ENBE 2025

XXI International Meeting of the Portuguese Association for Evolutionary Biology

BOOK OF ABSTRACTS

18th-19th December 2025

Bragança

ENBE 2025

XXI International Meeting of the Portuguese Association for Evolutionary Biology

Bragança

Title: Book of abstracts of the XXI International Meeting of the Portuguese Association for Evolutionary Biology

Editors: M. Alice Pinto, Ana Rita Lopes & Dora Henriques

Published by: Instituto Politécnico de Bragança

ISBN: 978-972-745-368-9

Design: Ana Rita Lopes

ENBE 2025

XXI International Meeting of the Portuguese Association for Evolutionary Biology

18th-19th December 2025

Bragança, Portugal

Edited by

M. Alice Pinto

Dora Henriques

Ana Rita Lopes

Instituto Politécnico de Bragança, Portugal

December, 2025

POSTER 38| HARNESSING MITOCHONDRIAL DIVERGENCE IN *APIS MELLIFERA* TO DETERMINE THE ORIGIN OF MEDITERRANEAN HONEY

Honrado, M (1,2); Henriques, D (1); Santos, J (1); Yadró García, CA (1); MEDIBEES Consortium; Pinto, MA (1) Amaral, JS (1)

(1) CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; (*2*) LAQV-REQUIMTE & Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, Aveiro, 3810-193, Portugal

Keywords: *Apis mellifera*, honey, mitochondrial DNA, entomological origin

Abstract

The Mediterranean region holds one of the highest levels of *Apis mellifera* subspecific diversity, exhibiting a rich evolutionary history shaped by long-term geographic isolation, climatic variation, and, more recently, by human-driven processes. As part of the international project MEDIBEES, we developed a DNA-metabarcoding approach to characterise the entomological origin of honey, focusing on mitochondrial lineages A (African), M (Western European), C (Eastern European), and O (Middle Eastern). To build a robust reference database, DNA was extracted from 1280 honeybees representing 16 *A. mellifera* subspecies (*A.m. sahariensis, A.m. intermisa, A.m. siciliana, A.m. ruttneri, A.m. iberiensis, A.m. ligustica, A.m. macedonica, A.m. adami, A.m. cecropia, A.m. cypria, A.m. caucasica, A.m. meda, A.m. anatoliaca, A.m. syriaca, A.m. jemenitica, A.m. lamarcki*) and whole genomes were sequenced. Mitochondrial genomes were assembled using MitoZ, resulting in 769 assemblies, which were individually aligned against the reference mitochondrial genome using MEGA. Only mitogenomes corresponding to native *subspecific* ancestry were retained, producing a final curated database of 355 sequences. This database was then used to calculate the fixation index (F_{ST}) pairwise values, and a 400 bp sliding window was used to identify single-nucleotide polymorphisms (SNPs) that effectively differentiate (F_{ST}>0.98) the four lineages. Three promising regions emerged for primer design: one in the COI gene, one in the ND1 gene, and one in the CYTB gene. The resulting primers were first validated using 36 honeybee samples of known lineage representing all 16 subspecies and subsequently applied to 83 honey samples from Lebanon, Jordan, Türkiye, Italy, Algeria, Malta, UAE, and Oman. Among the three markers, ND1 showed the highest discriminatory power, correctly assigning all reference honeybee samples to their respective lineage. The COI and CYTB primers also showed strong potential, and the observed error rates (5.8% in COI and 1.7% for CYTB) provide useful thresholds for interpreting the DNA-metabarcoding results in honey samples. Overall, this study demonstrates how patterns of mitochondrial divergence shaped by long-term evolutionary processes can be harnessed for applied DNA-metabarcoding on food authenticity.

Acknowledgements/Funding

M. Honrado and C. Yadró acknowledge the PhD scholarship from FCT (2021.08119.BD, 2021.06948.BD). This research was funded by the project MEDIBEES - Monitoring the Mediterranean Honey Bee Subspecies and their Resilience to Climate Change for the Improvement of Sustainable Agro-Ecosystems. MEDIBEES is part of the PRIMA programme supported by the European Union. This research was also supported by national funds through FCT/MCTES (PIDDAC): CIMO UID/00690/2025 (10.54499/UID/00690/2025) and UID/PRR/00690/2025 (10.54499/UID/PRR/00690/2025); SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020).