017). The robustness of the tree was assessed with 2000 replicates of ultrafast bootstraps (UFBoot) (Minh et al. 2013) and the SH-aLRT branch test (Guindon et al. 2010) (-pers 0.2 -nstop 500 -B 2000 -bnni -alrt 2000 -runs 10), respectively. The ML tree was visualized with iTOL (v7.2.1) (Letunic and Bork 2024).

Result and discussion

The mitogenomes of *C. glandicolana* and *C. kurokoi* are 15,395 bp and 15,246 bp long, respectively (Figure 2), similar in size to that of *C. pomonella* (15,253 bp). All sites of the two newly sequenced mitogenomes have sequencing coverage of at least 182× (Figures S1 and S2). They also show similar base composition, with 39.8%/39.9% adenine (A), 12.3%/12.7% cytosine (C), 8.1%/8.4% guanine (G), and 39.8%/39.0% thymine (T). This is similar to *C. pomonella* which has 39.9%, 12.0%, 7.9%, and 40.2% for A, C, G, and T, respectively. Both *C. glandicolana* and *C. kurokoi* consist of 37 genes, including 13 PCGs, 22 tRNAs, two rRNA genes, and an AT-rich noncoding D-loop region. The boundaries of nearly all genes predicted by MitoZ and MITOS2 were identical or highly similar (1 or 2 bp mismatch), except for the ND5 gene. The ND5 gene predicted by MITOS2 was 84 bp longer than expected compared to closely related species, so we adopted the ND5 boundaries predicted by MitoZ. All 13 PCGs terminate with the TAA stop codon; for

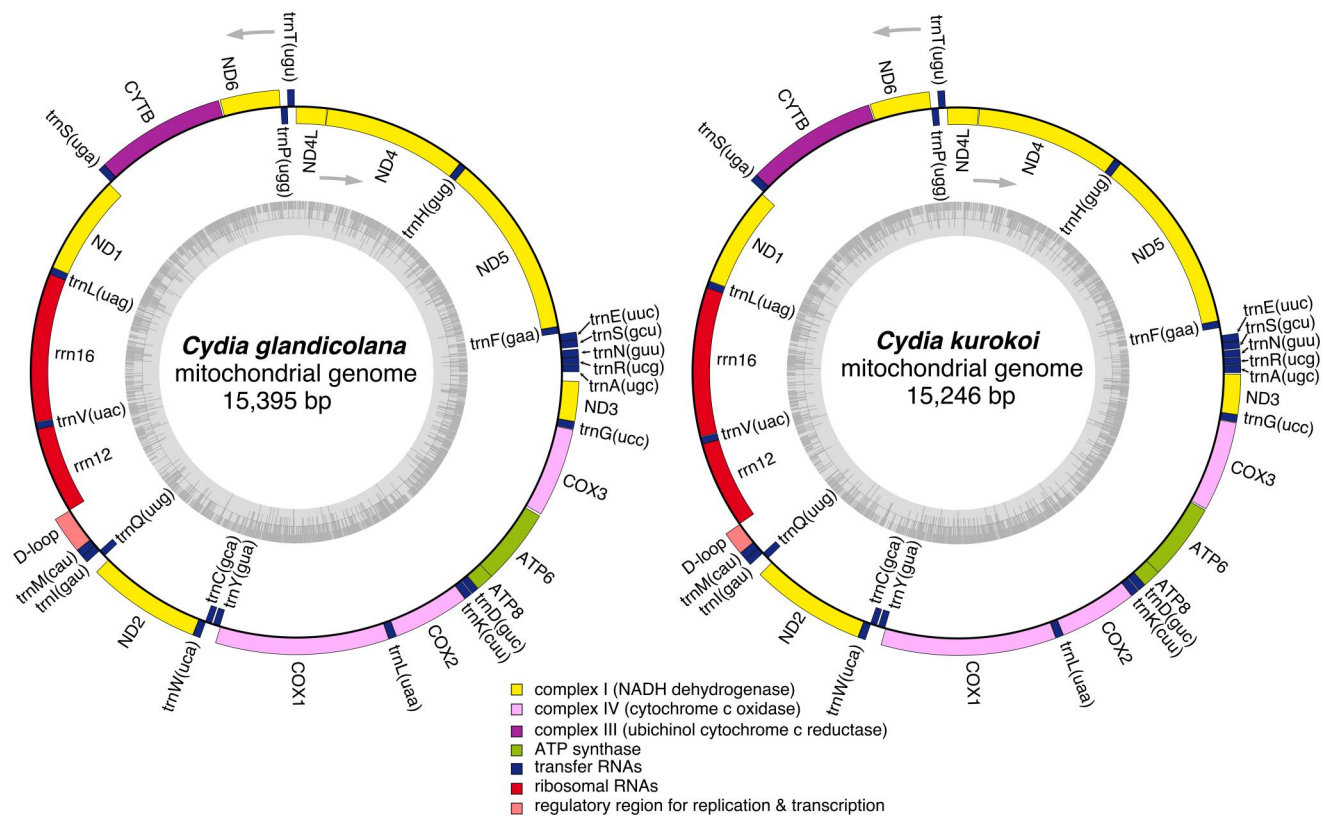


Figure 2. The mitochondrial genomes of *Cydia glandicolana* and *Cydia kurokoi*. Protein-coding genes are color-coded as yellow for complex I (NADH dehydrogenase) genes, pink for complex IV (cytochrome c oxidase) genes, green for ATP synthase genes, and purple for cytochrome b (CYTB), a component of complex III (ubiquinol cytochrome c reductase). tRNA genes are shown in navy blue, rRNA genes (ribosomal RNAs) in red, and the control region (D-loop) in peach pink, representing a regulatory region for mitochondrial DNA replication and transcription. Arrows indicate the direction of transcription.

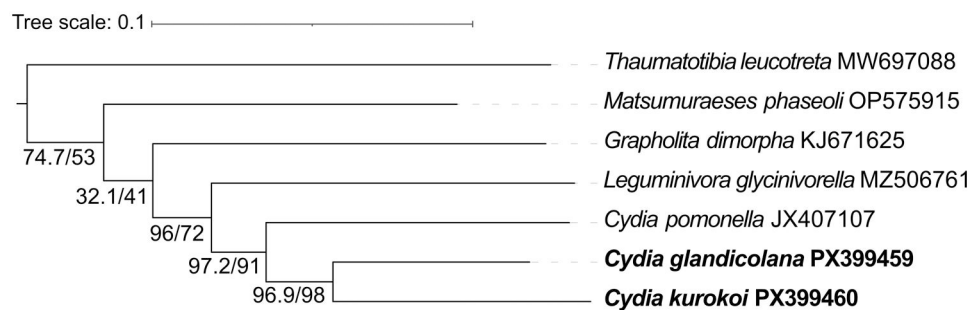


Figure 3. Phylogenetic relationships within the tribe Grapholitini. The tree was inferred using a partitioned IQ-TREE analysis of 13 mitochondrial PCGs. Newly sequenced taxa are shown in bold. The following sequences were used: JX407107 (Shi et al. 2013), KJ671625 (Niu et al. 2016), MZ506761, OP575915, and MW697088. The outgroup (DQ073916; Lee et al. 2006) is removed from the final visualized tree for clarity. Numbers on the branches are SH-aLRT support (%) / ultrafast bootstrap support (%).

COX2 and ND4 the stop codon is completed by post-transcriptional addition of adenosine residues at the mRNA 3' end. Interestingly, the start codons of COX1 for both species cannot be determined, similar to that of *C. pomonella*. In *C. glandicolana*, *rrn16* and *rrn12* have A + T contents of 83.4% and 84.7%, respectively, while they are 83.5% and 85.7% for *C. kurokoi*, respectively. Their D-loop regions are 333 bp and 210 bp long, with A + T contents of 94.0% and 93.3%, respectively. Finally, *C. glandicolana* and *C. kurokoi* show the same gene order as that of *C. pomonella*, suggesting that the evolution of *Cydia*

mitogenomes is either under evolutionary constraint or as a result of short divergent times.

Our partitioned ML analysis, based on limited taxon sampling, recovered the three *Cydia* species as forming a monophyletic group with strong SH-aLRT support (97.2%) but moderate UFBoot support (91%). The analysis also suggested that this clade is sister to the genus *Leguminivora* within Grapholitini, supported by strong SH-aLRT support (96%) but lower UFBoot support (72%), consistent with the findings of Hu et al. (2023) (Figure 3). Given that UFBoot values below

95% often indicate uncertainty (Minh et al. 2020), these results should be interpreted with caution. Indeed, a more comprehensive recent study (Hu et al. 2023) questioned the monophyly of *Cydia*. Since *Cydia* comprises over 20 species (Hu et al. 2023), further studies with broader taxon sampling and perhaps genome-wide data are necessary to clarify its phylogenetic relationships and guide future taxonomic revisions.

Conclusions

In this study, we present the first mitogenomes for *C. glandicolana* and *C. kurokoi*, which provide important molecular resources for species identification and pest control. Our phylogenetic analyses support the monophyly of *Cydia* and its sister relationship with the genus *Leguminivora* within Graptolithini, despite the limited taxon sampling. While this limitation should be acknowledged, the current data provide a valuable foundation for future taxonomic revisions of *Cydia* and further studies on the evolutionary history of Tortricidae.

Author contributions

CRedit: **Hairong Tan**: Conceptualization, Data curation, Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing; **Jiale Yang**: Data curation, Investigation, Methodology, Writing – review & editing; **Zufei Shu**: Data curation, Software, Visualization; **Yingming Zhang**: Resources, Validation, Visualization, Writing – review & editing; **Guanliang Meng**: Conceptualization, Methodology, Software, Supervision, Writing – original draft, Writing – review & editing.

Ethical approval

C. glandicolana and *C. kurokoi* are neither endangered nor protected species; therefore, no special permission was required for their collections. All research activities were conducted in accordance with the guidelines of the Bureau of Guangdong Chebaling National Nature Reserve and the Chinese Academy of Sciences.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the project titled DNA Barcoding Database Construction for Animal Species supported by Guangdong Chebaling National Nature Reserve (E5499811).

Data availability statement

The mitogenome sequences supporting the findings of this study are publicly available from GenBank with accessions PX399459 and PX399460 for *C. glandicolana* and *C. kurokoi*, respectively. The associated BioProject is PRJNA1331711, with BioSample and SRA numbers SAMN51637648/SRR35513741, and SAMN51637649/SRR35513740, respectively.

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