

Responses to the Reviewer's Questions

PhD Dissertation of Bassam Hussein Ibrahim Abubaker

*“REVEALING DIATOM DIVERSITY, RARE TAXA AND THEIR ENVIRONMENTAL DRIVERS
THROUGH COMPLEMENTARY eDNA METABARCODING AND MICROSCOPIC
APPROACHES”*

Reviewer: Tamás Felföldi, PhD

1. Traditionally, diatoms are still playing a prominent role in water quality assessment and other applications, such as paleolimnology and forensic investigations, due to their diverse morphological characteristics and well-preserved silica cell walls. Could you suggest another algal group that could potentially replace diatoms in eDNA-based water quality assessment and provide more accurate information?

Response:

Among eukaryotic algae, green algae (Chlorophyta), particularly desmids, could be considered a promising alternative group for molecular water-quality assessment. They are highly diverse in freshwater ecosystems, and many taxa respond sensitively to environmental gradients such as nutrient concentrations, pH, conductivity, light availability, and hydrological conditions. Desmids are especially useful because many species have relatively narrow ecological requirements and are therefore informative indicators of trophic and chemical conditions. Their diversity can also be characterized molecularly using markers such as 18S rRNA, ITS, or rbcL. However, I would not consider any single algal group a universally more accurate replacement for diatoms. Diatoms have the major advantage of decades of ecological calibration, well-established species-environment relationships, standardized assessment indices, and relatively well-developed reference libraries. A more promising direction may therefore be multi-taxon metabarcoding of the broader algal or phytoplankton community, combining diatoms with green algae and other groups. Because different groups respond to different environmental pressures and at different temporal scales, integrating them could provide complementary information and potentially produce a more comprehensive and robust assessment of ecological status than relying on one indicator group alone.

2. Is there an explanation for why the rbcL gene was selected as the primary taxonomic marker for diatoms, while the 18S rRNA gene or ITS is used for other groups of algae?

Response:

The choice of a taxonomic marker depends on the organismal group, the required taxonomic resolution, primer performance, and the quality of the available reference database. For diatoms, rbcL has become one of the preferred markers because it provides a useful balance between conserved regions, which allow reliable amplification across a broad range of taxa, and variable regions, which provide sufficient sequence divergence for discrimination among many closely related species. Another important advantage is the strong representation of rbcL

sequences in curated diatom reference resources such as diat.barcode, which improves the reliability of taxonomic assignment. In comparison, the 18S rRNA gene is more conserved and is therefore very useful for broad detection of eukaryotic algae, but it often has lower resolution among closely related diatom species. ITS generally evolves more rapidly and can provide high species-level resolution, but its high variability can make universal primer design, sequence alignment, and consistent amplification across diverse taxa more difficult, and reference coverage may be less uniform. For these reasons, the short 263-bp rbcL fragment used in this dissertation represents a practical compromise among amplification efficiency, taxonomic resolution, compatibility with high-throughput sequencing, and reference-database coverage.

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