# New species discovery using DNA barcoding approach – a case study in *Merodon aureus* group (Diptera: Syrphidae)

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## INTRODUCTION

Hoverflies (Diptera, Syrphidae) are recognized as insect pollinators of exceptional importance in both natural and agricultural ecosystems. Considering the accelerating pollinators decline, the accurate taxa identification within this insect family is prudent. Nevertheless, due to the presence of multiple cryptic taxa, resolving the *Merodon aureus* group of hoverfly species has been challenging. The traditional taxonomy based on the morphological approach has its limitations, especially in the cases of the cryptic taxa and morphological differences between developmental stages and / or sexes. However, the implementation of the integrative taxonomy approach involving molecular markers, geometric morphometry and ecological data helps to resolve species of the *M. aureus* group into subgroups and species complexes.

**With the aim to** identificate and delineate hoverfly specimens collected in Georgia to a species level within *M. aureus* group**,** the molecular analysis of both the 3' and 5' ends of the mitochondrial cytochrome c oxidase I gene (COI) was conducted.

## MATERIAL & METHODS

Total genomic DNA was extracted from insect mid and hind legs following SDS DNA extraction protocol described in Chen et al. (2010). We used C1-J-2183 and TL2-N-3014 primer pair for amplification and sequencing of the 3'COI (Simon et al. 1994), LCO1490 and HCO2198 (Folmer et al. 1994) for the 5' COI fragment. The edited and aligned 3' and 5' COI gene sequences were concatenated and combined into a single sequence matrix with the final length of 1260 bp, which was used as input for maximum parsimony and maximum likelihood tree constructions. Trees were rooted on both *Eumerus amoenus* and *Merodon luteofasciatus*.

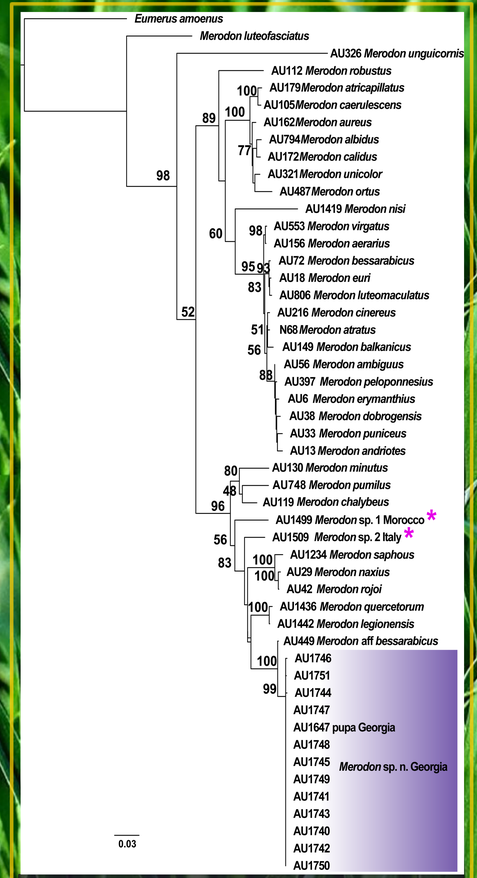


**Figure 1.** Maximum-likelihood COI tree of *Merodon aureus* species group constructed using RAxML 8.2.8 (Stamatakis 2014) through the CIPRES Science Gateway web portal (Miller et al. 2010). Bootstrap values ≥ 50 are presented near nodes. * for details please see Šašić Zorić et al. (2020).

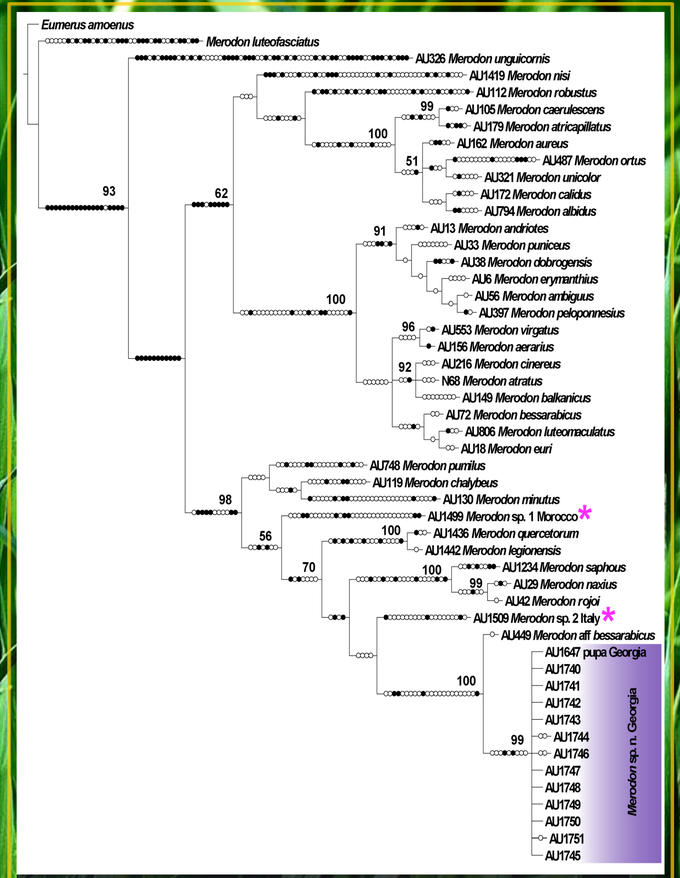


**Figure 2.** Strict concensus COI tree of four equally parsimonius trees for the *Merodon aureus* species group. Bootstrap values ≥50 are presented near nodes. Filled circles ● stand for unique changes, open circles ○ stand for non-unique changes. Analysis was preformed using NONA software (Goloboff 1999) implemented in Winclada ASADO (Nixon 2008). * for details please see Šašić Zorić et al. (2020).

## RESULTS

Specimens from Georgia are resolved as monophyletic on both maximum parsimony and maximum likelihood trees with high bootstrap support value (99). The BLAST search of the NCBI nucleotide database confirmed the belonging of these specimens to the *M. aureus* species group, while the tree topologies indicate that they were genetically most similar to the *M.* aff. *bessarabicus* from Turkey. Furthermore, this clade comprises the specimen in the juvenile stage from Georgia, that has not been successfully identified to a species level in the previous studies.

## CONCLUSION

In this study we discovered one new candidate species within the *M. aureus* group and defined the species status of the juvenile specimen from Georgia. The new species morphologically belong to the *M. cinereus* subgroup. However, our study showed a high genetic similarity between this species and morphologically different *M.* aff. *bessarabicus* from Turkey which belong to the *M. bessarabicus* subgroup. Such high discordance between molecular and morphological divergence has already been noticed within the *M. aureus* group, and in this particular case, it is probably a result of the geographical proximity, as well as the introgression during the evolutionary past of the two species. In order to fully address causes of observed discordance additional morphological and molecular analyses will be needed.

**References:**

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