balkan.bio
2025

6TH BALKAN CONFERENCE ON BIOSCIENCES

BOOK OF ABSTRACTS AND PROGRAM

ISBN 978-619-281-077-1

30-31 OCT 2025
PLOVDIV, BULGARIA

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P15 Assessing the efficiency of 23S rRNA metabarcoding in characterising phytoplankton community structure using mock communities

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Keywords: metabarcoding, eDNA, phytoplankton, mock communities

Molecular tools combined with bioinformatics are increasingly applied in biodiversity studies and biomonitoring. Metabarcoding of environmental DNA has proven effective for characterizing phytoplankton community structure and dynamics in aquatic ecosystems. However, accurate taxonomic identification is hindered by incompleteness of reference barcode libraries and the difficulties in selecting a universally suitable barcode for both prokaryotic and eukaryotic microalgae. The presented research is part of a study that aims to assess the applicability and success of metabarcoding in characterizing phytoplankton communities by testing it on artificial mock communities of known composition. DNA was extracted from 11 phytoplankton strains from Thonon Culture Collection (4 Cyanobacteria, 4 Bacillariophyta, and 3 Chlorophyta). The 23S rRNA barcode region was amplified with primers targeting cyanobacterial and chloroplast ribosomes. Three mock communities were set up by combining amplicons in different ratios: (1) an equimolar mix (30 ng per strain), (2) a mix with varying DNA concentrations (1–50 ng), and (3) a mock with three abundant (50 ng), four low-abundance (1 ng), and four rare (0.1 ng) taxa. Each mock was sequenced in triplicate using Illumina technology. Reads were processed using DADA2, and taxonomy was assigned with Phytool, a reference library dedicated to microalgae. Ten of the eleven strains were detected in expected proportions, including all three rare taxa in Mock 3, demonstrating sensitivity to low-abundance species. All but 2 unexpected taxa were excluded by applying pipeline filtering thresholds, highlighting the importance of cutoffs in distinguishing true rare taxa from potential false positives. Half of the detected taxa were assigned to the species level, but only two matched the mock input. Others were misassigned, likely due to barcode limitations or strain misidentification. The high number of unassigned sequences reflects the limited coverage of the Phytool library for freshwater phytoplankton. Overall, 23S rRNA metabarcoding with DADA2-Phytool pipeline is a sensitive and effective method for phytoplankton community profiling. However, improving library coverage and marker selection

are essential in enhancing taxonomic resolution and reliability in ecological studies.

Acknowledgments: This work has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101079234 (BIOLAWEB project).