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Insights into freshwater ciliate diversity through high throughput DNA metabarcoding

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Abstract

The freshwater bodies of India are highly biodiverse but still understudied, especially concerning ciliates. Ciliates constitute a significant portion of eukaryotic diversity and play crucial roles in microbial loops, nutrient recycling, and ecosystem maintenance. The present study aimed to elucidate ciliate diversity in three freshwater sites in the Delhi region of India: Okhla Bird Sanctuary (OBS), Sanjay Lake (SL), and Raj Ghat pond (RJ). This study represents the first investigation into the taxonomic diversity and richness of freshwater ciliates in India using a high-throughput DNA metabarcoding approach. For the analysis, total environmental DNA was extracted from the three freshwater samples, followed by sequencing of the 18S V4 barcode region and subsequent phylogenetic analyses. Operational taxonomic units (OTU) analyses revealed maximum species diversity in OBS (106), followed by SL (104) and RJ (99) sites. Ciliates from the classes Oligohymenophorea, Prostomatea, and Spirotrichea were dominant in the three sites. The study discusses the ability of the metabarcoding approach to uncover unknown and rare species. The study highlights the need for refined reference databases and cautious interpretation of the high-throughput sequencing-generated data while emphasizing the complementary nature of molecular and morphological approaches in studying ciliate diversity.

Keywords: ciliates; diversity; DNA metabarcoding; freshwater; India

Introduction

Ciliates represent a remarkably diverse clade of eukaryotic microorganisms, showcasing the utmost morphological complexity and differentiation among single-celled organisms (Chen et al. 2017). With an extensive species diversity, there are over 4000 described free-living ciliates (Gao et al. 2017, Canals et al. 2020). These microorganisms exhibit distinctive structural and functional features, including intricate subcellular and organellar structures, functionally diverse macronuclear and micronuclear genomes, sexual reproduction through conjugation, whole-genome duplications, unique patterns of cortical structure with semi-autonomous mechanisms of inheritance and morphogenesis. Additionally, there is evidence of epigenetic mechanisms, reflecting a broad array of ecological niches, lifestyles, coupled with rapid evolutionary radiation at the population level due to their short generation time (Clamp and Lynn 2017). Ciliates play a vital role in freshwater ecosystems, being the richest and relatively most abundant group (Carvalho da Silva and Fernandes 2023). They serve as major contributors to the microbial loop, acting as recyclers, and remineralizers of organic material. They also prey upon bacteria and smaller protists, contributing significantly to maintaining ecosystem balance (Abraham et al. 2019).

The taxonomic identifications of ciliates traditionally relies on microscopic techniques involving observation of live or fixed cells, with a focus on ciliature or silver-line system (Foissner 2016). However, relying solely on microscopy for identification presents certain limitations, including the time-intensive process of culturing ciliates, complex staining procedures, and the existence of morphospecies and cryptic species that can only be distinguished at the genetic level, posing challenges to accurate identifications (Rajter et al. 2022). Consequently, the identification of ciliates and other microorganisms has proposed DNA-based methods as an alternative taxonomic approach to unveil their vast diversity (Medinger et al. 2010, Cahoon et al. 2018, Pitsch et al. 2019, Antil et al. 2023).

DNA barcoding is a method of identifying species on the based on short DNA sequences linked to morphologically identified species (Hebert et al. 2003). DNA metabarcoding, or metabarcoding, is a method of identifying multiple species from bulk samples by relying on reference barcode sequences (Cordier et al. 2021). Environmental DNA can be directly extracted from soil or water and sequenced using high-throughput sequencing techniques such as 454-pyrosequencing, Ion torrent, and Illumina (Goodwin et al. 2017, Burki et al. 2021). The choice of a barcoding marker that provides the desired taxonomic resolution is crucial for ciliate metabarcoding studies. Hypervariable regions of the 18S ribosomal DNA (V4 or V9), COI gene, ITS1-5.8S-ITS2 region, and D1-D2 region of the 28S rDNA have been suggested as barcoding genes for ciliates (Zhao et al. 2016, Abraham et al. 2019, Antil et al. 2023). The method generates millions of short sequence reads (metabarcodes) of less than 500 bps in a single run (Slatko et al. 2018). These barcodes have created opportunities for discoveries from diverse environments, including the detection of rare, overlooked, and unculturable taxa (Andreoli et al. 2009, de Vargas et al. 2015, Pernice et al. 2016, Boenigk et al. 2018, Oliverio et al. 2020, Mugnai

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