Filling gaps in the DNA barcode library - Aquatic insects (Ephemeroptera, Plecoptera, Trichoptera) from semi-mountainous and mountainous rivers of Ecoregion 6 (North Macedonia)

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Abstract

This research provides pivotal molecular genetic data on the community structure of aquatic insects from semi-mountainous and mountainous rivers from the 6th Ecoregion that belongs to the territory of North Macedonia. The aim of this research is to fill the gaps for barcoding the aquatic macroinvertebrates from the Balkan Peninsula and check if the existing barcode library could provide improved identifications for the specimens that were not taxonomically determined to the lowest level possible. We analyzed 95 specimens from which total DNA was extracted and the COI barcode region amplified and sequenced. The taxa were selected from 20 different localities of the territory of western part of North Macedonia. The selected specimens were not determined to species-level in order to test the efficiency of the DNA barcoding methodology and what is missing in the DNA barcoding data library.

From the result from one plate (95 specimens) we obtained: 16 samples without barodes, or failed and 10 samples did not have a match in the BOLD database. In the remaining 69 samples, three were misidentified. In the total of 69 barcoded species new for the fauna of North Macedonia, 11 are mayflies: *Baetis melanonyx*, *Ecdyonurus vitoshensis*, *E. macani*;
stonfly *Isoperla vjose*; and caddisflies: *Agapetus delicatulus, Athripsodes bilineatus, Glossosoma klotho, Lepidostoma basale, Helicopsyche bacescui, Tinodes unicolor* and *Odontocerum hellenicum*. We have also four rarely found species: *Zwicknia bifrons, Drussus tenellus, Hydropsyche botsaneanui* and *Hydropsyche bulbifera*, and one species without barcode available as Ecdyonurus sp. SK2 (potential new species). We found 83% efficiency of DNA barcoding, where some samples failed or were with low or medium quality for some specimens, as for the representatives from the genera *Baetis, Oxietyra* and *Rhyacophila*.

In conclusion we can confirm that 10 of the selected vouchers need to be further identified by morphology and to be added in the BOLD barcode library, and maybe we’ll have the possibility to describe a new species as well.

**Keywords**

Macroinvertebrates, new records, Macedonian fauna, mayflies, stoneflies, caddisflies

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