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MITOGENOME REPORT

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The first complete mitochondrial genome of the genus *Homoderus* and insights into phylogeny of Lucanidae (Coleoptera: Lucanidae)

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ABSTRACT

Homoderus mellyi belongs to the Lucanidae family of Coleoptera. The first complete mitogenome of *Homoderus* is reported in this paper. The genome is 16,807 bp in length and contains the typical 37 genes with 22 transfer RNA genes, 13 protein coding genes, and 2 ribosomal RNA genes. The gene order is conserved across the lineage. The average base composition of the mitogenome is 36.6% for A, 20.8% for C, 11.6% for G, and 31.1% for T. The percentage of GC is 32.3%. The genome organization, nucleotide composition, and codon usage are similar to other beetles. Phylogenetic analysis shows that Lucanidae is monophyletic, and all subfamilies are monophyletic, respectively. The phylogenetic position of *H. mellyi* is consistent with other research.

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Introduction

The family Lucanidae Latreille, 1802, commonly known as stag beetle, contains about 1,700 species all over the world (Schoolmeesters 2021). It attracted lots of enthusiasts and researchers because of the distinctive morphology and behavior (Arrow 1937; Tetsuo and Shinji 1994; Kim and Farrell 2015; Kakizoe et al. 2023). They are characterized by the enormous size and the striking development of male mandibles. It is interesting that two lucanid species were found myrmecophilous (Kakizoe et al. 2023).

Homoderus mellyi Parry 1862 belongs to Lucaninae (Coleoptera: Lucanidae) (Parry 1862), which measures between 45 and 60 mm (Muafor and LeGall, 2012; Muafor et al. 2012). The male *H. mellyi* were uniformly colored, while the female has conspicuous stripes (Arrow 1937). *H. mellyi* has highly developed chewing mouthparts (Audino et al. 2007). The female's head is black. The specimen is distinguished by its four black spots on the pronotum, and the middle black spots are large than the outermost. The elytron are black with a yellow-red longitudinal stripe on each side (Figure 1). Specific permission is not needed as no endangered or protected species were involved.

They were found in many places in Central and West Africa (Nelson 1973; MAEs and Pauly 1998; Audino et al. 2007; Muafor et al. 2012) (Supplementary Figure S2). It is a part of West African forests, and contributes to the overall stability and resilience of the ecosystem. It is a kind of income source in Southwest Cameroon (Muafor et al. 2012). Locally they are relatively common, but their population is threatened from habitat destruction and exploitation of adults for insect trade (Muafor and LeGall 2012; Muafor et al. 2015). If carnitine is fed continuously during the larval stage, *H. mellyi* will become a giant beetle, whereas which were given isosorbide dinitrate (ISDN) will quickly become adults with small body size (Inoue et al. 2004). An X autosome rearrangement was detected in *H. mellyi* and other beetles (Dutrillaux and Dutrillaux 2023). However, there is no one complete mitochondrial genome from *Homoderus* so far.

Materials and methods

The specimen came from shipments mailed to China from Japan (Figure 1) and were collected by China Customs (Beijing). The specimen was female and deposited at Science and Technical Research Center of China Customs (http://www.chinacustoms-strc.cn, Lijie Zhang and zhanglijie8820@163.com) under the voucher number 2111000001008273-7. Permission was obtained from China Customs when specimens were obtained. The genomic DNA was extracted by a DNeasy Blood and Tissue kit (Qiagen, Germany) and deposited in a refrigerator at −20 °C at Institute of Zoology, Chinese Academy of Sciences.

The complete mitogenome of *H. mellyi* was sequenced by the Illumina HiSeq 6000 platform with 400 bp insert size and

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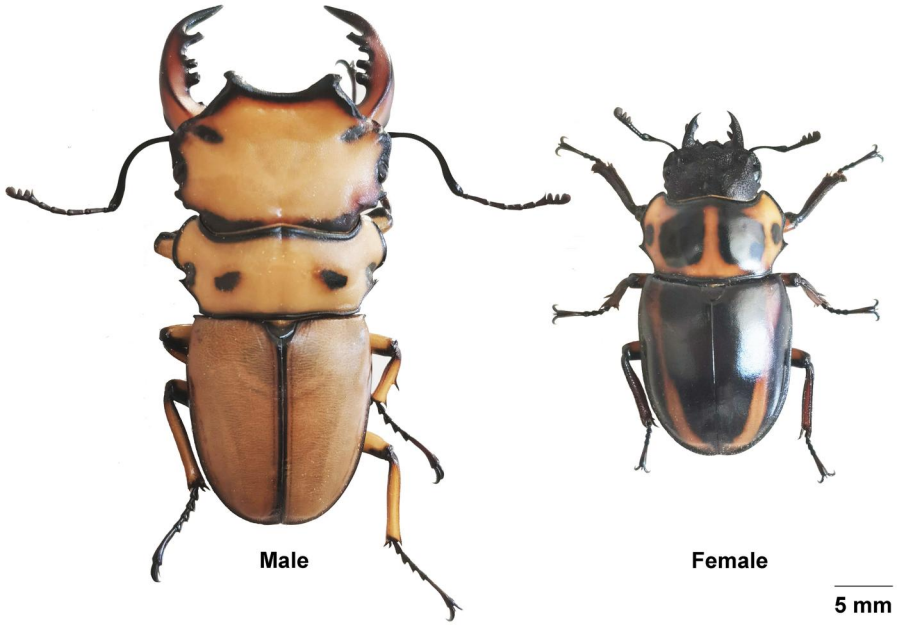

Figure 1. *Homoderus mellyi* Parry 1862 was seized from shipments mailed from Japan to China. The specimen is distinguished by its four black spots on the pronotum, and the Middle black spots are large than the outermost. The elytron are black with a yellow-red longitudinal stripe each side. Photographed by Lijie Zhang.

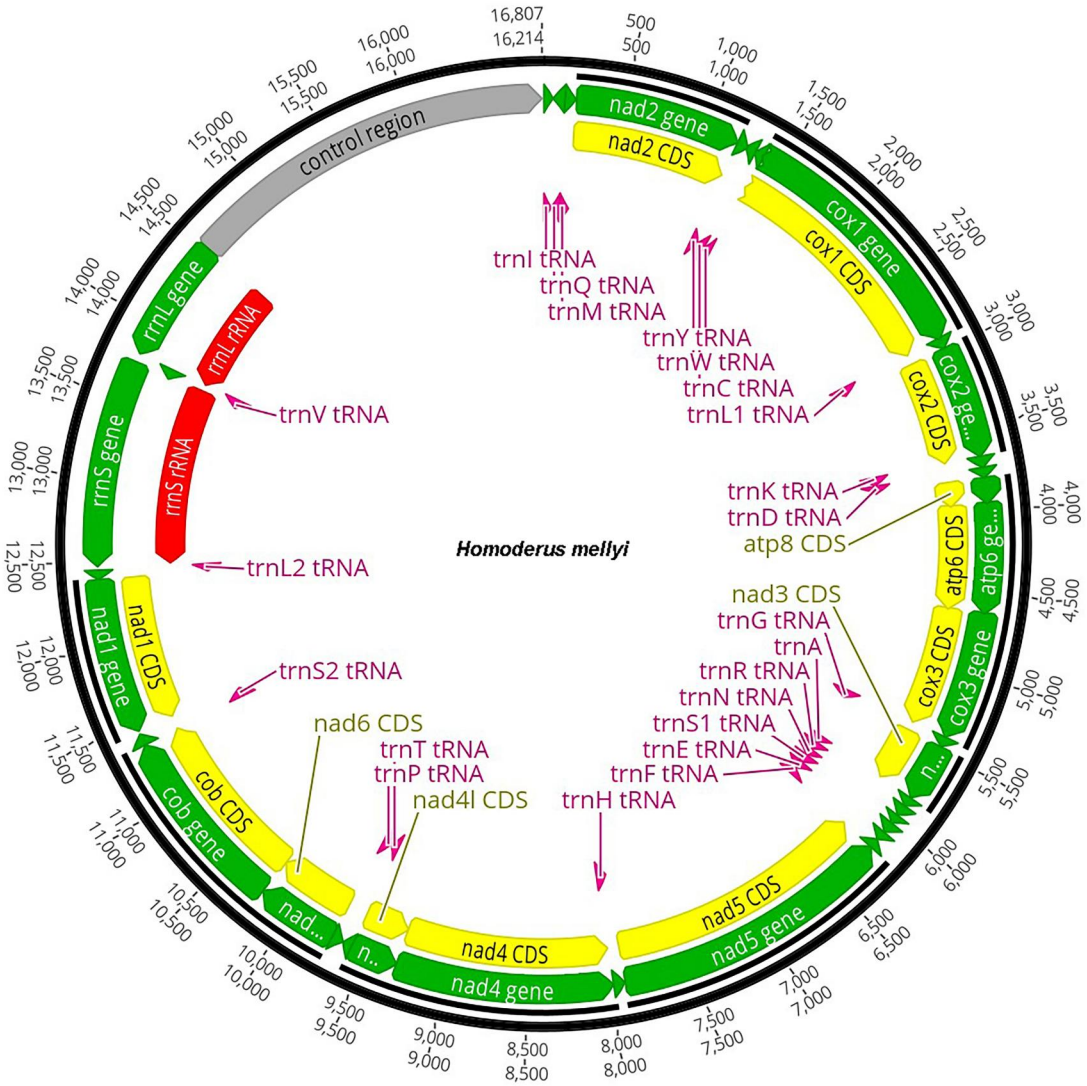

Figure 2. The complete mitochondrial genome map of *Homoderus mellyi* (GenBank: PP085183). Genes encoded on the majority strand (J-strand) are represented on the clockwise side of the circle, while genes encoded on the minority strand (N-strand) are shown on the anticlockwise side.

a pair-end 150 bp sequencing strategy. The sequencing depth and coverage map was drown following the website (https://www.protocols.io/run/generating-sequencing-depth-and-coverage-map-for-o-cswxwffn) (Ni et al. 2023). It is shown in Supplementary Figure S1. The sequence reads were first trimmed with Trimmomatic-0.38. (Bolger et al. 2014); then, the remaining high-quality reads were assembled using Megahit v1.2.9 (Li et al. 2015). The annotation of genes was performed by Geneious 8.0.5 (Kearse et al. 2012) with the reference of OL944342. The mitochondrial genome map was drawn using Geneious 8.0.5 (Kearse et al. 2012) (Figure 2).

Ten Lucanidae mitogenome sequences were downloaded from NCBI for phylogenetic tree reconstruction (https://www.ncbi.nlm.nih.gov/). The phylogenetic tree was constructed based on nucleotide sequences. Three Trogidae genomes were as outgroups. The following sequences were used: JX412734 (Timmermans et al. 2016), MH120283 (Görür and Davut 2019), KP735804 (Lin et al. 2017), OL944342 (Chen et al. 2021), PP085183 (in this study), MK109856, MK937809, MH120284, MH427658, MH120282, MF037205, OR888904, OL944349, OL944350. The last nine sequences were available on NCBI but still unpublished. After aligning each gene sequence with MAFFT v7.505 (Katoh et al. 2002), all alignments were concatenated with PhyloSuite v1.2.2 (Zhang et al. 2019). The 13 PCGs were combined using edge-unlinked branch lengths between partitions, and then the best IQ-TREE model was generated with ModelFinder (Kalyaanamoorthy et al. 2017), and finally the developmental tree reconstruction was performed with IQ-TREE (version 2.0.3) (Minh et al. 2020), with the GTR + F + I + G4 best model generated by ModelFinder. The number of bootstrap is 5000 and replicated 1000 times.

Results

Mitogenome organization

The mitogenome of *H. mellyi* was found to be a double-stranded circular molecule with 16,807 bp in length (GenBank accession number PP085183), containing the entire set of 37 genes usually present in most insect mtDNA (13 PCGs, 22 tRNA genes, and 2 rRNA genes) and a large non-coding region (control region). Twenty-three genes were transcribed on the majority strand (J-strand), whereas the others were oriented on the minority strand (N-strand). The overall organization of the mitogenome of *H. mellyi* is very compact, and numerous overlaps between genes were found (Figure 2). All PCGs except COX1 used ATN as start codon. Five PCGs (ATP6, COX3, ND4L, ND6, CYTB) start with an ATG, 3 PCGs (ND3, ND4, ND1) start with an ATA. Three PCGs (ND2, COX2, ND5) start with an ATT, while ATP8 starts with ATC and COX1 starts with truncated left end with ACC. Nine PCGs terminate with TAN stop codon, as 5 PCGs (ND2, COX1, ATP6, ND4L, ND6) stop with TAA, and 4 PCGs (ATP8, ND3, CYTB, ND1) stop with TAG. Three PCGs (COX3, ND5, ND4) terminate with TA, whereas COX2 terminates with incomplete T stop codons, which is frequently found in the mitogenome of beetles (Lee et al. 2020).

Phylogenetic analysis

To assess the phylogenetic relationships of *H. mellyi*, we selected the complete mitochondrial DNA sequences of ten Lucanidae species and three Trogidae species; *Trox sp.*, *Trogidae sp.* and *Omorgus chinensis* were set as the

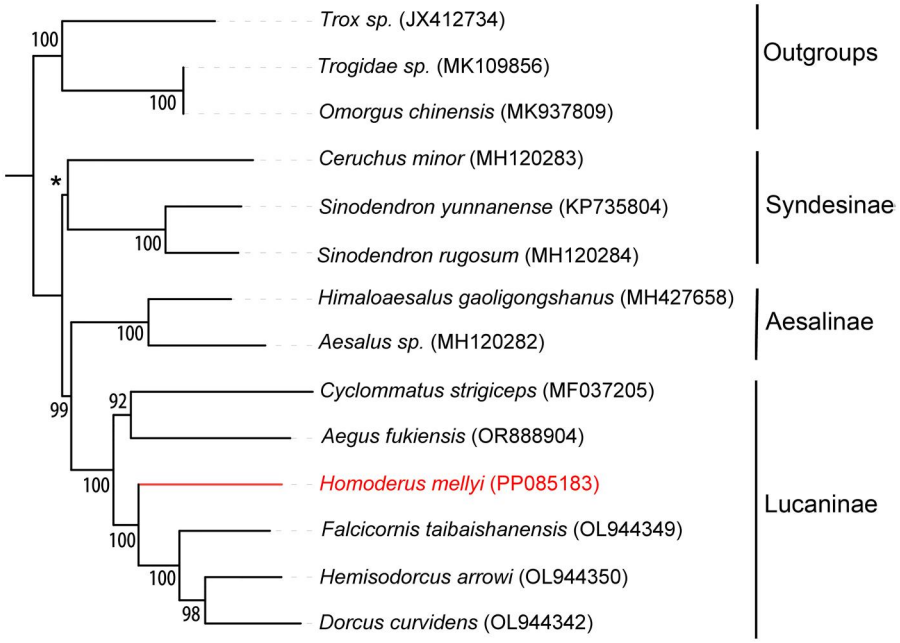

Figure 3. The IQ-tree based on the concatenated 13 PCGs of Lucanidae. Phylogenetic analysis was performed with IQ-TREE (version 2.0.3). The following sequences were used: *Trox sp.* (JX412734, Timmermans et al. 2016), *Trogidae sp.* (MK109856, unpublished), *Omorgus chinensis* (MK937809, unpublished), *Ceruchus minor* (MH120283, Görür and Davut 2019), *Sinodendron yunnanense* (KP735804, Lin et al. 2017), *Sinodendron rugosum* (MH120284, unpublished), *Himaloaesalus gaoligongshanus* (MH427658, unpublished), *Aesalus sp.* (MH120282, unpublished), *Cyclommatus strigiceps* (MF037205, unpublished), *Aegus fukiensis* (OR888904, unpublished), *Homoderus mellyi* (PP085183, this study), *Falcicornis taibaishanensis* (OL944349,unpublished), *Hemisodorcus arrowi* (OL944350, unpublished), *Dorcus curvidens* (OL944342, Chen et al. 2021). The bootstrap value based on 1000 replicated is represented on each node. *Trox sp.*, *Trogidae sp.* and *Omorgus chinensis* were used as outgroups to root the tree.

outgroups. The sequences were obtained from GenBank. *H. mellyi*, had a close relationship with the branches *Falcicornis taibaishannensis*, *Hemisodorcus arrowi* and *Dorcus curvidens*, with high support values. This newly sequenced complete mitochondrial genome provides valuable information for exploring the genetic diversity and phylogenetic relationships of the Lucanidae family.

The IQ-tree based on the 13 PCGs (Figure 3) shows that Lucanidae were recovered as monophyletic. The available subfamily rank of Lucanidae was resolved. This topology supports the monophyly of three subfamilies and the sister group relationships between Aesalinae and Lucaninae. Syndesinae was the basal branch, and was the sister group with other two subfamilies. It is consistent with the analysis using the mitogenomes of 11 representative species from Lucanidae (Lee et al. 2020).

Discussion and conclusion

Approximately 1,700 species have been described in the family Lucaninae (Schoolmeesters 2021). However, only 67 complete mitochondrial genomes have been deposited in the GenBank database so far (June 24, 2024). In this study, next-generation sequencing and assembly revealed that the complete mitogenome of *H. mellyi* is 16,807 bp in length, and the structural features of the mitochondrial genome and its phylogenetic position within the Lucanidae were described. The gene order and composition are identical to those of typical mitogenomes of other stag beetle (Wang et al. 2019; Zhai et al. 2020; Zhao et al. 2021; Choi et al. 2022). The iqtree based on the mitochondrial genome protein coding genes of *H. mellyi* and 10 other species supported the hypothesis that *H. mellyi* belongs to Lucaninae. In conclusion, this study provides important information for future taxonomic, systematic, and genetic studies on Lucanidae. The results will contribute to the inference of the phylogenetic relationships within the family Lucanidae.

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Ethical statement

This specimen is not an endangered or protected species. Therefore, ethical approval is not required in the study.

Author contributions

Lijie Jin drafted the manuscript, analyzed the data and designed the figures. Lijie Zhang collected the sample and photographed the specimen. Qiang Ding did the wet lab work. Lijie Zhang and Ming Bai designed the study, revised the manuscript, and approved the final version for publication. All authors discussed and critically revised the results and contributed to the final version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The genome sequence data supporting the findings of this study are openly available in the NCBI GenBank at https://www.ncbi.nlm.nih.gov/ under the accession number PP085183. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1063003, SRR27476234, and SAMN39326756, respectively.

References

Arrow GJ. 1937. Dimorphism in the males of stag-beetles (Coleoptera, Lucanidae). Transactions of the Royal Entomological Society of London. 86(13):239–245. doi:10.1111/j.1365-2311.1937.tb00244.x.

Audino LD, Nogueira JM, Silva PG, Neske MZ, Ramos AHB, Marcos LPM, Borba FS. 2007. Identificação dos coleópteros (Insecta: coleoptera) das regiões de Palmas (município de Bagé) e Santa Barbinha (município de Caçapava do Sul), RS.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 30(15):2114–2120. doi:10.1093/bioinformatics/btu170.

Chen YJ, Kim S, Wan X. 2021. Mitochondrial genomes of the *Dorcus velutinus* complex (Coleoptera: Lucanidae) with the large intergenic spacer showing unique short sequence repeats and their implications for systematics. J Asia-Pac Entomol. 24(1):493–501. doi:10.1016/j.aspen.2020.08.015.

Choi JH, Kim CJ, Kim MS, Kang JY, Kim IK. 2022. The mitochondrial genome of *Nigidius miwai* Nagel (Coleoptera: lucanidae), a carnivorous lucanid from South Korea. Mitochondrial DNA B Resour. 7(8):1443–1444. doi:10.1080/23802359.2022.2105171.

Dutrillaux B, Dutrillaux AM. 2023. Why are X autosome rearrangements so frequent in beetles? A Study of 50 cases. Genes. 14(1):150. doi:10.3390/genes14010150.

Inoue M, Sato EF, Nishikawa M, Hiramoto K, Kashiwagi A, Utsumi K. 2004. Free radical theory of apoptosis and metamorphosis. Redox Rep. 9(5):237–247. doi:10.1179/135100004225006010.

Görür V, Davut SA. 2019. Fungicide suspensions combined with hot-water treatments affect endogenous *Neofusicoccum parvum* infections and endophytic fungi in dormant grapevine canes. Phytopathol Mediterranea. 58(3):559–572.

Kakizoe S, Kakinuma S, Hoshino K, Hashim R, Abdullah NA, Maruyama M. 2023. Myrmecophily of the enigmatic stag beetle *Torynognathus chrysomelinus* (Coleoptera: Lucanidae: Lucaninae) with remarks on adult morphology, immature stages, and systematic position. Entomol Sci. 26(1): e12539. doi:10.1111/ens.12539.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 14(6):587–589. doi:10.1038/nmeth.4285.

Katoh K, Misawa K, Kuma K-i, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30(14):3059–3066., doi:10.1093/nar/gkf436.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious Basic:

an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28(12): 1647–1649. doi:10.1093/bioinformatics/bts199.

Kim SI, Farrell BD. 2015. Phylogeny of world stag beetles (Coleoptera: Lucanidae) reveals a Gondwanan origin of Darwin's stag beetle. Mol Phylogenet Evol. 86:35–48. doi:10.1016/j.ympev.2015.02.015.

Lee J, Park J, Xi H, Park J. 2020. Comprehensive analyses of the complete mitochondrial genome of *Figulus binodulus* (Coleoptera: Lucanidae). Journal of Insect Sciences. 20(5): 10. doi:10.1093/jisesa/ieaa090.

Li D, Liu CM, Luo R, Sadakane K, Lam TW. 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics. 31(10):1674–1676. doi:10.1093/bioinformatics/btv033.

MAEs JM, Pauly A. 1998. Lucanidae (Coleoptera) du Gabon. Bull Ann Soc Royale Belge d'Entomol. 134:279–285.

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 37(5):1530–1534. doi:10.1093/molbev/msaa015.

Muafor FJ, Gnetegha AA, LeGall P, Levang P. 2015. Exploitation, trade and farming of palm weevil grubs in Cameroon. Center for International Forestry Research (CIFOR). 178.

Muafor FJ, LeGall P. 2012. Commercial beetles of South-West Cameroon. A preliminary study report. Available online: https://horizon.documentation.ird.fr/exl-doc/pleins_textes/divers13-11/010057004.pdf.

Muafor FJ, Levang P, Angwafo TE, Gall PL. 2012. Making a living with forest insects: beetles as an income source in Southwest Cameroon. Int Forest Rev. 14(3):314–325. doi:10.1505/146554812802646693.

Nelson GJSZ. 1973. Comments on Leon Croizat's biogeography. Syst Zool. 22(3):312–320. doi:10.2307/2412312.

Ni Y, Li J, Zhang C, Liu C. 2023. Generating Sequencing Depth and Coverage Map for Organelle Genomes. doi:10.17504/protocols.io.4r3l27jkxg1y/v1.

Lin ZQ, Song F, Li T, Wu YY, Wan X. 2017. New mitogenomes of two Chinese stag beetles (Coleoptera, Lucanidae) and their implications for systematics. J Insect Sci (Tucson). 17(2):63. doi:10.1093/jisesa/iex041.

Parry FJS. 1862. Further descriptions and characters of undescribed Lucanoid Coleoptera. Proceedings of the Entomological Society of London 3:107–113.

Schoolmeesters P. 2021. Scarabs: world Scarabaeidae database.

Tetsuo M, Shinji N. 1994. The lucanid beetles of the world. Japan: Mushisha.

Timmermans MJ, Barton C, Haran J, Ahrens D, Culverwell CL, Ollikainen A, Dodsworth S, Foster PG, Bocak L, Vogler AP. 2016. Family-level sampling of mitochondrial genomes in Coleoptera: compositional heterogeneity and phylogenetics. Genome Biol Evol. 8(1):161–175. doi:10.1093/gbe/evv241.

Wang LJ, Huang JP, Shiao SF, Ko HP, Sung CH. 2019. Characterisation of the complete mitochondrial genome of *Lucanus chengyuani* (Coleoptera: Lucanidae). Mitochondrial DNA B Resour. 4(2):3460–3462. doi:10.1080/23802359.2019.1674703.

Zhai YN, Zhou SJ, Chen YJ, Wan X. 2020. The mitochondrial genome of a rare Chinese stag beetle *Kirchnerius guangxii* (Coleoptera: Lucanidae). Mitochondrial DNA Part B. 5(2):1633–1635. doi:10.1080/23802359.2020.1745708.

Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT. 2019. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Mol Ecol Resour. 20(1):348–355. doi:10.1111/1755-0998.13096.

Zhao Z, Wang J, Wu L, Bai Y, Wang C, Qi G, Li C, Cao Y. 2021. Characterization of the complete mitochondrial genome of *Pseudorhaetus sinicus* Boileau, 1899 (Coleoptera: Lucanidae). Mitochondrial DNA B Resour. 6(12):3398–3399. doi:10.1080/23802359.2021.1997656.