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# Genetic structure of *Pseudo-nitzschia pungens* (Bacillariophyceae) populations: Implications of a global diversification of the diatom

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ABSTRACT

*Pseudo-nitzschia pungens* is a planktonic marine diatom known to be widespread in tropical and temperate coastal waters. We examined the population genetic structure of tropical Southeast Asian populations of *P. pungens* and compared it with those of northern and southern temperate populations. The secondary structures of the nuclear encoded internal transcribed spacer (ITS) region of 164 strains of *P. pungens* were modeled and analyzed. The tree revealed three ITS entities: clade I (comprised of *P. pungens* var. *pungens*) was distributed mainly in northern temperate waters; clade II (comprised of both *P. pungens* var. *pungens* and var. *cingulata*) was mainly from the NE Pacific; and clade III (comprised of both *P. pungens* var. *pungens* and var. *aveirensis*) was restricted to tropical and warm-temperate waters. Hybrids of both *P. pungens* var. *pungens* and var. *cingulata* co-occurred in clades I and II. Sixty haplotypes were revealed from the sequences of 164 strains. Haplotype diversity inferred from the median-joining network was in accordance with phylogenetic analysis, further supporting the grouping of the *P. pungens* haplogroups. Our results revealed limited gene flow between *P. pungens* from tropical and temperate waters, and significant population structure, as estimated by an analysis of molecular variance (AMOVA), with 75% of the total ITS variation found among populations ($\Phi_{ST}$ = 0.75). This study suggests that distinct environmental clines, such as ocean thermohaline circulation, have a potential for fragmenting and dispersing global populations of *P. pungens*. Formation of the Isthmus of Panama, in particular, is speculated to play a role in this allopatric differentiation in *P. pungens* populations worldwide.


## 1. Introduction

Phytoplankton, as primary producers, have always been an important food source. Marine phytoplankton also play a role in the carbon cycle by absorbing atmospheric $CO_2$ and removing it to ocean depths (Falkowski et al., 2000). However, certain algal groups are known to be harmful when they bloom in the environment, causing a nuisance to other organisms. Certain species of the genus *Pseudo-nitzschia* are responsible for amnesic shellfish poisoning (ASP). The toxin responsible for this illness is domoic acid (DA). This neurotoxin affects not only humans, but also marine mammals and sea birds (reviewed by Lelong et al., 2012; Trainer et al., 2012). Outbreaks of toxic *Pseudo-nitzschia* blooms, with levels of DA in shellfish above the regulatory limit, have resulted in the closure of shellfish farming and harvesting in several countries (Kotaki et al., 1996; Amzil et al., 2001; Vale and Sampayo, 2001; Trainer et al., 2007).

*Pseudo-nitzschia pungens* (Cleve) Hasle was first described in 1993 (Hasle, 1993). In 1998, *P. pungens* var. *cingulata* Villac was described, characterized by apparent morphological differences in valve structure and cingular bands (Villac and Fryxell, 1998). A decade later, *P. pungens* var. *aveirensis* Lundholm, Churro, Carreira and Calado was described (Churro et al., 2009). The originally described *P. pungens* was then designated as the nominal variety (*P. pungens* var. *pungens*). Its taxonomic classification did not change. *Pseudo-nitzschia pungens* is one of the most studied species owing its wide distribution (Hasle, 2002; Casteleyn et al., 2010) and the ability of some strains to produce trace levels of DA (Rhodes et al., 1996; Trainer et al., 1998; Calu et al., 2009; Moschandreou et al., 2012). However, most *P. pungens* strains have been reported to be non-toxic (Bates et al., 1998; Villac and Fryxell, 1998; Li et al., 2005; Churro et al., 2009; Lim et al., 2010, 2012a; Quijano-Scheggia et al., 2010). *Pseudo-nitzschia pungens* is heterothallic (Chepurnov et al., 2005), and natural hybridization can take place in the environment (Casteleyn et al., 2009a; Holtermann et al., 2010).

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