

DNA barcoding of hoverflies (Diptera Syrphidae) – new species discovery in the *Merodon aureus* species group



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Introduction

The hoverfly genus *Merodon* Meigen, 1803 is known for its significant role in pollination, which makes it extremely important for research, in the age of pollinator population decline. It is the richest hoverfly genus in Europe, with 120 currently recognized species (Vujić et al. 2015). Species discovery within genus is facilitated by the application of DNA barcoding approach based on the analysis of sequence divergence of the short fragment on 5' end of mitochondrial cytochrome c oxidase subunit I gene (COI) (Hebert et al. 2003a, b).

This study aimed to generate DNA barcodes (5' COI gene sequences) and identify specimens collected in Morocco, Italy, Turkey and Georgia to a species level within the *M. aureus* species group.

Material and methods

The genomic DNA was extracted following SDS DNA extraction protocol (Chen et al. 2010). The amplification of the 5' end of the COI gene was conducted using the LCO1490 and HCO2198 primer pair (Folmer et al. 1994). Sequences were edited for base-calling errors using BioEdit 7.0.9.0. (Hall 1999) and aligned manually. The number of haplotypes was estimated using DNAsp v6.10.01 (Rozas et al. 2017). Maximum Parsimony (MP) analysis was performed in NONA (Goloboff 1999) spawned with the aid of ASADO (Nixon 2008). Maximum Likelihood (ML) tree was constructed in RAxML 8.2.8 (Stamatakis 2014) using the CIPRES Science Gateway web portal (Miller et al. 2010).

Results

We identified in total eight 5' COI gene haplotypes of 21 unidentified hoverfly specimens from Morocco, Italy, Turkey and Georgia, four of which belong to Moroccan specimens, two to Italian specimens, one corresponds to Turkish specimens and one to the specimen from Georgia. The blast search of the NCBI nucleotide database using those haplotypes showed the highest nucleotide sequence similarity with species belonging to the *Merodon aureus* species group.

To identify analysed specimens to a species within the *M. aureus* species group we applied methods of phylogenetic tree construction Maximum parsimony (MP) and Maximum likelihood (ML). The produced trees are similar in topology (Figure 1).

Discussion

In this study we discovered three new candidate species within the *Merodon aureus* species group based on analyses of the 5' COI gene sequences. All three species are resolved as reciprocally monophyletic clades with high bootstrap support values (100). Two of these, *M. sp. nova1* and *M. sp. nova2* have not been morphologically characterized, but the third, *M. aff. bessarabicus*, is morphologically very similar or cryptic to *M. bessarabicus* (A. Vujić, personal communication, 2020). Despite high morphological similarity, *M. aff. bessarabicus* is clearly genetically divergent from the latter species. Such high discordance between molecular and morphological divergence is unusual and can be either because cryptic species are differentiated by nonvisual mating signals and/or because of evolutionary mechanisms leading to

morphological similarity comprising recent divergence, niche conservatism and morphological convergence (Bickford et al. 2006; Fišer et al. 2018). Thus, additional analyses of subtle changes in morphological characters, behavior, ecology and geography are important to resolve cryptic species (Goulding and Dayrat 2016; Padial et al. 2010).

The juvenile specimen from Georgia (AU1647) is related to specimens from Turkey. It is placed within the same clade with Turkish specimens and probably also belongs to *M. aff. bessarabicus*. However, additional specimens from Georgia and Turkey will be needed to confirm its species status.

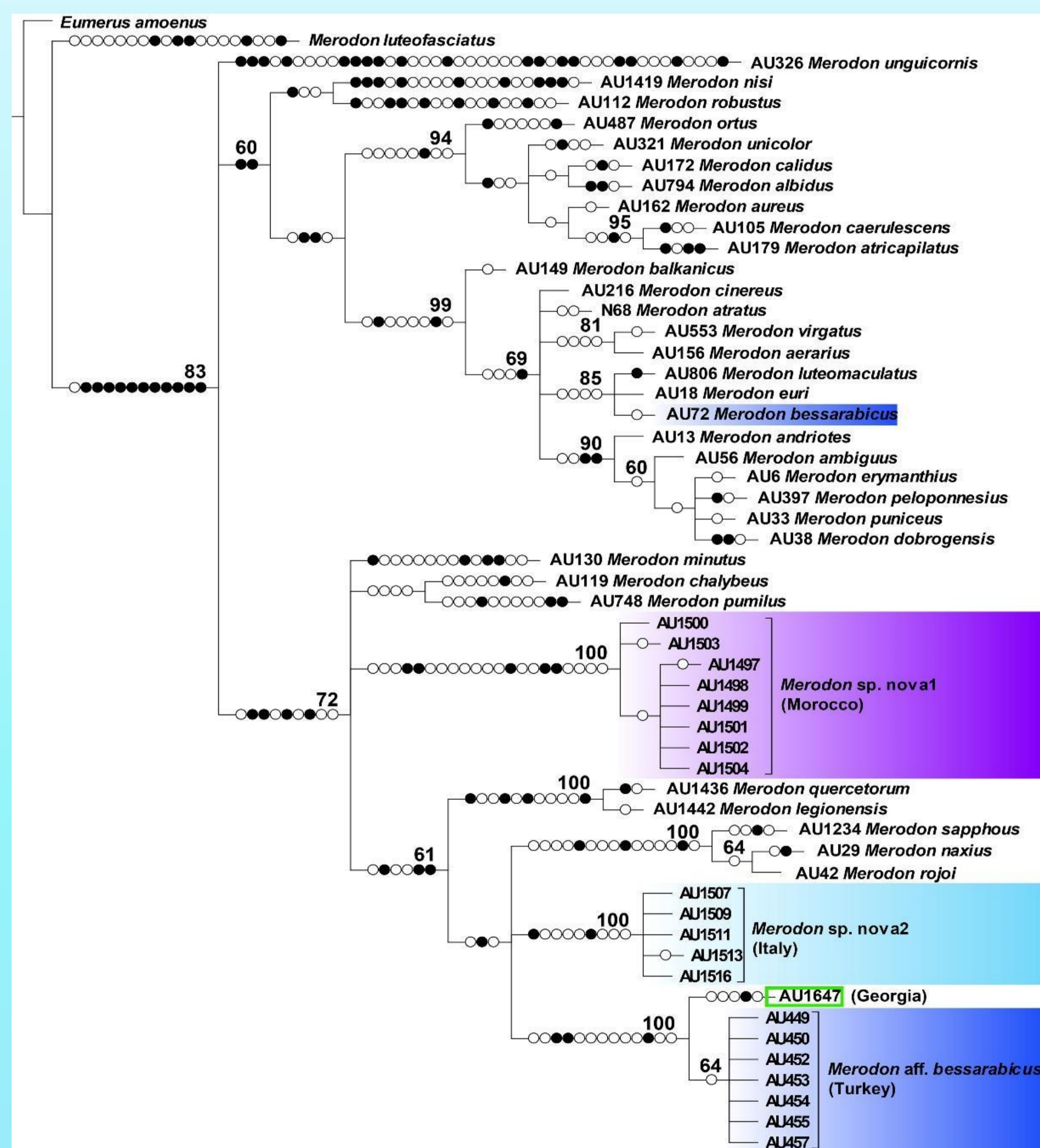


Figure 1. Strict consensus COI tree of six equally parsimonious trees for the *Merodon aureus* species group. Bootstrap values ≥ 50 are presented near nodes. Filled circles ● stand for unique changes, open circles ○ stand for non-unique changes.

Conclusions

In order to preserve existing diversity of pollinators and prevent further decline in their numbers it is important to accurately estimate their total diversity.

In this study, once again, we have shown the importance of molecular analysis in the discovery and delimitation of hoverfly species, and provided additional insight into the complex taxonomy of the *Merodon aureus* species group.

