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**SPATIOTEMPORAL CHANGES IN THE GENETIC DIVERSITY OF HARMFUL ALGAL BLOOMS CAUSED BY THE TOXIC DINOFLAGELLATE *ALEXANDRIUM MINUTUM***

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## **Abstract**

Organisms with sexual and asexual reproductive systems benefit from both types of reproduction. Sexual recombination generates new combinations of alleles whereas clonality favors the spread of the fittest genotype through the entire population. Therefore, the rate of sexual versus clonal reproduction has a major influence on the demography and genetic structure of natural populations. We addressed the effect of reproductive system on populations of the dinoflagellate *A. minutum*. More specifically, we monitored the spatiotemporal genetic diversity during and between bloom events in two estuaries separated by 150 km for two consecutive years. An analysis of population genetic patterns using microsatellite markers revealed surprisingly high genotypic and genetic diversity. Moreover, there was significant spatial and temporal genetic differentiation during and between bloom events. Our results demonstrate that (1) interannual genetic differentiation can be very high, (2) estuaries are partially isolated during bloom events, and (3) genetic diversity can change rapidly during a bloom event. This rapid genetic change may reflect selective effects that are nevertheless not strong enough to reduce allelic diversity. Thus, sexual reproduction and/or migration may regularly erase any genetic structure produced within estuaries during a bloom event.

## ***Introduction***

The demography and genetics of natural populations can vary according to the rate of sexual versus clonal reproduction. These two modes of reproduction occur simultaneously in many eukaryotes. These species can benefit both from the advantage of sexual reproduction, which lies in the genetic recombination that it generates, and from the theoretical short-term demographic advantage of clonal reproduction (Maynard-Smith 1978). The relative contribution of sexual and asexual reproduction can vary within a species depending on environmental conditions (Eckert 2001; Silvertown 2008). For example in aphids, sexual reproduction is associated with the production of resting eggs that can survive harsh conditions, whereas populations living in milder environments may reproduce asexually throughout the whole year (Rispe *et al.* 1998). Sexual reproduction thus seems required to produce a resistant form, regardless of the genetic variability generated. In the freshwater microcrustacean *Daphnia pulex* that practices facultative sexual reproduction (alternation between parthenogenesis and sexual reproduction) during the growing season, selection on clonal lines results in an increase in genotypic adaptation and an erosion in clonal diversity (Deng & Lynch 1996). The over-representation of certain clonal genotypes at the end of the growing season leads to greater genetic structuring and linkage disequilibrium. Similarly, most plankton species alternate between an active growing phase, during which the number of cells increases rapidly, and another phase, generally involving sexual reproduction, during which cells are in a dormant state. During the asexual reproduction phase, fitness differences among clonal lineages may cause rapid changes in genotype frequencies. In the cyclically parthenogenetic rotifer species *Brachionus plicatilis*, there is evidence that clonal selection occurs during the parthenogenetic phase (Gómez & Carvalho 2000). However, sexual reproduction as well as dormant stages may slow down the response to directional selection

and may act to maintain genetic variation when selection fluctuates (Hairston *et al.* 1996). Thus, in the freshwater copepod *Diaptomus sanguineus*, the dormant cells may act as a reservoir of genotypes (similar to a seed bank) produced in past environments, but they also may interact as a part of the selection-response dynamics in the current environment (Hairston *et al.* 1996).

An extreme case of alternation of an asexual growing phase and a dormant sexual phase is exemplified by unicellular phytoplankton bloom species involved in harmful algal blooms (HABs). Compared to multicellular organisms, these unicellular organisms are characterized by a ubiquitous distribution, suggesting very large population sizes and broad dispersal abilities (Cloern 1991; Finlay & Fenchel 2004; Norris 2000). Nevertheless, dispersal of marine phytoplankton is a hotly debated issue and published results are subject to controversies. Studies on marine phytoplankton dispersal differ vastly with respect to study scale, sampling strategy (e.g. sampling few individuals per global geographic region; population samples) and the resolution of the characters or markers used (e.g. morphological markers; highly variable, microsatellite-type molecular markers). For example, using morphological criteria, Cermeno and Falkowski (2009) argue that the biogeographical distribution of fossil diatom genera is ubiquitous and that these taxa thus have no geographic structure. However, more recent studies using microsatellite markers demonstrate that there is genetic differentiation within a single species, the toxic diatom *Pseudo-nitzschia pungens*, on a very large scale (i.e. samples from the Atlantic and Pacific Oceans) (Casteleyn *et al.* 2010). The issue of connectivity between populations remains unresolved because there is little information on the distance that a single phytoplankton cell can cover and because there are only a few studies on how the marine physical environment influences dispersal. Exploring these factors is necessary for a better understanding of the ecology and evolution of plankton

organisms (Palumbi 1994). Over the past several years, more and more studies have focused on spatiotemporal differentiation in dinoflagellate populations. For example, in the Gulf of Maine, genetic differentiation was found between two subpopulations investigated during offshore blooms, corresponding to the beginning and the end of a bloom event (which lasts about 2 months), but in different geographic locations (northern and southern, separated by hundreds of km) (Erdner *et al.* 2011). Other work (Richlen *et al.* 2012) shows spatiotemporal differentiation in the estuaries of Massachusetts (Nauset Marsh system and the Gulf of Maine) at two sites, but only one site was sampled throughout the whole bloom event (from beginning to end), precluding any comparison between dates of the same bloom at different sites. Whatever their scale, these previous studies demonstrate genetic differentiation between sites and high genetic diversity within a given bloom event. This differentiation of bloom populations and their high genetic diversity leads to questions on the role of the two life-cycle phases on population dynamics and the importance of sexual reproduction relative to clonal reproduction.

The dinoflagellate *Alexandrium minutum* (Halim 1960) is one of the main species involved in paralytic shellfish poisoning (PSP) blooms off the coasts of France. This microalga produces saxitoxins that can accumulate along the trophic food web. They produce PSP events that cause cases of human poisoning, typically by ingestion of contaminated shellfish. This species shows an alternation of asexual and sexual phases. The vegetative cell is haploid and sexual reproduction takes place when the environmental conditions become unfavorable (Figueroa *et al.* 2011). Sexual reproduction is the result of the fusion between two gametes, forming a planozygote (Probert *et al.* 2002). This stage changes into a resting cyst that sinks to the sediment (like seeds in a seed bank) (Wyatt & Jenkinson 1997). After a dormancy period of several months (Garcés *et al.* 2004; Figueroa *et al.* 2007), cysts may

germinate and give rise to vegetative haploid cells that reproduce asexually in the water column.

The objective of this study was to study the effect of asexual and sexual reproduction on the population genetic structure of this toxic dinoflagellate *A. minutum* on a small scale, both in space and in time. We used microsatellite markers to analyze monoclonal cultures of *A. minutum* isolated from samples taken at two sites located along the French coast of the English Channel, separated by roughly 150 km, for two consecutive annual bloom events and at three different periods during each bloom event. We specifically addressed the following questions: (1) To what degree are different estuaries within the same geographic region connected? (2) What role does sexual reproduction play in the dynamics of the bloom populations? (3) Finally, is there any temporal genetic structuring during bloom events?

## ***Materials and Methods***

### *Sampling sites and strain collection*

More than 1240 strains of dinoflagellates (different species) were collected in the Penzé estuary (48°37'37.57"N, 3°57'13.17"W) and the Rance estuary (48°31'49.61"N, 1°58'21.81"W, Table 1). These two estuaries are separated by approximately 150 km (Fig. 1). *A. minutum* blooms occurred concomitantly in both ecosystems in early summer. In 2010, blooms extend from 17 May to 12 July in the Rance estuary (maximum density of  $4.2 \times 10^4$  cells/L<sup>-1</sup> observed on June 22) and from 2 June to 7 July in the Penzé estuary (maximum density of  $3.7 \times 10^5$  cells/L<sup>-1</sup> observed on 8 June). In 2011, blooms lasted from 11 May to 20 June in Rance (maximum density of  $4.3 \times 10^5$  cells/L<sup>-1</sup> observed on 27 May) and from 1 June to 8 July in Penzé (maximum density of  $2.6 \times 10^5$  cells/L<sup>-1</sup> observed on 23 June).

Plankton samples were collected from these two sites during the developmental phase of the bloom (beginning), during the maintenance phase (middle) and during the decline phase (end of the bloom event) whenever possible in each of two study years (2010 and 2011). All strains were obtained by micropipetting one single cell into fresh medium; 530 of these strains survived and 265 strains of *A. minutum* were genotyped. They were maintained at 19°C with 70  $\mu\text{E}\cdot\text{m}^2\cdot\text{s}^{-1}$  of light in a 12:12 light:dark cycle. The medium F/2 (Marine Water Enrichment Solution, Sigma) was prepared using autoclaved natural seawater from the Penzé estuary. It was collected at least 3 months prior to use and stored in the dark. Strains used in this study (see supplementary Table S1) have been deposited at the Roscoff Culture Collection ([www.sb-roscoff.fr/Phyto/RCC](http://www.sb-roscoff.fr/Phyto/RCC)).

#### *Flow cytometry*

The DNA content of each monoclonal strain was checked by flow cytometry on whole *A. minutum* cells fixed in ethanol. A 250  $\mu\text{L}$  aliquot of each culture was centrifuged at 3000 rpm for 10 min. The supernatant was removed, cells were then re-suspended by vortexing in 250  $\mu\text{L}$  of 70% ethanol and kept overnight at 4°C. Cells were collected by centrifugation at 3000 rpm for 10 min to remove ethanol and then resuspended in 250  $\mu\text{L}$  of PBS. Triton X-100 (0.1% final), RNase A (0.01%) and Propidium Iodide (PI, 30  $\mu\text{g}\cdot\text{mL}^{-1}$ ) were added. Samples were incubated at 37°C for 30 min before flow cytometry analysis, performed using a FACS Canto II (Becton Dickinson, San Jose, CA, USA) flow cytometer equipped with a 488 nm laser and the standard filter setup. The DNA content of *A. minutum* strains was evaluated by comparison with Human Blood Cells (HBCs, 2C = 6.4 Mbp). Isolated nuclei of human blood cells were obtained by resuspending 10  $\mu\text{L}$  of blood in 1 mL of NIB buffer (Marie *et al.* 2000). Then, 10  $\mu\text{L}$  of this suspension was added to 250  $\mu\text{L}$  of *A. minutum* culture re-suspended in PBS buffer before staining. Then, all cultures were analyzed by flow



cytometry without HBC and mean fluorescence of PI were compared to check for ploidy level differences.

#### *DNA extraction, strain identification and genotyping*

For DNA extraction, strains were collected during their exponential growth stage (strains listed Table 1). DNA was extracted using either the CTAB method (Lebret *et al.* 2012) or the Nucleospin 96 Plant kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions, using 5-10 mg of dried tissue resuspended in 100 µL elution buffer.

Strains were screened using the intergenic region of ribosomal DNA (ITS1, 5.8S and ITS2). PCR mixes (14 µL in total) included 20 ng of DNA, 1X GoTaq Flexi green buffer (Promega, Madison, WI, USA), 150 µM of each dNTP (Thermo Fisher Scientific Inc., Waltham, MA, USA), 20 pmol of the forward primer 329F (5'-GTG AAC CTG CRG AAG GAT CA-3', complementary reverse of the eukaryote reverse primer 329-R (Moon-van der Staay *et al.* 2001), 20 pmol of the reverse primer DIR-R (5'- TAT GCT TAA AAT TCA GCA GGT-3' (Scholin & Anderson 1994), and 1.25 U GoTaq® Polymerase (Promega). Amplification was carried out in a DNA Engine Peltier Thermal Cycler (Bio-Rad, Hercules, CA, USA) with the following cycling conditions: an initial denaturation at 95°C for 5 min, followed by 35 PCR cycles (denaturation at 95°C for 45 s, annealing at 53°C for 45 s, and extension at 72°C for 45 s), followed by a final extension at 72°C for 10 min. The PCR products were purified using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) for subsequent bidirectional sequencing using a capillary sequencer type ABI 3130XL (Applied Biosystems).

The strains were genotyped using the 12 microsatellite markers developed by (Nagai *et al.* 2006). Amplification of microsatellite loci was carried out in a 10 µL final volume with the

following components: 20 ng DNA, 1x GoTaq® Flexi buffer (Promega), 2 mM MgCl<sub>2</sub>, 150 μM each dNTP (Thermo Fisher Scientific Inc.), 0.8 μg.μL<sup>-1</sup> bovine serum albumin, 30 pmol fluorescent-labeled forward primer (FAM, VIC, NED or PET), 30 pmol reverse primer, and 0.35 U GoTaq® Polymerase (Promega). PCR amplifications were carried out in a Bio-Rad DNA Engine Peltier Thermal Cycler using the following cycling conditions: an initial denaturation at 95°C for 5 min, followed by 10 PCR cycles (denaturation at 95°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 30 s) followed by 28 cycles (denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s), followed by a final extension at 72°C for 10 min. Then, 2 μL of PCR products, diluted 1:10, was added to 10 μL of loading buffer containing 0.5 μL of SM594 size standard (Mauger *et al.* 2012) and 9.5 μL of Hi-Di formamide (Applied Biosystems). The loading mix was denatured at 95°C for 3 min and run in an ABI 3130 XL capillary sequencer (Applied Biosystems) equipped with 50 cm capillaries. Genotypes were scored manually using GeneMapper version 4.0 (Applied Biosystems).

### *Genetic analyses*

To increase sample size, close sampling dates were pooled (i.e. PZ10D, PZ11B and RC11D, Table 1) and ultimately only 11 samples with a minimum sample size of 15 strains were used in the genetic analysis (i.e. RC10D was excluded from the analyses; see Table 1).

### *Genetic diversity and multilocus genotype diversity*

Because strains of *A. minutum* are haploid, the frequencies of null alleles were estimated by direct observation. After two re-amplifications, strains for which more than half of the loci did not amplify correctly were removed from the dataset. The absence of amplification at loci in other strains was considered as a null allele. Standard measures of genetic diversity (mean

number of alleles per locus ( $N_a$ ), Nei's unbiased estimator of genetic diversity ( $H_s$ ; Nei 1987), and allelic richness ( $R_a$ ) for each sampling date and site were calculated using Fstat (version 2.9.3.2; Goudet 2001). Allelic richness was estimated after randomly subsampling each sample (10,000 randomizations) to standardize the minimum sample size among populations. Test for differences among groups, Fstat (Goudet 2001) was used to test the difference between the  $H_s$  values obtained at the beginning phases (PZ11A, RC10A, RC11A) and at the end phases of bloom events (PZ10D, PZ11D, RC11D). Genclone (Arnaud-Haond *et al.* 2005) was used to test the efficiency of the microsatellite marker set to discriminate the maximum clonal diversity available in the data set and to calculate the number of distinct multilocus genotypes observed ( $G$ ).

#### *Linkage Disequilibrium*

Linkage disequilibrium (LD) was assessed using a single multilocus measurement of LD that is provided by the association index  $\bar{r}_d$  (Brown *et al.* 1980 modified by Agapow & Burt 2001) and was computed using MultiLocus ver 1.2. (Agapow & Burt 2001). Significance tests were based on comparisons of the observed value to those of randomized datasets generated from 1000 permutations (Burt *et al.* 1996). The global  $P$ -value was calculated using a generalized binomial procedure to combine independent data (Teriokhin *et al.*, 2007; De Meeûs *et al.*, 2009).

#### *Genetic differentiation*

$F_{st}$  values were calculated between all pairs of populations using GENETIX 4.05 (Belkhir *et al.*, 1996-2004), their significance was assessed using 1.000 permutations and  $D$  ( $D_{est}$ ; Jost, 2008) was estimated with SMOGD 1.25 (Crawford, 2010). Different levels of comparison were thus considered: among dates during a bloom event, among dates within a

site, and between sites. These data were summarized and statistically tested with a principal component analysis (PCA) performed using PCA-GEN software (version 1.2; Goudet 1999). The statistical significance associated with each axis was evaluated after 10,000 randomizations.

The partitioning of genetic variation at different hierarchical levels was examined using molecular analyses of variance (AMOVA) in Arlequin 3.5.1.2 (Excoffier & Lischer 2010). There was only one sampling date during the 2010 bloom in Rance (Table 1); it was therefore only possible to conduct the two following analyses. First, variation was assessed on the 2011 samples by determining the variance component attributable to between-sites differentiation and to among in-bloom phases within a site. Second, variation was examined in more detail only at Penzé by determining the variance component attributable to between-years differentiation and to among in-bloom phases within years. The significance ( $P < 0.05$ ) of the fixation indices was evaluated based on 10,000 permutations.

## ***Results***

### *Isolation and identification of strains*

Of the 1240 isolated cells, we obtained 530 usable strains for this study (i.e. 43% success rate). The survival rate by sampling date ranged from 27% to 66%. Of these 530 monoclonal strains, 364 (66%) were assigned to *A. minutum* showing a unique haplotype corresponding to 100% similarity with a 550 bp of the rDNA sequence (covering ITS1, the 5.8S rRNA gene, and ITS2) (McCauley *et al.* 2009; Casabianca *et al.* 2011) were selected (Table 1). Indeed, this unique sequence corresponds to an *A. minutum* sequence (AM1 strain) previously published in GenBank (EU707466). The comparison of *A. minutum* DNA content

shows that whole cells of *A. minutum* analyzed contain the same level of ploidy (32 Mbp, Fig. S1).

### *Genetic diversity*

After re-amplifying strains with dubious amplification, 5 of the 12 loci (Aminu39, Aminu44, Aminu10, Aminu15 and Aminu29) developed by Nagai *et al.* (2006) were removed from the dataset. We also removed strains for which more than half of the loci did not amplify correctly. The final dataset included seven loci amplified on 265 individual strains (Table S2). For these loci, the frequency of null alleles varied from 8% for locus Aminu11 to 22% for locus Aminu20. The efficiency of the microsatellite loci to discriminate the maximum clonal diversity available in the sample is given Fig. S2. Of the 265 strains analyzed, 265 (100%) had unique seven-locus genotypes; there were therefore no repeated genotypes in any of the analyzed strains.

The PCR products of these seven microsatellite markers showed one single band, i.e. one allele. All loci were polymorphic. The genetic diversity indices are given in Table 2. The number of alleles varied among bloom populations, with some populations having twice as many alleles as other populations (cf. RC11A and RC10A, Table 2), and with sampling effort. Allele richness, corrected for sampling size, varied from 6.49 (RC11A) to 9.01 (RC11C). The per-locus and per-population numbers of alleles ( $N_a$ ) ranged from 3 (locus A41) to 17 (locus A20) alleles. Whatever the sampling date during bloom events, Penzé and Rance showed high genetic diversities, with  $H_s$  values ranging from 0.79 to 0.88. These genetic diversity values did not differ between the beginning and the end phases of a bloom event within an estuary (permutation test of comparison among groups;  $P$ -value=0.647).

Tests of linkage disequilibrium revealed an overall  $P$ -value of 0.525. Of the 11 spatio-temporal samples, only one showed significant  $\bar{r}_d$  (i.e. linkage disequilibrium estimator)

values. This sample from Penzé (PZ11D) had a  $P$ -value of 0.047, with  $\bar{r}_d$  values of 0.068 (Table 3).

### *Genetic differentiation*

The results of the PCA (Fig. 2) show a significant overall  $F_{st}$  value of 0.073. The first two PCA axes were significant and explained 44% of the variance: axis 1 accounts for 26.39% of the variance ( $F_{st}= 0.019$ ,  $P=0.001$ ) and axis 2 accounts for 17.65% of the variance ( $F_{st}=0.013$ ,  $P=0.011$ ). Axis 1 separates samples from the beginning of the 2010 bloom (PZ10A-RC10A) from the rest of the samples (Fig. 2). Axis 2 separates samples according to the sampling date during a bloom and their geographic origin. Samples from the beginning of the bloom were less genetically differentiated than samples from the end of the bloom events. These last samples formed distinct clusters according to their geographic origin (Fig. 2; cf. PZ11C, PZ11D compared to RC11D and RC11C).

Genetic differentiation between pairs of samples ( $F_{st}$ ) ranged from -0.0035 (RC11A vs. RC11B) to 0.0770 (RC10A vs. PZ11C), whereas  $D_{est}$  ranged from 0.0589 (RC11B vs. RC11C) to 0.4695 (RC10A vs. PZ11C) (Table 3S). The lowest  $F_{st}$  values were observed for two samples from the same site in the same year at two consecutive in-bloom sampling dates, whereas the highest values were observed for samples between different sites and different years. The tests of differentiation at different spatial and temporal levels confirm the PCA results. They indicate that the early bloom samples in Penzé and Rance taken in 2010 (PZ10A and RC10A) differed significantly from all other samples (Table S3).

The first AMOVA helped gauge the importance of spatial variation with respect to phase variation within a bloom. This AMOVA could only be applied on the 2011 samples (Table 4A) and revealed that the genetic differentiation was significant between sites and among bloom phases within sites. More specifically, the percent of variation explained by the

genetic differentiation during the different phases of the bloom event was three times higher (3.87%) than the genetic differentiation between the Penzé and Rance sites (1.23%, Table 4A).

The second AMOVA was used to analyze the importance of temporal variation at two hierarchical levels: interannual and intrabloom (Table 4B). This temporal AMOVA could only be applied to the Penzé estuary. The results show again that the two levels of differentiation were significant. The percent variation explained by genetic differentiation between the different in-bloom periods was weaker (4.27%, Table 4B) than that explained by genetic differentiation between 2010 and 2011 (10.01%, Table 4B).

## ***Discussion***

### *Clonal reproduction versus sexual reproduction*

The reproductive system can play an important role in the functioning and evolution of populations by modifying their dynamics, their diversity and their population genetic structure. As in many other phytoplankton species, the dinoflagellate species *A. minutum* is characterized by high rates of clonal reproduction during the development of bloom events, followed by a period of sexual reproduction that produces resting cysts, coinciding with the maximum and the decline of cell concentrations, respectively (Garcés *et al.* 2004, Bravo *et al.* 2010). During periods of asexual division, differences in selective values between clonal lines can rapidly lead to differences in genotype frequencies. Numerical simulations show that selective sweeps can occur when rapidly growing clones become more abundant within a population (Rynearson & Armbrust 2000). During a bloom event, advantageous genotypes may increase in frequency, leading to an overall decrease in genetic diversity via genetic hitchhiking over the whole genome (Maynard-Smith *et al.* 1993). Even in the absence of

selection, low clonal diversity and linkage equilibrium provide evidence that sexual reproduction occurs in a population with clonal reproduction. At the end of a bloom, the most frequent clones would thus preferentially participate in the production of the following generation via sexual reproduction. However, in rapidly growing organisms with short generation times, the huge population sizes can be considered as virtually infinite and genotypic diversity can thus be maintained during the bloom events, even if organisms primarily reproduce clonally (Bengtsson 2003).

In this study, all the analyzed clones were genetically distinct, suggesting that bloom events involve huge effective population sizes. The high genetic diversity observed here indicates that asexual reproduction does not predominate within *A. minutum* populations. It however does suggest that blooms were initiated by numerous different sexual cysts. Our results corroborate the recent observations reported for *Alexandrium fundyense* showing unique multilocus genotypic frequencies of 81% to 97% (Erdner *et al.* 2011; Richlen *et al.* 2012). Likewise, a study on *Alexandrium tamarense* has shown that the probability of sampling clones with the same genotype is practically nil due to the large population sizes of blooms (Nagai *et al.* 2007). The high genotypic diversity as well as the high gene diversity values observed in our study ( $0.78 < H_s < 0.87$ ) are comparable to those of Mediterranean populations of the same species ( $0.62 < H_s < 0.89$ ; Casabianca *et al.* 2011). There are reports of similar high genotypic diversity values in other phytoplankton species, such as the haptophyte (*Prymnesium parvum*) and the Raphidophyceae species (*Gonyostomum semen*) using AFLP genetic markers (Barreto *et al.* 2011; Lebret *et al.* 2012).

The continuous germination of resting cysts during the entire bloom event (Lebret *et al.* 2012), as well as the specific cohort of these cysts produced during successive annual blooms and stored in the cyst bank, may enhance genetic diversity (in terms of genotypes and genes)



(Alpermann *et al.* 2009). Furthermore, we cannot exclude the possibility that sexual reproduction maintains genetic diversity, even during a bloom event. Observations in *in vitro* cultures suggest that the zygote (planozygote) may undergo meiosis without forming a resting cyst (Figueroa *et al.* 2007), thereby producing new vegetative cells. This type of reproduction may be promoted by the presence of the parasite *Parvilucifera sinerae* (Alveolata), and short-circuiting the resting cyst stage may possibly constitute a strategy to avoid the parasite (Figueroa *et al.* 2010). Two different species belonging to genus *Parvilucifera* were detected during the 2010 and 2011 sampling cruises (Lepelletier *et al.* submitted). However, unfortunately, there have been no investigations to identify this type of reproduction.

In our study, no global multilocus linkage disequilibrium was detected, suggesting that recombination is sufficient to break down linkages between loci, confirming that *A. minutum* reproduces sexually. Only one sample taken during the late bloom period in Penzé in 2011 (PZ11D) showed significant multilocus linkage disequilibrium, as estimated using  $\bar{r}_d$ .

#### *Spatiotemporal genetic structure of bloom events*

The results of the microsatellite analysis clearly indicate that *A. minutum* blooms are structured both in space and in time. Our study shows that there is a temporal genetic differentiation during and between *A. minutum* blooms as well as spatial differentiation between sites. Thus, the overall genetic differentiation observed between the Rance and Penzé estuaries separated by roughly 150 km was significant. This result suggests that gene flow via tidal currents is limited in *A. minutum*, as demonstrated in *A. tamarense* (Nagai *et al.* 2007). Given the short duration of *A. minutum* bloom events (30 days on average) and that they are geographically restricted to estuaries, the gene flow between populations is likely limited. Genetic differences observed between the two estuaries late bloom samples confirm the genetic isolation between estuaries during the bloom period. However, early bloom samples

showed weaker spatial differentiation (Table 4). This observation suggests that the Penzé and Rance estuaries communicate during spring tides, potentially transporting vegetative cells or cysts via the residual west-east tidal currents. During the bloom season, which preferentially occurs during neap tides, the two estuaries are likely isolated and the accumulation of bloom biomass is mainly due to a response to water flow and to constant, suitable environmental conditions (i.e. salinity temperature, irradiance and nutrient supply) (Anderson *et al.* 2012). In our study, interannual genetic differentiation was greater than intrabloom differentiation, suggesting that blooms are not only made up of newly formed cysts produced during the preceding year, but also of recruitment from migrant vegetative cells and/or from an older cyst bank (Estrada *et al.* 2010). Even if a multigenerational cyst bank gives rise to a bloom event, the germination of cysts may be influenced by environment conditions and, by chance or selection, samples become more differentiated than the more homogeneous cyst bank that produced them (McCue & Holtsford 1998). The constant germination of the cyst bank may thus lead to a decrease in the interannual  $F_{st}$ . Nevertheless, the cyst bank does not appear to contribute regularly to bloom events because the 2010 Penzé sample PZ10D was not genetically different from the early 2011 Penzé bloom sample (PZ11A). Therefore, the 2011 early bloom sample may have been composed at least partly of cysts produced in the preceding year.

In isolated estuaries, there were significant genetic changes during a bloom event (see AMOVA, Table 4A). Although no additional signatures of selection, such as multilocus linkage disequilibrium, could be detected with the neutral markers used in this study, we believe that, in these very large populations, genetic changes may nevertheless be driven by selection (see below). These genetic changes are concordant with those reported for *A. fundyense* blooms in Massachusetts where temporal intrabloom differentiation has also been

observed (Richlen *et al.* 2012). Two factors can account for this temporal differentiation. First, changes may be due to delayed germination of different cohorts of cysts buried in the sediments and/or continuous encystment by a small percentage of motile cells (Pitcher *et al.* 2007). In *Gonyostomum semen* (Raphidophyceae), Lebret *et al.* (2012) suggest that continuous germination of cysts during a bloom is a key element behind rapid genetic changes. Second, these changes may be the result of local abiotic or biotic selection as suggested for the diatom *Ditylum brightwellii* (Rynearson & Armbrust 2005; Rynearson *et al.* 2006). In these large populations, the effects of natural selection may predominate over those of genetic drift on genetic structure. Thus, in *D. brightwellii*, the succession of genetically distinct populations in different environmental conditions appears to reflect a tight coupling between environment and genetics (Rynearson *et al.* 2006). Of the various abiotic factors, seasonal temperature variation is one parameter that can explain the maintenance of genetic diversity (Maynard-Smith and Hoekstra 1980; Bell 1982). Recent studies on the diatom *Asterionella formosa* have demonstrated that variation in temperature may contribute to maintaining genetic diversity in blooms (Gsell *et al.* 2012). In this species, reaction norms of clone growth studied as a function of environmental temperature reveal considerable genetic diversity within a bloom. In our study, fluctuating temperatures may explain not only the maintenance of genetic diversity, but also genetic changes during a bloom event. However, biotic factors, such as predation and/or parasitism, may also play a role in determining population structure and dynamics (Montagnes *et al.* 2008). In *A. minutum*, eukaryotic parasites such as *Parvilucifera* spp. and *Amoebophrya* spp. can regulate blooms (Chambouvet *et al.* 2008; Figueroa *et al.* 2010; Lepelletier *et al.* submitted). The impact of parasitism can be tested by analyzing the fitness of different strains and their resistance to parasites according to date and sampling site. Whatever the factors involved, our study illustrates how sexual

reproduction and/or migration are likely to erase any selective effects that arise each year during the vegetative phase of a bloom event.

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### **Data accessibility**

Data used in this study (supplementary Table S1) have been deposited at the Roscoff Culture Collection ([www.sb-roscoff.fr/Phyto/RCC](http://www.sb-roscoff.fr/Phyto/RCC)).

### **Authors' contributions**

AD conceived the study, performed isolation and culture of strains, molecular data, analysis and drafted the manuscript, SM contributed to molecular analyses; EB performed isolation and culture of strains, DM performed cytometry analyses, MV participated in the design of the study, performed some statistical analysis and revised the manuscript, LG and CD coordinated the study, helped draft and revised the manuscript. All authors contributed to sample collection, read and approved the final manuscript.

**Figure legends:**

**Figure 1:** Maps of the study area showing sampling stations in the two estuaries: Penzé (PZ) and Rance (RC).

**Figure 2:** PCA of genetic differentiation between samples taken from the Penzé and Rance estuaries in 2010 and 2011.

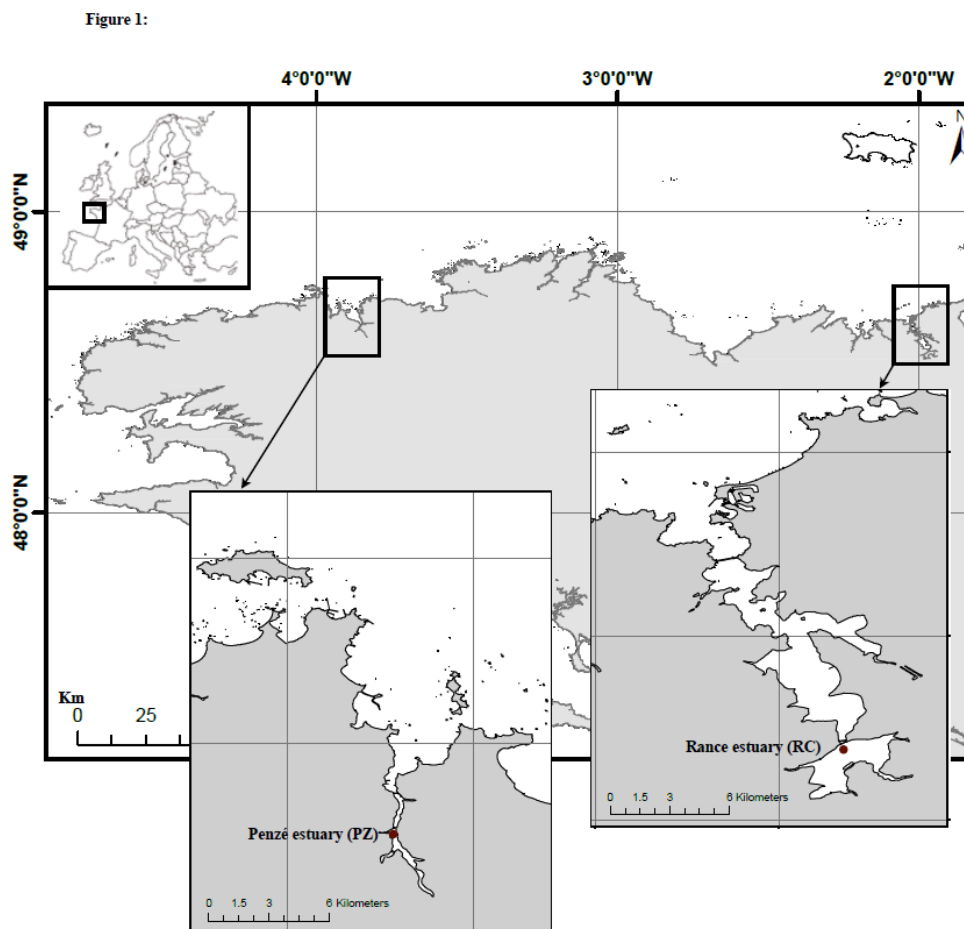


Figure 2:

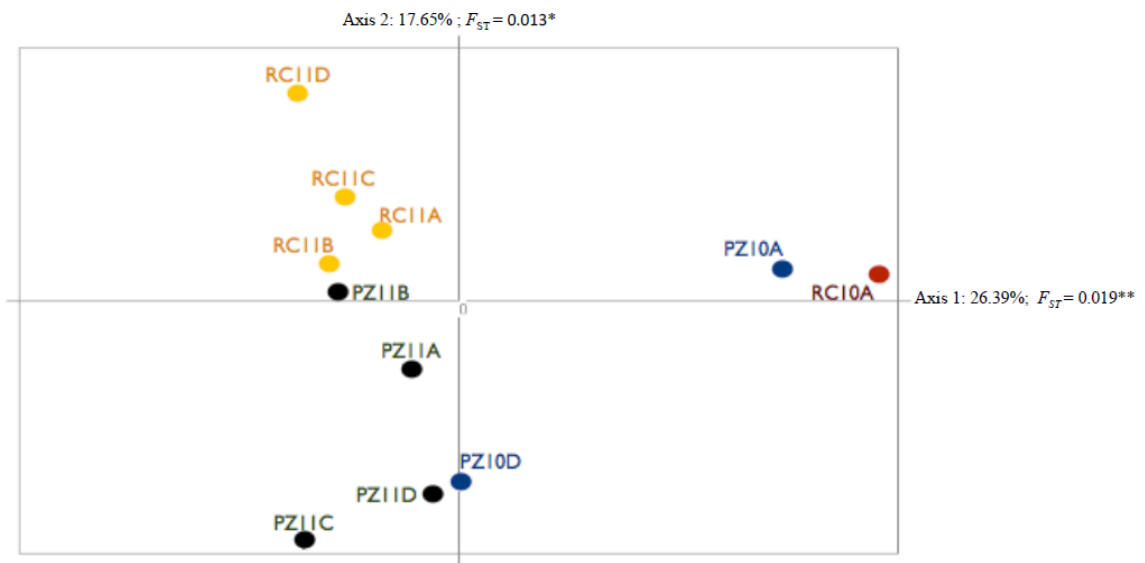




Table 1: Characteristics of the spatiotemporal sampling. Sample sizes (N) are given for strains that were isolated for culturing and for the surviving strains that were genotyped. Codes for samples: PZ for Penzé, RC for Rance; 10 for 2010 and 11 for 2011; A, B, C and D indicate respectively the different periods of the blooms: the beginning, the middle 1, the middle 2 and the end of the bloom. Cells were counted in a counting chamber using an inverted microscope (Olympus CKX41).

| Site/Year  | Sampling date | Sample code | Cells density<br>x 10 <sup>3</sup> cells/L | Temperature of<br>water (air<br>temperature min./<br>max.) (°C) | Number of<br>strains<br>isolated | Number of<br>surviving<br>strains (%) | Number of<br><i>A. minutum</i><br>strains after<br>screening | Number of<br>strains<br>genotyped |
|------------|---------------|-------------|--|---|----------------------------------|---------------------------------------|--|-----------------------------------|
| Penzé/2010 | 02 June       | PZ10A       | 8  | 14.5 (8.6/22)   | 82                               | 36 (43.9)                             | 32   | 24                                |
|            | 30 June       | PZ10D       | 102  | 19.0 (13.5/24.5)  | 29                               | 16 (55.2)                             | 09   | 09                                |
|            | 01 July       | PZ10D       | 63   | 19.3 (12.2/25.9)  | 55                               | 30 (54.5)                             | 14   | 08                                |
| Penzé/2011 | 01 June       | PZ11A       | 31   | 17.9 (2.7/18.6)   | 85                               | 55 (64.7)                             | 22   | 19                                |
|            | 15 June       | PZ11B       | 88   | 18.0 (12.3/20.5)  | 93                               | 46 (49.4)                             | 24   | 14                                |
|            | 17 June       | PZ11B       | 171  | 15.9 (8.4/16.3)   | 144                              | 36 (25)                               | 24   | 15                                |
|            | 22 June       | PZ11C       | 402  | 16.0 (11.5/18)  | 119                              | 49 (41.2)                             | 32   | 24                                |
|            | 11 July       | PZ11D       | ND   | 17.5 (9/23.8)   | 195                              | 30 (15.4)                             | 26   | 18                                |
| Rance/2010 | 02 June       | RC10A       | 36   | 16.3 (11.2/17.8)  | 65                               | 43 (66.2)                             | 43   | 40                                |
| Rance/2011 | 09 May        | RC11A       | 13   | 15.4 (9.8/19.7)   | 45                               | 26 (57.8)                             | 19   | 15                                |
|            | 23 May        | RC11B       | 168  | 16.8 (7.4/24.2)   | 96                               | 55 (55.2)                             | 40   | 25                                |
|            | 27 May        | RC11C       | 288  | 17.0 (10.8/15.1)  | 96                               | 57 (59.3)                             | 38   | 25                                |
|            | 03 June       | RC11D       | 66   | 18.5 (13/22.1)  | 83                               | 22 (26.5)                             | 17   | 10                                |
|            | 05 June       | RC11D       | 106  | 18.8 (13.8/14.7)  | 53                               | 29 (54.7)                             | 24   | 19                                |
| Total      |               |             |  |   | <b>1240</b>                      | <b>530 (42.7)</b>                     | <b>364</b>   | 265                               |



Table 2: Genetic diversity estimates in *A. minutum* at each of the 11 sampling sites and dates and for each of the seven microsatellite loci.  $N$ , number of individuals genotyped in each sample;  $N_a$ , number of alleles per locus;  $R_a$ , expected allelic richness based on the smallest sample size (10);  $H_s$ , Nei's gene diversity.

| Sample (N)            | PZ10 A (24) | PZ10 D (17) | PZ11 A (19) | PZ11 B (29)  | PZ11 C (24) | PZ11 D (18) | RC10 A (40)  | RC11 A (15) | RC11 B (25)  | RC11 C (25)  | RC11 D (29) |
|-----------------------|-------------|-------------|-------------|--------------|-------------|-------------|--------------|-------------|--------------|--------------|-------------|
| <b>Aminu22</b>        |             |             |             |              |             |             |              |             |              |              |             |
| N                     | 19          | 15          | 14          | 26           | 23          | 16          | 34           | 12          | 23           | 21           | 27          |
| $N_a$                 | 4           | 7           | 4           | 9            | 6           | 5           | 9            | 5           | 7            | 8            | 8           |
| $R_a$                 | 3.74        | 6.58        | 3.85        | 6.62         | 5.05        | 4.60        | 6.36         | 4.96        | 5.63         | 6.33         | 5.97        |
| $H_s$                 | 0.57        | 0.84        | 0.57        | 0.73         | 0.75        | 0.68        | 0.76         | 0.74        | 0.63         | 0.62         | 0.68        |
| <b>Aminu41</b>        |             |             |             |              |             |             |              |             |              |              |             |
| N                     | 22          | 16          | 17          | 27           | 24          | 17          | 36           | 12          | 23           | 19           | 24          |
| $N_a$                 | 10          | 3           | 4           | 7            | 3           | 4           | 10           | 3           | 6            | 10           | 5           |
| $R_a$                 | 7.93        | 3.00        | 3.68        | 5.29         | 2.67        | 3.82        | 6.63         | 2.98        | 4.74         | 8.26         | 4.00        |
| $H_s$                 | 0.84        | 0.69        | 0.64        | 0.71         | 0.56        | 0.68        | 0.77         | 0.59        | 0.63         | 0.85         | 0.62        |
| <b>Aminu11</b>        |             |             |             |              |             |             |              |             |              |              |             |
| N                     | 22          | 16          | 17          | 28           | 22          | 17          | 33           | 14          | 23           | 25           | 28          |
| $N_a$                 | 12          | 9           | 9           | 12           | 12          | 11          | 14           | 10          | 11           | 14           | 13          |
| $R_a$                 | 10.02       | 8.20        | 8.54        | 9.04         | 9.55        | 9.82        | 9.85         | 9.48        | 9.34         | 10.67        | 10.38       |
| $H_s$                 | 0.94        | 0.90        | 0.93        | 0.89         | 0.91        | 0.94        | 0.91         | 0.95        | 0.93         | 0.93         | 0.94        |
| <b>Aminu20</b>        |             |             |             |              |             |             |              |             |              |              |             |
| N                     | 19          | 14          | 10          | 28           | 19          | 17          | 24           | 11          | 21           | 18           | 23          |
| $N_a$                 | 10          | 9           | 7           | 17           | 13          | 10          | 9            | 5           | 13           | 13           | 12          |
| $R_a$                 | 8.22        | 8.55        | 7.00        | 12.28        | 11.23       | 8.68        | 6.88         | 4.99        | 10.68        | 11.03        | 9.30        |
| $H_s$                 | 0.78        | 0.91        | 0.91        | 0.96         | 0.97        | 0.84        | 0.79         | 0.84        | 0.95         | 0.95         | 0.89        |
| <b>Aminu43</b>        |             |             |             |              |             |             |              |             |              |              |             |
| N                     | 19          | 16          | 18          | 24           | 23          | 14          | 28           | 14          | 22           | 19           | 20          |
| $N_a$                 | 13          | 10          | 13          | 12           | 13          | 8           | 15           | 8           | 14           | 9            | 10          |
| $R_a$                 | 10.92       | 9.17        | 10.90       | 9.84         | 10.05       | 7.63        | 10.90        | 7.70        | 10.96        | 8.00         | 8.99        |
| $H_s$                 | 0.95        | 0.93        | 0.94        | 0.93         | 0.92        | 0.89        | 0.93         | 0.91        | 0.94         | 0.89         | 0.93        |
| <b>Aminu48</b>        |             |             |             |              |             |             |              |             |              |              |             |
| N                     | 17          | 15          | 19          | 25           | 23          | 18          | 37           | 15          | 20           | 25           | 27          |
| $N_a$                 | 11          | 12          | 11          | 10           | 8           | 7           | 14           | 5           | 8            | 10           | 9           |
| $R_a$                 | 9.82        | 10.96       | 9.18        | 7.71         | 6.69        | 6.36        | 8.74         | 4.78        | 6.73         | 7.74         | 6.54        |
| $H_s$                 | 0.94        | 0.96        | 0.87        | 0.83         | 0.83        | 0.78        | 0.85         | 0.64        | 0.80         | 0.85         | 0.68        |
| <b>Aminu08</b>        |             |             |             |              |             |             |              |             |              |              |             |
| N                     | 22          | 15          | 17          | 27           | 23          | 14          | 28           | 13          | 23           | 24           | 28          |
| $N_a$                 | 9           | 10          | 11          | 13           | 10          | 11          | 13           | 11          | 13           | 15           | 11          |
| $R_a$                 | 7.38        | 9.36        | 9.82        | 9.18         | 8.22        | 10.33       | 9.67         | 10.58       | 9.90         | 11.07        | 7.91        |
| $H_s$                 | 0.83        | 0.94        | 0.94        | 0.87         | 0.88        | 0.96        | 0.91         | 0.97        | 0.88         | 0.93         | 0.83        |
| <b>Mean over loci</b> |             |             |             |              |             |             |              |             |              |              |             |
| $N_a$                 | <b>9.86</b> | <b>8.57</b> | <b>8.43</b> | <b>11.43</b> | <b>9.29</b> | <b>8.00</b> | <b>12.00</b> | <b>6.71</b> | <b>10.29</b> | <b>11.29</b> | <b>9.71</b> |
| (SE)                  | 0.30        | 0.34        | 0.42        | 0.28         | 0.41        | 0.35        | 0.22         | 0.44        | 0.32         | 0.24         | 0.28        |
| $R_a$                 | <b>8.29</b> | <b>7.97</b> | <b>7.57</b> | <b>8.56</b>  | <b>7.64</b> | <b>7.32</b> | <b>8.43</b>  | <b>6.49</b> | <b>8.28</b>  | <b>9.01</b>  | <b>7.58</b> |
| (SE)                  | 0.29        | 0.32        | 0.38        | 0.27         | 0.40        | 0.34        | 0.21         | 0.43        | 0.31         | 0.21         | 0.29        |
| $H_s$                 | <b>0.84</b> | <b>0.88</b> | <b>0.83</b> | <b>0.85</b>  | <b>0.83</b> | <b>0.82</b> | <b>0.85</b>  | <b>0.81</b> | <b>0.82</b>  | <b>0.86</b>  | <b>0.79</b> |
| (SE)                  | 0.16        | 0.11        | 0.19        | 0.11         | 0.17        | 0.14        | 0.09         | 0.19        | 0.17         | 0.13         | 0.17        |

Table 3: Multilocus measurements of linkage disequilibrium using  $\bar{r}_d$  (Agapow and Burt 2001).

| Sample (N)     | PZ10A (24) | PZ10D (17) | PZ11A (19) | PZ11B (29) | PZ11C (24) | PZ11D (18) | RC10A (40) | RC11A (15) | RC11B (25) | RC11C (25) | RC11D (29) |
|----------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| $\bar{r}_d$    | -0.008     | 0.068      | -0.034     | -0.017     | 0.037      | 0.005      | 0.003      | 0.027      | -0.037     | -0.049     | 0.009      |
| <b>P-value</b> | 0.566      | 0.047      | 0.870      | 0.730      | 0.063      | 0.416      | 0.423      | 0.240      | 0.902      | 0.971      | 0.348      |

Table 4: Spatial (A) and temporal (B) analyses of bloom events

A: Spatial AMOVA: Variation among bloom phases between the Penzé and Rance sites sampled in 2011 ( $F_{st} = 0.05$ .  $P < 10^{-5}$ )

| Source of variation                               | df  | Sum of squares | Variance component | Percentage variation (%) | P-value |
|---|-----|----------------|--------------------|--------------------------|---------|
| Between the Penzé and Rance sites sampled in 2011 | 1   | 13.91          | 0.04               | 1.23                     | 0.008   |
| Among bloom phases within site                    | 6   | 44.51          | 0.12               | 3.87                     | 0.002   |
| Within bloom phase                                | 318 | 896.08         | 2.82               | 94.88                    |         |
| Total   |     | 954.49         | 2.97               |                          |         |

B: Temporal AMOVA: Variation among bloom phases between years 2010 and 2011 at Penzé site ( $F_{st} = 0.14$ .  $p < 10^{-5}$ )

| Source of variation                  | df  | Sum of squares | Variance component | Percentage variation (%) | P-value       |
|--------------------------------------|-----|----------------|--------------------|--------------------------|---------------|
| Between years 2010 and 2011 in Penzé | 1   | 41.24          | 0.33               | 10.01                    | $p < 10^{-5}$ |
| Among bloom phases within year       | 4   | 33.04          | 0.14               | 4.27                     | $p < 10^{-5}$ |
| Within bloom phase                   | 226 | 648.12         | 2.86               | 85.72                    |               |
| Total                                |     | 722.41         | 3.34               |                          |               |

SUPPORTING INFORMATION FOR:

# SPATIOTEMPORAL CHANGES IN THE GENETIC DIVERSITY OF HARMFUL ALGAL BLOOMS CAUSED BY THE TOXIC DINOFLAGELLATE *ALEXANDRIUM MINUTUM*

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**Figure S1.** Quantification of DNA in *Alexandrium minutum* cultures in comparison with nuclei isolated from human blood cells (HBC, 6.4 Mbp), after staining with propidium iodide in PBS buffer. HBC and *A. minutum* are discriminated on the forward scatter (X-axis, logarithmic scale), a proxy of size, versus red fluorescence (Y-axis, linear scale) dot plot (A). Quantification of DNA (X-axis, linear scale) by fluorescence in two monoclonal strains (B) and (C).

**Figure S2.** Resolving power of the microsatellite marker set to discriminate the maximum clonal diversity available in the data set.

## Supplementary Tables (3)

**Table S1.** Roscoff Culture Collection (RCC) number, origin, population code and date of isolation of strains used in this study. RCC web site: [www.sb-roscoff.fr/Phyto/RCC](http://www.sb-roscoff.fr/Phyto/RCC).

| Strain number | Site  | Population code | Date       |
|---------------|-------|-----------------|------------|
| RCC 3144      | Penzé | PZ10A           | 02-June-10 |
| RCC 3145      | Penzé | PZ10A           | 02-June-10 |
| RCC 3146      | Penzé | PZ10A           | 02-June-10 |
| RCC 3147      | Penzé | PZ10A           | 02-June-10 |
| RCC 3148      | Penzé | PZ10A           | 02-June-10 |

|          |       |       |            |
|----------|-------|-------|------------|
| RCC 3149 | Penzé | PZ10A | 02-June-10 |
| RCC 3150 | Penzé | PZ10A | 02-June-10 |
| RCC 3151 | Penzé | PZ10A | 02-June-10 |
| RCC 3152 | Penzé | PZ10A | 02-June-10 |
| RCC 3153 | Penzé | PZ10A | 02-June-10 |
| RCC 3154 | Penzé | PZ10A | 02-June-10 |
| RCC 3155 | Penzé | PZ10A | 02-June-10 |
| RCC 3156 | Penzé | PZ10A | 02-June-10 |
| RCC 3157 | Penzé | PZ10A | 02-June-10 |
| RCC 3158 | Penzé | PZ10A | 02-June-10 |
| RCC 3159 | Penzé | PZ10A | 02-June-10 |
| RCC 3160 | Penzé | PZ10A | 02-June-10 |
| RCC 3161 | Penzé | PZ10A | 02-June-10 |
| RCC 3162 | Penzé | PZ10A | 02-June-10 |
| RCC 3163 | Penzé | PZ10A | 02-June-10 |
| RCC 3164 | Penzé | PZ10A | 02-June-10 |
| RCC 3165 | Penzé | PZ10A | 02-June-10 |
| RCC 3166 | Penzé | PZ10A | 02-June-10 |
| RCC 3167 | Penzé | PZ10A | 02-June-10 |
| RCC 3168 | Penzé | PZ10D | 30-June-10 |
| RCC 3169 | Penzé | PZ10D | 30-June-10 |
| RCC 3170 | Penzé | PZ10D | 30-June-10 |
| RCC 3171 | Penzé | PZ10D | 30-June-10 |
| RCC 3172 | Penzé | PZ10D | 30-June-10 |
| RCC 3173 | Penzé | PZ10D | 30-June-10 |
| RCC 3174 | Penzé | PZ10D | 30-June-10 |
| RCC 3175 | Penzé | PZ10D | 30-June-10 |
| RCC 3176 | Penzé | PZ10D | 30-June-10 |
| RCC 3177 | Penzé | PZ10D | 01-July-10 |
| RCC 3178 | Penzé | PZ10D | 01-July-10 |
| RCC 3179 | Penzé | PZ10D | 01-July-10 |
| RCC 3180 | Penzé | PZ10D | 01-July-10 |
| RCC 3181 | Penzé | PZ10D | 01-July-10 |
| RCC 3182 | Penzé | PZ10D | 01-July-10 |
| RCC 3183 | Penzé | PZ10D | 01-July-10 |
| RCC 3023 | Penzé | PZ10D | 01-July-10 |
| RCC 3249 | Penzé | PZ11A | 01-June-11 |
| RCC 3250 | Penzé | PZ11A | 01-June-11 |
| RCC 3251 | Penzé | PZ11A | 01-June-11 |
| RCC 3252 | Penzé | PZ11A | 01-June-11 |
| RCC 3253 | Penzé | PZ11A | 01-June-11 |
| RCC 3254 | Penzé | PZ11A | 01-June-11 |
| RCC 3255 | Penzé | PZ11A | 01-June-11 |
| RCC 3256 | Penzé | PZ11A | 01-June-11 |
| RCC 3257 | Penzé | PZ11A | 01-June-11 |
| RCC 3258 | Penzé | PZ11A | 01-June-11 |
| RCC 3259 | Penzé | PZ11A | 01-June-11 |
| RCC 3260 | Penzé | PZ11A | 01-June-11 |

|          |       |       |            |
|----------|-------|-------|------------|
| RCC 3261 | Penzé | PZ11A | 01-June-11 |
| RCC 3262 | Penzé | PZ11A | 01-June-11 |
| RCC 3263 | Penzé | PZ11A | 01-June-11 |
| RCC 3264 | Penzé | PZ11A | 01-June-11 |
| RCC 3265 | Penzé | PZ11A | 01-June-11 |
| RCC 3266 | Penzé | PZ11A | 01-June-11 |
| RCC 3267 | Penzé | PZ11A | 01-June-11 |
| RCC 3297 | Penzé | PZ11B | 15-June-11 |
| RCC 3298 | Penzé | PZ11B | 15-June-11 |
| RCC 3299 | Penzé | PZ11B | 15-June-11 |
| RCC 3300 | Penzé | PZ11B | 15-June-11 |
| RCC 3301 | Penzé | PZ11B | 15-June-11 |
| RCC 3302 | Penzé | PZ11B | 15-June-11 |
| RCC 3303 | Penzé | PZ11B | 15-June-11 |
| RCC 3304 | Penzé | PZ11B | 15-June-11 |
| RCC 3305 | Penzé | PZ11B | 15-June-11 |
| RCC 3306 | Penzé | PZ11B | 15-June-11 |
| RCC 3307 | Penzé | PZ11B | 15-June-11 |
| RCC 3308 | Penzé | PZ11B | 15-June-11 |
| RCC 3309 | Penzé | PZ11B | 15-June-11 |
| RCC 3310 | Penzé | PZ11B | 15-June-11 |
| RCC 3311 | Penzé | PZ11B | 17-June-11 |
| RCC 3312 | Penzé | PZ11B | 17-June-11 |
| RCC 3313 | Penzé | PZ11B | 17-June-11 |
| RCC 3314 | Penzé | PZ11B | 17-June-11 |
| RCC 3315 | Penzé | PZ11B | 17-June-11 |
| RCC 3316 | Penzé | PZ11B | 17-June-11 |
| RCC 3317 | Penzé | PZ11B | 17-June-11 |
| RCC 3318 | Penzé | PZ11B | 17-June-11 |
| RCC 3319 | Penzé | PZ11B | 17-June-11 |
| RCC 3320 | Penzé | PZ11B | 17-June-11 |
| RCC 3321 | Penzé | PZ11B | 17-June-11 |
| RCC 3322 | Penzé | PZ11B | 17-June-11 |
| RCC 3323 | Penzé | PZ11B | 17-June-11 |
| RCC 3324 | Penzé | PZ11B | 17-June-11 |
| RCC 3325 | Penzé | PZ11B | 17-June-11 |
| RCC 3326 | Penzé | PZ11C | 22-June-11 |
| RCC 3327 | Penzé | PZ11C | 22-June-11 |
| RCC 3328 | Penzé | PZ11C | 22-June-11 |
| RCC 3329 | Penzé | PZ11C | 22-June-11 |
| RCC 3330 | Penzé | PZ11C | 22-June-11 |
| RCC 3331 | Penzé | PZ11C | 22-June-11 |
| RCC 3332 | Penzé | PZ11C | 22-June-11 |
| RCC 3333 | Penzé | PZ11C | 22-June-11 |
| RCC 3334 | Penzé | PZ11C | 22-June-11 |
| RCC 3335 | Penzé | PZ11C | 22-June-11 |
| RCC 3336 | Penzé | PZ11C | 22-June-11 |
| RCC 3337 | Penzé | PZ11C | 22-June-11 |

|          |       |       |            |
|----------|-------|-------|------------|
| RCC 3338 | Penzé | PZ11C | 22-June-11 |
| RCC 3339 | Penzé | PZ11C | 22-June-11 |
| RCC 3340 | Penzé | PZ11C | 22-June-11 |
| RCC 3341 | Penzé | PZ11C | 22-June-11 |
| RCC 3342 | Penzé | PZ11C | 22-June-11 |
| RCC 3343 | Penzé | PZ11C | 22-June-11 |
| RCC 3344 | Penzé | PZ11C | 22-June-11 |
| RCC 3345 | Penzé | PZ11C | 22-June-11 |
| RCC 3346 | Penzé | PZ11C | 22-June-11 |
| RCC 3347 | Penzé | PZ11C | 22-June-11 |
| RCC 3348 | Penzé | PZ11C | 22-June-11 |
| RCC 3349 | Penzé | PZ11C | 22-June-11 |
| RCC 3350 | Penzé | PZ11D | 11-July-11 |
| RCC 3351 | Penzé | PZ11D | 11-July-11 |
| RCC 3352 | Penzé | PZ11D | 11-July-11 |
| RCC 3353 | Penzé | PZ11D | 11-July-11 |
| RCC 3354 | Penzé | PZ11D | 11-July-11 |
| RCC 3355 | Penzé | PZ11D | 11-July-11 |
| RCC 3356 | Penzé | PZ11D | 11-July-11 |
| RCC 3357 | Penzé | PZ11D | 11-July-11 |
| RCC 3358 | Penzé | PZ11D | 11-July-11 |
| RCC 3359 | Penzé | PZ11D | 11-July-11 |
| RCC 3360 | Penzé | PZ11D | 11-July-11 |
| RCC 3361 | Penzé | PZ11D | 11-July-11 |
| RCC 3362 | Penzé | PZ11D | 11-July-11 |
| RCC 3363 | Penzé | PZ11D | 11-July-11 |
| RCC 3364 | Penzé | PZ11D | 11-July-11 |
| RCC 3365 | Penzé | PZ11D | 11-July-11 |
| RCC 3366 | Penzé | PZ11D | 11-July-11 |
| RCC 3367 | Penzé | PZ11D | 11-July-11 |
| RCC 3105 | Rance | RC10A | 02-June-10 |
| RCC 3106 | Rance | RC10A | 02-June-10 |
| RCC 3107 | Rance | RC10A | 02-June-10 |
| RCC 3108 | Rance | RC10A | 02-June-10 |
| RCC 3109 | Rance | RC10A | 02-June-10 |
| RCC 3110 | Rance | RC10A | 02-June-10 |
| RCC 3111 | Rance | RC10A | 02-June-10 |
| RCC 3112 | Rance | RC10A | 02-June-10 |
| RCC 3113 | Rance | RC10A | 02-June-10 |
| RCC 3022 | Rance | RC10A | 02-June-10 |
| RCC 3114 | Rance | RC10A | 02-June-10 |
| RCC 3115 | Rance | RC10A | 02-June-10 |
| RCC 3116 | Rance | RC10A | 02-June-10 |
| RCC 3117 | Rance | RC10A | 02-June-10 |
| RCC 3118 | Rance | RC10A | 02-June-10 |
| RCC 3119 | Rance | RC10A | 02-June-10 |
| RCC 3120 | Rance | RC10A | 02-June-10 |
| RCC 3121 | Rance | RC10A | 02-June-10 |

|          |       |       |            |
|----------|-------|-------|------------|
| RCC 3122 | Rance | RC10A | 02-June-10 |
| RCC 3123 | Rance | RC10A | 02-June-10 |
| RCC 3124 | Rance | RC10A | 02-June-10 |
| RCC 3125 | Rance | RC10A | 02-June-10 |
| RCC 3126 | Rance | RC10A | 02-June-10 |
| RCC 3127 | Rance | RC10A | 02-June-10 |
| RCC 3128 | Rance | RC10A | 02-June-10 |
| RCC 3129 | Rance | RC10A | 02-June-10 |
| RCC 3130 | Rance | RC10A | 02-June-10 |
| RCC 3131 | Rance | RC10A | 02-June-10 |
| RCC 3132 | Rance | RC10A | 02-June-10 |
| RCC 3133 | Rance | RC10A | 02-June-10 |
| RCC 3134 | Rance | RC10A | 02-June-10 |
| RCC 3135 | Rance | RC10A | 02-June-10 |
| RCC 3136 | Rance | RC10A | 02-June-10 |
| RCC 3137 | Rance | RC10A | 02-June-10 |
| RCC 3138 | Rance | RC10A | 02-June-10 |
| RCC 3139 | Rance | RC10A | 02-June-10 |
| RCC 3140 | Rance | RC10A | 02-June-10 |
| RCC 3141 | Rance | RC10A | 02-June-10 |
| RCC 3142 | Rance | RC10A | 02-June-10 |
| RCC 3143 | Rance | RC10A | 02-June-10 |
| RCC 3184 | Rance | RC11A | 09-May-11  |
| RCC 3185 | Rance | RC11A | 09-May-11  |
| RCC 3186 | Rance | RC11A | 09-May-11  |
| RCC 3187 | Rance | RC11A | 09-May-11  |
| RCC 3188 | Rance | RC11A | 09-May-11  |
| RCC 3189 | Rance | RC11A | 09-May-11  |
| RCC 3190 | Rance | RC11A | 09-May-11  |
| RCC 3191 | Rance | RC11A | 09-May-11  |
| RCC 3192 | Rance | RC11A | 09-May-11  |
| RCC 3193 | Rance | RC11A | 09-May-11  |
| RCC 3194 | Rance | RC11A | 09-May-11  |
| RCC 3195 | Rance | RC11A | 09-May-11  |
| RCC 3196 | Rance | RC11A | 09-May-11  |
| RCC 3197 | Rance | RC11A | 09-May-11  |
| RCC 3198 | Rance | RC11A | 09-May-11  |
| RCC 3199 | Rance | RC11B | 23-May-11  |
| RCC 3200 | Rance | RC11B | 23-May-11  |
| RCC 3201 | Rance | RC11B | 23-May-11  |
| RCC 3202 | Rance | RC11B | 23-May-11  |
| RCC 3203 | Rance | RC11B | 23-May-11  |
| RCC 3204 | Rance | RC11B | 23-May-11  |
| RCC 3205 | Rance | RC11B | 23-May-11  |
| RCC 3206 | Rance | RC11B | 23-May-11  |
| RCC 3207 | Rance | RC11B | 23-May-11  |
| RCC 3208 | Rance | RC11B | 23-May-11  |
| RCC 3209 | Rance | RC11B | 23-May-11  |

|          |       |       |            |
|----------|-------|-------|------------|
| RCC 3210 | Rance | RC11B | 23-May-11  |
| RCC 3211 | Rance | RC11B | 23-May-11  |
| RCC 3212 | Rance | RC11B | 23-May-11  |
| RCC 3213 | Rance | RC11B | 23-May-11  |
| RCC 3214 | Rance | RC11B | 23-May-11  |
| RCC 3215 | Rance | RC11B | 23-May-11  |
| RCC 3216 | Rance | RC11B | 23-May-11  |
| RCC 3217 | Rance | RC11B | 23-May-11  |
| RCC 3218 | Rance | RC11B | 23-May-11  |
| RCC 3219 | Rance | RC11B | 23-May-11  |
| RCC 3220 | Rance | RC11B | 23-May-11  |
| RCC 3221 | Rance | RC11B | 23-May-11  |
| RCC 3222 | Rance | RC11B | 23-May-11  |
| RCC 3223 | Rance | RC11B | 23-May-11  |
| RCC 3224 | Rance | RC11C | 27-May-11  |
| RCC 3225 | Rance | RC11C | 27-May-11  |
| RCC 3226 | Rance | RC11C | 27-May-11  |
| RCC 3227 | Rance | RC11C | 27-May-11  |
| RCC 3228 | Rance | RC11C | 27-May-11  |
| RCC 3229 | Rance | RC11C | 27-May-11  |
| RCC 3230 | Rance | RC11C | 27-May-11  |
| RCC 3231 | Rance | RC11C | 27-May-11  |
| RCC 3232 | Rance | RC11C | 27-May-11  |
| RCC 3233 | Rance | RC11C | 27-May-11  |
| RCC 3234 | Rance | RC11C | 27-May-11  |
| RCC 3235 | Rance | RC11C | 27-May-11  |
| RCC 3236 | Rance | RC11C | 27-May-11  |
| RCC 3237 | Rance | RC11C | 27-May-11  |
| RCC 3238 | Rance | RC11C | 27-May-11  |
| RCC 3239 | Rance | RC11C | 27-May-11  |
| RCC 3240 | Rance | RC11C | 27-May-11  |
| RCC 3241 | Rance | RC11C | 27-May-11  |
| RCC 3242 | Rance | RC11C | 27-May-11  |
| RCC 3243 | Rance | RC11C | 27-May-11  |
| RCC 3244 | Rance | RC11C | 27-May-11  |
| RCC 3245 | Rance | RC11C | 27-May-11  |
| RCC 3246 | Rance | RC11C | 27-May-11  |
| RCC 3247 | Rance | RC11C | 27-May-11  |
| RCC 3248 | Rance | RC11C | 27-May-11  |
| RCC 3268 | Rance | RC11D | 03-June-11 |
| RCC 3269 | Rance | RC11D | 03-June-11 |
| RCC 3270 | Rance | RC11D | 03-June-11 |
| RCC 3271 | Rance | RC11D | 03-June-11 |
| RCC 3272 | Rance | RC11D | 03-June-11 |
| RCC 3273 | Rance | RC11D | 03-June-11 |
| RCC 3274 | Rance | RC11D | 03-June-11 |
| RCC 3275 | Rance | RC11D | 03-June-11 |
| RCC 3276 | Rance | RC11D | 03-June-11 |



|          |       |       |            |
|----------|-------|-------|------------|
| RCC 3277 | Rance | RC11D | 03-June-11 |
| RCC 3278 | Rance | RC11D | 05-June-11 |
| RCC 3279 | Rance | RC11D | 05-June-11 |
| RCC 3280 | Rance | RC11D | 05-June-11 |
| RCC 3281 | Rance | RC11D | 05-June-11 |
| RCC 3282 | Rance | RC11D | 05-June-11 |
| RCC 3283 | Rance | RC11D | 05-June-11 |
| RCC 3284 | Rance | RC11D | 05-June-11 |
| RCC 3285 | Rance | RC11D | 05-June-11 |
| RCC 3286 | Rance | RC11D | 05-June-11 |
| RCC 3287 | Rance | RC11D | 05-June-11 |
| RCC 3288 | Rance | RC11D | 05-June-11 |
| RCC 3289 | Rance | RC11D | 05-June-11 |
| RCC 3290 | Rance | RC11D | 05-June-11 |
| RCC 3291 | Rance | RC11D | 05-June-11 |
| RCC 3292 | Rance | RC11D | 05-June-11 |
| RCC 3293 | Rance | RC11D | 05-June-11 |
| RCC 3294 | Rance | RC11D | 05-June-11 |
| RCC 3295 | Rance | RC11D | 05-June-11 |
| RCC 3296 | Rance | RC11D | 05-June-11 |

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**Table S2.** Microsatellite data amplified on 265 individual strains at seven loci. Population codes are given in Table 1.

| <b>Individuals</b> | <b>Sample codes</b> | <b>Aminu22</b> | <b>Aminu41</b> | <b>Aminu11</b> | <b>Aminu20</b> | <b>Aminu43</b> | <b>Aminu48</b> | <b>Aminu08</b> |
|--------------------|---------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| PZ374              | PZ10A               | 199            | 202            | 255            | 0              | 201            | 215            | 185            |
| PZ352              | PZ10A               | 199            | 200            | 228            | 279            | 201            | 0              | 190            |
| PZ330              | PZ10A               | 197            | 164            | 240            | 248            | 0              | 0              | 180            |
| PZ350              | PZ10A               | 197            | 202            | 228            | 279            | 0              | 176            | 185            |
| PZ344              | PZ10A               | 197            | 200            | 228            | 0              | 215            | 217            | 185            |
| PZ341              | PZ10A               | 197            | 232            | 248            | 248            | 230            | 234            | 185            |
| PZ362              | PZ10A               | 197            | 200            | 238            | 279            | 188            | 0              | 190            |
| PZ339              | PZ10A               | 197            | 232            | 0              | 0              | 202            | 0              | 190            |
| PZ351              | PZ10A               | 197            | 202            | 236            | 250            | 197            | 214            | 190            |
| PZ359              | PZ10A               | 197            | 162            | 240            | 279            | 188            | 0              | 199            |
| PZ331              | PZ10A               | 195            | 202            | 236            | 0              | 205            | 240            | 187            |
| PZ360              | PZ10A               | 179            | 0              | 236            | 279            | 221            | 0              | 190            |
| PZ353              | PZ10A               | 0              | 218            | 0              | 279            | 220            | 0              | 185            |
| PZ345              | PZ10A               | 0              | 0              | 287            | 0              | 188            | 252            | 185            |
| PZ355              | PZ10A               | 0              | 232            | 248            | 279            | 221            | 176            | 190            |
| PZ356              | PZ10A               | 0              | 207            | 238            | 279            | 197            | 214            | 190            |
| PZ340              | PZ10A               | 0              | 163            | 248            | 279            | 188            | 176            | 197            |
| PZ333              | PZ10A               | 195            | 202            | 237            | 236            | 200            | 239            | 187            |
| PZ334              | PZ10A               | 197            | 200            | 238            | 253            | 204            | 239            | 189            |
| PZ337              | PZ10A               | 197            | 202            | 255            | 273            | 189            | 240            | 191            |
| PZ338              | PZ10A               | 199            | 202            | 239            | 269            | 0              | 242            | 0              |
| PZ348              | PZ10A               | 199            | 204            | 242            | 265            | 0              | 240            | 189            |
| PZ354              | PZ10A               | 197            | 206            | 229            | 267            | 187            | 238            | 0              |
| PZ369              | PZ10A               | 197            | 202            | 267            | 247            | 0              | 206            | 174            |
| PZ482              | PZ10D               | 201            | 200            | 244            | 271            | 197            | 239            | 179            |
| PZ484              | PZ10D               | 197            | 204            | 229            | 261            | 204            | 0              | 184            |
| PZ485              | PZ10D               | 199            | 204            | 225            | 263            | 200            | 231            | 216            |
| PZ487              | PZ10D               | 199            | 200            | 225            | 0              | 211            | 232            | 226            |
| PZ488              | PZ10D               | 199            | 204            | 225            | 248            | 200            | 234            | 216            |
| PZ489              | PZ10D               | 0              | 0              | 225            | 231            | 0              | 210            | 0              |
| PZ491              | PZ10D               | 197            | 204            | 239            | 261            | 197            | 248            | 0              |
| PZ492              | PZ10D               | 197            | 200            | 249            | 251            | 200            | 0              | 187            |
| PZ493              | PZ10D               | 168            | 202            | 240            | 0              | 187            | 228            | 220            |
| PZ520              | PZ10D               | 199            | 200            | 242            | 260            | 190            | 240            | 185            |
| PZ521              | PZ10D               | 197            | 200            | 252            | 262            | 202            | 242            | 194            |
| PZ524              | PZ10D               | 195            | 202            | 242            | 248            | 205            | 239            | 185            |
| PZ514              | PZ10D               | 195            | 202            | 258            | 0              | 197            | 239            | 190            |
| PZ509              | PZ10D               | 182            | 202            | 242            | 261            | 198            | 225            | 190            |
| PZ500              | PZ10D               | 179            | 202            | 240            | 261            | 206            | 218            | 187            |
| PZ516              | PZ10D               | 0              | 200            | 0              | 279            | 206            | 229            | 199            |

|       |       |     |     |     |     |     |     |     |
|-------|-------|-----|-----|-----|-----|-----|-----|-----|
| PZ522 | PZ10D | 199 | 200 | 240 | 263 | 187 | 240 | 216 |
| PZ579 | PZ11A | 182 | 200 | 228 | 0   | 204 | 239 | 0   |
| PZ594 | PZ11A | 197 | 202 | 228 | 248 | 199 | 234 | 194 |
| PZ582 | PZ11A | 197 | 200 | 228 | 273 | 202 | 239 | 190 |
| PZ591 | PZ11A | 197 | 202 | 237 | 0   | 194 | 239 | 187 |
| PZ584 | PZ11A | 197 | 202 | 257 | 259 | 196 | 239 | 185 |
| PZ593 | PZ11A | 197 | 200 | 248 | 0   | 202 | 237 | 179 |
| PZ587 | PZ11A | 199 | 202 | 236 | 0   | 201 | 239 | 190 |
| PZ589 | PZ11A | 199 | 202 | 259 | 261 | 209 | 244 | 185 |
| PZ583 | PZ11A | 199 | 200 | 242 | 0   | 207 | 239 | 185 |
| PZ572 | PZ11A | 197 | 200 | 257 | 261 | 204 | 256 | 234 |
| PZ575 | PZ11A | 0   | 0   | 240 | 0   | 202 | 235 | 187 |
| PZ577 | PZ11A | 0   | 206 | 0   | 265 | 171 | 242 | 186 |
| PZ578 | PZ11A | 0   | 0   | 239 | 0   | 206 | 239 | 217 |
| PZ580 | PZ11A | 197 | 202 | 242 | 253 | 197 | 236 | 225 |
| PZ581 | PZ11A | 197 | 202 | 237 | 237 | 202 | 240 | 189 |
| PZ586 | PZ11A | 0   | 202 | 259 | 261 | 208 | 244 | 189 |
| PZ590 | PZ11A | 0   | 155 | 240 | 0   | 0   | 229 | 187 |
| PZ595 | PZ11A | 210 | 200 | 236 | 265 | 204 | 234 | 0   |
| PZ597 | PZ11A | 197 | 200 | 0   | 0   | 198 | 241 | 191 |
| PZ689 | PZ11B | 197 | 202 | 239 | 269 | 0   | 251 | 179 |
| PZ691 | PZ11B | 197 | 200 | 229 | 267 | 196 | 239 | 210 |
| PZ696 | PZ11B | 194 | 202 | 259 | 259 | 198 | 239 | 0   |
| PZ697 | PZ11B | 197 | 200 | 237 | 270 | 200 | 242 | 187 |
| PZ701 | PZ11B | 195 | 202 | 239 | 274 | 196 | 238 | 189 |
| PZ704 | PZ11B | 195 | 200 | 257 | 261 | 197 | 239 | 187 |
| PZ708 | PZ11B | 199 | 200 | 229 | 261 | 197 | 239 | 226 |
| PZ711 | PZ11B | 197 | 0   | 242 | 263 | 198 | 241 | 184 |
| PZ712 | PZ11B | 0   | 176 | 242 | 259 | 204 | 241 | 197 |
| PZ714 | PZ11B | 195 | 196 | 229 | 265 | 212 | 210 | 179 |
| PZ715 | PZ11B | 195 | 202 | 248 | 253 | 206 | 218 | 0   |
| PZ716 | PZ11B | 197 | 202 | 248 | 283 | 204 | 240 | 200 |
| PZ719 | PZ11B | 197 | 202 | 239 | 253 | 204 | 0   | 187 |
| PZ721 | PZ11B | 197 | 200 | 239 | 264 | 201 | 240 | 201 |
| PZ742 | PZ11B | 197 | 200 | 239 | 262 | 202 | 240 | 190 |
| PZ743 | PZ11B | 199 | 202 | 229 | 0   | 200 | 240 | 189 |
| PZ749 | PZ11B | 212 | 202 | 237 | 261 | 204 | 239 | 187 |
| PZ959 | PZ11B | 197 | 196 | 248 | 261 | 206 | 0   | 189 |
| PZ960 | PZ11B | 197 | 200 | 229 | 265 | 202 | 246 | 189 |
| PZ961 | PZ11B | 193 | 202 | 257 | 257 | 210 | 240 | 189 |
| PZ962 | PZ11B | 195 | 200 | 223 | 248 | 0   | 246 | 187 |
| PZ963 | PZ11B | 197 | 202 | 238 | 248 | 204 | 240 | 211 |
| PZ964 | PZ11B | 197 | 232 | 227 | 271 | 0   | 240 | 187 |
| PZ965 | PZ11B | 0   | 223 | 244 | 265 | 202 | 240 | 184 |

|       |       |     |     |     |     |     |     |     |
|-------|-------|-----|-----|-----|-----|-----|-----|-----|
| PZ966 | PZ11B | 197 | 202 | 248 | 273 | 0   | 0   | 198 |
| PZ967 | PZ11B | 0   | 0   | 0   | 274 | 208 | 240 | 208 |
| PZ968 | PZ11B | 192 | 202 | 252 | 269 | 194 | 248 | 189 |
| PZ969 | PZ11B | 196 | 210 | 229 | 279 | 200 | 0   | 189 |
| PZ970 | PZ11B | 200 | 200 | 229 | 279 | 0   | 246 | 189 |
| PZ788 | PZ11C | 200 | 200 | 236 | 0   | 198 | 239 | 190 |
| PZ770 | PZ11C | 199 | 200 | 248 | 258 | 188 | 240 | 187 |
| PZ753 | PZ11C | 199 | 202 | 228 | 253 | 205 | 242 | 190 |
| PZ761 | PZ11C | 197 | 202 | 257 | 263 | 201 | 237 | 201 |
| PZ764 | PZ11C | 195 | 202 | 0   | 264 | 203 | 240 | 185 |
| PZ789 | PZ11C | 195 | 200 | 259 | 0   | 198 | 242 | 187 |
| PZ754 | PZ11C | 190 | 200 | 236 | 267 | 204 | 239 | 174 |
| PZ757 | PZ11C | 195 | 200 | 237 | 259 | 191 | 239 | 189 |
| PZ758 | PZ11C | 195 | 200 | 226 | 269 | 204 | 242 | 184 |
| PZ759 | PZ11C | 199 | 202 | 242 | 263 | 0   | 0   | 0   |
| PZ762 | PZ11C | 195 | 202 | 229 | 267 | 208 | 239 | 187 |
| PZ766 | PZ11C | 199 | 200 | 242 | 269 | 202 | 239 | 187 |
| PZ767 | PZ11C | 0   | 202 | 0   | 265 | 212 | 239 | 184 |
| PZ771 | PZ11C | 199 | 202 | 229 | 0   | 200 | 239 | 189 |
| PZ773 | PZ11C | 197 | 200 | 229 | 273 | 198 | 233 | 189 |
| PZ775 | PZ11C | 195 | 204 | 229 | 259 | 197 | 231 | 192 |
| PZ777 | PZ11C | 199 | 200 | 242 | 257 | 208 | 241 | 225 |
| PZ778 | PZ11C | 197 | 202 | 229 | 261 | 206 | 241 | 198 |
| PZ780 | PZ11C | 204 | 200 | 236 | 0   | 200 | 241 | 225 |
| PZ781 | PZ11C | 195 | 200 | 228 | 261 | 208 | 239 | 189 |
| PZ782 | PZ11C | 199 | 202 | 238 | 270 | 204 | 242 | 189 |
| PZ784 | PZ11C | 197 | 200 | 229 | 0   | 198 | 256 | 189 |
| PZ785 | PZ11C | 199 | 202 | 244 | 270 | 198 | 240 | 187 |
| PZ787 | PZ11C | 199 | 202 | 239 | 271 | 204 | 242 | 184 |
| PZ665 | PZ11D | 199 | 210 | 242 | 248 | 209 | 242 | 0   |
| PZ661 | PZ11D | 199 | 202 | 253 | 248 | 192 | 239 | 187 |
| PZ645 | PZ11D | 199 | 202 | 228 | 248 | 207 | 237 | 190 |
| PZ648 | PZ11D | 197 | 200 | 228 | 248 | 197 | 239 | 0   |
| PZ682 | PZ11D | 197 | 202 | 252 | 0   | 203 | 239 | 0   |
| PZ643 | PZ11D | 197 | 202 | 244 | 270 | 200 | 239 | 190 |
| PZ679 | PZ11D | 197 | 200 | 228 | 254 | 207 | 240 | 207 |
| PZ660 | PZ11D | 183 | 200 | 248 | 248 | 0   | 239 | 227 |
| PZ655 | PZ11D | 0   | 202 | 0   | 248 | 0   | 236 | 190 |
| PZ637 | PZ11D | 197 | 200 | 239 | 248 | 200 | 229 | 233 |
| PZ639 | PZ11D | 198 | 0   | 237 | 269 | 199 | 239 | 229 |
| PZ640 | PZ11D | 199 | 200 | 255 | 253 | 200 | 229 | 211 |
| PZ641 | PZ11D | 197 | 202 | 242 | 257 | 204 | 242 | 221 |
| PZ644 | PZ11D | 199 | 200 | 239 | 269 | 200 | 239 | 189 |
| PZ646 | PZ11D | 197 | 200 | 248 | 261 | 204 | 229 | 0   |
| PZ647 | PZ11D | 207 | 200 | 242 | 271 | 0   | 239 | 187 |

|       |       |     |     |     |     |     |     |     |
|-------|-------|-----|-----|-----|-----|-----|-----|-----|
| PZ658 | PZ11D | 0   | 162 | 238 | 263 | 0   | 260 | 237 |
| PZ683 | PZ11D | 197 | 210 | 229 | 274 | 204 | 240 | 185 |
| RC275 | RC10A | 0   | 0   | 208 | 279 | 214 | 214 | 0   |
| RC277 | RC10A | 195 | 200 | 229 | 261 | 202 | 239 | 192 |
| RC280 | RC10A | 207 | 202 | 238 | 237 | 175 | 235 | 184 |
| RC281 | RC10A | 197 | 200 | 223 | 0   | 202 | 241 | 175 |
| RC283 | RC10A | 197 | 204 | 229 | 255 | 202 | 250 | 189 |
| RC297 | RC10A | 197 | 215 | 249 | 271 | 202 | 211 | 213 |
| RC304 | RC10A | 182 | 226 | 251 | 0   | 0   | 231 | 213 |
| RC322 | RC10A | 183 | 199 | 224 | 259 | 175 | 0   | 187 |
| RC317 | RC10A | 202 | 202 | 238 | 0   | 0   | 176 | 205 |
| RC307 | RC10A | 197 | 200 | 238 | 248 | 0   | 0   | 0   |
| RC321 | RC10A | 197 | 204 | 236 | 0   | 194 | 0   | 0   |
| RC271 | RC10A | 197 | 200 | 248 | 279 | 188 | 176 | 0   |
| RC293 | RC10A | 197 | 210 | 242 | 261 | 198 | 240 | 0   |
| RC323 | RC10A | 197 | 200 | 0   | 279 | 180 | 217 | 180 |
| RC301 | RC10A | 197 | 200 | 278 | 0   | 0   | 226 | 185 |
| RC316 | RC10A | 197 | 202 | 244 | 0   | 0   | 214 | 187 |
| RC311 | RC10A | 197 | 202 | 248 | 248 | 188 | 176 | 199 |
| RC310 | RC10A | 197 | 200 | 0   | 246 | 188 | 180 | 205 |
| RC315 | RC10A | 197 | 202 | 0   | 0   | 0   | 240 | 205 |
| RC296 | RC10A | 197 | 200 | 240 | 248 | 0   | 214 | 213 |
| RC325 | RC10A | 197 | 200 | 278 | 279 | 184 | 176 | 230 |
| RC327 | RC10A | 195 | 202 | 240 | 279 | 221 | 176 | 0   |
| RC295 | RC10A | 195 | 202 | 228 | 248 | 0   | 226 | 0   |
| RC298 | RC10A | 195 | 200 | 248 | 0   | 0   | 240 | 0   |
| RC305 | RC10A | 195 | 202 | 238 | 0   | 198 | 181 | 185 |
| RC313 | RC10A | 195 | 200 | 248 | 248 | 0   | 214 | 205 |
| RC308 | RC10A | 195 | 0   | 0   | 0   | 0   | 240 | 205 |
| RC303 | RC10A | 193 | 200 | 0   | 0   | 201 | 181 | 185 |
| RC314 | RC10A | 193 | 0   | 0   | 0   | 217 | 214 | 190 |
| RC324 | RC10A | 193 | 204 | 238 | 279 | 188 | 176 | 205 |
| RC328 | RC10A | 190 | 200 | 238 | 279 | 180 | 176 | 185 |
| RC287 | RC10A | 182 | 200 | 0   | 265 | 213 | 176 | 0   |
| RC300 | RC10A | 182 | 0   | 238 | 248 | 221 | 236 | 0   |
| RC282 | RC10A | 182 | 181 | 248 | 279 | 0   | 176 | 199 |
| RC302 | RC10A | 156 | 218 | 240 | 0   | 203 | 226 | 0   |
| RC294 | RC10A | 0   | 202 | 236 | 248 | 196 | 214 | 0   |
| RC273 | RC10A | 0   | 210 | 278 | 279 | 188 | 176 | 190 |
| RC292 | RC10A | 0   | 200 | 223 | 0   | 201 | 214 | 197 |
| RC320 | RC10A | 0   | 223 | 248 | 0   | 188 | 176 | 199 |
| RC326 | RC10A | 0   | 210 | 223 | 0   | 220 | 176 | 205 |
| RC913 | RC11A | 195 | 200 | 244 | 261 | 0   | 237 | 189 |
| RC915 | RC11A | 193 | 0   | 229 | 0   | 200 | 240 | 221 |
| RC922 | RC11A | 193 | 202 | 229 | 259 | 196 | 240 | 185 |

|        |       |     |     |     |     |     |     |     |
|--------|-------|-----|-----|-----|-----|-----|-----|-----|
| RC925  | RC11A | 200 | 200 | 239 | 259 | 196 | 231 | 208 |
| RC931  | RC11A | 197 | 202 | 239 | 255 | 206 | 240 | 191 |
| RC937  | RC11A | 197 | 204 | 248 | 261 | 201 | 240 | 189 |
| RC938  | RC11A | 197 | 202 | 242 | 269 | 200 | 243 | 184 |
| RC939  | RC11A | 195 | 200 | 238 | 261 | 193 | 240 | 206 |
| RC919  | RC11A | 197 | 202 | 236 | 259 | 198 | 240 | 185 |
| RC921  | RC11A | 0   | 200 | 253 | 248 | 203 | 237 | 187 |
| RC926  | RC11A | 0   | 200 | 250 | 248 | 205 | 239 | 0   |
| RC927  | RC11A | 197 | 200 | 237 | 0   | 205 | 239 | 0   |
| RC930  | RC11A | 197 | 200 | 238 | 0   | 205 | 240 | 172 |
| RC932  | RC11A | 199 | 0   | 0   | 0   | 203 | 240 | 213 |
| RC934  | RC11A | 0   | 0   | 238 | 248 | 203 | 240 | 210 |
| RC1003 | RC11B | 197 | 200 | 248 | 253 | 202 | 0   | 188 |
| RC1013 | RC11B | 197 | 200 | 239 | 268 | 204 | 238 | 187 |
| RC1014 | RC11B | 0   | 202 | 239 | 271 | 200 | 240 | 225 |
| RC1016 | RC11B | 199 | 200 | 0   | 258 | 200 | 239 | 187 |
| RC974  | RC11B | 197 | 202 | 236 | 0   | 200 | 240 | 224 |
| RC975  | RC11B | 197 | 200 | 238 | 265 | 198 | 328 | 187 |
| RC978  | RC11B | 199 | 202 | 229 | 263 | 201 | 0   | 0   |
| RC979  | RC11B | 200 | 200 | 229 | 255 | 0   | 0   | 216 |
| RC980  | RC11B | 197 | 200 | 238 | 253 | 0   | 240 | 189 |
| RC981  | RC11B | 197 | 200 | 263 | 278 | 195 | 240 | 187 |
| RC982  | RC11B | 195 | 200 | 245 | 269 | 200 | 242 | 187 |
| RC983  | RC11B | 197 | 202 | 239 | 261 | 202 | 242 | 187 |
| RC984  | RC11B | 197 | 200 | 239 | 261 | 207 | 0   | 0   |
| RC986  | RC11B | 197 | 210 | 244 | 269 | 202 | 240 | 197 |
| RC987  | RC11B | 199 | 202 | 240 | 252 | 198 | 240 | 184 |
| RC988  | RC11B | 195 | 196 | 229 | 0   | 191 | 236 | 216 |
| RC992  | RC11B | 197 | 200 | 237 | 259 | 0   | 240 | 187 |
| RC999  | RC11B | 197 | 202 | 237 | 261 | 209 | 240 | 205 |
| RC1008 | RC11B | 197 | 200 | 238 | 258 | 203 | 239 | 172 |
| RC1001 | RC11B | 197 | 162 | 244 | 0   | 205 | 239 | 185 |
| RC1005 | RC11B | 197 | 200 | 240 | 258 | 198 | 244 | 190 |
| RC1015 | RC11B | 191 | 200 | 0   | 0   | 218 | 230 | 230 |
| RC994  | RC11B | 188 | 0   | 248 | 259 | 194 | 239 | 230 |
| RC991  | RC11B | 180 | 0   | 229 | 269 | 197 | 0   | 187 |
| RC1011 | RC11B | 0   | 208 | 255 | 273 | 203 | 239 | 185 |
| RC1019 | RC11C | 194 | 223 | 239 | 0   | 0   | 242 | 199 |
| RC1020 | RC11C | 0   | 200 | 242 | 0   | 0   | 258 | 187 |
| RC1021 | RC11C | 197 | 202 | 236 | 259 | 198 | 242 | 184 |
| RC1022 | RC11C | 197 | 202 | 257 | 267 | 202 | 240 | 216 |
| RC1024 | RC11C | 197 | 202 | 237 | 261 | 200 | 242 | 201 |
| RC1026 | RC11C | 197 | 196 | 237 | 273 | 0   | 240 | 206 |
| RC1028 | RC11C | 0   | 200 | 198 | 261 | 204 | 240 | 195 |
| RC1032 | RC11C | 0   | 212 | 224 | 0   | 196 | 238 | 184 |

|        |       |     |     |     |     |     |     |     |
|--------|-------|-----|-----|-----|-----|-----|-----|-----|
| RC1034 | RC11C | 197 | 218 | 228 | 275 | 202 | 226 | 202 |
| RC1040 | RC11C | 229 | 0   | 230 | 0   | 202 | 233 | 189 |
| RC1042 | RC11C | 197 | 200 | 237 | 255 | 197 | 233 | 189 |
| RC1043 | RC11C | 197 | 200 | 239 | 261 | 0   | 242 | 187 |
| RC1044 | RC11C | 200 | 200 | 237 | 255 | 200 | 247 | 191 |
| RC1047 | RC11C | 197 | 0   | 248 | 251 | 202 | 238 | 176 |
| RC1049 | RC11C | 0   | 180 | 237 | 263 | 196 | 238 | 187 |
| RC1050 | RC11C | 197 | 0   | 229 | 269 | 200 | 242 | 164 |
| RC1055 | RC11C | 197 | 0   | 229 | 276 | 200 | 238 | 216 |
| RC1059 | RC11C | 197 | 197 | 255 | 0   | 204 | 240 | 216 |
| RC1061 | RC11C | 215 | 0   | 239 | 0   | 0   | 244 | 175 |
| RC1062 | RC11C | 197 | 0   | 239 | 265 | 193 | 218 | 187 |
| RC1035 | RC11C | 195 | 204 | 252 | 0   | 197 | 242 | 185 |
| RC1036 | RC11C | 183 | 210 | 236 | 261 | 0   | 242 | 172 |
| RC1056 | RC11C | 206 | 200 | 253 | 253 | 205 | 242 | 0   |
| RC1039 | RC11C | 197 | 202 | 242 | 253 | 207 | 239 | 187 |
| RC1060 | RC11C | 195 | 202 | 252 | 258 | 202 | 240 | 187 |
| RC854  | RC11D | 197 | 202 | 248 | 0   | 0   | 242 | 187 |
| RC855  | RC11D | 197 | 224 | 241 | 260 | 204 | 240 | 189 |
| RC857  | RC11D | 197 | 200 | 237 | 265 | 0   | 238 | 194 |
| RC858  | RC11D | 0   | 0   | 243 | 0   | 202 | 235 | 195 |
| RC859  | RC11D | 196 | 0   | 229 | 267 | 202 | 0   | 180 |
| RC860  | RC11D | 198 | 202 | 255 | 261 | 196 | 240 | 0   |
| RC861  | RC11D | 197 | 0   | 255 | 261 | 196 | 240 | 190 |
| RC863  | RC11D | 195 | 202 | 239 | 267 | 201 | 240 | 190 |
| RC864  | RC11D | 197 | 202 | 237 | 285 | 0   | 240 | 185 |
| RC867  | RC11D | 197 | 0   | 239 | 267 | 0   | 257 | 187 |
| RC873  | RC11D | 197 | 200 | 257 | 0   | 0   | 240 | 187 |
| RC874  | RC11D | 195 | 200 | 248 | 267 | 199 | 240 | 187 |
| RC875  | RC11D | 207 | 202 | 259 | 261 | 204 | 240 | 180 |
| RC876  | RC11D | 202 | 202 | 0   | 0   | 204 | 0   | 190 |
| RC877  | RC11D | 197 | 202 | 251 | 259 | 191 | 240 | 187 |
| RC878  | RC11D | 200 | 201 | 237 | 253 | 0   | 264 | 190 |
| RC880  | RC11D | 197 | 202 | 238 | 238 | 0   | 242 | 227 |
| RC881  | RC11D | 197 | 202 | 238 | 271 | 208 | 237 | 187 |
| RC884  | RC11D | 195 | 202 | 257 | 267 | 204 | 240 | 187 |
| RC885  | RC11D | 197 | 202 | 237 | 261 | 0   | 240 | 222 |
| RC889  | RC11D | 0   | 204 | 243 | 261 | 201 | 228 | 179 |
| RC894  | RC11D | 197 | 200 | 229 | 251 | 193 | 240 | 187 |
| RC895  | RC11D | 196 | 200 | 239 | 262 | 193 | 240 | 187 |
| RC897  | RC11D | 196 | 200 | 243 | 262 | 201 | 242 | 185 |
| RC898  | RC11D | 195 | 200 | 237 | 261 | 0   | 240 | 185 |
| RC883  | RC11D | 197 | 202 | 236 | 0   | 205 | 239 | 190 |
| RC886  | RC11D | 197 | 202 | 236 | 0   | 199 | 240 | 190 |

|       |       |     |     |     |     |     |     |     |
|-------|-------|-----|-----|-----|-----|-----|-----|-----|
| RC887 | RC11D | 191 | 200 | 242 | 255 | 205 | 239 | 174 |
| RC882 | RC11D | 197 | 0   | 236 | 255 | 197 | 239 | 187 |

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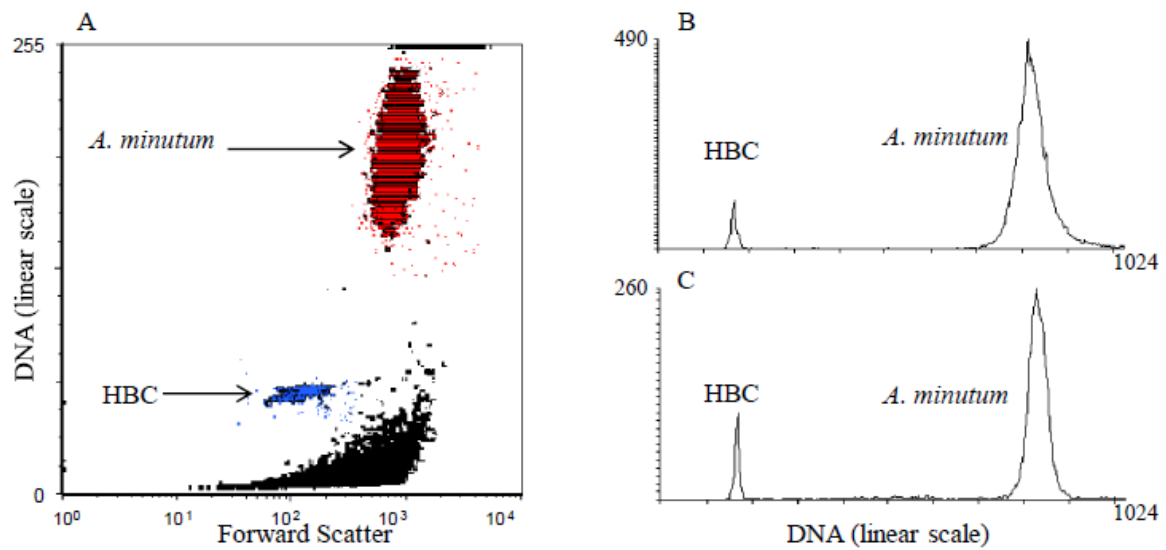


**Table S3:** Pairwise differentiation between the 11 populations of *Alexandrium minutum*.  $F_{st}(\theta)$  values are given above diagonal and Jost's  $D_{est}$  below diagonal. Significant  $F_{st}$  values (1000 permutations) are depicted in bold. \*:  $F_{st}$  values that remain significant after a Bonferroni correction. Population codes are given in Table 1.

|       | PZ10A         | PZ10D         | PZ11A         | PZ11B          | PZ11C          | PZ11D         | RC10A          | RC11A         | RC11B          | RC11C          | RC11D          |
|-------|---------------|---------------|---------------|----------------|----------------|---------------|----------------|---------------|----------------|----------------|----------------|
| PZ10A |               | <b>0.0396</b> | <b>0.0265</b> | <b>0.0378*</b> | <b>0.0639*</b> | <b>0.0403</b> | <b>0.0161</b>  | <b>0.0577</b> | <b>0.0456*</b> | <b>0.0443*</b> | <b>0.0486*</b> |
| PZ10D | <i>0.3228</i> |               | 0.0124        | <b>0.0247</b>  | 0.0170         | 0.0122        | <b>0.0495*</b> | 0.0281        | <b>0.0234</b>  | <b>0.0254</b>  | <b>0.0495*</b> |
| PZ11A | <i>0.1847</i> | <i>0.2007</i> |               | 0.0073         | <b>0.0211</b>  | 0.0019        | <b>0.0536*</b> | <b>0.0331</b> | 0.0100         | 0.0104         | <b>0.0236</b>  |
| PZ11B | <i>0.2748</i> | <i>0.2279</i> | <i>0.0929</i> |                | 0.0102         | <b>0.0234</b> | <b>0.0522*</b> | 0.0062        | 0.0008         | 0.0059         | 0.0103         |
| PZ11C | <i>0.3810</i> | <i>0.2206</i> | <i>0.1694</i> | <i>0.0576</i>  |                | <b>0.0259</b> | <b>0.0770*</b> | <b>0.0401</b> | <b>0.0260</b>  | <b>0.0429*</b> | <b>0.0534*</b> |
| PZ11D | <i>0.2135</i> | <i>0.2096</i> | <i>0.0956</i> | <i>0.2102</i>  | <i>0.1968</i>  |               | <b>0.0623*</b> | <b>0.0318</b> | <b>0.0197</b>  | <b>0.0366</b>  | <b>0.0581*</b> |
| RC10A | <i>0.1365</i> | <i>0.4011</i> | <i>0.3541</i> | <i>0.3287</i>  | <i>0.4695</i>  | <i>0.3464</i> |                | <b>0.0457</b> | <b>0.0544*</b> | <b>0.0582*</b> | <b>0.0763*</b> |
| RC11A | <i>0.2989</i> | <i>0.2938</i> | <i>0.2065</i> | <i>0.1187</i>  | <i>0.2487</i>  | <i>0.2071</i> | <i>0.2295</i>  |               | -0.0035        | <b>0.0259</b>  | 0.0179         |
| RC11B | <i>0.2696</i> | <i>0.2414</i> | <i>0.1631</i> | <i>0.0766</i>  | <i>0.1448</i>  | <i>0.1700</i> | <i>0.3007</i>  | <i>0.0684</i> |                | -0.0011        | 0.0163         |
| RC11C | <i>0.3100</i> | <i>0.2402</i> | <i>0.1157</i> | <i>0.0994</i>  | <i>0.2331</i>  | <i>0.2323</i> | <i>0.3723</i>  | <i>0.2081</i> | <i>0.0589</i>  |                | <b>0.0169</b>  |
| RC11D | <i>0.2225</i> | <i>0.3240</i> | <i>0.1177</i> | <i>0.1145</i>  | <i>0.2802</i>  | <i>0.2610</i> | <i>0.3815</i>  | <i>0.1453</i> | <i>0.1225</i>  | <i>0.1156</i>  |                |

**Figure S1.** Quantification of DNA in *Alexandrium minutum* cultures in comparison with nuclei isolated from human blood cells (HBC, 6.4 Mbp), after staining with propidium iodide in PBS buffer. HBC and *A. minutum* are discriminated on the forward scatter (X-axis, logarithmic scale), a proxy of size, versus red fluorescence (Y-axis, linear scale) dot plot (A). Quantification of DNA (X-axis, linear scale) by fluorescence in two monoclonal strains (B) and (C).

**Figure S1:**



**Figure S2.** Resolving power of the microsatellite marker set to discriminate the maximum clonal diversity available in the data set.

