Patterns of spatial and temporal variation in estuarine meiofaunal communities assessed through DNA metabarcoding: a case study in the Lima estuary (NW Portugal)

Maria Fais[1, 2, 3*], Sofia Duarte[1, 2], Pedro E. Vieira[1, 2], Ronaldo G. Sousa[1, 2], Mehrdad Hajibabaei[4], Carlos A. Canchaya[3] and Filipe O. Costa[1, 2]

- [1] Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Portugal
- [2] Institute of Science and Innovation for Bio-Sustainability, University of Minho, Portugal
- [3] Department of Biochemistry, Genetics and Immunology, University of Vigo, Spain
- [4] Centre for Biodiversity Genomics, Biodiversity Institute of Ontario, University of Guelph, Canada

Meiobenthic organisms are key components of estuarine environments, providing invaluable functions and services. In particular, meiofaunal organisms participate actively in nutrient cycles and energy flux, supporting higher trophic levels, as well as the stabilization of sediments. Due to their small size (between 30 μm and 1mm) and high dispersal potential, meiofauna was considered to have a ubiquitous distribution. However, with the recent progress of DNA-based technologies, in particular, DNA metabarcoding, the cosmopolitan meiofauna paradox is being questioned, suggesting that meiobenthic communities can comprise also endemic and narrow-range species. Several studies on the spatial variability of meiofauna at small-scale (within meters) and at meso-scale (meters to km) have been carried out in an attempt to elucidate community structure, both using traditional approaches (such as morphological identification) and DNA-based tools. All these studies focused on the importance of environmental features (i.e., salinity, sediments' size, percentage of organic matter) in the distribution of particular taxonomic groups, as well as of their bio-ecological characteristics, which may overall explain the spatial structure of the meiobenthic communities. However, little attention has been given to the temporal variation of meiofauna communities in estuaries, and to the best of our knowledge, no research on the spatial-temporal dynamics of Iberian estuarine meiofauna has been yet performed using DNA-

based tools. Although DNA metabarcoding is assumed to i) allow a faster detection, ii) identify hard morphologically-intractable meiobenthic organisms or communities, and iii) target different taxonomic groups simultaneously, further refinement is required to better tailor this method for meiofauna profiling. A well-defined choice of marker loci and primer pairs, and the design of robust protocols for sampling effort and for the bioinformatics data processing and analysis is needed to avoid the increase of technical bias during the workflow. The main goal of the present study was to investigate spatial and temporal variations of the estuarine meiofauna communities using DNA metabarcoding. Sediments were collected in four sampling stations in the Lima estuary (North-West of Portugal), in June 2017 and June 2018, from the first 5 cm of sediment directly to 50 mL sterile falcon tubes (3 cm ø). Three samples were collected at the high and middle intertidal zones in each sampling station. For each site and yearly sampling campaign, we assessed salinity, total organic matter (TOM) and grain size. DNA was extracted from 10.0 g (± 0.50 g) of sediment from each collected sample, using the DNeasy PowerMax Isolation kit (QIAGEN®). Two different primer pairs were used for the production of amplicon libraries and their high-throughput sequencing (Illumina-MiSeq): a sub-region from the mitochondrial cytochrome c oxidase gene (COI) and the V4 hypervariable region of the nuclear ribosomal 18S rRNA gene (18S). Raw reads were quality-filtered by the removal of sequencing adapters and trimming of low-quality bases. Further de-multiplexing and read processing were carried out in mothur, using customized procedures. Reads were clustered into OTUs using a 97% similarity threshold. Then, the representative sequences of each OTU were BLASTed against the GenBank non-redundant nucleotide database and taxonomically assigned in MEGAN v.6.13. In terms of OTUs richness and turnover, our results showed that our communities globally changed along space and time. Meiobenthic structure, in general, displayed well-separated temporal and spatial clusters, with some generalist OTUs occurring along with the stations for both sampling years. Qualitatively, the two primer pairs detected different taxonomic groups attributed to meiofauna in a complementary manner. Nematoda, Platyhelminthes, Annelida, Arthropoda Crustacea, Amoebozoa and Ciliophora were the most abundant phyla, with different temporal and spatial proportions. Our results indicated that Lima estuarine meiofaunal communities are strongly influenced by the salinity, sediment grain size, and total organic matter, affecting meiofauna distribution among the sampling stations. Moreover, further differences on a time scale were found between the sampling years, probably as a consequence of greater rainfall during 2018 (https://www.pordata.pt). With a careful sampling design, laboratory and data analysis protocols, DNA metabarcoding can constitute a powerful approach for meiobenthic communities profiling, although further efforts are required for preparing dedicated meiofauna references library.

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* Correspondence: Mx. Maria Fais, Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Braga, Braga, 4710-057, Portugal, maria_fais@hotmail.it