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LETTER

INTERSPECIFIC PLASTIDIAL RECOMBINATION IN THE DIATOM GENUS *PSEUDO-NITZSCHIA*

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Plastids are usually uni-parentally inherited and genetic recombination between these organelles is seldom observed. The genus *Pseudo-nitzschia*, a globally relevant marine diatom, features bi-parental plastid inheritance in the course of sexual reproduction. This observation inspired the recombination detection we pursued in this paper over a ~1,400-nucleotide-long region of the plastidial *rbc*L, a marker used in both molecular taxonomy and phylogenetic studies in diatoms. Among all the *rbc*L-sequences available in web-databases for *Pseudo-nitzschia*, 42 haplotypes were identified and grouped in five clusters by Bayesian phylogeny. Signs of hybridization were evident in four of five clusters, at both intra- and interspecific levels, suggesting that, in diatoms, (i) plastidial recombination is not absent and (ii) hybridization can play a role in speciation of *Pseudo-nitzschia* spp.

Pseudo-nitzschia H. Peragallo is a globally distributed genus of planktonic, potentially toxigenic diatoms (Lelong et al. 2012, Lundholm et al. 2012, Trainer et al. 2012). In order to detect signs of genetic recombination in plastids of *Pseudo-nitzschia* spp., we (i) analyzed all available sequences for the single-copy locus *rbc*L (RuBisCO large subunit) of this genus, (ii) identified unique haplotypes (Table S1 in the Supporting Information); (iii) built a Bayesian phylogeny (Fig. 1a), (iv) identified sub-sets of closely related sequences; and (v) performed recombination detection on each sub-set. Among plastidial genes, *rbc*L was chosen since the largest data set of sequences was available in the GenBank database for this molecular locus. Six out of 42 sequences analyzed in the present study were derived herein according to Amato et al. (2007), while the other 36 were downloaded from the GenBank database and, among the latter sequences, 20 were not associated to any paper (Table S1). All sequences were accurate and no-stop codon was detected in protein translations. Detection of chimeric sequences was carried out on distinct phylogenetic clades composed of more than three haplotypes and using SplitsTree v.4 (Huson 1998) (http://www.splitstree.org/), Pairwise Homoplasy Index test (or PHI-test; Bruen et al. 2006), and Recombination Detection Program (RDP) v.4 (Martin et al. 2010; http://web.cbio.uct.ac.za/~darren/rdp.html). The latter software included seven different algorithms for recombination detection (see details and specific references in Appendix S1 in the Supporting Information). Such a multiple approach was used since previous methodological studies suggested that definitive conclusions about the presence of recombination should not be taken on the basis of a single method (e.g., Posada 2002, Martin et al. 2011).

Within the 18 *Pseudo-nitzschia* species analyzed, 42 *rbc*L haplotypes were detected, distributed in five main clades supported by Bayesian phylogeny (Fig. 1a), which matched the delineation of species and species-groups in the genus *Pseudo-nitzschia* (Lelong et al. 2012, Lundholm et al. 2012, Trainer et al. 2012). Split-decomposition analysis produced Parsimony-supported networks in four out of five phylogenetic clades (differently colored in Fig. 1a). The presence of non-univocal paths connecting *rbc*L haplotypes suggested the presence of either homoplasy or recombination between them. The Phi-test showed that recombination was statistically supported in the green and pink networks (Fig. 1, b and d), suggesting a complex and reticulate evolutionary history of the *rbc*L gene at both intra- and interspecific level.

Statistically significant individual recombination events (i.e., chimeric sequences) were found in the blue, pink and red groups (Fig. 1, c–e; Table S2 in the Supporting Information). Among the latter, recombination signals were particularly strong in two events, one in the pink and one in the red clades, which were detected, respectively, by three and seven out of seven distinct recombination detection algorithms (Fig. 2, a, pink clade and b, red clade; Table S2). In both events, complementary phylogenies were significantly incongruent, i.e., different regions in the same recombinant sequence descended from distinct and relatively genetically distant parents. Mismatching topologies of trees produced with outer and inner alignment-win-