## 16S rRNA gene sequence based Wolbachia screening in Merodon hoverflies

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## Abstract

Wolbachia is a genus of bacterial endosymbionts found in arthropods and nematodes with the broadest range of host reproductive phenotypes. It can affect the pathways through which mitochondria are inherited and influence mitochondrial variation, including DNA barcodes. This way Wolbachia can compromise mitochondrial gene-based identification system. The first record of Wolbachia in Merodon hoverflies was published in Šašić Zorić et al. (2019) when the COI-based relationships of the M. aureus species group were discussed in the light of infection. Assuming that Wolbachia has effects on COI sequence variability, in this study we aimed to introduce Wolbachia screening as an integral part of the multilocus DNA barcoding procedure for hoverflies. The screening was based on 16S rRNA gene sequences and was an integral part of the study dedicated to development of NGS based multilocus DNA barcoding for Merodon hoverflies. Sequencing was performed on Illumina's MiSeq sequencing platform. The amplicon sequence variants (ASVs) approach was used to recover the exact biological sequences. The results showed that Wolbachia was present in 40 Merodon species, while 16S sequences were not recovered for 20 species. Thus, the infection rate was 66.7 %. Additionally; nine Wolbachia positive species had multiple 16S haplotypes, up to eight in M. unicolor. Multiple 16S haplotypes indicate possible infection by multiple Wolbachia strains, however without sequencing Wolbachia multilocus sequence typing (MLST) genes and comparison with PubMLST database (https://pubmlst.org) it is not possible to determine strains. Based on the results we can conclude that Wolbachia is present in many Merodon species and this should be taken into account when delimiting and identifying Merodon species.

**Keywords:** bacterial endosymbiont, DNA barcode compromised, NGS

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