

Title: Development of a novel molecular tool for the rapid assessment of changes in biodiversity of benthic nematodes assemblages

Authors:

Ana Patrícia Avó - MARE- Marine and Environmental Sciences Centre, University of Évora, Portugal

Tim Daniell – Ecological Sciences, The James Hutton Institute, Dundee, DD2 5DA, Scotland, UK

Roy Neilson – Ecological Sciences, The James Hutton Institute, Dundee, DD2 5DA, UK

Solange Oliveira – ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, University of Évora, Portugal

Helena Adão - MARE- Marine and Environmental Sciences Centre, University of Évora, Portugal

Keywords: assessment of biodiversity changes, benthic nematodes, dT-RFLP, new molecular tool

Abstract:

Nowadays molecular approaches are being used in population estimation of terrestrial nematode communities offering a more efficient and faster alternative over microscopy-based methods. A molecular profiling tool was developed using directed Terminal-Restriction Fragment Length Polymorphism (dT-RFLP) to characterize soil nematode assemblages by relative abundance of feeding guilds, and validated by comparison with traditional morphological method. Combining morphological and molecular analysis of benthic nematodes assemblages, the main aim of this study was to develop and validate the dT-RFLP tool for benthic nematodes. Estimation of population size was derived using real time PCR (qPCR).

A molecular phylogenetic analysis of benthic nematodes was created based on a database of 18S rDNA sequences related to individuals identified to species level. dT-RFLP results showed that the digest strategy developed for soil nematodes was not suitable for benthic nematodes. A new dT-RFLP strategy for benthic assemblages was designed by using the sequence database coupled with cloning and sequencing the whole assemblage from five samples. Several solutions were identified by the DRAT software and tested empirically to select the optimum solution that separates the assemblages. qPCR results showed differences in gene copy number between two sampling sites, which is consistently with the results of nematode density obtained by traditional methods. The application of these high-throughput molecular approaches for benthic nematodes will improve sample throughput and their implementation more efficient and faster as an indicator of marine ecosystem health.