



Conference Abstract

The first step towards a DNA barcode reference database for mayflies (Ephemeroptera) of Slovakia

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Abstract

Mayflies (Ephemeroptera) represent a small but diverse order of amphibiotic insects, whose larvae contribute to several essential processes in freshwater habitats, such as bioturbation and bioirrigation, decomposition, nutrient cycling, and also serve as a primary source of nutrients for numerous organisms. Due to their cosmopolitan distribution and high-quality water requirements, they are also important indicators of ecosystem health and an integral part of biomonitoring protocols. Although the Slovak mayfly fauna is well researched, studies on genetic diversity, including DNA barcoding, are still lacking. The absence of the comprehensive DNA barcode reference libraries from various biogeographical regions and the presence of so-called cryptic lineages may prevent further efficient use and application of new approaches to aquatic ecosystem biomonitoring (Biomonitoring 2.0) based on eDNA analyses. Therefore, in the initial stage of our research, we bring the first insight into the genetic diversity of mayflies (based on mtDNA COI-5P barcoding fragment) from 47 localities of Slovakia mostly situated in the biogeographically significant Western Carpathians' territory. A total of 403 sequences of 42 morphologically determined species were added to the BOLD (Barcode of Life Data System) database, representing more than 1/3 of the mayfly fauna of Slovakia and covering 10 of 16 families. Sequences of these species were finally assigned to 62 BINs (Barcode Index Numbers) in BOLD (Fig. 1), whereby sequences of 12 species were divided into more than one BIN, indicating the presence of cryptic lineages. The largest

number of BINs was represented by widely distributed species such as *Baetis rhodani* Pictet, 1843-1845 (6 BINs), *Habroleptoides confusa* Sartori & Jacob, 1986 (4 BINs) and *Ecdyonurus venosus* (Fabricius, 1775) (3 BINs). The sequences of the remaining nine species were split into two BINs. Maximum intraspecific variability (calculated by K2P) of some representatives was surprisingly high [e.g., *E. venosus* – 27.1 %; *Baetis muticus* (Linnaeus, 1758) – 23.6 %; *Caenis luctuosa* (Burmeister, 1839) – 23.34 %, *Baetis rhodani* – 18.66 % and *B. vernus* Curtis, 1834 – 15.25 %] and far exceeded the level of intraspecific variability of the COI fragment based on the BOLD standards. The sequences of 23 individuals determined as *Habroleptoides confusa*, *Baetis rhodani*, *B. buceratus* Eaton, 1870, *Caenis beskidensis* Sowa, 1973 and *Torleya major* (Klapálek, 1905) created seven unique BINs, which represent the distant phylogenetic lineages of already existing BINs, that are currently unique to Slovakia. The coexistence of *Baetis rhodani* individuals of two different BINs was confirmed at five localities. Our study indicates clear importance of more detailed sampling and DNA barcoding due to the presence of unexpected intraspecific genetic diversity of mayflies captured in a relatively small area of the Western Carpathians.

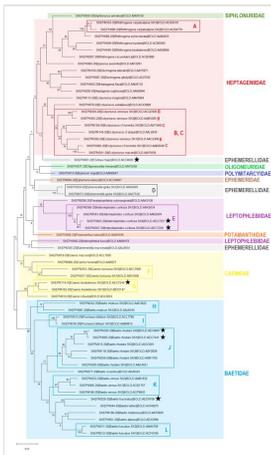


Figure 1. [doi](#)

Maximum-likelihood cladogram of 62 BINs (42 species, 10 families) based on the COI analyses using the GTR model. Individual branch nodes correspond to bootstrap analyses (1000 permutations). Rectangles A – L highlight species representing more than one BIN, and the asterisk indicates unique BINs created in the BOLD database.

Keywords

Mayflies, Slovakia, DNA barcoding, COI, BIN, Cryptic lineages

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