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Short Communication

Heterologous microsatellite-based genetic diversity in blue mussel (*Mytilus chilensis*) and differentiation among localities in southern Chile

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ABSTRACT. Mussels (*Mytilus* spp.) are one of the most cultivated and commercialized bivalves in southern Chile; culture is currently supplied almost entirely from wild-caught seed obtained from relatively few collection centers. The genetic diversity and differentiation of the blue mussel in southern Chile was investigated by sampling six locations: one natural bank and five seed collection centers. Nine polymorphic microsatellite (SSR) loci were genotyped (*Mgu1, Mgu3, MT203, MT282, Mg15, Mg56, Med737, MIT02* and *MGE005*). We found 75 different alleles, six of which were private alleles. Of the analyzed loci, 45 of 54 tests performed deviated from Hardy-Weinberg equilibrium after sequential Bonferroni correction ($P < 0.05$), revealing significant heterozygote deficiencies. The polymorphic information content (PIC) ranged from 0.322 (*MGE005*) to 0.893 (*Mgu1*). Despite the long distance between some sampling sites (up to 1360 km), genetic differentiation among the sites was low ($F_{ST} = 0.043$, $P < 0.0001$). The Bayesian cluster analysis (STRUCTURE) indicated two probable clusters, while the non-parametric cluster analysis (AWclust) identified two to four clusters. Both analyses showed a high level of admixture within clusters. Our results indicate that blue mussels in southern Chile show lower genetic diversity than in other countries, low inbreeding levels, and limited genetic differentiation among locations.

Keywords: *Mytilus*, mussels, genetic diversity, genetic differentiation, microsatellites, southern Chile.

Diversidad genética del mejillón (*Mytilus chilensis*) y diferenciación entre localidades del sur de Chile usando marcadores microsatélites heterólogos

RESUMEN. Los mejillones (*Mytilus* spp.) constituyen unos de los bivalvos más cultivados y comercializados en el sur de Chile. Su cultivo está actualmente abastecido completamente con semillas obtenidas desde el medio natural, de relativamente pocos centros de captación. La diversidad y diferenciación genética de los mejillones en el sur de Chile fue investigada muestreando seis lugares, un banco natural y cinco centros de captación de semilla. Se genotiparon nueve loci microsatélite (SSR) polimórficos (*Mgu1, Mgu3, MT203, MT282, Mg15, Mg56, Med737, MIT02* y *MGE005*). Se encontraron 75 alelos diferentes, seis de ellos fueron alelos privados. En los loci analizados, 45 de los 54 test realizados mostraron desviación significativa ($P < 0,05$) del equilibrio de Hardy-Weinberg después de la corrección secuencial de Bonferroni, revelando un significativo déficit de heterocigotos. El contenido de información polimórfica (PIC) varió entre 0,322 (*MGE005*) y 0,893 (*Mgu1*). A pesar de la gran distancia entre algunos sitios de muestreo (hasta 1.360 km), la diferenciación genética entre ellos fue baja ($F_{ST} = 0,043$; $P = 0,00$). El análisis Bayesiano de agrupamiento (STRUCTURE) indicó que la estructura poblacional más probable consiste en dos grupos, en tanto el análisis de agrupamiento no paramétrico (AWclust) identificó entre dos y cuatro grupos, ambos análisis mostraron un alto nivel de mezcla dentro de los grupos. Estos resultados indican que los mejillones del sur presentan menor diversidad genética que en otros países, baja endogamia y diferenciación genética entre localidades.

Palabras clave: *Mytilus*, mejillones, diversidad genética, diferenciación genética, microsatélites, sur de Chile.

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Mussels from the genus *Mytilus*, widely used for human consumption, are among the most cultivated and marketed bivalves. Chilean mussel culture yielded 10.8% of the world's *Mytilidae* production in 2012 (FAO, 2015). The distribution range for this species extends along the Chilean coast from Arauco (35ºS) to Cape Horn (55ºS) (Hernández & González, 1976), but nearly 100% of production comes from Chiloé Island and the mainland in the 41-44ºS region (SERNA-PESCA, 2015).

The name *Mytilus chilensis* (Hupé, 1854) was initially given to the native Chilean blue mussel, but the names *M. edulis chilensis* (Toro, 1998) and *M. galloprovincialis chilensis* (Cárcamo *et al.,* 2005) were later proposed. Also, the species *M. galloprovincialis* described in the coasts of Chile was found to be distinct from the endemic *Mytilus* according to various molecular evidence (Tarifeño *et al.*, 2005; Toro *et al.*, 2005; Astorga & Toro, 2010), other authors (Westfall & Gardner, 2010) reported that the blue mussel now found in Chile is a mix of native Southern-hemisphere and introduced Northern-hemisphere *M. galloprovincialis* and *M. edulis* and their respective hybrids. Borsa *et al.* (2012) proposed the taxonomic status *M. galloprovincialis planulatus* and *M. edulis platensis* for these species, respectively. Since 2013, the WoRMS no longer recognizes the name *Mytilus chilensis* Hupé, 1854 (Boxshall *et al.*, 2014) for the native Chilean smooth-shelled mussel, replacing it with the name *Mytilus edulis platensis* d'Orbigny, 1842 (Borsa *et al*., 2012). However, the name *Mytilus chilensis* is still used commercially, in statistics, in certifications, and on food product labels (GAA, 2013; FAO, 2015), and also by authors (Gazeau *et al*., 2013; Riisgard *et al*., 2013; Astorga, 2014; Larraín *et al.*, 2014; Oyarzún *et al*., 2014; Ouagajjou & Presa, 2015). Moreover, there is evidence suggesting that *M. chilensis* is a valid, distinct species within the genus based on the strong genetic and reproductive differences *versus* the native Chilean mussel, *M. galloprovincialis* (Mediterranean and Atlantic populations) and *M. edulis* found when these taxa were evaluated with a 54-SNP panel developed by Zbawicka *et al.* (2014) as marker for *Mytilus* taxa (R. Wenne *pers. comm.),* with microsatellite markers (Ouagajjou *et al.,* 2011), mitochondrial cytochrome oxidase I gene (COI) (Seguel, 2011), and sperm morphology (Oyarzún *et al*., 2014). Therefore, we use the name *M. chilensis* for the native and predominant blue mussel species that inhabits the Chilean coast.

The long larval phase of the *Mytilus* lifecycle extends its dispersal capacity to large geographic areas, increasing gene flow, preventing differentiation among populations, and preserving high levels of diversity within populations (Toro *et al.,* 2006). Currently, aquaculture farms are supplied solely with seed collected from the wild in the area of the Reloncaví estuary and Chiloé Island (Bagnara & Maltraín, 2008). In 2012-2013 a significant decrease in seed supply from the collection centers was reported for the zone.

Understanding the genetic diversity and differentiation of economically-important species is fundamental for supporting management and conservation programs, enhancing production, and exploring the possibility of developing DNA-based traceability systems using allocation algorithms. In Chile, studies of *Mytilus* genetic diversity and differentiation have been performed using RAPDs and allozyme markers (Toro *et al.,* 2004, 2006), producing no evidence of discrete stocks, with the possible exception of a Magallanes population (Punta Arenas 53ºS). Microsatellite markers are an important tool used to assess genetic diversity levels and population structures in marine species (Liu & Cordes, 2004); these markers have been applied to the *Mytilidae* family for species such as *Perumytilus purpuratus* (Perez *et al*., 2008; Briones *et al*., 2013), *M. galloprovincialis* (Diz & Presa, 2008, 2009), *M. edulis*, and *M. trossulus* (Kijewski *et al.,* 2009; Shields *et al.,* 2010). In Chilean blue mussels, SSR have been used by Vidal *et al.* (2009) and Ouagajiou *et al.* (2011) in cross-species amplification and allelic variation assessment, respectively, on a limited number of individuals from a single location, but to date there are no published studies of genetic diversity or differentiation among locations using this tool.

The current study aims to investigate the genetic diversity and differentiation of *Mytilus chilensis* in southern Chile using nine heterologous SSR loci. This survey complements previous population studies conducted in the region based on allozyme and RAPDs analysis, with more informative SSR markers.

Mussel samples (n = 50 by location) were collected in southern Chile from three zones: 1) Reloncaví (Quillaipe: 1-QI, Pichicolo: 1-PI, Caleta La Arena: 1-LA, and Canutillar: 1-CN), 2) Chiloé (Canal Coldita-Piedra Blanca: 2-CB), and 3) Magallanes (Isla Peel: 3-IP). In zones 1 and 2, where mussel aquaculture activities are carried out, we sampled seed collection centers, and in zone 3, the source was a natural bank. Detailed information about sampling, DNA extraction, genus, and species identification can be found in Larraín *et al.,* (2012, 2014). Only *M. chilensis* individuals, identified according to designation at the RFLP-PCR Me 15-16 *Aci* I marker (Inoue *et al*., 1995; Santaclara *et al.,* 2006), were used for microsatellite analysis. Nine SSR loci were genotyped. The repeat motifs obtained from GenBank sequences by accession number were: mononucleotide (*Mg15*) (Cruz *et al.,*

2005), perfect dinucleotide (*Mgu3, MT203, MT282, MIT02, MGE005*) (Presa *et al.*, 2002; Gardeström *et al.*, 2007; Yu & Li, 2007; Vidal *et al.*, 2009), compound dinucleotide (*Mgu1, Med737*) (Presa *et al.*, 2002; Lallias *et al.*, 2009), and complex (*Mg56*) (Cruz *et al.*, 2005). Detailed information about primers and PCR conditions used to amplify eight of the motifs (*Mgu1, Mgu3, MT203, MT282, Mg15, Mg56, Med737* and *MIT02*) are described in Larraín *et al.* (2014). The other locus used in this work was *MGE005* (Yu & Li, 2007). For this last locus, primers (F: 5´-AGACCAAGGTA TTGCAACCATGTG-3 and R: 5´-TCGAAAGCATG GTACCTGGTCA-3´) were obtained using AmplifX (http://crn2m.univ-mrs.fr/recherche/brue/jullien-nicolas/ programmation/amplifx) and the Primer Blast NCBI utility (http://www.ncbi.nlm.nih.gov/tools/ primer-blast/). The primers were commercially synthesized by Integrated DNA technologies Inc. (IDT) (Singapore). The PCR thermal profile to amplify the *MGE005* locus was 95°C for 5 min, followed by 35 cycles at 95°C (1 min), 67ºC (30 s), 72°C (50 s), and a final 10-min extension step at 72°C. PCR amplification was carried out in a 15-µL reaction mixture containing 1.5 µL of 10x PCR buffer, 1.5 mM $MgCl_2$, 100 µM each of dNTP, 0.3 µM of each primer, 0.5 U Taq DNA Polymerase (RBC Bioscience®), and 40 ng of DNA. A negative control with template DNA replaced by water was performed for each set of amplifications. To evaluate amplification, PCR products were visualized on an agarose gel (1.8%) in TBE buffer with 10 mg mL^{-1} of ethidium bromide under ultraviolet light. For genotyping, polyacrylamide gels (6%) with silver staining were used to resolve alleles (Larraín, 2012); for every gel, the size of amplified fragments was estimated from a 10-bp DNA ladder (Invitrogen®) or HyperLadder V (Bioline®).

The Excel add-in MS tools (Park, 2001) and CONVERT software (Glaubitz, 2004) were used to reformat diploid genotypic data. MICRO-CHECKER (Van Oosterhout *et al.*, 2004) was used to test for the presence of null alleles, stuttering, and large allele dropout. Genetic diversity was determined by the observed number of alleles per locus (*Na*), observed (*Ho*) and expected (*He*) heterozygosities, polymorphic information content (*PIC*), and the presence of private alleles, using MS tools (Park, 2001). Genepop 4.0.10 (Raymond & Rousset, 1995; Rousset, 2008) was used to test genotypic linkage disequilibria (*LD*) between each pair of loci, to evaluate genotypic distributions for conformation to Hardy-Weinberg equilibrium (HWE), and to estimate Wright's fixation indices ($F_{IS,}$ F_{ST}, and F_{IT}) according to Weir & Cockerham (1984). Exact *P*-values were estimated by the Markov chain method (Guo & Thompson, 1992), using default software conditions. Sequential Bonferroni correction was used for multiple tests (Holm, 1979; Rice, 1989). The relatedness index r_{xy} (Queller & Goodnight, 1989) for each pair of individuals was obtained using Identix software (Belkhir *et al.*, 2002). Genetic differentiation among locations was determined with Genetix 4.05 (Belkhir *et al.*, 1996) to estimate pairwise F_{ST} and its statistical significance and to perform a three-dimensional factorial correspondence analysis (3D-FCA). The Mantel test (Mantel, 1967) was performed to test an isolation-by-distance model of genetic differentiation. Correlation coefficient (*r*) calculations were performed with Genetix 4.05, and the significance of the associations was tested with 10,000 iterations. We compared the genetic distance matrices (F_{ST} / (1-F_{ST})) with the logarithm of the minimal geographic distance estimated by the coastline (Abbott *et al.*, 2013), as recommended for a two-dimensional habitat (latitude and longitude) (Rousset, 2008). The genetic structure was investigated with parametric-with -STRUCTURE 2.3.4 (Pritchard *et al.*, 2000)- and non-parametric -AWclust (Gao & Stramer, 2008)- frameworks. The Bayesian approach (STRUCTURE) was performed using the standard burn-in period length (50,000) and number of MCMC reps after burn-in (100,000) (Falush *et al.*, 2007), achieving convergence with our data. Ten repeated runs were performed, selecting K = 6, the admixture ancestry, and the correlated allele frequency models. The number of clusters (K) that best fit the data was inferred using Delta K values as described by Evanno *et al.* (2005) and calculated using the STRUCTURE Harvester webpage (Earl & von Holdt, 2012). The R package AWclust -allele sharing distance and Ward's minimum variance hierarchical clustering- (Gao & Starmer, 2008) was used, converting the multiallelic SSR data according to Wei *et al.* (2013).

Null alleles were present in all locations for loci *Mgu1*, *Mgu3*, *MT203*, *Mg15*, *Mg56*, and *MIT02* and in some locations for loci *MT282* and *Med737* but not detected in any location for locus *MGE005*. The frequency of non-amplifying individuals (putative null allele homozygotes) was less than 5% for all loci, and thus all were used in further analysis (Dąbrowski *et al.*, 2014).

All loci showed common allele distributions among sites. Six private alleles, *i.e.*, alleles unique to a single site, were found at low frequency (0.01-0.02) in the seed collection centers Pichicolo (1-PI) and Caleta La Arena (1-LA) at locus *Mgu3*, Canal Coldita-Piedra Blanca (2-CB) at locus *Mg56*, Quillaipe (1-QI) at locus *Med737*, and Quillaipe (1-QI) and Canutillar (1-CN) at locus *MGE005* (Annex 1). A total of 75 alleles were detected at the nine loci among the analyzed individuals (Table 1). All loci were polymorphic in all locations as the frequency of the most common allele did not

Table 1. Global genetic diversity estimators by locus in samples (n = 300) of blue mussel (*Mytilus chilensis*) in southern Chile using nine SSR loci. *Na*: number of alleles, *Ho*: the observed heterozygosity, *He*: the expected heterozygosity, *PIC*: polymorphic information content and the fixation indices F_{IS}, F_{ST}, F_{IT}, according to Weir & Cockerham (1984).

Locus	*Na*	*Ho*	*He*	*PIC*	F_{IS}	F_{ST}	F_{IT}
Mgu1	15	0.407	0.903	0.893	0.542	0.021	0.551
Mgu3	6	0.097	0.610	0.540	0.802	0.227	0.847
MT203	10	0.453	0.782	0.757	0.413	0.015	0.422
MT282	4	0.543	0.616	0.543	0.111	0.010	0.119
Mg15	4	0.416	0.694	0.630	0.395	0.012	0.403
Mg56	11	0.470	0.760	0.725	0.369	0.026	0.385
Med737	10	0.483	0.756	0.721	0.319	0.073	0.369
MIT02	5	0.365	0.617	0.544	0.411	0.000	0.409
MGE005	10	0.320	0.330	0.322	0.032	0.000	0.031
Global	75	0.395	0.674	0.631	0.393	0.043	0.419

exceed 0.95; average number of alleles per locus was 8.3 ± 3.8, ranging from four for *MT282* and *Mg15* in all locations to 15 for *Mgu1* in Quillaipe (1-QI) and Pichicolo (1-PI) (Annex 2). *PIC* values ranged from 0.322 at locus *MGE005* to 0.893 at locus *Mgu1* (Table 1). With the exception of locus *MGE005*, which was moderately informative, all other loci were highly informative (*PIC* > 0.5) (He *et al.*, 2012). *Ho* ranged from 0.097 (*Mgu3*) to 0.543 (*MT282*), while *He* values ranged from 0.330 (*MGE005*) to 0.903 (*Mgu1*) (Table 1). Significant deviations from HWE were observed in 45 of the 54 tests (9 loci × 6 locations), performed after correction for multiple testing (Holm, 1979; Rice, 1989). Significant deviations corresponded to positive F_{IS} values observed in various loci, indicating heterozygote deficiencies. Tests for linkage disequilibrium (*LD*) showed no significant deviations for any of the 216 tests performed (36 locus combinations × 6 locations) after sequential Bonferroni correction for multiple tests (Holm, 1979; Rice, 1989).

Although average relatedness (r_{xy}) between pairs of individuals from the same site was significantly higher than between pairs of randomly-mixed individuals from different locations, at -0.019 ± 0.0013 and -0.137 ± 0.0009, respectively ($P < 0.0001$), neither value was significantly different from zero.

Pairwise F_{ST} values among all six locations indicate (Table 2) that the highest F_{ST} (0.1112) value was between Canal Coldita-Piedra Blanca (2-CB) and Caleta La Arena (1-LA). Fourteen of the 15 tests ($P < 0.05$) showed significant pairwise F_{ST} values after correction for multiple comparisons (Holm, 1979; Rice, 1989). The global F_{ST} value indicates that 4.3% of the total allele frequency variance lies among sample sites and is highly significant ($P < 0.0001$), while 95.7% is explained by the variation within sites. The first three components of the 3D-FCA (Fig. 1) account for 81.78% of the total variation in multilocus genotypes, 43.38% of which is explained by Axis 1, which separates Caleta La Arena (1-LA) from the other five sites, 22.18% by Axis 2, which separates Quillaipe (1-QI) and Canal Coldita-Piedra Blanca (2-CB) from the other sites, and 16.22% by Axis 3, which separates the most southern sample (3-IP). The Mantel test showed no significant correlation (r = -0.0130, P = 0.3610) between genetic and geographic distance.

Bayesian clustering analysis indicated that K = 2 (Fig. 2a) is the most likely value for inferred *Mytilus chilensis* clusters in the zone. The analysis showed that individuals from 1-LA were grouped together in cluster II and 2-CB in cluster I, with high average proportions of membership at 0.966 and 0.824, respectively (Table 3a), coinciding with the separation of these sites as shown by 3D-FCA. All other sites appeared to be highly admixed with individuals sharing membership between both clusters. The gap analysis from AWclust showed 3 clusters, by a narrow margin with widely overlapping error bars for K = 2 and 4 (Fig. 2b). As in the STRUCTURE analysis, a large proportion (0.72) of 1-LA individuals were grouped into a single cluster (cluster III) (Table 3b).

Microsatellites are one of the most informative genetic markers at the intra-specific level, used extensively to study marine species. However, in *Mytilus*, a relatively low number of SSR markers have been described compared with other aquaculture species such as salmon or trout, which have more than 2000 mapped SSR markers (Guyomard *et al.*, 2012).

Genetic diversity is essential because it allows for population and species survival and adaptation to changing environmental conditions (Mohanty *et al.*, 2014). The total of 75 alleles detected after genotyping

Table 2. Pairwise F_{ST} values between the blue mussel's *(Mytilus chilensis)* locations in southern Chile. Zone and location codes: 1) Reloncaví (Quillapie: 1-QI, Pichicolo: 1-PI, Caleta La Arena: 1-LA and Canutillar: 1-CN), 2) Chiloé (Canal Coldita-Piedra Blanca: 2-CB), and 3) Magallanes (Isla Peel: 3-IP). *P < 0.05 after correction with sequential Bonferroni correction.

	1-PI	1-LA	1-CN	2-CB	3-IP
1-QI	0.0191*	0.0390*	0.0305*	0.0787*	0.0323*
1-PI		0.0650*	0.0049	0.0255*	0.0146*
1-LA			0.0712*	0.1112*	0.0740*
1-CN				0.0196*	0.0245*
2-CB					0.0250*

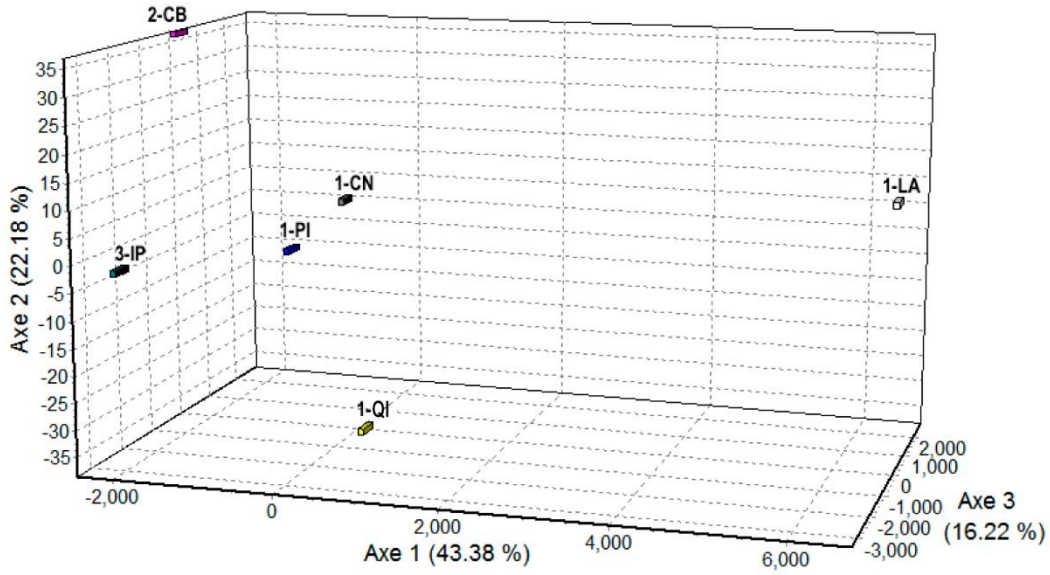

Figure 1. Three-dimensional factorial correspondence analysis plot (3D-FCA) for the six blue mussel *(Mytilus chilensis)* populations in southern Chile. Sample codes are indicated in Table 2.

all nine SSR loci across the six sampling locations suggests lower genetic diversity than in other SSR studies of *Mytilus* (Diz & Presa, 2009; Shields *et al.*, 2010). This is also evident from the global levels of observed and expected heterozygosities (*Ho*: 0.395 and *He*: 0.674) (Table 1). For example, in locus *Mgu3*, twelve alleles were found in Spain (Diz & Presa, 2009) and in a hybrid zone in Canada (Shields *et al.*, 2010), while in this study we found only six alleles.

The genetic diversity range ($0.330 \leq He \leq 0.903$) in *Mytilus chilensis* from the zone studied was wider than observed in *M. galloprovincialis* from Galicia, Spain ($0.72 \leq He \leq 0.80$) as assessed with six SSR loci (Diz & Presa, 2008). But the observed heterozygosity in this study exceeds that reported for allozymes in the same species and area (*Ho* = 0.29) (Toro *et al.*, 2006), as expected due to the highly polymorphic nature of SSR.

Deviations from HWE as shown by positive F_{IS} values observed at several loci, at many sites, revealed heterozygote deficiencies. Toro *et al.* (2006) also reported widespread heterozygote deficiencies in population STRUCTURE studies in the same species and area using allozymes. Null alleles cause heterozygous individuals to appear homozygous if they carry a visible allele and the null allele (Brookfield, 1996). This is the best-known reason for underestimation of heterozygosity with microsatellites (Shields *et al.*, 2010) and a common phenomenon in marine bivalve population genetics (Chapuis & Estoup, 2007). Although we used only loci with <5% of non-amplifying individuals (putative null allele homozygotes) (Dąbrowski *et al.*, 2014), null alleles could be a reason for the heterozygote deficiencies observed.

The heterologous nature of the microsatellites used in this study -SSR developed for *M. edulis*, *M. trossulus*, and *M. galloprovincialis* were applied to Chilean blue mussels- could explain the presence of null alleles. Using the SSR developed in *M. chilensis*, not available

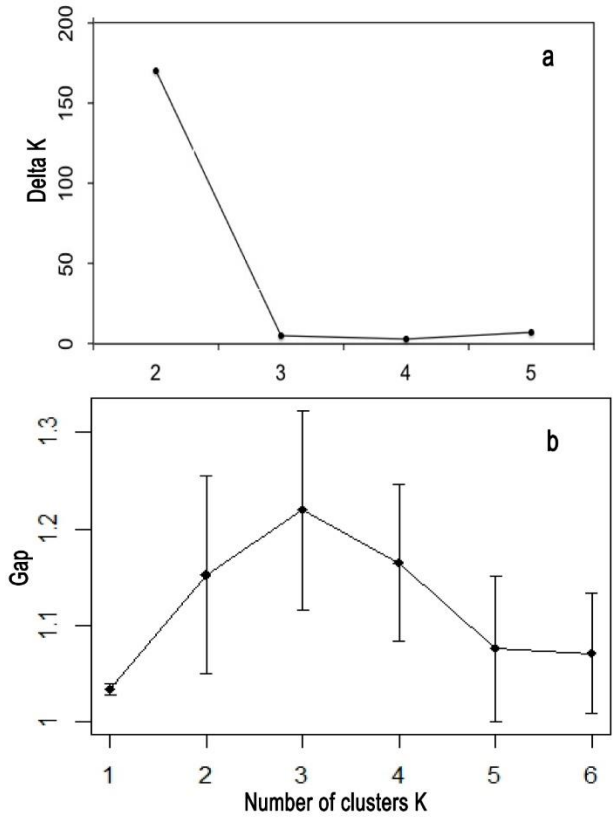

Figure 2. Number of clusters identified by a) Parametric approach implemented in STRUCTURE. Plot of the Delta K calculated as $\Delta K = \frac{mean\ |L''(K)|}{sd\ [L(K)]}$. The modal value of this distribution is the true K or the uppermost level of structure, b) non-parametric approach implemented in AWclust. Plot of the gap statistics between the observed and expected Wk results for K = 1-6 in *M. chilensis*. The largest gap indicates the most appropriate number of clusters for the data.

until after the completion of this experimental work, may have reduced the frequency of null alleles found (Ouagajjou *et al.*, 2011). However, these species are genetically and phenotypically close in both hemispheres, as evidenced by the controversy over the taxonomic status of the Chilean blue mussel and the widely-described presence of null alleles in *Mytilus* SSR (Presa *et al.*, 2002; Gardeström *et al.*, 2007; Yu & Li, 2007; Shields *et al.*, 2010; Ouagajjou *et al.*, 2011). Also, in other studies in the literature, correcting allele frequencies for null alleles between *Mytilus* populations did not change the results of statistical tests to estimate population differentiation (Gardeström *et al.*, 2007; Diz & Presa, 2008) or had a only minor effect on the population differentiation estimates, genetic distance determinations, and allocation of individuals to populations (Hauser *et al.*, 2006; Chapuis & Estoup, 2007). Given the above, along with the consideration that null alleles may not be the only reason for the observed heterozygote deficiencies, we decided not to correct the allele frequencies. Other possible reasons for heterozygote deficiencies found in *Mytilus* population studies are, among others, inbreeding, aneuploidy, molecular imprinting, and selection (Beaumont, 1991; Toro *et al.*, 2006; Diz & Presa, 2009). Although the relatedness between pairs of individuals from the same site was significantly higher than between individuals from different sites, in both cases relatedness was no higher than expected by chance (data not shown), providing no evidence of mating between related individuals (inbreeding).

The results of the microsatellite analyses revealed a common distribution of alleles between blue mussel locations in southern Chile. The global F_{ST} (0.040) obtained in this survey was low but significant, and it is within the range reported by Toro *et al.* (2004) using allozymes (F_{ST} = 0.03), although it is higher than the value reported by Cárcamo *et al.* (2005), also using allozymes (F_{ST} = 0.012), and those found in mussel populations on the Iberian Peninsula (F_{ST} = 0.0240 and 0.0122) using microsatellites. However, a greater differentiation between localities cannot be discounted due to cryptic allelic homoplasy described for SSR markers (Diz & Presa, 2008, 2009).

Although individuals taken from seed collection centers in zones 1 and 2 are intended for transport to grow-out centers and never will spawn in the place where they were recruited, they account for the composition of the fixed individuals in each location and may be informative regarding the location's genetic diversity and differentiation. Despite the geographical barriers of the coastline (fjords) and the considerable geographical distance between the most southern sample from Isla Peel (3-IP) in zone 3 and the Reloncaví and the Chiloé zones (zones 1 and 2 respectively), the mussel's reproductive system (external fertilization), the prolonged pelagic larval stage of this species (40-45 days) (Toro *et al.*, 2004), the intense aquaculture activities in the Reloncaví and Chiloé zones (transportation of juveniles from seed collection centers to ongrowing centers), and the ocean currents (Cape Horn and Chilean Coastal Current) (Strub *et al.*, 1998) promote dispersal, preventing higher levels of genetic differentiation.

Furthermore, in studies of other bivalves in the area with lower production levels than *Mytilus chilensis*, such as the clam (*Venus antiqua*) and the ribbed mussel (*Aulacomya atra*) (Mena *et al.*, 2001), higher genetic differentiation values were found using allozymes (F_{ST} = 0.107 and 0.147, respectively). This finding led us to infer that some of the genetic homogeneity between locations in the current study could be attributed to the

Table 3. Number of blue mussel *(Mytilus chilensis)* individuals in each location (n) and proportion of membership of individuals from each location to each of: a) the two clusters inferred by STRUCTURE, and b) the three clusters inferred by AWclust. Sample codes are indicated in Table 2.

a		STRUCTURE-inferred clusters	
Locations	n	I	II
1-QI	50	0.391	0.609
1-PI	50	0.623	0.377
1-LA	50	0.034	0.966
1-CN	50	0.731	0.269
2-CB	50	0.824	0.176
3-IP	50	0.634	0.366

b		AWclust-inferred clusters		
Locations	n	I	II	III
1-QI	50	0.26	0.16	0.58
1-PI	50	0.42	0.24	0.34
1-LA	50	0.08	0.20	0.72
1-CN	50	0.32	0.46	0.22
2-CB	50	0.48	0.36	0.16
3-IP	50	0.26	0.36	0.38

effect of extensive seed transfer, as reported in Galicia (Diz & Presa, 2009). The extent of pairwise genetic differentiation among the six sampled locations was small, but pairwise F_{ST} showed significant allelic differentiation, revealing unpatterned genetic heterogeneity among the local population, or genetic patchiness. This effect results in geographically close populations that may differ genetically as much as very distant populations, as has been reported in other marine organisms (Larson & Julian, 1999; Appleyard *et al.,* 2002).

The numbers of clusters inferred from the parametric (K = 2) and non-parametric methods (K = 3) were different; however, they were not in conflict because the gap statistic to determine the discrete number of clusters in AWclust gave widely overlapping error bars for K = 2, 3, and 4, providing a better resolution of the population structure (Fig. 2b). The parametric clustering approach in STRUCTURE provided more information (*i.e.*, about the probability an individual's membership in each cluster), but it relies on assumptions like HWE that were not always met during this study. Therefore, the non-parametric method for population structure analysis is more appropriate. According to Gao & Starmer (2008), the two methods can be complementary in structure analysis.

Our results for blue mussels using nine microsatellite loci indicate low inbreeding levels and lower levels of genetic diversity in Chile than in Spain or Canada; this latter is recognized as a hybrid zone, so higher levels of allelic richness and heterozygosity are expected. Furthermore, we found low but significant genetic differentiation among locations in southern Chile. These findings may be partly explained by reproduction conditions, prolonged pelagic larval stage, oceanographic conditions, and intense Chilean blue mussel aquaculture in the region.

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Annex 1. Allele frequencies for nine SSR loci genotyped in blue mussels (*Mytilus chilensis*) from six locations in southern Chile. Zone and location codes: 1) Reloncaví (Quillaipe: 1-QI, Pichicolo: 1-PI, Caleta La Arena: 1-LA and Canutillar: 1-CN), 2) Chiloé (Canal Coldita-Piedra Blanca: 2-CB), and 3) Magallanes (Isla Peel: 3-IP).

Allele size [bp]		Location					
Locus		1-QI	1-PI	1-LA	1-CN	2-CB	3-IP
Mgu1	115	4.00	-	17.00	2.00	6.00	3.00
	151	6.00	3.00	2.00	3.00	14.00	8.00
	153	6.00	12.00	24.00	9.00	18.00	19.00
	155	21.00	17.00	8.00	8.00	7.00	26.00
	163	6.00	12.00	3.00	13.00	4.00	1.00
	171	2.00	1.00	6.00	-	3.00	3.00
	173	6.00	4.00	6.00	4.00	-	2.00
	175	10.00	2.00	10.00	13.00	16.00	2.00
	177	11.00	12.00	14.00	24.00	15.00	11.00
	181	13.00	20.00	8.00	11.00	-	10.00
	187	7.00	5.00	-	3.00	4.00	6.00
	189	2.00	6.00	1.00	-	2.00	-
	197	1.00	2.00	1.00	6.00	8.00	4.00
	217	5.00	2.00	-	2.00	3.00	5.00
	225	-	2.00	-	2.00	-	-
Mgu3	141	-	1.00	-	-	-	-
	143	31.00	62.00	1.00	74.49	83.00	58.00
	147	51.00	36.00	47.00	23.47	15.00	33.00
	151	-	-	48.00	-	-	-
	157	9.00	-	1.00	1.02	1.00	7.00
	159	9.00	1.00	3.00	1.02	1.00	2.00
MT203	178	36.00	45.00	25.00	34.00	50.00	44.00
	184	6.00	13.00	25.00	12.00	6.00	6.00
	188	30.00	21.00	17.00	18.00	12.00	18.00
	192	2.00	1.00	-	1.00	6.00	-
	194	6.00	-	6.00	7.00	7.00	4.00
	198	7.00	4.00	10.00	8.00	10.00	10.00
	202	7.00	11.00	1.00	11.00	4.00	10.00
	206	3.00	1.00	3.00	7.00	1.00	5.00
	216	3.00	3.00	9.00	2.00	4.00	2.00
	220	-	1.00	4.00	-	-	1.00
MT282	335	28.00	41.00	33.00	33.00	37.00	40.00
	347	18.00	14.00	2.00	10.00	5.00	17.00
	353	49.00	41.00	60.00	52.00	55.00	41.00
	381	5.00	4.00	5.00	5.00	3.00	2.00
Mg15	115	9.78	2.08	3.06	7.14	2.04	3.00
	120	38.04	31.25	32.65	29.59	28.57	43.00

Continuation

Allele size [bp]		Location					
Locus		1-QI	1-PI	1-LA	1-CN	2-CB	3-IP
	123	10.87	30.21	35.71	36.73	39.80	29.00
	135	41.30	36.46	28.57	26.53	29.59	25.00
Mg56	247	-	-	-	-	1.00	-
	294	1.04	6.12	8.00	2.04	6.00	2.00
	301	7.29	4.08	7.00	8.16	1.00	3.00
	337	2.08	9.18	4.00	5.10	11.00	9.00
	344	26.04	32.65	26.00	41.84	51.00	30.00
	351	45.83	24.49	46.00	26.53	17.00	32.00
	372	3.13	2.04	1.00	5.10	3.00	2.00
	399	-	1.02	-	1.02	1.00	1.00
	425	4.17	3.06	3.00	1.02	3.00	4.00
	441	10.42	13.27	4.00	8.16	4.00	17.00
	464	-	4.08	1.00	1.02	2.00	-
Med737	122	1.00	-	-	-	-	-
	134	-	1.00	1.00	3.00	-	-
	140	57.00	40.00	52.00	51.00	13.00	19.00
	142	4.00	-	7.00	4.00	4.00	1.00
	146	18.00	35.00	21.00	22.00	35.00	8.00
	152	8.00	3.00	2.00	3.00	2.00	6.00
	164	8.00	12.00	11.00	14.00	29.00	37.00
	168	2.00	4.00	2.00	1.00	13.00	12.00
	174	2.00	3.00	4.00	-	4.00	13.00
	184	-	2.00	-	2.00	-	4.00
MIT02	197	4.00	1.00	2.00	6.00	7.14	7.00
	221	7.00	14.00	3.00	5.00	10.20	2.00
	227	51.00	51.00	52.00	52.00	39.80	45.00
	237	2.00	4.00	4.00	-	2.04	3.00
	243	36.00	30.00	39.00	37.00	40.82	43.00
MGE005	113	2.00	-	-	-	-	-
	119	2.00	2.00	-	-	1.00	-
	122	3.00	3.00	3.00	-	3.00	5.00
	125	4.00	7.00	5.00	7.00	3.00	4.00
	128	2.00	2.00	1.00	-	2.00	3.00
	134	5.00	-	3.00	11.00	3.00	2.00
	137	79.00	82.00	83.00	77.00	83.00	85.00
	140	2.00	2.00	4.00	3.00	3.00	-
	143	1.00	2.00	1.00	1.00	2.00	1.00
	149	-	-	-	1.00	-	-

Annex 2. Diversity parameters for nine SSR loci analyzed in six mussel locations (*Mytilus chilensis*) from southern Chile. Sample codes are indicated in Annex 1. Note: The number of alleles (*Na*), the number of private alleles (*Ap*), the observed heterozygosity (*Ho*), the expected heterozygosity (*He*), and the fixation index *Fis* according to Weir & Cockerham are provided for each locus and sample site. * Significant departures from Hardy-Weinberg expectations are corrected with sequential Bonferroni correction.

Locus/Location	1-QI	1-PI	1-LA	1-CN	2-CB	3-IP
Mgu1						
A(Ap)	14(0)	14(0)	12(0)	13(0)	12(0)	13(0)
Ho	0.500	0.300	0.560	0.340	0.500	0.240
He	0.902	0.886	0.871	0.883	0.888	0.866
Fis (W&C)	0.448*	0.664*	0.360*	0.617*	0.440*	0.725*
Mgu3						
A(Ap)	4(0)	4(1)	5(1)	4(0)	4(0)	4(0)
Ho	0.160	0.040	0.200	0.041	0.040	0.100
He	0.634	0.491	0.553	0.394	0.291	0.555
Fis (W&C)	0.750*	0.919*	0.641*	0.897*	0.864*	0.821*
MT203						
A (Ap)	9(0)	9(0)	9(0)	9(0)	9(0)	9(0)
Ho	0.380	0.420	0.440	0.420	0.500	0.560
He	0.769	0.729	0.830	0.817	0.717	0.753
Fis (W&C)	0.508*	0.426*	0.473*	0.488*	0.305*	0.259*
MT282						
A (Ap)	4(0)	4(0)	4(0)	4(0)	4(0)	4(0)
Ho	0.420	0.560	0.580	0.620	0.600	0.480
He	0.653	0.649	0.534	0.614	0.563	0.649
Fis (W&C)	0.359*	0.139*	-0.088	-0.009	-0.067	0.263*
Mg15						
A (Ap)	4(0)	4(0)	4(0)	4(0)	4(0)	4(0)
Ho	0.413	0.396	0.490	0.469	0.347	0.380
He	0.671	0.685	0.690	0.709	0.679	0.674
Fis (W&C)	0.387*	0.425*	0.293*	0.341*	0.492*	0.439*
Mg56						
A (Ap)	8(0)	10(0)	9(0)	10(0)	11(1)	9(0)
Ho	0.521	0.469	0.460	0.408	0.380	0.580
He	0.710	0.807	0.712	0.743	0.698	0.775
Fis (W&C)	0.269*	0.421*	0.357*	0.453*	0.458*	0.254*
Med737						
A (Ap)	8(1)	8(0)	8(0)	8(0)	7(0)	8(0)
Ho	0.634	0.706	0.673	0.675	0.764	0.792
He	0.595	0.650	0.630	0.628	0.719	0.758
Fis (W&C)	0.245*	0.152*	0.378*	0.499*	0.269*	0.371*
MIT02						
A (Ap)	5(0)	5(0)	5(0)	4(0)	5(0)	5(0)
Ho	0.280	0.300	0.400	0.320	0.449	0.440
He	0.769	0.729	0.830	0.817	0.717	0.753
Fis (W&C)	0.543*	0.530*	0.313*	0.463*	0.328	0.284
MGE005						
A (Ap)	9(1)	7(0)	7(0)	6(1)	8(0)	6(0)
Ho	0.320	0.320	0.280	0.360	0.340	0.300
He	0.373	0.323	0.381	0.393	0.310	0.275
Fis (W&C)	0.143	0.011	0.092	0.085	-0.099	-0.093
Mean acrossloci ± SD						
A (*Ap*)	7.1 ± 3.3	7.2 ± 3.4	6.9 ± 2.5	7.0 ± 3.2	7.0 ± 2.9	6.9 ± 3.1
Ho	0.386 ± 0.117	0.378 ± 0.167	0.426 ± 0.123	0.369 ± 0.154	0.413 ± 0.167	0.398 ± 0.159
He	0.662 ± 0.141	0.657 ± 0.167	0.639 ± 0.170	0.647 ± 0.170	0.620 ± 0.200	0.661 ± 0.175
Fis (W&C)	0.406*	0.410*	0.313*	0.426*	0.332*	0.369*