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Serracanta Fernández, Maria. COI DNA barcoding in ants, keystones of the global Earth ecosystem. 2022. 1 pag. (833 Grau en Genètica)

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COI DNA barcoding in ants, keystones of the global Earth ecosystem

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1. Introduction

With >13.000 species that represent ~15% of the total terrestrial biomass, **Ants** (*Hymenoptera: Formicidae*) are one of the most diverse and ecologically relevant groups of organisms. In the face of the ongoing planetary crisis, ant cataloguing and quantification has become a priority on the conservation agenda. There is currently an urgent need to test the validity of identification methods [1–3].



Mymrica rubra worker

COI (Cytochrome oxidase subunit1) **DNA barcoding** is a theoretically and methodologically sound species identification tool [4]. It uses small amounts of DNA obtainable from eggs, tissues, or organismal traces to determine the identity of the source species by means of comparative sequence analysis. Currently, the method has become the gold standard in ant diversity characterisation across the world. However, the diagnostic potential of the method has not yet been fully explored [5].

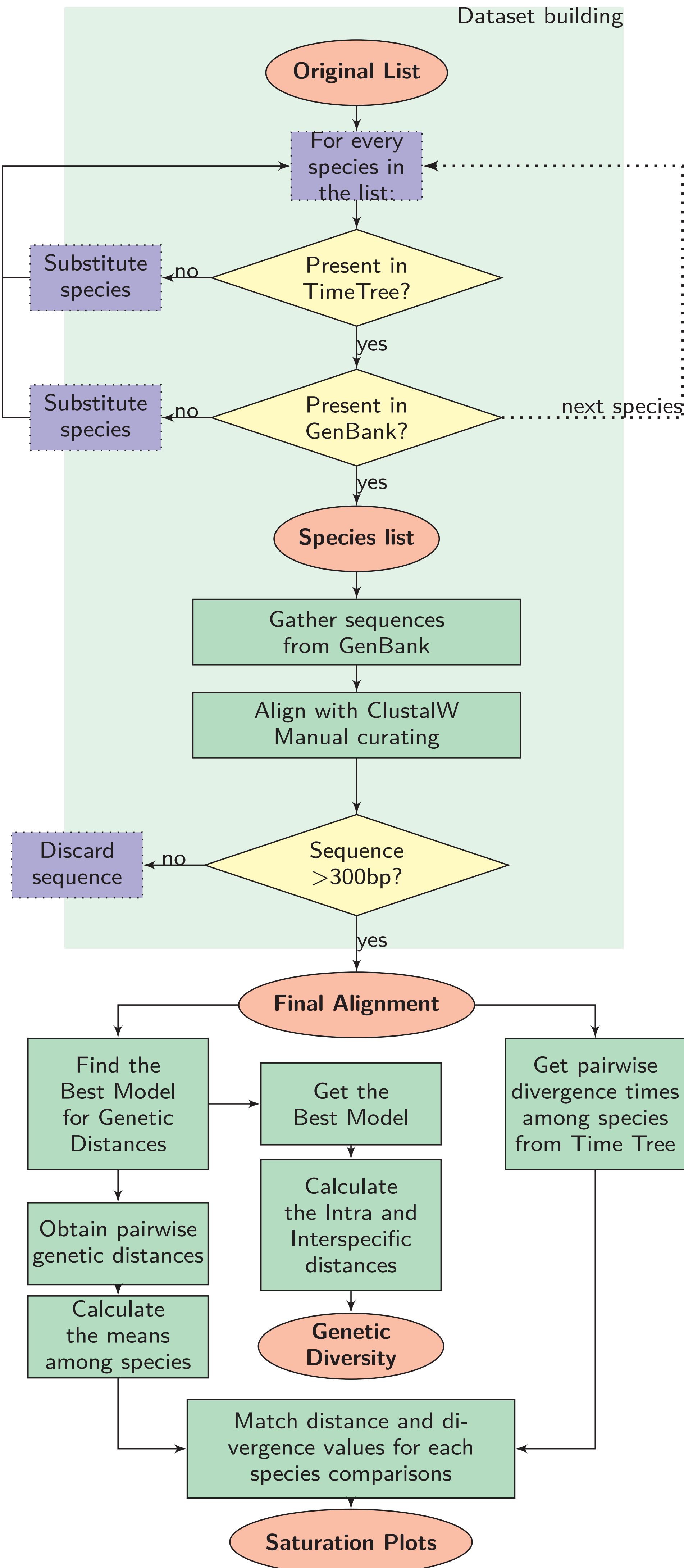
Objective: To test the ant cataloguing utility of the COI DNA barcode from shallow (inter-specific) to deep (inter-subfamilial) levels of evolutionary divergence applying evolutionary models.

2. Methods

2.1 Literature Research

- Databases: PubMed and ResearchGate
- Keywords: *Ants*, *Formicidae*, *Barcoding*, *COI*, *CO1*, *Cytochrome oxidase*, and combinations thereof using boolean operators AND, OR, NOT.

2.2 Bioinformatic Pipeline



Self-coded Python and R scripts were applied.

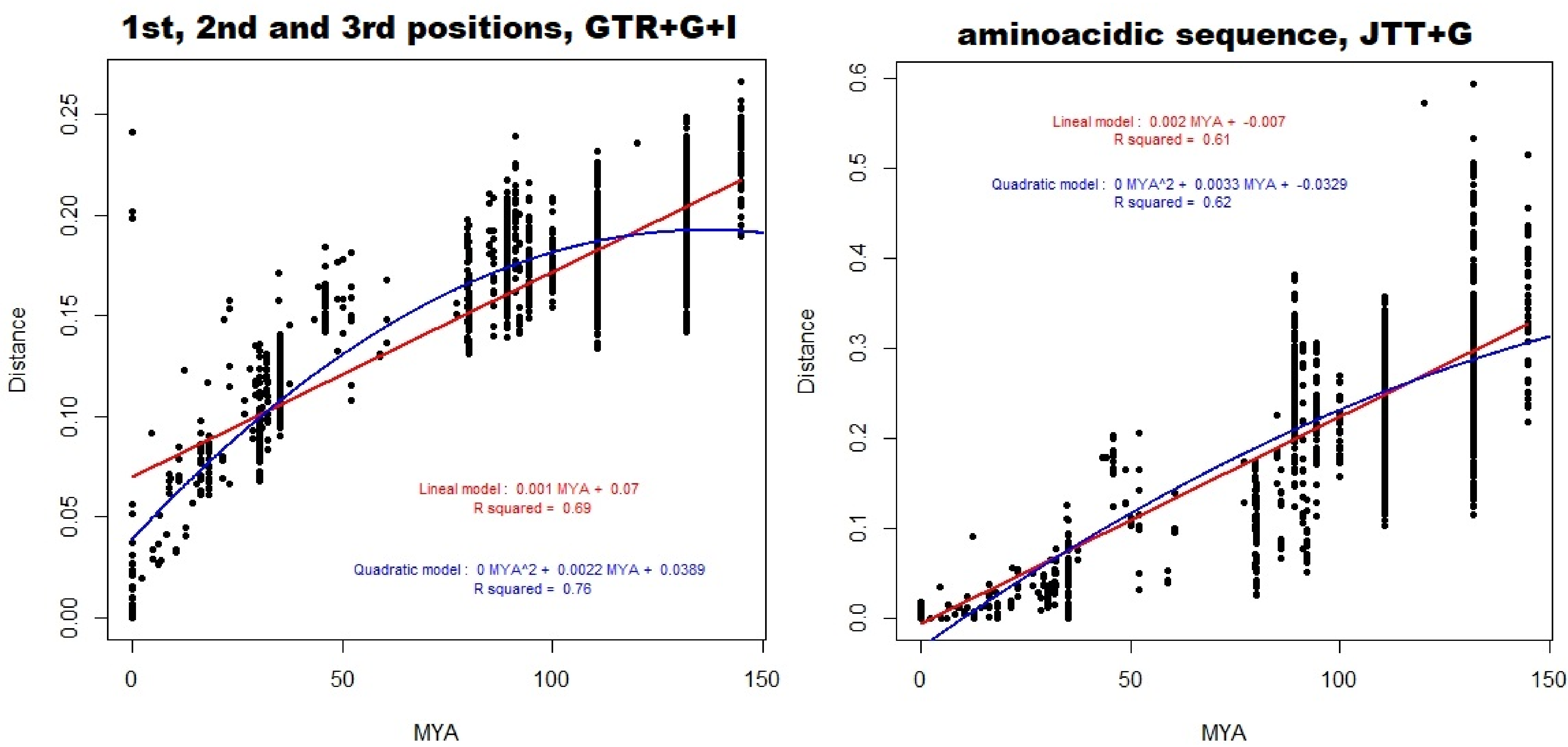
All figures self-generated

3. Modelling of Sequence Evolution

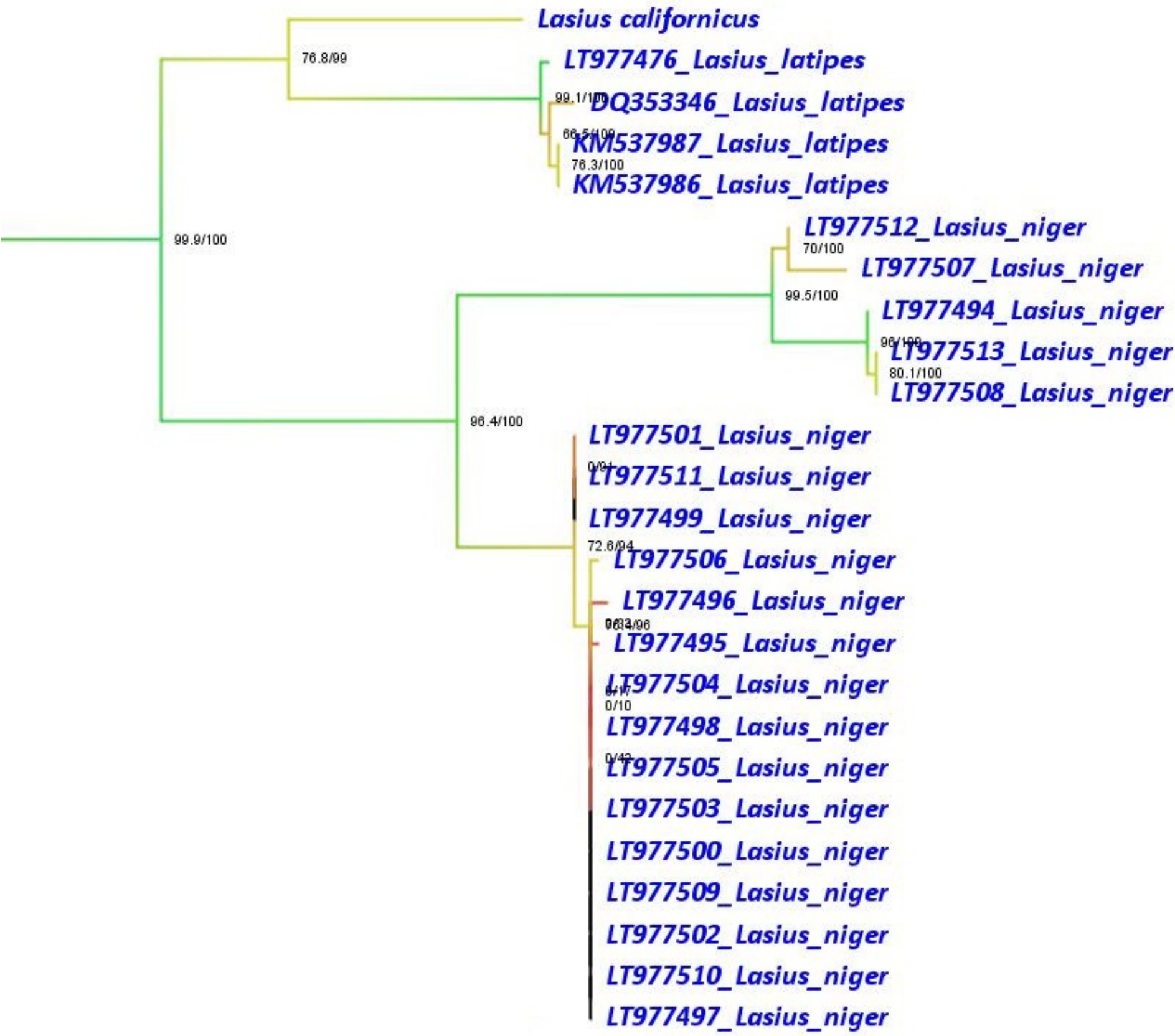
3.1 Saturation Analysis

ACATGT → Multiple-hits → ACATGT

3.2 Modelling Divergence against Time



4. Conclusions



1. The COI barcode succeeded at classifying at interspecies level.
2. Below genus, taxonomic classification is best performed using the **COI nucleotide barcode** under the best-fit **GTR+G+I** model. Above this rank, the model became increasingly unable to account for the accumulating unobserved substitutions.
3. The **COI amino acid barcode** under the best-fit **JTT+G** model showed robustness to the multiple hits problem, thereby is the approach of choice for ranks higher than genus.
4. Ant COI barcoding should be preceded by full sequence saturation analysis.

5. References

[1] S. Schär, M. Menchetti, E. Schifani, J. C. Hinojosa, L. Platania, L. Dapporto, and R. Vilà, "Integrative biodiversity inventory of ants from a sicilian archipelago reveals high diversity on young volcanic islands (hymenoptera: Formicidae)," *Organisms Diversity & Evolution*, 2020.

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[3] O. Skaldina and J. Sorvari, "Biomarkers of ecotoxicological effects in social insects," *Perspectives in Environmental Toxicology*, 2017.

[4] P. Hebert and A. Cywinska, "Biological identification through dna barcodes," *Proceedings of the Royal Society of London*, 2003.

[5] R. Collins and R. Cruickshank, "The seven deadly sins of dna barcoding," *Molecular Ecology Resources*, 2012.

GTR+G+I: General Time Reversal+Gamma Distribution+Invariant Sites, JTT: Jones-Taylor-Thornton