Complete mitochondrial genome of *Prismognathus prossi* (Coleoptera: Lucanidae) with phylogenetic implications

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The complete mitochondrial genome of a Chinese stag beetle, *Prismognathus prossi*, was generated using the Illumina next-generation sequencing. The mitogenome sequence is 15,984 bp in length, the nucleotide composition is A 36.6%, C 17.5%, T 34.3% and G 11.6% with the AT-content of 70.9%. The sequence has similar features with other reported insect mitogenomes, consisting of 13 protein-coding genes (PCGs), 22 transfer RNA genes, two ribosomal RNAs and a control region. All of the protein-coding genes start with the typical ATN initiation codon except for *COI*. Maximum Likelihood (ML) and Bayesian Inference (BI) indicated that *P. prossi* share an affinity with *Lucanus mazama*, *Lucanus fortunei* and *Cyclommatus vitalisi*.

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1. Introduction

Insect mitogenome has been one of the most useful molecular markers and it has been widely applied to infer systematics at many taxonomic levels (Cameron 2014, Breeschoten *et al.* 2016, Timmermans *et al.* 2016, Li *et al.* 2017, Liu *et al.* 2017, López-López & Vogler 2017). The number of sequenced mitochondrial genomes in Coleoptera are far from enough to clarify the biodiversity and complex evolutionary pathways between them (Gillett *et al.* 2014, Crampton-Platt *et al.* 2015, Timmermans *et al.* 2016, Yuan *et al.* 2016). In particular, complete mitogenomic data of Lucanidae is available for only few species despite their great evolutionary and systematic interest. So far, just 18 lucanid mitogenomes including seven complete ones have been reported (Sheffield *et al.* 2009, Kim *et al.* 2013, Wu *et al.* 2016, Lin *et al.* 2017, Liu *et al.* 2017).

In this study, we sequenced the complete mitogenome of the lucanid *Prismognathus prossi* Bartolozzi & Wan, 2006 for the phylogenetic studies. This species is easily recognized in the field by its impressive mandibles and canthus in males (Fig. 1). It is distributed in the southern China with limited records in provinces of Sichuan, Guizhou and Guangxi (Bartolozzi & Wan 2006, Wan & Yang 2007, Fujita 2010, Huang & Chen 2017). Like many other stag beetles, *P. prossi* has also confronted habitat loss or fragmentation, global climate change and overhunting by enthusiasts and specimen merchants. The mitogenomic data is not indispensable only for understanding the systematics of Lucanidae, but it could also provide the basic genetics infor-

Table 1. Primers and their sequences used in this study for *Prismognathus prossi*.

Gene	Primer name	Sequence (5'-3')	Reference
COI	COI-F1 COI-R1	CAACATTTATTTTGATTTTTTGG TCCAATGCACTAATCTGCCATATTA	Simon *et al.* 1994
Cytb	Cytb-F2 Cytb-R2	GAGGAGCAACTGTAATTACTAA AAAAGAAARTATCATTCAGGTTGAAT	Balke *et al.* 2004
rRNA-L	16SF1 16SR1	CCGGTTTGAACTCAGATCATG TAATTTATTGTACCTTGTGTATCAG	Hosoya *et al.* 2001

mation for follow-up studies, and for formulating plans for assessing the conservation status of *P. prossi* throughout its range.

2. Materials and methods

2.1. Sample collection and DNA extraction

The voucher specimen of *P. prossi* was collected from Mt. Fanjingshan, Guizhou Province in August, 2014 by Yunfei Wu. It was deposited in the Museum of Anhui University. Total genomic DNA was extracted from the muscular tissue of *P. prossi* using the Qiagen DNAeasy Kit (Qiagen, Düsseldorf, Germany).

2.2. Primer design, polymerase chain reaction, amplification and sequencing

The complete mitogenome of *P. prossi* was assembled from amplified fragments with the primers listed in Table 1. The PCR amplifications were carried out in the volume of 25 μL containing 1 μL in 10 μM of each primer (forward and reverse), 2 μL template DNA, 12.5 μL 2X EasyTaq SuperMix (+dye), and 8.5 μL sterile double-distilled water. The PCR was performed under the following conditions: an initial denaturation at 94 °C for 2 min, followed by 35–37 cycles of denaturation at 94 °C for 40 s, annealing at 52–58 °C for 50 s, and elongation at 70 °C for 1 min, and then a final extension step at 72 °C for 7 min. The annealing temperature was determined by the length of the fragment. The sequencing was conducted with the Illumina HiSeq 2000 platform (BGI Genomics, Shenzhen, China).

The cluster strands created by the bridge amplification were primed and all four fluorescently labeled and 3-OH-blocked nucleotides were added to the flow cell with DNA polymerase. The cluster strands were extended in single nucleotides. Following the incorporation step, the unused nucleotides and DNA polymerase molecules were washed away, a scan buffer added to the flow cell, then the optics system scanned each lane of the flow cell in imaging units (tiles). Once imaging was completed, chemicals that affect cleavage of the fluorescent labels and the 3-OH blocking groups were added to the flow cell, which prepared the cluster strands for another round of fluorescent nucleotide incorporation.

2.3. Mitogenome assembly, annotation and analysis

The mitogenome of *P. prossi* was assembled using SOAP denovo (BGI Company, Shenzhen, China) and preliminary annotations were made with the MITOS WebServer (http://mitos.bioinf.uni-leipzig.de/index.py). Secondary structure of tRNA genes was inferred using tRNAscan-SE 2.0 (http://lowelab.ucsc.edu/tRNAscan-SE/). Those that were not identified by tRNAscanoSE, as well as 16S ribosomal RNA *(rRNA-L*) and 12S ribosomal RNA (*rRNA-S*), were determined according to sequence similarity with related species.

The protein-coding genes (PCGs) were determined by ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) under the invertebrate mitochondrial genetic code. The values of nucleotide composition, codon usage, and relative synonymous codon usage (RSCU) of PCGs were calculated with MEGA version 6.05 (Tamura *et al.* 2013). PCGs were translated with DNAMAN

Table 2. Mitogenome organization of *Prismognathus prossi*.

Gene	Strand	Region	Length (bp)	Start codon	Stop codon	Anti-codon	Intergenic nucleotides (bp)
trnI	J	1~64	64	–	–	GAT	–3
trnQ	N	62~130	69	–	–	TTG	3
trnM	J	73~202	69	–	–	CAT	0
nad2	J	203~1216	1,014	ATC	TAA	–	2
trnW	J	1022~1083	65	–	–	TCA	–8
trnC	J	1276~1335	60	–	–	GCA	0
trnY	N	1336~1398	63	–	–	GTA	1
cox1	J	1400~2935	1,536	AAT	TAA	–	–5
trnL(CUN)	J	2931~2995	65	–	–	TAA	0
cox2	J	2996~3683	688	ATA	T	–	0
trnK	J	3684~3753	70	–	–	CTT	0
trnD	J	3754~3816	63	–	–	GTC	0
atp8	J	3817~3972	156	ATT	TAA	–	–4
atp6	J	3975~4635	661	ATT	T	–	0
cox3	J	4657~5419	784	ATG	T	–	0
trnG	J	5420~ 5481	62	–	–	TCC	–3
nad3	J	5479~5835	357	ATA	TAG	–	–2
trnA	J	5834~5898	65	–	–	TGC	–1
trnR	J	5898~5961	64	–	–	TCG	–1
trnN	J	5961~6025	65	–	–	GTT	–1
trnS(*AGN*)	J	6026~6092	67	–	–	TCT	0
trnE	J	6093~6155	63	–	–	TTC	–2
trnF	N	6154~6216	63	–	–	GAA	–3
nad5	N	6214~7930	1,717	ATT	T		0
trnH	N	7931~ 7993	63	–	–	GTG	–4
nad4	N	7990~9329	1,340	ATG	T	–	–7
nad4l	N	9323~9610	298	ATG	TAA	–	2
trnT	J	9613~9675	63	–	–	TGT	3
trnP	N	9679~9740	62	–	–	TGG	4
nad6	J	9745–10227	483	ATG	TAA	–	–1
cob	J	10227~11369	1,143	ATG	TAG	–	2
trnS(*UCN*)	J	11368~11432	65	–		TGA	19
nad1	N	11452~12405	954	ATA	TAG	–	–3
trnL(*CUN*)	N	12403~12465	63	–	–	TAG	0
rrnL	N	12466~13726	1,261	–	–	–	1
trnV	N	13728~13796	69	–	–	TAC	–1
rrnS	N	13796~14542	747	–	–	–	0
Control region	–	14543~15984	1,442	–	–	–	0

v7.0.2.176 (Lynnon Biosoft, Vaudreuil-Dorion, Canada). The mitogenome was mapped with CGView (Grant & Stothard 2008). Composition skew analysis was conducted according to formulas AT skew = [A – T] / [A + T] and GC skew = [G – C] / [G + C] (Perna & Kocher 1995).

2.4. Phylogenetic analyses

Phylogenetic analyses, based on 12 of the 13 protein-coding genes (for *Aegus angustus*, *nad2* was not available), included the newly sequenced *P. prossi* and 18 other stag beetles from Genbank (Table 2) as well as three scarabid outgoup species. The model of nucleotide substitution was selected according to the Akaike Information Criterion (AIC) with jModelTest v2.1.4 (Posada 2008). Phylogenetic trees based on GTR + I + G model were generated via ML analysis using RAxML (Stamatakis 2014) and Bayesian inference (BI) with MrBayes v3.2.5 (Huelsenbeck & Ronquist 2003). Node supports in the ML tree

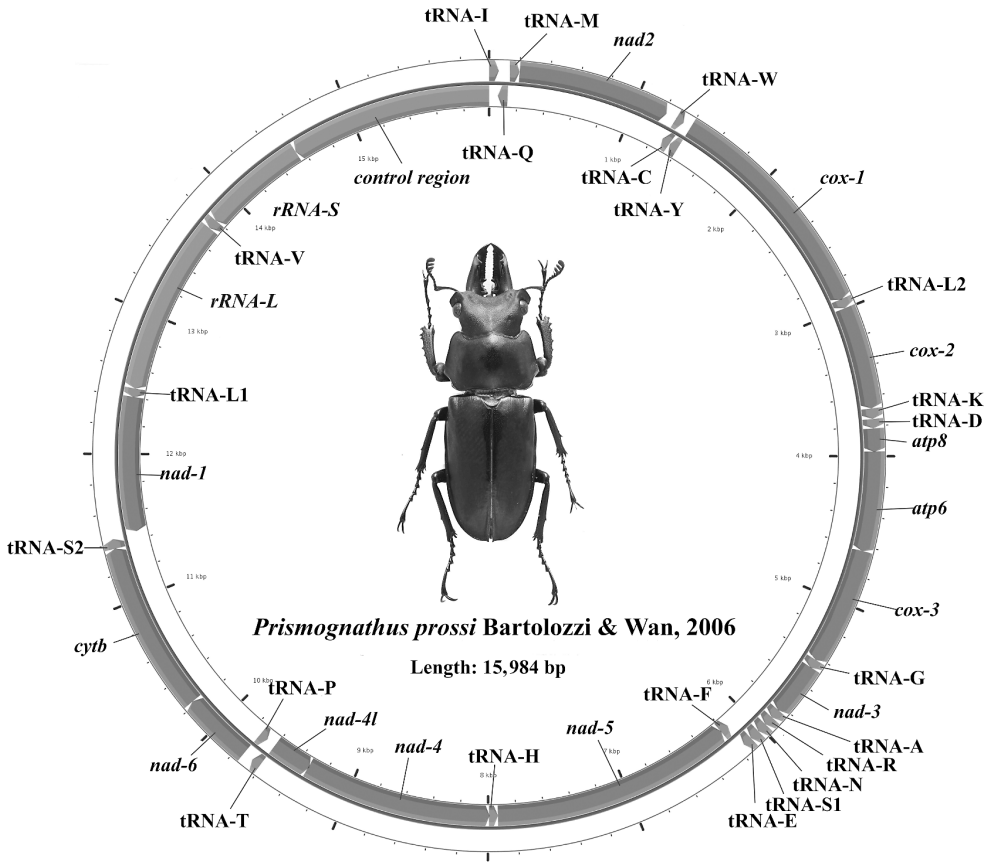

Fig. 1. Mitochondrial genome map of *Prismognathus prossi*. Abbreviations for the genes: *cox1–3*, cytochrome C oxidase subunits 1 to 3; *cytb*, cytochrome B; *nad1–6*, NADH dehydrogenase subunits 1 to 6; *atp6* and *atp8*, subunits 6 and 8 of ATPase; *rRNA-L* and *rRNA-S*, ribosomal ribonucleic acid 16S and 12S; tRNA A–Y, transfer ribonucleic acid A to Y.

were estimated through bootstrap analysis with 1,000 replications. The BI was conducted with two simultaneous Markov chain Monte Carlo runs of 2 million generations, sampled every 1,000 steps, and with the first 25% discarded as burn-in.

ML and BI phylogenies were reconstructed using a supermatrix of the 12 PCGs; only 12 of the 13 PCGs were used, because the *nad2* gene of *Aegus angustus* was not available. Values of BI ≥ 0.95 and 0.75 ≤ BI < 0.95 were considered as strong and weak support, respectively. Correspondingly, values of ML ≥ 75% were considered as strong support, values of 50% ≤ ML < 75% as moderate support and ML < 50% as weak support (Kim *et al.* 2015).

Phylogenetic trees were viewed and edited in Figtree v1.4.3 (Rambaut 2016).

3. Results

3.1. Structure of mitogenome sequence of *Prismognathus prossi*

The complete mitogenome of *P. prossi* (Fig. 1, Table 3, Genbank number MF614014) is 15,984 bp in length. It consists of 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (*rRNA-L* and *rRNA-S*), 13 protein-coding genes (PCGs) and a control region. Four PCGs, two rRNAs and seven tRNAs are located on the N-strand; the other nine PCGs and 15 tRNAs are on the J-strand.

The nucleotide composition of the mitogenome of *P. prossi* is 36.6% A, 11.6% G, 17.6% C and 34.3% T with an AT content of 70.85%. The sequence has both positive AT-skew (0.076)

Table 3. Information about sequences of 22 beetle species of this study.

Family / Tribe	Species	Genbank accession no.
Scarabaeidae		
Meolonthini	*Rhopaea magnicornis* Blackburn	FJ859903
Cetoniini	*Protaetia brevitarsis* (Lewis)	KC775706
Euchirini	*Cheirotonus jansoni* (Jordan)	KC428100
Lucanidae		
Aegini	*Aegus angustus* Bomans	JX313668
Aegini	*Odontolabis fallaciosa* Boileau	MF908524
Aegini	*Neolucanus perarmatus* Didier	MF401425
Lucanini	*Prismognathus prossi* Bartolozzi et Wan	MF614014
Lucanini	*Cyclommatus vitalisi* Pouillaude	MF037205
Lucanini	*Lucanus mazama* (LeConte)	FJ613419
Lucanini	*Lucanus fortunei* Saunders	MF614013
Dorcini	*Prosopocoilus gracilis* (Saunders)	KP735805
Dorcini	*Pseudorhaetus sinicus* (Boileau)	KP987575
Dorcini	*Dorcus curvidens hopei* Nomura	MF612067
Dorcini	*Dorcus parallelipipedus* (Linnaeus)	JX412841
Dorcini	*Rhaetus westwoodi* (Parry)	MG159815
Dorcini	*Macrodorcas seguyi* DeLisle	MF612068
Dorcini	*Hemisodorcus rubrofemoratus* (Snellen van Vollenhoven)	JX313682
Dorcini	*Hexarthrius vitalisi* Didier	JX313676
Dorcini	*Prosopocoilus confucius* (Hope)	KU552119
Dorcini	*Prosopocoilus blanchardi* (Parry)	KF364622
Dorcini	*Serrognathus platymelus* (Saunders)	MF612070
Sinodendrini	*Sinodendron yunnanense* Král	KP735804

and GC-skew (0.884). The start codon of *cox1* is AAT whereas the other 12 PCGs are ATN codons. Eight PCGs are stopped with TAA or TAG codons whereas the incomplete stop codons of T are involved in the remaining five genes. The length of all 22 tRNAs are ranging from 61 to 71 bp, The length of the control region of *P. prossi* is 1,442 bp with AT content of 69.56%. It is located between *rRNA-S* and *tRNA-Q* (Fig. 1).

3.2. Phylogenetic analysis

As can be seen from Fig. 2, both methods yielded a similar topology indicating that *P. prossi* has an affinity with *Lucanus fortunei* and *L. mazama* (0.75 BPP / 55% MLB), and these three species share a common ancestor with *Cyclommatus vitalisi* (1 BPP / 93% MLB).

4. Discussion

The mitogenome of *P. prossi* has the same structure as other stag beetles (Sheffield *et al.* 2009, Kim *et al.* 2013, Wu *et al.* 2016, Lin *et al.* 2017, Liu *et al.* 2017). The five genes that have incomplete stop codons of T in PCGs are generally found in insects and other invertebrates (Masta & Boore 2004, Wu *et al.* 2014, Cheng *et al.* 2016). The single *tRNA-S* lacked the cloverleaf secondary structure owing to the structure of the dihydrouridine (DHU) arm, which is also in accordance with previous studies (Cameron 2014).

The phylogenetic analyses suggest that *P. prossi* is related to *L. mazama* and *L. fortunei* and this triplet has a common ancestor with *C. vitalisi*. These phylogenetic analyses are consistent with the studies inferred from multi-genes including COI, 16S rDNA, Cytb, ITS2 and EF1-a (Kim & Farrell 2015, Wu & Wan 2016). Due to the inadequate taxa sampling, however, this study cannot explore the affinity of *P. prossi* with other taxa conclusively, and thus information about more taxa are required in order to establish the definite phylogenetic relationships of Lucanidae.

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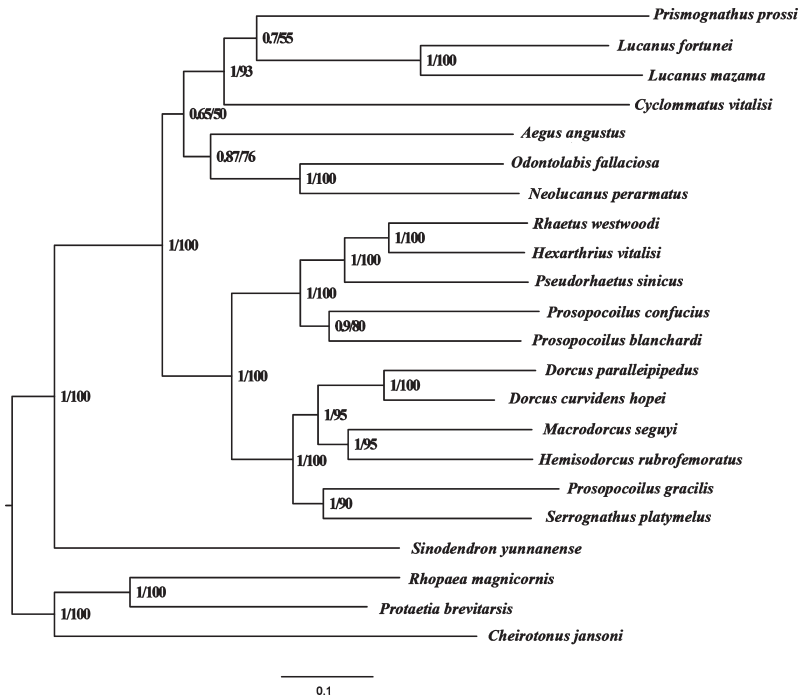

Fig. 2. Topology based on Maximum likelihood inference (ML, bootstrap values to right of each slash) based on 12 of the 13 protein-coding genes (for *Aegus angustus*, *nad2* was not available) of *Prismognathus prossi* and 18 other lucanids with three scarab species (*Rhopaea magnicornis*, *Protaetia brevitarsis* and *Cheirotonus jansoni*) as outgroups. Bayesian inference (BI, posterior probabilities to left of each slash) produced a similar topology. The line with 0.1 is a scale for scaled branches and shows the evolutionary distance of each species.

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