



Extensive intragenomic variations of the 18S rDNA V4 region in the toxigenic diatom species *Pseudo-nitzschia multistriata* revealed through high-throughput sequencing

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ABSTRACT

Metabarcoding analysis is an effective technique for monitoring the domoic acid-producing *Pseudo-nitzschia* species in marine environments, uncovering high-levels of molecular diversity. However, such efforts may result in the overinterpretation of *Pseudo-nitzschia* species diversity, as molecular diversity not only encompasses interspecies and intraspecies diversities but also exhibits extensive intragenomic variations (IGVs). In this study, we analyzed the V4 region of the 18S rDNA of 30 strains of *Pseudo-nitzschia multistriata* collected from the coasts of China. The results showed that each *P. multistriata* strain harbored about a hundred of unique 18S rDNA V4 sequence varieties, of which each represented by a unique amplicon sequence variant (ASV). This study demonstrated the extensive degree of IGVs in *P. multistriata* strains, suggesting that IGVs may also present in other *Pseudo-nitzschia* species and other phytoplankton species. Understanding the scope and levels of IGVs is crucial for accurately interpreting the results of metabarcoding analysis.

1. Introduction

The cosmopolitan diatom genus *Pseudo-nitzschia* has garnered global attention not only because its vital roles as primary producers in the biogeochemical cycles of marine ecological environments (Nelson et al., 1995; Lundholm et al., 2002; Armbrust, 2009; Bates et al., 2019; Falcione et al., 2020), but also because many *Pseudo-nitzschia* species have the potential to cause harmful algal blooms (HABs) associated with the production of the neurotoxin domoic acid (DA). The toxin is responsible for amnesic shellfish poisoning (ASP), also known as domoic acid poisoning, which causes serious negative impact on marine ecosystems and human health (Bates et al., 2018; Cembella et al., 2023), also kill seabirds and marine mammals (Lelong et al., 2012). The year 1987 marked the first case of ASP attributed to *Pseudo-nitzschia* species

(*P. multiseriata*), which was diagnosed in 107 patients and caused the death of three in Prince Edward Island, eastern Canada (Perl et al., 1990; Lelong et al., 2012). Since then, toxin-producing *Pseudo-nitzschia* species have been identified in all continents (Bates et al., 1989; Lelong et al., 2012). Significant reduction in the adverse effects of the toxin-producing *Pseudo-nitzschia* species is largely attributable to the establishment of surveillance programs worldwide that monitor the density of *Pseudo-nitzschia* species in seawater and the levels of DA in molluscan shellfish and finfish safe for human consumption (Lelong et al., 2012).

The practical need for monitoring toxin-producing *Pseudo-nitzschia* species motivated taxonomists to identify and characterize *Pseudo-nitzschia* species using a range of morphology-based and molecular marker-based methods (Bates et al., 2018). In morphology-based identification of *Pseudo-nitzschia* species, both light microscopy (LM) and electron

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