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The mitochondrial genome of *Iberobaenia* (Coleoptera: Iberobaeniidae): First rearrangement of protein coding genes in the beetles

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Abstract:

The complete mitochondrial genome of the recently discovered beetle family Iberobaeniidae is described and compared with known coleopteran mitogenomes. The mitochondrial sequence was obtained by shotgun metagenomic sequencing using the Illumina Miseq technology and resulted in an average coverage of 130x and a minimum coverage of 35x. The mitochondrial genome of Iberobaeniidae includes 13 protein-coding genes, 2 rRNAs, 22 tRNAs genes and one putative control region, and showed a unique rearrangement of protein coding genes. This is the first rearrangement affecting the relative position of protein-coding and ribosomal genes reported for the order Coleoptera.

Running Title: The mitochondrial genome of *Iberobaenia*

Coleoptera are the most diverse order of insects, with about 179 extant families (Bouchard *et al.* 2011) and more than 350000 described species. More than 250 complete or almost complete mitochondrial genomes have been sequenced to date, including representatives from \approx 100 families. Despite the high diversity and ancient origin of Coleoptera, estimated at around 300 MYA (Hunt *et al.*, 2007; Misof *et al.*, 2014), all known coleopteran mitogenomes share the same gene order and orientation for the protein-coding and rRNA genes, while rearrangements found to date affect only the position of the tRNA genes (Timmermans & Vogler, 2012). The genus *Iberobaenia* Bocak, Kunderata, Andújar & Vogler 2015 has been recently discovered and phylogenetic analyses conducted on mitochondrial and nuclear genes revealed the distinctiveness of the new lineage within the superfamily Elateroidea, resulting in the description of a new beetle family, Iberobaeniidae (Bocak *et al.* in press).

For sequencing the complete mitochondrial genome of *Iberobaenia* we followed the mitochondrial metagenomic approach (MMG) (Dettai *et al.*, 2012; Zhou *et al.*, 2013; Andújar *et al.*, 2015; Crampton-Platt *et al.*, 2015), which allows to assemble complete mitochondrial genomes from shotgun sequencing of specimen mixtures. The raw DNA extracted from a larval specimen of *Iberobaenia* sp. (Sierra de Cabra, Córdoba, Spain; Voucher specimen deposited in the Natural History Museum of London–BMNH 1042541) was pooled in equimolar concentration with DNA from 20 other Coleoptera from distant lineages mostly in the suborder Adephaga not connected to the current study. The dsDNA concentration was measured using a Qubit 2.0 Fluorometer (Life Technologies Corp., Carlsbad, CA, USA). A TruSeq DNA library was constructed with the pooled DNA and sequenced using the Illumina aMiSeq platform (2x300 bp; \sim 800–950 bp insert size) in 45% of an Illumina flow cell.

The Illumina output was quality assessed with FastQC 0.10.1 (Babraham Institute 2013) and processed with Trimmomatic 0.30 (Lohse *et al.* 2012) for Illumina adapter removal. Reads were subsequently filtered using Blast 2.2.27 (Altschul *et al.* 1990) against a reference database including 245 nearly complete coleopteran mitochondrial genomes retrieved from GenBank. Surviving mitochondrial reads were subsequently assembled using Celera Assembler 7.0 (Myers 2000), Newbler 2.7 (Margulies *et al.* 2006) and IDBA-UD (Peng *et al.* 2012). Celera analyses were done using the RunCa command with the bogart method for unitigger computations, and Unitig Repeat/Unique Toggling was conducted from unitigs longer than 500 bp; other parameters were set to default options. Newbler analyses were run using -mi 95 -ml 150 and IDBA using --maxk 300 --mink 50.

Mitochondrial contigs longer than 500 bp obtained with the three assemblers were pooled and super-contiged using Geneious 7.1.9 (<http://www.geneious.com>), saving the 50% majority rule consensus from the resulting contigs. The mitogenome of *Iberobaenia* was identified using the *cox1* barcode sequence obtained from the same specimen with PCR-Sanger sequencing (Genbank accession no. XXX), and was annotated using gene predictions with MITOS (Bernt et al., 2013) and manually refined in Geneious. Additionally, mitochondrial Illumina reads were mapped against the mitogenome of *Iberobaenia* in Geneious (using a 99% similarity threshold).

The widely overlapping contigs obtained from the three independent assemblers, after super-contiging and circularization, resulted in a complete mitochondrial sequence of 16931bp (Accession no. KT825140) including 13 protein-coding, 2 rRNAs and 22tRNAs genes. The overall base composition was 46.9% for A, 14.1% for C, 7.5% for G, 31.5% for T. All protein-coding genes were encoded on the H-strand with exception of *nad1*, *nad4*, *nad4l* and *nad5*. All tRNA genes were encoded on the H-strand with the exception of *tRNA-Cys*, *tRNA-Phe*, *tRNA-His*, *tRNA-Val*, *tRNA-Gln*, *tRNAPro* and *tRNA-Leu2*. Our predictive annotation resulted in the canonical ATN start codon (isoleucine) for all genes (ATA: *nad2*, *cox1*, *cob*, *atp6*, *atp8* and *nad3*; ATG: *cox2* and *cox3*; ATT: *nad5*, *nad4* and *nad4l*; ATC: *nad6*) but *nad1*, where a TTG start codon was identified as previously reported in other Coleoptera (Sheffield et al., 2008). With regard to the 3' end, *nad2* was predicted with an incomplete stop codon "T", three genes showed the stop codon TAG (*nad5*, *nad1* and *cob*) and all other genes were inferred with the stop codon TAA. The longest gene was *nad5* (1647bp) and the shortest *atp8* (150bp). A maximum-likelihood phylogenetic tree was obtained in RaxML (Stamatakis, 2006) including some of the closest relatives to Iberobaniidae according to Bocak et al. (in review) (Fig. 1). The best tree was selected over 100 alternative runs and node support was calculated with 10000 bootstrap replicates.

The structure of the mitogenome of *Iberobaenia* showed a unique gene rearrangement with regard to the constant pattern found in all other mitochondrial genomes for the order Coleoptera (Timmermans & Vogler, 2012). The rearrangement affected the protein-coding, ribosomal and tRNAs genes. The gene order for the mitogenome of *Iberobaenia* sp., including tRNAs, is: I, M, *nad2*, W,C , Y, *cox1*, L1, *cox2*, K, D, *atp8*, *atp6*, *cox3*, *nad3*, N, R, A, E, F, G, S, *nad5*, H, *nad4l*, *nad1*, control region, Q, *nad4*, T, P, *nad6*, *cob*, S2, L2, *rrnL*, V, *rrnS*. The high sequencing read coverage (average:130x; max: 213x; min: 35x) provides high reliability to the obtained sequence. This is the first mitochondrial gene rearrangement reported in the order Coleoptera excluding those affecting only to tRNAs (Timmermans &

Vogler, 2012). This finding adds to the unique features of the enigmatic beetle family Iberobaeniidae recently described from the Iberian Peninsula.

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Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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Figure captions:

Figure 1. Maximum-likelihood phylogenetic tree including some of the closest relatives to Iberobaniidae according to Bocak et al. (in review). Numbers on nodes represent bootstrap support. Right: Schematic representation of *Iberobaenia* sp. male specimen.

