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 UFBoot (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 *Wasmania 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
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/](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.

**References**

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(2):313–319.

Bolton B. 2014. An online catalog of the ants of the world; [version 2013 Jan 1; cited 2014 Dec 1], [accessed 2021 26 March]. http://www.antcat.org/catalog/

Duan X-Y, Peng X-Y, Qian Z-Q. 2016. The complete mitochondrial genomes of two globally invasive ants, the Argentine ant Linepithema humile and the little fire ant Wasmannia auropunctata. Conservation Genet Resour. 8(3):275–277.

Forel A. 1885. Indian ants of the Indian Museum, Calcutta. J Asiat Soc Bengal, Part 2. Nat Sci. 54:176–182.

Gotzek D, Clarke J, Shoemaker D. 2010. Mitochondrial genome evolution in fire ants (Hymenoptera: Formicidae). BMC Evol Biol. 10:300.

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. 2018. UFBoot2: improving the ultrafast bootstrap approximation. Mol Biol Evol. 35(2):518–522.

Hoffmann B, Davis P, Gott K, Jennings CD, Joe S, Krushelnicky P, Miller R, Webb G, Widmer M. 2011. Improving ant eradications: details of more successes, a global synthesis, and recommendations. Aliens. 31:16–23.

Huson DH, Richter DC, Rausch C, Dezulian T, Franz M, Rupp R. 2007. Dendroscope: an interactive viewer for large phylogenetic trees. BMC Bioinform. 8:460.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 30(4):772–780.

Lee C-C, Wang J, Matsuura K, Yang C-C S. 2018. The complete mitochondrial genome of yellow crazy ant. Mitochondrial DNA B Resour. 3(2):622–623.

Sharaf MR, Monks J, Polaszek A, Aldawood AS. 2016. A remarkable new species of the genus Lepisiota Santschi (Hymenoptera: Formicidae) from Oman and the United Arab Emirates with a key to the Arabian species. J Nat Hist. 50:29–30.

Sithole H, Smit IPJ, Parr CL. 2009. Preliminary investigations into a potential ant invader in Kruger National Park, South Africa. Afric J Ecol. 48:429.

Tay WT, Elfekih S, Polaszek A, Court LN, Evans GA, Gordon KHJ, De Barro PJ. 2017. Novel molecular approach to define pest species status and trophic interactions from historical Bemisia specimens. Sci Rep. 7(1):429.

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 44(W1):W232–235.

Wachkoo AA, Bharti H, Akbar SA. 2021. Taxonomic review of the ant genus Lepisiota Santschi, 1926 (Hymenoptera: Formicidae: Formiciniae) from India. Bonn Zool Bull. 70(2):227–245.

Wu J, Wang C. 1995. The ants of China. Beijing: China Forestry Publishing House, x + p. 214. pp