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Complete mitochondrial genome of *Parasa sinica*: New insights into the phylogeny of Limacodidae

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ABSTRACT

In this study, the whole mitochondrial genome (mitogenome) of *Parasa sinica* was sequenced. It contains 15,306 base pairs (bp), 13 protein-coding genes (PCGs), two ribosomal RNAs (rRNAs), 22 transfer RNAs (tRNAs), and one non-coding regulatory area (CR), all of which are shared by other lepidopterans. It follows the same gene order as ordinary lepidopterans. Further, out of these 37 genes, 23 are present on the heavy strand whereas the remaining 14 are located on the light strand. The A + T composition of the mitogenome is relatively high. Although *P. sinica* has a negative AT-skew and GC-skew, the GC-skew value is significantly lower than the AT-skew value. All PCGs, with the exception of CO1, carry the same start codon (ATN). All tRNAs exhibit the usual cloverleaf secondary structure. We identified the conserved motif "ATAGA + poly-T" found in other lepidopteran insects at the beginning of the CR. We collected the concatenated PCGs sequences in the mitochondrial genome of 15 species of Zygaenoidea, with the sequences of Geometridae as outgroups, including *P. sinica*, and constructed phylogenetic trees using Bayesian inference (BI) and maximum likelihood (ML) methods. The monolineage of each superfamily is usually well supported. Based on phylogenetic analysis, *P. sinica* is a member of family Limacodidae, strongly supporting the monophyly of the Zygaenoidea groups.

1. Introduction

Parasa sinica is a pest, also known as the small green prickly moth and the brown edge green prickly moth. It is distributed in China and a number of countries worldwidel. According to the morphological classification, it belongs to Limacodidae [1]. *P. sinica* has a wide range of hosts and feeds on the leaves of many garden trees, such as walnut, apple, and purple plum. *P. sinica* not only affects the normal growth of trees, but also personal safety. Upon contact with its poisonous hair, the skin turns red, swollen, itchy and painful [2]. Currently, artificial control, crown spraying and light trapping are used to control pests in China [3]. We sequenced the mitochondrial genome of *P. sinica* to determine its evolutionary position relative to other relevant species.

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Mitochondria are semi-autonomous organelles containing double stranded circular genomes. The insect mitochondrial genome contains a double-stranded closed loop molecule measuring 15–20 kb in size. It typically carries two ribosomal RNA genes (rRNAs), 13 protein coding genes (PCGs), 22 transfer RNA genes (tRNAs), and one or more coding regions (CRs) with transcription and replication signals [4]. Insect mitochondrial genome has the characteristics of simple structure, stable composition, high copy number, maternal inheritance, rapid rates of evolution and low recombination [5]. Compared with a single gene or a few genes, the mitochondrial genome, as a complete genome, contributes significantly to the study of insect species evolution, phylogeny and population genetics. It is an important molecular marker, which has been used widely in Syrphidae [6], *Zygaenidae* [7], Noctuidae [8], Lycaenidae [9] and other insect families [10,11]. Lepidoptera is the second largest order of insects after Coleoptera, and comprises nearly 160,000 species [12]. Lepidopteran insects are widely distributed in all continents except Antarctica. The vast majority of species live on land, and a few live in water. Lepidopteran insects are important agricultural and forestry pests, pollinators and economic insects, which have a profound impact on human society [13,14].

Zygaenoidea belongs to Lepidoptera and is included in the clade Lepidoptera Ditrysia. Currently, it comprises about 3300 species in 12 families [15]. The wide geographical distribution and interesting chemical defense system of Zygaenoidea insects have attracted many experts in Lepidoptera [16]. However, the phylogenetic analysis of Zygaenoidea was confined to the morphological level for a long time due to limitations in molecular data. More than 200 complete or nearly complete mitochondrial genomes of Lepidoptera have been identified, including only four are Zygaenoidea's moths [17,18]. At present, the internal phylogenetic relationship of Zygaenoidea is still under investigation, and the phylogenetic relationship with other Lepidoptera groups is still disputed. This study provides a basis for clarifying the internal phylogenetic relationship of Zygaenoidea.

Limacodidae belongs to Lepidoptera Zygaenoidea. It comprises more than 1500 known species worldwide [19]. They are widely distributed in the world's major animal geographical regions, with the most abundant species found in the tropics and subtropics [20]. About 230 species are known in China [21]. Other regions have been added in recent years [22]. The taxonomic position of Limacodidae in Lepidoptera has been disputed. Currently, the system proposed by Holloway is widely used in the classification of Limacodidae in Southeast Asia. Accordingly, Limacodidae is divided into five groups based on the shape of antennae, the type of mating bursa of female external genitalia, the front wing veins and the morphological characteristics of larvae. However, the classification system of the five groups of Limacodidae is still not very clear, suggesting the need for further study. Thus, the internal phylogenetic relationship of Zygaenoidea is still unclear. We analysed the mitochondrial genome sequence of *P. sinica*. The findingreveal the phylogenetic relationship of Limacodidae, which is of great significance for further internal classification system of Limacodidae. Additionally, this analysis provides a foundation for elucidating the internal phylogenetic relationship of Zygaenoidea.

2. Materials and methods

2.1. Specimen collection and DNA extraction

P. sinica specimens collectedfrom farms in Yancheng City, Jiangsu Province, China, were preserved in 95 % ethanol. The total DNA from a single specimen's thorax muscle tissue was extracted using the Genomic DNA Extraction Kit (Sangon Biotech, China) according to the manufacturer's instructions. Extracted DNA was preserved at 20 °C for subsequent analysis.

2.2. PCR amplification and sequencing

In this experiment, conventional PCR and long PCR were used together to obtain a complete mitogenome. Based on mitogenome sequences obtained from other lepidopteran insects, the current PCR primers were designed [23]. The fragments were amplified using Aidlab Red Taq (Beijing, China), as directed by the manufacturer. The amplifications were carried out in 50 μL reaction volumes on a MASTERCYCLER (Eppendorf) and Mastercycler Gradient. The PCR amplification steps (10 min) included 35 cycles of denaturation at 94 °C (for 30 s), annealing at 48–60 °C (depending on the primer combinations, for 35 s), elongation at 68 °C (for 1–3 min, depending on the anticipated length of the amplification fragments), and extension at 72 °C. PCR results were identified on a 1.5 % TAE agarose gel using appropriate DNA markers. The purification step was completed using a DNA gel extraction kit (Transgene, China). The T-vector (Eide Biotech, China) was ligated with the purified PCR products before sequencing each at least three times.

2.3. Sequence assembly, annotation and analysis

The mitogenome was put together using the SeqMan (DNAStar) program, and the sequence was annotated using NCBI BLAST. Using MAFFT, the PCGs and rRNA genes annotated using BLAST were aligned with homologous sequences from other members of Limacodidae [24]. We used Geneious v9 to create the circular mitogenome map [25]. The tRNAscan-SE v1.21 was used to predict the majority of the secondary structures of the tRNA genes [26]. By comparison with sequences from other species, we identify the remaining tRNAs that were not predicted. In light of the formulas (AT skew = [A − T]/[A + T]; GC skew = [G − C]/[G + C]), the composition skewness was determined to directly distinguish genes [27]. MEGA version 6.06 was used to calculate base composition and codon usage [28]. Finally, we used the Tandem Repeats Finder program (http://tandem.bu. edu/trf/trf.html) to predict the tandem repeats of the A + T-rich region [29]. MITOS software is used to annotate genes.

2.4. Phylogenetic analysis

Sequences from various families were accessed from GenBank in Zygaenoidea and used in our phylogenetic studies to determine the affiliation of *P. sinica*, as shown in Table 1. *Phthonandria atrilineata* (EU569764), which belong to Geometridae were used as an outgroup. The default settings of 13 mitochondrial PCGs were compared via MAFFT [30], and the results were imported into Gblocks v 0.91b to identify conserved regions [31]. A replacement saturation study was carried out in DAMBE v 5.2.73 to determine the suitability of the dataset for tree construction [32]. Next, EasyCodeML software was used to change the Gblocks-generated files' format. The concatenated collection of amino acid and nucleotide sequences was used for phylogenetic analysis using maximum likelihood (ML) methods with raxmlGUI [33] and Bayesian inference (BI) using MrBayes v3.2.2 [34]. Based on the Akaike Information Criterion (AIC), we used MrModeltest v2.3 and found that GTR + I + G was the best-fit model for nucleotide analyses [35]. Moreover, we found that MtArt + I + G + F was the best-fit model for amino acid analyses under the AIC using ProtTest v3.4 [36]. Ten million iterations of the Bayesian MCMC were conducted, with samples obtained every 1000 generations. A posterior probability of more than 0.9 was considered plausible [37]. Convergence was attained over 10 million generations.

3. Results and discussion

3.1. Genome organization and base composition

The *P. sinica* mitogenome is a complete typical circular sequence of 15,306 bp. In addition to a CR, it contains 37 functional genes, including 13 PCGs, 22 tRNAs, and 2 rRNAs. Among these 37 genes, 14 are encoded on the light strand while the remaining 23 are found on the heavy strand (Fig. 1, Table 2). Table 3 presents the basic nucleotide composition of *P. sinica*: (A) 40.2 %, (T) 40.8 %, (G) 11.5 %, and (C) 7.5 %. Further, the A + T content of the whole mitogenome is high (80.9 %), including the A + T levels of PCGs, tRNAs, rRNAs, and CR (79.3 %, 82.7 %, 85.1 %, and 93.0 %, respectively). The A + T content of *P. sinica* ranges from 77.8 % (*Ochrogaster lunifer*) to 82.7 % (*Coreana raphaelis*) of known lepidopteran mitogenomes [38,39]. Further, AT-skew and GC-skew values often depict the base composition of a mitogenome [40,41]. The AT-skew and GC-skew in the whole mitogenome are 0.007 and 0.210, respectively (Table 3). Although both the AT-skew and the GC-skew of *P. sinica* are negative, the GC-skew value is significantly lower than the AT-skew value. Also, practically all AT-skew and GC-skew values are negative, with the exception of PCGs, whose AT-skew value is 0.001 and tRNAs, whose AT-skew value is 0.008, suggesting that the utilization of As and Gs throughout the whole mitogenome is higher than that of Ts and Cs.

3.2. Protein-coding genes

Among the 13 PCGs, the ND5 (1735 bp) is the longest and the ATP8 (162 bp) is shorter, as shown in Table 2. Nine genes (CO1, CO2, CO3, ND2, ND3, ND6, ATP6,ATP8 and CytB) are encoded on the J-strand, and the remaining four genes (ND1, ND4, ND4L and ND5) on the N-strand (Table 2, Fig. 1). Except for CO1 (CGA), the starting codon of all protein coding genes (PCGs) is ATN (ATG for CO3, ND1, ND4, ND4L, ATP6, CytB; ATT for ND2, ND3, ND5, ND6, CO2; ATA for ATP8). Five of the 13 PCGs—CO1, CO2, ND2, ND4, and ND5-terminate with an incomplete stop codon T; eight—terminate in a full stop codon TAA; and one PCG ends with an incomplete stop codon TA. The final PCG is ATP6. PCGs with 11169 bp long have the lowest A + T concentration of all of the genes (Table 3). The prevalence of codon families and the Relative Synonymous Codon Use (RSCU) in PCGs are depicted in Fig. 2. The results show that five codon families (UUU, AUU, UUA, AAU and AUA) were the most frequently used (more than 200 times) in Chinese insect species. In addition, six codon families did not appear in the genome (CUC, CUG, CCG, UAG, CGC, AGG) (Table 4, Fig. 2).

Table 1
List of species used to construct the phylogenetic tree.

Family	Species	GBAN*
Zygaenidae	*Phauda flammans*	NC_047243
	Eterusia aedea	MZ964410
	Histia rhodope	OM891519
	Pidorus atratus	NC_037909
	Illiberis pruni	MZ726799
	Illiberis ulmivora	MT075808
	Amesia sanguiflua	NC_046467
	Rhodopsona rubiginosa	NC_025761
Limacodidae	*Quasithosea sythoffi*	MW813978
	Iragoides fasciata	MK250437
	Mahanta tanyae	MK396080
	Thosea sinensis	NC_061059
	Parasa consocia	OK149235
	Narosa nigrisigna	NC_041304
	Monema flavescens	NC_032683
	Cnidocampa_flavescens	KY628213

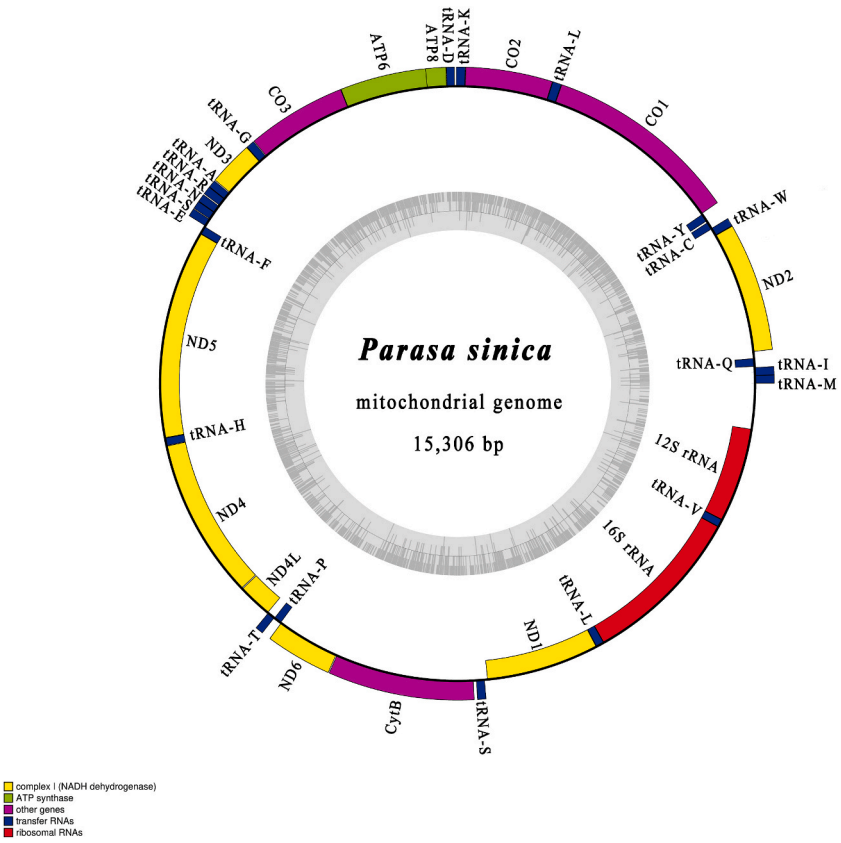

Fig. 1. Circular mitogenome map of the *P. sinica.* Protein coding, ribosomal and tRNA genes are shown with standard abbreviations. Gene orientations are indicated by the arrow directions. Complex I is indicated in yellow, ATP synthase is indicated in green, transfer RNA gene is indicated in blue, two rRNA genes are indicated in red and other genes are indicated in purple.

3.3. Transfer and ribosomal RNAs

The mitogenome of *P. sinica* contains 22 tRNA genes, the majority of which are common and range in length from around 63 to 70 bp (Table 2, Fig. 1). The J-strand carries 14 tRNA genes, whereas the N-strand contains eight enduring tRNA genes. All lepidopteran species carry the same tRNA anticodons in all directions. The MITOS WebServer theoretically supports the tRNA gene lineup. Fig. 3 displays the inferred component compact of these tRNAs. The 22 tRNAs have an unconditioned lightning flash of 1480 bp, a disdainful A T content of 82.7 %, a tyrannical AT-skew of 0.008, and a detrimental GC-skew of −0.148.

As shown in Table 3, two rRNAs were found to exhibit negative AT-skews (−0.031) and negative GC-skews (−0.377), which matched the genome's second-highest A + T content of 85.1 %. Two 2137 bp long rRNA genes were detected on the N-strand. The rrnL gene is located between tRNA-L1 and tRNA-V (1352 bp), whereas the rrnS gene is found between tRNA-V and the CR (Table 2, Fig. 1).

3.4. Control region

The replication and transcription of the mitogenome are thought to be under the control of the control region [42]. In *P. sinica*, the 374 bp-long CR is located between the rrnS and the tRNA-M. The mitogenome's A + T content is the highest (93.0 %) in this region (Tables 2 and 3). The AT-skew and GC-skew for CR are both negative, indicating that Ts and Cs are more common than As and Gs. This trait, which typifies the general mitogenome trend, is shared by all known lepidopteran species. The conserved motif "ATAGA + poly-T," which is the source of N-strand DNA replication, was found close to the beginning of the CR with 19 poly T (Fig. 3). Similar to other lepidopteran mitogenomes, the CR had a terminal poly-A region [43].

3.5. Phylogenetic analyses

A number of mitochondrial and nuclear genes have been combined, resulting in topologies with significantly lower support values than phylogenetic analyses based on joining all protein-coding genes in mitogenomes [44]. In the current investigation, we used the combined PCG sequences from 16 correctly classified hymenopteran insects, including *P. sinica*, for which the whole mitogenome sequence is also available in GenBank, to determine the phylogeny.

Table 2
Annotation of the complete mitogenome of *P. sinica*.

Gene	Direction	Location	Size	Anticodon	Start codon	Stop codon	Intergenic nucleotides
tRNA-M	F	1–68	68	CAT	–	–	−3
tRNA-I	F	66–133	68	GAT	–	–	6
tRNA-Q	R	140–208	69	TTG	–	–	53
ND2	F	262–1270	1009	–	ATT	T	0
tRNA-W	F	1271–1338	68	TCA	–	–	−8
tRNA-C	R	1331–1396	66	GCA	–	–	12
tRNA-Y	R	1409–1476	68	GTA	–	–	4
CO1	F	1481–3011	1531	–	CGA	T	0
tRNA-L2	F	3012–3083	72	TAA	–	–	0
CO2	F	3084–3765	682	–	ATT	T	0
tRNA-K	F	3766–3837	72	CTT	–	–	12
tRNA-D	F	3850–3914	65	GTC	–	–	0
ATP8	F	3915–4076	162	–	ATA	TAA	−7
ATP6	F	4070–4746	677	–	ATG	TA	0
CO3	F	4747–5529	783	–	ATG	TAA	2
tRNA-G	F	5532–5599	68	TCC	–	–	0
ND3	F	5600–5953	354	–	ATT	TAA	4
tRNA-A	F	5958–6020	63	TGC	–	–	0
tRNA-R	F	6021–6087	67	TCG	–	–	6
tRNA-N	F	6094–6158	65	GTT	–	–	0
tRNA-S1	F	6159–6224	66	TCT	–	–	2
tRNA-E	F	6227–6292	66	TTC	–	–	5
tRNA-F	R	6298–6365	68	TGA	–	–	0
ND5	R	6366–8100	1735	–	ATT	T	0
tRNA-H	R	8101–8166	66	GTG	–	–	1
ND4	R	8168–9502	1335	–	ATG	T	5
ND4L	R	9508–9795	288	–	ATG	TAA	2
tRNA-T	F	9798–9863	66	TGT	–	–	0
tRNA-P	R	9864–9929	66	TGG	–	–	2
ND6	F	9932–10456	525	–	ATT	TAA	4
CytB	F	10461–11609	1149	–	ATG	TAA	22
tRNA-S2	F	11632–11701	70	TGA	–	–	22
ND1	R	11724–12662	939	–	ATG	TAA	0
tRNA-L1	R	12663–12730	68	TAG	–	–	0
16S ribosomal RNA	R	12731–14082	1352	–	–	–	0
tRNA-V	R	14083–14147	65	TAC	–	–	0
12S ribosomal RNA	R	14148–14932	785	–	–	–	0
A + T-rich region		14933–15306	374	–	–	–	–

Table 3
Composition and skewness in the *P. sinica* mitogenome.

P. sinica	Size(bp)	A%	G%	T%	C%	A + T%	AT-skew	GC-skew
Mitogenome	15306	40.2	11.5	40.8	7.5	80.9	−0.007	−0.210
PCGs	11169	39.7	8.4	39.6	12.3	79.3	0.001	−0.192
tRNAs	1480	41.7	7.4	41.0	9.9	82.7	0.008	−0.148
rRNAs	2137	41.2	4.3	43.9	10.2	85.1	−0.031	−0.377
CR	374	43.1	2.9	50.0	4.0	93.0	−0.075	−0.154

To elucidate the taxonomy of *P. sinica* and its genetic relationship with other Zygaenoids, concatenated PCGs sequences from the mitochondrial genomes of 15 Zygaenoidea species were subjected to phylogenic analysis using the BI and ML methods, along with sequences derived from members of the Geometridae as outgroups, including *P. sinica*. We present a cladogram based on the four trees generated by BI and ML for the nucleotide and amino acid datasets, which exhibited roughly identical topologies (Fig. 4). The monophyly of each superfamily is generally highly supported, with the posterior probability typically greater than 0.9 and the bootstrap support levels higher than 75 %.

These results provide a strong support for the inclusion of *P. sinica* under Limacodidae and the phylogenetic relationship within Zygaenoidea. Similar to many superfamilies in the suborder Ditrysia, it lacks distinct features, and many characteristics are difficult to understand based onmorphological systematics. Different studies have classified the family Zygaenoidea into 7 to 13 families according to their own standards, and its monophyly and internal phylogenetic implications are still disputed. Fracker (1954) first proposed "Zygaenoidea", which includes Chalcosinae = Chalcodidaesic, Procridinae = Pyromorphidae, Epipyropidae, Megalopygidae and Limacodidae [45]. This classification system laid the foundation for subsequent taxonomic studies of Zygaenoidea. Niehuis et al. carried out phylogenetic analysis based on mitochondrial genome fragment sequence and partial nuclear gene sequence, and divided Zygaenoidea into 7 families, namely Phaudidae, Zygaenidae, Somabrachyidae, Himantopteridae, Limacodidae, Lacturidae and

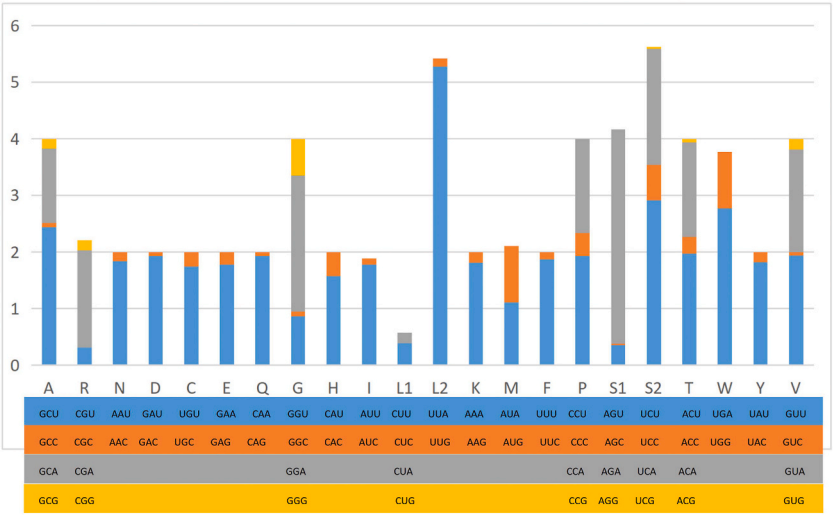

Fig. 2. Relative synonymous codon usage (RSCU) in the mitogenome of *P. sinica*.

Table 4
Codon number and RSCU in *P. sinica* mitochondrial PCGs.

Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU
UUU(F)	324	1.87	UCU(S)	92	2.91	UAU(Y)	149	1.82	UGU(C)	27	1.74
UUC(F)	22	0.13	UCC(S)	20	0.63	UAC(Y)	15	0.18	UGC(C)	4	0.26
UUA(L)	443	5.28	UCA(S)	65	2.05	UAA(*)	7	0.23	UGA(W)	83	2.77
UUG(L)	12	0.14	UCG(S)	1	0.03	UAG(*)	0	0	UGG(W)	2	1
CUU(L)	33	0.39	CCU(P)	56	1.93	CAU(H)	54	1.57	CGU(R)	7	0.31
CUC(L)	0	0	CCC(P)	12	0.41	CAC(H)	15	0.43	CGC(R)	0	0
CUA(L)	15	0.18	CCA(P)	48	1.66	CAA(Q)	56	1.93	CGA(R)	39	1.72
CUG(L)	0	0	CCG(P)	0	0	CAG(Q)	2	0.07	CGG(R)	4	0.18
AUU(I)	381	1.78	ACU(T)	65	1.97	AAU(N)	207	1.84	AGU(S)	11	0.35
AUC(I)	24	0.11	ACC(T)	10	0.3	AAC(N)	18	0.16	AGC(S)	1	0.03
AUA(M)	238	1.11	ACA(T)	55	1.67	AAA(K)	86	1.81	AGA(S)	86	3.79
AUG(M)	25	1	ACG(T)	2	0.06	AAG(K)	9	0.19	AGG(S)	0	0
GUU(V)	62	1.94	GCU(A)	72	2.44	GAU(D)	58	1.93	GGU(G)	40	0.86
GUC(V)	2	0.06	GCC(A)	2	0.07	GAC(D)	2	0.07	GGC(G)	4	0.09
GUA(V)	58	1.81	GCA(A)	39	1.32	GAA(E)	64	1.78	GGA(G)	111	2.4
GUG(V)	6	0.19	GCG(A)	5	0.17	GAG(E)	8	0.22	GGG(G)	30	0.65

Heterogynidae,(((Somabrachyidae + Himantopteridae) + Limacodidae)+(Phaudidae + Lacturidae)) + Zygaenidae [46]. Liu et al. constructed a phylogenetic tree based on the nucleotide sequences of 13 PCGs in the mitochondrial genome, and found a close genetic relationship between Zygaenoidea, Papilionidae and Tortricidea [47]. Lu et al. reported sister group relationship between Cossoidae and Zygaenoidea based on the whole mitochondrial genome sequence [48]. Zhang et al. reported that Phaudidae was restored as a single family of Zygaenidae, and Phaudidae Flammans belonged to Phaudidae [49]. However, our study indicated that Phaudidae Flammans belongs to Zygaenidae and Phaudidae may not be a single family. Further studies are needed to establish the internal phylogenetic relationship of Zygaenoidea.

In this study, *Phauda flammans*, *Illiberis ulmivora*, *I. pruni*, *Pidorus atratus*, *Histia rhodope*, *Eterusia aedea*, *Rhodopsona rubiginosa* and *Amesia sanguiflua* were classified under Zygaenidae. The genus-level relationships in Zygaenidae were determined as follows: (*Phauda flammans* + [*I. ulmivora* + *I. pruni*] + [*Pidorus atratus* + {*Histia rhodope* + *Eterusia aedea*}+ {*Rhodopsona rubiginosa* + *Amesia sanguiflua*}]) (Fig. 4). Otherwise, *Narosa nigrisigna*, *Iragoides fasciata*, *Thosea sinensis*, *Quasithosea sythoffi*, *P. sinica*, *P. consocia, Monema flavescens and Cnidocampa flavescens* belong to Limacodidae. In Limacodidae, genus-level relationships were determined as follows: (*Narosa nigrisigna* + ([*Iragoides fasciata* + {*Thosea sinensis* + *Quasithosea sythoffi*}] + [{*Parasa sinica* + *Parasa consocia*} + {Monema flavescen + *Cnidocampa flavescens* }])) (Fig. 4).

Data availability statement

Data associated with this study has been deposited at GenBank under the accession number MK122617.

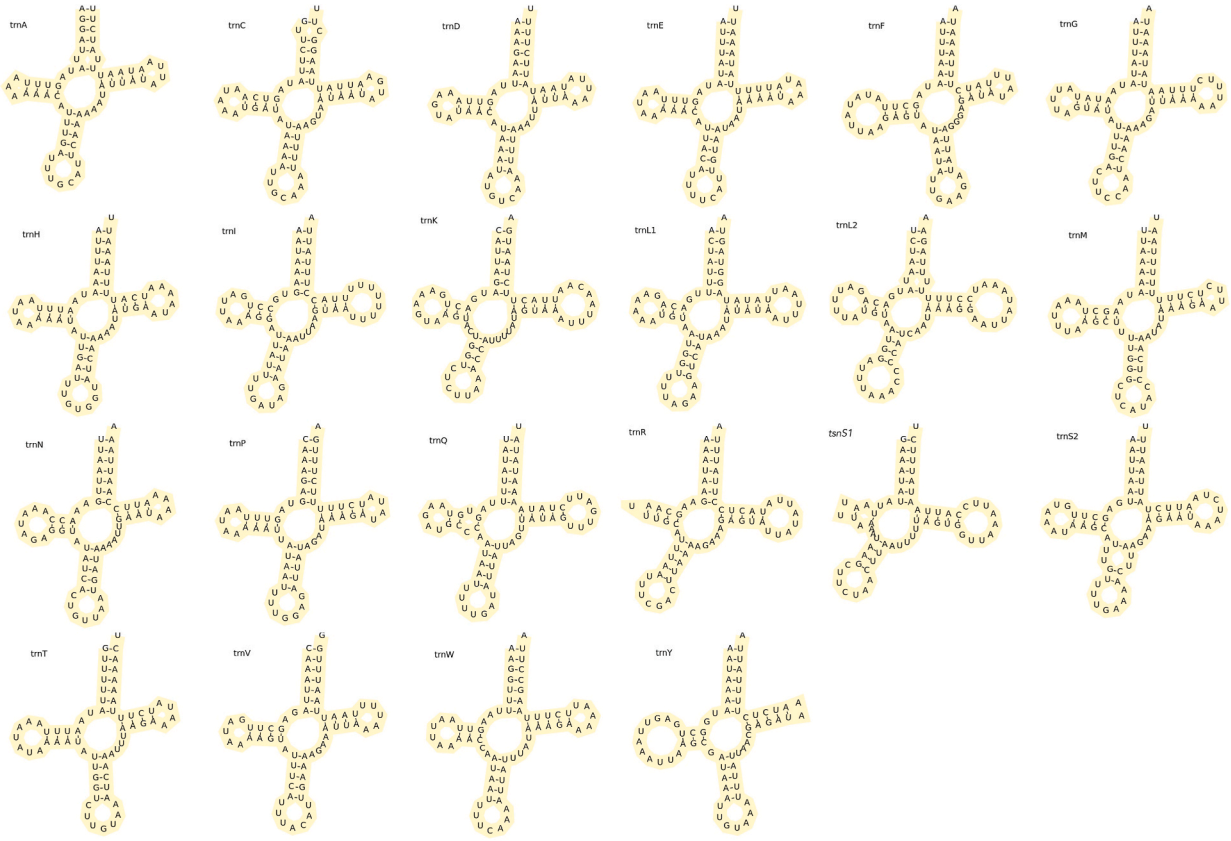

Fig. 3. Putative secondary structures for the tRNA genes of the *P. sinica* mitogenome.

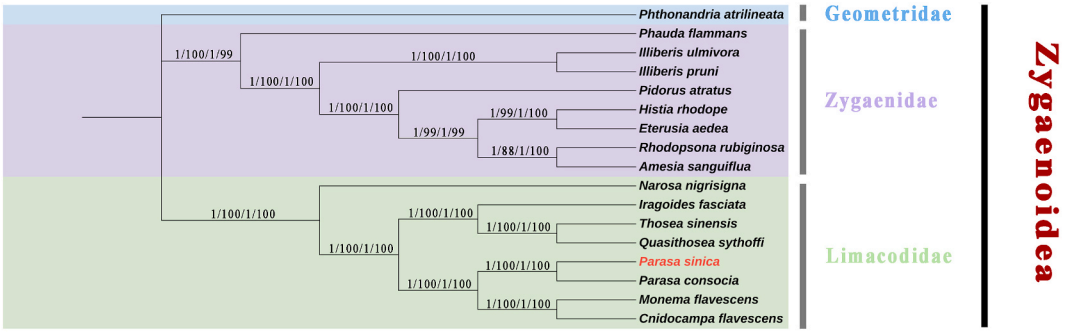

Fig. 4. Phylogenetic tree inferred from nucleotide and amino acid sequences of 13 PCGs using the ML analysis and BI analysis. Numbers on the branches are BI posterior probability (nucleotide)/ML bootstrap support (nucleotide)/BI posterior probability (amino acid)/ML bootstrap support (amino acid).

CRediT authorship contribution statement

Si-Pei Zhang: Conceived and designed the experiments, analysed and interpreted the data, wrote the paper. **Jie Zhang:** Conceived and designed the experiments, analysed and interpreted the data, wrote the paper. **Jie Xu:** Conceived and designed the experiments, analysed and interpreted the data, wrote the paper. **Qing-Hao Wang:** Analysed and interpreted the data. **Yang Ye:** Analysed and interpreted the data. **Gang Wang:** Analysed and interpreted the data. **Hua-Bin Zhang:** Contributed reagents, materials, analysis tools or data. **Dai-Zhen Zhang:** Contributed reagents, materials, analysis tools or data. **Bo-Ping Tang:** Conceived and designed the experiments. **Qiu-Ning Liu:** Conceived and designed the experiments.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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