UNIVERSITÉ DE GENÈVE
FACULTÉ DES SCIENCES

SECTION DE BIOLOGIE
DEPARTEMENT DE GENETIQUE
ET EVOLUTION

PD. Dr. Maria Holzmann
Lecturer and Research Assistant

Ligne directe: + 41 22 379 30 84
E-mail: Maria.Holzmann@unige.ch

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Review of the doctoral dissertation of Nguyen Ngoc Loi

The review was carried out at the request of PD. Dr. Joana Pawlowska-Tomkowicz and Prof. Dr. Jan Pawlowski.

The document is a doctoral thesis by Nguyen Ngoc Loi titled "Exploring the Marine Biodiversity with Environmental DNA," prepared under the supervision of PD. Dr. Joanna Pawłowska-Tomkowicz and the co-supervision of Prof. Dr. Jan Pawłowski at the Institute of Oceanology, Polish Academy of Sciences. The thesis consists of a collection of thematically related scientific publications and is divided into several main sections.

A. Structure and language of the dissertation

The work is presented in English with extensive summaries in English and Polish. It consists of a series of four publications, three of which have been published in 2022-2024 and one that is submitted. The dissertation includes information about fundings and collaboration. The following publications are included in the dissertation:

1. **Taxonomic and abundance biases affect the record of marine eukaryotic plankton communities in sediment DNA archives.** Ngoc-Loi Nguyen, Joanna Pawłowska, Marek Zajaczkowski, Agnes Weiner, Tristan Cordier, Danielle Grant, Stijn De Schepper and Jan Pawłowski. Submitted to Molecular Ecology Resources.
2. **Metabarcoding reveals high diversity of benthic foraminifera linked to water masses circulation at coastal Svalbard.** Ngoc-Loi Nguyen, Joanna Pawłowska, Inès Barrenechea Angeles, Marek Zajaczkowski and Jan Pawłowski. Published in Geobiology, 21(1), 133–150 (2023).
3. **Assigning the unassigned: a signature-based classification of rDNA metabarcodes reveals new deep-sea diversity.** Inès Barrenechea Angeles, Ngoc-Loi Nguyen, Mattia Greco, Koh Siang Tan, and Jan Pawlowski. Published in PLoS One, 19(2), e0298440 (2024).
4. **Sedimentary ancient DNA: a new paleo-genomic tool for reconstructing the history of marine ecosystems.** Ngoc-Loi Nguyen, Dhanushka Devendra, Natalia Szymańska, Mattia Greco, Inès Barrenechea Angeles, Agnes K. M. Weiner, Jessica Louise Ray, Tristan Cordier, Stijn De Schepper, Jan Pawłowski and Joanna Pawłowska. Published in Frontiers in Marine Science 10:1075 (2023).

The dissertation is well written and edited. Tables and Figures including results of metabarcoding data are provided.

B. Assessment of the candidate's individual contribution

All publications appeared (or will appear) in peer-reviewed journals and Nguyen Ngoc Loi is the first author of three publications and coauthor (2nd author) of one publication. The candidate has significantly contributed to each publication by investigation, conceptualization, data analysis and curation, methodology, visualization, writing and editing each publication. The candidate has further successfully cooperated with different coauthors for each publication. I consider the individual contribution of Nguyen Ngoc Loi as important and significant for each publication and for his dissertation.

C. Topics and Goal of the dissertation

The present work is dealing with analysis of environmental DNA (eDNA) from marine sediments and the water column. Marine sediments contain DNA from both benthic and planktonic organisms, providing a repository for studying marine biodiversity. Environmental DNA found in these sediments can reveal the taxonomic composition and biases of current communities and help reconstruct past biodiversity.

The methodology includes

Study sites and sample collection
DNA extraction, amplification and sequencing
Data quality control and analysis
Identification and phylogenetic analysis of organisms

The thesis addresses:

-***Investigation of eukaryotic biodiversity in water columns and surface sediments***

Not much data is available about how marine biodiversity is recorded in the sediment, especially for plankton taxa-
The Ph.D. student investigated metabarcoding data of different planktonic and benthic eucaryote communities in Nordic Seas. The results suggest that the genetic composition of plankton communities varies through the water column and differs from what accumulates in the sediment. Only 40% of ASV's detected in the water were also present in the sediment.

Conclusion: The composition and structure of plankton communities recorded in sedimentary eDNA differ from those observed in the water column, highlighting potential biases in reconstructing past marine biodiversity.

-***Studying the diversity of foraminifera and their response to environmental factors***

-Arctic marine communities are undergoing rapid changes due to global warming and modifications of oceanic water masses circulation. Studies on Arctic foraminifera are based exclusively on morphological determination. The Ph. D. student investigated for the first time the genetic diversity of the foraminiferal community by obtaining metabarcoding data from different sediment samples in the Svalbard Archipelago. The analyzed data revealed high phylogenetic diversity compared to traditional morphological studies, high genetic novelty as more than half of the ASVs could not be assigned to any known group and community variation influenced by different water masses.

Conclusion: This study provides comprehensive data on foraminiferal biodiversity in the Svalbard area and shows how the foraminiferal community responds to Arctic environmental gradients.
-The diversity of deep sea meiofauna and eukaryotic microbiota is largely unexplored.

For another study, eDNA metabarcodes from 311 deep-sea sediment samples were used to analyze dep-sea foraminifera from the Pacific Clarion-Clipperton Fracture Zone. 61 new foraminiferal lineages were identified placed in 27 phylogenetic clades based on unique genetic signatures. Most novel lineages were found in other deep-sea areas, with limited overlap with coastal datasets which suggests limited migration between shallow and deep-sea habitats.

Conclusion: Deep-sea benthic foraminifera form a unique group highly adapted to abyssal environments. The signature-based approach can help investigate the distribution and ecology of deep-sea foraminifera, useful for future environmental monitoring.

-**Summarizing advances and limitations in marine sedaDNA research.**

Sedimentary ancient DNA (sedaDNA) offers a new approach to reconstruct the history of marine ecosystems over geological time scales. The work reviews the advances and current state of marine sedaDNA research, focusing on planktonic and benthic organisms. The candidate reviewed 55 original studies on marine sedaDNA research.
The key findings are taphonomic processes affecting DNA preservation in marine sediments, methodological challenges related to analysis, potential biases and limitations and possible applications including reconstruction of marine ecosystem history, understanding biodiversity changes and developing conservation strategies.

Conclusion: sedaDNA approaches provide unique insights into biodiversity changes over geological timescales, recent anthropogenic impacts, and the evolution of marine ecosystems. Continued development in this field could enhance paleoceanographic studies and aid in marine ecosystem management.

Overall Conclusion

The thesis demonstrates the potential of eDNA metabarcoding in marine biodiversity research, showing its applications in both present and past ecosystems. It highlights the importance of addressing methodological challenges to fully leverage the capabilities of eDNA in understanding marine biodiversity across time and space.

Evalution summary

Research Significance and Originality

The thesis by Nguyen Ngoc Loi provides significant contributions to the field of marine biodiversity, particularly through the use of environmental DNA (eDNA) metabarcoding to study marine ecosystems. The research is original and innovative, addressing important gaps in our understanding of marine biodiversity and the preservation of DNA in marine sediments.

Methodological Strengths

1. **Advanced Techniques:** The use of high-throughput sequencing and eDNA metabarcoding represents cutting-edge methodologies in marine biology research.
2. **Comprehensive Analysis:** The thesis employs a robust and comprehensive approach, combining field sampling, DNA extraction, sequencing, and bioinformatics analysis.
3. **Novel Insights:** The identification of novel foraminiferal lineages and the use of molecular indicators to assess environmental gradients are notable achievements.

Key Findings and Contributions

1. **Eukaryotic Diversity:** The research highlights significant differences in the taxonomic composition of eDNA from water columns and sediments, contributing to our understanding of how plankton DNA is archived in sediments.
2. **Arctic Foraminifera:** The discovery of high genetic diversity and novel taxa in the Arctic foraminifera expands our knowledge of biodiversity in polar regions and the influence of water mass characteristics.
3. **Deep-Sea Foraminifera:** The identification of unique deep-sea foraminiferal lineages underscores the distinctiveness of abyssal ecosystems and their limited connectivity with shallower habitats.

4. **Marine sedaDNA:** The review article synthesizes current advancements and methodological challenges in marine sedaDNA research, providing a valuable resource for future studies in this field.

Limitations and Challenges

1. **Taphonomic Biases:** The thesis acknowledges the biases in DNA preservation and representation in marine sediments, which can affect the interpretation of past biodiversity.
2. **Methodological Improvements:** Further refinement in eDNA extraction and sequencing techniques is needed to reduce biases and enhance the accuracy of biodiversity assessments.
3. **Environmental Factors:** The influence of various environmental factors on eDNA degradation and preservation requires more detailed investigation.

Overall Assessment

Nguyen Ngoc Loi's doctoral thesis is a well-structured and comprehensive study that makes substantial contributions to the field of marine biodiversity research. The innovative use of eDNA metabarcoding provides new insights into marine ecosystems, addressing significant scientific questions and methodological challenges. The research findings have important implications for biodiversity conservation, environmental monitoring, and paleoceanography. The thesis demonstrates a high level of scientific rigor, originality, and relevance and I recommend the top grade for the candidate.

Maria Holzmann