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Microsatellite Markers for Population Genetic Applications in the Domoic Acid-producing Diatom *Pseudo-nitzschia australis* Frenguelli (Bacillariophyceae)

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Microsatellites are commonly used markers in population genetics and are increasingly being employed to determine population structure in phytoplankton populations. We have developed seven polymorphic microsatellite markers for the domoic-acid producing diatom *Pseudo-nitzschia australis*. Using these markers, thirty *P. australis* isolates were genotyped, 10 isolates were from Monterey Bay, California and 20 were from off the northern coast of Oregon. The number of alleles per locus ranged from two to eight and observed heterozygosities ranged from 0.11 to 0.70. All but two of the isolates were genetically distinct and initial population differentiation analysis indicated no significant differences between the Pacific Northwest isolates and the Monterey Bay isolates. *Pseudo-nitzschia australis* microsatellites appear to be species specific based on cross amplification tests with *Pseudo-nitzschia fraudulenta* (Cleve) Hasle, *Pseudo-nitzschia seriata* (Cleve) H.Peragallo, *Pseudo-nitzschia pungens* (Grunow ex Cleve) and *Pseudo-nitzschia multiseries* (Hasle) Hasle.

Key words: *Pseudo-nitzschia australis*; microsatellite; domoic acid; population genetics.

Introduction

Pseudo-nitzschia has been described as a cosmopolitan genus, species of which having been observed around the world (Hasle 2002; Lelong et al. 2012; Trainer et al. 2012). The neurotoxin domoic acid (DA) has been shown to be produced by ~13 of the approximately 37 described species (Trainer et al. 2012). While this genus has the capacity to contaminate shellfish with a potent neurotoxin, it generally accounts for a relatively small portion of the entire carbon biomass of the total phytoplankton assemblage (Horner et al. 2000; Trainer et al. 2009). Additionally, very little is known regarding the population dynamics of individual *Pseudo-nitzschia* species that have the potential to cause DA events on local scales, information which would be extremely valuable in predicting domoic acid events and developing mitigation strategies.

Since 1991, *Pseudo-nitzschia australis* has either been directly or indirectly implicated as the causative organism in several domoic acid events on the U.S. West coast (e.g. Bill et al. 2006; Buck et al. 1992; Fritz et al. 1992; Horner and Postel 1993; Scholin et al. 2000; Trainer et al. 2001, 2007). Specifically, in 2003 this species caused the first shellfish harvesting closure due to domoic acid in Puget Sound, WA (Bill et al. 2006) and it was theorized that oceanic *P. australis* had been advected into the Puget Sound region. An understanding of the genetic structure of toxigenic populations of this species on regional and local scales will aid resource managers by providing a clearer understanding of the geographic distribution and relationship among *P. australis* populations.

Microsatellites are markers commonly used in population genetics due to their co-dominant inheritance, abundance in the genome, and the benefit of rapid and cost-efficient

screening (Balloux and Goudet 2002). Furthermore, microsatellites reveal high genetic diversity per locus and provide high statistical power in identifying distinct populations and distinguishing between sexual and clonal reproduction (Halkett et al. 2005). Microsatellite markers have been used for population genetic studies on *Pseudo-nitzschia pungens* (Adams et al. 2009; Casteleyn et al. 2009; Evans and Hayes 2004; Evans et al. 2005), *Pseudo-nitzschia multiseries* (Evans et al. 2004), and *Pseudo-nitzschia multistriata* (Tesson et al. 2011, 2013, 2014). While distinct populations of *P. pungens* have been found all over the world (Adams et al. 2009; Casteleyn et al. 2010), this species is considered non-toxic in some places (Evans and Hayes 2004) and weakly toxic in others (Trainer et al. 1998). Conversely, *P. multiseries* from the Washington coast has been shown to produce high concentrations of DA (Baugh et al. 2006). *Pseudo-nitzschia multiseries* was also identified as the cause of human poisoning due to domoic-acid laden mussels from Prince Edward Island, Canada, in 1987 (Bates et al. 1998). Further, Evans et al. (2004) described genetic diversity as well as a high level of variation in DA production in 25 isolates of *P. multiseries* over a large geographic scale, demonstrating that strains isolated from the same location can vary greatly in their toxicity. Similarly, *P. australis* is a known toxigenic species with a broad geographic range, but information on the genetic structure of its natural populations is lacking. Here, we describe seven polymorphic microsatellite loci that can be used in population genetic analysis of *P. australis*, enabling the quantification of genetic diversity and identification of distinct populations.

Results

Pseudo-nitzschia australis Microsatellites

A total of 171 cloned fragments from the microsatellite enriched library were PCR amplified and sequenced. Of the 171 cloned fragments 17 were unique and contained microsatellites with sufficient flanking sequence for primer design. PCR products of the expected size were found in nine of the 17 sequences and seven of these were polymorphic microsatellite loci in 30 *P. australis* isolates. Table 1 shows the core locus microsatellite repeat sequence, primer sequences, optimal annealing temperature, allele size range, observed number of alleles, and observed and expected heterozygosity for each microsatellite locus. All loci were neutral and there was no evidence for scoring errors due to stuttering or large allele drop-out at any locus. Also, there was evidence of null alleles at only locus *PnAu3*, due to an observed homozygote excess in most allele size classes, with no evidence for null alleles at the other loci.

Genetic Variation in *Pseudo-nitzschia australis*

Genotypes were obtained at all seven loci for 29 of the 30 field isolates. For the single isolate that could not be genotyped at all loci, data were missing at two loci (Table 2). The observed numbers of alleles per locus ranged from two to eight. Although only two alleles were observed in *PnAu6* and *PnAu7*, additional alleles may be revealed as more isolates are tested. The observed heterozygosities (H_O) ranged from 0.11 to 0.70 (Table 1) and suggest a high degree of genetic diversity at most loci within the 30 field isolates. Twenty nine of the 30 field isolates had unique genotypes. The two isolates (CA001 and CA003) with the same genotype were collected in California on the same day in 2009.

When all isolates were combined there were no deviations from Hardy-Weinberg equilibrium (HWE) at any given locus ($P < 0.05$), but there was a highly significant deviation from HWE overall (Table 3). When treated as separate samples, locus *PnAu3* deviated from

HWE in both the OR and CA isolates, and there was still a significant ($P < 0.05$) overall deviation from HWE in the OR sample but not in the CA sample. F_{IS} was much further from 0 (F_{IS} ranges from -1 to 1) in all samples at locus *PnAu3* than at any other locus. Positive values of F_{IS} can indicate a heterozygote deficiency, but the only a significant ($P < 0.05$) deficit of heterozygotes at *PnAu3* was observed when the CA isolates were analyzed as a single sample (Table 3). Significant deviations from linkage equilibrium ($P < 0.05$) were observed at four of the 21 locus pairs when all isolates were analyzed together (*PnAu2*/*PnAu4*, *PnAu1*/*PnAu5*, *PnAu4*/*PnAu5*, *PnAu2*/*PnAu6*). There were significant deviations at two locus pairs in the OR sample (*PnAu2*/*PnAu4* and *PnAu1*/*PnAu6*) and at three locus pairs in the CA sample (*PnAu1*/*PnAu2*, *PnAu2*/*PnAu4*, *PnAu2*/*PnAu6*). Tests for multilocus linkage ($\overline{r}_d$) indicated that there was no significant linkage among markers ($P > 0.05$) for tests of all isolates combined as well as the individual Oregon and California samples. Genetic differentiation (F_{ST} = 0.013) between the OR and CA samples was not significant ($P > 0.05$).

Cross Reactivity of Primers

All of the *P. australis* primer pairs were tested for cross amplification with DNA from *P. fraudulenta*, *P. seriata*, *P. pungens* and *P. multiseries*. No amplification of microsatellite loci was observed in any of these other species using the *P. australis* primer pairs.

Discussion

130 The core microsatellite sequences in *P. australis* show a similar complexity to those identified in
131 other species of *Pseudo-nitzschia* (Evans et al. 2004; Evans and Hayes 2004). *Pseudo-nitzschia*
132 *australis* microsatellite loci (4.4 alleles/locus) were found to be less polymorphic than those for
133 *P. pungens* (10.7 alleles/locus) and *P. multistriata* (7.3 alleles/locus) but showed similar
134 polymorphism to *P. multiseries* (4.8 alleles/locus). However, many more isolates were analyzed
135 for *P. pungens* (up to 213, Evans and Hayes 2004) than were analyzed for *P. multiseries* (25,
136 Evans et al., 2004), *P. multistriata* (66, Tesson et al. 2011) or *P. australis* (30, this study). More
137 alleles may be revealed at all loci as additional *P. australis* isolates are analyzed from the Pacific
138 Northwest and other parts of the world. Nevertheless, there does appear to be a high degree of
139 diversity among the 30 isolates analyzed in the present study.
140 The lack of deviation from HWE at any of the six individual loci in the combined group
141 of 30 *P. australis* isolates (Table 3) suggests no cryptic diversity in *P. australis* from the Pacific
142 Northwest. Although deviations from HWE were observed at two loci in the OR sample and a
143 single locus in the CA sample (Table 3), these may have been a result of the small number of
144 individual isolates that comprised the OR and CA samples. In contrast, in a sample of 66 *P.*
145 *multistriata* isolates Tesson et al. (2011) found that seven microsatellite loci deviated from HWE
146 with observed heterozygosity higher than expected in five of the seven loci. Similarly, Tesson et
147 al. (2014) found higher observed heterozygosities than expected in 19 of 22 samples with an
148 overall negative F_{IS} that indicated outbreeding and an overall excess of heterozygotes.
149 Additionally, Adams et al. (2009) found deviations from HWE at most loci in four samples of *P.*
150 *pungens* from the Pacific Northwest of similar size to the *P. australis* sample in the current
151 study. The four original *P. pungens* samples were subdivided based on population structure
152 analysis where two new sub-samples were created from each original sample for a total of eight

153 sub-samples. Adams et al. (2009) found only a single deviation from HWE (unpublished data) in
154 one of the new sub-samples, suggesting cryptic diversity among *P. pungens* in the four original
155 samples. Moreover, population differentiation tests between *P. pungens* sub-samples showed
156 significant differentiation (Adams et al. 2009) supporting the hypothesis of the existence of
157 cryptic diversity of *P. pungens* in the Pacific Northwest.
158 In addition to the lack of cryptic diversity in Pacific Northwest *P. australis*, population
159 differentiation tests also showed that there was no significant difference, based on F_{ST}, between
160 the OR and CA samples and suggests that all 30 *P. australis* isolates may belong to a single well-
161 mixed coastal population. Similarly, Casteleyn et al. (2009) observed a lack of population
162 genetic structure in *P. pungens* var. *pungens* in isolates collected over a ~650 km range in the
163 North Sea. However, Tesson et al. (2014) observed two distinct populations of *P. multistriata*
164 that coexisted in multiple years, but in different relative proportions from year to year, at a single
165 location in the Gulf of Naples. Tesson et al. (2014) also found a large portion of strains that
166 were classified as hybrids of the two population and suggest that the two populations could be
167 relatively new arrivals to the system that have not merged entirely through sexual reproduction.
168 That the OR and CA isolates in the present study likely belong to the same population could be
169 explained by oceanic current systems on the U.S. West Coast (Hickey 1989; Hickey and Banas
170 2003). The southward flowing California Current System and the northward flowing California
171 Undercurrent could act to re-circulate a single population of *P. australis*, as well as other
172 phytoplankton species, on the U.S. West Coast to create a well-mixed assemblage.
173 There have been several domoic acid contamination events in shellfish attributed to *P.*
174 *australis* along the U.S. West coast since 1991, both on the outer coast and in inland waters. In
175 2003, it was hypothesized that either a coastal population of toxic *P. australis* was advected into

176 Puget Sound or that environmental conditions allowed a *P. australis* population already in Puget
177 Sound to bloom and contaminate shellfish with domoic acid (Bill et al. 2006). The microsatellite
178 markers described here could be used to identify "resident" populations and ecotypes of *P.*
179 *australis* in coastal and inland waters, including Puget Sound.

180 Previous studies on diatoms have indicated that certain ecotypes may be more prone to
181 adapting to local environmental conditions than others. *Ditylum brightwellii*, while not a toxin
182 producing diatom, was suggested to maintain distinct populations in Puget Sound through
183 hydrologic influences and differential selection with some populations associated with water of
184 Puget Sound origin and others associated with water originating from the Strait of Juan de Fuca
185 (Rynearson and Armbrust 2004). However, Rynearson et al. (2006) described a transition
186 between two distinct populations of *D. brightwellii*, where both populations were observed in the
187 Strait of Juan de Fuca and in Puget Sound. Similarly, Thessen et al. (2007) demonstrated that
188 certain strains of three *Pseudo-nitzschia* species (*P. calliantha*, *P. fraudulenta*, and *P.*
189 *multiseries*) exhibited differential growth under a variety of environmental conditions. They also
190 suggested that in combination with high genetic variability, *Pseudo-nitzschia* spp. could take
191 advantage of multiple environmental regimes as at least one of the strains they tested was able to
192 grow optimally in each of their experimental treatments (Thessen et al. 2007). Likewise, the two
193 populations of *P. multistriata* that were observed by Tesson et al. (2014) occurred during the
194 same time periods, and therefore the same environmental conditions, in multiple years in the
195 Gulf of Naples . Like the transition zone described in (Rynearson et al. 2006) this could indicate
196 that, depending on the conditions, distinct populations *Pseudo-nitzschia* spp. can also coexist in
197 the same environmental regime. Therefore, the effects of environmental conditions on
198 potentially toxic populations of *Pseudo-nitzschia* spp. may be species and/or strain specific.

The bloom dynamics of a particular species of phytoplankton in a given region (whether they had been advected or are "resident") can depend on the prevailing oceanographic conditions in the region, the genetic composition of the population, and the adaptability of certain ecotypes in that population. This implies that if toxic coastal *P. australis* were advected into Puget Sound and transported across distinct hydrographic features, like a mixing zone such as Admiralty Inlet in Puget Sound (Rynearson and Armbrust 2004), they may or may not be able adapt to the prevailing oceanographic conditions depending on the genetic make-up of the advected population. Therefore, to mitigate shellfish DA contamination from *P. australis* it is important to take an integrated approach that includes monitoring oceanographic conditions, testing for marine toxins, and analyzing the regional and local population structure of *P. australis*.

Conclusions

While the results of the population genetic testing in this study should be interpreted with caution due to the relatively small number of isolates and the short time frames in which they were collected, the data suggest that *P. australis* from northern Oregon and Monterey Bay, CA may belong to a single population. More isolates will need to be collected from the entire U.S. West Coast and analyzed to further support this hypothesis. Future population genetic testing should also include testing isolates for domoic acid content under varying conditions. The identification of toxic coastal and/or resident *P. australis* populations may help to characterize the conditions under which they produce the highest toxin concentrations, thereby improving our ability to understand and mitigate *P. australis* impacts to shellfish harvesting and human health.

222 **Methods**

223

224 **Sample collection and cell isolation:** A total of 20 monoclonal *P. australis* cultures were
225 established from a surface water plankton sample collected off of the northwest coast of the State
226 of Oregon (Fig. 1). The sample was collected using a net (25 cm diameter x 60 cm length; 20-
227 µm Nitex® mesh) deployed by hand over the side of the NOAA Ship McArthur II in July 2008.
228 Presumptive (based on cell morphology) *P. australis* cells or chains of cells were isolated with
229 drawn out capillary pipets under 100x magnification on a Nikon TMS inverted microscope. The
230 cells were rinsed three times in sterile seawater and placed into individual wells of a 48-well
231 plate containing f/2 medium (0.75 mL) (Guillard and Ryther 1962). Additional samples from
232 Monterey Bay, CA used for analysis included five live *P. australis* cultures that were collected in
233 May (1 isolate), September (3 isolates), and November (1 isolate) 2009 as well as archived *P.*
234 *australis* DNA extracts from three cultures originally isolated in July 2003 and two from March
235 2004 (Fig. 1).

236 **Culture maintenance, identification, harvesting and DNA extraction:** The plates
237 containing the isolates from Oregon were maintained in a temperature and light controlled
238 incubator at 12 °C on a 12 hours on:12 hours off light:dark cycle and at a photon flux density of
239 approximately 85 µEinsteins$\cdot m^{-2} \cdot s^{-1}$. The isolates were examined microscopically for
240 contamination with other algal species every two days and cultures were re-isolated when
241 necessary. Cultures from Oregon and Monterey Bay were transferred to glass culture tubes
242 (20x150 mm) containing 15 mL f/2 medium, maintained under the incubation conditions
243 described above, and allowed to grow to high densities. Morphological characteristics (Hasle et
244 al. 1996) for *P. australis* were confirmed using scanning electron microscopy. The California

245 isolates were confirmed to be *P. australis* by ITS sequence analysis (pers. comm, G. Jason
246 Smith).

247 Cultures were harvested onto nitrocellulose filters (Millipore HA, 25 mm diameter, 0.45
248 µm pore size, Millipore, Billerica, MA, USA) and stored at -80 °C. DNA was extracted from the
249 material on the filters using Qiagen (Valencia, CA, USA) DNeasy Plant Mini kits with the
250 following modifications. To prepare the material for the extraction procedure, 500 µL Qiagen
251 lysis buffer, 5.0 µL RNase A (Qiagen, 100 mg•mL^{-1}) and 2.5 µL Proteinase K (Qiagen, 20
252 mg•mL^{-1}) were added directly to the tube containing the filter with the harvested culture. The
253 tubes were then vortexed at the highest setting for 30 sec and centrifuged for 5 min (5223 x *g*) to
254 pellet the cells. The filters were removed and the tubes were vortexed for 2-3 sec to resuspend
255 the material. Following incubation at 65 °C for one hour, the Qiagen procedure was followed
256 according to the manufacturer's instructions, with the exception of only one elution of 70 µL of
257 Qiagen buffer AE. DNA was quantified by PicoGreen staining on an FLx800 plate reader
258 (Biotek Instruments, Inc, Winooski, VT, USA) and DNA concentration was normalized to 0.2
259 ng/µL.

260 **Development of microsatellite libraries:** Monoclonal *P. australis* cultures (100 mL)
261 were grown at 12 °C on a 12:12 light:dark cycle and at a photon flux density of approximately
262 85 µEinsteins·m^{-2}·s^{-1} for 18 days. The cultures were harvested onto 47 mm Millipore HA filters
263 (0.45 µm pore size). The freshly harvested material was scraped off of the filter with a sterile
264 spatula, transferred to a 1.5 mL microcentrifuge tube and DNA was extracted following the
265 procedure described above.

266 Genomic DNA libraries enriched in microsatellite DNA loci were constructed following
267 the procedure described in Glenn and Schable (2005). Briefly, the DNA was digested with *RsaI*

and *PvuII* (one restriction enzyme per isolate replicate), ligated to a linker oligonucleotide, and hybridized to biotinylated microsatellite probes [$(GT)_8$ or $(CT)_8$]. The DNA fragments that were hybridized to a biotinylated probe were captured onto Streptavidin coated M-280 Dynabeads (Invitrogen, Carlsbad, CA, USA), rinsed and PCR amplified. The resulting PCR products were cloned using the TOPO TA cloning system (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol with the following modifications: 4 µL of PCR product was used in cloning reactions, reactions were incubated on ice for 25 min., and three volumes of transformed cells (10 µL, 25 µL, and 100 µL) were spread onto separate plates for each cloning reaction. The colonies were PCR amplified directly using M13 forward and reverse primers.

Cycle sequencing was performed using the Life Technologies (Carlsbad, CA, USA) BigDye® Terminator v3.1 Cycle Sequencing kit; unincorporated dye was removed from the reactions using Agencourt CleanSEQ (Beckman Coulter, Brea, CA, USA) following the manufacturers protocol. PCR primers were designed to target microsatellite repeats from the sequence data using the Geneious v5.0.3 (Biomatters Ltd., Auckland, New Zealand) software package and tested with DNA f rom one isolate using a Bio-Rad iCycler gradient thermocycler (Bio-Rad, Hercules, CA, USA) to determine optimal annealing temperatures. PCR reactions were carried out in 20 µL volumes containing 2.0 ng template DNA, 12.5 mM Tris-HCl (pH 8.5), 62.5 mM KCl, 0.125% Triton X-100, 2.0 mM MgCl2, 0.25 mM dNTPs, 75 ng of each primer, and 0.5 units Taq polymerase (Apex Taq DNA Polymerase, Genesee Scientific, San Diego, CA, USA) using a thermal profile of 94° C for 3 minutes, followed by 35 cycles of 94 °C for 1 min, a gradient of 50 °C-65 °C for 1 min, 72 °C for 30s with a final extension of 72 °C for 5 min. The PCR products were visualized on a 1.5% agarose gel. Primer sets that produced fragments of the expected size were labeled using a forward 5' fluorescent (6-FAM) tag. These primer sets were used to test for polymorphic loci using DNA from six isolates. DNA was PCR

amplified at the optimal annealing temperature and the PCR products were analyzed using a Life Technologies 3100 Genetic Analyzer. Primer sets that revealed polymorphic loci were used to genotype the remaining *P. australis* field samples.

Tests for cross reactivity of primers: Primer sets were tested for interspecific cross reactivity with a single strain each of *P. fraudulenta*, *P. seriata*, *P. pungens* and *P. multiseries* collected during the same NOAA Ship McArthur II cruise in July 2008 in which the *P. australis* strains in the present study were collected. The identity of these strains was confirmed with scanning electron microscopy and cultures of these four strains were maintained, harvested and extracted using the methods above. DNA from these cultures along with DNA from a *P. australis* culture was PCR amplified using optimal annealing temperatures and the products were visualized on a 1.5% agarose gel.

Microsatellite data analysis: The program LOSITAN was used to assess the neutrality of the seven microsatellite markers and the program MICRO-CHECKER (Van Oosterhout et al. 2004) was used to check for errors due to stuttering, large allele dropout and the presence of null alleles by testing all isolates combined in a single group. Population genetic analyses were performed in two ways. The isolates first were treated as a single sample, and then the analyses were repeated with the isolates separated into a sample from Oregon (OR) and a sample from California (CA). Due to the small sample size and that the California isolates were collected in different years, only cursory analysis of genetic variation was performed and should be interpreted with caution.

Basic population genetic summary statistics were calculated using GENEPOP version 4.2 (Raymond and Rousset 1995; Rousset 2008) including allele frequencies and locus allele counts. Tests for deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD)

between pairs of loci were also calculated using GENEPOP. Concordance of genotypic distributions to Hardy-Weinberg expectations was determined by exact tests (Guo and Thompson 1992) in GENEPOP, with Markov chain parameters of 1000 for dememorization number, 300 batches, and 1000 iterations per batch. Multilocus linkage was evaluated by calculating the $\overline{r}_d$ statistic using the software package MULTILOCUS version 1.3 (Agapow and Burt 2001). One thousand randomizations of the data were performed to test whether the observed $\overline{r}_d$ was significantly larger than expected. The F_{IS} statistic, a measure of the extent of genetic inbreeding within subpopulations which is used to evaluate deviation from Hardy-Weinberg equilibrium (HWE; Wright 1951) was estimated using FSTAT version 2.9.3.2 following Weir and Cockerham (1984). F_{IS} ranges from -1 to 1, where negative numbers indicate heterozygote excess and positive numbers indicate heterozygote deficits. ARLEQUIN 3.1 (Excoffier et al. 2005) was used to perform exact tests for pairwise differentiation between the Oregon and California samples as well as to calculate F_{ST} (Wright 1951) which is an estimator of genetic population differentiation that ranges from zero to one, with zero indicating no difference and one being completely diverged.

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Figure Legends

Figure 1. Locations where *P. australis* isolates were obtained (black filled circles).

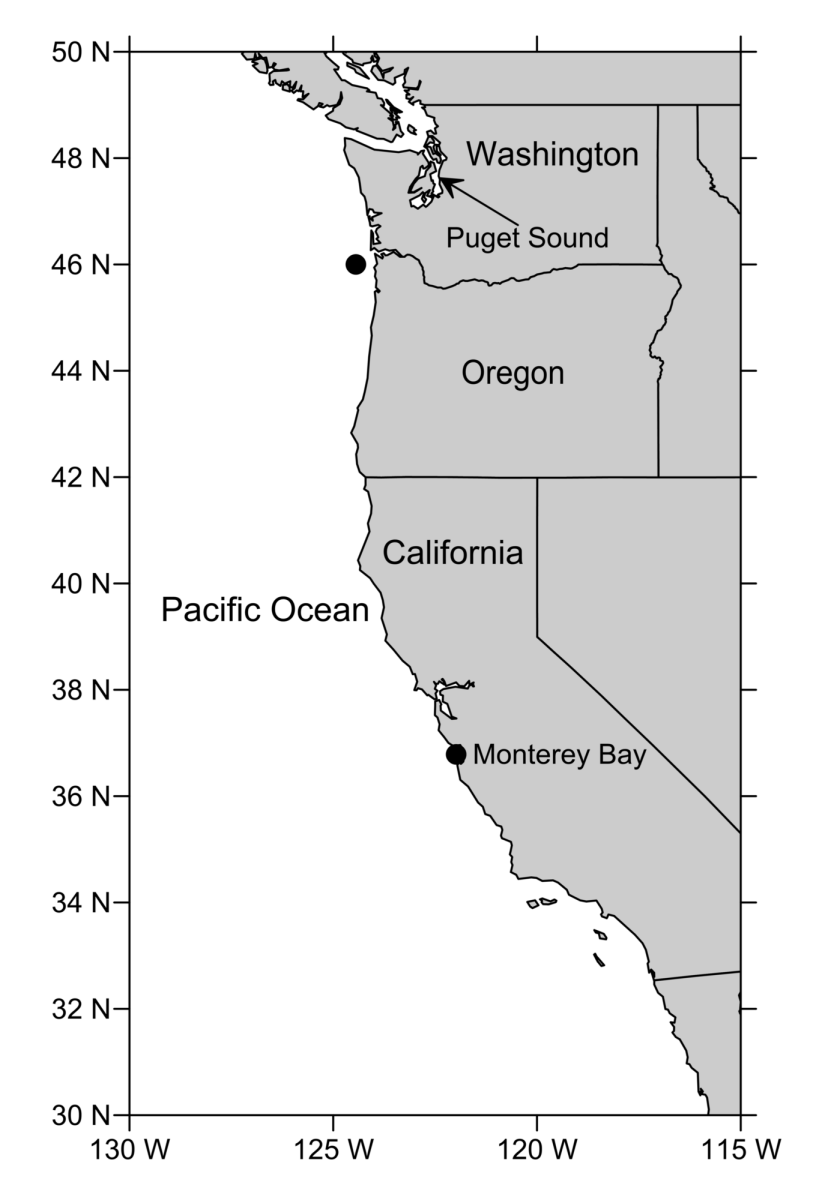
50 N
48 N
46 N
44 N
42 N
40 N
38 N
36 N
34 N
32 N
30 N
130 W
125 W
120 W
115 W
Washington
Puget Sound
Oregon
California
Pacific Ocean
Monterey Bay

Table 1. Attributes of seven microsatellite loci for *Pseudo-nitzschia australis* from the U.S. West Coast.

Locus	n	Core sequence*	Primer sequences (5′–3′)	T_A	bp	N_A	H_O	H_E	GenBank Accession No.
PnAu1	30	$(GT)_9N_2(GT)_8$	F: GCACGAGGGCACGAACAGGG R: GCGTGATGACGGGGGAACTGG	64	213-262	8	0.70	0.76	KU728057
PnAu2	30	$(GT)_{10}$	F: AGCCATCGCTCGGGGTTTGC R: ACTGCGTGTCGCAACAGTCCA	64	261-279	8	0.63	0.69	KU728058
PnAu3	29[a]	$(GT)_{11}$	F: TGCTCAGCCTGGCGGTCAGA R: AAGGCGCGGCTTTCGACTGA	64	266-284	5	0.50	0.74	KU728059
PnAu4	30	$(GT)_{13}$	F: CGATCTAGCGACGAACGAGATCCA R: AGCAGCTCCAGTTCCAGTGT	64	125-129	3	0.53	0.48	KU728060
PnAu5	29[a]	$(ACT)_3N_3(ACT)_{11}$	F: GCAATGAAATCAACACCATCTGCCA R: ACGTACACGTACCAGGCATCTCA	64	170-182	3	0.11	0.10	KU728061
PnAu6	30	$(GT)_7$	F: CCGCCCGCATCAAAGGCGAA R: GCTTCCGAGCGAAAGGCCA	64	197-203	2	0.50	0.44	KU728062
PnAu7	30	$(GT)_7N_2(GT)_3$ $N_2(GT)_3$	F: CGGACGCCATTTGGTGGGGG R: TCACTCATTCATTGGTTGTTTCA	64	197-205	2	0.23	0.21	KU728063

*N indicates bases that are within the core but that do not form part of a repeat unit.

[a] this locus did not amplify in one isolate

The number of individuals tested (n), annealing temperature (T_A, °C), allele size range (bp), number of alleles (NA) and observed (H_O) and expected (H_E) heterozygosities are given. GenBank Accession numbers are given in parentheses below each locus.

Table 1. Genotypes (allele sizes in base pairs) of the *Pseudo-nitzschia australis* isolates from the U.S. West Coast. 10 from Monterey Bay (CA) and 20 from waters off the coast of northern Oregon (OR). n.d. indicates no data

Sample Name	PnAu1	PnAu2	PnAu3	PnAu4	PnAu5	PnAu6	PnAu7
CA001	237/245	262/279	268/284	127/129	182/182	197/197	197/205
CA002	223/227	275/275	268/268	127/129	182/182	197/203	197/197
CA003	237/245	262/279	268/284	127/129	182/182	197/197	197/205
CA004	227/249	267/275	268/268	125/127	179/182	197/197	197/197
CA005	227/227	275/275	268/284	127/129	182/182	197/203	197/205
CA006	227/227	275/275	266/266	127/129	182/182	197/203	197/197
CA007	213/237	275/279	266/268	127/127	182/182	197/203	197/197
CA008	235/245	275/279	268/268	127/127	170/182	197/203	197/197
CA009	223/262	263/279	268/268	127/127	182/182	203/203	197/205
CA010	223/245	275/275	268/268	125/129	182/182	197/203	197/205
OR001	227/227	275/275	268/268	127/129	182/182	197/197	197/197
OR002	223/235	261/279	266/268	125/127	179/182	197/197	197/197
OR003	227/235	275/279	273/282	127/127	182/182	197/197	197/197
OR004	227/235	273/279	268/268	127/127	182/182	197/203	197/205
OR005	227/227	263/269	268/268	127/127	182/182	197/197	197/197
OR006	223/245	262/267	268/268	127/127	182/182	197/203	197/205
OR007	213/213	275/279	266/268	127/127	182/182	197/203	197/197
OR008	227/227	275/275	268/273	127/129	182/182	197/203	197/197
OR009	213/227	275/275	268/282	127/127	182/182	197/203	197/197
OR010	227/227	263/275	266/268	125/129	182/182	197/197	197/197
OR011	227/227	269/269	268/268	125/129	182/182	197/197	197/197
OR012	223/227	262/275	266/268	127/129	182/182	197/197	197/197
OR013	227/227	262/275	266/268	127/127	182/182	197/197	197/197
OR014	227/231	275/279	268/268	127/127	182/182	197/197	197/197
OR015	213/227	275/275	266/268	127/127	182/182	197/203	197/197
OR016	235/237	273/279	266/284	127/127	182/182	197/203	197/197
OR017	227/245	275/279	268/268	127/127	182/182	197/203	197/197
OR018	227/235	275/275	n.d.	127/129	n.d.	197/203	197/197
OR019	213/245	263/275	268/268	127/129	182/182	203/203	197/197
OR020	223/227	275/275	266/266	127/129	182/182	197/197	197/197

Table 3. *P*-values for HWE deviation tests along with F_{IS} statistics for each microsatellite locus. Samples include the 30 combined isolates (All Isolates) and samples where the isolates were separated into groups depending on their origin (OR or CA). HWE *P*-values that are in bold indicate significant deviations from HWE at a particular locus in a given sample. n.i. indicates no information (i.e. just two alleles were detected but one allele was represented by only one copy, see Table 2)

Locus	All Isolates	OR	CA
PnAu1			
HWE	0.395	0.599	0.230
F_{IS}	0.077	0.064	0.071
PnAu2			
HWE	0.134	**0.035**	0.183
F_{IS}	0.081	0.095	0.085
PnAu3			
HWE	0.060	**0.002**	**0.005**
F_{IS}	0.327	0.196	0.5636[a]
PnAu4			
HWE	0.904	0.222	0.544
F_{IS}	-0.114	-0.024	-0.248
PnAu5			
HWE	1.000	n.i.	1.000
F_{IS}	-0.025	n.i.	-0.029
PnAu6			
HWE	0.886	1.000	1.000
F_{IS}	-0.139	-0.103	-0.200
PnAu7			
HWE	1.000	1.000	1.000
F_{IS}	-0.115	-0.027	-0.286
Overall			
HWE	***	**0.023**	0.203
F_{IS}	0.063	0.064	0.049

[a] significant heterozygote deficiency,
*** "highly significant"