



DNA Barcoding and Morphological Identification of Benthic Nematodes Assemblages of Estuarine Intertidal Sediments: Advances in Molecular Tools for Biodiversity Assessment

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Concerns regarding the status of marine ecosystems have increased in part due to traditional and emerging human activities in marine waters, driving a demand for approaches with high sample throughput capability to improve ecosystem monitoring. Nematodes are already used as indicator species in biodiversity assessments and biomonitoring of terrestrial and marine systems, with molecular approaches offering the opportunity to utilize these organisms further in large scale ecological surveys and environmental assessments. Based on an available nematode dataset for estuarine sediments of the Mira estuary (SW coast, Portugal), we evaluated the diversity of the nematode community of this system, using the molecular markers 18S rRNA and COI genes. These approaches were compared to voucher specimens from a morphological characterization of the same samples allowing validation and comparison between nematode communities. The spatial and temporal variability of the density and diversity of the nematode assemblages was analyzed based on morphological characterization to allow the validation and efficiency of the genetic characterization. A PCO ordination plot showed a distinct separation of the assemblages between sampling occasions confirmed by PERMANOVA analysis, which showed significant differences, although no significant differences were detected between sampling sites. The morphological characterization identified 50 genera of which only 26 and 25 distinct 18S rRNA and COI DNA barcodes, respectively, were obtained. 90.2% of the morphologically identified specimens representing eleven different genera, successfully generated DNA barcodes for both 18S rRNA and COI genes. This study confirmed that the success of the 18S rRNA gene PCR amplification is higher than of COI gene with 43 species amplified against 34. The study highlights a limitation of available sequences for both targets in databases when compared to the known diversity of marine nematodes. The gene sequences of