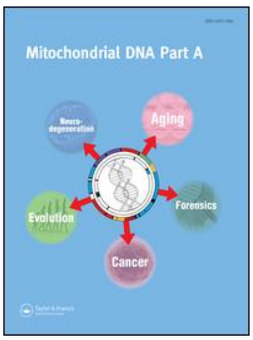

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MITOGENOME ANNOUNCEMENT

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

Carmelo Andujar[a,b], Paula Arribas[a,b], Benjamin Linard[a], Robin Kundrata[c], Ladislav Bocak[c] and Alfried P. Vogler[a,b]

[a]Department of Life Sciences, Natural History Museum, London, UK; [b]Department of Life Sciences, Imperial College London, Ascot, UK; [c]Department of Zoology, Faculty of Science, Palacky University, Olomouc, Czech Republic

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 130 × and a minimum coverage of 35×. The mitochondrial genome of Iberobaeniidae includes 13 protein-coding genes, 2 rRNAs, 22 tRNAs genes, and 1 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.

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Coleoptera, Elateroidea, gene rearrangement, mitochondrial metagenomics, mitochondrion

Coleoptera are the most diverse order of insects, with about 179 extant families (Bouchard et al. 2011) and more than 350 000 described species. More than 250 complete or almost complete mitochondrial genomes have been sequenced to date, including representatives from ≈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 et al. 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. 2015).

For sequencing the complete mitochondrial genome of *Iberobaenia,* we followed the mitochondrial metagenomic approach (MMG) (Andújar et al. 2015; Crampton-Platt et al. 2015; Dettai et al. 2012; Zhou et al. 2013), which allows us 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). A TruSeq DNA library was constructed with the pooled DNA and sequenced using the Illumina MiSeq platform (Illumina Inc., San Diego, CA) (2 × 300 bp; ~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 we 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 to -ml 150 and IDBA using –maxk 300 to –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 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

CONTACT Carmelo Andujar candujar@um.es Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK

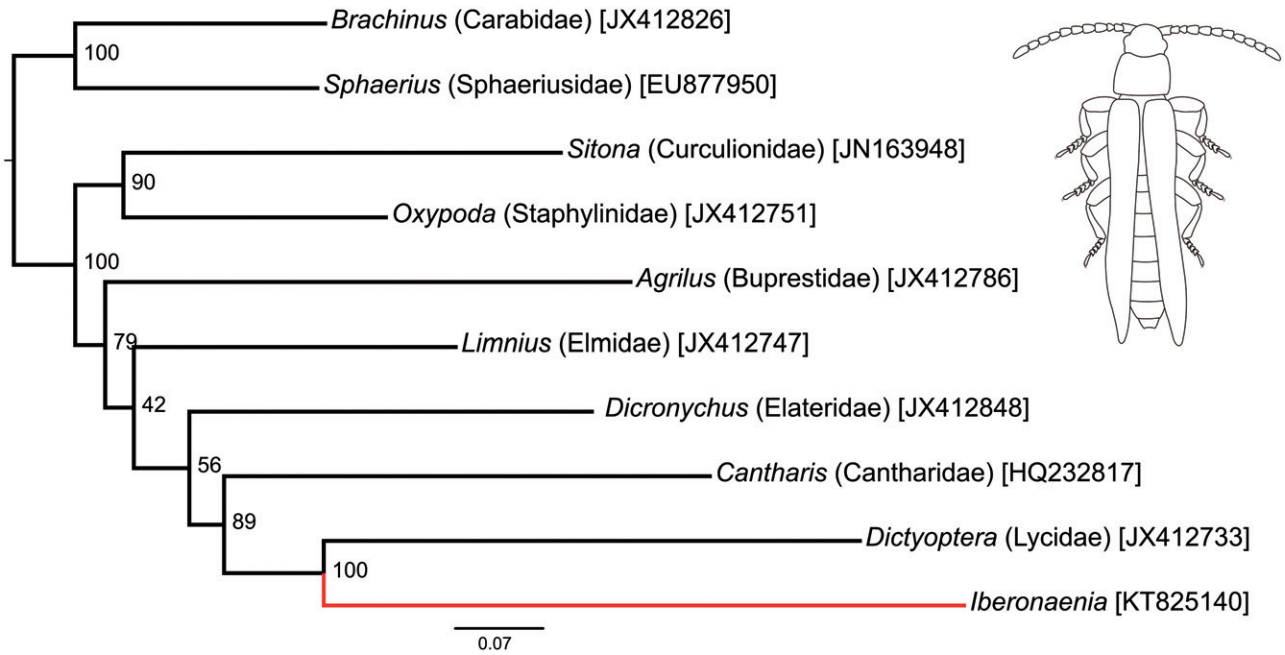

Figure 1. Maximum-likelihood phylogenetic tree including some of the closest relatives to Iberobaniidae according to Bocak et al. (2015). Numbers on nodes represent bootstrap support. *Right*: schematic representation of *Iberobaenia* sp. male specimen.

16 931 bp (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, and 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* (1647 bp) and the shortest gene was *atp8* (150 bp). A maximum-likelihood phylogenetic tree was obtained in RaxML (Stamatakis 2006) including some of the closest relatives to Iberobaniidae according to Bocak et al. (2015) (Figure 1). The best tree was selected over 100 alternative runs and node support was calculated with 10 000 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: 130×; max: 213×; min: 35×) 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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Disclosure statement

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol. 215:403–410.

Andújar C, Arribas P, Ruzicka F, Crampton-Platt A, Timmermans MJTN Vogler AP. 2015. Phylogenetic community ecology of soil biodiversity using mitochondrial metagenomics. Mol Ecol. 24:3603–3617.

Babraham Institute. 2013. FastQC: A quality control tool for high throughput sequence data; [cited 2015 Jul 24]. Available at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: Improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 69:313–319.

Bocak L, Kundrata R, Andújar C, Vogler AP. 2015. The discovery of Iberobaeniidae (Coleoptera: Elateroidea), a new family of beetles from Spain, with immatures detected by environmental DNA sequencing. Proc R Soc Lond B 283:20152350.

Bouchard P, Bousquet Y, Davies AE, Alonso-Zarazaga Ma, Lawrence JF, Lyal CHC, Newton AF, Reid CAM, Schmitt M, Ślipiński SA, Smith ABT. 2011. Family-group names in Coleoptera (Insecta). ZooKeys. 972:1–972.

Crampton-Platt A, Timmermans MJTN Gimmel ML, Kutty SN, Cockerill TD, Vun Khen C Vogler AP. 2015. Soup to tree: The phylogeny of beetles

inferred by mitochondrial metagenomics of a Bornean rainforest sample. Mol Biol Evol. 32:2302–2316.

Dettai A, Gallut C, Brouillet S, Pothier J, Lecointre G, Debruyne R. 2012. Conveniently pre-tagged and pre-packaged: Extended molecular identification and metagenomics using complete metazoan mitochondrial genomes. PloS One. 7:e51263

Hunt T, Bergsten J, Levkanicova Z, Papadopoulou A, John OS, Wild R, Hammond PM, Ahrens D, Balke M, Caterino MS, et al. 2007. A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. Science. 318:1913–1916.

Lohse M, Bolger AM, Nagel A, Fernie AR, Lunn JE, Stitt M, Usadel B. 2012. RobiNA: a user-friendly, integrated software solution for RNA-Seq-based transcriptomics. Nucleic Acids Res. 40:W622–W627.

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, et al. 2006. Genome sequencing in open microfabricated high density picoliter reactors. Nature. 437:376–380.

Misof B, Liu S, Meusemann K, Peters RS, Donath a, Mayer C, Frandsen PB, Ware J, Flouri T, Beutel RG, et al. 2014. Phylogenomics resolves the timing and pattern of insect evolution. Science. 346:763–767.

Myers EW. 2000. A whole-genome assembly of Drosophila. Science. 287:2196–2204.

Peng Y, Leung HCM, Yiu SM, Chin FYL. 2012. IDBA-UD: A de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics. 28:1420–1408.

Sheffield NC, Song H, Cameron SL, Whiting MF. 2008. A comparative analysis of mitochondrial genomes in Coleoptera (Arthropoda: Insecta) and genome descriptions of six new beetles. Mol Biol Evol. 25:2499–2509.

Stamatakis A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 22:2688–2690.

Timmermans MJTN, Vogler AP. 2012. Phylogenetically informative rearrangements in mitochondrial genomes of Coleoptera, and monophyly of aquatic elateriform beetles (Dryopoidea). Mol Phylogenet Evol. 63:299–304.

Zhou X, Li Y, Liu S, Yang Q, Su X, Zhou L, Tang M, Fu R, Li J, Huang Q. 2013. Ultra-deep sequencing enables high-fidelity recovery of biodiversity for bulk arthropod samples without PCR amplification. GigaScience. 2:1–12.