Abstract

The aim of this pilot study was to investigate the potential of eDNA techniques to detect the presence of the two dragonfly species *Cordulegaster heros* and *Cordulegaster bidentata*. Both species are classified as "near threatened" according to the IUCN Red List and are strictly protected in several countries. Monitoring these species with traditional sampling methods is often difficult, time-consuming and invasive. In this pilot study, we first collected tissue samples from *C. heros* and *C. bidentata* to sequence the traditional DNA-barcode gene fragment COI. We then collected further dragonfly COI sequences from BOLD to design species-specific primers. This, however, was impossible given the enormous variability of COI. Therefore, we refrained from species-specific eDNA assays and followed eDNA metabarcoding protocol using universal (BF2/UF2) and a newly designed dragonfly specific primer. For the evaluation of the method, we took water samples from places where *Cordulegaster* specimens are known to occur. After the extraction, we used two sequential PCR steps for obtaining the desired amplicon (two-step PCR) using universal primers in the first step, and group (dragonfly) specific primers or universal primers. Amplicons were sequenced on an Illumina MiSeq platform and then analysed the data with the JAMP pipeline. With the newly designed primers and we could effectively detect the
targeted dragonfly species from tissue samples, and also from filtered environmental samples. The detection of the species with the traditional method is time consuming and involves the destruction of the specimens. In comparison, with the eDNA method we could easily detect these near threatened odonates and other dragonfly species in a non-invasive way.

**Keywords**

eDNA, Odonata, new primer, metabarcoding

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