



# Rich molecular diversity of ribosomal RNA genes in *Pseudo-nitzschia* species (Bacillariophyceae) revealed through single-strain high throughput sequencing

Hui Wang<sup>a,b,c,d</sup>, Kuiyan Liu<sup>a,b,c,d</sup>, Ziyang He<sup>a,b,c,d</sup>,  
Yang Chen<sup>a,b,c,d</sup>, Chui Pin Leaw<sup>e</sup>, Nansheng Chen<sup>a,b,d,f,\*</sup>

<sup>a</sup> CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

<sup>b</sup> Laboratory of Marine Ecology and Environmental Science, Qingdao Marine Science and Technology Center, Qingdao 266200, China

<sup>c</sup> College of Marine Science, University of Chinese Academy of Sciences, Beijing 100039, China

<sup>d</sup> Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao 266071, China

<sup>e</sup> Bachok Marine Research Station, Institute of Ocean and Earth Sciences, University of Malaya, 16310 Bachok, Kelantan, Malaysia

<sup>f</sup> Department of Molecular Biology and Biochemistry, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia V5A 1S6, Canada

## ARTICLE INFO

### Keywords:

Intragenomic variations  
Metabarcoding  
Molecular diversity  
Homogenization  
*Pseudo-nitzschia*  
SSU rDNA

## ABSTRACT

Ribosomal RNA genes (rDNAs) are widely used as molecular markers in phytoplankton identification and diversity analysis, showing significant efficacy in metabarcoding studies. Recent analysis of amplicon sequencing results of the 18S rDNA V4 domain in multiple strains of the toxigenic *Pseudo-nitzschia multistriata* revealed that, in addition to the presence of a dominant copy of 18S rDNA V4 in each strain, there also exists a large number of low-abundance 18S rDNA V4 sequence variants, indicating the existence of high levels of intragenomic variations (IGVs). Many of these low-abundance variants identified in *P. multistriata* also show high similarity to that of other *Pseudo-nitzschia* species. In this study, we analyzed the molecular diversity of 18S rDNA V4 sequences of additional 56 clonal strains of nine *Pseudo-nitzschia* species collected along the coast of China and the South China Sea using high-throughput amplicon sequencing. The results showed that each strain harbors high levels of IGVs as expected. Although a common dominant sequence variant is often shared by all strains of the same species, these variants are usually also found at low frequencies in other species, suggesting that IGVs in these diatoms, like most protists, are linked to the biological processes of genome evolution. Understanding the nature of IGVs is not only critical for ascertaining the species evolution, but also for precise interpretation of the molecular diversity revealed in metabarcoding studies.

## 1. Introduction

Species of the pennate diatom genus *Pseudo-nitzschia* are mostly cosmopolitan and play a crucial role as primary producers in the marine biogeochemical cycles [1–6]. *Pseudo-nitzschia* species have attracted worldwide attention after a deadly incident resulting in three deaths and more than a hundred hospitalizations in Prince Edward Island in Canada in 1987 [7]. Of the 61 species described thus far, 29 have been demonstrated to be toxigenic by producing the neurotoxin domoic acid (DA), which can accumulate in shellfish and cause amnesic shellfish poisoning (ASP), and affects marine lives and human health [6–11]. In China, sampling effort has resulted in the identification of over 40

*Pseudo-nitzschia* species, ten of which are known to be toxigenic (*P. bipertita*, *P. cacciantha*, *P. cuspidata*, *P. fraudulenta*, *P. fukuyoi*, *P. lundholmiae*, *P. multiseries*, *P. pseudodelicatissima*, *P. punctonis*, and *P. simulans*) [9,12–17]. The cosmopolitan presence of toxigenic *Pseudo-nitzschia* species prompted the establishment of a series of monitoring plans for toxigenic *Pseudo-nitzschia* species and DA in aquatic products to mitigate their adverse impacts [8]. Because the toxigenic potential of *Pseudo-nitzschia* species varies (reviewed in [7]), it is important to identify and monitor the specific toxic species.

Ribosomal gene markers including the SSU (18S), LSU (28S), ITS1, ITS2, and 5.8S rDNAs have been widely used for distinguishing species of *Pseudo-nitzschia*, each having its own advantages and disadvantages

\* Corresponding author at: CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China.

E-mail address: [chenn@qdio.ac.cn](mailto:chenn@qdio.ac.cn) (N. Chen).

<https://doi.org/10.1016/j.algal.2024.103869>

Received 8 June 2024; Received in revised form 13 December 2024; Accepted 15 December 2024

Available online 17 December 2024

2211-9264/© 2024 Elsevier B.V. All rights reserved, including those for text and data mining, AI training, and similar technologies.