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Integrating molecular approaches to explore the diversity and biogeography of Mediterranean deep-sea polychaetes

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ABSTRACT

The Mediterranean Sea is one of the most studied marine areas in the world, however its deep ecosystems and its benthic fauna remain largely unexplored. Despite their crucial ecological role, polychaetes are still poorly understood from a taxonomic and biogeographical perspective, in particular molecular databases lack barcode sequences for Mediterranean polychaetes below 100 m depth. This PhD project aim to investigate the diversity and biogeography of deep-sea polychaetes in the western Mediterranean by integrating different molecular approaches: integrative taxonomy, barcoding and eDNA metabarcoding. Samples were collected from strategic locations, including canyons and seamounts. The first barcoding library for Mediterranean deep-sea polychaetes ('Deepoly') was constructed, with a total of 190 sequences (COI and 16S) belonging to 13 families and 44 species, revealing numerous cryptic species and species complexes that require further study. For the family Onuphidae, taxonomic uncertainties and species diversity were partially addressed in an integrative taxonomic study, which revealed the presence of two potentially endemic *Nothria* species, while distinct biogeographical affinities were observed for *Paradiopatra* species, showing that the "biodiversity pump" effect may also affect the deep Mediterranean biota. Using the 'Deepoly' reference library as a basis for taxonomic assignment, it was possible to establish a protocol for eDNA metabarcoding studies of Mediterranean deep-sea annelids. Despite challenges in primer design, bioinformatics pipelines and taxonomic assignments, the protocol allowed a genus-level characterisation of a rich polychaete community. The results highlighted the presence of two trophic levels – deposit feeders and predators - as well as significant small-scale species variability. This research has highlighted the usually overlooked diversity of Mediterranean deep-sea polychaetes. The Mediterranean is vulnerable to climate change and conservation strategies are needed. The complex biogeographic composition of the Mediterranean deep-sea fauna must be taken into account when planning conservation and management strategies for these unique environments. The integration of eDNA with conventional techniques provides critical insights for biodiversity monitoring and will enhance our ability to address environmental challenges to conserve deep-sea ecosystems.