The first mitochondrial genomes for Pyralinae (Pyralidae) and Glaphyriinae (Crambidae), with phylogenetic implications of Pyraloidea

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Abstract

In the present study, we report five complete and one nearly complete mitochondrial genomes of the Pyraloidea including the first representatives from the Pyralinae (Pyralidae) and Glaphyriinae (Crambidae). We also conduct a comparative analysis of mitogenomic features of this group. Our results show that Pyraloidea mitogenomes evolved under a common trend found in lepidopteran mitogenomes and share several typical genomic characters. The extra conserved blocks are identified in the Pyraloidea control region, and diverse missing codons formed another unique trait within Pyraloidea mitogenome. Furthermore, we reconstruct the mitogenomic phylogeny of Pyraloidea and confirm the phylogenetic position of Pyralinae and Glaphyriinae within the Pyraloidea using BI and ML method based on multiple mitochondrial datasets.

Introduction

As a species-rich superfamily within the Lepidoptera, the Pyraloidea comprises more than 15,576 species with a world wide geographical distribution[1]. The Pyraloidea are of particular interest because it contains a large number of notorious pest of commercial crops, forests and ornamental plants, stored foodstuffs with significant economic importance. They are of further interest because of their diverse life history adaptations including larvae with phytophagous, detritivorous, coprophagous, parasitic habits, and even aquatic habitats, has prompted the idea that pyraloids could be an ideal model for biodiversity [2, 3]. An efficient taxonomy, management, and pest control of these important moths rely on a sound and comprehensive classification and phylogeny. Initially, apart from Crambidae and Pyralidae, several other families, including the Pterophoridae, Thyrididae, Hyblaeidae, Alucitidae, and Tineodidae, were historically recognized within the Pyraloidea. With a better understanding of moth and butterfly phylogenies, the current consensus view holds that most of the families formerly included in the Pyraloidea should belong to their own superfamily, and there is strong evidence from molecular studies for the sister relationship of Pyraloidea and Macroheterocera [4]. Additionally, the monophyly for Pyraloidea and two members, the Pyralidae and Crambidae, is supported by both morphological and molecular analyses [5, 6]. And the relationships among

subfamilies for Pyralidae and Crambidae has also been investigated deeply based on nuclear gene data. However, due to limited samplings only a few mito-phylogenetic analyses have been involved in subfamily-level relationships [7].

The mitochondrial genome (mitogenome) is a common and practical system for comparative genomic and phylogenetic research. Recently, owing to the improvement of polymerase chain reaction (PCR) and sequencing technology, in particular the application of next-generation sequencing, mitogenome data have soared in many animal lineages, especially insects [8, 9]. To date, over 380 lepidopteran mitogenomes have been determined, while only 30 pyraloids mitogenomes representing three subfamilies of Pyralidae and eight subfamilies of Crambidae are now available (www.ncbi.nlm.nih.gov/nuccore/). A comparative study of mitogenome evolution and phylogeny of Pyraloidea highlights the requirement for broadening taxon sampling in further studies.

In the present study, we report five complete and one nearly complete pyraloids mitogenomes including the first representatives from the Pyralinae (Pyralidae) and Glaphyriinae (Crambidae). In addition, we summarize the evolutionary pattern of pyraloids mitogenomic features including base composition, codon usages, secondary structures of transfer RNA (tRNA) and ribosomal RNA (rRNA) genes, and control region. Furthermore, the molecular phylogeny of the Pyraloidea was reconstructed using multiple mitogenomic data, which confirms for the first time the phylogenetic position of Pyralinae and Glaphyriinae.

Materials and methods

Specimen collection and DNA extraction

All specimens were collected in August 2014 by a light trap at Xunyangba (33.33˚N, 108.33˚E), Ningshan County, Shaanxi Province, China, preserved in 95% ethanol and stored at -20˚C. All these specimens were identified by Ping You [10]. For each species, Voucher specimens have been deposited in the Insect Collection (Accession Number SNU-Lep-20140017-22), College of Life Sciences, Shaanxi Normal University, Xi'an, China 710062. The total DNA was extracted using a TIANamp Micro DNA Kit (Tiangen Biotech, Beijing, China), according to the manufacturer's protocol.

PCR amplification and sequencing

Six mitogenomes were amplified with overlapping fragments using conserved primers [11]. PCRs were performed using FastPfu Fly DNA Polymerase (TransGen Biotech, Beijing, China) as previously described [12]. After purification with PCR Purification Kit (Sangon Biotech, Shanghai, China), all PCR products were sequenced directly with a primer-walking strategy.

Genome annotation and sequence analysis

Contiguous sequences were assembled using Staden Package v1.7.0 [13]. PCGs and rRNA genes were identified based on homologous regions of published Pyraloidea mitogenomes using the Clustal X in MEGA 5 [14]. The tRNAscan-SE [15] was used to predict tRNA genes and their secondary structures. Secondary structures of the two rRNA genes were predicted according to the models for *Paracymoriza prodigalis* [12]. The base composition and codon usage were calculated using MEGA 5.

Phylogenetic analysis

Mitogenomic phylogeny of Pyraloidea was reconstructed based on four datasets (PCG123: 13 PCGs including all codon positions; PCG123R: 2 rRNAs, 22 tRNAs and 13PCGs including all

codon positions; PCG12: 13 PCGs without third codon positions; PCG12R: 2 rRNAs, 22 tRNAs and 13PCGs without third codon positions) using Bayesian inference (BI) and maximum likelihood (ML) methods. Three species from Thyrididae (*Pyrinioides aurea*, KT337662) [16], Alucitidae (*Alucita montana*, KJ508059) and Pterophoridae (*Emmelina monodactyla*, KJ508063) [17] were selected as outgroups. Each of 37 mitochondrial gene sequences was aligned with Clustal. Considering partitioning strategy of previous studies [18], a similar partitioning scheme (tRNA genes, rRNA genes, and each codon site of PCGs) was employed for phylogenetic analysis. The optimal model (GTR+I+Γ) for each partition was selected using Akaike information criterion in jModelTest [19]. The BI analyses were implemented in MrBayes 3.1.2 [20] with four MCMC chains running for five million generations. Each set was sampled every 200 generations with a burn-in of the first 25% of steps. The ML analyses were performed using RAxML 7.0.3 [21] with 1000 bootstrap replicates.

Results and discussion

General features of Pyraloidea mitogenomes

Full or partial mitogenomes of the six pyraloid moths (*Endotricha consocia*, *Hypsopygia regina*, *Orybina plangonalis*, *Evergestis junctalis*, *Tyspanodes striata*, *Maruca vitrata*) were generated and deposited in GenBank (Table 1). In addition, 38 complete or nearly complete mitogenomes of the Pyraloidea were integrated into a combined dataset for conducting comparative analyses. As reported in most metazoan mitogenomes, all the pyraloid mitogenomes contained 37 mitochondrial genes including 13 protein-coding genes (PCGs), 22 tRNA and 2 rRNA genes, and a putative control region (namely A+T-rich region for insects) (S1 Fig) [8, 22]. The length of five newly sequenced complete mitogenomes fell into the range of previously reported pyraloid mitogenomes (from 14,960-bp *Glyphodes pyloalis* to 15,594-bp *Ephestia kuehniella*). The gene order of pyraloid mitogenomes was highly conserved and identical with the typical gene order of Ditrysia mitogenomes. Compared with the predicted ancestral gene order for insects, however, a gene rearrangement occurred in the tRNA cluster (trnI-trnQ-trnM) among most Ditrysia mitogenomes, which is also proved as a synapomorphy for all the Ditrysia lineages [17]. Of this rearrangement event, the tandem duplication and random loss model [23] appears to be the most reasonable mechanism with the following events: firstly, the tRNA cluster (ancestral gene order trnI-trnQ-trnM) duplicated followed by the random deletion of the supernumerary genes including trnI, trnQ (the first copy) and trnM (the second copy).

The base composition is a common genome-level character for exploring mitogenome evolution [24]. We assessed this feature of pyraloid mitogenomes by calculating A+T content, AT-skew, and GC-skew. In general, lepidopteran mitogenomes exhibit a strong bias to A and T and the negative GC-skew [8]. And our analyses confirmed that the base composition of pyraloid mitogenomes is similar to the typical trend of lepidopteran mitogenomes. Within the Pyraloidea, Crambidae and Pyralidae there was no significant difference in A+T content and GC-skew, while the AT-skew presented a distinct tendency (S2 Fig). All the Pyralidae species demonstrated the negative AT-skew (< -0.035, except for *Lista haraldusalis* -0.007), while Crambidae species showed a higher AT-skew (> -0.0249) than that of Pyralidae. Additionally, comparative analyses of A+T content and strand asymmetry at the subfamily level revealed that A+T content and AT-/GC-skew largely overlapped, suggesting that base composition evolved under an identical pattern among subfamilies from the same family. Overall, the relatively consistent patterns of base composition for the Pyraloidea mitogenomes not only reflects similar substitution pressures but appears to result from the conserved genome organization.

Protein-coding genes and codon usage

The total size of 13 PCGs in Pyraloidea mitogenome was intermediate, ranging from 11,134 bp (*Ephestia kuehniella*) to 11,230 bp (*Chilo suppressalis*). And the average A+T content of PCGs

Table 1. GenBank accession numbers of the species used in the present study.

Family	Subfamily	Species	GenBank
Pyralidae	Epipaschiinae	*Lista haraldusalis*	KF709449
	Galleriinae	*Galleria mellonella*	KT750964
		Corcyra cephalonica	HQ897685
	Phycitinae	*Amyelois transitella*	KT692987
		Ephestia kuehniella	KU877167
		Plodia interpunctella	KP729178
		Meroptera pravella	MF073207
	Pyralinae	*Hypsopygia regina**	KP327714
		*Endotricha consocia**	MF568544
		*Orybina plangonalis**	MF568543
Crambidae	Acentropinae	*Elophila interruptalis*	KC894961
		Paracymoriza distinctalis	KF859965
		Paracymoriza prodigalis	JX144892
		Parapoynx crisonalis	KT443883
	Crambinae	*Chilo auricilius*	KJ174087
		Chilo sacchariphagus	KU188518
		Chilo suppressalis	JF339041
		Diatraea saccharalis	FJ240227
		Pseudargyria interruptella	KP071469
	Glaphyriinae	*Evergestis junctalis**	KP347976
		Hellula undalis	KJ636057
	Pyraustinae	*Loxostege sticticalis*	KR080490
		Ostrinia furnacalis	AF467260
		Ostrinia nubilalis	AF442957
		Ostrinia penitalis	KM395814
	Schoenobiinae	*Scirpophaga incertulas*	KF751706
	Scopariinae	*Eudonia angustea*	KJ508052
	Spilomelinae	*Cnaphalocrocis medinalis*	JN246082
		Dichocrocis punctiferalis	JX448619
		Glyphodes pyloalis	KM576860
		Glyphodes quadrimaculalis	KF234079
		Haritalodes derogata	KR233479
		*Maruca vitrata**	KP327715
		Nomophila noctuella	KM244688
		Pycnarmon lactiferalis	KX426346
		Spoladea recurvalis	KJ739310
		Tyspanodes hypsalis	KM453724
		*Tyspanodes striata**	KP347977
Alucitidae		*Alucita montana*	KJ508059
Pterophoridae		*Emmelina monodactyla*	KJ508063
Thyrididae		*Pyrinioides aurea*	KT337662

Note

* represents the new mitochondrial genomes.

was also similar to other moths [25], ranging from 75.1% (*Scirpophaga incertulas*) to 81.1% (*Paracymoriza distinctalis*), while the A+T content largely differed among three codon positions. The third codon position showed a far higher A+T content than that of the first and second codon positions, which is similar to other moths. Overall, all of these features of PCGs make a major contribution to the strong AT bias of whole genome.

Most of the PCGs in Pyraloidea mitogenomes possessed canonical start codons (ATN) with the exception of COI, which used CGA as a start codon. Apart from COI in most species, there were several genes with other start codons: GTG for ND3 (*Pycnarmon lactiferalis*) and TTG for ND1 (*Galleria mellonella*). Estimated among 13 PCGs, start codons of ATP6, COIII, ND4, and ND4L were the most conserved, and in contrast, COII, ND3, and ND1 held diverse start codons (Table 2). Compared with start codons, the stop codon in Pyraloidea mitogenomes

Table 2. The missing codons in Pyraloidea mitochondrial genomes.

Family	Subfamily	Species	Missing codon						
Pyralidae	Epipaschiinae	*Lista haraldusalis*	AGG(S)	CGC(R)	CUG(L)				
	Galleriinae	*Galleria mellonella*	AGG(S)	AGC(S)	CGC(R)	ACG(T)	CUG(L)		
		Corcyra cephalonica	AGG(S)	CCG(P)	CUG(L)				
	Phycitinae	*Amyelois transitella*	AGG(S)	CGC(R)					
		Ephestia kuehniella	AGG(S)	CCG(P)					
		Plodia interpunctella	AGC(S)	GGC(G)	ACG(T)	CUG(L)			
	Pyralinae	*Hypsopygia regina*	AGG(S)	ACG(T)	UCG(S)				
		Orybina plangonalis	AGG(S)	ACG(T)					
Crambidae	Acentropinae	*Elophila interruptalis*	AGG(S)						
		Paracymoriza distinctalis	AGC(S)	CGG(R)	CGC(R)	GCG(A)	CCG(P)	UCG(S)	CUG(L)
		Paracymoriza prodigalis	AGC(S)	GGC(G)	CGC(R)	UCG(S)			
		Parapoynx crisonalis	AGG(S)	CGC(R)	CAG(Q)	GCG(A)	ACG(T)	UCG(S)	CUG(L)
	Crambinae	*Chilo auricilius*	AGC(S)	CGC(R)	CCG(P)	ACG(T)	CUG(L)		
		Chilo sacchariphagus	AGG(S)	CGC(R)	CUG(L)				
		Chilo suppressalis	GUC(V)						
		Diatraea saccharalis	AGG(S)	CUG(L)	CGC(R)	CCG(P)			
		Pseudargyria interruptella	ACG(T)	CUG(L)					
	Glaphyriinae	*Evergestis junctalis*	AGG(S)	AGC(S)	ACG(T)	CCG(P)	CUG(L)		
		Hellula undalis	AGG(S)	CGC(R)	CCG(P)	UCG(S)	CUG(L)		
	Pyraustinae	*Loxostege sticticalis*	AGG(S)	AGC(S)	CGG(R)	CGC(R)	UGC(C)	ACG(T)	CUC(L)
		Ostrinia furnacalis	ACG(T)	CUG(L)					
		Ostrinia nubilalis	AGG(S)	CUG(L)	CUC(L)				
		Ostrinia penitalis	-						
	Schoenobiinae	*Scirpophaga incertulas*	AGG(S)	ACG(T)					
	Scopariinae	*Eudonia angustea*	-						
	Spilomelinae	*Dichocrocis punctiferalis*	AGG(S)	CGC(R)					
		Cnaphalocrocis medinalis	AGG(S)	AGC(S)	CGC(R)	GCG(A)	ACG(T)	UCG(S)	GUC(V)
		Glyphodes pyloalis	AGG(S)	CGC(R)	GCG(A)	CUG(L)			
		Glyphodes quadrimaculalis	AGG(S)	CGG(R)	UGG(W)	CUG(L)			
		Haritalodes derogata	AGG(S)	AGC(S)	CUG(L)				
		Maruca vitrata	AGG(S)	AGC(S)	CGC(R)	CCG(P)	CUG(L)		
		Nomophila noctuella	AGG(S)	AGC(S)	CGG(R)	CGC(R)	GUC(V)	CUG(L)	CUC(L)
		Pycnarmon lactiferalis	AGG(S)	CGC(R)	UGC(C)	GUC(V)	CUG(L)	CUC(L)	
		Spoladea recurvalis	AGG(S)	GGC(G)	AGC(S)	CGG(R)	CGC(R)	UGG(W)	CCG(P)
		Tyspanodes hypsalis	AGG(S)	CUG(L)					
		Tyspanodes striata	AGG(S)	GGC(G)	CCG(P)				

was more straightforward. ND2, ATP8, ATP6, COIII, ND6, and CYTB mainly used TAA as the stop codon, and the incomplete stop codon T or TA chiefly occurred in COI, COII, ND5, and ND4. Another typical stop codon TAG scattered among COII, ND3, ND4, ND4L, and ND1.

The relative synonymous codon usage of five newly sequenced species is shown in S3 Fig. It is obvious that the usage of codons with high A/T bias is more frequent than that with G/C bias. The most frequently used codons in Pyraloidea mitogenome was identical, namely UUU, UUA, AUU (Ile), AUA (Met), UAU (Tyr) and AAU (Asn). Missing codons were constantly presented in most Pyraloidea mitogenomes with different degrees, ranged from 1 codon (*Elophila interruptalis*, *Chilo suppressalis*) to 7 codons (*Spoladea recurvalis*, *Nomophila noctuella*, *Loxostege sticticalis*, *Cnaphalocrocis medinalis*, *Parapoynx crisonalis*, *Paracymoriza distinctalis*). Generally, these missing codons showed high G/C content. Codons that were most commonly missed were those coding for the amino acid Ser, Leu, Arg. Additionally, we evaluated the lineage-specificity of missing codons, but no remarkable signal or pattern of evolution were found. On the whole, the codon usage fully reflected the A/T-preference base composition for Pyraloidea mitogenomes.

Transfer RNA and ribosomal RNA genes

A common set of 22 tRNA genes were found in all the complete mitogenomes of Pyraloidea. These tRNA genes illustrated a consistent length and base composition among pyralids. Although most tRNAs could be folded into the typical clover-leaf structure, the exception (trnS^{AGN}) existed widely in Pyraloidea mitogenomes. For trnS^{AGN}, the dihydrouridine (DHU) stem was replaced by an unstable loop, which has been observed in many insect mitogenomes [26–28]. Additionally, we identified mismatched base pairs in different stems of tRNA (S4 Fig), a general feature for animal mitogenomes, and meanwhile these mismatched nucleotides might be modified during post-transcriptional processing [29].

In order to explore the evolutionary pattern of tRNA in Pyraloidea, we calculated the percentage of identical nucleotides. As shown in S5 Fig, the Crambidae and Pyralidae show similar levels of nucleotide conservation. Comprehensive analyses combining the base composition and gene arrangement revealed that the J-strand tRNAs were more conserved than N-strand tRNAs, but the identical degree did not closely link to A+T content or absolute location of mitogenome, which has been reported in other insect lineages [28]. Furthermore, S4 Fig shows that the acceptor and anticodon stem were more conserved than DHU and TψC stem, and anticodon loop also presented the highest nucleotide similarity.

Among Pyraloidea mitogenomes, the average size of rrnL (~780 bp) and rrnS (~1361 bp) is comparable to those of other moths [16,30], however a number of unique insertion sequences were identified in both of the two rRNA genes for a few species. To confirm an accurate position of these insert fragments, we predicted the secondary structures of rrnL (S6 Fig) and rrnS (S7 Fig). As observed in other insect rRNAs [11], rrnL and rrnS contain five domains (46 helices) and three domains (27 helices), respectively. According to S6 and S7 Figs, the insert fragments are mainly in domain II of rrnS and domain III of rrnL. The largest inserting-sequences were found in *Orybina plangonalis*, which included several short repeated sequences. In fact, these sequences were excluded from the stable stem region of secondary structure, so the inserted or deleted sequences of hypervariable regions did not significantly influence the function of rRNAs. In contrast, the conserved regions show a high similarity in both sequences and secondary structure. In rrnL, helices H579, H1925, and H2547 were the most conserved and stable, and helices H944, H984 and H1399 lacked variation in rrnS. It appears that rrnL evolved under a more conserved pattern than rrnS.

Non-coding regions

In most cases, the animal mitogenome is compact and economic [22]. The largest non-coding region is generally considered to be the control region (CR). Even though the CR of Pyraloidea mitogenomes is small in size (~339.8 bp average) without any large tandem repeat sequences, it remains the longest non-coding region. The CR regulates the replication and transcription of mitogenome, and many conserved blocks (CBS) were considered to play a key role in the function of CR [31]. In insect mitogenomes, the CBS are diverse in different lineages [31, 32]. In lepidopteran mitogenomes, four conserved elements have been found in nearly all the Ditrysia species, i.e. ATAGA (CBS-1) followed by a large poly-T stretch, microsatellite structures (AT)n, and a poly-A stretch, though only three of them were identified in Pyraloidea mitogenomes (S8 Fig), which lacked the poly-A stretch. However, comparative analyses of Pyraloidea CRs indentified two other CBS: A(T)TTTA (CBS-2) and ACCRT (CBS-3). The CBS-2 was located upstream of (AT)n, while the CBS-3 occurred at the 3' end of the CR. It should be emphasized that a clear function of these conserved elements is uncertain and thus should be included in future studies.

In addition to CRs, two other intergenic gaps (trnS-ND1 and tnrQ-ND2) existed in all Pyraloidea mitogenomes. The first gap (trnS-ND1) contained a conserved motif ATACTAW, which is involved in regulatory functions as the binding site of the transcription termination factor (DmTTF) [33]. Alignment of the second gap (trnQ-ND2) and ND2 gene showed the relatively high sequence similarity suggesting that this gap may be the debris of duplicate ND2 genes and this duplicate event should occur before the divergence of Ditrysia.

Phylogenetic analyses

In spite of the Pyraloidea having a large number of important pests, the molecular phylogeny of the group is still ambiguous, especially for the Crambidae. Adding mitogenomes from new subfamilies and genera provide more data to investigate the phylogenetic relationships of the Pyraloidea. The inferred phylogenetic trees based on four datasets using Bayesian inference (BI) and maximum likelihood (ML) methods showed a similar topology (S9 Fig), which is consistent with other researches basically [6]. Monophyly of the families (Pyralidae and Crambidae) and subfamilies is well-supported, as is suggested by the morphological characters [34]. Comparative analyses of the trees from four datasets revealed that rRNA genes could contribute to improving the node support values, and the third codon positions of PCGs also provided phylogenetic information. Thus, the PCG123R dataset is more appropriate for reconstructing the molecular phylogeny of the Pyraloidea.

The Pyralidae is a relatively robust group, which contains five subfamilies. A previous nuclear genes study confirmed the phylogenetic relationships at the subfamily level [6], but this differs from some morphological studies [5]. In this study, we validated a stable molecular topology: (((Pyralinae + Epipaschiinae) + Phycitinae) + Galleriinae) with high support values. The main difference between morphological and molecular results is the phylogenetic position of the Pyralinae [5,6]. The Crambidae was divided into two large lineages: PS clade (Pyraustinae and Spilomelinae) and non-PS clade (the other subfamilies) [6], and supported by our results. The placement *S. incertulas* in this paper is the same as in [7] and [12]; both studies placed *Scirpophaga* as sister group to the Crambinae. It differs from the nuclear gene-based hypothesis [6], where *Scirpophaga* is either sister group to the Midilinae or *Rupela* + Acentropinae. Unlike the nuclear-gene based study [12]. Neither Midilinae or *Rupela* were included in this study and may account for the results.This significant difference could also be explained by long-branch attraction [35], and could be corrected by increasing the sampling number for

Schoenobiinae and by combining both the nuclear and mitogenomic data. Overall, most lineages inferred by mitogenomic data confirmed the current view of Pyraloidea phylogeny.

Supporting information

S1 Fig. The gene map of the newly sequenced mitochondrial genomes of Pyraloidea.
(TIF)

S2 Fig. The base composition and strand asymmetry in Pyraloidea mitochondrial genomes. (A) AT% vs. AT-Skew. (B) AT% vs. AT-Skew.
(TIF)

S3 Fig. The relative synonymous codon usage of five newly sequenced species. Red codons are not presented in mitochondrial PCGs.
(TIF)

S4 Fig. Predicted secondary structures of tRNAs found in *Orybina plangonalis* mitochondrial genomes.
(TIF)

S5 Fig. Nucleotide conservation of tRNAs in Pyraloidea mitochondrial genomes.
(TIF)

S6 Fig. Predicted secondary structure of the rrnL of *Orybina plangonalis*.
(TIF)

S7 Fig. Predicted secondary structure of the rrnS of *Orybina plangonalis*.
(TIF)

S8 Fig. The organisation of control region in Pyraloidea mitochondrial genomes.
(TIF)

S9 Fig. Phylogeny of 35 Pyraloidea species based on the maximum likelihood (ML) and Bayesian inference (BI) analysis of four mitochondrial datasets (PCG123R, PCG12R, PCG123, and PCG12). The asterisk represents that all the bootstrap support values of ML and posterior probability of BI are 100 and 1 for four datasets, respectively.
(TIF)

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References

1. van Nieukerken EJ, Kaila L, Kitching IJ, Kristensen NP, Lees DC, Minet J, et al. Animal biodiversity: an outline of higher-level classification and survey taxonomic richness. Zootaxa, 2011; 3148: 1–237.

2. Beck J, Brehm G, Fiedler K. Links between the environment, abundance and diversity of Andean moths. Biotropica, 2015; 43(2): 208–217.

3. You P, Wang S, Li H, Chen K. Genus *Paracymoriza* Warren from China (Lepidoptera: Crambidae: Nymphulinae). Aquatic Insects, 2003; 25(3): 211–217.

4. Mitter C, Davis DR, Cummings MP. Phylogeny and evolution of Lepidoptera. Annual Review of Entomology, 2017; 62: 265–283. https://doi.org/10.1146/annurev-ento-031616-035125 PMID: 27860521

5. Solis MA, Mitter C. Review and preliminary phylogenetic analysis of the subfamilies of the Pyralidae (sensu stricto) (Lepidoptera: Pyraloidea). Systematic Entomology, 1992; 17(1): 79–90.

6. Regier JC, Mitter C, Solis MA, Hayden JE, Landry B, Nuss M, et al. A molecular phylogeny for the pyraloid moths (Lepidoptera: Pyraloidea) and its implications for higher-level classification. Systematic Entomology, 2012; 37(4): 635–656.

7. Ma H, Zheng X, Peng M, Bian H, Chen M, Liu Y, et al. Complete mitochondrial genome of the meadow moth, *Loxostege sticticalis* (Lepidoptera: Pyraloidea: Crambidae), compared to other Pyraloidea moths. Journal of Asia-Pacific Entomology, 2016; 19(3): 697–706.

8. Cameron SL. Insect mitochondrial genomics: implications for evolution and phylogeny. Annual Review of Entomology, 2014; 59: 95–117. https://doi.org/10.1146/annurev-ento-011613-162007 PMID: 24160435

9. Tang M, Tan M, Meng G, Yang S, Su X, Liu S, et al. Multiplex sequencing of pooled mitochondrial genomes-a crucial step toward biodiversity analysis using mito-metagenomics. Nucleic Acids Research, 2014; 42(22): e166. https://doi.org/10.1093/nar/gku917 PMID: 25294837

10. Li H, You P, Xiao Y, Bai H, Wang S, Zhu Y, et al. Microlepidoptera of Qinling Mountains (Insect: Lepidoptera). Beijing: Science Press; 2012.

11. Simon C, Buckley TR, Frati F, Stewart JB, Beckenbach AT. Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. Annual Review of Ecology Evolution & Systematics, 2006; 37: 545–579.

12. Ye F, Shi Y, Xing L, Yu H, You P. The complete mitochondrial genome of *Paracymoriza prodigalis* (Leech, 1889) (Lepidoptera), with a preliminary phylogenetic analysis of Pyraloidea. Aquatic Insects, 2013; 35(3–4): 71–88.

13. Staden R, Beal KF, Bonfield JK. The Staden package, 1998. Methods in Molecular Biology, 2000; 132: 115–130. PMID: 10547834

14. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 2011; 28(10): 2731–2739. https://doi.org/10.1093/molbev/msr121 PMID: 21546353

15. Lowe TM, Eddy SR. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Research, 1997; 25(5): 955–964. PMID: 9023104

16. Zhu W, You P. Complete mitochondrial genome of *Camptochilus aurea* (Lepidoptera: Thyrididae). Mitochondrial DNA, 2015; 27(6): 4528–4529. https://doi.org/10.3109/19401736.2015.1101547 PMID: 26540644

17. Timmermans MJ, Lees DC, Simonsen TJ. Towards a mitogenomic phylogeny of Lepidoptera. Molecular Phylogenetics & Evolution, 2014; 79: 169–178.

18. Ye F, Lan X, Zhu W, You P. Mitochondrial genomes of praying mantises (Dictyoptera, Mantodea): rearrangement, duplication, and reassignment of tRNA genes. Scientific Reports, 2016; 6: 25634. https://doi.org/10.1038/srep25634 PMID: 27157299

19. Posada D. jModelTest: phylogenetic model averaging. Molecular Biology & Evolution, 2008; 25(7): 1253–1256.

20. Huelsenbeck JP Ronquist FR. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics, 2001; 17(8): 754–755. PMID: 11524383

21. Stamatakis A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 2014; 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033 PMID: 24451623

22. Boore JL. Animal mitochondrial genomes. Nucleic Acids Research, 1999; 27(8): 1767–1780. PMID: 10101183

23. Moritz C, Brown WM. 1987 Tandem duplications in animal mitochondrial DNAs: variation in incidence and gene content among lizards. Proceedings of the National Academy of Sciences of the United States of America, 1987; 84(20): 7183–7187. PMID: 3478691

24. Li Q, Wei S, Tang P, Wu Q, Shi M, Sharkey MJ, et al. Multiple lines of evidence from mitochondrial genomes resolve phylogenetic relationships of parasitic wasps in Braconidae. Genome Biology and Evolution, 2016; 8(9): 2651–2662. https://doi.org/10.1093/gbe/evw184 PMID: 27503293

25. 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. Molecular Phylogenetics & Evolution, 2015; 85: 230–237.

26. Yong HS, Song SL, Lim PE, Chan KG, Chow WL, Eamsobhana P. Complete mitochondrial genome of *Bactrocera arecae* (Insecta: Tephritidae) by next-generation sequencing and molecular phylogeny of Dacini tribe. Scientific Reports, 2015; 5: 15155. https://doi.org/10.1038/srep15155 PMID: 26472633

27. Shi Y, Chu Q, Wei D, Qiu Y, Shang F, Dou W, et al. The mitochondrial genome of booklouse, *Liposcelis sculptilis* (Psocoptera: Liposcelididae) and the evolutionary timescale of Liposcelis. Scientific Reports, 2016; 6: 30660. https://doi.org/10.1038/srep30660 PMID: 27470659

28. Zhang H, Ye F. Comparative mitogenomic analyses of praying mantises (Dictyoptera, Mantodea): origin and evolution of unusual intergenic gaps. International Journal of Biological Sciences, 2017; 13(3): 367–382. https://doi.org/10.7150/ijbs.17035 PMID: 28367101

29. Lavrov DV, Brown WM, Boore JL. A novel type of RNA editing occurs in the mitochondrial tRNAs of the centipede *Lithobius forficatus*. Proceedings of the National Academy of Sciences of the United States of America, 2000; 97(25): 13738–13742. https://doi.org/10.1073/pnas.250402997 PMID: 11095730

30. Gong Y, Shi B, Kang Z, Zhang F, Wei S. The complete mitochondrial genome of the oriental fruit moth *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae). Molecular Biology Reports, 2012; 39(3): 2893–2900. https://doi.org/10.1007/s11033-011-1049-y PMID: 21670960

31. Zhang D, Szymura JM, Hewitt GM. Evolution and structural conservation of the control region of insect mitochondrial DNA. Journal of Molecular Evolution, 1995; 40(4): 382–391. PMID: 7769615

32. Li X, Li W, Ding S, Cameron SL, Mao M, Shi L, et al. 2017 Mitochondrial genomes provide insights into the phylogeny of Lauxanioidea (Diptera: Cyclorrhapha). International Journal of Molecular Sciences, 2017; 18(4): 773.

33. Taanman JW. The mitochondrial genome: structure, transcription, translation and replication. Biochimica and Biophysica Acta, 1999; 1410(2): 103–123.

34. Solis MA, Maes KVN. Preliminary phylogenetic analysis of the subfamilies of Crambidae (Pyraloidea Lepidoptera). Belgian Journal of Entomology, 2003; 4(1): 53–95.

35. Philippe H, Zhou Y, Brinkmann H, Rodrigue N, Delsuc F. Heterotachy and long-branch attraction in phylogenetics. BMC Evolutionary Biology, 2005; 5: 50. https://doi.org/10.1186/1471-2148-5-50 PMID: 16209710