Morphology, phylogeny and diversity of the diatom genus *Pseudo-nitzschia* in the northern Adriatic Sea

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INTRODUCTION

The diatom genus *Pseudo-nitzschia* H. Peragallo 1900 contains more than 30 chain forming and potentially toxic species. Most of them are discernible only on the basis of ultrastructural or genetic differences. Ultrastructural investigations, combined with genetic characterization with different molecular markers have revealed several cryptic and pseudo-cryptic species within the genus *Pseudo-nitzschia* in the northern Adriatic. This ubiquitous genus is present in phytoplankton assemblages of the northern Adriatic Sea throughout the entire year and is often found to be dominating the diatom community. However, the actual species composition and species succession is, due to the limitations of light microscopical determination, still unknown and requires further examination.

RESULTS

Pseudo-nitzschia spp. were the dominant diatoms present in 60% of all samples on a yearly basis, with a maximum contribution of up to 97% (maximal abundance $1.6 \cdot 10^6$ cells L^{-1}) (Fig.8) to the total diatom abundance (Ljubešić et al., 2011, Marić et al., 2011) in the northern Adriatic. Morphological analyses revealed *Pseudo-nitzschia fraudulenta* (Fig.1), *P. mannii* (Fig.6) and the potentially toxic *P. pseudodelicatissima* (Fig.7), *P. calliantha* (Fig.2) and *P. pungens* (Fig.4) as dominant species in different blooms (Ljubešić et al., 2011). In order to further elucidate the phylogeny and diversity of *Pseudo-nitzschia* species, monoclonal cultures were established. Subsequent phylogenetic analysis based on sequences of 18S rDNA (Fig.5) and morphological analysis of the frustules confirmed *P. fraudulenta* (Fig.1) and *P. delicatissima* (Fig.3) in the northern Adriatic and showed further cryptic diversity in the genus. *P. delicatissima** showed variations in very conserved regions of the 18S rDNA, suggesting several new species.

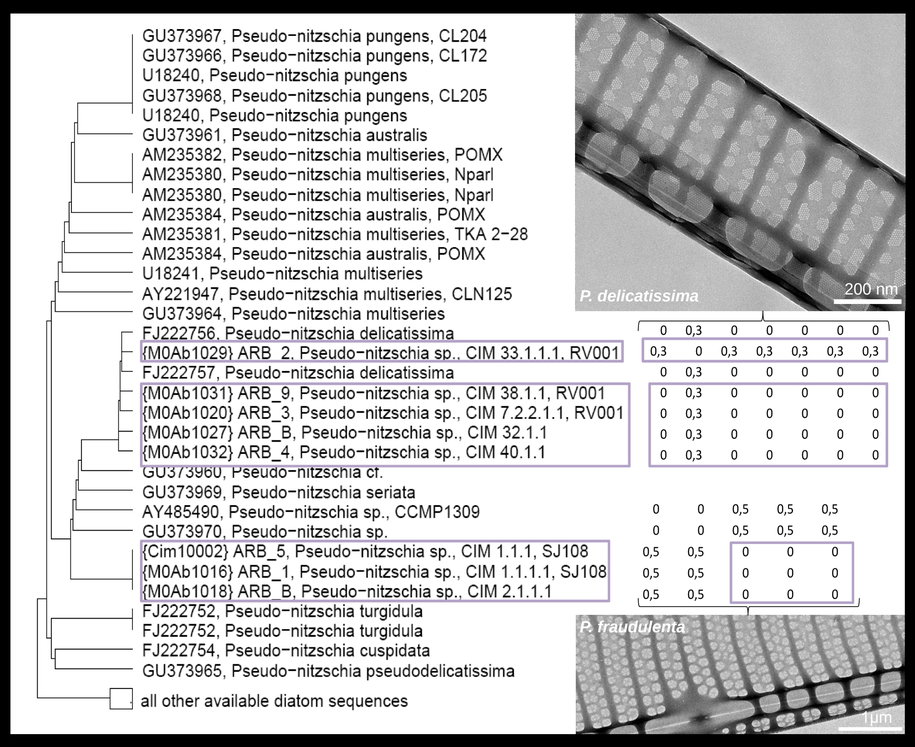

Figure 5. Neighbourjoining representation of the so far available *Pseudo-nitzschia* sequences (18S rRNA). On the right hand side are the sequence diffferences given. Up within the *P. delicatissima* and down within the *P. fraudulenta* strains from the northern Adriatic. As outgroup all available Diatom 18S rRNA sequences were incorporated (6.9.2011, NCBI).

CONCLUSIONS

Morphoplogical and molecular analyis revealed 6 different *Pseudo-nitzschia* species in the northern Adriatic Sea. Microscopical and molecular analysis suggested the existence of more *Pseudo-nitzschia* species. This number is not jet final and more work with monoclonal cultures, with different molecular markers and sequencing is in process at the Center for Marine Research culture collection in Rovinj.

CITED LITERATURE

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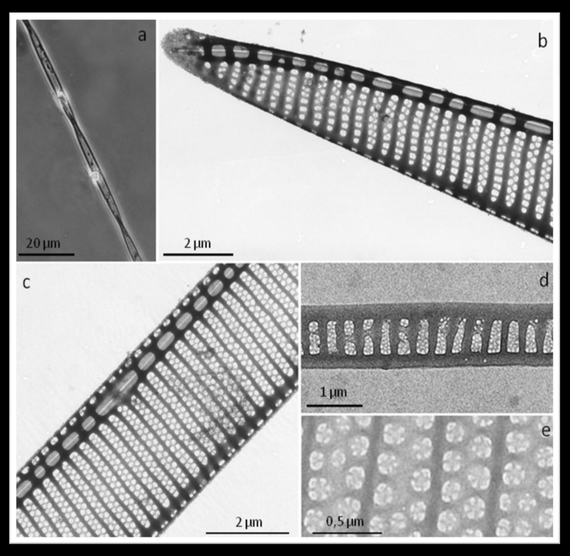

Figure 1. *Pseudo-nitzschia fraudulenta.* Light micrograph of a stepped colony in valvar view (a). Tip of the valve (b), middle of the valve with central interspace (c), girdle band (d) and poroid pattern (e) TEM (Ljubešić et al., 2011).

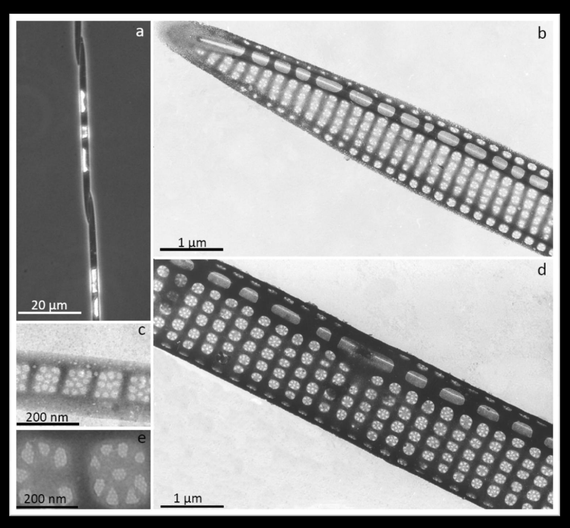

Figure 2. *P. calliantha* LM of a stepped colony in girdle view (a). Tip of the valve (b), girdle band (c) large central interspace (d) and poroid pattern (e) TEM.

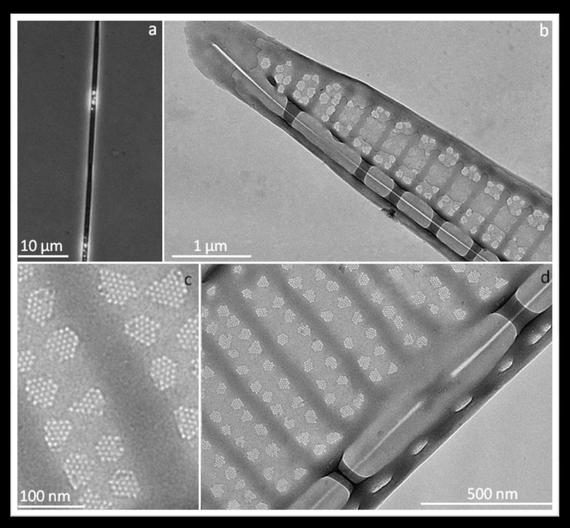

Figure 3. *P. delicatissima**. Light micrograph of a stepped colony (a). Tip of the valve (b) and middle of the valve (d), poroid structure (c) TEM.

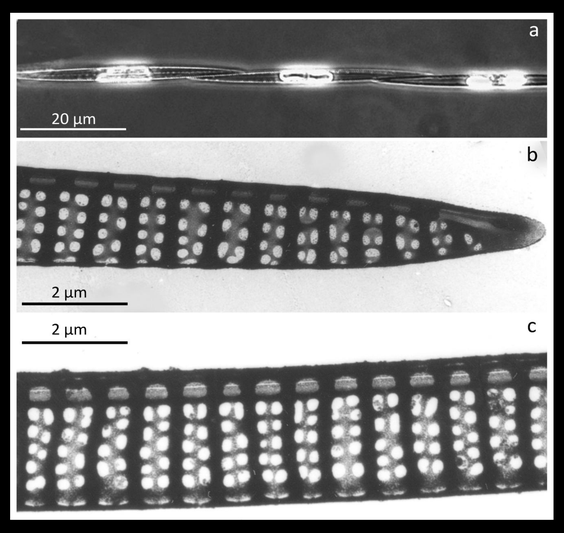

Figure 4. *P. pungens.* Light micrograph of a stepped colony in valval view (a). Tip of the valve (b) and middle of the valve (c) TEM.

MATERIALS AND METHODS

Samples were collected monthly at 10 stations in the northern Adriatic Sea from 2008-2010. Water samples were taken with 5 L Niskin bottles. Net samples (53 µm mesh size) were vertically towed for 15 m and preserved in formaldehyde. Phytoplankton cells were identified and enumerated on an inverted light microscope (Zeiss Axiovert 200) (Utermöhl, 1958). Single live chains of *Pseudo-nitzschia* were manually isolated with a micropipette from a net samples and grown into monoclonal batch cultures in f/2 medium. Cultures were incubated at a temperature of 18 °C, 12:12 dark-light cycle. Monoclonal cultures were harvested by centrifugation. DNA was isolated with the Qiagen plant tissue kit (Qiagen). Partial 18S rRNA sequences were amplified using the primers described in Zimmermann et al., (2011) and sequenced on an ABI PRISM 3100 Avant Genetic Analyzer (Applied Biosystems). The resulting sequences from 2 sequencing runs for each direction were compared to exclude sequencing mistakes by majority rule (3:1). The resulting sequence was aligned into an alignment of near full length 18S rDNA genes.
For transmission electron microscopy (TEM, SEM), *Pseudo-nitzschia* frustules were first acid-cleaned (in HNO_3 and H_2SO_4) and rinsed with distilled water. The micrographs were taken with a FEI Morgagni 268D and a FEI TECNAI transmission electron microscopes; and a 515 Philips scanning electron microscope. The ultrastructure and morphometry of the valves were analysed according to recent literature (Lundholm et al., 2003).

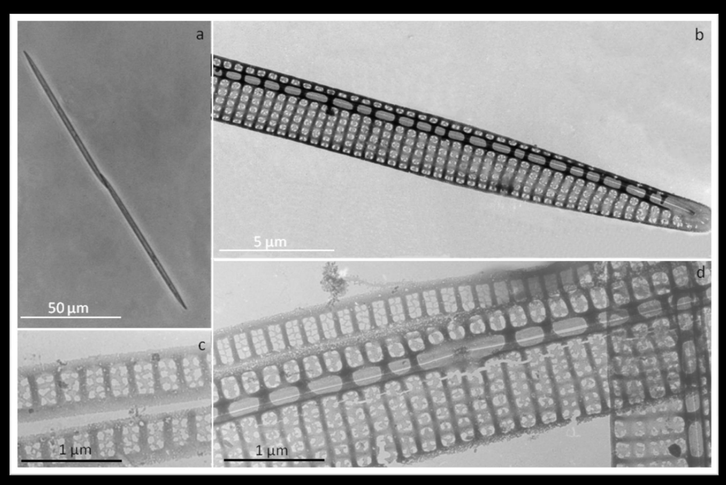

Figure 6. *P. mannii.* External view of a stepped colony in LM (a). Valve tip (b) and middle part with central interspace (d). Girdle bands (c) TEM.

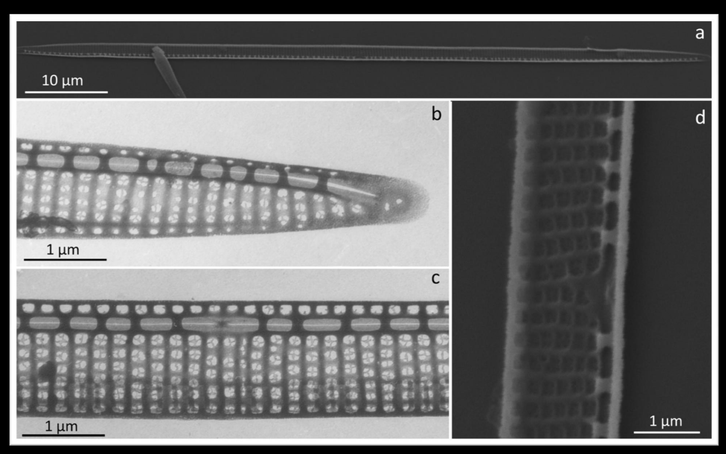

Figure 7. *P. pseudodelicatissima.* External view of whole valve SEM (a). Top (b) and middle (c) of the valve TEM. Internal view of middle of the valve SEM (d) (Ljubešić et al., 2011).

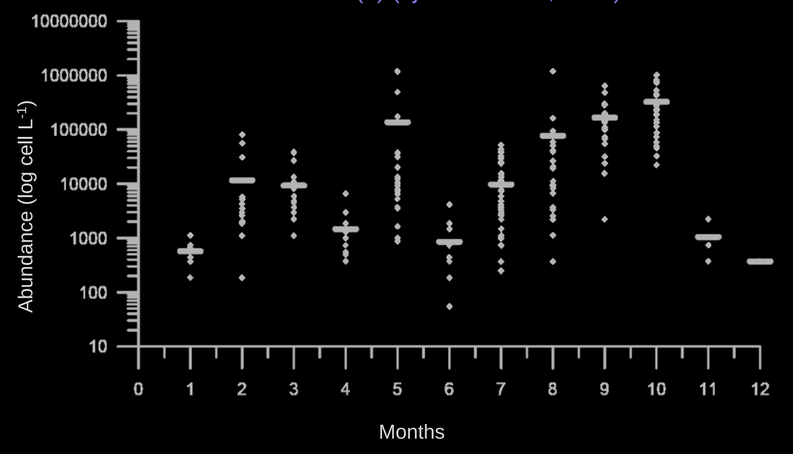

Figure 8. Temporal distribution of monthly mean of *Pseudo-nitzschia* sp. abundance in the northern Adriatic Sea.

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