Università degli Studi di Urbino *“Carlo Bo”*
Dipartimento di Scienze Pure e Applicate (DiSPeA)
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PhD EVALUATION REPORT

To whom it may concern,
this report evaluates the PhD thesis of Nguyen Ngoc Loi entitled “*Exploring the marine biodiversity with environmental data*” and submitted to the Institute of Oceanology, Polish Academy of Sciences - Department of Paleoceanography (Poland). The thesis has been submitted on March 2024 and has been prepared under the supervision of Dr. Hab. Joanna Pawłowska-Tomkowicz and the co-supervision of Dr. Jan Pawłowski.

The present PhD thesis has been conceived as a collection of thematically related scientific publications (i.e., papers and Ms under revision). The thesis focuses on the potential exploitation of environmental DNA (eDNA) retrieved from marine sedimentary archives as an important tool by which reconstructing marine biodiversity and inferring short- and long-term responses of marine ecosystems to global and environmental changes. The PhD candidate organizes the thesis in six main chapters (i.e., summary, four research articles and a final synthesis with the recommendation for future works). I particularly appreciate causal link among consecutive chapters, where the open questions have been properly addressed. The candidate has correctly conceived the PhD thesis that is also very well written, organized, clear, concise, and easy to read. Indeed, the candidate has successfully published three of these chapters (i.e., Research Articles: RA II, III and IV) and submitted one (i.e., RA I).

The topics cover by the PhD thesis are definitively novelty and well-thought and represent a main advance of our knowledge in the field. In the abstract and summary, the candidate frames the background and the gaps that required to be filled in. Specifically, in the preface the candidate well-defines key questions that are then perfectly reflected in the PhD thesis’s structure and represent crucial points in such areas in light of the current demand for tools for evaluating marine eukaryote diversity and their exploitation in paleo-environmental reconstructions.
In the first chapter, the candidate after providing a general view on eDNA, briefly introduces purpose of research, the study area, the methodology for eDNA, the content of each RA and a conclusion. All RAs have been developed to tackle specific methodological questions. The statistical analysis applied for the RAs are very done and the candidate shows a complete capability

in performing advance statistical analysis using different software. Additionally, all the figures and tables have been well prepared and contain the minimum required information. The only very minor suggestion, if feasible, would be to add the supplementary figures at the end of the RAs or at the end of the PhD thesis.

In the second chapter that has been submitted in Molecular Ecology Resources, it is unveiled the potential bias of the eDNA preservation of plankton organisms by comparing surface, 100 m depth, bottom water and sediment. The present RA provides compelling evidence of the unequally preservation of plankton in the sediment, where nano- and pico-plankton are commonly underrepresented. The candidate also offers tentative explanations of it. It can be stressed that the candidate reported 40 h as half-time in offshore systems but Collins et al. (2018) reported 48 h for inshore waters and also stressed the difficultly to predict at very low eDNA concentrations. The candidate can also report/cite alternative estimation of sinking velocity like those using foraminifera (e.g., J Exp Biol. 2021 Jan 15; 224(2): jeb230961) or phytoplankton (/doi.org/10.1007/s00227-012-1945-2). Particularly, the latter provides sinking velocities quite slow for phytoplankton cells in the order of few centimetres per day; this further supports the lower capability of small phytoplankton organisms (i.e., pico- and nanoplankton) to settle down in the sediments and your statements.

The third chapter published in Geobiology in 2023 with the candidate as first author focuses on the high diversity of benthic foraminiferal metabarcodes in the Svalbard and how they reflect water masses and be used for their identification in a context of climate changes (e.g., Atlantification of the Arctic). The present RA provides evidence of a much higher diversity of benthic foraminifera in the Arctic than previously thought (i.e., based on more traditional morphological approach). It also sheds light on the different taxonomic composition among sediment fractions, the reason behind it and how the use of sieved or unsieved material could be tackled; this represents an important methodological advance in eDNA research and its application. Additionally, the RA documents the changes in α- and β-diversity of benthic foraminifera to track water masses by identifying potential ASVs bioindicators.

The fourth chapter published in PlosOne in 2024 provides a methodological tool, namely signature-based classification for increasing the assignment of the large unassigned foraminiferal metabarcodes in the deep-sea. This approach enables the recognition of 61 new lineages constrained in 27 clades. The RA also compares the distribution of them by comparing them with the available dataset (e.g., Pacific and Atlantic deep-sea areas and shallow water environments) and covering therefore a variety of marine settings. The candidate correctly reported that this methodological approach is not capable of solving the problem in the paucity of reference database but it can provide useful information on the distribution and ecology of deep-sea unassigned benthic foraminiferal taxa.

The fifth chapter has been published in Frontiers in Marine Science and sees the candidate as first author. Here, the candidate presents a sort of review-scientometric paper where the implications, opportunities, limitations and trends of sedaDNA. It is really appreciated the network analysis with VOSviewer but it would have been, at least tentatively, interpreted the clusters (i.e., different colours) and also used the keyword co-occurrence network map with respect to total link strength with the score of the average publication year of the documents. Regardless of it, the RA is still very informative and represents a comprehensive view by stressing the potential biases during each step from coring to bioinformatics.

Despite some very minor points within PhD thesis, I do recognize the great efforts by the candidate in collecting a large body of data and evidence, his critical skill and problem solving, the capability of performing a set of analyses and his good overview over the literature. In light of the above and based on my expertise, I do think that the present PhD thesis is worthy of defence and the candidate absolutely masters the topic.

If you have any doubt regarding my evaluation, please do not hesitate to contact me.

Yours sincerely,

Prof. Fabrizio Frontalini PhD
Dipartimento di Scienze Pure e Applicate (DiSPeA) - Università degli Studi di Urbino "Carlo Bo"
Campus Scientifico Enrico Mattei, Località Crocicchia, 61029 Urbino - Italy
fabrizio.frontalini@uniurb.it - Tel: (+39) 0722 304309 Mobile phone: (+39) 3928457666
International School on Foraminifera Urbino www.isf.tmsoc.org/