SCIENTIFIC NOTE

DNA BARCODE IDENTIFICATION OF AUSTRALIAN COLLECTED MUSHROOM FLY *Coboldia fuscipes* (MEIGEN, 1830) (SCATOPSIDAE: DIPTERA)

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Summary

Mushroom fly *Coboldia fuscipes* was recently found contaminating Elizabeth Macarthur Agricultural Institute (EMAI) insectary cultures. Species identification secured via DNA barcoding was followed by phylogenetic analysis to determine the phylogenetic relationship of this introduced pest. We found that DNA barcodes of EMAI-collected *C. fuscipes* were matched to North American accessions of the species, which might imply a Nearctic rather than Palearctic source origin of the pest in Australia.

Keywords: phylogenetic relationship, Cytochrome *c* Oxidase Subunit I (COI), genetic bottlenecks, invasion.

INTRODUCTION

Coboldia fuscipes is known for a Palearctic habit (Fauna Europaea 2022) where it is the most important fly pest of oyster mushroom (Yi *et al*. 2008). It is now in the Southern hemisphere being present in Australia including the Australian Capital Territory, New South Wales, Queensland, South Australia, Victoria and Western Australia (AFD 2022). Recently we found unwanted *C. fuscipes* in an Elizabeth Macarthur Agricultural Institute research insectary. We thought it a possible exotic, so did DNA barcoding followed by a phylogenetic relationship analysis. Here we present the output of that investigation.

METHODS

Samples were collected in Elizabeth Macarthur Agricultural Institute (EMAI) insectary cultures. Total DNA was extracted from the whole body of individual adult specimens with 5% Chelex®100 (Sigma-Aldrich®) based on the manufacturer's instructions with some modifications. The Cytochrome *c* Oxidase Subunit I (COI) was then amplified by Polymerase Chain Reaction (PCR), using the universal primers LCO1490 and HCO2198 (Folmer *et al*. 1994). PCR was performed in a 25 µL reaction containing 1× MyFi™ Mix (Bioline, Australia), ~20 ng DNA and 0.2 µM each primer. Thermal cycling conditions were as follows: initial denaturing step of 94 °C for 1 min, followed by 5 cycles of 94 °C for 30 s, 45 °C for 1 min 30 s, 72 °C for 30 sec, then followed by a further 35 cycles of 94 °C for 30 s, 51 °C for 1 min 30 s, 72 °C for 1 min, with a final extension step for 5 min at 72 °C. PCR products were purified using magnetic beads AMPure XP (Beckman Coulter Life Sciences, Australia). DNA sequencing was performed with ABI PRISM BigDye terminator cycle sequencing Version 2.0 by Macrogen Inc. (Korea). Sequence quality analysis and editing were performed using SEQUENCHER 5.4.6 software (Gene Codes, Ann Arbor, MI, USA). Edited sequences were deposited as accessions at GenBank and submitted to the Identification Engine at BOLD (Barcode of Life Data System) v3 to return a list of nearest matched existing DNA barcode sequences (Ratnasingham and Hebert 2007). Sixty-nine *C. fuscipes* DNA barcode sequences obtained from BOLD were included with EMAI sequences in an alignment aligned by Muscle in Mega 6.06. A maximum likelihood phylogenetic tree was constructed to test the phylogenetic relationship of investigated sequences (Tamura *et al.* 2013).

RESULTS AND DISCUSSION

We sequenced three specimen samples collected from our insectary and obtained high quality COI sequences of 658bp (primer truncated) length. Two out of three sample sequences shared a haplotype (submitted to GenBank as accession number: MZ723345) and were 100% identical to publicly available *C. fuscipes* sequences at BOLD; the third sample sequence (GenBank accession number: MZ723346) was three nucleotides different and 99.85% match to the existing *C. fuscipes* sequences at BOLD. The COI maximum likelihood phylogenetic tree (Figure 1) showed very low genetic diversity within 71 tested sequences. There are only two to three nucleotides different between samples, with the overall mean distance of all sequence pairs being 0.34. The three Australian *C. fuscipes* COI sequences sit within tested sequences collected from other countries.

Mushroom fly, *Coboldia fuscipes* is one of the most important fly pests of Oyster mushroom (*Philomycus*

spp.) (Yi *et al.* 2008, Zhang *et al.* 2016). DNA-based phylogenetic analysis of EMAI collected *C. fuscipes* resulted in a low mitochondrial genetic diversity suggesting that the species experienced genetic bottlenecks prior to or early in the invasion process. Although it is present in most of Australia major states and territories (the Australian Capital Territory, New South Wales, Queensland, South Australia, Victoria and Western Australia) there is little reported about its distribution and damage to the Australian mushroom industry. Its establishment in the Sydney basin should be closely monitored as the region is the major mushroom production area in New South Wales.

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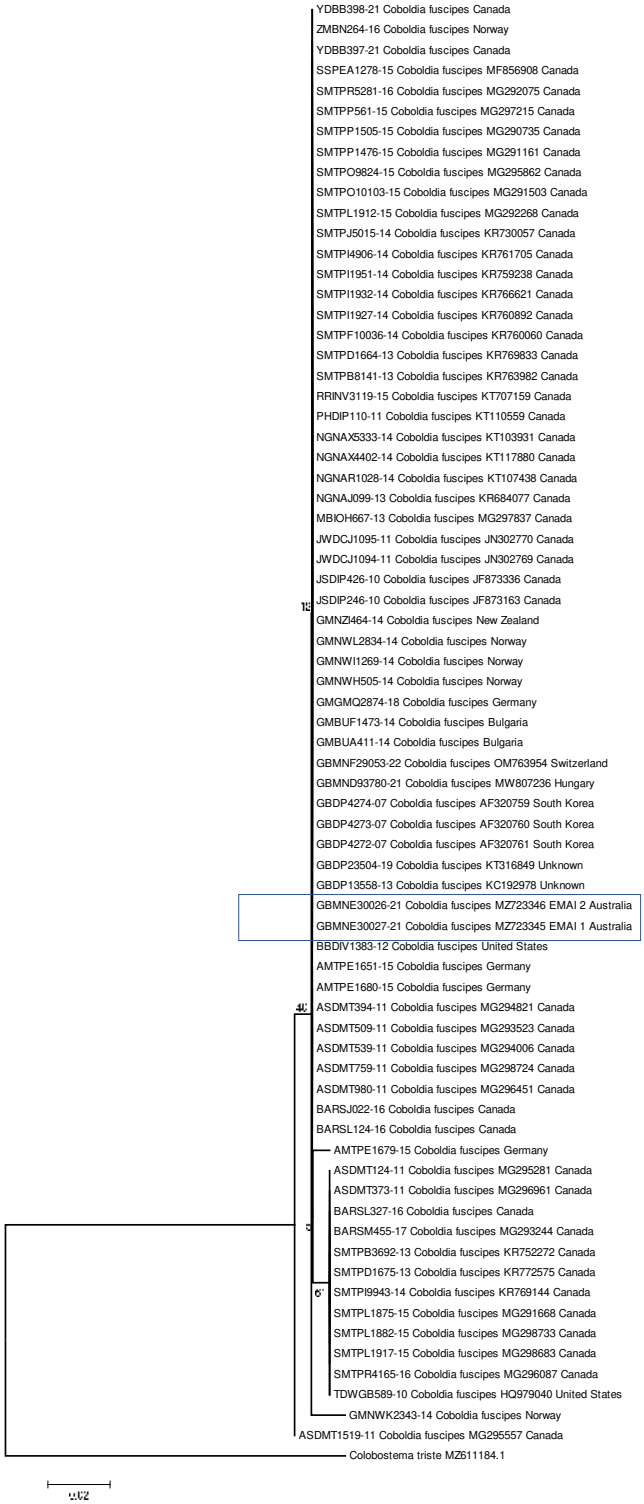

Figure 1. Maximum likelihood phylogenetic tree based on the COI gene (657bp) of *Coboldia fuscipes* constructed by Mega 6.06 (Evolution model Tamura 3-parameter). *Colobostema triste* (Gene bank accession MZ611184.1) is used as the outgroup