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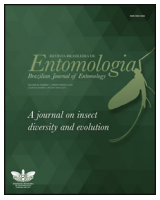

The first complete mitochondrial genomes of three dobsonfly species (Megaloptera: Corydalidae) from Pakistan with phylogenetic implications

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ABSTRACT

Megaloptera is a small holometabolous insect order that includes two genera and three species of Corydalidae in Pakistan. Here we sequenced the complete mitochondrial genomes of these three Pakistani corydalids: *Nevromus intimus* (McLachlan, 1869) (16,614 bp), *Protohermes motuoensis* Liu & Yang, 2006 (16,238 bp), and *Protohermes walkeri* Navás, 1929 (16,514 bp). It also represents the first set of complete mitogenomes sequenced for Neuropterida in Pakistan. The gene order was found to be similar to other published dobsonfly mitogenomes except the variable length of the non-coding region in each species. The phylogenetic analysis using 13 protein-coding genes by Maximum likelihood and Bayesian inference yielded largely consistent topologies, in which the phylogenetic positions of the three species herein studied are recovered.

Introduction

The insect mitochondrial genome (mitogenome or mtDNA) is a closed-circular double-stranded molecule of 14–20 kilobases (kb) in size that encodes 37 genes, including 13 protein-coding genes (PCGs: nad1–6, nad4L, atp6, atp8, cox1–3, cob), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs: rrnS, rrnL), and a variable-length of non-coding A+T-rich region (Boore, 1999; Cameron, 2014). To date, the mitogenomes of 28 recognized insect orders have been sequenced (Cameron, 2014) and are extensively used for analyzing the evolutionary history, biogeographical history, population phylogeographic relationships, and molecular phylogenetic studies at various taxonomic levels (Cameron, 2014; Cameron et al., 2009; Ma et al., 2012; Simon and Hadrys, 2013; Timmermans et al., 2014; Jiang et al., 2020, 2022). The gene arrangements within mtDNA are a key feature that provides valuable information regarding the evolutionary relationships at different taxonomic ranks and can be variable among insects (Boore et al., 1995; Boore, 1999).

To date, more than 160 complete or nearly complete mitogenomes of Neuropterida species have been sequenced and are available on GenBank (https://www.ncbi.nlm.nih.gov/). The mitochondrial phylogenomics have greatly improved our knowledge on the phylogeny and biogeography of various groups of Neuropterida (Jiang et al., 2016; Wang et al., 2017; Yang et al., 2018; Jiang et al., 2022; Shen et al., 2022). However, no complete mitochondrial genome is available on the Pakistani species of Neuropterida until now. Here, the complete mitochondrial genomes of three Pakistani Megaloptera species are first sequenced and analyzed. This research provides a basic foundation for future molecular phylogenetic studies on Megaloptera and other groups of Neuropterida in Pakistan.

Materials and Methods

Sampling and DNA extraction

Adult specimens of *Nevromus intimus* (Punjab: Murre [1♂, 30.VII.2019, 33.92192°N, 73.40353°E, 1928 m]), *Protohermes motuoensis* (Khyber Pakhtunkhwa: Swat [1♂, 11.IX.2019, 35.054092°N, 72.564847°E, 760 m]) and *Protohermes walkeri* (Azad Kashmir: Bagh [1♀, 04.VIII.2019, 34.5844°N, 73.4638°E, 1628 m]) were collected from the northern areas of Pakistan

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in 2019. The specimens were stored in 95% ethanol and refrigerated at -20°C. The specimens are deposited in the Entomological Museum of China Agricultural University (CAU), Beijing, China. Total genomic DNA was extracted from the thoracic muscle tissues or several legs of each individual sample preserved in ethanol, using the TIANamp Genomic DNA Kit (TIANGEN BIOTECH CO., LTD, Beijing, China) following manufacturers' instructions.

Library preparation, and Illumina sequencing

The sequencing library was generated using Truseq Nano DNA Sample Prep Kits (Illumina, USA) with insert sizes from 350 bp sequence data with 150 bp paired-end reads. Raw reads were trimmed of adapters using Trimmomatic (Bolger et al., 2014). GenBank accession numbers of all sequences used in this study are provided in Table 1. The clustering of the index-coded samples was performed on a cBot Cluster Generation System using Novaseq PE Cluster Kit v.2.5 (Illumina).

Genome assembly and annotation

We did the reference-guided assembling by mapping to the mitogenome of *Nevromus exterior* Navás, 1927 and *Protohermes concolorus* Yang & Yang, 1988 as the reference sequences (Hua et al., 2009; Jiang et al., 2015) through GENEIOUS v. 9.0 (Kearse et al., 2012) (https://www.geneious.com), with the parameters set as follows: 95% minimum overlap identity, 4 maximum ambiguity, and minimum overlap of 25 bp. The other parameters use the default settings. The preliminary mitogenome annotations were conducted using the MITOS web server, under default settings, and the invertebrate genetic code for mitochondria (Bernt et al., 2013). For the mitochondrial protein-coding genes, we first removed the stop codon of each sequence. Then, the nucleotide sequences were aligned based on their corresponding amino acid translations using the MAFFT algorithm implemented in TranslatorX (Abascal et al., 2010). Poorly aligned sites were removed with Gblocks v 0.91 (Talavera and Castresana, 2007), under options for a less stringent selection. For the mitochondrial tRNA and rRNA genes, each of them was aligned using MAFFT (Katoh and Standley, 2013) under the iterative refinement method incorporating the most accurate local pairwise alignment information (E-INS-i), and ambiguously aligned sites were pruned using Gblocks with the same settings mentioned above. All alignments were checked in MEGA 7 (Kumar et al., 2016). Finally, all alignments were concatenated using FASconCAT_v1.0 (Kück and Meusemann, 2010) to construct the full dataset of PCGs. Mitogenome maps were constructed using CG View server V 1.0 (http://stothard.afns.ualberta.ca/cgview_server/) (Grant and Stothard, 2008).

Phylogenetic analyses

To test the phylogenetic positions of the three Pakistani dobsonflies, the phylogenetic analyses were carried out based on two mitogenomic datasets: PCG123 (nucleotide data of 13 PCGs) and PCG_AA (amino acid sequences of the 13 PCGs) of 49 species (44 ingroup taxa of Corydalinae and 5 outgroup taxa respectively belonging to Chauliodinae, Sialidae, and Raphidioptera), including the three species from Pakistan (Table 1). We used PartitionFinder v.2.1.1 (Lanfear et al., 2016) to determine the best partitioning schemes for the datasets under the Bayesian Information Criterion (BIC). Detailed information on the partitions and the best models selected are summarized in Table 2.

Phylogenies were inferred based on Bayesian inference (BI) and Maximum likelihood (ML). ML analysis was performed in IQ-TREE (Nguyen et al., 2015; Trifinopoulos et al., 2016) and the BI analysis was performed in MrBayes v.3.2.7a (Ronquist et al., 2012) implemented on XSEDE (Extreme Science and Engineering Discovery Environment) through the CIPRES Science Gateway (Miller et al., 2010) (http:// www.phylo.org/) with various data partition schemes and best-fitting models determined by PartitionFinder. The maximum likelihood method was performed on IQ-tree website server (http://iqtree.cibiv.univie.ac.at) with 1000 bootstrap replicates. The BI analysis contains four simultaneous Markov chain Monte Carlo (MCMC) runs of 2 million generations. Trees were sampled every 1000 generations and the burn-in fraction set to 25%. Analyses terminated when the standard deviation of clade frequencies fell below 0.01, which indicates that stationarity had been reached. A majority-rule consensus tree was calculated with posterior probabilities (PPs) for each node. The phylogenetic relationships were also reconstructed with the MrBayes and IQ-TREE plugin in PhyloSuite 1.2.2 (Zhang et al., 2020). Trees were visualized and edited with FIGTREE v1.3.1 (Rambaut, 2009).

Results

The complete mitogenome of *Nevromus intimus* is 16,614 bp in length, which is similar to *Protohermes motuoensis* (16,238 bp) and *Protohermes walkeri* (16,585 bp), and the mitochondrial genome maps are presented in Fig. 1. It contains 13 PCGs, two rRNAs, 22 tRNAs, and a variably lengthed control region: 1,867 bp (*Protohermes walkeri*), 1,527 bp (*Protohermes motuoensis*), and 1,858 bp (*Nevromus intimus*) (Tables 3-5). Genetic divergence was calculated with the Kimura 2-parameter (K2P) model (Kimura, 1980) in MEGA 7.0 based on COI. The intraspecific genetic distance of *P. motuoensis* was 0.010 between the specimens respectively from Pakistan and China (Table S1).

For the phylogenetic analyses, the PCG123 dataset includes 11061 bp nucleotides for each taxon, and the PCG_AA dataset includes 3687 bp amino acids for each taxon. Four phylogenetic trees were obtained from the ML and BI analysis of the above two datasets (Fig. 2, Figs. S1-S4). The 13 *Neoneuromus* species form a monophyletic group with high nodal supports and *Nevromus* was recovered as sister group with high support values in all analysis as in Tu et al. (2021). Similarly, 28 *Protohermes* species for a monophyletic group with high nodal supports in all analysis except for some branches with low support in ML analysis in PhyloSuite. In all analysis, the phylogenetic placement of the newly sequences species of *Nevromus* and *Protohermes* are consistent with Tu et al. (2021) and Jiang et al. (2022), respectively. However, the basal-most portion of *Chloroniella* in Corydalinae was only supported in BI analysis by PhyloSuite (Fig. 2), which is consistent with the phylogenetic results in Jiang et al. (2022), whereas in the ML tree through IQ-tree website server, the BI tree through the CIPRES Science Gateway, and ML tree in PhyloSuite, *Chloroniella* was recovered as sister to *Protohermes* (Figs. S1-S4).

Discussion

Our study provides the first detailed mitochondrial genome data on Megaloptera from Pakistan. In particular, we added the mitogenomic data of *P. walkeri* and *N. intimus* that have not yet been sequenced. The sister-group relationship between *P. walkeri* and *P. niger*, and their grouping with the other species of the *P. costalis* species-group support our previous placement of *P. walkeri* in the *P. costalis* group based on morphological characters, such as the widely separated lateral ocelli, the short, subcylindrical male ectoproct, and the female sternum 9 with a pair of sac-like lobes (Hassan et al., 2020). Moreover, the morphological similarity between *P. walkeri* and *P. niger* is also noteworthy. *Protohermes niger* is a spectacular species with sexual

Table 1
List of taxa included in this study.

S.N.	Order	Family	Species	Number (bp)	Accession number	Locality	References
1	**Megaloptera**	**Corydalidae**	*Acanthacorydalis orientalis*	15753	KF840564	China (Sichuan)	Wang et al. (2014)
2			*Chloroniella peringueyi*	15764	MW642269	South Africa (Elandsbos River)	Jiang et al. (2022)
3			*Corydalus cornutus*	15687	FJ171323	Florida	Beckenbach and Stewart (2009)
4			*Platyneuromus soror*	15750	MW642273	Mexico (Veracruz)	Jiang et al. (2022)
5			*Protohermes motuoensis*	16238	**ON209202**	**Pakistan (Khyber Pakhtunkhwa)**	**Present study**
6			*Protohermes motuoensis*	15849	MW642284	China (Tibet)	Jiang et al. (2022)
7			*Protohermes walkeri*	16522	**2573900**	**Pakistan (Azad Kashmir)**	**Present study**
8			*Protohermes cangyuanensis*	15826	MW642275	China (Yunnan)	Jiang et al. (2022)
9			*Protohermes concolorus*	15851	EU526394	China (Yunnan)	Hua et al. (2009)
10			*Protohermes concolorus*	15852	MW642276	China (Yunnan)	Jiang et al. (2022)
11			*Protohermes costalis*	10413	MW642321	China (Guangxi)	Jiang et al. (2022)
12			*Protohermes davidi*	15851	MW642277	China (Sichuan)	Jiang et al. (2022)
13			*Protohermes dichrous*	15827	MW642278	Malaysia (Borneo)	Jiang et al. (2022)
14			*Protohermes differentialis*	15255	MW642303	China (Guangxi)	Jiang et al. (2022)
15			*Protohermes flavinervus*	15854	MW642279	China (Yunnan)	Jiang et al. (2022)
16			*Protohermes grandis*	15840	MW642280	Japan (Oita)	Jiang et al. (2022)
17			*Protohermes gutianensis*	15434	MW642304	China (Fujian)	Jiang et al. (2022)
18			*Protohermes hainanensis*	15841	MW642281	China (Hainan)	Jiang et al. (2022)
19			*Protohermes infectus*	15848	MW642282	China (Tibet)	Jiang et al. (2022)
20			*Protohermes latus*	15852	MW642283	China (Tibet)	Jiang et al. (2022)
21			*Protohermes basimaculatus*	15838	MW642274	China (Yunnan)	Jiang et al. (2022)
22			*Protohermes niger*	15749	MW642285	China (Yunnan)	Jiang et al. (2022)
23			*Protohermes similis*	15844	MW642286	China (Sichuan)	Jiang et al. (2022)
24			*Protohermes sinensis*	14774	MW642305	China (Hubei)	Jiang et al. (2022)
25			*Protohermes sinuolatus*	15851	MW642287	Laos (Attapeu)	Jiang et al. (2022)
26			*Protohermes spectabilis*	15869	MW642288	Malaysia (Borneo)	Jiang et al. (2022)
27			*Protohermes subnubilus*	15511	MW642306	China (Yunnan)	Jiang et al. (2022)
28			*Protohermes tonkinensis*	14291	MW642320	China (Yunnan)	Jiang et al. (2022)
29			*Protohermes xanthodes*	15846	MW642289	China (Hunan)	Jiang et al. (2022)
30			*Neoneuromus sikkimmensis*	15767	MW965204	China (Yunnan)	Tu et al. (2021)
31			*Neoneuromus latratus*	15772	MW965206	China (Yunnan)	Tu et al. (2021)
32			*Neoneuromus coomani*	15766	MW965203	Laos (Champasak)	Tu et al. (2021)
33			*Neoneuromus indistinctus*	15770	MW965202	China (Yunnan)	Tu et al. (2021)
34			*Neoneuromus maclachlani*	15770	MW965201	China (Hubei)	Tu et al. (2021)
35			*Neoneuromus vanderweelei*	15767	MW965200	China (Yunnan)	Tu et al. (2021)
36			*Neoneuromus fenestralis*	15769	MW965199	China (Yunnan)	Tu et al. (2021)
37			*Neoneuromus maculatus*	15772	MW965197	Vietnam (Thua Thien Hue)	Tu et al. (2021)
38			*Neoneuromus niger*	15772	MW965196	Vietnam (Kon Tum)	Tu et al. (2021)
39			*Neoneuromus similis*	15771	MW965198	China (Fujian)	Tu et al. (2021)
40			*Neoneuromus orientalis*	15771	MW965205	China (Anhui)	Tu et al. (2021)
41			*Neoneuromus ignobilis*	15768	MW642272	China (Zhejiang)	Tu et al. (2021)
42			*Neoneuromus tonkinensis*	15776	KP126231	Vietnam (Vinh Phuc)	Jiang et al. (2015)
43			*Nevromus exterior*	15763	KP126232	Vietnam (Bac Kan)	Jiang et al. (2015)
44			*Nevromus intimus*	16614	**ON209201**	**Pakistan (Punjab)**	**Present study**
45			*Neochauliodes fraternus*	15768	MW642326	China (Zhejiang)	Jiang et al. (2022)
46			*Neochauliodes formosanus*	14476	MW642325	China (Guangdong)	Jiang et al. (2022)
47		**Sialidae**	*Sialis jiyuni*	15590	MW642261	China (Sichuan)	Jiang et al. (2022)
48			*Sialis koreana*	15537	MW642300	Korea	Jiang et al. (2022)
49	**Raphidioptera**	**Raphidiidae**	*Mongoloraphidia harmandi*	16006	FJ859902	Japan	Cameron et al. (2009)

S.N.: serial number; bp: base pair.

Table 2
Best-fit partitioning schemes and models of evolution determined by PartitionFinder.

Subset Partitions	Best Model	Alignment
COX1, ND3, ATP6, COX2, CYTB, COX3	GTR+I+G	814-2334 6892-7212 1-651 2335-3012 3799-4935 3013-3798;
ND2, ATP8, ND6	GTR+I+G	5878-6891 652-813 10540-11043;
ND1, ND5, ND4L, ND4	GTR+I+G	4936-5877 8842-10539 8548-8841 7213-8547;

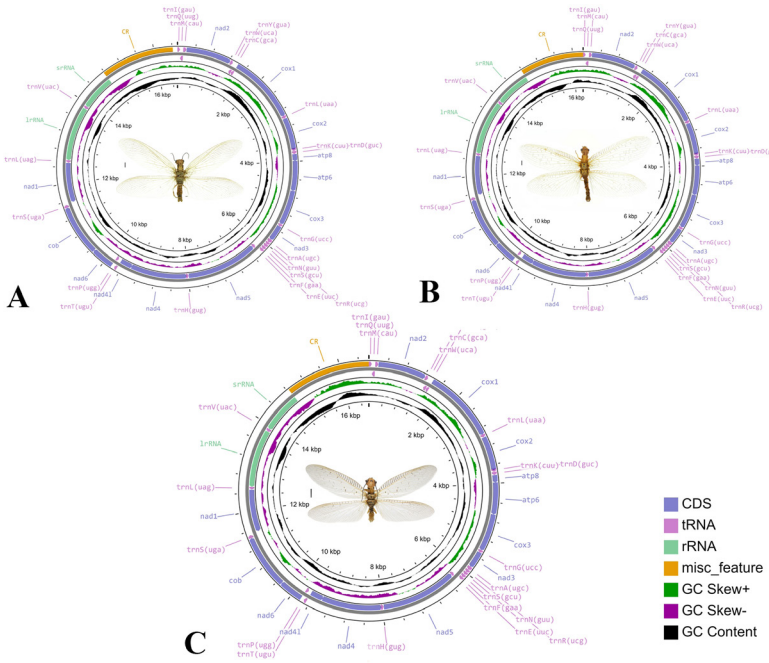

Figure 1 The maps of the complete mitochondrial genome of three Pakistani dobsonfly species. A. *Protohermes walkeri*; B. *Protohermes motuoensis*; C. *Nevromus intimus*.

Table 3
Organization of the complete mt genome in *Protohermes walkeri*.

Gene	Direction	Position (bp)	Size (bp)	IGN*	Anticodon	Start Codon	Stop Codon
$tRNA^{Ile}$	F	1—63	63		GAT		
$tRNA^{Gln}$	R	61—129	69	-3	TTG		
$tRNA^{Met}$	F	136—204	69	6	CAT		
ND2	F	205—1222	1018	0		ATT	TGT
$tRNA^{Trp}$	R	1223—1288	66	0	TCA		
$tRNA^{Cys}$	F	1281—1345	65	-8	GCA		
$tRNA^{Tyr}$	R	1346—1410	65	0	GTA		
COI	F	1403—2944	1542	-8		ATT	TAA
$tRNA^{Leu(UUR)}$	F	2946—3010	65	1	TAA		
COII	F	3013—3696	684	2		ATG	TAA
$tRNA^{Lys}$	F	3698—3767	70	1	CTT		
$tRNA^{Asp}$	F	3768—3835	68	0	GTC		
ATP8	F	3836—3991	156	0		ATT	TAA
ATP6	F	3988—4643	656	-4		ATG	TAA
COIII	F	4662—5448	787	18		ATG	GCT
$tRNA^{Gly}$	F	5449—5511	63	0	TCC		
ND3	F	5512—5863	352	0		ATT	ACT
$tRNA^{Ala}$	F	5864—5928	65	0	TGC		
$tRNA^{Arg}$	F	5946—6007	62	17	TCG		
$tRNA_{Asn}$	F	6007—6073	67	-1	GTT		
$tRNA^{Ser(AGN)}$	F	6074—6140	67	0	GCT		
$tRNA^{Glu}$	F	6141—6205	65	0	TTC		
$tRNA^{Phe}$	R	6204—6266	63	-2	GAA		
ND5	R	6267—7992	1726	0		AAA	AAT
$tRNA^{His}$	R	7993—8056	64	0	GTG		
ND4	R	8057—9395	1339	0		ACA	CAT
ND4L	R	9389—9685	297	-7		TTA	CAT
$tRNA^{Thr}$	F	9688—9750	63	2	TGT		
$tRNA^{Pro}$	R	9751—9815	65	0	TGG		
ND6	F	9818—10333	516	2		ATT	TAA
CytB	F	10330—11464	1135	-4		ATG	ATT
$tRNA^{Ser(UCN)}$	F	11465—11531	67	0	TGA		
ND1	R	11544—12497	954	12		TTA	CAA
$tRNA^{Leu(CUN)}$	R	12499—12561	63	1	TAG		
lrRNA	R	12562—13875	1314	0		ATT	TAA
$tRNA^{Val}$	R	13876—13945	70	0	TAC		
srRNA	R	13946—14717	772	0		ATC	TAA
Control region	–	14718—16585	1867	0		GCC	ATA

*bp: base pair. *IGN: intergenic nucleotides.*

Table 4
Organization of the complete mt genome in *Protohermes motuoensis*.

Gene	Direction	Position (bp)	Size (bp)	IGN*	Anticodon	Start Codon	Stop Codon
$tRNA^{Ile}$	F	1—63	63		GAT		
$tRNA^{Gln}$	R	61—129	69	-3	TTG		
$tRNA^{Met}$	F	134—201	68	4	CAT		
ND2	F	202—1219	1018	0		ATT	TTT
$tRNA^{Trp}$	R	1220—1285	66	0	TCA		
$tRNA^{Cys}$	F	1278—1341	64	-8	GCA		
$tRNA^{Tyr}$	R	1342—1406	65	0	GTA		
COI	F	1399—2940	1542	-8		ATT	TAA
$tRNA^{Leu(UUR)}$	F	2942—3006	65	1	TAA		
COII	F	3009—3692	684	2		ATG	TAA
$tRNA^{Lys}$	F	3694—3763	70	1	CTT		
$tRNA^{Asp}$	F	3764—3830	67	0	GTC		
ATP8	F	3831—3989	159	0		ATA	TAA
ATP6	F	3983—4657	675	-7		ATG	TAA
COIII	F	4657—5443	787	-1		ATG	GTT
$tRNA^{Gly}$	F	5444—5506	63	0	TCC		
ND3	F	5507—5858	352	0		ATC	ATT
$tRNA^{Ala}$	F	5859—5923	65	0	TGC		
$tRNA^{Arg}$	F	5941—6003	63	17	TCG		
$tRNA^{Asn}$	F	6003—6068	66	-1	GTT		
$tRNA^{Ser(AGN)}$	F	6069—6135	67	0	GCT		
$tRNA^{Glu}$	F	6136—6200	65	0	TTC		
$tRNA^{Phe}$	R	6199—6261	63	-2	GAA		
ND5	R	6262—7987	1726	0		AAA	AAT
$tRNA^{His}$	R	7988—8050	63	0	GTG		
ND4	R	8051—9389	1339	0		AAA	CAT
ND4L	R	9383—9679	297	-7		TTA	CAT
$tRNA^{Thr}$	F	9682—9744	63	2	TGT		
$tRNA^{Pro}$	R	9745—9809	65	0	TGG		
ND6	F	9812—10327	516	2		ATT	TAA
CytB	F	10327—11461	1135	-1		ATG	ATT
$tRNA^{Ser(UCN)}$	F	11462—11528	67	0	TGA		
ND1	R	11541—12494	62	12		TTA	CAA
$tRNA^{Leu(CUN)}$	R	12496—12557	62	1	TAG		
lrRNA	R	12558—13869	1312	0		ATT	CTT
$tRNA^{Val}$	R	13870—13939	70	0	TAC		
srRNA	R	13940—14713	774	0		ATC	TTA
Control region	–	14712—16238	1527	-2		GCC	TAA

*bp: base pair. *IGN: intergenic nucleotides.*

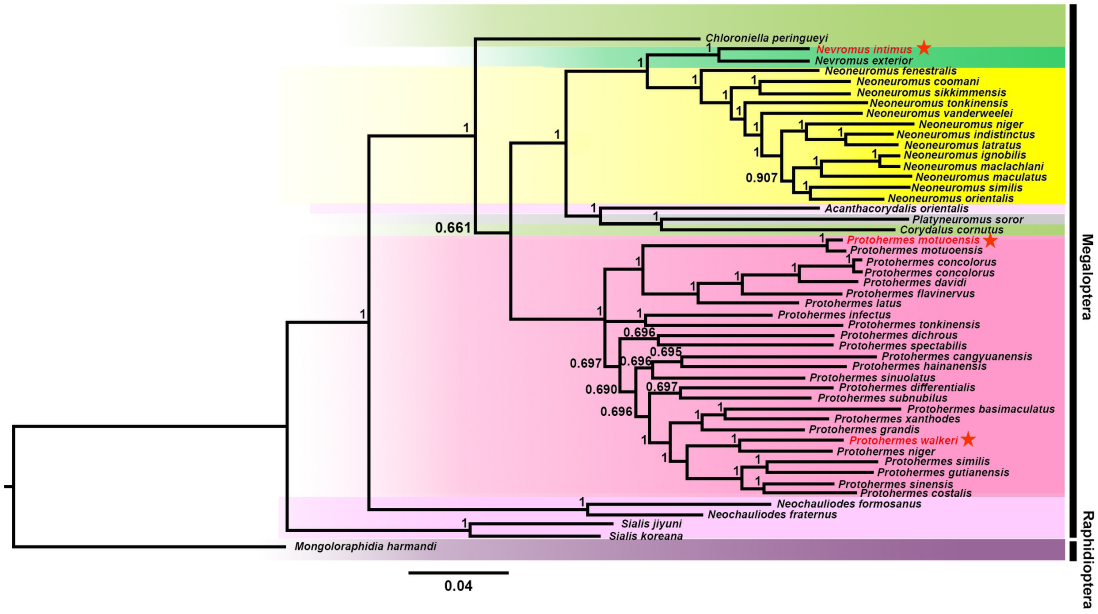

Figure 2 Phylogenetic relationships among the selected species of Megaloptera based on mitogenomic data. BI tree for Megaloptera based on concatenated 13 PCGs with branch supports shown as Bayesian posterior probabilities. The colors represent different genera.

Table 5
Organization of the complete mt genome in *Nevromus intimus*.

Gene	Direction	Position (bp)	Size (bp)	IGN*	Anticodon	Start Codon	Stop Codon
$tRNA^{Ile}$	F	1—65	65		GAT		
$tRNA^{Gln}$	R	66—134	69	0	TTG		
$tRNA^{Met}$	F	134—202	69	-1	CAT		
ND2	F	203—1219	1017	0		ATT	TAA
$tRNA^{Trp}$	R	1218—1284	67	-2	TCA		
$tRNA^{Cys}$	F	1277—1340	64	-8	GCA		
$tRNA^{Tyr}$	R	1341—1404	64	0	GTA		
COI	F	1397—2937	1541	-8		ATT	GTA
$tRNA^{Leu(UUR)}$	F	2938—3001	64	0	TAA		
COII	F	3004—3687	684	2		ATG	TAA
$tRNA^{Lys}$	F	3688—3757	70	0	CTT		
$tRNA^{Asp}$	F	3758—3824	67	0	GTC		
ATP8	F	3825—3983	159	0		ATT	TAA
ATP6	F	3977—4651	675	-7		ATG	TAA
COIII	F	4651—5437	787	-1		ATG	GTT
$tRNA^{Gly}$	F	5438—5499	62	0	TCC		
ND3	F	5500—5851	352	0		ATT	ACT
$tRNA^{Ala}$	F	5852—5914	63	0	TGC		
$tRNA^{Arg}$	F	5929—5992	64	14	TCG		
$tRNA^{Asn}$	F	5992—6056	65	-1	GTT		
$tRNA^{Ser(AGN)}$	F	6057—6122	66	0	GCT		
$tRNA^{Glu}$	F	6123—6187	65	0	TTC		
$tRNA^{Phe}$	R	6186—6248	63	-2	GAA		
ND5	R	6249—7974	1726	0		AAA	AAT
$tRNA^{His}$	R	7975—8037	63	0	GTG		
ND4	R	8038—9376	1339	0		ACA	CAT
ND4L	R	9370—9666	297	-7		TTA	CAT
$tRNA^{Thr}$	F	9669—9731	63	2	TGT		
$tRNA^{Pro}$	R	9732—9797	66	0	TGG		
ND6	F	9800—10315	516	2		ATT	TAA
CytB	F	10315—11449	1135	-1		ATG	TTT
$tRNA^{Ser(UCN)}$	F	11451—11517	67	1	TGA		
ND1	R	11530—12483	954	12		TTA	CAA
$tRNA^{Leu(CUN)}$	R	12485—12546	62	1	TAG		
lrRNA	R	12547—13859	1313	0		ATT	ATG
$tRNA^{Val}$	R	13860—13929	70	0	TAC		
srRNA	R	13930—14757	828	0		TTA	TAA
Control region	–	14758—16614	1858	0		TAA	TAA

*bp: base pair. *IGN: intergenic nucleotides.*

dimorphism, as the male is blackish in body and wing coloration, but the female is yellowish (Chang et al., 2013). The females of *P. niger* have almost identical cephalic and pronotal marking patterns (different from most other *Protohermes* species) with *P. walkeri*. Thus, the present molecular data also suggests consistent interspecific relationship to the morphological inference concerning above two *Protohermes* species. For *P. motuoensis*, which is a species ranging along southern Himalayas (Hassan et al., 2020), the low genetic divergence of COI between the specimens respectively from Pakistan and southeastern Tibet confirms that they are conspecific despite long geographic distance of their distribution. By adding the mitogenome of *N. intimus*, the mitogenomic data are available for only two species of *Nevromus*. *Nevromus intimus* was assigned in a monophyletic group with *Nevromus aspoeck* Liu, Hayashi & Yang and *N. exterior* based on morphological data (Liu et al., 2012). Further molecular data are needed to investigate the phylogenetic position of *N. intimus* in *Nevromus*. The mitochondrial genome data of insects from Pakistan are still scarce. Our study may promote future works on molecular taxonomy and phylogeny of Neuropterida and other insect groups from Pakistan.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

Conceptualization, MAH and XL; writing—original draft preparation, MAH, RS and MA; writing—review and editing, MAH and XL; supervision, XL; figures and tables, MAH and RS; funding acquisition, XL. All authors have read and agreed to the published version of the manuscript.

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Supplementary Material

The following online material is available for this article:

Figure S1 - Phylogenetic relationships among the selected species of Megaloptera based on mtDNA. 1. BI analysis with MrBayes in PhyloSuite.

Figure S2 - ML analysis performed in IQ-TREE embedded in PhyloSuite.

Figure S3 - ML tree through IQ-tree website.

Figure S4 - BI tree through the CIPRES Science Gateway.

Table S1 - Genetic divergence among samples based on mtDNA.