Hidden in the Sediment: DNA Metabarcoding Reveals Freshwater Gastrotricha Diversity Across Italy

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Freshwater gastrotrichs are microscopic meiofaunal invertebrates within the phylum Gastrotricha, which currently comprises more than 900 described species in the orders Macrodasyida and Chaetonotida. Although 380 species are reported from freshwater habitats, much of their diversity likely remains undocumented. DNA metabarcoding can provide complementary data for a more comprehensive assessment of gastrotrich biodiversity. In this study, we applied a replicated sampling strategy combined with DNA metabarcoding to investigate freshwater gastrotrich diversity across 12 habitat types in four Italian regions. For each biotope, at least two sites were examined, and three sediment replicates were collected per site. DNA was amplified using two markers: a fragment of the 18S rRNA mini-barcode and a mtCOI mini-barcode. In parallel, all samples were analyzed using conventional morphological identification. Gastrotrich DNA was detected in all biotopes, including those where no specimens were observed morphologically or in dry sediment samples, highlighting the ability of metabarcoding to detect rare, cryptic, or low-abundance taxa, as well as resting stages. The molecular data revealed 525 gastrotrich amplicon sequence variants (ASVs), of which 139 (26.5%) were assigned to species or genus level, while the remainder were classified only at family or order level. The taxonomic assignment of mtCOI sequences was found to improve markedly, with a 14.1% increase in genus- or species-level classifications when using a custom database combining newly generated reference sequences with GenBank sequences, as opposed to a widely used curated database (MIDORI2). Overall, DNA metabarcoding proves to be a powerful approach for assessing gastrotrich biodiversity, although its full potential remains constrained by incomplete molecular reference data. Expanding taxonomic knowledge and reference sequence coverage is therefore essential to uncover hidden diversity and to achieve a more comprehensive understanding of freshwater meiofaunal communities.