Bioinvasion threatens the genetic integrity of native diversity and a natural hybrid zone: smooth-shelled blue mussels (*Mytilus* spp.) in the Strait of Magellan

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ABSTRACT

Smooth-shelled blue mussels of the *Mytilus edulis* species complex are widely distributed bivalve molluscs whose introductions threaten native marine biodiversity (non-indigenous species - NIS). The aim of the present study was to identify the species and hybrids of *Mytilus* present in the Magellan Region (southern Chile). Results indicate that three mussel species of the *Mytilus edulis* complex are found in the region – *M. edulis*, *M. chilensis* (or the Southern hemisphere lineage of *Mytilus galloprovincialis*), and *M. galloprovincialis* of Northern hemisphere origin. For the first time, alleles of the introduced *M. trossulus* are reported from the Southern hemisphere. In the Strait of Magellan the native Pacific blue mussel, *Mytilus chilensis* and the native Atlantic blue mussel, *Mytilus edulis*, meet and mix at a natural hybrid zone (about 125 km in length). This is the first record of a natural *Mytilus* hybrid zone in the Southern hemisphere and is also the first record of the co-occurrence of genes from all four *Mytilus* species in any one region. These results contribute to the knowledge of the biodiversity and delimitation of mussel species in southern South America, and highlight how introduced species may threaten the genetic integrity of native species through hybridisation and introgression.

INTRODUCTION

The Magellan Region in southern Chile is characterised by a unique system of fjords and channels. Specifically, the Strait of Magellan is a complex natural channel that connects the Pacific and Atlantic Oceans. Along this Strait, molluscs of the genus *Mytilus* are the most important macroinvertebrates of the benthic fauna (Aldea & Rosenfeld, 2011). They have been a key ecological component of the coast line since the Holocene (Estevez *et al.*, 2001; Rabassa *et al.*, 2009; Gordillo, Bayer & Martinelli, 2010) and the fossil record indicates that a smooth-shelled blue mussel (*Mytilus* sp.) may date back to the late Miocene (~ 10 M years BP) in this region (Martínez & del Río, 2002; Aguirre, Hlebszevitsch & Deflatore, 2008).

South America is arguably the last continent for which the specific status of native blue mussels is still unresolved, largely because of differences concerning taxonomic identity (McDonald, Seed & Koehn, 1991; Daguin & Borsa, 2000; Westfall, Wimberger & Gardner, 2010; Ouagajjou *et al.*, 2011; Borsa *et al.*, 2012; Westfall & Gardner, 2013; Oyarzún *et al.*, 2014). There is however, general agreement that the shoreline of Chile has been subject to recent (localised) invasion by Northern hemisphere *M. galloprovincialis*, and that hybridisation between this invader and the native mussel occurs in several geographically limited locations. Hybridisation has been described as an invasion of the genome (Mallet, 2005) and given that wherever *Mytilus* spp, meet and mix they form hybrids and that this process may be accompanied by introgression, the occurrence of invasive blue mussels in sympatry with native blue mussels constitutes a major threat to the diversity and genetic integrity of the native mussels. The situation for southern South America, in the region of the Strait of Magellan, and also along the Atlantic coast of Argentina is still largely unresolved and requires more work, although mussels on the Atlantic coast are referred to as being *M. edulis*-like (McDonald, Seed & Koehn, 1991). This little-studied region is an area of high conservation value given the biotic differences between the Pacific and Atlantic oceans, and is one of the three classically recognised Antarctic provinces (Thatje & Mutschke, 1999). To date, the only study of its type has reported the presence of *M. edulis*, *M. galloprovincialis* and their hybrids, with samples taken in different locations within the middle part of the Strait of Magellan (Toro *et al.*, 2005). Therefore, considering the occurrence of these two *Mytilus* species and the very high abundance of mussels in this area, there is a high probability of some sort of ecological and genetic interaction between the native Atlantic blue mussels and the native Pacific blue mussels in the region of the Strait of Magellan where coastal waters meet and mix (Salinas, Contreras & Fierro, 2004). With this in mind we used three RFLP assays which have been developed specifically to genetically identify *Mytilus* diversity. The objective of this study was to identify the species and hybrids of *Mytilus* present in the Magellan Region (Chile) to better understand their interactions, their distributions and the role that bioinvasion may play in threatening the genetic integrity of the native mussels on the Atlantic and Pacific shores via hybridisation and introgression in this area of high conservation value.

MATERIAL & METHODS

Mussel collection

All mussels were collected live from the shallow subtidal by SCUBA divers. Samples of mussels (10-35 per site) were collected between August and October 2014 from nine sites in the region of the Strait of Magellan, Chile (Table 1). Five sampling sites were located in the Strait of Magellan, two in the archipelago of Tierra del Fuego and two in the Patagonian Channel (Fig. 1). In total, 208 mussels were sampled with a size range (shell length) between 31.7 mm and 57.8 mm.

Table 1. Site survey information, number of *Mytilus* specimens collected (*N*), date of collection, geographical coordinates of sites and map codes corresponding to Fig. 1.

Site	Map code	Coordinates	*N*	Date
Buque Quemado	BQ	52° 19' 52.6" S; 69° 29' 14.2" W	30	22.10.2013
San Gregorio	SG	52° 34' 01.8" S; 70° 04' 13.8" W	35	22.10.2013
Bahía Zenteno	BZ	52° 48' 50.2" S; 70° 47' 12.9" W	10	14.08.2013
Muelle Loreto	ML	53° 08' 53.7" S; 70° 54' 02.6" W	29	20.10.2013
Estero Fanny	EF	53° 05' 04.8" S; 72° 18' 39.6" W	30	17.10.2013
Caleta Pescadores	CP	53° 21' 06.2" S; 70° 57' 27.6" W	30	21.10.2013
Isla Piazzi	IP	51° 44' 38.4" S; 74° 10' 02.1" W	15	20.09.2013
Isla London	IL	54° 45' 57.6" S; 71° 58' 29.7" W	15	15.09.2013
Paso Pomar	PP	54° 51' 22.9" S; 70° 44' 38.9" W	14	13.08.2013
Total number of mussels			208	

Molecular markers and taxonomic determination

Me 15/16

The sizes of the Me15/16 PCR fragments are species-specific and used to distinguish between *M. trossulus*, *M. edulis* and *M. galloprovincialis* (Inoue *et al.*, 1995). For this codominant nuclear DNA marker the assumption is that each species is represented by a unique (homozygous) signature and that a double band (heterozygous) signature is representative of a hybrid between the two species that are identified from the combined pattern. Three PCR products differing in length, (1) 180 bp for *M. edulis*, (2) 168 bp for *M. trossulus* and (3) 126 bp for *M. galloprovincialis* and *Mytilus chilensis* have been observed at the Me15/16 locus (Inoue *et al.*, 1995). Double bands correspond to hybrids. Me15 5´-CCA GTA TAC AAA CCT GTG AAGA - 3´ and Me16 5´- TGT TGT CTT AAT AGG TTT GTA AGA - 3´ primers were used to amplify a segment of the Glu gene that encodes an adhesive protein of the mussel byssus. Standard PCR amplifications were carried out in 25 µl reaction mixtures containing 2 µl DNA template, 0.2 mM total dNTP, 2mM $MgCl_2$, 0.4 mM each primer, 1U of Taq (Invitrogen™), the manufacturer-supplied PCR buffer, and sterile distilled water. The PCR conditions involved an initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, annealing at 55°C for 30s and elongation at 72°C for 1 min, followed by further elongation at 72°C for 5 min.

16s RFLP

This RFLP assay of variation at the 16s rRNA gene identified four taxonomic groups: (1) 342, 167 and 28 bp fragments for Southern hemisphere *M. galloprovincialis* (or *Mytilus chilensis* as indicated by Fernández-Tajes *et al.*, 2011); (2) 342 and 195 bp fragments for Northern hemisphere *M. galloprovincialis*; (3) 342, 85, 82 and 28 bp fragments for the Northern hemisphere shared *M. edulis*/*M. galloprovincialis* haplotype; and (4) 370, 85 and 82 bp fragments for *M. trossulus* (Westfall, Wimberger & Gardner, 2010). A 527 bp fragment of the 16s rRNA gene was amplified using primers 16Sar-L 5´-CGC CTG TTT ATC AAA AAC AT-3´ and 16SbrH 5´- CCG GTC TGA ACT CAG ATC ACGT - 3´ (Palumbi *et al.*, 1991). Standard PCR amplifications were carried out in 25 µl reaction mixtures containing 2 µl DNA template, 0.2 mM total dNTP, 2 mM $MgCl_2$, 0.4 mM each primer, 1U of Taq (Invitrogen™), the manufacturer-supplied PCR buffer, and sterile distilled water. The PCR conditions were an initial denaturation at 95°C for 3 min, followed by 30 cycles of 95°C for 30 s, annealing at 52°C for 30s and elongation at 72°C for 45 s, followed by further elongation at 72°C for 3 min. A 20 µl triple restriction endonuclease digest containing 10 U *Eco*RV, 5 U *Nhe*I, 5 U *Spe*I (Invitrogen™), 10X buffer M, 100 mg BSA and 10 µL PCR product was incubated overnight at 37 °C. Only the female mitotype was assayed: the male mitotype was eliminated due to length variation of the 16s rRNA PCR amplicon.

COIXba RFLP

Fernández-Tajes *et al.*, (2011) developed a RFLP assay based on PCR amplification of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene and digestion with *Xba* I for identification of *M. chilensis*. A 233 bp fragment was amplified using primers COIXbaF 5´-CCG CCA TTG TCT GTA TAC CC-3´ y COIXbaR 5´-TAA TGC CCC CTC CTA AAA CC-3´. The PCR conditions were an initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, annealing at 52°C for 30 s and elongation at 72°C for 60 s, followed by further elongation at 72°C for 3 min. Digestion was carried out in a 20 µl reaction volume containing 5 µl of PCR product, 10 U of restriction endonuclease *Xba* I, 2µl BSA and 2 µl of the 10X buffer M supplied by the manufacture (Invitrogen™), and was incubated overnight at 37°C. This enzyme has one recognition site in *M. chilensis*, which yields two fragments of 134 and 99 bp, while no restriction target sites exist in *M. galloprovincialis*.

RESULTS

Table 2. Genotype and haplotype information for *Mytilus* samples collected from the Strait of Magellan, Chile

Locations	Code	*N*	Genotype Me15/16	RFLP haplotype 16S	RFLP haplotype COIXba
Buque Quemado	BQ	29	Me	–	–
		1	Me/Mg	MgSH	Mc
San Gregorio	SG	5	Me	–	–
		18	Mg	MgSH	Mc
		11	Me/Mg	MgSH	Mc
		1	Mt/Mg	MgSH	Mc
Bahía Zenteno	BZ	2	Me	–	–
		6	Mg	MgSH	Mc
		2	Me/Mg	MgSH	Mc
Muelle Loreto	ML	2	Mg	MgNH	MgNH
		20	Mg	MgSH	Mc
		1	Me/Mg	MgNH	MgNH
		4	Me/Mg	MgSH	Mc
		2	Mt/Mg	MgSH	Mc
Caleta Pescadores	CP	1	Mg	MgNH	MgNH
		29	Mg	MgSH	Mc
Isla Piazzi	IP	13	Mg	MgSH	Mc
		2	Me/Mg	MgSH	Mc
Estero Fanny	EF	2	Mg	MgNH	MgNH
		28	Mg	MgSH	Mc
Isla London	IL	1	Mg	MgNH	MgNH
		14	Mg	MgSH	Mc
Paso Pomar	PP	14	Mg	MgSH	Mc

N: number of individuals; Me: *Mytilus edulis*; MgNH: Northern Hemisphere *M. galloprovincialis*; MgSH: Southern Hemisphere *M. galloprovincialis*; Mg: MgNH or MgSH; Mc: *Mytilus chilensis*.
It was not necessary to carry out this analysis if the Me15/16 assay identified the mussel as being 'pure' *M. edulis*.

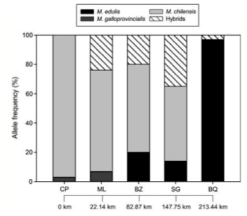

Figure 2. *Mytilus* samples in the Strait of Magellan, Chile: allele frequencies (Me15/16) for individual species and for hybrids. The geographical distance of coast line was obtained used Google Earth. BQ: Buque Quemado, SG: San Gregorio, BZ: Bahía Zenteno, ML: Muelle Loreto (Punta Arenas), CP: Caleta Pescadores.

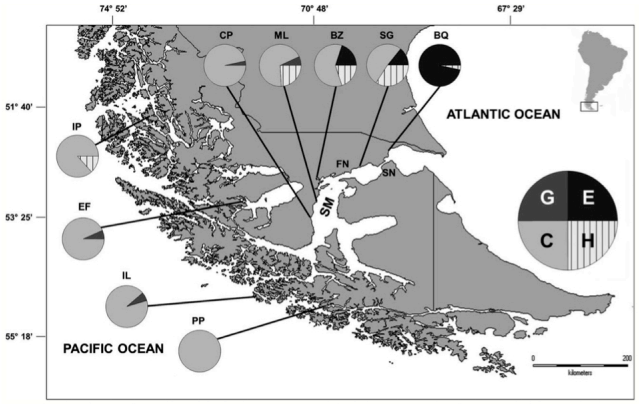

Figure 1. Location of sampling sites within the Magellan Region, Chile. Allele composition of *Mytilus* samples are shown as frequencies of alleles for the species-specific nuclear and mitochondrial DNA RFLP assays: C = *Mytilus chilensis* or Southern Hemisphere *Mytilus galloprovincialis*; G = Northern Hemisphere *Mytilus galloprovincialis*; E = *Mytilus edulis*; H = hybrid mussels. Sampling sites: BQ = Buque Quemado, SG = San Gregorio, BZ = Bahía Zenteno, ML = Muelle Loreto (Punta Arenas), CP = Caleta Pescadores, IP = Isla Piazzi, EF = Estero Fanny, IL = Isla London, PP = Paso Pomar. Other codes – SM = Strait of Magellan, FN = First Narrow, SN = Second Narrow.

CONCLUSION

An enhanced understanding of the environmental variation within the region, whether gradual or stepped from east to west, or perhaps mosaic-like (i.e. patchy and therefore difficult to predict) will provide a valuable framework against which to judge the threat posed by bioinvasive mussels. The location of the international shipping port at Puenta Arenas (ML in Fig. 1) near the centre of the Strait of Magellan (and also near the centre of the natural hybrid zone) poses a major threat to the conservation of the Magellanes region, to the Antarctic region and also to the Pacific and Atlantic coasts of southern South America. The burgeoning cruise ship traffic using the port for access to the Chilean fiords, to Patagonia, to the subantarctic islands, and to Antarctica itself poses a major threat to the protection of native biodiversity in these different biogeographic regions. In addition, the long history of international maritime traffic moving through the area is likely to have contributed to the biosecurity threats faced by the region. Once established, invasive mussels are highly unlikely to be eradicated, although there are cases with positive results (Aquenal 2008; Hopkins *et al.*, 2011a). Natural internal borders (e.g. Forrest, Gardner & Taylor, 2009) may slow or halt the spread of an invader, or genetic mechanisms such as gamete incompatibility or hybrid unfitness may also reduce the introgression of alien genes (e.g. Brannock & Hilbish, 2010). Possible courses of action to prevent or minimise spread of non-native mussels may include increased monitoring (Roy *et al.*, 2015) for secondary spread associated with pleasure craft or local working boats (e.g. coastal fishing vessels) that do not travel too far beyond their home port (Murray, Pakhomov & Therriault, 2011), increased surveillance of niche areas such as anodes, sea chests, velocity probes and echo sounders (Coutts & Taylor, 2004; Piola & Johnston, 2008; Murray, Pakhomov & Therriault, 2011) and improved hull cleaning procedures (Hopkins *et al.*, 2011b).
The identification of two invasive blue mussel species in the Strait of Magellan, and the genetic threat to the native mussels and the ecological threat to the shallow subtidal community in the area, highlight the Magellanes region as a hot spot in urgent need of biosecurity and conservation research and management options.

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