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MITOGENOME ANNOUNCEMENT

Draft mitogenomes of the invasive ant *Lepisiota frauenfeldi* (Mayr 1855) (Hymenoptera: Formicidae)

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ABSTRACT

We present the draft mitochondrial genomes (mitogenomes) of two *Lepisiota frauenfeldi* (Mayr 1855) workers from two separate invasive populations detected in Western Australia (Perth OK569858) and Queensland (Brisbane OK5569859), Australia. The draft mitogenomes ranged between 16,657 and 17,090 bp and contained 37 genes (13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), and two ribosomal RNA (rRNA) genes). As with other arthropod mitogenomes, we observed high A + T content (A: 39.4–39.8%, T: 40.55–41.5%). We confirmed the species identity by molecular diagnostics based on the partial mtCOI gene that showed >99% similarity between the Australian populations and other *L. frauenfeldi* sequences reported to date, and in the process identified putative origins of the invasive populations as Pakistan and India for the WA and Qld incursions respectively that suggested separate introductions.

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Browsing ant; multiple introductions; invasion biology

The genus *Lepisiota* currently includes 87 species and 49 subspecies (Bolton 2014) with multiple new species recently being recognized (e.g. Wu and Wang 1995; Sharaf et al. 2016) from different continents. Various *Lepisiota* species can now be considered to be 'horizon' invasive species due to their increasing association with trade movements and invasive traits (Sithole et al. 2009; Hoffmann et al. 2011). Despite their importance, *Lepisiota* taxonomy remains incomplete, and species identifications can therefore benefit from molecular diagnostics via the DNA barcoding approach. The partial mitochondrial DNA COI (mtCOI) genes of numerous *Lepisiota* specimens now exist in public sequence repositories (e.g. the Barcode of Life DATA (BOLD) data systems; GenBank) to allow greater confidence in species diagnostics.

Lepisiota specimens were collected from Western Australia (Perth airport, (31° 56′S, 115° 57′E)) and Queensland (Brisbane Port, (27° 22′S, 153° 10′E)). This is a pest ant species and no ethical approval or permission to collect was required. A specimen from each of the two collections was deposited in the Australian National Insect Collection (ANIC Database No. 32-146151; 32-146152; Contact details: Dr. Federica Turco <federica.turco@csiro.au>). Total genomic DNA from one specimen each was extracted using the Qiagen DNeasy Blood and tissue DNA extraction kit (Qiagen, Hilden, Germany) following the modified protocol as detailed in Tay et al. (2017). Individual gDNA libraries were prepared using the Illumina Nextera DNA sample preparation kit prior to sequencing on the Illumina MiSeq sequencer. Mitogenome assembly was carried out using the Geneious v11.1.5 (Biomatters Ltd, Auckland, NZ) bioinformatics software and annotated using Mitos (Bernt et al. 2013). BOLD/GenBank deposited *Lepisiota* and *Plagiolepis* sequences were downloaded and aligned with the mtCOI genes from the WA and Qld samples. Aligned partial mtCOI gene sequences were trimmed to 546 bp. We used MAFFT Align (Katoh & Standley 2013) within Geneious v11.1.5, specifying default settings (Algorithm: Auto; Scoring matrix: 200PAM/k = 2; Gap open penalty: 1.53; Offset value: 0.123) for sequence alignment, followed by phylogenetic inference using IQ-Tree (Trifinopoulos et al. 2016) with branch support estimated using UFBoots (Hoang et al. 2018). Visualization of phylogram was by Dendroscope 3 (Huson et al. 2007).

The two mitogenomes shared 98.99% nucleotide identity (*ca.* 16,448 bp compared) and 99.67% identity between the full COI gene (1530 bp). We identified 13 PCGs in both mitogenomes, with gene orders and orientation similar to that reported in, e.g. *Anoplolepis gracilipes* (Lee et al. 2018) and *Acropyga myops* (MH158408). tRNA re-arrangements (e.g. tRNA-Val; tRNA-Asn) and orientation differences (e.g. tRNA-Met, tRNA-Tyr, tRNA-Pro) were detected when compared with mitogenomes of *Wasmannia auropunctata* (Duan et al. 2016) and *S. invicta* (Gotzek et al. 2010). The mitogenomes were AT-rich (e.g. OK5569858: 39.8% (A), 41.5% (T), 5.5% (G), 13.3% (C)) and included an estimated 712 bp putative origin of replication, located between the small (12S) rRNA and tRNA-Met gene.

Molecular diagnostics based on 546 bp of partial mtCOI gene suggested our specimens were *L. frauenfeldi*, with high

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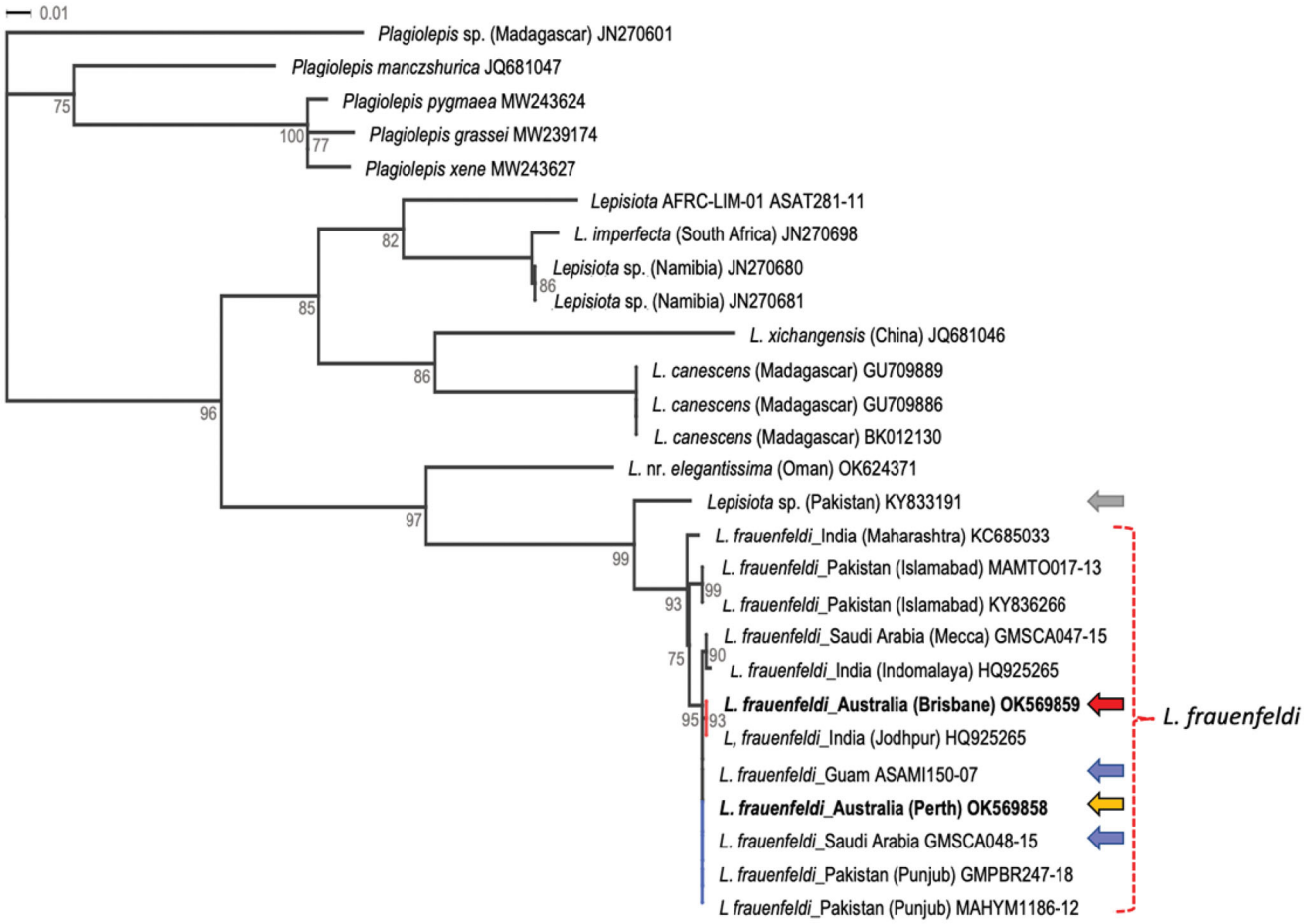

Figure 1. Partial mtCOI gene (546 bp) Maximum Likelihood (ML) phylogeny inferred using IQ-Tree with 1,000 UFBoot approximations. Branch node confidence >75% is shown. The Queensland and the Perth individuals are indicated by red and orange arrows, respectively. A Guam and two Saudi Arabia (Mecca) specimens representing invasive populations with potential Indian/Pakistan origins are indicated by blue arrows. *Note*: A *Paratrechina* sp. from Pakistan (KY833191; gray arrow) shared >95% partial mtCOI sequence identity with *L. frauenfeldi*, suggesting a taxonomic revision of *Paratrechina* may be needed.

nucleotide similarity (>98.56%) to the partial mtCOI gene sequences of other reported *L. frauenfeldi*. Phylogenetic analysis with *Plegiolepis* species as outgroups showed the *Lepisiota* genus to represent a diverse group of formicine ants with African/Chinese/Middle East species clustered as sister clades to the *L. frauenfeldi* species from India/Pakistan with large nucleotide difference (*p*-dist: 11.35–14.51%). The WA and Qld *L. frauenfeldi* mtCOI sequences clustered confidently (100%) with other *L. frauenfeldi* sequences, with the Qld and WA populations each sharing 100% mtCOI sequence identity with a sequence from India (Jodhpur) and Pakistan (Punjab), respectively (Figure 1). Unique mitogenomes between the WA and Qld populations also supported separate introduction events for these two incursions in Australia. While *L. frauenfeldi* was thought to be of Palearctic origin (Forel 1885), and there is some conjecture about its status within India (Wachkoo et al. 2021), partial mtCOI sequences of voucher specimens from India (e.g. KC685033; HQ925261) and Pakistan (e.g. KY836266; GMPBR247-18) suggest the species' native range possibly extends throughout Afghanistan, and the Indian subcontinent. However, two *L. frauenfeldi* specimens from the Arabian Peninsula (e.g. Saudi Arabia (Mecca), Sharaf et al. 2016) and one from Guam (Figure 1, blue arrows) are introduced populations potentially of Indian/Pakistan origins. Despite the invasive and putative native *L. frauenfeldi* individuals (e.g. WA-Guam-Saudi Arabia-Pakistan; Qld-India; Figure 1) sharing 100% partial mtCOI sequence identity, a better understanding of their invasion biology will benefit from sampling populations throughout its presumed native range so that analyses of complete mitogenomes and genome-wide DNA markers can be undertaken.

Author contributions

WTT, BDH, and AP designed the study. BDH and AP acquired relevant specimens for this study, LNC, AP, WTT carried out laboratory work to generate DNA data, WTT, BDH, AP interpreted the results and wrote the manuscript, all authors contributed to improving the final submitted version for intellectual content and integrity.

Disclosure statement

The authors declare no conflict of interest.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/> under the accession no. OK569858 - OK569859. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA773948 and PRJNA773860, SRR16549499 and SRR16546820, and SAMN22546381 and SAMN22518038, respectively.

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