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DNA metabarcoding reveals overlooked freshwater Gastrotricha diversity

Freshwater Gastrotricha are among the least documented components of the freshwater meiofauna, and their diversity is likely substantially underestimated. We evaluated their diversity in Italian inland waters using DNA metabarcoding and compared the molecular results with taxonomic identifications based on light microscopy. Twelve freshwater lentic biotopes distributed across four Italian regions were surveyed. From each biotope, bulk sediment samples were collected for taxonomic identification by light microscopy, while sediment cores (upper 5–10 cm) were collected for DNA metabarcoding. Meiofaunal organisms were separated from the sediment by Ludox density-gradient centrifugation, recovered by filtration on a 32-µm mesh sieve, and processed for DNA extraction. Two mini-barcode markers targeting the V4 region of the 18S rRNA gene and a portion of the Folmer mtCOI fragment were amplified and sequenced using Illumina MiSeq paired-end technology. Amplicon sequence variants (ASVs) were generated in QIIME2 and taxonomically assigned using marker-specific bioinformatic pipelines and curated reference databases. DNA metabarcoding recovered gastrotrich ASVs from every surveyed biotope, including five in which no Gastrotricha were detected by light microscopy and one dry pond, demonstrating its ability to reveal diversity overlooked by morphological analyses. The 18S and COI datasets yielded 401 and 327 gastrotrich ASVs, respectively. Taxonomic resolution improved substantially through the use of curated reference databases, allowing more reliable assignments while highlighting current gaps in molecular reference data for freshwater Gastrotricha. Overall, DNA metabarcoding proved to be a powerful tool for assessing freshwater gastrotrich diversity, substantially expanding biodiversity detection compared with light microscopy alone. Our findings demonstrate the value of integrating molecular and morphological approaches to document overlooked components of freshwater meiofaunal communities and highlight the need to further expand curated molecular reference databases for Gastrotricha.