

## ORIGINAL ARTICLE

# The complete mitochondrial genome of *Odontolabis fallaciosa* (Coleoptera: Lucanidae) with its phylogenetic implications

Qian Wang, Jing Liu, Ziqi Lin, Xia Wan\*

Department of Ecology, School of Resources and Engineering, Anhui University, Hefei 230601, P. R. China

\*Corresponding author, E-mail: wanxia@ahu.edu.cn

**Abstract** In this study, the complete mitochondrial genome of *Odontolabis fallaciosa* was sequenced for the first time using the next generation sequencing method. Like most reported mitogenomes of scarab beetles, it was a double-stranded circular molecule, consisting of 13 protein-coding genes (PCGs), 22 transfer RNA genes, 2 ribosomal RNA genes, and a control region. The mitogenome of *O. fallaciosa* was 19,614 bp in length. The base nucleotide composition in the mitogenome of *O. fallaciosa* was: A (40.0%), T (30.5%), C (18.5%) and G (11.0%), with the total AT content of 70.5%. The phylogenetic analysis of twelve species of stag beetles and other three scarab beetles showed that *O. fallaciosa* and *Aegus angustus* share a common ancestor. The mitogenomic data could provide a reliable reference for the future study on conservation, phylogeny and even evolution of these species.

**Key words** Mitogenome, stag beetle, the next generation sequencing, phylogenetic analysis.

## 1 Introduction

*Odontolabis fallaciosa* Boileau, 1901 is a well-recognized and popular stag beetle due to its large size, vivid body colour and amazing mandibles' variation, and is often thought to be typical 'Beetle Warrior' in the enthusiasts' vision. Currently, this species has been excessively collected in the interest of private collections or pets, which has seriously affected the amount of wild populations. Mitochondrial genome, as one of useful genetic tools, has been applied to estimate genetic diversity, population size, gene flow and also has been ultimately incorporated into the conservation and phylogeny (Timmermans & Vogler, 2012; Cameron, 2014; Li *et al.*, 2015; Song *et al.*, 2016). It is necessary for us to obtain the genetic data of *O. fallaciosa* for the future conservational and phylogenetic work.

In this study, we firstly sequenced the mitogenome of *O. fallaciosa* and reconstructed the phylogenetic relationships within other stag beetles, which provided a reliable data for the conservation of this species and subsequent further phylogenetic studies.

## 2 Material and methods

### 2.1 Sample collection and DNA extraction

The specimen of *O. fallaciosa* was collected from Jinxiu, Guangxi, China by Wen Jin in July 2014, the sequence was submitted to GenBank and assigned accession number MF908524. Total genomic DNA was extracted from head muscle

urn:lsid:zoobank.org:pub:0EFD0829-F424-45C4-8796-711ABB11BF51

Received 1 December 2017, accepted 16 April 2018

Executive editor: Fuqiang Chen

using the QIAquick Tissue Kit (Qiagen, Germany) according to the manufacturer's protocol.

## 2.2 PCR amplification and sequencing

The complete mitogenome was assembled from amplified fragments. The PCR amplification was performed in a 25 µL reaction that containing 1 µL DNA, 2 µL primers, 10 µL sterile double-distilled water (ddH<sub>2</sub>O), and 12.5 µL 2×EasyTaq SuperMix (+dye). All primers used for amplification are listed in Table 1. The PCR amplifications were performed under the following conditions: a denaturation step at 94°C for 2 min, followed by 34–37 cycles at 94°C for 40s, 52–55°C for 40–50s, and 70–72°C for 50–60s, and final extension at 72°C for 7 min. Sequencing was conducted with the Illumina HiSeq 2000 platform. Cluster strands created by bridge amplification were primed and all four fluorescently labelled, and 3-OH blocked nucleotides were added to the flow cell with DNA polymerase. The cluster strands were extended in single nucleotides. The insert size of the library is 450 bp. 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 effect cleavage of the fluorescent labels and the 3-OH blocking groups were added to the flow cell, which prepares the cluster strands for another round of fluorescent nucleotide incorporation.

**Table 1. Details on primers used in this study.**

Gene	Primer name	Sequence(5'–3')	Reference
<i>cox1</i>	COI-F1	CAACATTTATTTTGATTTTTTGG	Simon <i>et al.</i> , 1994
	COI-R1	TCCAATGCACTAATCTGCCATATTA	Simon <i>et al.</i> , 1994
<i>cytb</i>	Cytb-F2	GAGGAGCAACTGTAATTACTAA	Balke <i>et al.</i> , 2004
	Cytb-R2	AAAAGAAARTATCATTGAGGTTGAAT	Balke <i>et al.</i> , 2004
<i>rrnL</i>	16S-F1	CCGGTTTGAAGTCAGATCATG	Hosoya <i>et al.</i> , 2001
	16S-R1	TAATTTATTGTACCTTGTGTATCAG	Hosoya <i>et al.</i> , 2001

## 2.3 Assembly, annotation and analysis

The complete mitogenome was assembled using SOAP denovo (BGI Company, China), then annotation was performed by MITOS. Thirteen PCGs and two rRNA genes were determined by comparing with related complete mitogenome of stag beetles species using the CLUSTAL W programs. The PCGs were confirmed by the ORF finder (<http://www.ncbi.nlm.nih.gov/orf/gorf.html>). tRNAscan-SE (<http://lowelab.ucsc.edu/tRNAscan-SE/>) was used to identify transfer RNA (tRNA) genes and their secondary structures. Unidentified tRNAs were compared with sequences from other species. The nucleotide compositions of different regions were analyzed by using MEGA 6 (Tamura *et al.*, 2013). The skews of the compositions were determined using the formulas: AT skew = (A – T)/(A + T); GC skew = (G – C)/(G + C) (Junqueira *et al.*, 2004).

## 2.4 Phylogenetic analyses

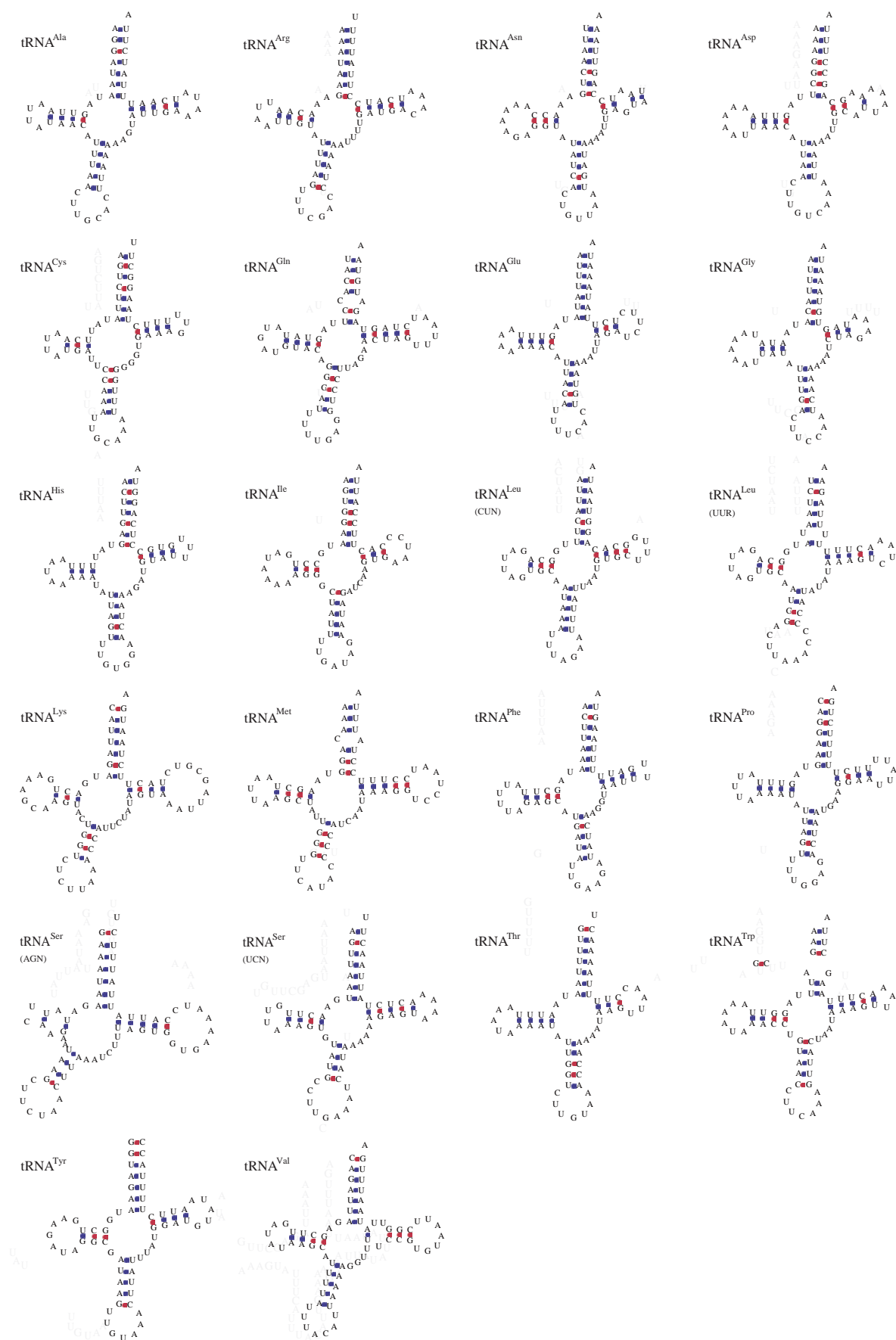
Phylogenetic analysis including the newly sequenced *O. fallaciosa*, eleven other stag beetles (ingroup) and three scarab species (outgroup) retrieved from GenBank was performed using a concatenated dataset of the 12 PCGs except for *ND2* because this PCG was failing to obtained in *Aegus angustus*. Phylogenetic tree was generated from BI analysis with MrBayes 3.2.5 (Huelsenbeck *et al.*, 2001), under the GTR + I + G model. The BI was conducted with two simultaneous Markov chain Monte Carlo (MCMC) runs of 2 million generations, sampled every 1,000 steps, with the first 25% discarded as burn-in. Phylogenetic tree was viewed and edited in Figtree v 1.4.3 (Rambaut, 2016).

# 3 Results and discussion

## 3.1 Genome organization and base composition

The complete mitogenome of *O. fallaciosa* was 19,614 bp in length, as a closed circle (Fig. 1). It was composed of 13 PCGs (*cox1-3*, *ND1-6*, *atp6*, *atp8*, *ND4L*, and *Cytb*), 22 tRNA genes, 2 rRNA genes (*rrnS* (12S) and *rrnL* (16S)) and one control region as other typical stag beetles (Sheffield *et al.*, 2009; Kim *et al.*, 2013; Du *et al.*, 2016; Wu *et al.*, 2016; Yang *et al.*



Figure 2. The secondary structure of the 22 transfer RNAs (tRNAs) in the *O. fallaciosus* mt genome.

with TAA or TAG, with the remains sharing single T and TA as termination codons (Table 3). The relative synonymous codon usage (RSCU) of the third position showed that the frequency of AU codons in two and fourfold degeneracy was greater than GC (Fig. 3).

**Table 3. Mitogenome organization of *O. fallaciosa*.**

Gene	Strand	Region	Length (bp)	Start codon	Stop codon	Anticodon	Intergenic nucleotides (bp)
<i>trnI</i>	J	1–64	64	–	–	GAT	–3
<i>trnQ</i>	N	62–129	68	–	–	TTG	–1
<i>trnM</i>	J	129–197	69	–	–	CAT	0
<i>nad2</i>	J	198–1205	1008	ATC	TAG	–	2
<i>trnW</i>	J	1208–1273	66	–	–	TCA	–8
<i>trnC</i>	N	1266–1327	62	–	–	GCA	0
<i>trnY</i>	N	1328–1391	64	–	–	GTA	1
<i>cox1</i>	J	1393–2928	1536	AAC	TAA	–	–5
<i>trnL(UUR)</i>	J	2924–2988	65	–	–	TAA	0
<i>cox2</i>	J	2989–3679	691	ATT	T	–	–3
<i>trnK</i>	J	3677–3747	71	–	–	CTT	0
<i>trnD</i>	J	3748–3812	65	–	–	GTC	0
<i>atp8</i>	J	3813–3968	156	ATC	TAA	–	–4
<i>atp6</i>	J	3965–4631	667	ATA	T	–	1
<i>cox3</i>	J	4633–5416	784	ATG	T	–	0
<i>trnG</i>	J	5417–5478	62	–	–	TCC	0
<i>nad3</i>	J	5479–5830	352	ATG	T	–	0
<i>trnA</i>	J	5831–5895	65	–	–	TGC	–1
<i>trnR</i>	J	5895–5960	66	–	–	TCG	–1
<i>trnN</i>	J	5960–6022	63	–	–	GTT	0
<i>trnS(AGN)</i>	J	6023–6089	67	–	–	TCT	0
<i>trnE</i>	J	6090–6151	62	–	–	TTC	–2
<i>trnF</i>	N	6150–6212	63	–	–	GAA	–1
<i>nad5</i>	N	6212–7929	1718	ATT	TA	–	0
<i>trnH</i>	N	7930–7992	63	–	–	GTG	–1
<i>nad4</i>	N	7992–9328	1337	ATG	TA	–	–7
<i>nad4L</i>	N	9322–9609	288	ATG	TAA	–	2
<i>trnT</i>	J	9612–9674	63	–	–	TGT	0
<i>trnP</i>	N	9675–9739	65	–	–	TGG	5
<i>nad6</i>	J	9745–10242	498	ATG	TAA	–	–1
<i>cytb</i>	J	10242–11384	1143	ATG	TAG	–	–2
<i>trnS(UCN)</i>	J	11383–11447	65	–	–	TGA	18
<i>nad1</i>	N	11466–12419	954	ATA	TAG	–	–3
<i>trnL(CUN)</i>	N	12417–12479	63	–	–	TAG	–6
<i>rrnL</i>	N	12474–13745	1272	–	–	–	–1
<i>trnV</i>	N	13745–13814	70	–	–	TAC	0
<i>rrnS</i>	N	13815–14562	748	–	–	–	0
<i>Control region</i>	–	14563–19614	5052	–	–	–	0

### 3.3 Features in Control regions

The control region was located between the *trnI* and *rrnS* genes. The length of the control region of *O. fallaciosa* was 5,052 bp, with high AT contents of 66.9%. There was no marked non-coding region, which was similar to that of *Prosopocoilus gracilis*, *Prosopocoilus confucius* and *Sinodendron yunnanense*. Whereas remarkable non-coding regions

were found in the two species *Lucanus mazama* and *Prosopocoilus blanchardi* except the control region (Sheffield *et al.*, 2009; Kim *et al.*, 2015). Besides, there was no gene rearrangement in these two species, which has been only found in the species of *S. yunnanense* until now (Lin *et al.*, 2017).

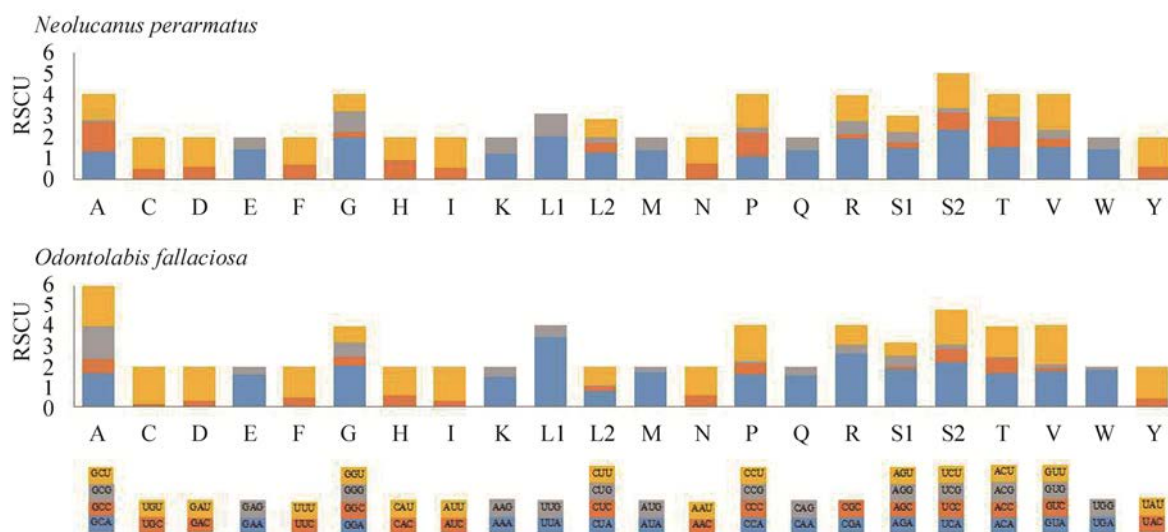


Figure 3. Relative synonymous codon usage (RSCU) in the *O. fallaciosa*. Lower bars give color codes. Y axis gives RSCU.

### 3.4 Phylogenetic implication

The phylogeny relationship was reconstructed using a supermatrix of 12 PCGs of the 12 stag beetles and other three scarab beetles in Figure 4. The topology indicated that *S. yunnanense* was sister to other ingroup members. In consideration of the gene rearrangement only occurring to the species, we supposed that the mitogenome possibly play an important role during the evolution of *S. yunnanense* so that it could divergent fully from other representatives of Lucanidae. The relationships among other species, such as *P. gracilis*, *P. confucius*, *P. blanchardi* and *L. mazama*, were consistent with our previous conclusion of them (Lin *et al.*, 2017). Interestingly, the newly sequenced species *O. fallaciosa* shared a common ancestor with *A. angustus* in spite of their remarkable difference in morphology. Hosoya & Araya (2005) once reached a similar conclusion based on 16S mtrRNA. Their work showed that the member of *Aegus*, *A. laevicollis subnitidus*, formed one cluster with two taxa of *Neolucanus* that was sister to *Odontolabis* both on the morphological and

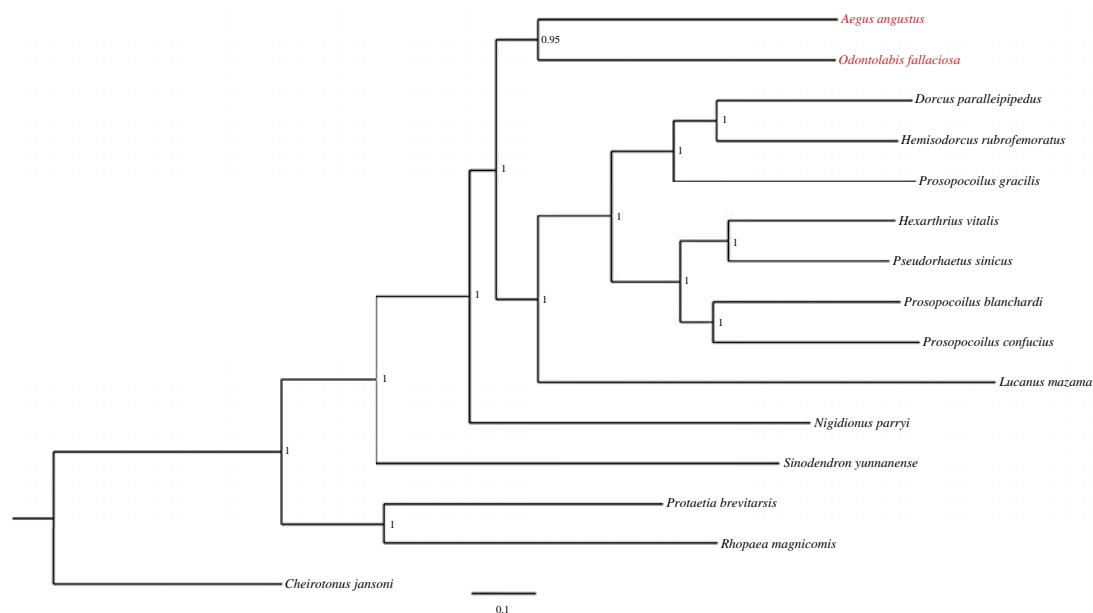


Figure 4. The BI phylogenetic tree of 15 beetles based on 12 PCGs. GTR+I+G was selected as the best model.

molecular studies (Zhang & Wan, 2013, Master dissertation). The previously morphological studies considered that the genus *Aegus* should have close phylogenetic affinity of the genus *Dorcus* (Benesh, 1960; Maes, 1992; Krajcik, 2001; Bartolozzi & Sprecher-U., 2006). However, the ecological habits of larvae (Araya, 1994) and almost symmetrical, finger-shaped spermatheca of female genitalia among of the members in *Aegus*, *Odontolabis* and *Neolucanus* from Asian region (Wan & Yang, 2007, Doctoral dissertation) could imply their more closed associations in comparison to other stag beetles. Therefore, the phylogenomic analyses based on more many samples of the three genera could be very useful to reveal their systematics due to the implication in this study had shed light on their relationships.

**Funding** This study was supported by the National Natural Science Foundation of China (31201745, 31071954, 31572311).

**Acknowledgments** We would like to express our gratitude to Dr. Hu Li and Dr. Fan Song (Department of Entomology, China Agricultural University) for their valuable advices about the data analyses in this study.

## References

- Araya, K. 1994. On the habitat of Lucanid larvae (Coleoptera, Lucanidae) in Southeast Asia. *Nature and Insects*, 29: 2–10.
- Bartolozzi, L., Sprecher-Uebersax, E. 2006. Lucanidae. In: Lobl, I., Smetana, A. (eds.), *Catalogue of Palaearctic Coleoptera, Vol. 3: Scarabaeoidea – Scirtoidea – Dascilloidea – Buprestoidea – Byrrhoidea*. Apollo Books, Vester Skerninge, Denmark. pp. 63–76.
- Balke, M., Ribera, I., Vogler, A.P. 2004. MtDNA phylogeny and biogeography of Copelatinae, a highly diverse group of tropical diving beetles (Dytiscidae). *Molecular Phylogenetics Evolution*, 32: 866–880.
- Benesh, B. 1960. Coleopterorum Catalogus (Supplementum). Editio secunda. Pars 8. Lucanidae. W Junk, The Hague, 178 pp.
- Boileau, H. 1901. Description de Lucanides nouveaux. *Annales de la Société Entomologique de Belgique*, 45: 12–22.
- Cameron, S.L. 2014. Insect mitochondrial genomics: implications for evolution and phylogeny. *Annual Review of Entomology*, 59: 95–117.
- Du, C., He, S., Song, X., Liao, Q., Zhang, X.Y., Yue, B.S. 2016. The complete mitochondrial genome of *Epicauta chinensis* (Coleoptera: Meloidae) and phylogenetic analysis among Coleopteran insects. *Gene*, 578: 274–280.
- Hosoya, T., Honda, M., Araya, K. 2001. Genetic variation of 16S rRNA gene observed in *Ceruchus lignarius* and *Dorcus rectus rectus* (Coleoptera: Lucanidae). *Entomological Science*, 4: 335–344.
- Hosoya, T., Araya, K. 2005. Phylogeny of Japanese stag beetles (Coleoptera: Lucanidae) inferred from 16S mtrRNA gene sequences, with reference to the evolution of sexual dimorphism of mandibles. *Zoological Science*, 22: 1305–1318.
- Huelsenbeck, J.P., Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17: 754–755.
- Junqueira, A.C., Lessinger, A.C., Torres, T.T., Da, S.F., Vettore, A.L., Arruda, P. 2004. The mitochondrial genome of the blowfly *Chrysomya chloropyga* (Diptera: Calliphoridae). *Gene*, 339: 7–15.
- Kim, M.J., Kim, K.G., Kim, S.R., Kim, I. 2013. Complete mitochondrial genome of the two-spotted stag beetle, *Metopodontus blanchardi* (Coleoptera: Lucanidae). *Mitochondrial DNA*, 26: 307–309.
- Kim, S.I., Farrell, B.D. 2015. Phylogeny of world stag beetles (Coleoptera: Lucanidae) reveals a Gondwanan origin of Darwin's stag beetle. *Molecular Phylogenetics Evolution*, 86: 35–48.
- Krajcik, M. 2001. *Lucanidae of the World. Catalogue. Part I. Checklist of the Stag Beetles of the World (Coleoptera: Lucanidae)*. Stampata in proprio, Most, Czech Republic. 108 pp.
- Li, H., Shao, R., Song, N., Song, F., Jiang, P., Li, Z. 2015. Higher-level phylogeny of Paraneopteran insects inferred from mitochondrial genome sequences. *Scientific Reports*, 5: 8527. doi: 10.1038/srep08527.
- Lin, Z.Q., Song, F., Li, T., Wu, Y.Y., Wan, X. 2017. New mitogenomes of two Chinese stag beetles (Coleoptera, Lucanidae) and their implications for systematics. *Journal of Insect Science*, 17: 1–9. doi: 10.1093/jisesa/iex041.
- Maes, J.M. 1992. Lista de los Lucanidae (Coleoptera) del mundo. *Revista Nicaraguense de Entomología*, 22: 1–121.
- Rambaut, A. 2016. FigTree: Tree Figure Drawing Tool, Version 1.4.3. Institute of Evolutionary Biology, University of Edinburgh, Available from <http://tree.bio.ed.ac.uk/>.
- Sheffield, N.C., Song, H., Cameron, S.L., Whiting, M.F. 2008. A comparative analysis of mitochondrial genomes in Coleoptera (Arthropoda: Insecta) and genome descriptions of six new beetles. *Molecular Biology Evolution*, 25: 2499–2509.
- Sheffield, N.C., Song, H., Cameron, S.L., Whiting, M.F. 2009. Nonstationary evolution and compositional heterogeneity in beetle mitochondrial phylogenomics. *Systematic Biology*, 58: 381–394.
- Song, F., Li, H., Jiang, P., Zhou, X.G., Liu, J.P., Sun, C.H., Vogler, A.P., Cai, W.Z. 2016. Capturing the phylogeny of holometabola with mitochondrial genome data and bayesian site-heterogeneous mixture models. *Genome Biology Evolution*, 8: 1411–1426.

- Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, 87: 651–701.
- Tamura, K., Stecher, G., Peterson, D., Filipska, I., Kumars, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology Evolution*, 30: 2725–2729.
- Timmermans, M.J., Vogler, A.P. 2012. Phylogenetically informative rearrangements in mitochondrial genomes of Coleoptera, and monophyly of aquatic elateriform beetles (Dryopoidea). *Molecular Phylogenetics Evolution*, 63: 299–304.
- Wu, Y.Y., Cao, Y.Y., Fang, J., Wan, X. 2016. The first complete mitochondrial genome of stag beetle from China, *Prosopocoilus gracilis* (Coleoptera, Lucanidae). *Mitochondrial DNA*, 27: 1–2.
- Yang, J., Ye, F., Huang, Y. 2016. Mitochondrial genomes of four katydids (Orthoptera: Phaneropteridae): New gene rearrangements and their phylogenetic implications. *Gene*, 575: 702–711.