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Millennial persistence of freshwater gastrotrich DNA in ancient pond sediments revealed by metabarcoding

Freshwater gastrotrichs are microscopic meiofaunal metazoans whose diversity is still poorly documented and whose representation in sedimentary ancient DNA (sedaDNA) studies remains largely unexplored. Here, we investigated whether DNA metabarcoding can recover gastrotrich signals from ancient freshwater sediments across millennial timescales, combining zoology, paleoecology, palynology, molecular biology, and bioinformatics.
A sediment core was collected from a dried pond within the Castelporziano Presidential Estate (Rome, Italy) and analysed at three dated horizons spanning the last ~5700 years (489, 1403, and 5690 years BP). Palynological evidence linked all three layers to freshwater wetland conditions through the presence of pollen from plant taxa characteristic of these habitats. DNA metabarcoding targeted two mini-barcode markers of the 18S rRNA and mtCOI genes. Taxonomic assignment was enhanced using a customized reference database combining publicly available sequences with additional freshwater gastrotrich sequences generated in our laboratory.
All gastrotrich ASVs recovered from the three layers belonged to freshwater taxa, fully consistent with the paleoenvironmental reconstruction based on palynological data. In total, 34 ASVs were assigned to Gastrotricha, of which 21 were identified to genus or species level using the customized database. The oldest layer displayed the highest genetic diversity. Notably, gastrotrich DNA was recovered from sediments dated to 5690 years BP, demonstrating the remarkable preservation potential of sedaDNA from freshwater meiofauna over millennial timescales. This finding raises the possibility that resistant life stages, such as resting eggs, may contribute to long-term DNA persistence, although further studies are needed to test this hypothesis. Overall, our results open new perspectives for reconstructing the long-term dynamics of overlooked freshwater meiofaunal communities and highlight the value of integrative zoology in extending biodiversity research beyond present-day ecosystems.