New Mitogenomes of Two Chinese Stag Beetles (Coleoptera, Lucanidae) and Their Implications for Systematics

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Abstract

Although conspicuous and well-studied, stag beetles have been slow to join the genomic era. In this study, mitochondrial genomes of two stag beetles, Sinodendron yunnanense and Prosopocoilus confucius, are sequenced for the first time. Both of their genomes consisted of 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), 2 ribosomal RNAs (rRNAs), and a control region. The mitogenome of S. yunnanense was 16,921 bp in length, and P. confucius was 16,951 bp. The location of the gene trnL(UUR), between the A+T-rich and control region in S. yunnanense, is the first observed in Lucanidae. In P. confucius, an unexpected noncoding region of 580 bp was discovered. Maximum likelihood and Bayesian inference on the 13 mitochondrial PCGs were used to infer the phylogenetic relationships among 12 representative stag beetles and three scarab beetles. The topology of the two phylogenetic trees was almost identical: S. yunnanense was recovered as the most basal Lucanid, and the genus Prosopocoilus was polyphyletic due to P. gracilis being recovered sister to the genera Dorcus and Hemisodorcus. The phylogenetic results, genetic distances and mitogenomic characteristics call into question the cohesion of the genus Prosopocoilus. The genetic resources and findings herein attempts to redress understudied systematics and mitogenomics of the stag beetles.

Key words: mitogenomes, gene rearrangement, noncoding region, Chinese stag beetles, phylogenomics
rare in the field. The species *P. confucius* is a large but otherwise typical member of the genus, also with limited distribution in South China. We aim to create a more robust understanding of the evolution of lucanid beetles by developing mitochondrial phylogenomics, with a particular focus on *Prosopocoilus*, including a comparison among *P. confucius*, *P. blanchardi* and *P. gracilis*.

### Materials and Methods

#### Sample Collection and DNA Extraction

The specimen of *S. yunnanense* was collected by Dr. Hong-liang Shi from Lanping, Yunnan, China, in July 2012, and the specimen of *P. confucius* was collected from Wuming, Guangxi, China, by Yinghua Wu in July 2011. Total genomic DNA was extracted from the

#### Table 1 Details on primers used in this study

| Gene | Primer name | Sequence (5'–3') | Reference |
|------|-------------|------------------|-----------|
| cox1 | COI–F1      | CAACATTTATTTTGATTTTTTGG | Simon et al. 1994 |
|      | COI–R1      | TCCAATGCACTAATCTGCCATATTA | Simon et al. 1994 |
| cytb | Cytb–F2     | GAGGAGCAACTGTAATTACTAA | Balke et al. 2004 |
|      | Cytb–R2     | AAAAGAAATATCATCTCAGGTGGAAT | Balke et al. 2004 |
| rrnL | 16S–F1      | CCGGTGGGAACTCAAGATCATG | Hosoya et al. 2001 |
|      | 16S–R1      | TAATTATTTTCTTGTGTATCAG | Hosoya et al. 2001 |
| nad4 | PcND4F1     | AATCATGACCCTGAAACAG | This study |
|      | PcND4R1     | AGGGAGATTCTTTGAGGGTG | This study |

Fig. 1 Mitochondrial genome maps of *Sinodendron yunnanense*. The abbreviations for the genes are as follows: *cox1–3* refer to the cytochrome C oxidase subunits; *cytb* refers to cytochrome B; and *nad1–6* refers to NADH dehydrogenase subunits; *atp6* and *atp8* refer to subunits 6 and 8 of ATPase; *rrnL* and *rrnS* refer to ribosomal RNA of 12S and 16S. From outer to innermost, the first circle shows the gene map, the second circle shows GC content, and the third shows GC skew calculated as \((G - C)/(G + C)\). There is a unique gene rearrangement: *trnL2* (UUR) located between the A + T-rich and control region.
muscle of a single *S. yunnanense* using the Qiagen DNAeasy Kit. For *P. confucius*, a small portion of the muscle from a specimen preserved in 100% ethanol was used for total DNA extraction with the Blood & Tissue Kit (Qiagen, Germany). The voucher specimens (*S. yunnanense*: MAHUS012; *P. confucius*: MAHU00102) were deposited at the Museum of Anhui University, China, and the sequences were submitted to GenBank with the accession numbers KP735804 (*S. yunnanense*) and KP552119 (*P. confucius*).

**Primer Design, Polymerase Chain Reaction Amplification, and Sequencing**

Complete mitogenomes were assembled from amplified fragments, with all primers used for amplification listed in Table 1. PCR amplification reactions were carried out in 25 μl volumes containing 10 μM of each primer (forward and reverse) 1 μl, 2 μl template DNA, 12.5 μl 2 × EasyTaq SuperMix (+dye), and 8.5 μl sterile double-distilled water to make up a final volume of 25 μl. The polymerase chain reaction amplifications were 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 seconds, annealing at 52–58 °C for 50 seconds, and elongation at 70 °C for 1 min, and then a final extension step at 72 °C for 7 min. The temperature of annealing was determined by the length of fragments. Sequencing was conducted with the Illumina HiSeq 2000 platform. Cluster strands created by 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 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.

**Mitogenome Assembly, Annotation, and Analysis**

The mitogenomes were assembled using SOAP denovo (BGI company, Shenzhen, China) and preliminary annotations made with the MITOS WebServer (http://mitos.bioinf.uni-leipzig.de/index.py). tRNA genes and their secondary structures were inferred using tRNAscan-SE 1.21 (http://lowelab.ucsc.edu/tRNAscan-SE/). Those not identified by tRNAscanSE, in addition to 16S ribosomal RNA (rrnL, lrRNA), and 12S ribosomal RNA (rrnS, srRNA), 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. Nucleotide compositions, codon usage, and relative synonymous codon usage (RSCU) values of PCGs were calculated with MEGA version 6.05 (Tamura et al. 2013). PCGs were translated with DNAMAN v7.0.2.176 (Lynnon Biosoft, Vaudreuil-Dorion, Canada). Mitogenomes were mapped with CGView (Grant and Stothard 2008, available at http://stothard.afns.ualberta.ca/cgviewserver/). Composition skew analysis was conducted according to formulas AT skew $\text{AT skew} = \frac{[A/C]_T}{[A + C]}$ and GC skew $\text{GC skew} = \frac{[G/C]_T}{[G + C]}$ (Perna and Kocher 1995).

Phylogenetic Analyses
Phylogenetic analyses of stag beetles, including the newly sequenced S. yunnanense and P. confucius and 13 species retrieved from GenBank, were performed using a concatenated dataset of the 13 PCGs. Models of nucleotide substitution were selected according to the Akaike Information Criterion (AIC) with jModelTest v2.1.4 (Posada 2008). Phylogenetic trees were generated from ML analysis using RAxML (Stamatakis 2014) and Bayesian inference (BI) with MrBayes v3.2.5 (Huelsenbeck and Ronquist 2001), both under the GTR + I + G model. Node support in the ML tree was estimated through bootstrap analysis with 1,000 replicates. The BI was conducted with two simultaneous Markov chain Monte Carlo runs of 2 million generations, sampled every 1,000 steps, with the first 25% discarded as burn-in. Phylogenetic trees were viewed and edited in Figtree v1.4.3 Rambaut 2016.

Results and Discussion

Genome Composition and Base Content
The complete mitogenomes of S. yunnanense and P. confucius were 16,921 bp (Fig. 1), and 16,951 bp (Fig. 2), respectively. Details of the 13 PCGs, 22 tRNAs, 2 rRNAs, and control region are given in Tables 2 and 3. Their compositions were typical of the Coleoptera (Sheffield et al. 2009, Kim et al. 2015, Wu et al. 2016, Du et al. 2016, Yang et al. 2016). Of the 37 genes, 23 (9 PCGs and 14 tRNAs) genes were located on the J-strand, with the remaining 4 PCGs, 2 rRNAs, and 8 tRNAs on the N-strand. Typical of metazoan mitogenomes (Cameron 2014a), the single tRNA gene trnS(AGN) lacked the cloverleaf secondary structure, due to the structure of the dihydrouridine arm. The lengths of the tRNAs ranged from 61 to 71 bp.

![Table 2 Mitogenome organization of Sinodendron yunnanense](https://example.com/table2.png)

| Gene   | Strand | Region | Length (bp) | Start codon | Stop codon | Anticodon | Intergenic nucleotides (bp) |
|--------|--------|--------|-------------|-------------|------------|-----------|----------------------------|
| trnI   | J      | 1–65   | 65          | –           | –          | GAT       | –3                         |
| trnQ   | N      | 63–131 | 69          | –           | –          | TTG       | –1                         |
| trnM   | J      | 131–199| 69          | –           | –          | CAT       | 0                          |
| nad2   | J      | 200–1213| 1014        | –           | TAA        | –         | 2                          |
| trnW   | J      | 1,216–1,281| 66        | –           | –          | TCA       | –8                         |
| trnC   | N      | 1,274–1,335| 62        | –           | –          | GCA       | 0                          |
| trnY   | N      | 1,336–1,401| 66        | –           | –          | GTA       | 1                          |
| cox1   | J      | 1,403–2,938| 1536      | AAT         | TAA        | –         | 46                         |
| cox2   | J      | 2,985–3,672| 688       | ATG         | T          | –         | 0                          |
| trnK   | J      | 3,673–3,743| 71        | –           | –          | CTT       | –1                         |
| trnD   | J      | 3,743–3,806| 64        | –           | –          | GTC       | 0                          |
| atp8   | J      | 3,807–3,962| 156       | ATT         | TAA        | –         | –7                         |
| atp6   | J      | 3,956–4,636| 681       | ATG         | TAA        | –         | –1                         |
| cox3   | J      | 4,636–5,424| 789       | ATG         | TAA        | –         | 2                          |
| trnG   | J      | 5,427–5,489| 63        | –           | –          | TCC       | 0                          |
| nad3   | J      | 5,490–5,843| 335       | ATT         | TAG        | –         | –2                         |
| trnA   | J      | 5,842–5,906| 65        | –           | –          | TGC       | –1                         |
| trnR   | J      | 5,906–5,971| 66        | –           | –          | TCG       | –1                         |
| trnN   | J      | 5,971–6,035| 65        | –           | –          | GTT       | –1                         |
| trnS(AGN)| J    | 6,035–6,103| 69        | –           | –          | ACT       | –1                         |
| trnE   | J      | 6,103–6,165| 63        | –           | –          | TCC       | –2                         |
| trnF   | N      | 6,164–6,228| 65        | –           | –          | GAA       | 0                          |
| nad5   | N      | 6,229–7,951| 1723      | ATA         | T          | –         | –3                         |
| trnH   | N      | 7,949–8,011| 63        | –           | –          | GTG       | 0                          |
| nad4   | N      | 8,012–9,347| 1336      | ATG         | T          | –         | –7                         |
| nad4L  | N      | 9,341–9,631| 291       | ATT         | TAA        | –         | 2                          |
| trnT   | J      | 9,634–9,697| 64        | –           | –          | TGT       | 0                          |
| trnP   | N      | 9,698–9,760| 63        | –           | –          | TGG       | 1                          |
| nad6   | J      | 9,762–10,259| 498      | ATC         | TAA        | –         | –1                         |
| cytB   | J      | 10,259–11,401| 1143     | ATG         | TAG        | –         | –2                         |
| trnS(UCN)| J    | 11,400–11,464| 65       | –           | –          | TGA       | 19                         |
| nad1   | N      | 11,484–12,434| 951     | ATA         | TAG        | –         | 1                          |
| trnL(CUN)| N    | 12,436–12,499| 64       | –           | –          | TAG       | 0                          |
| rrnL   | N      | 12,500–13,777| 1278    | –           | –          | TAC       | 0                          |
| rrnV   | N      | 13,778–13,847| 70       | –           | –          | TAC       | 0                          |
| rrnS   | N      | 13,848–14,614| 767     | –           | –          | –         | 0                          |
| A + T–rich region | – | 14,615–16,301| 1687   | –           | –          | –         | 0                          |
| trnL(UUR)| J   | 16,302–16,367| 66       | –           | –          | TAA       | 0                          |
| Control region | – | 16,368–16,921| 554      | –           | –          | –         | 0                          |
nucleotides in both species. In both species, rRNA was located between trnL (CUN) and trnV, and was 1,278 bp in S. yunnanense, and 1,275 bp in P. confucius. sRNA was 767 bp in length and located upstream of the A+T-rich region in S. yunnanense, whereas 747 bp and upstream of the control region in P. confucius.

The mitogenome nucleotide composition was A (38.8%), C (15.8%), T (36.2%), and G (9.1%) in S. yunnanense, and A (36.5%), C (21.9%), T (31.3%), and G (10.3%) in P. confucius, with an AT content of 75% and 67.8%, respectively. The higher AT content of S. yunnanense is relative to the A+T-rich region. In five lucanid species, the heavy strand of the mitogenome has a positive AT-skew (0.035 to 0.107) and negative GC-skew (–0.362 to –0.271). In contrast, S. yunnanense rRNA genes have negative AT-skew and positive GC-skew. Interestingly, the AT-skew and GC-skew of PCGs were negative, and positive in the tRNA genes (Table 4). It has been proposed that nucleotide composition strand bias might be related to replication and transcription mechanisms (Hassanin et al. 2005, Song et al. 2016a).

**Table 3 Mitogenome organization of Prosopocoilus confucius**

| Gene     | Strand | Region | Length (bp) | Start codon | Stop codon | Anticodon | Intergenic nucleotides (bp) |
|----------|--------|--------|-------------|-------------|------------|-----------|----------------------------|
| trnl     | J      | 1–62   | 62          | –           | –          | GAT       | 0                          |
| noncoding region | – | 63–642 | 580         | –           | –          | –         | 0                          |
| trnQ     | N      | 643–711| 69          | –           | – TAG      | TTG       | –1                         |
| trnM     | J      | 711–778| 68          | –           | – CAT      |          | 0                          |
| nad2     | J      | 779–1,792| 1014    | ATA TAG     | –          |          | 2                          |
| trnW     | J      | 1,795–1,860| 66       | –           | – TCA      | –         | –8                         |
| trnC     | N      | 1,853–1,913| 61       | –           | – GCA      | –         | –1                         |
| trnY     | N      | 1,913–1,977| 65       | –           | – GTA      | 1         |                            |
| cox1     | J      | 1,979–3,309| 1531    | AAT T       | –          |          | 0                          |
| trnL(UUR)| J      | 3,510–3,573| 64       | –           | TAA        |          | 0                          |
| cox2     | J      | 3,574–4,261| 688      | ATA T       | –          |          | 0                          |
| trnK     | J      | 4,262–4,322| 71       | –           | – CTI      | –         | –1                         |
| trnD     | J      | 4,332–4,393| 62       | –           | – GTC      | 0         |                            |
| atp8     | J      | 4,394–4,549| 156      | ATT TAG     | –          |          | –7                         |
| atp6     | J      | 4,543–5,211| 669      | ATG TAA     | –         | –        | –1                         |
| cox3     | J      | 5,211–5,995| 785      | ATG TA      | –          |          | –1                         |
| trnG     | J      | 5,995–6,056| 62       | –           | – TCC      | 0         |                            |
| nad3     | J      | 6,057–6,410| 354      | ATA TAG     | –          |          | –2                         |
| trnA     | J      | 6,409–6,473| 65       | –           | – TGC      | –         | –1                         |
| trnR     | J      | 6,473–6,536| 64       | –           | – TCG      | –         | –1                         |
| trnN     | J      | 6,536–6,602| 67       | –           | – GTT      | –         | –1                         |
| trnS(AGN)| J      | 6,602–6,670| 69       | –           | – TCT      | –         | –1                         |
| trnE     | J      | 6,670–6,732| 63       | –           | – TTC      | –         | –2                         |
| trnF     | N      | 6,731–6,794| 64       | –           | – GAA      | 0         |                            |
| nad5     | N      | 6,795–8,511| 1717     | ATT T       | –          |          | 0                          |
| trnH     | N      | 8,512–8,578| 67       | –           | – GTG      | 0         |                            |
| nad4     | N      | 8,579–9,914| 1336     | ATG T       | –          |          | –7                         |
| nad4L    | N      | 9,908–10,195| 288     | ATG TAA     | –          |          | 2                          |
| trnT     | J      | 10,198–10,261| 64      | –           | TGT        | 0         |                            |
| trnP     | N      | 10,262–10,327| 66      | –           | TGG        | 5         |                            |
| nad6     | J      | 10,333–10,830| 498     | ATG TAA     | –          |          | –1                         |
| cyt b    | J      | 10,830–11,972| 1143   | ATG TAA     | –          |          | –2                         |
| trnS(UCN)| J      | 11,971–12,035| 65     | –           | – TGA      | 18        |                            |
| nad1     | N      | 12,054–13,004| 951    | ATA TAG     | –          |          | 0                          |
| trnL(CUN)| J      | 13,005–13,067| 63     | –           | – TAG      | 0         |                            |
| rrlL     | N      | 13,068–14,342| 1275   | –           | –         | –        | –8                         |
| trnV     | N      | 14,333–14,403| 69     | –           | – TAC      | 0         |                            |
| rnrS     | N      | 14,404–15,150| 747    | –           | –         | –        | –14                        |
| Control region | – | 15,137–16,951| 1815 | —           | –         | –        | 0                          |

**Table 4 AT-content, AT-skew, and GC-skew of five Lucanidae mitochondrial genomes**

| Genus       | Species | Genome | PCGs | tRNAs | rRNAs |
|-------------|---------|--------|------|-------|-------|
| Lucanus      | mazama  | 67.125 | 0.074| –0.272| –0.141|
| Prosopocoilus| confucius| 67.351 | 0.077| –0.362| –0.072|
| Prosopocoilus| gracilis| 66.091 | 0.106| –0.332| –0.077|
| Prosopocoilus| blanchardi| 67.015 | 0.107| –0.285| –0.074|
| Sinodendron  | yunnanense| 75.061 | 0.035| –0.271| –0.057|

PCGs and Codon Usage

All PCGs of S. yunnanense and P. confucius had the conventional start codon for invertebrate mitochondrial PCGs (ATN) with the

nucleotides in both species. In both species, trnL (CUN) and trnV, and was 1,278 bp in S. yunnanense, and 1,275 bp in P. confucius. sRNA was 767 bp in length and located upstream of the A+T-rich region in S. yunnanense, whereas 747 bp and upstream of the control region in P. confucius.

The mitogenome nucleotide composition was A (38.8%), C (15.8%), T (36.2%), and G (9.1%) in S. yunnanense, and A (36.5%), C (21.9%), T (31.3%), and G (10.3%) in P. confucius, with an AT content of 75% and 67.8%, respectively. The higher AT content of S. yunnanense is relative to the A+T-rich region. In five lucanid species, the heavy strand of the mitogenome has a positive AT-skew (0.035 to 0.107) and negative GC-skew (–0.362 to –0.271). In contrast, S. yunnanense rRNA genes have negative AT-skew and positive GC-skew. Interestingly, the AT-skew and GC-skew of PCGs were negative, and positive in the tRNA genes (Table 4). It has been proposed that nucleotide composition strand bias might be related to replication and transcription mechanisms (Hassanin et al. 2005, Song et al. 2016a).
exception of cox1 (AAT) as found previously (Sheffield et al. 2008). In *S. yunnanense*, 10 (eight in *P. confucius*) of the 13 PCGs shared the typical termination codons TAA and TAG, whereas in the remaining genes an incomplete stop codon T or TA was inferred. It is not uncommon in insects that incomplete codon structures signal a halt of protein translation (Wu et al. 2014, Li 2014, Cheng et al. 2016). The RSCU analysis indicated that codons including A or T at the third position were overrepresented in comparison to other synonymous codons (Fig. 3), which could reflect nucleotide bias.

**Intergenic Spacers and Noncoding Region**

There was frequent occurrence of intergenic spacers in the two new mitogenomes, interspersed throughout the PCGs and RNA genes, and ranging from 1 to 580 bp in length (Tables 2 and 3). Among them, a 7-bp conserved motif (TACTAAA) upstream of *nad1* was proposed as a binding site for the mitochondrial transcription termination factor, and noted in various beetles and other insects (Cameron and Whiting 2008, Sheffield et al. 2009, Cameron 2014b, Kim et al. 2015, Wu et al. 2016, Yang et al. 2016).
A notable result in *P. confucius* was a 580 bp noncoding region located between *trnI* and *trnQ*, with three structural sections: (C)_{14} in the initial third; (A)_{14} in the center, and a (C)_{9} in the latter third. This noncoding region is also present and at the same position in *P. blanchardi*, albeit longer (4,051 bp) and more complex (with 17 tandem repetitive sequence, each of which is composed of two subunits; Kim et al. 2015). These features appear to reflect both congeneric and phylogenetic distances (Fig. 6). Moreover, this noncoding region did not occur in *P. gracilis*, despite being a longstanding member of *Prosopocoilus*. Indeed, the mitogenome of this species was structured more typical of insects. Absence of this noncoding region in *P. gracilis* and paraphyly of *Prosopocoilus* in relation to this species (Fig. 6) raises a question on its taxonomic status.

New Gene Rearrangement

Gene arrangements of most insect mitogenomes are of the type exemplified by *Drosophila yakuba*, considered to be the ancestral state (Cameron 2014a). However, gene rearrangement events have been reported in many lineages (Timmermans and Vogler 2012, Song et al. 2016b), with a moderate frequency in Coleoptera (Wei and Chen 2011, Li et al. 2013). Although as the most diverse animal order (with >390,000 described species; Zhang 2013), there are merely ~80 reported mitogenomes of Coleopterans, belonging to 32 families and representing all four suborders (as of February 2016). Gene rearrangement events have been reported in 10 of these, in the form of reversal or absence of tRNAs; in Anthicidae (1), Byrrhoidea (3), Chrysomelidae (3), Curculionidae (1), Lycidae (1), and Mordellidae (1) (Timmermans and Vogler 2012, Nie and Yang 2014).

**Fig. 5** The TDRL model was introduced to interpret the gene rearrangement in *S. yunnanense*. (A) The putative ancestral arrangement. (B) Genes in gray were duplicated to form the intermediate. (C) Some regions were lost to form the current arrangement in *S. yunnanense*. The X in B indicates the randomly lost regions.

**Fig. 6** Phylogenetic reconstruction of 15 beetles using a concatenated dataset of mitochondrial PCGs. (A) ML and (B) BI.
In this study, we report a novel gene rearrangement of *S. yunnanense*, a first for Lucanidae. *trnL* (UUR), ancestrally located between *cox1* and *cox2*, was transllocated to a position between A+T-rich region and control region (Fig. 4). Moreover, intergenic spacers were observed between *trnL* (UUR) and *trnL*, and *cox1* and *cox2*, which is thought to be evidence of random gene losses (San et al. 2006). The TDRL model (tandem duplication followed by random loss; Moritz et al. 1987) was introduced to interpret the transposition of *trnL* (UUR) (Fig. 5). Additionally, recombination is another possible mechanism since illicit priming is unlikely due to the lack of a replication origin in this region (Wei et al. 2009). Data for more representatives are needed to determine the universality of the rearrangement in Lucanidae and to better understand the causal mechanisms.

Phylogenetic Analysis

ML and BI phylogenies were reconstructed using a supermatrix of the 13 PCGs. The phylogeny showed that Lucanidae was a monophyletic group and *S. yunnanense* was sister to other Lucanidae (Fig. 6). Similarly, the genetic distance between this species and others was high (0.285–0.359) (Supp Table 1 [online only]). These results are consistent with previous studies based on morphological characters (Holloway 1960, 2007) and molecular data (Kim and Farrell 2015).

The species *P. confucius* and *P. blanchardi* were recovered as sister taxa (1.00 BPP, 83% MLB), and had low genetic distance (0.241; Supp Table 1 [online only]). This result is consistent with their traditional taxonomy. These two species have long been considered archetypal *Prosopocoilus*, sharing characters such as large, stout and shiny body, with straight and well developed mandibles in the males. However, the genus *Prosopocoilus* formed a polyphyly due to the distinctive positioning of *P. gracios* (1.00 BPP, 100% MLB), sister to the pair *Dorcas paraleepipeda* and *Hemisodorcus rubrofemoratus*. The genetic distance among the three taxa of *Prosopocoilus* is 0.241, 0.287, and 0.296 (Supp Table 1 [online only]), whereas the value between *P. gracis* and *D. paraleepipeda* is 0.267, and 0.260 with *H. rubrofemoratus* (Supp Table 1 [online only]). The status of *P. gracis* has been revised often in morphological taxonomy. *P. gracis* was first erected by Benesh (1950). Seguy (1954) established the genus *Epadorus*, including *P. gracis* and a congener, despite a lack of convincing generic characters (see Zhong et al. 2014). Actually, its genital characters are quite distinct, and much more similar to those of species of *Serrognathus* (Wan 2007). Huang and Chen (2013) resurrected the genus *Epadorus* for *P. gracis* and its congeners after morphological analysis although they acknowledged characters shared by *Epadorus* and *Serrognathus* species.

In our study, the phylogenomic analysis supported the morphological conclusion, i.e., *P. gracis* should be removed from *Prosopocoilus*. Instead, *P. gracis* probably belongs in *Dorcas* (s.l.). This is just one of many issues warranting more comprehensive work in Lucanidae phylogenetics, including higher-level classification, doubtful monophilies of some key genera, and species/subspecies boundaries and nomenclature, all of which would benefit from more comprehensive genetic data.

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Supplementary Data

Supplementary data are available at Journal of Insect Science online.

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