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Development of molecular approaches to assess the diversity and abundance of marine nematodes assemblages for assessment of good environmental status of the estuarine and marine ecosystems

Ana Patrícia Avó ;Tim Daniell; Roy Neilson; Solange Oliveira & Helena Adão (pat.avo@uevora.pt)

In Europe, the concerns with the status of marine ecosystems have increased, and the Marine Directive has as main goal the achievement of Good Environmental Status (GES) of EU marine waters by 2020. Molecular tools are seen as promising and emerging approaches to improve ecosystem monitoring, and have led ecology into a new era, representing perhaps the most source of innovation in marine monitoring techniques. Benthic nematodes are considered ideal organisms to be used as biological indicator of natural and anthropogenic disturbances in aquatic ecosystems underpinning monitoring programmes on the ecological quality of marine ecosystems, very useful to assess the GES of the marine **Terminal-Restriction** dT-RFLP (directed environment. Fragment Polymorphism) allows to assess the diversity of nematode communities, but also allows studying the functioning of the ecosystem, and combined with relative realtime PCR (qPCR), provides a high-throughput semi-quantitative characterization of nematode communities. These characteristics make the two molecular tools good descriptors for the good environmental status assessment. The main aim of this study is to develop and optimize the dT-RFLP and qPCR in Mira estuary (SW coast, Portugal).

A molecular phylogenetic analysis of marine and estuarine nematodes is being performed combining morphological and molecular analysis to evaluate the diversity of free-living marine nematodes in Mira estuary. After morphological identification, barcoding of 18S rDNA and COI genes are being determined for each nematode species morphologically identified. So far we generated 40 new sequences belonging to 32 different genus and 17 families, and the study has shown a good degree of concordance between traditional morphology-based identification and DNA sequences. These results will improve the assessment of marine nematode diversity and contribute to a more robust nematode taxonomy. The DNA sequences are being used to develop the dT-RFLP with the ability to easily process large sample numbers (hundreds and thousands), rather than typical of classical taxonomic or low throughput molecular analyses.