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Mitochondrial genome of *Poecilimon cretensis* (Orthoptera: Tettigoniidae: Phaneropterinae): Strong phylogenetic signals in gene overlapping regions

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Abstract

We report the complete mitochondrial genome of the Cretan bush cricket *Poecilimon cretensis*. The mitogenome consists of 13 protein-coding regions, 22 tRNAs, two rRNAs, and one control region. The length of mitogenome in *P. cretensis* varies between15477 and 15631 bp, mainly due to variability in control region. The start and stop codons of protein coding genes exhibit the general pattern in Phaneropterinae. Phylogenetic tree constructed with the mitogenome obtained during this study and 12 mitogenomes of Phaneropterinae downloaded from GenBank, placed *P. cretensis* in Barbitistini as sister group to *Poecilimon luschani*. Data indicate that the gene overlapping pattern exhibit strong phylogenetic signals.

Key words: Orthoptera, Phaneropterinae, *Poecilimon cretensis*, mitogenome

Introduction

Genus *Poecilimon* Fischer holds a number of the records. It is the largest genus of Barbitistini including nearly half of the species and the largest genus of Phaneropterinae in Palaearctic (Cigliano *et al*. 2022; Borissov *et al*. 2023). With more than 150 species/subspecies, *Poecilimon* is an Anatolio-Greek-Balkan genus in distribution, though a few species also occur in the peripheral area (Çıplak 2004). Additionally, some species of the genus may become pests in certain conditions or years (Çıplak 2021). Though it is a large genus and includes economically important species, genetic data relating to the genus are very scarce, therefore prevents making robust statements about evolution of the genus, and the higher taxa it belongs. So far, mitogenome of a single species, *Poecilimon luschani,* is produced and uploaded to databases (Öztürk & Çıplak 2019). The present study aims; (i) to describe the mitogenome of *Poecilimon cretensis* Werner, the only species of the genus occurring in Crete, an island considered as a part of the possible origin place of the genus (Çıplak 2004), (ii) to compare with available mitogenomes of other related species belonging to the genus *Poecilimon*, tribe Barbististini and subfamily Phaneropterinae, and (iii) to determine the phylogenetic position of the species.

Materials and methods

The samples of *P. cretensis* were collected from Crete Island of Greece (GREECE: Kreta, Chania, Chora Sfakion (35°12′4″N, 24°8′34″E), 100 m, 25.05.2016, coll. Martina Heller; Collection of Heller- CH8219, CH8220, CH8221, CH8224 and CH8225). Total DNA was extracted from alcohol preserved muscle tissue of the hind

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legs of five specimens using DNeasy Blood & Tissue Kit (Qiagen Inc.). Total genomic DNA extracts of five *P. cretensis* specimens were multiplexed and sequenced using the Illumina HiSeq 2500 next generation sequencing (NGS) platform for the 150 bp paired-end reads via Novogene Inc.(China). The raw NGS reads of five specimens provided by the company were first de-multiplexed and then each filtered by removing reads with adaptor/barcode contamination and low quality (N10 bp; low quality scores b30; poly-Ns N5 bp) (Zhou *et al.* 2013; Tang *et al*. 2014). The sequence quality was checked using FASTQC (Andrews 2010). The processed and unmapped forward and reverse reads were imported to the TRUBA clusters (The Scientific and Technological Research Council of Turkey, TUBITAK) and assembled using the short-read assembler MINIA with default settings (Salikhov *et al*. 2014). The contigs obtained were blasted against the custom database covering Orthoptera mitogenome data generated from GenBank. The mitogenomes were annotated using metazoa (RefSeq 63) reference on MITOS2 (Donath *et al*. 2019). The secondary structures of tRNAs were checked using ARWEN v.1.2 (Laslett *et al*. 2008). The annotated mitogenomes were imported to GENEIOUS v. 9.0.5 (Biomatters Ltd., Auckland, New Zealand), and aligned with annotated *P. luschani* reference genome (see Öztürk & Çıplak 2019) for final manual control.

For phylogenetic analysis, we acquired 13 mitogenomes belonging to subfamily Phaneropterinae; one from *P. cretensis* produced during this study plus 10 belong to other genera of the subfamily as ingroup and one from each of Mecopodinae and Pseudophyllinae subfamilies as outgroup (Table 1). We generated multiple sequence alignments separately for each of the 13 protein coding genes (PCG), 22 tRNAs and 2 rRNAs separately for a total of 13 sequences using MAFFT v.7 (Katoh & Standley 2013). Then we removed the stop codons of each PCG matrices in MEGA v.X (Kumar *et al*. 2018). Individual gene alignments were concatenated using SEQUENCEMATRIX v.1.7.8 (Vaidya *et al*. 2011) into the final 15,026 bp in length. We then reconstructed a maximum likelihood tree using IQ-TREE2 (Minh *et al*. 2020) with ModelFinder (Kalyaanamoorthy *et al*. 2017) function, and trees were visualized using FIGTREE v.1.4.2.

TABLE 1. GenBank accession numbers of the sequences used in the phylogenetic analysis

Species	Acc.No.	Reference
Ruidocollaris obscura	NC_028160	Yang *et al*. 2016
Holochlora fruhstorferi	NC_033993	Zhou *et al*. 2017
Sinochlora longifissa	NC_021424	Liu *et al*. 2013
Isophya major	MK759880	Öztürk & Çıplak 2019
Poecilimon cretensis	WILL BE ADDED	THIS STUDY
Poecilimon luschani	MK757458	Öztürk & Çıplak 2019
Phaneroptera gracilis	NC_034756	Wang *et al*. 2017
Deflorita sp.	KX057719.1	Zhou et al., 2017
Elimaea cheni	NC_014289	Zhou *et al*. 2010
Ducetia japonica	NC_031652	Zhou *et al*. 2017
Kuwayamaea brachyptera	NC_028159	Yang *et al*. 2016
Mecapoda niponensis	NC_021379	Zhou *et al*. 2013
Phyllomimus detersus	NC_028158	Yang *et al*. 2016

Results and Discussion

Length of the complete mitogenomes in five specimens of *P. cretensis*, assembled against *P. luschani* (MK757458) varies between15477 and 15631 bp. This difference mainly arises from the length of AT rich control region which varies between 676 and 830 bp. The matrix established by these five mitogenomes has 414 variable base positions. The mitogenome of *P. cretensis* consists of 13 protein coding genes, 22 tRNAs, two rRNAs, and one AT rich control region (Table 2) and the gene order is identical to published pancrustacean mitogenome (Cameron 2014; Öztürk & Çıplak 2019). The mitogenome with 15542 bp-length was used in descriptive analyses (Figure 1). The rate of AT/GC is 67.7/33.3 similar to other Orthoptera (Fenn *et al*. 2008; Öztürk & Çıplak 2019). Of the 13 protein coding genes *cox1*, *atp8*, *nad3*, *nad5* and *nad6* have ATT start codon; *nad2, cox2*, *atp6*, *cox3*, *nad4*, *nad4l* and *cytb* have ATG, and *nad1* has TTG. All start codons, other than that of *nad3,* are the same as *P. lushani,* and fit to the ATN

pattern of Phaneropterinae and other orthopteran as well (Fenn *et al*. 2008; Öztürk & Çıplak 2019). Stop codons are TAA in *nad2, cox1, cox2, atp8, atp6, cox3, nad3*, *nad4l* and *nad1,* TAG in *cytb*, incomplete TA- in *nad6* and incomplete T-- in *nad4* and *nad5*. Stop codons of PCG other than *nad6*, *cytb* and *nad1* are the same as *P. luschani* and the common pattern of TAN or T-- pattern observed in all Phaneropterinae (Öztürk & Çıplak 2019). The tRNA genes constitute 1449 bp of the mitogenome in *P. cretensis* and the lengths of them vary between 62 and 71 bp (Table 2). All of them except for *trnS1* (without D-stem) formed clover-leaf structure as observed in other insects (Kim *et al*. 2005). The rRNA genes constitute 2086 bp of the mitogenome.

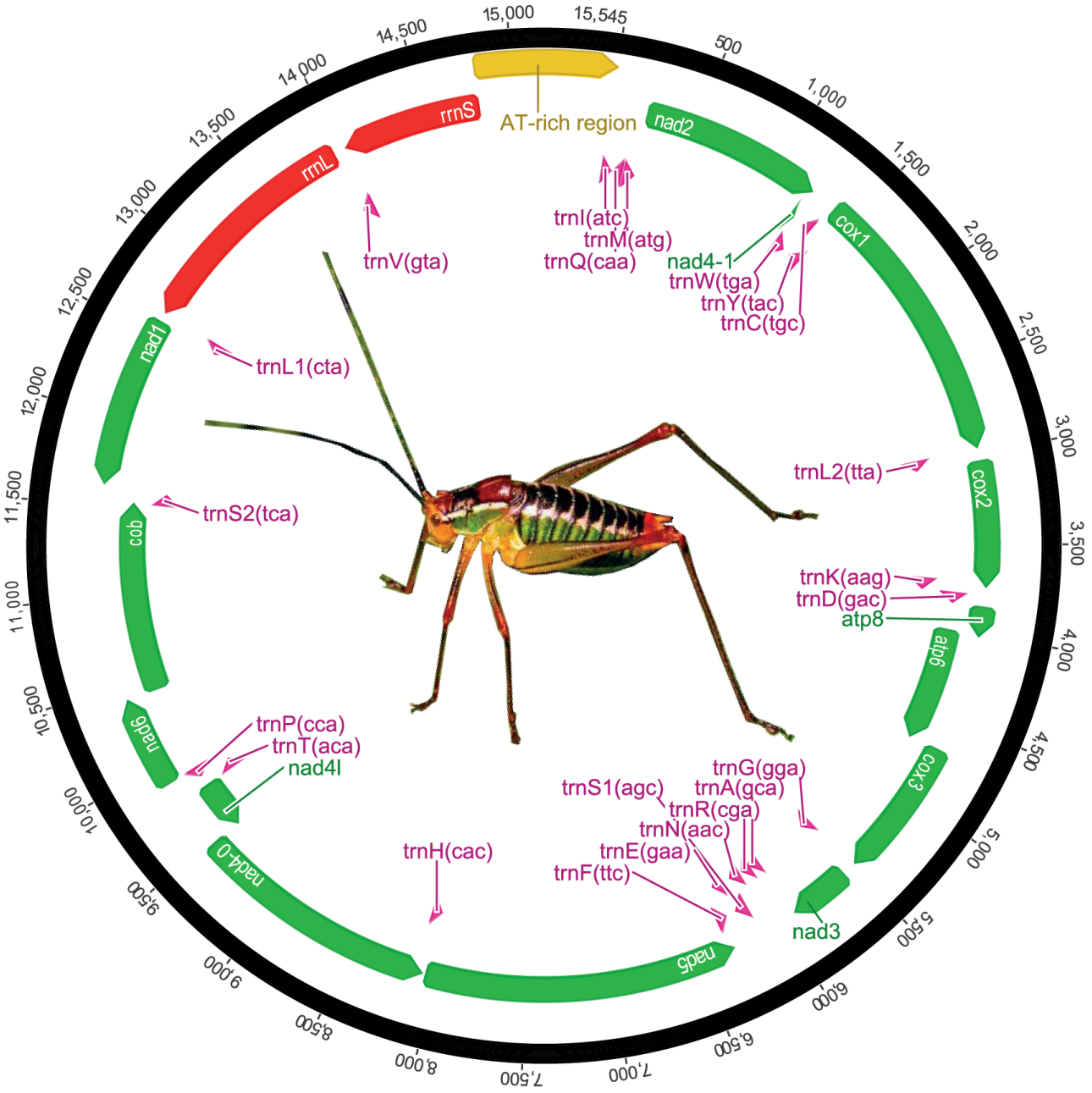

FIGURE 1. The map of mitochondrial genome and habitus of *Poecilimon cretensis*

The topology of the calculated phylogenetic tree largely recovers that presented by Öztürk & Çıplak (2019), but the nodal supports here are lower. As expected *P. cretensis* occurs as sister group to *P. luschani* and then these two to *Isophya major* (Figure 2). The overlapping and non-coding intergenic sequences pattern of *P. cretensis* and *P. luschani* are very similar especially for the PCG with adjacent genes (Table 3). Differences between two species for the overlapping regions are: (i) *cytb-trnS2* overlaps by two bp in *P. cretensis* but one bp in *P.luschani*, and (ii) *trnW-trnC* overlaps for eight bp in *P. cretensis* but nine bp in *P. luschani*. The overlapping pattern of the adjacent genes is

also largely similar with the other member of the tribe Barbitistini, *Isophya major* and with other Phaneropterinae, with Orthoptera as well, for certain positions (Fenn *et al*. 2008; Öztürk & Çıplak 2019). This case indicates to strong phylogenetic signals for the overlapping regions and to some degree non-coding intergenic sequences.

TABLE 2. The mitogenome profile for *Poecilimon cretensis* (genes are listed according to their order in genome; S, strand; intergenic spacers (IGS) are indicated by "+" and the overlapping regions (OR) by "-")

Gene	S	Position	Size (bp)	IGS/OR	IGS/OR sequence
trnI	J	1-64	64	+7	CTACGTA
trnQ	N	72-139	68	+8	TATATTCC
trnM	J	148-213	66	+6	CTGTTA
nad2	J	220-1236	1,017	-2	AA
trnW	J	1235-1302	68	+5	CCTTA
trnC	N	1298-1359	62	+3	TAC
trnY	N	1363-1428	66	-8	ATTCTACC
cox1	J	1391-2962	1542	-1	A
trnL2	J	2962-3026	65	+4	GTAA
cox2	J	3031-3741	711	-20	CATCAGATGGCTGAAAGTAA
trnK	J	3722-3791	70	-1	A
trnD	J	3791-3856	66	0	
atp8	J	3857-4024	168	-7	ATGATAA
atp6	J	4018-4695	678	-1	A
cox3	J	4695-5486	792	+11	CATTATTCCTT
trnG	J	5498-5562	65	-3	ATA
nad3	J	556-5916	357	+5	CTTTT
trnA	J	5922-5986	65	0	
trnR	J	5987-605	64	+19	GTGTAACAATTATAGTAAT
trnN	J	607-6134	65	-2	GA
trnS1	J	6133-6199	67	+1	A
trnE	J	6201-6267	67	+2	TA
trnF	N	6266-6329	64	0	
nad5	N	633-8061	1732	0	
trnH	N	8062-8125	64	0	
nad4	N	8126-9464	1339	-7	TTAACAT
nad4l	N	9458-9754	297	+4	TCCT
trnT	J	9759-9821	63	+1	T
trnP	N	9821-9885	65	+1	T
nad6	J	9887-10405	519	-1	A
cytb	J	10405-11541	1137	-2	AG
trnS2	J	1154-11608	69	+22	CTATGTTACTAAATTCATTACA
nad1	N	11631-12587	957	-6	TACTAT
trnL1	N	12582-12646	65	0	
rrnL	N	12647-1395	1304	0	
trnV	N	13951-14021	71	0	
rrnS	N	14022-14803	782	0	
A + T		14804-15542	739	0	

TABLE 3. The number of intergenic (+) or overlapping (-) bases in three species of Barbitistini (CRE, *Poecilimon cretensis*; LUS, *P. luschani*; MAJ, *Isophya major*. The gene order in first panel of the table is followed by first line of the second panel. The yellow cells indicate the pattern shared by all species of the tribe, and the blue cells the pattern shared two species of *Poecilimon*).

Gene	CRE	LUS	MAJ	**Gene**	**CRE**	**LUS**	**MAJ**
trnI	+7	+14	+4	*trnN*	-4	−4	−3
trnQ	+8	+9	+7	*trnS1*	0	0	−1
trnM	+5	+5	+5	*trnE*	-4	−4	−4
nad2	-3	−3	−3	*trnF*	-1	−1	−1
trnW	-8	-9	−10	*nad5*	0	0	0
trnC	+3	+3	+1	*trnH*	-2	−2	−2
trnY	-8	−8	−8	*nad4*	-7	−7	−7
cox1	+1	+1	+2	*nad4l*	+4	+4	+4
trnL2	+1	0	+1	*trnT*	-2	−2	−2
cox2	-20	−20	−20	*trnP*	+1	+1	+1
trnK	-3	−3	−1	*nad6*	-1	-1	-1
trnD	-2	−2	0	*cytb*	-2	−1	−2
atp8	-7	−7	−7	*trnS2*	+21	+21	+24
atp6	+2	+3	−1	*nad1*	0	0	0
cox3	+11	+2	0	*trnL1*	0	0	0
trnG	-4	−4	−4	*rrnL*	0	0	0
nad3	+5	+4	+13	*trnV*	0	0	0
trnA	-1	−1	+8	*rrnS*	0	0	0
trnR	+17	+17	+159	*A + T*	0	0	0

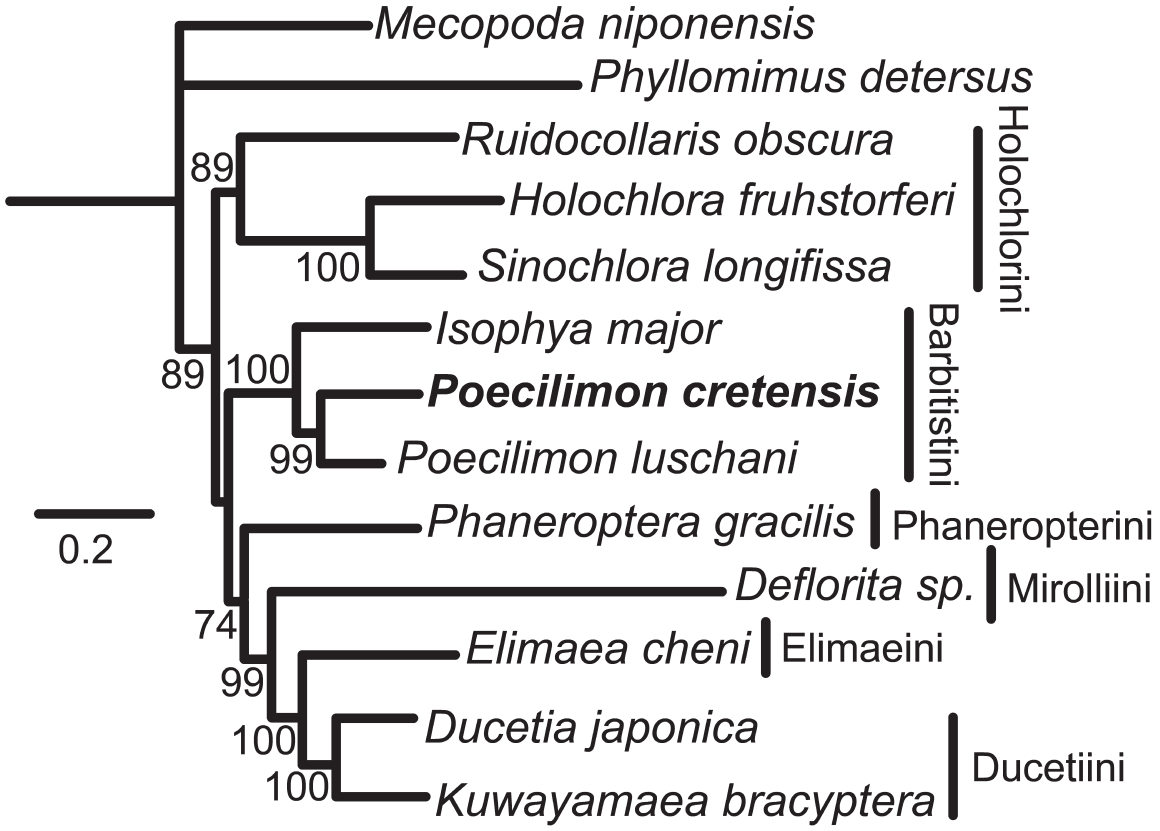

FIGURE 2. Phylogenetic tree inferred by maximum likelihood using W-IQ-Tree from 13 Phaneropterinae mitogenomes representing different tribes (number along the nodes indicate bootstrap support).

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Author contributions

The author contributions were as follows: **M. Y. Karakaş,** analysis; **Ö. Yahyaoğlu**, analysis, figure preparation and interpretation; **O. Uluar**, analysis and interpretation; **M. Budak**, analysis and interpretation; **B. Çıplak**, conception, design, analysis, interpretation and writing. All authors read and contributed to the manuscript.

Disclosure statement

The authors declare no potential conflict of interest.

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