Structural Analysis and Phylogenetic Relationship of a Lepidopteran *Scopelodes kwangtungensis* Based on its Complete Mitochondrial Genome Sequence

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ABSTRACT

In this study, we used next-generation sequencing to obtain the complete sequence of the mitochondrial genome of a lepidopteran *Scopelodes kwangtungensis* and analyzed its gene composition and structural characteristics. The complete mitochondrial genome of *S. kwangtungensi*s is 14871 bp in length and comprises 13 protein-coding genes, 22 tRNA genes, two rRNA genes, and one control region. It has A+T and G+C contents of 80.1% and 19.9%, respectively, indicating a clear A+T bias. Within the coding regions, the most common start codon is ATG, the most common stop codon is TAA, and leucine is the most commonly occurring amino acid. To establish the phylogenetic status of *S. kwangtungensi*s, we used the maximum likelihood and Bayesian inference methods to construct phylogenetic trees based on the sequences of the 13 protein-coding genes. The results revealed *S. kwangtungensi*s to be closely related to *Monema flavescens*, of which it forms a sister branch. These results augment the current mitochondrial genome data for the lepidopteran family Limacodidae and provide information for the systematic classification of this group.

INTRODUCTION

The lepidopteran *Scopelodes kwangtungensis*, a moth in the family Limacodidae, was first described by Hering in 2015 (Pan and Wu, 2015). It is distributed primarily in western and southern China, including the provinces of Gansu, Yunnan, and Hubei, where it is among the main pests of fruit trees, garden shrubs, and cash crops (Jiao *et al.*, 2019). Notably, the larvae of this species bear numerous urticating hairs and toxin-producing glands, which can cause dermatitis and a severe burning pain when coming into contact with human skin. Given that the species is widely distributed in densely populated green belt areas, it potentially poses a considerable and largely overlooked threat to human health (Han, 2013; Cao and Peng, 2009). Adult *S. kwangtungensi*s have a wingspan of approximately 55 mm, the hair at the end of the lower lip is white and long, and the chest is dark brown. Its abdomen is orange-yellow, with black bands on each section from the third section, and dark brown in the distal segment. The forewings are dark brown and covered with silver-gray scales, whereas the hind wings are light to dark brown, one third of the base and the posterior margin are partly yellowish brown (Wu *et al.*, 2009), and the outer half of the wing vein is yellowish. The larvae of *S. kwangtungensi*s have a yellow ventral surface, a green dorsal surface, and two rows of dense spines. The eighth section of the larvae has red, white, and blue horizontal stripes on the dorsal surface and black spots on the tail.

The mitochondrial genome is a double-stranded circular DNA molecule that is the only genetic material that can be replicated and transcribed independently outside the animal nucleus. Moreover, given its simple structure, compact arrangement, and low rate of mutation, mitochondrial genomes tend to be widely used in molecular systematics and analysis of pedigree geography (Qiu, 2023). The mitochondrial genomes of insects are generally 14–20 kb in size and typically comprise 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, and a single control region. Given that mitochondrial genomes have undergone rapid evolution and have a relatively stable gene composition,

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they are widely used to analyze the phylogeny of insects. To date, the mitochondrial genome sequences of more than 25 species in the family Limacodidae have been deposited in the GenBank database, which will contribute to gaining a further understanding the evolutionary history of Limacodidae (Xu *et al*., 2023; Cameron, 2014).

The elucidation of mitochondrial genomes has become increasingly crucial within the field of biology. Mitochondria, serving as the energy centers within cells, house genetic material that is not only vital for the provision of cellular energy but also harbors crucial information for phylogenetic studies and genetics. The lepidopteran species *Scopelodes kwangtungensis* (*S. kwangtungensis*) represents a significant insect pest, posing a substantial threat to fruit trees, garden shrubs, and economically important crops. A comprehensive understanding of the structure and features of its mitochondrial genome holds paramount importance for gaining insights into its biology, genetics, and evolutionary history. Furthermore, mitochondrial genome research serves a dual purpose by facilitating the development of molecular biology identification techniques, the formulation of pest control strategies, and the exploration of potential drug development avenues. Thus, this study aims to provide foundational data for molecular and biological research related to *S. kwangtungensis* by analyzing its mitochondrial genome. Additionally, it contributes valuable information to the broader fields of biology and ecology. Through this research, we aspire to make significant contributions to advancements in pest control and ecological preservation.

MATERIALS AND METHODS

Experimental materials, DNA extraction, and sequencing

An adult specimen of *S. kwangtungensis* was collected in July 2021 in Lancang County, Yunnan Province (99°55′55″E, 22°33′21″W). This specimen has been preserved at the School of Ecology, Lanzhou University, China (http://www.lzu.edu.cn/; Fang Li: lf2454822793@163.com; voucher number: SK0001). Total genomic DNA was extracted from a wing sample using a Tiangen Genome DNA Kit (TIANGEN, China) in accordance with the manufacturer's instructions. The quality and purity of the extracted DNA were determined by agarose gel electrophoresis and NanoDrop 2000 spectrophotometry (NanoDrop Technologies, Wilmington, DE, USA). Qualified total genomic DNA was sent to Wuhan Bena Biotechnology Co., Ltd. for preparation of a DNA library using an MGIEasy Digestion DNA Library Prep Kit (192 RXN) This library DNA was subsequently subjected to next-generation high-throughput sequencing (double-ended 150 bp) using the DNBSEQ-T7 sequencing

platform. fastp software (Chen *et al*., 2018) was used to filter the original data and remove sequencing adapters, low-quality terminals, N > 10% reads, and fragments of less than 25 bases.

Assembly, annotation, and feature analysis

Get Organelle v1.7.5 software (Jin *et al*., 2020), with parameters set to "- R 30 -k 21, 45, 65, 85, 105 -w 50," was used to assemble the mitochondrial genome into a loop configuration, and the looped mitochondrial genome was uploaded to the MITOS (Bernt *et al*., 2013) website (http://mitos.bioinf.uni-leipzig.de/) for functional annotation. The genetic code was set to insects, and the default parameters of MITOS were used for other settings. Having obtained the annotation results, further manual correction was performed using Geneious (Kearse *et al*., 2012). The base composition, codon usage frequency, AT skew, and GC skew of each coding gene in the mitochondrial genome were calculated using MEGA11 (Tamura *et al*., 2021).

Phylogenetic analysis

To establish the phylogenetic status of *S. kwangtungensis* (family, Limacodidae), we compared the mitochondrial genome assembled in this study with those of five species in the family Tortricidae, five species in the family Zygaenidae, three species in the family Limacodidae, three species in the family Gelechiidae, two species of the family Lymantriidae, and four species in the family Noctuidae, using sequences published in the NCBI database (Table I). The species in the family Tortricidae served as an outgroup. A maximum likelihood (ML) phylogenetic tree was constructed based on the sequences of the 13 PCGs using IQ-TREE v1.6.8 (Nguyen *et al*., 2015) in PhyloSuite software. Initially, the best model was screened using model finder (Kalyaanamoorthy *et al*., 2017), after which, 100000-branch confidence was calculated using the ultrafast bootstrap method (ultrafast bootstrapping) (Minh *et al*., 2013). Mbayes v3.2.6 (Huelsenbeck and Ronquist, 2001) was used to construct a Bayesian inference (BI) phylogenetic tree using the partition model, in which the initial 10% of the sampled data were discarded. The phylogenetic tree was visualized using FigTree v1.4.4 (https://github.com/rambaut/figtree).

RESULTS

Gene structure and composition

The whole mitochondrial genome of *S. kwangtungensis* is a circular DNA molecule of 14871 bp (Fig. 1) containing 37 genes, namely, 13 PCGs, 22 RNAs, two rRNAs, and a single AT-enriched region. The nucleotide composition of the genome is A (39.5%), T (40.6%), C (8.0%), and G

**Table I. Origins of mitochondrial genomes of Ditrysia.**

Taxon (Species)	Size (bp)	AT%	AT-Skew	GC-Skew	Accession number
Ditrysia					
Tortricidae (outgroup)					
Grapholita dimorpha	15813	80.9	-0.01112	-0.1875	NC024582
Rhyacionia leptotubula	15876	80.3	-0.00125	-0.228426	NC 019619
Adoxophyes orana	15343	79.9	-0.00125	-0.21	NC 021396
Choristoneura conflictana	15387	81	0.004938	-0.168421	NC 039421
Choristoneura murinana	15488	81.4	0.002457	-0.16129	NC 037396
Zygaenidae					
Amesia sanguiflua	15203	79.8	0.002506	-0.227723	NC 046467
Eterusia aedea	15196	79.6	-0.00503	-0.211823	NC 038208
Histia rhodope	15209	78.5	0.014013	-0.240741	NC 039447
Rhodopsona rubiginosa	15248	79	0.010127	-0.228571	NC 025761
Phauda flammans	15470	82	-0.02195	-0.206704	NC 047243
Limacodidae					
Monema flavescens	15396	80.5	0.013665	-0.22449	NC 032683
Scopelodes kwangtungensis	14871	80.1	-0.01373	0.19598	OQ848600
Parasa consocia	15296	80.6	0	-0.237113	NC 034993
Narosa nigrisigna	15292	81.2	-0.03941	-0.180851	NC 041304
Gelechiidae					
Dichomeris ustalella	15410	81.1	-0.03822	-0.174603	NC 029810
Helcystogramma macroscopa	15394	80.9	0.013597	-0.189474	NC 029844
Mesophleps albilinella	15274	80.5	-0.06087	-0.230769	NC 029811
Lymantriidae					
Orgyia postica	15258	77.2	-0.0285	0.303965	NC 057614
Somena scintillans	15410	80.9	-0.00865	-0.25	NC 039764
Noctuidae					
Helicoverpa assulta	15373	80.8	-0.00248	-0.193717	NC 035890
Heliothis subflexa	15323	80.7	0.001239	-0.19171	NC 028539
Mythimna loreyi	15320	80.9	-0.01607	-0.2	NC 057500
Spodoptera litura	15388	81	0.012346	-0.193717	NC 022676

(11.9%), with an A+T content of 80.1%. Among the 37 genes, 25 genes, along with the AT-enriched region, are contained within the heavy chain, of which the A+T content is 73.0%. The remaining 12 genes are contained in the light chain, the A+T content of which is 80.2%. The 22 tRNA genes range in size from 54 to 72 bp. Of the two rRNA genes, the length of the *rrnl* (16S rRNA) gene is 648 bp and has an A+T content of 76.7%, whereas the *rrns* (12S rRNA) gene is 787 bp in length and has an A+T content of 85.0% (Table II). Within the entire mitochondrial genome there are five gene overlaps and 24 gene gaps, the largest of which is the interval between *rrnl* and *trn*L1 (76 bp). The total length of gene overlap is 21 bp, among which there is a large overlap between *trn*C and *trn*W, with eight bases in the overlapping region.

Protein-coding genes

The full length of the 13 protein-coding genes in the mitochondrial genome of *S. kwangtungensis* is 12584 bp. Among these genes, (four *nad*1, *nad*4, *nad*4L, and *nad*5), are located in the heavy chain, with the remaining nine being located in the light chain. In terms of codon usage, apart from *cox*1, for which the start codon is CGA, the start codon of all coding genes is ATN. Deletion of the termination codon is typically attributable to polyadenosine acidification. In the mitochondrial genome of *S. kwangtungensis*, the termination codons of *nad*4 and *nad*5 have an incomplete (T) codo
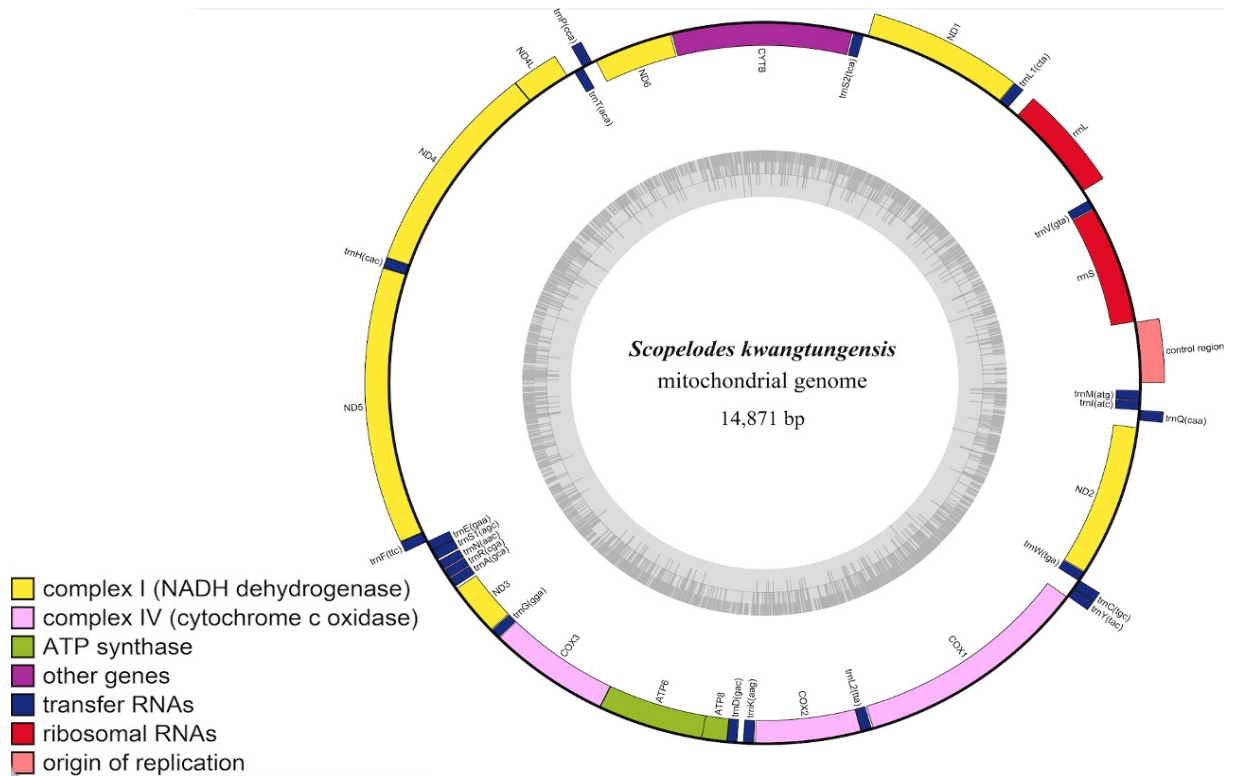
Fig. 1. Circular map of the mitogenomes of *S. kwangtungensis*.

Table II. Characteristics of the mitochondrial genome of *S. kwangtungensis*.

| Gene | Position | | Size | Intergenic nucleotid | Condon | | |
	From	To			Start	Stop	Strand
D-loop	1	401	401	48			H
rrns	404	1191	788	2			L
*trn*V	1194	1258	65	2			L
rrnl	1328	1976	649	69			H
*trn*L1	2053	2120	68	76			H
*nad*1	2122	3060	939	1	ATG	TAA	H
*trn*S2	3086	3152	67	25			L
cytb	3159	4310	1125	6	ATG	TAA	L
*nad*6	4319	4822	504	8	ATA	TAA	L
*trn*P	4846	4913	68	23			H
*trn*T	4914	4977	64	0			L
*nad*4L	5025	5312	288	47	ATG	TAA	H
*nad*4	5313	6648	1336	0	ATG	T	H
*trn*H	6649	6713	65	0			H
*nad*5	6714	8445	1732	0	ATT	T	H
*trn*F	8446	8513	68	0			H
*trn*E	8512	8579	68	-2			L

Table continued on next column.........

| Gene | Position | | Size | Intergenic nucleotid | Condon | | |
	From	To			Start	Stop	Strand
*trn*S1	8580	8647	68	0			L
*trn*N	8657	8722	66	9			L
*trn*R	8725	8789	65	2			L
*trn*A	8792	8857	66	2			L
*nad*3	8871	9221	351	13	ATT	TAA	L
*trn*G	9225	9290	66	3			L
*cox*3	9293	10078	786	2	ATG	TAA	L
*atp*6	10078	10758	681	-1	ATG	TAA	L
*atp*8	10752	10913	162	-7	ATT	TAA	L
*trn*D	10914	10979	66	0			L
*trn*K	11016	11087	72	36			L
*cox*2	11092	11769	678	4	ATG	TAA	L
*trn*L2	11770	11838	69	0			L
*cox*1	11846	13369	1524	7	CGA	TAA	L
*trn*Y	13374	13438	65	4			H
*trn*C	13439	13503	65	0			H
*trn*W	13496	13563	68	-8			L
*nad*2	13566	14579	1014	2	ATC	TAA	L
*trn*Q	14626	14694	69	46			H
*trn*I	14692	14755	64	-3			L
*trn*M	14757	14823	67	1			L

Table III. Nucleotide composition of protein-coding genes and rRNA in *S. kwangtungensis*.

Gene	Lenth (bp)	T (%)	C (%)	A (%)	G (%)	AT %	GC %	AT-skew	Gc-skew
*atp*6	681	43.2	12.9	35.8	8.1	79	21	-0.171	0.229
*atp*8	162	48.8	8.6	40.1	2.5	88.9	11.1	-0.178	0.550
*cox*1	1524	38.2	15.1	33.4	13.3	71.6	28.4	-0.126	0.063
*cox*2	678	40	13.4	37.3	9.3	77.3	22.7	-0.068	0.181
*cox*3	786	40.2	14.4	33.7	11.7	73.9	26.1	-0.162	0.103
cytb	1152	41.1	14.9	33.9	10.2	75	25.1	-0.175	0.187
*nad*1	939	49	7.1	28.9	15	77.9	22.1	-0.410	-0.357
*nad*2	1014	46.2	10.7	37.4	5.8	83.6	16.5	-0.190	0.297
*nad*3	351	48.1	11.1	33.6	7.1	81.7	18.2	-0.301	0.220
*nad*4	1336	46.3	6.7	34.8	12.3	81.1	19	-0.248	-0.295
*nad*4L	288	51.7	5.6	29.5	13.2	81.2	18.8	-0.429	-0.404
*nad*5	1732	47.7	5.9	34.2	12.1	81.9	18	-0.283	-0.344
*nad*6	504	49.4	8.1	36.3	6.2	85.7	14.3	-0.265	0.133
rrnl	649	38.4	8.6	38.4	14.6	76.8	23.2	0	-0.259
rrns	788	44.2	4.7	40.9	10.3	85.1	15	-0.075	-0.373

the remaining genes use a TAA as the stop codon, which is a commonly used termination codon in metazoan

mitochondrial genomes. Although the genes differ with respect to base contents, they are all characterized by lower G + C contents and higher A + T contents (Table III).

Codon usage frequency

To determine the ratio of the expected frequency of amino acids to the observed frequency using synonymous codons, we analyzed the mitochondrial genome of S. kwangtungensis using MEGA (Table IV, Fig. 2) As a result, we identified 27 preferentially used codons (relative synonymous codon usage ≥ 1) in the 13 PCGs (Behura and Severson, 2013). The genome sequence of 12584 bp encodes 3704 amino acids, the most commonly occurring of which is leucine (Leu), with a content of 14.23%, whereas the least used amino acid is cysteine (Cys), with a content of 1.03%.

tRNAs, rRNAs, and the control region

Similar to other moths in the family Limacodidae, the mitochondrial genome of *S. kwangtungensis* contains 22 tRNAs, ranging in length between 64 and 72 bp. Of the two rRNAs, 12s rRNA (*rrns*) is located in the 404–1191-bp region of the light chain with a length of 788 bp, whereas 16s rRNA (*rrnl*) is located in the 1328–1976-bp of the heavy chain with a length of 649 bp. The control region spans the sequence between base pairs 1 and 401.

Table IV. Frequency of codon usage in 13 protein-coding genes.

| Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU
UUU(F)	27.3	1.84	UCU(S)	7.4	2.42	UAU(Y)	12.1	1.87	UGU(C)	2.5	1.74
UUC(F)	2.4	0.16	UCC(S)	1.3	0.43	UAC(Y)	0.8	0.13	UGC(C)	0.4	0.26
UUA(L)	33.6	4.98	UCA(S)	7.6	2.5	UAA(*)	0.8	2	UGA(W)	7.3	2
UUG(L)	1.5	0.23	UCG(S)	0	0	UAG(*)	0	0	UGG(W)	0	0
CUU(L)	2.9	0.43	CCU(P)	4.7	1.97	CAU(H)	4.2	1.59	CGU(R)	0.7	0.69
CUC(L)	0.5	0.08	CCC(P)	1.2	0.52	CAC(H)	1.1	0.41	CGC(R)	0.1	0.08
CUA(L)	1.9	0.28	CCA(P)	3.5	1.45	CAA(Q)	4.6	1.85	CGA(R)	3.2	3.15
CUG(L)	0	0	CCG(P)	0.2	0.06	CAG(Q)	0.4	0.15	CGG(R)	0.1	0.08
AUU(I)	31.5	1.85	ACU(T)	6.2	2.19	AAU(N)	18.2	1.83	AGU(S)	1.4	0.45
AUC(I)	2.5	0.15	ACC(T)	1.2	0.43	AAC(N)	1.7	0.17	AGC(S)	0.1	0.03
AUA(M)	21.1	1.85	ACA(T)	3.9	1.38	AAA(K)	7.8	1.82	AGA(S)	6.6	2.17
AUG(M)	1.8	0.15	ACG(T)	0	0	AAG(K)	0.8	0.18	AGG(S)	0	0
GUU(V)	5.3	1.89	GCU(A)	5.2	2.25	GAU(D)	4.4	1.9	GGU(G)	3.3	0.86
GUC(V)	0.2	0.05	GCC(A)	0.5	0.2	GAC(D)	0.2	0.1	GGC(G)	0.2	0.04
GUA(V)	5.1	1.81	GCA(A)	3.5	1.49	GAA(E)	5.1	1.69	GGA(G)	10.2	2.64
GUG(V)	0.7	0.25	GCG(A)	0.2	0.07	GAG(E)	0.9	0.31	GGG(G)	1.8	0.46
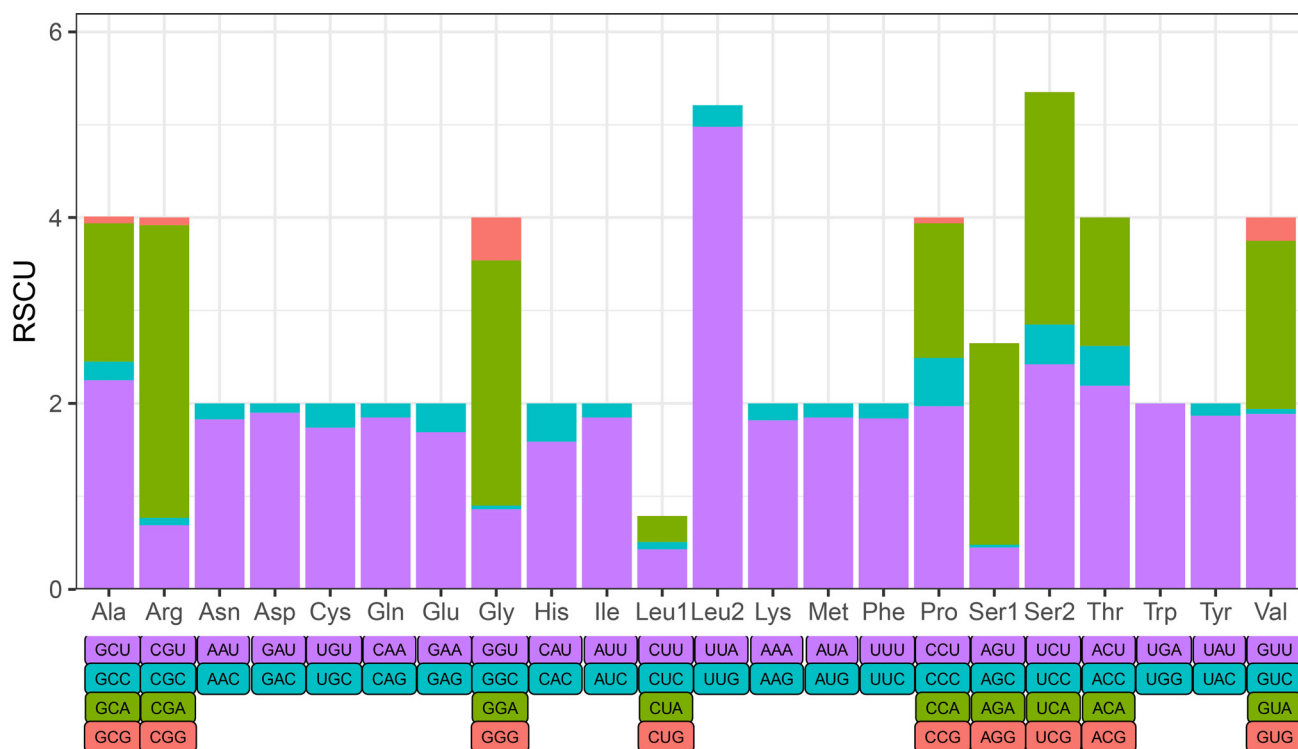
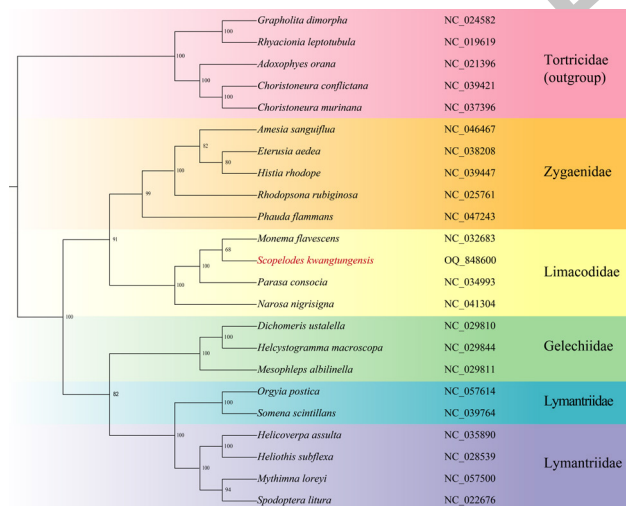Fig. 2. Relative synonymous codon usage in mitochondrial protein-coding genes of *S. Kwangtungensis.*

Fig. 3. Phylogenetic relationship of 23 species of Ditrysia based on the concatenated data set of 13 PCGs. Number of each node indicates the ML bootstrap support value. Alphanumeric terms indicate the GenBank accession numbers.

Phylogenetic relationships

ML and BI phylogenetic trees, obtained to established the phylogenetic status of *S. kwangtungensis*, were constructed based on the nucleotide sequences of the 13 PCGs. These trees revealed that *S. kwangtungensis* belongs to the family Limacodidae and is classified as a branch of *Monema flavescens*, *Parasa consocia,* and *Narosa nigrisigna*, which are sister branches of the family Zygaenidae (Fig. 3).

DISCUSSION

With recent developments in next-generation sequencing technology, the mitochondrial genomes of insects are increasingly studied in diverse contexts, including species identification, pest control, population polymorphism, and phylogeny. The class Insects comprises the largest proportion of described species in the animal kingdom, and has been extensively studied by scientists. Owing to its matrilineal inheritance, high level of evolution, and low rate of intramolecular genetic recombination, the mitochondrial genome of insects is widely used in phylogeny, population genetics, and evolutionary biology (Yu *et al.*, 2022). Lepidoptera is the third largest order of insects, with only the orders Diptera and Coleoptera containing a larger number of known species. Lepidoptera play important beneficial roles in almost all terrestrial ecosystems, but also include many agricultural pests and

disease vectors that have a significant impact on human production and welfare.

In this study, we successfully assembled the mitochondrial genome of *S. kwangtungensis*, which was established to contain the 13 PCGs, two rRNAs, 22 tRNAs, and single control region typically found in insects. There is an overlap region of 7 bp (ATGATAA) between *atp*6 and *atp*8. Among the 13 PCGs, leucine (UUA) is the most commonly used amino acid, which is consistent with the findings of Chen *et al*. (2022).

It should be noted, however, that while the mitochondrial genome can be used for phylogenetic analysis, it represents only the genetic information of the mitochondria and not the entire organism's genetic information. Therefore, for a more comprehensive understanding of the systematic phylogenetic position of *S. kwangtungensis*, it is advisable to conduct further research by incorporating additional genetic data and morphological characteristics. Additionally, including more mitochondrial genome data from multiple individuals of *S. kwangtungensis* and related species in the analysis can further enhance the accuracy of phylogenetic analysis.

CONCLUSION

The entire mitochondrial genome of *S. kwangtungensis* was obtained using second-generation sequencing. It contains 13 protein-coding genes, 22 tRNA genes, two rRNA genes, and a single control region. Phylogenetic analysis revealed that *S. kwangtungensis* is closely related to *Monema flavescens*, of which it forms a sister branch.

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IRB approval
The study was approved by the Ethical Committee of the Mudanjiang Normal University.

Ethical statement
The animal study protocol was approved by the Ethics Committee of Mudanjiang Normal University. All efforts were made to minimize pain and discomfort to the specimens during research.

Data availability statement
The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession No. OQ848600.

Statement of conflict interest
The authors have declared no conflict of interest.

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