Unfolding the mitochondrial genome structure of green semilooper (Chrysodeixis acuta Walker): An emerging pest of onion (Allium cepa L.)

Soumia P. S. 1*, Dhananjay V. Shirsat 1, Ram Krishna 1, Guru Pirasanna Pandi G. 2, Jaipal S. Choudhary 3, Naiyar Naaz 3, Karuppaiah V. 3*, Pranjali A. Gedam 1, Anandhan S. 1*, Major Singh 1

1 ICAR-Directorate of Onion and Garlic Research (DOGR), Pune, Maharashtra, India, 2 ICAR-National Rice Research Institute (NRRI), Cuttack, Odisha, India, 3 ICAR- Research Complex for Eastern Region (RCER), Research Centre, Ranchi, India

* soumiaps@gmail.com (SPS); anandhans@gmail.com (AS)

Abstract

Onion is the most important crop challenged by a diverse group of insect pests in the agricultural ecosystem. The green semilooper (Chrysodeixis acuta Walker), a widespread tomato and soybean pest, has lately been described as an emergent onion crop pest in India. C. acuta whole mitochondrial genome was sequenced in this work. The circular genome of C. acuta measured 15,743 base pairs (bp) in length. Thirteen protein-coding genes (PCGs), 22 tRNA genes, two rRNA genes, and one control region were found in the 37 sequence elements. With an average 395 bp gene length, the maximum and minimum gene length observed was 1749 bp and 63 bp of nad5 and trnR, respectively. Nine of the thirteen PCGs have (ATN) as a stop codon, while the other four have a single (T) as a stop codon. Except for trnS1, all of the tRNAs were capable of producing a conventional clover leaf structure. Conserved ATAGA motif sequences and poly-T stretch were identified at the start of the control region. Six overlapping areas and 18 intergenic spacer regions were found, with sizes ranged from 1 to 20 bp and 1 to 111 bp correspondingly. Phylogenetically, C. acuta belongs to the Plusiinae subfamily of the Noctuidae superfamily, and is closely linked to Tri-choplusia ni species from the same subfamily. In the present study, the emerging onion pest C. acuta has its complete mitochondrial genome sequenced for the first time.

Introduction

Onion (Allium cepa L.) of the family Alliaceae has been cultivated for more than 5000 years [1]. Though onion is one of the important vegetables, it is also being used as a spice for flavoring various cuisines globally [2–4]. Being a bulbous crop, onion is mainly grown for its bulbs. However, the green leaves are also consumed worldwide as raw, cooked, semi-cooked, or in processed forms as a condiment, nutritional, nutraceutical, and medicinal purposes [3, 5, 6].
India is the second-largest producer (26738.000 MT), consumer, and exporter of onion globally (https://www.fao.org/faostat/en/#data/QCL). But unfortunately, the productivity per hectare is very low compared to countries where onion is grown commercially. The onion agroecosystem has a highly diverse insect pest complex, among which onion thrips, *Thrips tabaci* is the key pest of onion [7]. Besides thrips, several defoliators of the order lepidoptera are found at damaging levels in onions causing monetary loss globally [1, 2]. These insect pests affect almost every stage of the onion and cause a potential yield loss of 20 to 90% in onion production [8, 9]. Insects can coevolve with the host plants, a relatively frequent phenomenon attributed to the prevailing environmental condition and host plant availability [10–12]. Subsequently, insects pose a serious threat by expanding their geographic distribution and host range.

Green semilooper *Chrysodeixis acuta* Walker, an important established pest of soybean and tomato across India, is also becoming an emerging onion pest recently. Onion is commonly intercropped with soybean in India’s southern and central agricultural zones to limit insect pests by colonizing biocontrol agents [13]. *C. acuta* was recorded in onions for the first time in Maharashtra, India, in 2017–2018 [14]. The glossy green larvae are highly polyphagous, multivoltine species with huge potential to spread to new locations and adapt to new climatic or ecological conditions, which is likely why it has recently infested onions [14]. *C. acuta* has a wide range of hosts, including various vegetables, grains, and fruit crops [15–17]. *C. acuta*, like most defoliators, feeds on onion leaves; early instars scrape the leaves’ mesophyll tissue, leaving papery white structures, while later instars bore enormous feeding holes. Though this pest currently causes minimal damage to onion crops, significant defoliation by these caterpillars is expected where the soybean-onion cropping system is practiced [14]. Excessive defoliation can lower the net photosynthetic area, resulting in reduction of bulb size.

There is very little information available on the geographic variability and genetic structure of *C. acuta*. *Chrysodeixis* belongs to the *Plusiinae* subfamily and the *Noctuidae* family of moths. This subfamily is taxonomically compact and moderately large. More than 500 species have been identified globally [18] with 59 species belonging to 25 genera and three tribes [19] Nonetheless, the NCBI GenBank (https://www.ncbi.nlm.nih.gov/) only contains mitochondrial genome data only for four species from plusiinae subfamily: *Diachrysia nadeja* (MT916722), *Trichoplusia ni* (MK714850), *Ctenoplusia albostriata* (MN495624), and *Ctenoplusia limbirena* (KM244665). However, no pest species from the genus *Chrysodeixis* was sequenced to date even though some of the economically important pests under this genus are *Chrysodeixis chalcites*, *Chrysodeixis eriosoma*, *Chrysodeixis includens*, and *Chrysodeixis acuta*.

*Plusiinae* subfamily larvae are usually referred to as semiloopers. They are distinguished by 3 pairs of abdominal legs (prolegs) with biordinal crochets. In contrast, adult moths are robust, small to medium-sized, with a characteristic metallic spot in the center of the forewing. Morphological techniques cannot reliably distinguish these pests. However, taxonomic identification of the particular pest species is the fundamental step to devise a suitable management strategy [20]. As a result, the mitochondrial (*mt*) genome of insects is frequently utilized as a molecular marker to disclose basic information at the genomic level for phylogenetic inference, molecular evolution, identification of species, geographic distribution, and population dynamics [21, 22]. Therefore, in the present study, the whole *mt* genome of *C. acuta* was assembled for the first time using next-generation sequencing (NGS), which might provide a solution for species evolution and phylogeny. Furthermore, the extensive genetic information obtained from this investigation regarding this new onion pest may aid in devising effective control or preventative strategies.
Materials and methods

Sample collection
Green semilooper, *Chrysodeixis acuta* larvae were collected from onion plants at the Indian Council of Agriculture Research—Directorate of Onion and Garlic Research (ICAR-DOGR), situated at the (latitude: 27˚19’00.2 N, longitude: 82˚25’00.1 E, 553.8 meters above sea level) Pune, Maharashtra, India. The insects were initially reared in the laboratory. After completion of the life cycle, newly emerged adults from a single egg mass were collected, one pair was preserved in the insect repository of the Center with voucher specimen number: DOGR Voucher 15 following standard procedures. To further confirm the species identity, larval specimens were analyzed by 'DNA barcoding' at ICAR-DOGR, Pune, India and the sequence was submitted under the accession number MT644267 in NCBI GenBank and GBMNC55083-20 in Barcode of Life Data System (BOLD).

Sample preparation and DNA extraction
Laboratory reared fully grown fourth instar larvae of *C. acuta* were kept in a refrigerator at -20˚C in 100 percent ethanol until the experiment. According to the manufacturer’s instructions, total genomic DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN, Germany). The quality and amount of extracted DNA were assessed using a 1% agarose gel and with SmartSpec 3000 UV/Visible Spectrophotometer at 260 and 280 nm (Bio-Rad, Hercules, California, USA). By referring to the REPLI-g Mitochondrial DNA Kit (QIAGEN, Germany) protocol, mitochondrial DNA was isolated from genomic DNA.

Sequencing and mitogenome analysis
The mt genome was sequenced using the Illumina Next Seq 500 sequencing platform and Trimmomatic (v0.38) [23] was used to eliminate sequences of adaptor, ambiguous reads (reads having >5% unidentified nucleotides), and junk sequences (reads having > 10% quality threshold (QV) 20 phred score) from sequenced raw data to get high-quality clean reads. The high-quality reads aligned to the reference sequences with the help of BWA MEM (version 0.7.17) [24]. The consensus sequence was extracted using SAM toolsmpileup [25]. The consensus sequence was employed to identify protein-coding and RNA genes in the sample. The MITOS algorithm was used for genes prediction from the invertebrates’ mitochondrial genome [26].

The protein-coding and rRNA genes were manually annotated and verified comparing them with four mitogenome sequences of *Diachrysia natija* (MT916722), *Trichoplusia ni* (MK714850), *Ctenoplusia albostriata* (MN495624), and *Ctenoplusia limbirena* (KM244665) of subfamily Plusiinae available in GenBank. Using tRNAscanSEv 2.0 and a 15.0 covariance limit value, the Mito/Chloroplast model was utilized to recheck the MITOS downloaded two-dimensional tRNA structures [27]. The CGview server was used to generate the circular map of the complete mt genome, GC concentration, and GC skew [28]. Finally, protein-coding sequence regions of mitochondria were assembled and aligned in Mega version X using ClustalW with basic parameters [29].

The A+T contents, codon use, and relative synonymous codon usage (RSCU) of the PCGs were analyzed by using MEGA version X. The GC and AT skews were calculated utilizing the formulas (GC skew = [G-C] / [G+C] and AT skew = [A-T] / [A+T]) given by Perna and Kocher [30]. The overlapping and intergenic spacer region between genes were manually calculated. The synonymous (Ks) and nonsynonymous substitution rates (Ka) for each Protein-coding gene were calculated using the DnaSP 6.0 and Jukes-Cantor adjusted Ka/Ks (JKa/
JKs) software programmes [31]. The control region tandem repeats were predicted by using Tandem Repetitions Finder tool with default settings [32]. The assembled and annotated C. acuta mitogenome has been submitted under the accession number OL892047 in NCBI GenBank.

**Phylogenetic analysis**

All of the available complete Nocutidae mitogenomes (55 mitogenomes) were chosen for the phylogenetic study. One of the 55 Nocutidae mitogenomes was sequenced in this study, and the rest samples were obtained from the NCBI database.

The phylogenetic study used concatenated nucleotide sequences from 13 PCGs datasets. The PCG was aligned using MAFFT 7 (https://mafft.cbrc.jp/alignment/server/) based on codons for amino acids [33]. Similarly, to remove ambiguously aligned sites from PCG alignments, GBlocks v.0.91b (http://molevol.cmima.csic.es/castresana/Gblocks/Gblocksdocumentation.html) was used [34]. MEGA 10.0 was used as the final quality check for all of the alignments. All gene alignments were concatenated using PhyloSuite 1.2.1 [35]. PCG123 matrix with single sequences in the following order: \textit{nad2}, \textit{cox1}, \textit{cox2}, \textit{atp8}, \textit{atp6}, \textit{cox3}, \textit{nad3}, \textit{nad5}, \textit{nad4}, \textit{nad4L}, \textit{nad6}, \textit{cytb}, and \textit{nad1}. For phylogenetic reconstruction, these thirteen PCGs were concatenated. The best partitioning schemes were chosen using Partition Finder 2.1.1 with the Bayesian Information Criterion (BIC) and greedy algorithm (www.phylo.org) [36]. According to BIC, the GTR+I+G model was ideal for nucleotide alignment analysis. PHYLML online web server utilized the optimum substitution model obtained from Model Test to infer maximum likelihood (ML) analysis, and model parameter values were calculated [37]. Bayesian analyses were performed by utilizing Markov Chain Monte Carlo (MCMC) method with software, Mr. Bayes v.3.1.2 [38] having two independent runs of $2 \times 10^6$ generations with four chains with trees sampled each 1000th generation. Mr. Bayes’ “sump” command was used to evaluate the similar values for every post-analysis trees and the convergence and burn-in parameters. The top 100 trees from each run were utilized for burn-in, and the remaining trees were used to form a consensus tree with a 50% majority rule.

**Results and discussion**

**Organization of the genome**

The semilooper’s circular genome was 15,399 nucleotides in length. There are 37 sequence elements in total, comprising 13 protein-coding genes (PCGs) which include Cytochrome c oxidase, NADH dehydrogenase, Cytochrome B, ATPase, and two rRNA genes with 22 tRNA genes, and a control region. Every gene having an average gene length of 395 bp; the maximum and minimum gene lengths were 1749 and 63 bp, respectively (Fig 1). The mitochondrial genome of C. acuta was sequenced, and its molecular and phylogenetic features were studied in this work. In general, the mitogenome of insects are circular double-stranded molecule of 14–19 kb size which includes 37 genes: 13 PCGs, 2 rRNA genes and 22 tRNA genes [39, 40]. Gene content, order, and orientation are identical to those of reported noctuid mitogenomes and are typical of Lepidoptera [41, 42]. The semilooper’s circular mt genome measured 15,399 bp in size, slightly bigger than \textit{Trichoplusia ni} (15,239 bp), which belongs to the same Plusiinae subfamily [43]. Similarly, the mt genome of green semilooper is closely related to the other defoliator pests infesting onions, such as \textit{S. exigua} (15,365 bp) and \textit{S. litura} (15,374 bp) [44, 45]. C. acuta is the third insect pest from the Plusiinae subfamily to have its whole mt genome sequenced after \textit{T. ni} [43] and Macdunnoughia hybrida [42].
Protein-coding genes (PCGs)

The mt genome of *C. acuta* has 13 genes coding protein scattered in a circular chromosome. These PCGs contain three cytochrome c oxidase (cox1, cox2, and cox3 genes), seven NADH dehydrogenase (nad1, nad2, nad3, nad4, nad5, nad6, and nad4l genes), one cytochrome B (cytB gene), and two ATPase (atp6 and atp8 genes) (Table 1). The H-strand included nine of the 13 PCGs (cox1, cox2, cox3, nad2, nad3, nad6, atp6, atp8, and cytB), whereas the L-strand had the remaining four (nad1, nad4, nad4l, and nad5). In *C. acuta*, four start codons (ATA, ATT, ATG, and ATC) were found. Seven genes (cox2, cox3, atp6, nad1, nad4, nad4l, and cytB) adopted ATG as the start codons, 3 genes (cox1, atp8 and nad5) used ATT, two (nad2 and nad6) used ATA, while ATC by single gene (nad3). Nine PCGs employed the TAA termination codon (cox3, atp6, atp8, nad1, nad3, nad4l, nad5, nad6, and cytB). The incomplete termination codon T was found in four genes: cox1, cox2, nad2, and nad4 (Table 1). Table 2 summarizes the values for the relative synonymous codon use (RSCU) and amino acid usage in the PCGs of *C. acuta*. Phenylalanine (Phe, F), Leucine (Leu, L), Isoleucine (Ile, I), Asparagine (Asn, N), and Serine (Ser, S) were found to be the five most commonly occurring amino acids, whereas Cysteine (Cys, C), and Arginine (Arg, R) were found to be the rarest (Table 2). The protein-coding genes are found scattered on both heavy and light strands, covering 71.93% of the total mt genome of *C. acuta*, and—11,077 bp long. These PCGs include cytochrome c oxidase, NADH dehydrogenase, Cytochrome B, and ATPase, commonly found in most insect species belonging to Lepidopteran order [21, 46–48].

Insect species like *Ctenoplusia albostriata* [49], *Diachrysia nadeja* [50], *Trichoplusia ni* [43], *Laelia suffusa* [51], *Cerura menciana* [22] and *Eudocima salaminia* [48] all have the same genes with little differences in size and location. The start codons for the cox1 gene in lepidopteran insects are not uniform [52], which has been widely debated and has long been a source of contention [53]. The canonical start codon ATN—was used to initiate all of *C. acuta*’s protein-coding genes. The initial codon for cox1 was ATT, instead of “CGA” utilized in several insect species [54]. In *C. acuta*, two termination codons were discovered. Four genes cox1, cox2, nad2, and nad4, used the incomplete termination codon "T"; nevertheless, other genes

![Fig 1. Mitochondrial genome map of *Chrysodeixis acuta*.](https://doi.org/10.1371/journal.pone.0273635.g001)
were terminated by TAA. Our findings are consistent with those of Liu et al. [55], Dai et al. [22], Dai et al. [56], Li et al. [57] Chen et al. [58] and Riyaz et al. [48] who also reported the presence of single "T" as a termination codon for the cox1 and cox2 genes in the majority of Lepidopteran species. This incomplete termination codon "T" in lepidopteran mt genes might get polyadenylated to TAA codon during translation [46, 47, 59]. Numerous studies have demonstrated that the nucleotides 'A' and 'T' are generally overrepresented in metazoan mitogenomes, which causes bias in the corresponding encoded amino acids [21]. Codons having 'A' or 'T' at the third prime position were found to be over used in this study when compared to

| Name | Start position | Stop position | Strand | Length | Intergenic Spacers | Anticodon | Start codon | Stop codon |
|------|----------------|---------------|--------|--------|--------------------|-----------|-------------|------------|
| trnM | 1              | 68            | +      | 68     |                    |           | atg         |            |
| trnI | 69             | 135           | +      | 67     | 0                  |           | atc         |            |
| trnQ | 133            | 201           | -      | 69     | -2                 |           | caa         |            |
| nad2 | 276            | 1149          | +      | 874    | 74                 |           | ata         | t          |
| trnW | 1261           | 1327          | +      | 67     | 111                |           | tga         |            |
| trnC | 1320           | 1384          | -      | 65     | -7                 |           | tgc         |            |
| trnY | 1386           | 1450          | -      | 65     | 1                  |           | tac         |            |
| cox1 | 1443           | 2988          | +      | 1546   | -7                 |           | att         | t          |
| trnL2 | 2989          | 3055          | +      | 67     | 0                  |           | tta         |            |
| cox2 | 3056           | 3737          | +      | 682    | 0                  |           | atg         | t          |
| trnK | 3738           | 3808          | +      | 71     | 0                  |           | aag         |            |
| trnD | 3818           | 3886          | +      | 69     | 9                  |           | gac         |            |
| atp8 | 3887           | 4048          | +      | 162    | 0                  |           | att         | taa        |
| atp6 | 4042           | 4719          | +      | 678    | -6                 |           | atg         | taa        |
| cox3 | 4730           | 5515          | +      | 786    | 10                 |           | atg         | taa        |
| trnG | 5521           | 5585          | +      | 65     | 5                  |           | gga         |            |
| nad3 | 5595           | 5939          | +      | 345    | 9                  |           | atc         | taa        |
| trnA | 5939           | 6003          | +      | 65     | -1                 |           | gca         |            |
| trnR | 6004           | 6066          | +      | 63     | 0                  |           | cga         |            |
| trnN | 6067           | 6133          | +      | 67     | 0                  |           | aac         |            |
| trnS1 | 6137           | 6202          | +      | 66     | 3                  |           | agg         |            |
| trnE | 6203           | 6268          | +      | 66     | 0                  |           | gaa         |            |
| trnF | 6268           | 6364          | -      | 67     | 29                 |           | ttc         |            |
| nad5 | 6365           | 8113          | -      | 1749   | 0                  |           | att         | taa        |
| trnH | 8114           | 8179          | -      | 66     | 0                  |           | cac         |            |
| nad4 | 8252           | 9590          | -      | 1339   | 72                 |           | atg         | t          |
| nad4l | 9597          | 9887          | -      | 291    | 6                  |           | atg         | taa        |
| trnT | 9890           | 9955          | +      | 66     | 2                  |           | aca         |            |
| trnP | 9956           | 10020         | -      | 65     | 0                  |           | cca         |            |
| nad6 | 10028          | 10561         | +      | 534    | 7                  |           | ata         | taa        |
| cytB | 10581          | 11732         | +      | 1152   | 19                 |           | atg         | taa        |
| trnS2 | 11735         | 11800         | +      | 66     | 2                  |           | tca         |            |
| nad1 | 11820          | 12758         | -      | 939    | 19                 |           | atg         | taa        |
| trnL1 | 12760         | 12828         | -      | 69     | 1                  |           | cta         |            |
| rrnL | 12808          | 14166         | -      | 1359   | -20                |           |             |            |
| trnV | 14215          | 14279         | -      | 65     | 48                 |           | gta         |            |
| rrnS | 14280          | 15060         | -      | 781    | 0                  |           |             |            |
| Control region | 15061 | 15399 | +      | 339    | 0                  |           |             |            |

Table 1. Summary table for characteristics of the complete mitogenome of Chrysodeixis acuta.
other similar codons. For example, the valine codons GTC and GTG were rare, whereas GTT and GTA’s synonymous codons were prevalent (Table 2). In the mitogenome of *Leucoma salicis* [60] and *Eucrate crenata* [47] a similar tendency was observed. The pattern of codons for the frequently used amino acids like Phe, Leu, Ile, Asn and Ser of *C. acuta* are similar to most noctuid mitogenomes [48, 61, 62].

## Transfer RNAs (tRNA) and ribosomal RNAs (rRNA)

The mitochondrial genome of *C. acuta* had 22 tRNA genes strewn over the circular genome. From the total, 14 tRNAs were present within H- strand, whereas the rest 8 were present within L- strand (Fig 1 and Table 1). The 22 tRNAs’ aggregate sequence was 1,464 bp, 9.51% of the entire mt genome. Except for the trnS1 (AGN) gene, which codes for the serine (Ser, S) amino acid, the secondary structures of the remaining mt tRNA of *C. acuta* were predicted; all genes revealed the characteristic clover leaf shape (Fig 2). The two rRNA genes rrnL of 1359 bp and rrnS of 781 bp are found on the L- strand, accounting for 13.90% of the mt genome (Fig 1).

The length of 22 transfer RNA genes in the *C. acuta* mt genome ranged from 63 bp (trnR) to 71 bp (trnK), which is similar with the mt genomes of *L. suffusa* [51], *D. pyloalis* [54], and *C. menciana* [22]. Except for trnS1 (AGN), which lacks the dihydrouridine (DHU) arm and forms a simple loop, all tRNA secondary structures resembled the conventional clover leaf shape (Fig 2). Many insect mitogenomes showed similar results, including *Bombyx mori* [63], *Actias selene* [64], *Spodoptera frugiperda* [65], *Spodoptera litura* [45], *Chrysochroa fulgidissima* [66], *B. zonata* [67] *Idioscopus nitidulus* [68] *Acronicta runcic* [69] and *Eudocima salamina* [48].

There was an unusual (G-U and U-U) mismatch pair in the tRNA genes. Ten of the tRNA genes were found to contain 14 G-U mismatches in their secondary structures, forming a weak bondThe amino acid acceptor stems of trnAla(UUR), trnAla1, tRNAAsn1, and tRNA-Ser2 contained five U-U mismatches (Fig 2). Sun et al. [60] and Riyaz et al. [48] also observed a similar trend in *L. salicis* and *E. salamina* respectively. The control (AT-rich) region varies in

---

**Table 2. Codon usage and relative synonymous codon usage (RSCU) within *Chrysodeixis acuta* mitochondrial genome.**

| Codon   | Count | RSCU | Codon   | Count | RSCU | Codon   | Count | RSCU | Codon   | Count | RSCU |
|---------|-------|------|---------|-------|------|---------|-------|------|---------|-------|------|
| UUU(F)  | 26.3  | 1.92 | UCU(S)  | 10.2  | 3.3  | UAU(Y)  | 14.2  | 1.88 | UGU(C)  | 2.3   | 2    |
| UUC(F)  | 1.2   | 0.08 | UCC(S)  | 0.6   | 0.2  | UAC(Y)  | 0.9   | 0.12 | UGC(C)  | 0     | 0    |
| UUA(L)  | 35.4  | 5.27 | UCA(S)  | 4.9   | 1.59 | UAA(∗)  | 0.7   | 2    | UGA(W)  | 7.1   | 1.96 |
| UUG(L)  | 0.6   | 0.09 | UCG(S)  | 0.1   | 0.02 | UAG(∗)  | 0     | 0    | UGG(W)  | 0.2   | 0.04 |
| CUU(L)  | 2.9   | 0.44 | CUC(P)  | 6.5   | 2.69 | CAU(H)  | 4.8   | 1.85 | CGU(R)  | 1.4   | 1.33 |
| CUC(L)  | 0     | 0    | CCC(P)  | 0.8   | 0.32 |CAC(H)  | 0.4   | 0.15 | CGC(R)  | 0     | 0    |
| CU(A)   | 1.4   | 0.21 |CCA(P)  | 2.4   | 0.99 |CAA(Q)  | 4.7   | 1.91 | CGA(R)  | 2.7   | 2.59 |
| CUG(L)  | 0     | 0    | CCG(P)  | 0     | 0    |CAG(Q)  | 0.2   | 0.09 | CGG(R)  | 0.1   | 0.07 |
| AUU(I)  | 33.8  | 1.94 | ACU(T)  | 6.4   | 2.24 | AAU(N)  | 18.7  | 1.92 | AGU(S)  | 2.4   | 0.77 |
| AUC(I)  | 1     | 0.06 | ACC(T)  | 0.4   | 0.14 | AAC(N)  | 0.8   | 0.08 | AGC(G)  | 0     | 0    |
| AU(A)   | 20    | 1.86 | ACA(T)  | 4.6   | 1.62 | AAA(K)  | 6.8   | 1.81 | AGA(S)  | 6.5   | 2.11 |
| AUG(M)  | 1.5   | 0.14 | ACG(T)  | 0     | 0    | AAG(K)  | 0.7   | 0.19 | AGG(S)  | 0     | 0    |
| GUU(V)  | 5.8   | 1.99 | GCU(A)  | 6.5   | 2.67 | GAU(D)  | 4.7   | 1.94 | GGU(G)  | 4.8   | 1.22 |
| GUC(V)  | 0.1   | 0.03 | GCC(A)  | 0.2   | 0.06 |GAC(D)  | 0.2   | 0.06 | GGC(G)  | 0.2   | 0.06 |
| GUA(V)  | 5.1   | 1.75 | GCA(A)  | 2.9   | 1.21 | GAA(E)  | 5.4   | 1.87 | GGA(G)  | 9.2   | 2.36 |
| GUG(V)  | 0.7   | 0.24 | GCG(A)  | 0.2   | 0.06 | GAG(E)  | 0.4   | 0.13 | GGG(G)  | 1.4   | 0.35 |

Average codons = 284

https://doi.org/10.1371/journal.pone.0273635.t002
size across members of the Noctuidae family, although it includes comparable sequence components in most species. In many Lepidopteran species, the ATAGA motif found at the start of the control region is a conserved motif sequence. Similarly, Li et al. [51], Dai et al. [22], Chen et al. [69] and Liu et al. [70] have also reported the ATAGA motif in the control region followed by the Poly-T stretch and microsatellite A/T repeats elements along with Poly-A stretch at the end of the control region.

Overlapping and intergenic spacer regions

The whole circular mitochondrial genome map of C. acuta has six overlapping sections ranging from 1 to 20 bp with a total length of 43 bp. The overlapping area between the trnL and rrnL genes is the longest one measuring 20 bp. Other overlapping regions include 7 bp between trnC and trnW, cox1 and trnY; 6 bp between atp6 and atp8, 2 bp between trnQ and trnI, and a single base pair between trnA and nad3 genes (Table 1). The mt genome of C. acuta comprises 18 intergenic spacer sequences, ranged 1 to 111 bp which constituted a total 427 bp. The longest intergenic spacer was 111 bp, present between nad2 and trnW is an A/T rich
region. The largest intergenic spacer sequences are 74 bp and 72 bp situated between \textit{trnQ} and \textit{nad2} genes, and \textit{trnH} and \textit{nad4} genes respectively. The other intergenic spacers are less than 50 bp size (Table 1). The spacer region between \textit{trnS2} (UCN) and \textit{nad1} includes two motifs ‘ATACTAA’ and “TACTAAAAATAAAAT” of 7 and 14 bp respectively (Fig 3). The intergenic spacer region between the genes \textit{trnS2} (UCN) and \textit{nad1} includes the ‘ATACTAA’ motif, which is commonly present in most lepidopteran species; even though the size of the intergenic spacer region varies [52, 69–71]. This spacer region is generally considered a constitutive synapomorphic feature of the lepidopteran mitochondrial genomes because this region won’t present in non-lepidopteran insects species [72]. The similar motif were located in the \textit{Dysgonia stuposa} [73], \textit{L. salicis} [60], \textit{C. menciana} [22] and \textit{Ctenoptilum vasava} [52]. The motif ‘TACTAAAAATAAAAT” was located in the intergenic spacer region between the genes \textit{trnS2} (UCN) and \textit{nad1} of the \textit{C. acuta}, were also common in lepidopteran species (Fig 3). However, this motif was not reported in other than lepidopteran families.

The control region

The control region (AT-rich) of \textit{C. acuta} \textit{mt} genome comprises 339 bp is positioned between \textit{rrnS} and \textit{trnM} genes. It is one of the longest mitochondrial non-coding region which cover about 2.20% of the whole mitochondrial genome and contains 93.51% AT nucleotides. There have been no reports of noticeable extensive repeats in the AT-rich control region. However, many short repetitive sequences are distributed across the region, including the ATAGA motif, followed by a 19-bp Poly-T stretch, a microsatellite-like (AT)$_{10}$ and a 9-bp poly-A stretch downstream of \textit{trnM} (Fig 4). Similar sequence elements were found in the mitochondrial genomes of lepidopteran species, \textit{Laelia suffusa} [51], \textit{Cerura menciana} [22], \textit{Hestina persimilis} and \textit{Hestinalis nama} [74].

Phylogenetic analysis

The phylogenetic tree of thenoctuidae family comprising 55 Lepidoptera species based on 13 PCGs nucleotide sequence datasets constructed by utilizing Maximum Likelihood (ML), and Bayesian Inference (BI) methods. The phylogenetic tree, as shown in Fig 5, revealed that the \textit{mt} genome of \textit{C. acuta} taxonomic status was closest with \textit{T. ni} (GenBank accession No. MK 714850), \textit{C. limbirena} (GenBank accession No. KM 244665), and \textit{C. albostriata} (GenBank

---

**Fig 3.** Alignment of the intergenic spacer region between \textit{trnS2} (UCN) and \textit{nad1} of several lepidopteran insects of Plusiinae subfamily. ATACTAA motif is underlined and the TACTAAAAATAAAAT is shaded.

https://doi.org/10.1371/journal.pone.0273635.g003

**Fig 4.** Features present A+T rich region of \textit{Chrysodeixis acuta}. The ATAGA motif is shaded, Poly-T strand is underlined, Poly-A stretch is double underlined; the single microsatellite A/T repeat sequence is dotted underlined.

https://doi.org/10.1371/journal.pone.0273635.g004
accession No. MN 495624) from the same sub-family Plusiinae. Hadeninae, Noctuinae, Amphipyrinae, Plusiinae, and Heliothinae sub-families of the Noctuidae family are closely related. The sub-family of C. acuta, Plusiinae is closely related to the Heliothinae and Amphipyrinae. However, the Hadeninae and Noctuinae are sister groups from the same superfamily (Fig 5). According to the overall findings of the phylogenetic analysis, species that belong to the same family but different subfamilies are placed together.

The phylogenetic analysis discovered that C. acuta is most closely correlated to the cabbage looper, Trichoplusia ni, from the same subfamily Plusiinae, notably in mt genome similarities, 13 PCGs, 22 tRNAs, and two rRNAs. However, the size of the control region appears to be different [43]. The five sub-families comprising Hadeninae, Noctuinae, Amphipyrinae, Plusiinae, and Heliothinae from the Noctuidae family are closely related. The sub-family of C. acuta, Plusiinae is closely related to the Heliothinae and Amphipyrinae. However, the Hadeninae and Noctuinae are sister groups from the same superfamily (Fig 5). The phylogenetic tree confirmed that the previously characterized species C. albostriata [49], D. nadeja [50], and T. ni [43] belonged to the sub-family Plusiinae and are related to the C. acuta which confirms that the C. acuta also belonged to the Plusiinae sub-family. The phylogenetic relationships were reconstructed based on the concentrated data of the 13 PCGs, which supports the traditional morphology-based view of the relationship within the Noctuidae family. The phylogenetic relationships were reconstructed based on the concentrated data of the 13 PCGs, which supports the traditional morphology-based view of the relationship within the Noctuidae family.
Nucleotide composition

The mitochondrial genomes of the insects generally reflect real strand discrimination in nucleotide compositions [75]. The strand dissimilarity is measured as the AT and GC-skews [30]. The J-strand displayed strand bias with negative GC-skew and positive AT-skew pattern (S1 Fig) in a comparative study of nucleotide percentages vs skewness of the 55 Noctuidae family members, including C. acuta. The relative synonymous codon usages (RUSC) were evaluated to identify the preference for a specific synonymous (Table 2). The codon usages pattern of C. acuta suggests that the two-fold and four-fold degenerate codons overuse the A/T at the third codon position (S2 Fig). Two-fold and four-fold degenerate codon usage was obviously A/T biased over G/C in the third position [76].

Gene evolution rate

For the analysis of gene evolution rate, the rate of non-synonymous substitution (Ka, pi modified), synonymous substitution (Ks, pi modified), and the Ka/Ks ratio, as well as the Jukes-Cantor adjusted Ka/Ks (JKa/JKs) ratio, of 13 PCGs from 55 mitogenome sequences from Noctuidae family species, including C. acuta was used. Based on synonymous and non-synonymous nucleotide substitution analysis, the Ka/Ks values of the 13 PCGs of 55 Noctuidae species ranged from 0.0463 to 1.0947 (Table 3). The Ka/Ks values of only two genes nad2 and cytB were more than one, 1.0947 and 1.0654, respectively, indicating the highest evolutionary rates. While cox1 recorded lowest (0.0463) among the 13 PCGs. Similarly, Jukes-Cantor adjusted Ka/Ks also showed that the cytB and nad2 had highest evolutionary rate with JKa/JKs values 2.0443 and 1.6206, respectively. However, cox1 and atp6 recorded the lowest JKa/JKs values of 0.0349 and 0.0916, respectively. Overall ratios of Ka/Ks and JKa/JKs for the genes cox1, cox2, atp6, nad3, nad4, and nad4L were less than 0.5, indicating that these genes in the Noctuidae family evolved under purifying selects (S3 Fig). Though this gene has a relatively slow evolutionary rate, this could be used as the candidate barcoding marker for species identification in Noctuidae family. Nucleotide substitution in the mitogenome indicates the evolution at the molecular level [77]. Nucleotide substitution was previously thought to represent a directional bias between various genes in the mitochondrial genome of insects, according to previous research by Cameron [39].

### Table 3. Rates of non-synonymous substitutions (Ka, pi modified), synonymous substitutions (Ks, pi modified) and the Ka/Ks ratio as well as Jukes-Cantor adjusted Ka/Ks (JKa/JKs) ratio in each PCG of 55 mitogenome sequences of Noctuidae family species including Chrysodeixis acuta from this study.

| Protein-coding genes | Rates of non-synonymous substitutions (Ka) | Rates of synonymous substitutions (Ks) | Ka/Ks ratio | Rates of non-synonymous substitutions Jukes-Cantor adjusted J(Ka) | Rates of synonymous substitutions Jukes-Cantor adjusted J(Ks) | JKa / JKs ratio |
|----------------------|-----------------------------------------|--------------------------------------|-------------|-------------------------------------------------------------|-------------------------------------------------------------|---------------|
| nad2                 | 0.33444                                 | 0.30550                              | 1.09473     | 0.65868                                                     | 0.40644                                                     | 1.62061       |
| cox1                 | 0.01457                                 | 0.31435                              | 0.04635     | 0.01479                                                     | 0.42349                                                     | 0.03492       |
| cox2                 | 0.05820                                 | 0.33466                              | 0.17391     | 0.11659                                                     | 0.46371                                                     | 0.25143       |
| atp8                 | 0.15659                                 | 0.21912                              | 0.71463     | 0.18637                                                     | 0.26938                                                     | 0.69185       |
| atp6                 | 0.0402                                 | 0.3316                               | 0.121261    | 0.04187                                                     | 0.4573                                                      | 0.0916        |
| cox3                 | 0.27558                                 | 0.37520                              | 0.73449     | 0.76770                                                     | 0.55221                                                     | 1.39023       |
| nad3                 | 0.10409                                 | 0.38134                              | 0.27296     | 0.18199                                                     | 0.56431                                                     | 0.32250       |
| nad6                 | 0.27856                                 | 0.28836                              | 0.96601     | 0.43713                                                     | 0.37632                                                     | 1.16159       |
| nad5                 | 0.1939                                 | 0.2619                               | 0.740482    | 0.45273                                                     | 0.3326                                                      | 1.3611        |
| nad4                 | 0.04544                                 | 0.24551                              | 0.18508     | 0.04748                                                     | 0.30501                                                     | 0.15567       |
| nad4L                | 0.10840                                 | 0.24782                              | 0.43741     | 0.17589                                                     | 0.31478                                                     | 0.55877       |
| cytB                 | 0.40883                                 | 0.38370                              | 1.06549     | 1.15181                                                     | 0.56342                                                     | 2.04432       |
| nad1                 | 0.19112                                 | 0.26457                              | 0.72238     | 0.49746                                                     | 0.33763                                                     | 1.47339       |
Conclusions
Many agricultural pests and economically important insects are found in the order Lepidoptera. Most economically important defoliator pests belong to the Noctuidae family. The mitogenome was utilised to elucidate the evolutionary position of Lepidoptera at several taxon levels, especially for Noctuoidea [78]. The mitogenomes of the Noctuidae family have been studied extensively; however, very few reports are available on the Plusiinae subfamily. The first complete mt genome sequence of Chrysodeixis acuta is revealed in this work. The circular mitogenome of C. acuta is 15,399 bp length and contains 37 sequence elements. It comprises 13 protein-coding genes, ATPase genes, Cytochrome B gene, cytochrome c oxidase gene, NADH dehydrogenase gene, two ribosomal RNA genes, 22 transfer RNA genes, and a control region. The complete mt genome adds to the genetic richness of this species and elucidates crucial information for further evolutionary and phylogenetic studies of the Noctuidae family. In addition, other potential pests of the genus Chrysodeixis, such as Chrysodeixis chalcites, Chrysodeixis eriosoma, and Chrysodeixis inclusens, infesting various crops, would benefit from the mitochondrial genomic organization of Chrysodeixis acuta.

Supporting information
S1 Fig. AT% vs AT-skew and GC% vs GC-skew in the 63 Noctuidae family species including Chrysodeixis acuta from this study. Values are calculated on J-strands for full length of mt genomes. The X-axis provides the skews values, while the Y axis provides the A+T/G+C values. Names of species are colored according to their taxonomic placement. (TIF)

S2 Fig. The AT content percentage of 0-fold degenerate sites, 2-fold degenerate sites and 4-fold degenerate sites in each protein coding gene of 55 mitochondrial genome sequences of Noctuidae family species including C. acuta from this study. The black line with short line on the top of each bar represents the standard deviation value (SD). (TIF)

S3 Fig. Ratio of non-synonymous substitutions (Ka, pi modified) & synonymous substitutions (Ks, pi modified) (Ka/Ks) as well as Jukes-Cantor adjusted (JKa/JKs) ratio in each PCG of 55 mitogenome sequences of Noctuidae family species including C. acuta from this study. (TIF)

Acknowledgments
The authors are thankful to the Head of the Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India, and Dr. P. R. Shashank, Division of Entomology, ICAR-IARI, New Delhi, India, for insect species confirmation. We also gratefully acknowledge the editors and anonymous reviewers for their valuable suggestions and comments.

Permissions: The researchers were granted permission to collect the insect sample as part of the routine monitoring of the experimental trials for emerging and invasive pest species by the Director ICAR-Directorate of Onion and Garlic Research, Pune, Maharashtra, India.

Author Contributions
Conceptualization: Soumia P. S., Guru Pirasanna Pandi G., Anandhan S.
Data curation: Soumia P. S., Dhananjay V. Shirsat, Ram Krishna, Guru Pirasanna Pandi G., Jaipal S. Choudhary, Naiyar Naaz, Anandhan S.

Formal analysis: Soumia P. S., Dhananjay V. Shirsat, Ram Krishna, Guru Pirasanna Pandi G., Jaipal S. Choudhary, Naiyar Naaz, Anandhan S.

Funding acquisition: Major Singh.

Methodology: Soumia P. S., Dhananjay V. Shirsat, Guru Pirasanna Pandi G., Anandhan S.

Project administration: Soumia P. S., Anandhan S., Major Singh.

Resources: Major Singh.

Software: Soumia P. S.

Supervision: Soumia P. S., Anandhan S., Major Singh.

Validation: Soumia P. S.

Visualization: Soumia P. S.

Writing – original draft: Soumia P. S., Dhananjay V. Shirsat, Ram Krishna, Guru Pirasanna Pandi G., Jaipal S. Choudhary, Naiyar Naaz, Karuppaiah V., Pranjali A. Gedam, Anandhan S.

Writing – review & editing: Soumia P. S., Dhananjay V. Shirsat, Ram Krishna, Guru Pirasanna Pandi G., Karuppaiah V., Pranjali A. Gedam, Anandhan S.

References
1. Jadhav VG, Baviskar PP, Pathrikar DT, Bhosale GV. Export performance of Onion in India. Pharma Innov. 2022; 11:428–430.
2. Soumia PS, Karuppaiah V, Mahajan V, Singh M. Beet Armyworm Spodoptera exigua: emerging threat to onion production. Natl. Acad. Sci. Lett. 2020; 43(5):473–6. https://doi.org/10.1007/s40009-020-00892-5
3. Khandagale K, Krishna R, Roylawar P, Ade AB, Benke A, Shinde B, et al. Omics approaches in Allium research: Progress and way ahead. PeerJ. 2020; 9; 8:e9824. https://doi.org/10.7717/peerj.9824 PMID: 32974094
4. Gedam PA, Thangasamy A, Shirsat DV, Ghosh S, Bhagat KP, Sogam OA, et al. Screening of onion (Allium cepa L.) genotypes for drought tolerance using physiological and yield based indices through multivariate analysis. Front. Plant Sci. 2021; 12: 122. https://doi.org/10.3389/fpls.2021.600371 PMID: 33633759
5. Bahram-Parvar M, Lim LT. Fresh-cut onion: A review on processing, health benefits, and shelf-life. Compr. Rev. Food Sci. Food Saf. 2018; 17:290–308. https://doi.org/10.1111/1541-4337.12331 PMID: 33350082
6. Mahmood N, Muazzam MA, Ahmad M, Hussain S, Javed W. Phytochemistry of Allium cepa L. (Onion): An Overview of Its Nutritional and Pharmacological Importance. Sci. Inquiry Rev. 2021; 5:42–59. https://doi.org/10.32350/sir.53.04
7. Diaz-Montano J, Fuchs M, Nault BA, Fail J, Shelton AM. Onion thrips (Thysanoptera: Thripidae): a global pest of increasing concern in onion. J. Econ. Entomol. 2011; 104:1–13. https://doi.org/10.1603/ec10269 PMID: 21404832
8. Mishra RK, Jaiswal RK, Kumar D, Saabale PR, Singh A. Management of major diseases and insect pests of onion and garlic: A comprehensive review. J. Plant Breed Crop Sci. 2014; 6:160–170. https://doi.org/10.5897/JPCBS2014.0467
9. Moretti EA, Harding RS, Scott JG, Nault BA. Monitoring onion thrips (Thysanoptera: Thripidae) susceptibility to spinetoram in New York onion fields. J. Econ. Entomol. 2019; 112:1493–1497. https://doi.org/10.1093/jeeco/toz032 PMID: 30805650
10. Pelissie B, Crossley MS, Cohen ZP, Schoville SD. Rapid evolution in insect pests: the importance of space and time in population genomics studies. Curr. Opin. Insect Sci. 2018; 26:8–16. https://doi.org/10.1016/j.cois.2017.12.008 PMID: 29764665
Complete mitochondrial genome of green semi-looper (Chrysodeixis acuta)

11. Lehmann P, Ammenüt T, Barton M, Battisti A, Eigenbrode SD, Jepsen JU, et al. Complex responses of global insect pests to climate warming. Front. Ecol. Environ. 2020; 18(3):141–50. https://doi.org/10.1002/fee.2160

12. Srinivasa R, Tamó M, Malini P. Emergence of Maruca vitrata as a major pest of food legumes and evolution of management practices in Asia and Africa. Annu. Rev. Entomol. 2021; 66:141–161. https://doi.org/10.1146/annurev-ento-021220-084539 PMID: 33417822

13. Kumar B. Insect Pest Management. In Pests and Their Management, Springer, Singapore, 2018; 1015–1078.

14. Karuppaia V, Soumia PS, Shinde PS, Singh M. Occurrence of green semi-looper Chrysodeixis acuta Walker (Lepidoptera: Noctuidae) in onion (Allium cepa L.) (Amaryllidaceae). Fla. Entomol. 2019; 102:783–784. https://doi.org/10.1653/024.102.0418

15. Raju GS, Khandwe N, Nema KK. Seasonal incidence, biology and behaviour of green semi-looper, Chrysodeixis acuta (Walker) on Soybean. Ann. Plant Prot. Sci. 2014; 22:306–309.

16. Brahman SK, Awasthi AK, Singh S. Studies on insect pests of soybean (Glycine max) with special reference to seasonal incidence of Lepidopteran defoliators. J. Pharmacogn. Phytochem. 2018; 7:1808–1811.

17. Balabag NM, Anub RR, Sabado EM. Survey of Insects and Other Arthropods in Tomato (Lycopersicon esculentum Mill.) in Lanao del Sur Province, ARMM, Philippines. Int. J. Sci. Manag. Stud. 2019; 2:45–53. https://doi.org/10.51386/25815946/jiams-v2/3p104

18. Ronkay L, Ronkay G, Behounek G. A taxonomic atlas of the Eurasian and North African Noctuoidea. Plusiinae Witt Catalogue. 2008; 1:1–348.

19. Shashank PR, Singh L. Checklist of the subfamily plusiinae (Lepidoptera: noctuidae) from India. Indian J. Entomol. 2014; 76(3):229–40.

20. Sajjad M, Anand S, Anush R, Sabado EM. Description of the genus Thysanoplusia (Fabricius) (Lepidoptera: Noctuidae: Plusiinae) from Pakistan. Saudi J. Biol. Sci. 2020; 27(5):1375–9. https://doi.org/10.1016/j.sjsb.2019.12.006 PMID: 32346348

21. Salvato P, Simonato M, Battisti A, Negrisolo E. The complete mitochondrial genome of the bag-shelter moth Ochrogaster lunifer (Lepidoptera, Notodontidae). BMC Genom. 2008; 9(1):1–5. https://doi.org/10.1186/1471-2164-9-331 PMID: 19505943

22. Dai L, Qian C, Zhang C, Wang L, Lei G, Li J, et al. Characterization of the complete mitochondrial genome of Cerura menziana and comparison with other lepidopteran insects. PloS one. 2015; 10(8): e0132951. https://doi.org/10.1371/journal.pone.0132951 PMID: 26309239

23. Bolger AM, Lohse M, Usadel B. Trimomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014; 30(15):2114–20. https://doi.org/10.1093/bioinformatics/btu170 PMID: 24695404

24. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics. 2009; 25(14):1754–60. https://doi.org/10.1093/bioinformatics/btp324 PMID: 19451168

25. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAMtools. Bioinformatics. 2009; 25(16):2078–9. https://doi.org/10.1093/bioinformatics/btp352 PMID: 19505943

26. Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, et al. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol. Phylogenet. Evol. 2013; 69(2):313–9. https://doi.org/10.1016/j.ympev.2012.08.023 PMID: 22982436

27. Lowe TM, Chen PP. IRINAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res. 2016; 44(5):54–7. https://doi.org/10.1093/nar/gkw413 PMID: 27174935

28. Grant JR, Stothard P. The CGView Server: a comparative genomics tool for circular genomes. Nucleic Acids Res. 2008; 36:181–4. https://doi.org/10.1093/nar/gkn179 PMID: 18411202

29. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 2018; 35(6):1547. https://doi.org/10.1093/molbev/msy096 PMID: 29722887
34. Talavera G, Castresana J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 2007; 56(4):564–77. https://doi.org/10.1080/10635150701472164 PMID: 17654362

35. Zhang D, Gao F, Jakovljevic I, Zou H, Zhang J, Li WX, et al. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Mol Ecol Resour. 2020; 20(1):348–55. https://doi.org/10.1111/1755-0998.13096 PMID: 3159058

36. Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. 2017; 34(3):772–3. https://doi.org/10.1093/molbev/msw260 PMID: 28013191

37. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 2003; 52(5):696–704. https://doi.org/10.1080/10635150390235520 PMID: 14530136

38. Huelsenbeck JP, Ronquist F. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 2001; 17(8):754–5. https://doi.org/10.1093/bioinformatics/17.8.754 PMID: 11524383

39. Cameron SL. Insect mitochondrial genomics: implications for evolution and phylogeny. Annu. Rev. Entomol. 2014; 59:95–117. https://doi.org/10.1146/annurev-ento-011613-162007 PMID: 24160435

40. De Mandal S, Chhakchhuak L, Gurusubramanian G, Kumar NS. Mitochondrial markers for identification and phylogenetic studies in insects–A Review. DNA Barcodes. 2014; 2(1):1–9. https://doi.org/10.2478/dna-2014-0001

41. Zhao JR, Zhang SP, Tang YY, Wang WZ, Tang BP, Liu QN, Yang RP. Characterization and phylogenetic analysis of the complete mitochondrial genome of Parnassius bremeri (Lepidoptera: Noctuoidea: Noctuidae) and Other Noctuid Insects Reveals Conserved Genome Organization and Phylogeny. Ann. Entomol. Soc. Am. 2022; 115 (3):304–13. https://doi.org/10.1093/aesaa/aab055

42. Zhang Y, Xue S. Characterization and phylogenetic analysis of the mitochondrial genome of Macdunnoughia hybrida (Lepidoptera: Noctuidae: Plusiinae). Mitochondrial DNA B: Resour. 2021; 6(8):2294–6. https://doi.org/10.1080/23802359.2021.1945972 PMID: 34345683

43. Liu T, Li Z. The complete mitochondrial genome sequence of cabbage looper, Trichoplusia ni (Lepidoptera: Noctuidae). Mitochondrial DNA B: Resour. 2019; 4(1):2005–7. https://doi.org/10.1080/23802359.2019.1617063

44. Wu QL, Gong YJ, Gu Y, Wei SJ. The complete mitochondrial genome of the beet armyworm Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae). Mitochondrial DNA. 2013; 24(1):31–3. https://doi.org/10.3109/19401736.2012.716052 PMID: 22954158

45. Liu QN, Zhu BJ, Dai LS, Wang L, Qian C, Wei GQ, et al. The complete mitochondrial genome of the common cutworm, Spodoptera litura (Lepidoptera: Noctuidae). Mitochondrial DNA A. 2016; 27 (1):122–3. https://doi.org/10.3109/19401736.2013.873934 PMID: 24491094

46. Baeza JA. The complete mitochondrial genome of the Caribbean spiny lobster Panulirus argus. 2018; Sci. Rep. 8(1):1–10. https://doi.org/10.1038/s41598-018-36132-6 PMID: 30523272

47. Pang X, Han C, Guo B, Liu K, Lin X, Lu X. The First Complete Mitochondrial Genome of Eucrate crenata (Decapoda: Brachyura: Goneplacidae) and Phylogenetic Relationships within Infraorder Brachyura. Genes. 2022; 13(7):1127. https://doi.org/10.3390/genes13071127 PMID: 35885910

48. Riyaz M, Shah RA, Savarimuthu I, Kuppasamy S. Comparative mitochondrial genome analysis of Eudocima salamina (Cramer, 1777) (Lepidoptera: Noctuoidea), novel gene rearrangement and phylogenetic relationship within the superfamily Noctuoidea. Mol. Biol. Rep. 2021; 48(5):4449–63. https://doi.org/10.1007/s11033-021-06465-z PMID: 34109499

49. Xue S, Zhang Y, Gao S, Zhang M. Characterisation and phylogenetic analysis of the complete mitochondrial genome of Ctenoplusia abrostiata (Lepidoptera: Noctuidae: Plusiinae). Mitochondrial DNA B: Resour. 2019; 4(2):3509–10. https://doi.org/10.1080/23802359.2019.1675551 PMID: 33366062

50. Gao S, Xue S, Zhang Y, Wang J, Zhang K. Mitochondrial genome of Diachrysis nadeja (Lepidoptera: Noctuoidea: Noctuidae) and phylogenetic analysis. Mitochondrial DNA B: Resour. 2021; 6(2):406–7. https://doi.org/10.1080/23802359.2020.1870861 PMID: 33628786

51. Li J, Lu Q, Zhang XM, Han HL, Zhang AB. Characterization and Phylogenetic Analysis of the Complete Mitochondrial Genome of Laelia suffusa (Lepidoptera: Erebidae, Lymantriinae). J. Insect Sci. 2021; 21 (1):5. https://doi.org/10.1093/isesa/ieaa138 PMID: 33428744

52. Hao J, Sun Q, Zhao H, Sun X, Gai Y, Yang Q. The complete mitochondrial genome of Ctenoplusium vasava (Lepidoptera: Hesperiidae: Pyrginae) and its phylogenetic implication. Comp. Funct. Genomics. 2012; 2012:1.13. https://doi.org/10.1155/2012/328049 PMID: 22977351

53. Kim M, Baek JY, Kim MJ, Jeong HC, Kim KG, Bae CH, et al. Complete nucleotide sequence and organization of the mitogenome of the red-spotted apple butterfly, Parnassius bremeri (Lepidoptera:
Complete mitochondrial genome of green semilooper (Chrysodeixis acuta) and comparison with other lepidopteran insects. Mol. Cells. 2009; 28(4):347–63. https://doi.org/10.1007/s10059-009-0129-5 PMID: 19823774

54. Zhu BJ, Liu QN, Dai LS, Wang L, Sun Y, Lin KZ, et al. Characterization of the complete mitochondrial genome of Diaphania pyloalis (Lepidoptera: Pyralidae). Gene. 2013; 527(1):283–91. https://doi.org/10.1016/j.gene.2013.06.035 PMID: 23810944

55. Liu QN, Zhu BJ, Dai LS, Liu CL. The complete mitogenome of Bombyx mori strain Dazao (Lepidoptera: Bombyciidae) and comparison with other lepidopteran insects. Genomics. 2013; 101(1):64–73. https://doi.org/10.1016/j.ygeno.2012.10.002 PMID: 23070077

56. Dai LS, Zhu BJ, Qian C, Zhang CF, Li J, Wang L, Wei GQ, Liu CL. The complete mitochondrial genome of the diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae). Mitochondrial DNA A. 2016; 27(2):1512–3. https://doi.org/10.3109/19401736.2014.953116 PMID: 25187437

57. Li J, Zhao Y, Lin R, Zhang Y, Hu K, Li Y, et al. Mitochonrdial genome characteristics of Someina scintilans (Lepidoptera: Erebidae) and comparison with other Noctuoidea insects. Genomics. 2019; 111(6):1239–48. https://doi.org/10.1016/j.ygeno.2018.08.003 PMID: 3010612

58. Chen Q, Chen L, Liao CQ, Wang X, Wang M, Huang GH. Comparative mitochondrial genome analysis and phylogenetic relationship among lepidopteran species. Gene. 2022; 20:146516. https://doi.org/10.1016/j.gene.2022.146516 PMID: 35452707

59. Ojala D, Montoya J, Attardi G. tRNA punctuation model of RNA processing in human mitochondria. Nature. 1981; 290(5806):470–4. https://doi.org/10.1038/290470a0 PMID: 7219536

60. Sun YX, Wang L, Wei GQ, Qian C, Dai LS, Sun Y, Abbas MN, Zhu BJ, Liu CL. Characterization of the complete mitochondrial genome of Leucoma salicis (Lepidoptera: Lymantriidae) and comparison with other lepidopteran insects. Sci. Rep. 2016; 6(1):1–4. https://doi.org/10.1038/srep39153 PMID: 27974854

61. Nethavhani Z, Straeuli R, Hiscock K, Veldtman R, Morton A, Oberprieler RG, van Asch B. Mitogenomics and phylogenetics of twelve species of African Saturniidae (Lepidoptera). PeerJ. 2022; 10:e13275. https://doi.org/10.7717/peerj.13275 PMID: 35462770

62. Zhou N, Dong Y, Qiao P, Yang Z. Complete mitogenomic structure and phylogenetic implications of the genus Ostrinia (Lepidoptera: Crambidae). Insects. 2020; 11(4):232. https://doi.org/10.3390/insects11040232 PMID: 32272743

63. Dai LS, Zhu BJ, Liu QN, Wei GQ, Liu CL. Characterization of the complete mitochondrial genome of Bombyx mori strain H9 (Lepidoptera: Bombyciidae). Gene. 2013; 519(2):326–34. https://doi.org/10.1016/j.gene.2013.02.002 PMID: 23454477

64. Liu QN, Zhu BJ, Dai LS, Wei GQ, Liu CL. The complete mitochondrial genome of the wild silkworm moth, Actias selene. Gene. 2012; 505(2):291–9. https://doi.org/10.1016/j.gene.2012.06.003 PMID: 22688122

65. Liu QN, Chai XY, Bian DD, Ge BM, Zhou CL, Tang BP. The complete mitochondrial genome of fall armyworm Spodoptera frugiperda (Lepidoptera: Noctuidae). Genes Genomics. 2016; 38:205–216. https://doi.org/10.1007/s13258-015-0346-6

66. Hong MY, Jeong HC, Kim MJ, Jeong HU, Lee SH, Kim I. Complete mitogenome sequence of the jewel beetle, Chrysochroa fulgidissima (Coleoptera: Buprestidae) Full-length Research Article. Mitochondrial DNA A. 2016; 27(2):1512–3. https://doi.org/10.3109/19401736.2014.953116 PMID: 25187437

67. Choudhary JS, Naaz N, Prabhakar CS, Rao MS, Das B. The mitochondrial genome of the peach fruit fly, Bactrocera zonata (Saunders) (Diptera: Tephritidae): Complete DNA sequence, genome organization, and phylogenetic analysis with other tephritids using next generation DNA sequencing. Gene. 2015; 569(2):191–202. https://doi.org/10.1016/j.gene.2015.05.066 PMID: 26031235

68. Choudhary JS, Naaz N, Das B, Bhatt BP, Prabhakar CS. Complete mitochondrial genome of Idiosco pus nitidulus (Hemiptera: Cicadellidae). Mitochondrial DNA B. Resour. 2018; 3(1):191–192. https://doi.org/10.1080/23802359.2018.1437798 PMID: 33474113

69. Chen C, Li J, Ding W, Geng X, Zhang H, Sun Y. First complete mitochondrial genome of Acronicta (Lepidoptera: Noctuidae): genome description and its phylogenetic implications. Biologia. 2022; 77(1):93–103. https://doi.org/10.1007/s11756-021-00894-8

70. Liu J, Dai J, Jia J, Zong Y, Sun Y, Peng Y, Wang L, Qian C, Zhu B, Wei G. Characterization and phylogenetic analysis of the complete mitochondrial genome of Saturnia japonica. Biochem. Genet. 2022; 60(3):914–36. https://doi.org/10.1007/s10526-021-10129-9 PMID: 34553327

71. Miga M, Jahari PN, Siang CV, Kumarasivam KR, Shamiris MS, Tokimann S, Rajandas H, Mohamed F, Salieh FM. The complete mitochondrial genome data of the Common Rose butterfly, Pachliopta aristolochiae (Lepidoptera, Papilionoidea, Papilionidae) from Malaysia. Data in brief. 2022; 40:107740. https://doi.org/10.1016/j.dib.2021.107740 PMID: 35141362
72. Cameron SL, Whiting MF. The complete mitochondrial genome of the tobacco hornworm, Manduca sexta, (Insecta: Lepidoptera: Sphingidae), and an examination of mitochondrial gene variability within butterflies and moths. Gene. 2008; 408(1–2):112–23. https://doi.org/10.1016/j.gene.2007.10.023 PMID: 18065166

73. Sun Y, Zhu Y, Chen C, Zhu Q, Zhu Q, Zhou Y, et al. The complete mitochondrial genome of Dysgonia stuposa (Lepidoptera: Erebidae) and phylogenetic relationships within Noctuoidea. PeerJ. 2020; 8: e8780. https://doi.org/10.7717/peerj.8780 PMID: 32211241

74. Wu Y, Fang H, Wen J, Wang J, Cao T, He B. Mitochondrial Genomes of Hestina persimilis and Hestinalis nama (Lepidoptera, Nymphalidae): genome description and phylogenetic implications. Insects. 2021; 12(8):754. https://doi.org/10.3390/insects12080754 PMID: 34442319

75. Hassanin A, Leger NE, Deutsch J. Evidence for multiple reversals of asymmetric mutational constraints during the evolution of the mitochondrial genome of Metazoa, and consequences for phylogenetic inferences. Syst. Biol. 2005; 54(2):277–98. https://doi.org/10.1080/10635150590947843 PMID: 16021696

76. Wang Q, Tang G. The mitochondrial genomes of two walnut pests, Gastrolina depressa depressa and G. depressa thoracica (Coleoptera: Chrysomelidae), and phylogenetic analyses. PeerJ. 2018; 6: e4919. https://doi.org/10.7717/peerj.4919 PMID: 29888134

77. Zhang B, Nardi F, Hull-Sanders H, Wan X, Liu Y. The complete nucleotide sequence of the mitochondrial genome of Bactrocera minax (Diptera: Tephritidae). PLoS One. 2014; 9(6):e100558. https://doi.org/10.1371/journal.pone.0100558 PMID: 24964138

78. Yang X, Cameron SL, Lees DC, Xue D, Han H. A mitochondrial genome phylogeny of owlet moths (Lepidoptera: Noctuoidea), and examination of the utility of mitochondrial genomes for lepidopteran phylogenetics. Mol. Phylogenet. Evol. 2015; 85:230–7. https://doi.org/10.1016/j.ympev.2015.02.005 PMID: 25698356