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Genetics and taxonomy of Chilean smooth-shelled mussels, *Mytilus* spp. (Bivalvia: Mytilidae)

Génétique et taxinomie des moules à coquille lisse du Chili, Mytilus *spp. (Bivalvia: Mytilidae)*

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ABSTRACT

It has been previously established that native smooth-shelled mussels in southern South America possess close evolutionary affinities with Northern-Hemisphere *Mytilus edulis* L. 1758 (McDonald et al. (1991) [5]). This result has since been challenged by authors claiming that Chilean mussels should be considered a local subspecies of *M. galloprovincialis* Lmk. 1819. Moreover, morphological, physiological, ecotoxicological and molecular genetic studies on Chilean smooth-shelled mussels still frequently refer to '*M. chilensis*' Hupé 1854, even though the previous discovery of alien *M. galloprovincialis* and considerable heterogeneity in shell morphology among samples collected along the Chilean shores raise concerns that different *Mytilus* spp. species might have been included under '*M. chilensis*'. Here we reviewed the molecular and morphological data available on smooth-shelled mussels from Chile in an attempt to clarify both their genetic composition and their taxonomic status. Using multivariate analysis on sample × allozyme-frequency matrices, we confirmed the widespread occurrence of the Southern-Hemisphere form of *M. edulis* along the shores from the North Patagonia region of Chile to the southern tip of the South American continent. The populations sampled in southern central Chile showed some evidence of slight introgression from Southern-Hemisphere *M. galloprovincialis*. Morphological characterization of a sample from Dichato in southern central Chile was consistent with its previous genetic identification as Mediterranean *M. galloprovincialis*. The occurrence of Southern-Hemisphere *M. galloprovincialis* in Punta Arenas at the southern tip of the South American continent was also reported. Southern-Hemisphere *M. edulis*, including native Chilean smooth-shelled *Mytilus*, should be assigned subspecific rank and named *M. edulis platensis* d'Orbigny 1846.

Mots clés :
Génotypage moléculaire
Morphométrie
Espèce envahissante
Mytilus edulis platensis
Mytilus galloprovincialis

RÉSUMÉ

Il a été établi antérieurement que les moules à coquille lisse natives d'Amérique du Sud ont des affinités évolutives étroites avec *Mytilus edulis* L. 1758 de l'hémisphère Nord (McDonald et al. (1991) [5]). Ce résultat a depuis été contesté, certains auteurs proposant que les moules chiliennes soient considérées comme une sous-espèce endémique de *M. galloprovincialis* Lmk. 1819. De plus, des études morphologiques, physiologiques,

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écotoxicologiques et de génétique moléculaire sur les moules à coquille lisse chiliennes se réfèrent régulièrement au nom d'espèce '*M. chilensis*' Hupé 1854, bien que la découverte de *M. galloprovincialis* introduites, ainsi que la forte variabilité morphologique observée entre échantillons collectés le long des côtes chiliennes, suggèrent qu'un mélange d'espèces différentes soient ainsi désignées sous le terme '*M. chilensis*'. Nous avons ici réalisé une synthèse des données morphologiques et génétiques disponibles chez les moules chiliennes afin de clarifier leur composition génétique et leur statut taxinomique. À l'aide d'analyses multivariées de matrices de fréquences allozymiques par échantillon, nous confirmons la présence de la forme australe de *M. edulis* le long des côtes chiliennes, de la partie centrale méridionale du Chili jusqu'à la pointe sud de l'Amérique du Sud. Les populations échantillonnées dans la partie centrale méridionale du Chili montrent des traces d'introgression par la forme australe de *M. galloprovincialis*. Par ailleurs, la caractérisation morphologique d'un échantillon de Dichato (sud de la partie centrale du Chili) est en accord avec son identification moléculaire comme la forme méditerranéenne de *M. galloprovincialis*. La présence de la forme australe de *M. galloprovincialis* à Punta Arenas à l'extrémité sud du continent sud-américain est rapportée ici pour la première fois. La forme australe de *M. edulis*, qui inclut les moules à coquille lisse natives des côtes chiliennes, mérite le rang de sous-espèce et doit être désignée sous le nom *M. edulis platensis* d'Orbigny 1846.

1. Introduction

Three species of smooth-shelled mussels, *Mytilus edulis* L. 1758 [1], *M. galloprovincialis* Lmk. 1819 [2] and *M. trossulus* Gould 1850 [3] have been re-defined on the basis of allozyme-genotype and concurrent morphological variation worldwide [4–6]. Although hybridization occurs in virtually every known case where two of the species occur sympatrically, evidence of restriction to gene flow despite broadcast spawning and pelagic larval transport confirms the biological status of the three species [6]. *M. edulis* and *M. galloprovincialis* are present in the temperate and cold regions of both Hemispheres, while *M. trossulus* is confined to the boreal and sub-boreal regions [5]. Southern-Hemisphere *M. galloprovincialis* are allozymically and morphologically distinct from their Northern-Hemisphere counterparts, less so *M. edulis* [5]. All smooth-shelled mussels from Chile examined by J.H. McDonald et al. [5] were closely related to those from Argentina, the Falkland Islands and the Kerguelen Islands, and all were clustered with Northern-Hemisphere *M. edulis* by both their allozymic composition and their shell morphology. This result has since been challenged, with authors claiming that Chilean mussels should be considered a local subspecies of *M. galloprovincialis* [7]. Independently, a number of authors, e.g. [8–14], have persisted in employing the species name '*M. chilensis*' for smooth-shelled mussels sampled in Chile, ignoring previous work [5,6] and instead following Hupé [15]. Hupé [15] mentioned the presence of *M. chilensis* "*en la costa, en Valparaiso, etc.*" and recognized that *M. chilensis* "*tiene enteramente el aspecto del Mytilus edulis de las mares de Europa*", except that "*su forma es mas aplastada*". Given the morphological variation encountered within Northern-Hemisphere *M. edulis* [5], it remains to be proven that the reportedly flatter shell of Hupé's *M. chilensis* constitutes a character strong enough to distinguish it from *M. edulis* and assign it specific rank.

Evidence of invasion by alien Northern-Hemisphere *M. galloprovincialis* has been reported from localities in both the Northern and Southern-Hemispheres, including the northwestern and the northeastern shores of the Pacific Ocean, southern Africa, southeastern Australia, New Zealand, and Chile ([5,16–19] and references therein). Since Northern-Hemisphere *M. galloprovincialis* occurs in southern central Chile [16], presumably as the result of intentional introduction for aquaculture purposes [20], there is uncertainty as to the actual genetic composition of smooth-shelled mussels samples collected along the Chilean shores for a number of physiological, ecotoxicological, and morphological and even molecular genetic studies [8–14] undertaken since [5]. Because physiological response may vary considerably across *Mytilus* species [5], it is mandatory to ascertain the taxonomic status of the Chilean *Mytilus* material used prior to physiological analysis. Also, considerable morphological differences have been reported among samples of Chilean *Mytilus* spp. [11,14], to an extent that suggests that different species may have been present, even though the authors assumed an effect solely of environmental factors.

Here, we review the genetic and morphometric data published within the last two decades on smooth-shelled mussels from Chile, to assess the taxonomic status of populations and eventually detect more locations along the coasts of central and southern Chile where alien *M. galloprovincialis* may have settled. We advocate the systematic use of a genetic assay to identify smooth-shelled *Mytilus* material from Chile prior to their ecological, physiological or molecular study, or to any related biomonitoring survey.

2. Materials and methods

The list of smooth-shelled *Mytilus* samples considered in this review is presented in Table 1 and the sample locations have been reported on a map (Fig. 1). This list tentatively includes all samples from the Chilean coasts that have been genotyped at nuclear and mitochondrial markers and reported in the literature. Table 1 also includes a sample from Maullin (southern central Chile)

Table 1
Smooth-shelled *Mytilus* spp. samples examined in the present review, including samples from the Chilean coastline and reference samples from Northern- and Southern-Hemisphere *Mytilus edulis* and *M. galloprovincialis*.

Sample				*N*	Marker loci	Reference
Location	Coordinates	Abbreviation	Date			
Chilean Mytilus *spp.*						
Valdivia	39°51′S 73°27′W	*PVA*	Feb. 1997–May 1998	55–61	Allozymes	[7]
Puerto Montt	41°33′S 72°48′W	*PPM*	Feb. 1997–May 1998	68–71	Allozymes	[7]
Ancud	41°51′S 73°50′W	*PAN*	Feb. 1997–May 1998	31–41	Allozymes	[7]
Quellón	43°08′S 73°39′W	*PQE*	Feb. 1997–May 1998	37–72	Allozymes	[7]
Arauco	37°14′S 73°19′W	*Arauco*	–	109–112	Allozymes	[10]
Queule	~39°S ~73°W	*Queule*	–	80–108	Allozymes	[10]
Valdivia	~40°S ~73°W	*Valdivia*	–	102–116	Allozymes	[10]
Calbuco	~41°S ~73°W	*Calbuco*	–	110	Allozymes	[10]
Ancud	~42°S ~74°W	*Ancud*	–	99–110	Allozymes	[10]
Yaldad	~43°S ~73°W	*Yaldad*	–	111–128	Allozymes	[10]
Pto. Marin Balmaceda	~43°S ~73°W	*Marin*	–	99	Allozymes	[10]
Punta Arenas	53°08′S 70°55′W	*Arenas*	–	100–107	Allozymes	[10]
Dichato	36°33′S 72°57′W	*CHL*	Oct. 1998	9–76	*mac-1*, *Glu-5′*, *COI*	[16,21]
Maullin	41°37′S 73°36′W	*MAU*	Jan. 1999	7–52	*mac-1*, *Glu-5′*, *COI*	[21], present work
Concepcion	36°44′S 73°08′W	*CO*	1994–2009	19	*Me15/16*, *16S*	[18]
Colchogue	37°03′S 73°10′W	*CL*	1994–2009	20	*Me15/16*, *16S*	[18]
Southern-Hemisphere M. edulis						
Yaldad Bay, Chile	~43°S ~73°W	*40*	1986	25	Allozymes	[5]
Chiloe, Chile	42–43°S 73–74°W	*41*	Jan. 1988	23	Allozymes	[5]
Punta Arenas, Chile	~53°S ~71°W	*42*	Jan. 1988	25	Allozymes	[5]
Mar del Plata, Argentina	~38°S ~57°W	*44*	1985–1988	25	Allozymes	[5]
Falkland Islands[a]	51–52°S 58–61°W	*43*	1985–1988	25	Allozymes	[5]
Kerguelen Islands	~49°S ~69°E	*45*	July 1988	22	Allozymes	[5]
Kerguelen Islands	49°28′S 69°56′E	*KER*	June 1997	79–83	*mac-1*, *Glu-5′*, *COI*	[21,22]
Southern-Hemisphere M. galloprovincialis						
Huon River Estuary, Tasmania	~43°S ~147°E	*47*	1985–1988	23	Allozymes	[5]
Nedlands, Western Australia	32°03′S 115°44′E	*AUS*	July 1998	7–46	*mac-1*, *Glu-5′*, *COI*	[16,21]
Adventure Bay, Tasmania	43°21′S 147°22′E	*ADB*	Mar. 1997	26–28	*mac-1*, *Glu-5′*	[22]
Alonnah, Tasmania	43°18′S 147°14′E	*ALO*	Mar. 1997	25–59	*mac-1*, *Glu-5′*	[22]
Cloudy Bay Lagoon, Tasmania	43°25′S 147°12′E	*CBL*	Feb. 1997	5–32	*mac-1*, *Glu-5′*, *COI*	[21,22]
Hobart, Tasmania	42°53′S 147°20′E	*HOB*	Feb. 1997	8–31	*mac-1*, *Glu-5′*, *COI*	[21,22]
Partridge Narrows, Tasmania	43°24′S 147°06′E	*PAR*	Mar. 1997	25–30	*mac-1*, *Glu-5′*	[22]
Simpson's Bay, Tasmania	43°17′S 147°20′E	*SIM*	Mar. 1997	3–40	*mac-1*, *Glu-5′*, *COI*	[21,22]
Dunedin, New Zealand	45°55′S 170°28′E	*DUN* (=*NZL*)	June 1999	6–79	*mac-1*, *Glu-5′*, *COI*	[16,21]
Northern-Hemisphere M. edulis						
Aarhus, Denmark	56°10′N 10°14′E	AapXyc	1985–1988	11	Allozymes	[23,24]
Netherlands	–	*EH*	–	59–75	Allozymes	[7]
Gilleleje, Kattegat	56°07′N 12°18′E	*GIL*	Sep. 1996	16–26	*mac-1*, *Glu-5′*	[16]
Flødevigen, Skagerrak	58°25′N 08°45′E	*FLØ*	Jan. 1997	20–47	*mac-1*, *Glu-5′*, *COI*[b]	[16,21]
Grand Fort Philippe, N France	51°00′N 02°05′E	*GFP*	June 1997	42	*mac-1*, *Glu-5′*	[16]
Northern-Hemisphere *M. galloprovincialis*						
Vigo, Spain	~42°N ~09°W	*GV*	–	35–73	Allozymes	[7]
Palavas, Western Mediterranean	43°31′N 03°56′E	*Palavas*	1988–1990	75–100	Allozymes	[26]
Setubal, Portugal	38°29′N, 08°56′E	*STB*	Sep. 1997	19–26	*mac-1*, *Glu-5′*	[22]
Sète, Western Mediterranean	43°24′N 03°41′E	*SET*	May 1996	56–68	*mac-1*, *Glu-5′*	[16]
Chioggia, Adriatic Sea	45°13′N 12°18′E	*CHI*	June 1997	18–47	*mac-1*, *Glu-5′*	[22]
Bloubergstrand, South Africa	33°48′S 18°27′E	*SAF*	Nov. 1998	62–65	*mac-1*, *Glu-5′*	[16]
Southern Korean Peninsula	~35°N ~126°E	*KOR*	< 1999	19–30	*mac-1*, *Glu-5′*	[16]
Bodega Bay, California	38°19′N 123°04′W	*BOD*	Nov. 1996	23–34	*mac-1*, *Glu-5′*	[16]
Sète, Western Mediterranean	~43°N ~03°E	*G-Fr*	< 1998	17	*16 S*	[27]

[a] Sample consisting of a mixture of individuals from Stanley Harbour (51°42′S 57°49′W) and individuals from the West Falkland Island.
[b] *COI* sequences originally are from sample 'Tjärnö, Sweden' [25].

whose genotyping at nuclear-DNA markers *mac-1* and *Glu-5′* is presented here for the first time. Table 1 also presents reference samples of Northern- and Southern-Hemisphere *M. edulis* and *M. galloprovincialis* genotyped at the same marker loci.

C. Carcamo et al. [7] have analyzed 4 samples from the Chilean coasts, together with reference samples of Northern-Hemisphere *M. edulis* and *M. galloprovincialis*, at 23 polymorphic allozyme loci. Eight of these marker loci were common with the previously published worldwide

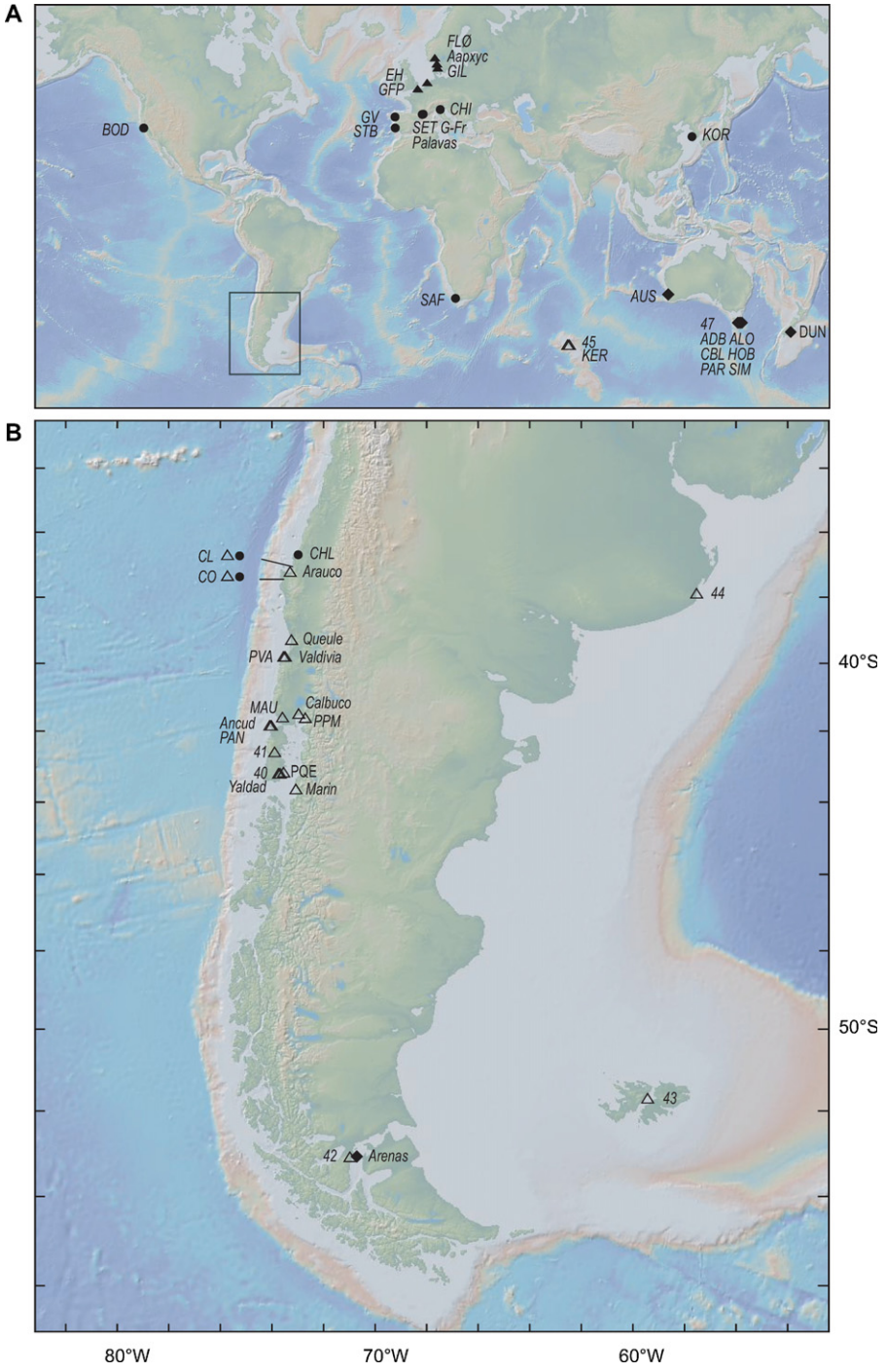

Fig. 1. Sampling sites for smooth-shelled *Mytilus* spp. A. Sampling locations for *Mytilus edulis* and *M. galloprovincialis* in the Northern and the Southern-Hemispheres. B. Map of the southern tip of South America, including all sampling sites for smooth-shelled *Mytilus* spp. in Chile ([5,7,10,16,18,20–24]; present study). *Full triangles* (▲): Northern-Hemisphere *M. edulis*; *full circles* (●): Northern-Hemisphere *M. galloprovincialis*; *open triangles* (△): Southern-Hemisphere *M. edulis*; *diamonds* (◆): Southern-Hemisphere *M. galloprovincialis*. Background topographic map from GeoMapApp [28] (http://www.geomapapp.org).

dataset of McDonald et al. [5]. Homologies between electromorphs from different studies [5,7,23,24,26] were inferred as detailed in the legend to Appendix A.

J.E. Toro et al. [10] have analyzed 8 samples from the Chilean coast using 7 allozyme loci. Four samples ('Ancud', 'Yaldad' 'Valdivia' and 'Pta. Arenas') were from the same locations as previous allozyme surveys [5,7], potentially allowing cross-comparisons at two loci scored in common (*Gpi*, *Pgm*). Three other loci scored by [10] (*GSR*, *ICD*, *ME*) had not been scored by [5], and we were unable to

Table 2
Smooth-shelled *Mytilus* spp. Summary of genetic characteristics at nuclear-DNA loci *mac-1* and *Glu-5′* ([16,22,29] and unpublished data) and mitochondrial locus *COI* [21] of two samples from Chile (*CHL*, *MAU*) and reference samples (*CBL*, *FLØ*, *GIL*, *KER*, *SET*), all analysed morphometrically (Fig. 4). *Allozymes*: genetic characterization of samples from the same or nearby locations, previously analyzed at 7–8 allozyme loci [5,24,30,31]; *E*, *G*: compound alleles characteristic of *Mytilus edulis* and *M. galloprovincialis*, respectively; N_A bulk of the *N* clade that includes all Northern-Hemisphere *M. edulis*, and a proportion of Northern-Hemisphere *M. galloprovincialis* female *COI* haplotypes; N_D well-supported subclade of the *N* clade that exclusively comprises Northern-Hemisphere *M. galloprovincialis* female *COI* haplotypes [19,21].

Sample	*Marker*											
	mac-1			*Glu-5′*			*COI*					Allozymes
	E	*G*	(*N*)	*E*	*G*	(*N*)	N_A	N_D	S_1	S_3	(N)	
CHL	0.04	0.96	(76)	–	1.00	(48)	0.22	0.78	–	–	(9)	nd
MAU	1.00	–	(52)	–	1.00	(28)	–	–	1.00	–	(7)	*E*
CBL	–	1.00	(32)	–	1.00	(29)	–	–	–	1.00	(5)	*G*
FLØ[a]	1.00	–	(47)	1.00	–	(35)	1.00	–	–	–	(20)	*E*
GIL	1.00	–	(26)	1.00	–	(16)	nd	nd	nd	nd	nd	*E*
KER	1.00	–	(83)	0.35	0.65	(79)	–	–	1.00	–	(83)	*E*
SET[b]	0.03	0.97	(68)	0.06	0.94	(39)	0.65	0.35	–	–	(17)	*G*

N: sample size; nd: no data.
[a] *COI* data from sample 'Tjärnö, Sweden' [25].
[b] Female-mitochondrial composition determined from *16S* RFLP haplotypes of sample *G-Fr* [27].

establish correspondence between either of the remaining loci, *LAP* or *PEP*, scored by [10] and any of the *Aap*, *Ap* or *Lap* loci of [5] or [7]. Correspondence between electromorphs was easily established at locus *Gpi*, where electromorphs *A* and (*B* + *C*) of [10] were found to be homologous to, respectively, compound electromorphs ≤ 96 and ≥ 98 of [5] (Appendix B). Locus-*Pgm* electromorphs *A*, *B* and (*C* + *D*) of [10] were found to be homologous to, respectively, electromorphs < 93, *100* and ≥ 106 [5] (Appendix B). Locus *Gpi* shows substantial electromorph-frequency differences between Southern-Hemisphere *M. edulis* and *M. galloprovincialis* ([5]; Appendix A) and therefore *Gpi* is potentially helpful to assess the occurrence of alien *M. galloprovincialis* in Chile. We noted that allelic frequencies at locus *Pgm* in sample 'Pta. Arenas' of [10] were not consistent with those reported earlier [5].

The sample from Maullin (Tables 1, 2) was analyzed for polymorphism at nuclear-DNA loci *mac-1* and *Glu-5′* and compared to other samples previously analyzed using these two markers [16,22,29,32] (Appendix C). The protocols for DNA extraction, PCR amplification and electrophoresis and staining of PCR products have been detailed previously [22].

Correspondence analysis (CA) [35] was performed to visualize samples characterized by their electromorph/allelomorph frequencies, by reducing the multidimensional allelic frequency space to a bidimensional space. Two CAs were run on allozyme-frequency data, the first one on the matrix of samples × allele-frequencies derived from Appendix A ('Matrix-A': 15 samples × 8 allozyme loci), and the second one on a matrix comprising all samples of Appendix A together with the samples of [10], all characterized by their electromorph frequencies at loci *Gpi* and *Pgm* (Appendix B) ('Matrix B': 22 samples × 2 allozyme loci). A third CA run was made on the nuclear-DNA dataset presented in Appendix C. Hierarchical clustering analysis [36] was used to delineate clusters of samples; for this, pairwise distances between samples were Euclidean distances in the space defined by the first five axes of the CA.

Principal component analysis (PCA) was performed on the shell measurements of the samples listed in Table 2. The left shell of each individual was characterized by 10 measurements according to [5]: length of anterior adductor muscle scar (*aam*), length of hinge plate (*hp*), shell height (*ht*), distance between umbo and posterior end of the ligament (*lig*), length of posterior adductor muscle scar (*pad*), distance between pallial line and ventral shell margin midway along shell (*pal*), distance between umbo and posterior end of anterior retractor scar (*ular*), width of anterior retractor muscle scar (*war*), shell width (*wid*), and width of posterior retractor muscle scar (*wpr*). Measurements were made to the nearest 0.1 mm using a digital caliper (Mitutoyo, Andover, UK) (all measurements except *aam* and *war*) or to the nearest 0.01 mm using an ocular micrometer fitted to a stereo microscope (Wild Heerbrugg, Aarau, Switzerland) equipped with a camera lucida (*aam* and *war*). To standardize the measurements for size, each was $\log_{10}$-transformed and divided by the $\log_{10}$-transformed shell length. PCA was run using ViSta [37]. Reference Northern-Hemisphere *M. edulis* (*F*, *G*), Northern-Hemisphere *M. galloprovincialis* (*S*) and Southern-Hemisphere *M. galloprovincialis* (*C*) shells were represented by average values for all 10 measurements in, respectively, samples *FLØ* (Flødevigen, Skagerrak; $N = 53$), *GIL* (Gilleleje, northern Denmark; $N = 35$), *SET* (Sète, southern France; $N = 55$), and *CBL* (Cloudy Bay Lagoon, Tasmania; $N = 96$). All shells, which have been deposited at Laboratoire de biologie des invertébrés marins et malacologie, Museum national d'histoire naturelle, Paris under collection numbers MNHN-IM-2008-73 to 75, ranged in size from 20.2 to 69.2 mm.

3. Results

The first axis of the CA run on Matrix-A opposed reference *M. galloprovincialis*, to reference *M. edulis* samples from the Northern-Hemisphere (Fig. 2A), explaining about 3/5 of the total inertia borne by the

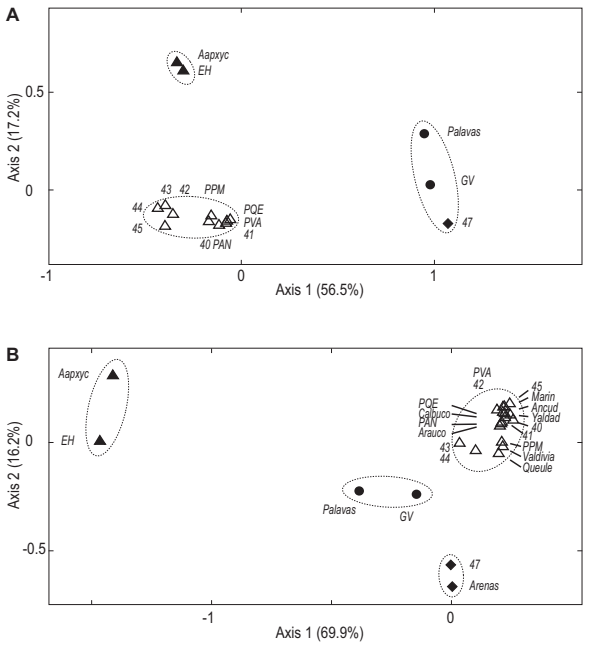

Fig. 2. Genetic relationships of Chilean *Mytilus* spp. Projection of samples from Chile together with reference samples of Northern-Hemisphere *Mytilus edulis* (*EH* and *Aapxyc*), Southern-Hemisphere *M. edulis* (*43–45*), Northern-Hemisphere *M. galloprovincialis* (*GV* and *Palavas*), and Southern-Hemisphere *M. galloprovincialis* (*47*). Samples were characterized by their electromorph frequencies at allozyme loci and the resulting matrix was subjected to correspondence analysis [35] using the FactoMineR package [36] under R [38]; percentages for each axis are their inertias [35]; ellipses delineate clusters of samples determined by hierarchical clustering [36], allowing the identification to species and subspecies of the tested samples. *Full triangles* (▲): Northern-Hemisphere *M. edulis*; *full circles* (●): Northern-Hemisphere *M. galloprovincialis*; *open triangles* (Δ): Southern-Hemisphere *M. edulis*; *diamonds* (♦): Southern-Hemisphere *M. galloprovincialis*. A. Analysis performed on Matrix-A (15 samples × 8 allozyme loci). B. Analysis performed on Matrix B (23 samples × 2 allozyme loci).

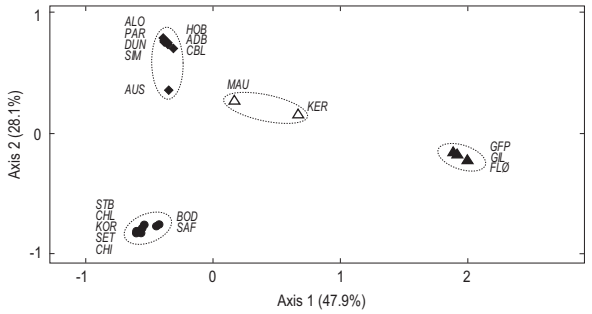

Fig. 3. Genetic relationships of Chilean *Mytilus* spp. Projection of samples from Chile together with reference samples of Northern-Hemisphere *Mytilus edulis* (*FLØ*, *GFP* and *GIL*), Southern-Hemisphere *M. edulis* (*KER*), Northern-Hemisphere *M. galloprovincialis* (*BOD*, *CHI KOR*, SET and *STB*), and Southern-Hemisphere *M. galloprovincialis* (*ADB*, *ALO*, *AUS*, *CBL*, *DUN*, *HOB*, *PAR* and *SIM*). Samples were characterized by their allelomorph frequencies at nuclear-DNA loci *mac-1* and *Glu-5′* (Appendix C) and the resulting matrix was subjected to correspondence analysis [35,36]. Ellipses delineate clusters of samples determined by hierarchical clustering [36], allowing the identification to species and subspecies of the tested samples. *Full triangles* (▲): Northern-Hemisphere *M. edulis*; *full circles* (●): Northern-Hemisphere *M. galloprovincialis*; *open triangles* (Δ): Southern-Hemisphere *M. edulis*; *diamonds* (♦): Southern-Hemisphere *M. galloprovincialis*.

dataset. The second axis, which explained approximately an additional fifth of the total inertia, differentiated Southern-Hemisphere samples from Northern-Hemisphere *M. edulis*. These Southern-Hemisphere samples formed a distinct, nearly continuous cluster elongated along Axis 1. The Southern-Hemisphere samples genetically closest to reference Northern-Hemisphere *M. edulis* were samples *43* and *44* from the South Atlantic [5]. They clustered with the samples from Punta Arenas and the Kerguelen Islands (*42* and *45*, respectively). The samples from southern central Chile (*40*, *41*, *PAN*, *PPM*, *PQE*, *PVA*) tended to show slight affinity towards the reference *M. galloprovincialis* pole (Fig. 2A) as already apparent from electromorph frequencies (Appendix A) where Southern-Hemisphere *M. galloprovincialis*-like alleles at locus *Est* were present at higher frequency in all southern central Chile samples than in sample *42* from Punta Arenas [5]. However, there was no evidence of the presence of alien *M. galloprovincialis* in the Chilean *Mytilus* samples in the Matrix-A dataset (Fig. 2A).

All additional samples from Chile analyzed by [10] but one clustered with the other Chilean samples, together with the Southern-Hemisphere *M. edulis* samples from the South Atlantic and from the Kerguelen Islands (Fig. 2B). Unlike the Punta Arenas sample of [5], sample 'Pta. Arenas' of [10] clustered with the reference sample of Southern-Hemisphere *M. galloprovincialis* (Fig. 2B).

The nuclear-DNA dataset presented here included two smooth-shelled *Mytilus* spp. samples from Chile. One sample, from Dichato (*CHL*), was previously identified as Mediterranean *M. galloprovincialis* [16]; the other one, from Maullin (*MAU*), clustered with reference Southern-Hemisphere *M. edulis* (Fig. 3).

Sharp morphological differences were evident between the shells of the two mussel samples from southern central Chile analyzed here (*CHL* and *MAU*; Table 2) (Fig. 4). Individuals of the *MAU* sample clustered with the Southern-Hemisphere *M. edulis* from Kerguelen whereas those from sample *CHL* formed a distinct cluster at the center of which the reference sample of Northern-Hemisphere *M. galloprovincialis* was positioned.

4. Discussion

Evidence of alien Mediterranean *M. galloprovincialis* in Chile so far comes from a single sample, from Dichato (southern central Chile), previously characterized at nuclear-DNA loci *mac-1* and *Glu-5′* [16] and at the mitochondrial locus *COI* [21], and here also shown to be morphologically identical to reference Northern-Hemisphere *M. galloprovincialis*. Additional evidence of Northern-Hemisphere *M. galloprovincialis* mitotypes has recently been reported in samples from Concepcion and Colchogue, two localities in southern central Chile [18]. All the other smooth-shelled *Mytilus* samples from Chile reviewed in the present study, but one, were identified as Southern-Hemisphere *M. edulis* since they clustered with

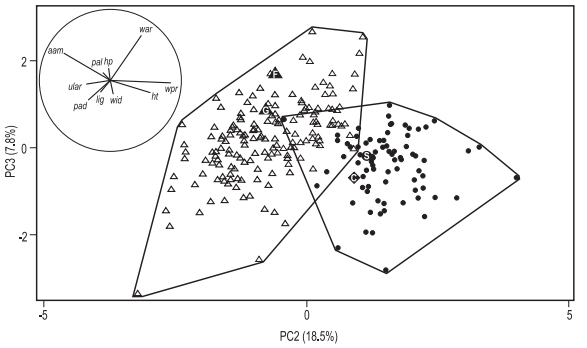

Fig. 4. Shell morphometrics of Chilean *Mytilus* spp. Projection on the plane defined by axes 2 and 3 of principal component analysis (PCA) [37] of individuals sampled in Dichato, Chile (sample *CHL* in Table 1: *full circles*; $N = 80$), Maullin, Chile (*MAU*: *thinner triangles*; $N = 56$) and Kerguelen (*KER*: *thicker triangles*; $N = 101$); insert: correlation circle indicating the relative contribution (proportional to size of arrow) of each shell measurement; the quality of representation of a shell measurement can be visualized by the distance between its projection on the plane and the correlation circle. The left shell of each individual was characterized by 10 measurements according to [5]: length of anterior adductor muscle scar (*aam*), length of hinge plate (*hp*), shell height (*ht*), distance between umbo and posterior end of the ligament (*lig*), length of posterior adductor muscle scar (*pad*), distance between pallial line and ventral shell margin midway along shell (*pal*), distance between umbo and posterior end of anterior retractor scar (*ular*), width of anterior retractor muscle scar (*war*), shell width (*wid*), and width of posterior retractor muscle scar (*wpr*). Reference Northern-Hemisphere *Mytilus edulis* (*F*, *G*), Northern-Hemisphere *M. galloprovincialis* (*S*) and Southern-Hemisphere *M. galloprovincialis* (*C*) shells were represented by average values for all 10 measurements in, respectively, samples *FLØ* (Flødevigen, Skagerrak; $N = 53$), *GIL* (Gilleleje, northern Denmark; $N = 35$), *SET* (Sète, southern France; $N = 55$), and *CBL* (Cloudy Bay Lagoon, Tasmania; $N = 96$) and incorporated as illustrative variables in the PCA.

reference samples from the South Atlantic and from the Kerguelen Islands, both genetically and by their shell morphology ([5,21], present study). The exception is a sample from Punta Arenas [10] at the southern tip of South America, which was here identified as Southern-Hemisphere *M. galloprovincialis* on the basis of allozyme frequencies at loci *Pgm* and *Gpi*. This sample has also been analyzed morphologically [11] and found to be significantly different from all the other samples from Chile (distribution of samples along principal component 1 [11]: Dixon's test for detecting outliers [39]; $Q = 0.545$; $N = 8$; $P < 0.05$). The mussels in this sample [10,11] were characterized by a concave and slightly pointed umbo [11], consistent with their allozyme identification as Southern-Hemisphere *M. galloprovincialis* (present work).

How can we explain the occurrence of Southern-Hemisphere *M. galloprovincialis* at a location and in a region where only Southern-Hemisphere *M. edulis* had been previously reported [5]? Southern-Hemisphere *M. galloprovincialis* are native from temperate Australia, Tasmania, and New Zealand [5,16,18,19,21], while Southern-Hemisphere *M. edulis* are native from southern South America, the Falkland Islands and the Kerguelen Islands [5,21], and possibly other islands in the Southern Ocean. The distribution areas of the two species are separated by a stretch of ocean of over 105° longitude, from New Zealand to Chile. An hypothesis is that the introduction of Southern-Hemisphere *M. galloprovincialis* to Punta Arenas is recent and has been caused by maritime traffic, since Punta Arenas is a port of call for global shipping lines that link New Zealand to South America (http://www.timetableimages.com/maritime/). The alternative hypothesis, that both species naturally co-occur in the Punta Arenas area, but that Southern-Hemisphere *M. galloprovincialis* had previously escaped detection there and all along the cold-temperate shores of South America is, in our view, much less likely. To test the hypothesis that the Southern-Hemisphere *M. galloprovincialis* sample of [10,11] consists of alien mussels would require genotyping them at marker loci able to distinguish different sub-populations within that population, e.g. the *COI* marker [21].

Valladares et al. [14] have similarly reported strong morphological differences between cultivated mussels from southern central Chile and wild mussels from the same area and from the Magellanic region of southern Chile. The authors ascribed these differences to differences in ecological pressure on cultivated vs. wild populations. However, no genetic assay was performed, that would help confirm that the cultivated populations analyzed by [14] were native mussels as assumed by the authors, and not alien *M. galloprovincialis*, despite earlier reports mentioning alien *M. galloprovincialis* in southern central Chile [16,20,21] and its introduction to mussel farms [20]. Cultivated Chilean mussels differed from wild mussels by umbo shape and orientation, and ligament length [14]. These features have proven useful for distinguishing *M. galloprovincialis* from *M. edulis* [5,40]. Therefore, genetic assays are necessary to ascertain that the cultivated smooth-shelled *Mytilus* samples from southern central Chile analyzed by [14] were not in fact *M. galloprovincialis*.

In conclusion, the present study confirmed the presence of Mediterranean *M. galloprovincialis* in southern central Chile, and uncovered the occurrence of Southern-Hemisphere *M. galloprovincialis* in Punta Arenas. The term '*M. chilensis*' employed by different authors for smooth-shelled mussels sampled in Chile actually concerns Southern-Hemisphere *M. edulis* and so-far unreported Southern-Hemisphere *M. galloprovincialis*, and potentially concerns alien Northern-Hemisphere *M. galloprovincialis*.

Since morphological characterization of mussel samples has apparently been insufficient for some authors to see mixtures of species in their samples [11], we advocate the systematic use of a genetic assay to identify smooth-shelled *Mytilus* material from Chile prior to their ecological, physiological or molecular study, or to any related biomonitoring survey. The single marker of choice for identifying smooth-shelled *Mytilus* spp. to species is *mac-1* ([16,22]; present study). In particular, *mac-1* allows the distinction of Southern-Hemisphere *M. edulis* and *M. galloprovincialis* from their Northern-Hemisphere counterparts [22]. Alternatively, a two-locus diagnostic has been proposed recently [18]. Several studies have employed *ITS* and *Glu-5′* (or *Me15/16*, which is part of the same gene [34]) to identify Chilean mussels [8,9] but *ITS* does not separate Southern-Hemisphere

M. edulis from either Northern-Hemisphere *M. edulis* or Northern-Hemisphere *M. galloprovincialis* [9,41] and *Glu-5′* (or *Me15/16*) does not separate Southern-Hemisphere *M. edulis* from *M. galloprovincialis* [18,22,34].

Southern-Hemisphere *M. edulis* are distinct from Northern-Hemisphere *M. edulis* at a proportion of nuclear loci ([5,22], present work) and at the mitochondrial locus [21], to an extent that warrants their recognition as a separate, geographically isolated entity. Therefore, it is sensible to assume subspecific rank for them. The valid subspecific name for Southern-Hemisphere *M. edulis* is *M. edulis platensis* d'Orbigny 1846 [42] by the principle of priority [43]. Under the same rationale, Southern-Hemisphere *M. galloprovincialis* should be assigned the subspecific name *M. galloprovincialis planulatus* Lmk 1819 [2]. Epithet *chilensis* being a junior synonym of *platensis* (as is *desolationis* Lamy 1936 [44]), it should be abandoned.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Appendix A

Electromorph frequencies at eight allozyme loci in seven samples of Chilean smooth-shelled mussels ('Yaldad Bay, Chile' (*40*), 'Chiloe, Chile' (*41*), 'Punta Arenas, Chile' (*42*), *AN*, *PPM*, *PQE* and *PVA*) [5,7] compared with three samples of Southern-Hemisphere *Mytilus edulis* ('Mar del Plata, Argentina' (*44*), 'Falkland Islands' (*43*) and 'Kerguelen Islands' (*45*)) [5]. Homology of electromorphs between [5] and [26] at loci *Ap*, *Gpi*, *Lap*, *Mpi*, *Odh* and *Pgm* has been established previously [22], and a similar procedure was followed here for locus *Aap* ('*Lap-1*' of [26]). Homology of electromorphs between [5,26] and [7] were uncovered by comparing samples *EH* and *GV* [7] with, respectively, *Aapxyc* [23] (='Aarhus, Denmark' [5]) and *Palavas* [26] on the basis of relative migrations and similarities in frequencies; when uncertainty about identity remained, electromorphs were pooled as indicated (' + ', '≤', '≥'). To complete the *Aapxyc* sample [23], electromorph frequencies at locus *Pgm* were taken from the geographically close *SWED* sample [24]. Sample sizes in brackets.

Locus, electromorph			Sample												
[5]	[7]	[26]	*40*	*41*	*42*	*43*	*44*	*45*	*47*	*PAN*	*PVA*	*PPM*	*PQE*	*Aapxyc*	*EH*	*Palavas*	*GV*
Aap	*Lap-1*	*Lap-1*	(25)	(23)	(25)	(25)	(25)	(22)	(23)	(41)	(60)	(71)	(71)	(11)	(65)	(93)	(38)
≤ 95	*93 + 96*	*2*	0.36	0.28	0.48	0.74	0.52	0.76	-	0.34	0.27	0.26	0.24	0.14	0.21	0.01	-
100	*100*	*3*	0.62	0.68	0.44	0.26	0.48	0.24	0.02	0.66	0.68	0.68	0.73	0.76	0.76	0.06	-
105	*102*	*4*	0.02	0.02	0.04	–	–	–	–	–	–	0.02	0.01	0.10	0.01	0.09	-
110	*104*	*5*	–	0.02	0.04	–	–	–	0.15	–	0.05	0.02	0.02	–	0.02	0.41	0.49
115 + 120	*≥ 108*	*6 + 7*	–	–	–	–	–	–	0.83	–	–	0.01	–	–	0.01	0.43	0.51
Ap	*Ap-1*	*Ap*	(25)	(23)	(25)	(25)	(25)	(22)	(23)	(41)	(59)	(69)	(72)	(11)	(71)	(92)	(70)
90 + 95	*93 + 96*	*1 + 2*	–	–	0.08	0.04	0.06	–	–	0.01	0.01	0.01	0.01	0.08	0.02	0.01	0.01
100	*100*	*3*	0.54	0.52	0.72	0.70	0.58	0.86	0.19	0.62	0.54	0.66	0.53	0.64	0.72	0.18	0.38

Appendix A (*Continued*)

Locus, electromorph			Sample													
[5]	[7]	[26]	*40*	*41*	*42*	*43*	*44*	*45*	*47*	*PAN*	*PVA*	*PPM*	*PQE*	*Aapxyc*	*EH*	*Palavas*	*GV*
103	*104*	*4*	–	–	–	–	–	–	–	–	0.01	–	–	0.02	0.01	–	–
105	*108*	*5*	0.42	0.33	0.18	0.22	0.30	0.12	0.23	0.29	0.33	0.30	0.40	0.22	0.22	0.47	0.46
108	*114*	*6*	0.04	0.11	0.02	0.04	0.06	0.02	0.56	0.06	0.09	0.03	0.07	–	0.04	0.16	0.13
117 + 120	*122 + 128*	*7 + 8*	–	0.04	–	–	–	–	0.02	0.01	0.02	0.01	–	–	–	0.18	0.03
Est	*Est-D*	*Est-D*	(25)	(23)	(25)	(25)	(25)	(22)	(23)	(41)	(61)	(71)	(71)	(11)	(75)	(99)	(72)
80	*82*	*1.2*	–	–	–	–	–	–	0.02	–	–	–	–	–	–	0.04	0.04
90	*90*	*4*	0.30	0.59	0.08	–	–	–	0.48	0.62	0.57	0.45	0.63	0.04	0.01	0.94	0.91
≥ 100	*≥ 100*	*≥ 6*	0.70	0.41	0.92	1.00	1.00	1.00	0.50	0.38	0.43	0.55	0.37	0.96	0.99	0.02	0.06
Gpi	*Gpi*	*Pgi*	(25)	(23)	(25)	(25)	(25)	(22)	(23)	(41)	(58)	(70)	(69)	(11)	(75)	(94)	(66)
≤ 96	*≤ 98*	*1 + 2*	–	0.02	0.14	0.12	0.26	0.10	0.12	0.11	0.13	0.05	0.21	0.20	0.07	0.01	0.06
98 + 100 + 102	*100 + 102 + 105*	*3 + 4 + 5*	1.00	0.98	0.86	0.84	0.74	0.90	0.85	0.89	0.87	0.95	0.79	0.26	0.38	0.82	0.85
≥ 105	*≥ 107*	*≥ 6*	–	–	–	0.04	–	–	0.02	–	–	–	–	0.54	0.55	0.18	0.09
Lap	*Lap-2*	*Lap-2*	(25)	(23)	(25)	(25)	(25)	(22)	(23)	(41)	(59)	(71)	(72)	(11)	(72)	(100)	(68)
92 + 94	*90 + 95*	*1 + 2*	0.16	0.15	0.38	0.32	0.28	0.10	0.12	0.17	0.25	0.28	0.24	0.08	0.17	0.03	0.10
96	*100*	*3*	0.82	0.81	0.62	0.68	0.72	0.90	0.79	0.82	0.75	0.68	0.73	0.70	0.58	0.46	0.54
98 + 100	*≥ 102*	*5 + 7*	0.02	0.04	–	–	–	–	0.08	0.01	0.01	0.04	0.03	0.22	0.25	0.51	0.36
Mpi	*Mpi*	*Mpi*	(25)	(23)	(25)	(25)	(25)	(22)	(23)	(40)	(59)	(68)	(70)	(11)	(59)	(75)	(56)
90 + 92	*25 + 100*	*2*	0.22	0.24	0.12	–	0.06	0.02	0.96	0.26	0.36	0.16	0.4	0.06	0.02	0.97	0.97
96 + 100	*200*	*3*	0.78	0.76	0.88	1.00	0.88	0.98	0.04	0.73	0.64	0.84	0.6	0.94	0.98	0.03	0.03
110	*300*	–	–	–	–	–	0.06	–	–	0.01	–	–	–	–	0.01	–	–
Odh	*Odh*	*Odh*	(25)	(23)	(25)	(25)	(25)	(22)	(23)	(31)	(58)	(68)	(37)	(11)	(64)	(99)	(35)
80 + 90	*80 + 100*	*1 + 3*	0.14	0.07	0.08	0.02	0.02	0.16	0.59	0.03	0.02	0.13	0.01	–	0.06	0.15	0.49
≥ 98	*≥ 112*	*≥ 4*	0.86	0.93	0.92	0.98	0.98	0.84	0.42	0.97	0.98	0.86	0.99	1.00	0.95	0.86	0.52
Pgm	*Pgm-2*	*Pgm*	(25)	(23)	(25)	(25)	(25)	(22)	(23)	(37)	(55)	(71)	(72)	(66)	(74)	(96)	(73)
≤ 93	*≤ 96*	*≤ 3*	0.02	–	–	0.04	0.06	–	0.29	0.03	–	0.06	0.01	0.09	0.19	0.17	0.15
100	*100*	*4*	0.88	0.80	0.82	0.54	0.56	0.90	0.69	0.82	0.84	0.80	0.84	0.70	0.57	0.57	0.55
≥ 106	*≥ 102*	*≥ 6*	0.10	0.20	0.18	0.42	0.38	0.10	0.02	0.15	0.16	0.15	0.15	0.21	0.24	0.27	0.30

Appendix B

Electromorph frequencies at two allozyme loci in eight samples of Chilean smooth-shelled mussels analyzed by [10]. Homology of electromorphs between [5] and [10] was established as indicated in ("Materials and Methods"). Sample sizes in brackets.

Locus, electromorph		Sample							
[5]	[10]	*Arauco*	*Queule*	*Valdivia*	*Calbuco*	*Ancud*	*Yaldad*	*Marin*	*Arenas*
Gpi	*GPI*	(112)	(80)	(102)	(110)	(110)	(111)	(99)	(107)
≤ 96	*A*	0.11	0.03	0.03	0.10	0.10	0.07	0.13	0.13
≥ 98	*B + C*	0.88	0.97	0.97	0.90	0.91	0.93	0.87	0.87
Pgm	*PGM*	(109)	(108)	(116)	(110)	(99)	(128)	(99)	(100)
≤ 93	*A*	0.03	0.07	0.06	0.02	0.01	0.01	0.01	0.30
100	*B*	0.80	0.75	0.78	0.79	0.83	0.80	0.84	0.47
≥ 106	*C +D*	0.18	0.19	0.16	0.20	0.16	0.20	0.16	0.25

Appendix C

Allelomorph frequencies at nuclear-DNA loci *mac-1*and *Glu-5'* in 20 samples of smooth-shelled *Mytilus* spp. including two samples collected in Chile (*CHL*, *MAU*). Size homologies between allelomorphs from different samples were ascertained by side-by-side electrophoretic runs. *mac-1* allelomorph nomenclature follows [33]; *Glu-5'* allelomorphs *G*, *E* and *E'* [29] are allelomorphs *300*, *350* and *380*, respectively, in [34]; reference samples from the Northern-Hemisphere (*FLØ*, *GIL*, *STB*, *SET*, *CHI*) from [16,22,29,32]. *N*, sample size.

Locus, Allelomorph	Sample																			
	FLØ	GIL	GFP	STB	SET	CHI	SAF	KER	AUS	ADB	ALO	CBL	HOB	PAR	SIM	KOR	DUN	BOD	CHL	MAU
mac-1																				
f1	–	–	–	0.02	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
f2	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–
f3	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–	–	–	–	–	0.01	–
b0	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–
b05	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–
b2	–	–	–	0.04	0.05	–	0.04	–	–	–	–	–	–	–	–	0.02	–	0.04	0.05	–
b1	–	–	0.01	0.15	0.21	0.28	0.09	–	0.04	–	–	–	–	–	–	0.42	–	0.32	0.32	–
b3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	0.01	–
b4	–	–	–	0.02	–	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–	–
b5	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
c1	–	–	0.01	0.10	0.07	0.06	0.10	–	–	–	–	–	–	–	–	0.05	–	0.04	0.08	–
c12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.01	–
c15	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–
c2	–	–	–	0.50	0.54	0.57	0.53	–	0.16	–	–	–	–	–	–	0.43	–	0.41	0.39	–
c3	–	–	–	0.02	–	0.01	0.04	–	–	–	–	–	–	–	–	–	–	–	0.01	–
c4	0.05	–	0.02	–	–	–	–	0.08	–	–	–	–	–	–	–	–	–	–	–	–
c6	–	–	–	–	0.01	–	0.01	–	–	–	–	–	–	–	–	–	–	0.01	–	–
a0	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
a0.5	–	–	–	–	–	–	–	–	–	–	0.01	–	–	–	–	–	0.04	–	–	–
a1	0.06	0.02	0.01	0.02	–	–	–	–	–	–	–	0.03	–	–	–	–	–	–	–	–
a15	–	–	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–	0.01	–	–	–
a2	0.10	0.15	0.20	0.02	–	–	0.02	0.20	0.62	0.95	0.99	0.92	0.98	1.00	0.99	0.02	0.92	–	0.03	0.26
a3	0.29	0.31	0.24	0.04	0.01	–	0.03	0.70	0.16	0.04	–	0.03	–	–	0.01	–	0.02	0.03	–	0.74
a4	0.07	0.17	0.18	–	–	–	–	0.01	–	–	–	0.02	0.02	–	–	–	–	–	0.01	–
a5	0.38	0.27	0.29	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	0.10	0.01	–
a6	–	0.08	0.04	–	0.01	–	0.02	–	0.01	–	–	–	–	–	–	–	–	–	0.01	–
a7	–	–	–	0.02	0.04	0.03	0.04	–	–	–	–	–	–	–	–	0.03	–	0.01	0.03	–
a8	–	–	–	0.04	0.06	0.03	0.01	–	–	–	–	–	–	–	–	0.02	–	0.01	0.05	–
a9	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
d	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
(N)	(47)	(26)	(42)	(26)	(68)	(47)	(62)	(83)	(38)	(28)	(59)	(32)	(31)	(30)	(40)	(30)	(79)	(34)	(76)	(51)
Glu–5'																				
E +E' + E" + i	1.00	1.00	1.00	–	0.01	–	0.05	0.35	–	–	–	–	–	–	–	–	–	–	–	–
G + i + ii	–	–	–	1.00	0.99	1.00	0.95	0.65	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00
T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–
(N)	(35)	(16)	(42)	(19)	(56)	(18)	(65)	(79)	(46)	(26)	(25)	(29)	(26)	(25)	(38)	(19)	(77)	(23)	(48)	(28)

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