

The evaluation of DNA barcoding for identification of dinoflagellates: a test using *Prorocentrum*

Introduction

Dinoflagellates comprise 75% of the species implicated in harmful algal blooms (HABs)¹, which have increased in frequency, intensity and geographical area over the past two decades². Establishing DNA Barcodes for protists, such as dinoflagellates, could provide a fast, accurate and universal method of species identification, potentially providing an efficient tool to monitor the presence of specific species, in an effort to eliminate the risks associated with HABs.

45-60 species of the 2000 extant dinoflagellates, representing at least 12 genera, are known to be toxic.^{1,3} Representatives of four HAB genera, including *Prorocentrum*, have been found in Atlantic Canadian waters. There are currently 58 recognized species of *Prorocentrum*, with strains for about 15 species maintained in algal culture collections.

For a limited number of studies using protists DNA barcoding has proven to be an effective taxonomic tool capable of species delimitation indicating the potential to extend this technique to other protists groups^(4,5,6).

Objectives

Test the efficacy of a 5' COI fragment as a suitable DNA barcode for unambiguous species identification within the dinoflagellate genus *Prorocentrum*

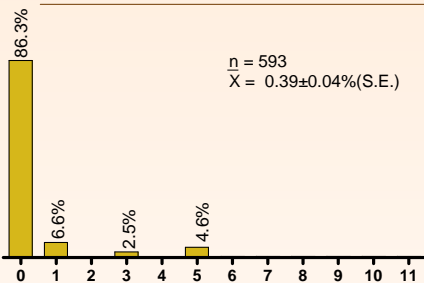
Materials and Methods

- Cultures were obtained from CCMP, CCAP, CCCM and NRC (Halifax)
- COI fragment was amplified from extracted DNA using nested PCR with dinoflagellate specific primers
- Sequence comparisons were used to estimate both inter and intraspecific divergences
- Five COI sequences included in this study were obtained from GenBank (AB000133, AB000134, EF036588, AF463414 and EF036587) and one *P. micans* sequence was obtained from a single cell isolated from an environmental sample

8 of the *Prorocentrum* species used in this study

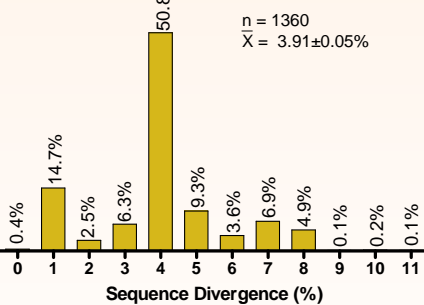


Results



Intraspecific variation of the COI gene among 5 species (59 strains) of *Prorocentrum* revealed a mean divergence of $0.39 \pm 0.04\%$

Intraspecific divergences > 1% were observed for comparisons with only two strains; one *P. lima* and one *P. micans* sequence that was obtained from GenBank



Interspecific variation of the COI gene among 9 species (63 strains) of *Prorocentrum* revealed a mean divergence of $3.91 \pm 0.05\%$

But interspecific divergences < 1% were observed for comparisons among three species: *P. balticum*, *P. dentatum* and *P. minimum*

	1	11	21	31	41	51	61
<i>balticum</i>	CACTCCGGGACATAAATCTACGTCCTATAGAAATAGAAATATATTCATCAGGAATAGGATATATCT						
<i>dentatum</i>	GAATCCGGGACATAAATCTACGTCCTATAGAAATAGAAATATATTCATCAGGAATAGGATATATCT						
<i>micans</i>	GAATCCGGGACATAAATCTACGTCCTATAGAAATAGAAATATATTCATCAGGAATAGGATATATCT						
<i>mexicanum</i>	CACTCCGGGACATAAATCTACGTCCTATAGAAATAGAAATATATTCATCAGGAATAGGATATATCT						
<i>minimum</i>	GAATCCGGGACATAAATCTACGTCCTATAGAAATAGAAATATATTCATCAGGAATAGGATATATCT						
<i>triestinum</i>	GAATCCGGGACATAAATCTACGTCCTATAGAAATAGAAATATATTCATCAGGAATAGGATATATCT						

Two of the 63 sequences exhibited sequence abnormalities:

For one of the two strains of *P. triestinum*, a gap of 63 nucleotides (21 codons) but no frameshift was observed.

For the sole *P. mexicanum* strain, a nucleotide deletion (site 23) and an additional two base deletion 44 bases downstream resulted in a frameshift between the sites. A nucleotide insertion (site 409) also resulted in a frameshift for the remainder of the sequence.

Discussion

This study found that, within the genus *Prorocentrum*, intraspecific divergences were typically less than interspecific divergences. Intraspecific differences greater than 1% are due to only two sequences. For one *P. lima* strain, 32 base pairs immediately following the 5' primer site showed no sequence identity to its conspecifics, and therefore, further examination of the culture is required. The second sequence originated from GenBank and with no strain information available, must be treated with skepticism.

Interspecific variation less than 1% was due to only three species comparisons (*P. minimum*, *P. balticum* and *P. dentatum*), which showed as little as 1 nucleotide change. *Prorocentrum* taxonomy, as with many protists, has been revised but remains tentative as new species are described and questions regarding species characteristics and ranking remain. Therefore, it is reasonable that some of the unexpectedly low divergences (< 1%), may be due to incorrect species identification, or may indicate that a single gene fragment does not provide sufficient resolution for species delineation in this group.

Conclusions

Currently DNA barcoding shows some promise for dinoflagellates, however there appears to be enough overlap in COI sequence variation among and between species that a second gene fragment may prove invaluable for resolution.

References

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