

THREE NOVEL SPECIES IN THE *PSEUDO-NITZSCHIA PSEUODELICATISSIMA* COMPLEX:
P. BATESIANA SP. NOV., *P. LUNDHOLMIAE* SP. NOV., AND *P. FUKUYOI* SP. NOV.
(BACILLARIOPHYCEAE) FROM THE STRAIT OF MALACCA, MALAYSIA¹

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A study on the morphology and phylogeny of 18 strains of *Pseudo-nitzschia* established from the Strait of Malacca, Peninsular Malaysia, was undertaken. Morphological data combined with molecular evidence show that they constitute three new species, for which the names, *P. batesiana* sp. nov., *P. lundholmiae* sp. nov., and *P. fukuyoi* sp. nov., are proposed. The three new species closely resemble species in the *P. pseudodelicatissima* complex *sensu lato*. Morphologically, *P. batesiana* differs from other species in the complex by having a smaller part of cell overlapping in the chain, whereas *P. lundholmiae* differs by having fewer poroid sectors and *P. fukuyoi* by having a distinct type of poroid sectors. Nucleotide sequences of the LSU rDNA (D1–D3) of the three new species reveal significant nucleotide sequence divergence (0.1%–9.3%) from each other and from other species in the *P. pseudodelicatissima* complex *s.l.* The three species are phylogenetically closely related to species in the *P. pseudodelicatissima* complex, with *P. batesiana* appearing as a sister taxon to *P. circumspora*, *P. ceciantha*, and *P. subpacificae*; whereas *P. lundholmiae* and *P. fukuyoi* are more closely related to *P. pseudodelicatissima* and *P. cuspidata*. The three species show 2–3 compensatory base changes (CBCs) in their ITS2 transcripts when compared to the closely related species. The ITS2 with its structural information has proven its robustness in constructing a better resolved phylogenetic framework for *Pseudo-nitzschia*.

Key index words: ITS; LSU rDNA; morphology; *P. batesiana* sp. nov.; *P. fukuyoi* sp. nov.; *P. lundholmiae* sp. nov.; *Pseudo-nitzschia*

Abbreviations: ASP, amnesic shellfish poisoning; CBC, compensatory base change; DA, domoic acid; HCBC, hemi-compensatory base change; SNP,

single-nucleotide polymorphism; TEM, transmission electron microscope

The genus *Pseudo-nitzschia* Peragallo has gained considerable research attention due to its association with amnesic shellfish poisoning (ASP). Species in the genus are identified on the basis of the frustule's morphological features. Precise species identification is crucial; as one third of the species are known to produce the neurotoxin, domoic acid (DA) (Lundholm 2011, Lelong et al. 2012, Trainer et al. 2012), and the majority of DA-producing *Pseudo-nitzschia* spp. are reported as cosmopolitan (Hasle 2002).

Traditional species assignment was always difficult, which required taxonomic knowledge in conjunction with the technical skills on electron microscopy. A combination of molecular approaches has increasingly been applied to delimit species of *Pseudo-nitzschia* (Vrieling et al. 1996, Cho et al. 2001, Orsini et al. 2004, Cerino et al. 2005, McDonald et al. 2007, Quijano-Scheggia et al. 2010). Several molecular phylogenetic attempts have been made to reveal the lineages of *Pseudo-nitzschia* species, and to detect species limits by DNA sequence variation, yet, some taxonomic positions of closely related species remain elusive (e.g., *P. cuspidata* and *P. pseudodelicatissima*; Lundholm et al. 2012).

Cryptic and pseudo-cryptic species diversity of *Pseudo-nitzschia* has greatly complicated its taxonomy. Nevertheless, compelling evidence provided by various aspects of morphology, phylogenetic reconstruction, and sexual reproduction has allowed descriptions of novel species (e.g., Amato and Montresor 2008, Quijano-Scheggia et al. 2009, Lundholm et al. 2012). Lundholm et al. (2003) first suggested that *P. pseudodelicatissima* [Hasle] Hasle emend. Lundholm, Hasle and Moestrup was a complex when they revealed several pseudo-cryptic species with close morphological resemblances and phylogenetic relationships, and later described or emended

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