MID-COI: a curated reference database for DNA-based monitoring of Central Italian freshwater macroinvertebrates

Belli B[1], Giusto C[1]*, Marcellini L[1], Canestrelli D[1], Bellati A[1]
*lead presenter: chiara.giusto@unitus.it
1 Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy

The Mediterranean Basin hosts particularly high levels of cryptic diversity due to the complex biogeographic history of the Apennine Peninsula. Within freshwaters, such hidden diversity represents a major challenge for biomonitoring programs based on benthic macroinvertebrates, which are widely used to assess ecological status under the EU Water Framework Directive. In this context, environmental DNA provides rapid and non-invasive tools for fine-level biodiversity assessment; however, their reliability strongly depends on the reference database used for taxonomic assignment in bioinformatic pipelines. Here, we developed a regional cytochrome c oxidase subunit I reference library focused on freshwater macroinvertebrates (MID-COI; Macro Invertebrates DNA reference library for the river benthos of Central Italy), including more than 600 taxonomically and geographically validated COI sequences. The library integrates selected public barcode records from BOLD with newly generated sequences and associated metadata, allowing the characterization of haplotypic diversity. Phylogenetic analyses showed a clustering pattern consistent with recognized morphology-based groups, supporting the taxonomic coherence of the reference database. On the other hand, the analysis of intraspecific distances and barcoding gap exploration revealed marked genetic structuring in several lineages, notably Ephemeroptera and Amphipoda, suggesting widespread cryptic diversity in Central Italian freshwaters. The absence of a consistent barcoding gap across taxa further indicates that fixed genetic-distance thresholds may lead to incomplete detection in metabarcoding studies. Overall, MID-COI supports more accurate taxonomic assignments, improved biodiversity detection, and stronger ecological assessment tools for Central Italian river systems.