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Pleistocene separation of mitochondrial lineages of *Mytilus* spp. mussels from Northern and Southern Hemispheres and strong genetic differentiation among southern populations

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A B S T R A C T

Smooth-shelled mussels, *Mytilus* spp., have an antitropical distribution. In the Northern Hemisphere, the *M. edulis* complex of species is composed of three genetically well delineated taxa: *M. edulis*, *M. galloprovincialis* and *M. trossulus*. In the Southern Hemisphere, morphological characters, allozymes and intron length polymorphisms suggest that *Mytilus* spp. populations from South America and Kerguelen Islands are related to *M. edulis* and those from Australasia to *M. galloprovincialis*. On the other hand, a phylogeny of the 16S rDNA mitochondrial locus demonstrates a clear distinctiveness of southern mussels and suggests that they are related to Mediterranean *M. galloprovincialis*. Here, we analysed the faster-evolving cytochrome oxidase subunit I locus. The divergence between haplotypes of populations from the two hemispheres was confirmed and was found to predate the divergence between haplotypes of northern *M. edulis* and *M. galloprovincialis*. In addition, strong genetic structure was detected among the southern samples, revealing three genetic entities that correspond to (1) South America and Kerguelen Island, (2) Tasmania, (3) New Zealand. Using the trans-Arctic interchange as a molecular clock calibration, we estimated the time since divergence of populations from the two hemispheres to be between 0.5 million years (MY) and 1.3 MY (average 0.84 MY). The contrasting patterns observed for the nuclear and the organelle genomes suggested two alternative, complex scenarios: two trans-equatorial migrations and the existence of differential barriers to mitochondrial and nuclear gene flow, or a single trans-equatorial migration and a view of the composition of the nuclear genome biased by taxonomic preconception.

1. Introduction

Smooth-shelled mussels (*Mytilus edulis* L., *M. galloprovincialis* Lmk., *M. trossulus* Gould) are among those emblematical marine invertebrates that have an antitropical distribution—i.e. present in the cold and temperate waters of both hemispheres but absent from the tropics (Briggs, 1987; Vermeij, 1992). As for other antitropically distributed species, the analysis of the genetic relationships between populations of the two hemispheres may allow to infer the sense and timing of trans-equatorial migration (Bowen and Grant, 1997). Although introgressive hybridization occurs in each pair of smooth-shell *Mytilus* species (Skibinski et al., 1983; Rawson and Hilbish, 1998; Bierne et al., 2003), the genetic composition of the three taxa is now globally well defined in the Northern Hemisphere (Koehn, 1991; Quesada et al., 1998a; Daguin, 2000).

Using allozyme and morphometric characters, McDonald et al. (1991) first assigned populations from South America and Kerguelen Islands to *M. edulis* and those from Australasia to *M. galloprovincialis*. Further analysis, which added nuclear DNA markers to the available allozyme loci, globally supported this assignment but highlighted the endemic particularities of southern *Mytilus* spp. (Daguin and Borsa, 2000; Borsa et al., 2007). For instance, Kerguelen mussels present a genome of mixed *M. edulis* and *M. galloprovincialis* ancestry while Tasmanian mussels possess a predominantly *M. galloprovincialis* genomic background introgressed by *M. edulis* alleles fixed at some loci, pointing to past introgression (Borsa et al., 2007). To date the divergence between southern and northern *Mytilus* spp., Hilbish et al. (2000) have inferred their phylogeny at the 16S rDNA mitochondrial locus (16S). Most haplotypes from the Southern Hemisphere populations cluster into a single clade closely related to the clade D typical of Mediterranean *M. galloprovincialis*, and the divergence between the two clades supports a southward migration in the Pleistocene; South

American populations are not differentiated from Australasian populations. In addition, some haplotypes typical of northern *M. edulis* were found in the Kerguelen population, suggesting a recent introduction (Hilbish et al., 2000). Levels of diversity and divergence observed at the 16S locus in *Mytilus* spp. are low and the resolution of the phylogenetic relationships among haplotypes is poor. The cytochrome oxidase subunit I (*COI*) gene evolves faster than the 16S rDNA (Mueller, 2006) and this holds true in *Mytilus* spp. (Wares and Cunningham, 2001; Riginos et al., 2004).

The aim of the present study was to pursue the work of Hilbish et al. (2000) by (i) increasing the sampling effort at the 16S locus, especially in the Kerguelen population, where the nuclear genome is supposed to be the result of reticulated evolution, (ii) analysing the faster-evolving *COI* locus and (iii) re-evaluating relative divergence times between southern and northern *Mytilus* spp. mussels, as well as attempting absolute dating with this new dataset.

2. Material and methods

2.1. Sampling and mtDNA sequencing

Mussels were collected between 1997 and 2004 at 13 sampling sites in Italy (CHG, equal to sample CHI of Daguin et al. (2001)), Chile (CHL, MAU, PAT), South Africa (AFR), Kerguelen Islands (KER), Australia (AUS), Tasmania (CBL, SIM, HOB) and New Zealand (DUN, WHB, GRS). Details on sampling locations are in Table 1 and in legend to Fig. 1. Pieces of gill and mantle were preserved in 95% ethanol. DNA was extracted using Chelex 100[®] chelating resin (Walsh et al., 1991). The 417 bp fragment of the 16S locus was amplified by polymerase chain reaction (PCR) using the two primers 16S-RA (5' CGCCTGTTATCAAAAACAT 3') and 16S-RB (5' CCGGTCTGAACACTCAGATCAGT 3') (Palumbi et al., 1991). The PCR program consisted of 5 min at 95 °C, then 35 cycles of 95 °C for 1 min, 54 °C for 45 s, 72 °C for 1 min and a final extension of 7 min at 72 °C. A 645 bp fragment of the *COI* gene was amplified using the forward primer LCO1490 (5' GGTCACAAATCATAAAGA TATTGG 3') (Folmer et al., 1994), the reverse primer AM-HCO (5' TAAACYTCAGGCTGCCAAAAAYCA 3') and the same PCR program as above with an annealing temperature of 59 °C. PCR products were sequenced directly. Only female mitochondrial sequences were considered in the present study. Chromatograms were inspected visually and the sequences were aligned using CLUSTALW (Thompson et al., 1994) implemented in BIOEDIT (Hall, 1999). 16S and *COI* gene sequences were submitted to EMBL (<http://www.embl-heidelberg.de/>) under accession numbers AM904568–AM904606 and AM905146–AM905225, respectively (Table 1).

2.2. Population genetics analysis

Haplotype diversity and levels of nucleotide diversity estimated from the number of polymorphic sites (θ_w ; Watterson, 1975) and from pairwise differences (θ_π ; Nei, 1987) were computed using DNAsp (Rozas et al., 2003). Genetic differentiation between populations was estimated with the Φ -analogues (Excoffier et al., 1992) of Wright's (1965) *F*-statistics, by using the software ARLEQUIN 3.0 (Excoffier et al., 2005). The null distribution of pairwise Φ_{ST} values under the hypothesis of no difference among populations was obtained by permutating haplotypes between populations. The *p*-value given by the test is the proportion of Φ_{ST} pseudo-values generated by random permutations that are larger than or equal to the observed value. In order to visualize the genetic similarities among samples, non-metric multidimensional scaling was used on the matrix of Φ_{ST} values (MDS; Kruskal and Wish, 1978).

2.3. Phylogenetic analysis

To our sequence set, we added the northern and southern *Mytilus* haplotypes of the 16S locus and northern haplotypes of the *COI* gene available from the literature and from the EMBL database. The sources of the complete sequence set are detailed in Table 1. These sequences were respectively 405 bp and 384 bp long.

Phylogenetic reconstructions were performed using distance and maximum-likelihood approaches. The most appropriate model of evolution as determined by MrAIC (Nylander, 2004) was the Tamura-Nei nucleotide substitution model (TN93; Tamura and Nei, 1993). Distance trees were constructed with the neighbour-joining (NJ) algorithm using TN93 distances with MEGA version 3.1 (Kumar et al., 2004). Maximum-likelihood (ML) trees were built using PHYML (Guindon and Gascuel, 2003). Levels of support of tree nodes were determined using 1000 and 100 bootstrap replications of the original data for NJ and ML methods, respectively.

2.4. Estimating the time of the trans-equatorial migration

Mutation rate constancy at the *COI* locus in smooth-shelled mussels *Mytilus* spp. was checked using Tajima's (1993) test of relative rate implemented in MEGA 3.1. To perform this test, we used *M. californianus* (Accession No. U68776) as outgroup, as it represents the closest known taxon to the smooth-shelled mussels. To estimate the net nucleotide divergence, *d*, we assumed that the ancestral coalescence time was similar to the present one and estimated θ_π from the available samples. Then, $\theta_\pi/2$ was subtracted from the average divergence d_{xy} (Nei and Li, 1979). Among the available samples, we used the five largest samples (all with size greater than 15 individuals), including two samples from the Southern Hemisphere (PAT and KER, AM905187–AM905211), two samples of *M. edulis* from Sweden (AY723897–AY723917) and Nova Scotia (AF241951–AF24195157, AY130016–AY130023, AY723881–AY723887, AY723889, AY723890) and a sample of *M. trossulus* (AY130061–AY130067, AF242027–AF242029, AF242031–AF242035). We used the opening of the Bering Strait, 3.5 million years (MY) ago as a calibration point to date the divergence between *M. trossulus* and North Atlantic *M. edulis* populations, following Wares and Cunningham (2001) and Riginos et al. (2004). Although the validity of this hypothesis can be discussed, it provides at least a basis for comparison with other studies.

3. Results

3.1. Population genetics of southern *Mytilus* spp. mussels

Among the 224 sequences at the 16S locus, 20 haplotypes with 19 polymorphic sites were identified; whereas among the 171 sequences of the *COI* gene, we scored 59 haplotypes, with 104 polymorphic sites. Consequently, the level of polymorphism expressed by nucleotide and haplotype diversities, was much higher for the *COI* locus than for the 16S locus (Table 2). Nucleotide variation at the 16S locus was low and mainly composed of singleton mutations, while the diversity of the *COI* locus was higher. Genetic differentiation between populations was observed at both loci but was stronger at the *COI* locus (global $F_{ST} = 0.8$, $p < 0.0001$) than at the 16S locus. Fig. 2 depicts the genetic differences between southern samples for the *COI* locus. Four groups of samples sharing low Φ_{ST} values appeared, three of which correspond to geographic regions: South America, Tasmania and New Zealand. The fourth group was composed of geographically separated samples (CHL, AFR, AUS). Non-significant Φ_{ST} values were observed among samples from New Zealand, between two samples from Tasmania (CBL and SIM), and between CHL and AFR (Fig. 2).

Table 1
Mytilus spp. nucleotide sequences of *COI* and *16S* loci used in the present study. *N*, sample size

Locus species	Sampling locations	<i>N</i>	Name of sequences	GenBank Accession Nos.	References
16S					
<i>M. trossulus</i>	Northeastern Pacific: Alaska, Oregon	5	Tshel, Tore, Tak	AY636148, AY636150–AY636153	Rawson (2005)
<i>M. trossulus</i>	Northeastern Pacific: Oregon	3	TU1, TU2, TU3	U22879–U22881	Rawson and Hilbish (1995)
<i>M. edulis</i>	Northwestern Atlantic: Delaware	3	E1, E3, E4	U22864, U22866, U22867	Rawson and Hilbish (1995)
<i>M. edulis</i>	Northwestern Atlantic: Delaware	6	EDe6-11	AF023542, AF023544, AF023545, AF023547, AF023549, AF023550	Rawson and Hilbish (1998)
<i>M. edulis</i>	—	1	Ew22F	AY350784	Cao et al. (2004)
<i>M. edulis</i>	—	1	E38	AJ293738	Quesada et al. (2003)
<i>M. galloprovincialis</i>	Mediterranean: Sète (France)	5	GFr1-5	AF023552, AF023554, AF023556, AF023558, AF023560	Rawson and Hilbish (1998)
<i>M. galloprovincialis</i>	Atlantic: Spain	3	GSp1-3	AF023563–AF023565	Rawson and Hilbish (1998)
<i>M. galloprovincialis</i>	Northeastern Pacific: San Diego, CA	2	GM-1, GM-2	U22884–U22885	Rawson and Hilbish (1995)
<i>M. galloprovincialis</i>	Northeastern Pacific: San Diego, CA	7	GAL2-4, GAL6-9	U22870–U22872, U22874–U22877	Rawson and Hilbish (1995)
<i>M. galloprovincialis</i>	—	1	Mgal-mtgenome	AY497292	Mizi et al. (2005)
<i>M. galloprovincialis</i>	Chioggia, Italy	9	CHG	AM904603–AM904606	Present study
<i>Mytilus</i> sp.	Northeastern Atlantic: Ex River, UK	2	HEX1, HEX2	AF023589–AF023590	Rawson and Hilbish (1998)
<i>Mytilus</i> sp.	Northeastern Atlantic: Whitsand Bay, UK	8	HWb1-8	AF023572, AF023574, AF023576, AF023577, AF023579, AF023581, AF023582, AF023584	Rawson and Hilbish (1998)
<i>Mytilus</i> sp.	Southern Ocean: Chile, Falklands, Kerguelen, Australia, New Zealand	20	CH, FA, K, AU, NZ	AF179443–AF179462	Hilbish et al. (2000)
<i>Mytilus</i> sp.	Dichato, Chile	8	CHL	AM904600–AM904602	Present study
<i>Mytilus</i> sp.	Maullin, Chile	10	MAU	AM904582–AM904584	Present study
<i>Mytilus</i> sp.	Canal de las Montañas, Patagonia, Chile	39	PAT	AM904585–AM904588	Present study
<i>Mytilus</i> sp.	Pater Noster Bay, South Africa	8	AFR	AM904595–AM904599	Present study
<i>Mytilus</i> sp.	Kerguelen islands	99	KER	AM904589–AM904590	Present study
<i>Mytilus</i> sp.	Nedlands, Australia	5	AUS	AM904591–AM904594	Present study
<i>Mytilus</i> sp.	Cloudy Bay lagoon, Tasmania	9	CBL	AM904577, AM904578	Present study
<i>Mytilus</i> sp.	Simpson's Bay, Tasmania	6	SIM	AM904579–AM904581	Present study
<i>Mytilus</i> sp.	Hobart, Tasmania	9	HOB	AM904576	Present study
<i>Mytilus</i> sp.	Wellington Harbour, New Zealand	10	WHB	AM904570–AM904572	Present study
<i>Mytilus</i> sp.	Dunedin, New Zealand	10	DUN	AM904568, AM904569	Present study
<i>Mytilus</i> sp.	George Sound, New Zealand	10	GRS	AM904573–AM904575	Present study
COI					
<i>M. trossulus</i>	Mahone Bay, Nova Scotia	3	669NS, 671NS, 672NS	AY130061–AY130063	Riginos et al. (2004)
<i>M. trossulus</i>	Penn Cove, WA, USA	4	764WA, 766WA, 768WA, 769WA	AY130064–AY130067	
<i>M. trossulus</i>	North eastern Pacific	2	MMT	AF242031–AF242032	Wares and Cunningham (2001)
<i>M. trossulus</i>	North America	6	JMT, PMT	AF242027–AF242029, AF242033–AF242035	Wares and Cunningham (2001)
<i>M. edulis</i>	Northwestern Atlantic: Newfoundland, Nova Scotia, Maine, North Carolina, Rhode Island, Massachusetts	46	NF, NS, ME, NC, RI, MA	AF241936–AF241981	Wares and Cunningham (2001)
<i>M. edulis</i>	Northwestern Atlantic: Massachusetts	4	MA	AY101417, AY101425, AY101426, AY101429	Riginos et al. (2004)
<i>M. edulis</i>	Northwestern Atlantic: Newfoundland, Nova Scotia, Maine, Massachusetts, North Carolina	40	NF, NS, ME, MA, NC	AY130011–AY130037, AY723880–AY723892	Riginos et al. (2004)
<i>M. edulis</i>	Northeastern Atlantic: Iceland, Norway, France	45	Ice, No, Fr	AF241982–AF242026	Wares and Cunningham (2001)
<i>M. edulis</i>	Northeastern Atlantic: Iceland, Norway, Sweden	28	Ice, No, Sw	AY723893–AY723901, AY723903–AY723917, AY101421–AY101424	Riginos et al. (2004)
<i>M. edulis</i>	Northeastern Atlantic: Ireland, Finland, France	21	Ire, Fi, Fr	AY723918–AY723922, AY130038–AY130053	—
<i>M. galloprovincialis</i>	Greece: Samos	7	GR	AY130054–AY130060	—
<i>M. galloprovincialis</i>	—	1	Mgal-mtgenome	AY497292	Mizi et al. (2005)
<i>M. galloprovincialis</i>	Chioggia, Italy	10	CHG	AM905220–AM905225	Present study
<i>Mytilus</i> sp.	Dichato, Chile	9	CHL	AM905174–AM905179	Present study
<i>Mytilus</i> sp.	Maullin, Chile	7	MAU	AM905180–AM905186	Present study
<i>Mytilus</i> sp.	Canal de las Montañas, Patagonia, Chile	20	PAT	AM905187–AM905195	Present study
<i>Mytilus</i> sp.	Pater Noster Bay, South Africa	7	AFR	AM905216–AM905219	Present study
<i>Mytilus</i> sp.	Kerguelen islands	83	KER	AM905196–AM905211	Present study
<i>Mytilus</i> sp.	Nedlands, Australia	7	AUS	AM905212–AM905215	Present study
<i>Mytilus</i> sp.	Cloudy Bay lagoon, Tasmania	5	CBL	AM905161–AM905164	Present study
<i>Mytilus</i> sp.	Simpson's Bay, Tasmania	3	SIM	AM905165–AM905167	Present study
<i>Mytilus</i> sp.	Hobart, Tasmania	8	HOB	AM905168–AM905173	Present study
<i>Mytilus</i> sp.	Wellington Harbour, New Zealand	7	WHB	AM905149–AM905154	Present study
<i>Mytilus</i> sp.	Dunedin, New Zealand	6	DUN	AM905146–AM905148	Present study
<i>Mytilus</i> sp.	George Sound, New Zealand	9	GRS	AM905155–AM905160	Present study

Hyphens indicate missing informations.

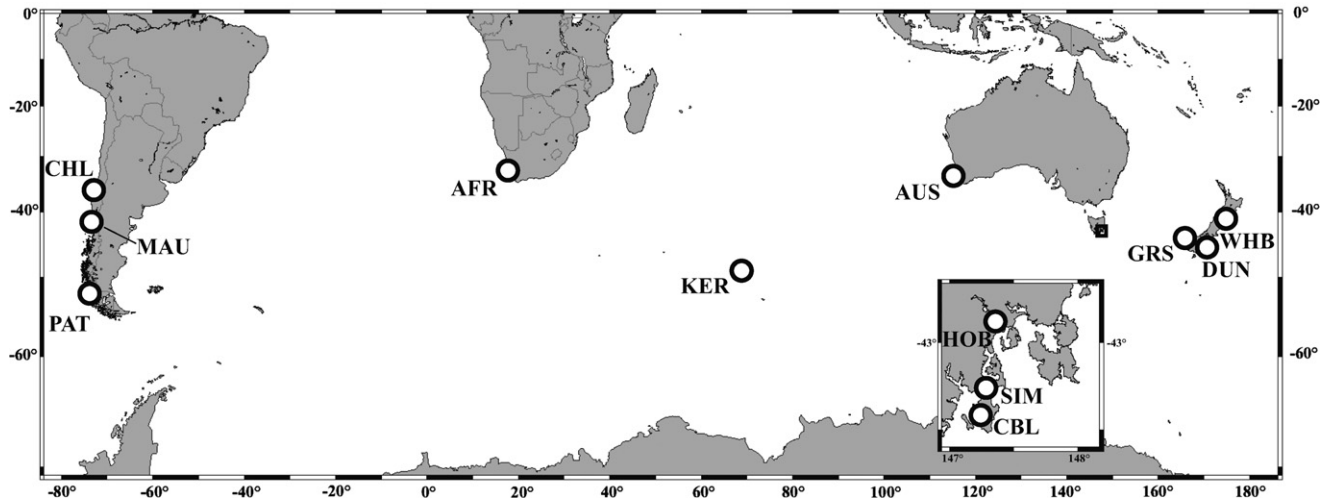


Fig. 1. Sampling locations of Southern Hemisphere *Mytilus* spp. mussels. CHL, Dichato, Chile (36°32'S; 72°56'W); MAU, Maullin, Chile (41°37'S; 73°37'W); PAT, Canal de las Montañas, Patagonia, Chile (51°51'S; 73°20'W); AFR, Pater Noster Bay, South Africa (32°48'S; 17°54'E); KER, Kerguelen islands (49°20'S; 70°10'E); AUS, Nedlands, Australia (34°S; 115°E); CBL, Cloudy Bay Lagoon, Tasmania (43°26'S; 147°13'E); SIM, Simpson's Bay, Tasmania, (43°17'S; 147°18'E); HOB, Hobart, Tasmania (42°50'S; 147°20'E); WHB, Wellington Harbour, New Zealand (41°20'S; 174°48'E); DUN, Dunedin, New Zealand (45°54'S; 170°30'E); GRS, George Sound, New Zealand (44°52'S; 167°20'E).

Table 2

Haplogroup frequencies in Southern Hemisphere *Mytilus* spp. populations and parameters of nucleotide sequence variability at mitochondrial loci *COI* and *16S*

Locus Haplogroup	Sample											
	CHL	MAU	PAT	AFR	KER	AUS	SIM	CBL	HOB	WHB	DUN	GRS
<i>16S</i>												
N	1	0	0	1	0	1	0	0	0	0.2	0	0
S	0	1	1	0	1	0	1	1	1	0.8	1	1
H_d	0.464	0.51	0.24	0.86	0.02	0.90	0.73	0.22	0	0.62	0.20	0.38
θ_π	0.00612	0.0013	0.0006	0.00788	0.00005	0.00719	0.00208	0.00053	0	0.0038	0.00048	0.00096
θ_w	0.00639	0.0017	0.0017	0.00647	0.00046	0.00806	0.0021	0.00088	0	0.0017	0.00085	0.00339
(N)	(8)	(10)	(39)	(8)	(99)	(5)	(6)	(9)	(9)	(10)	(10)	(10)
<i>COI</i>												
N	1	0	0	1	0	1	0	0	0	0.14	0	0
S1	0	1	1	0	1	0	0	0	0	0	0	0
S2	0	0	0	0	0	0	0	0	0	0.86	1	1
S3	0	0	0	0	0	0	1	1	1	0	0	0
H_d	0.889	1	0.75	0.81	0.85	0.81	1	0.90	0.893	0.95	0.60	0.83
θ_π	0.01559	0.00753	0.00453	0.01521	0.00341	0.01801	0.00413	0.00279	0.00604	0.02110	0.00270	0.00172
θ_w	0.01711	0.00949	0.00524	0.01392	0.00590	0.01455	0.00413	0.00298	0.00777	0.00285	0.00272	0.02974
(N)	(9)	(7)	(20)	(7)	(83)	(8)	(3)	(5)	(8)	(7)	(6)	(9)

Clades-N and -S (S1, S2, S3) were defined in the results section. H_d , haplotype diversity; θ_π , nucleotide diversity; θ_w , Watterson's estimate of polymorphism (1975); N, sample size. Abbreviations for samples as in legend to Fig. 1. It should be noted that the haplogroup frequencies of the sample AUS strongly differs between the two loci due to different subsets of individuals successfully sequenced.

3.2. Phylogenetic relationships

The phylogenetic relationships among *Mytilus* spp. haplotypes are presented in Fig. 3A (*16S* locus) and Fig. 3B (*COI* locus). The topology of the ML tree was identical to that of the NJ tree as far as nodes supported by bootstrap scores over 50% are concerned. Only the NJ trees were presented, with the bootstrap scores of each NJ and ML phylogenies indicated on the branches. Tree nodes generally were supported by higher bootstrap values in the *COI* tree than in the *16S* tree. The *M. trossulus* sample was not polluted by introgression and we were able to use it as a robust closely related outgroup. In agreement with previous results (Hilbish et al., 2000), the majority of haplotypes sampled in the Southern Hemisphere clustered in a single clade. We called this clade 'S' (for 'Southern'). Clade S was distinct from the haplogroup made of all the haplotypes sampled in the Northern Hemisphere and forming a clade here called N ('Northern'). This result was supported in the *COI* tree (Fig. 3B) with higher bootstrap values than in the *16S* tree (Fig. 3A).

Clade-N haplotypes were present in four populations of the Southern Hemisphere: Chile (CHL), South Africa (AFR), Western Australia (AUS) and New Zealand (WHB). Samples CHL, AFR and AUS were exclusively composed of clade-N haplotypes (Table 2) and sample WHB was polymorphic for clade-S and clade-N haplotypes, the clade-N haplotypes being sampled in lower frequency (Table 2). Finally, the analysis of the *COI* locus revealed that the clade S can be subdivided into three sub-clades, two of which were well supported, corresponding to three geographic regions: S1 (South America and Kerguelen), S2 (New Zealand) and S3 (Tasmania).

3.3. Dating the trans-equatorial migration

Tajima's relative rate tests were not significant ($p > 0.05$). Therefore, the existence of different rates of evolution between lineages was not demonstrated and we consequently assumed a clock-like rate of evolution. The Table 3 details the different steps that led to the estimation of the net nucleotide divergence between

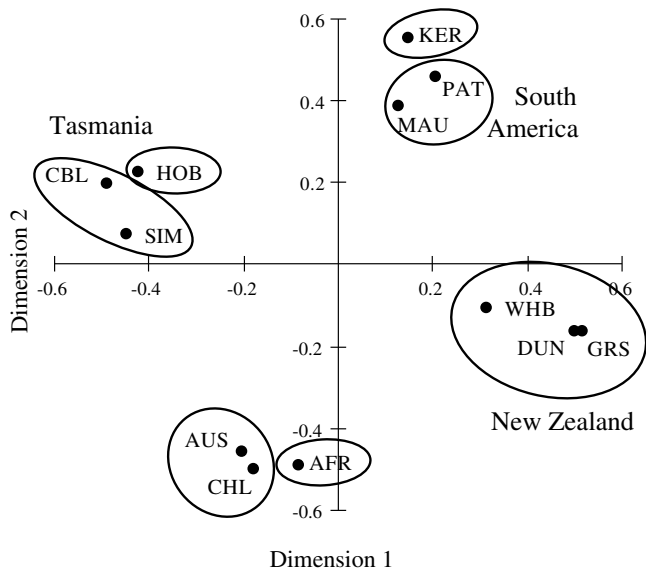


Fig. 2. Bidimensional representations of multidimensional scaling (MDS) based on ϕ_{st} values calculated between all pairs of southern samples at *COI* locus (stress 0.17). Circles group samples significantly differentiated from excluded sample and between which ϕ_{st} values are non-significant ($P > 0.05$). See Fig. 1 for sample abbreviations.

the *M. trossulus* sample and the other samples, $d_{(1)}$, and the one between the *M. edulis* samples (from Sweden and/or Nova Scotia) and PAT and KER, $d_{(2)}$. Since the ratio of the net divergences was 4.17 and assuming that the divergence time between *M. trossulus* and other populations was 3.5 MY, we estimated the trans-equatorial migration event to be 0.84 MY (0.54–1.31 MY) old.

4. Discussion

As previously emphasized in the Northern Hemisphere, the *COI* locus appeared to be a more informative marker than the *16S* locus for phylogeographical studies of *Mytilus* in the Southern Hemisphere. The divergence between Southern- and Northern-Hemisphere haplotypes predates the divergence of haplogroups typical of modern *M. edulis* and *M. galloprovincialis*. However, gene trees are not species trees (Nichols, 2001), particularly in a species complex with hybridization and introgression as observed in smooth-shelled *Mytilus*. The results obtained with the single mitochondrial genome do not allow us to infer a general history of population divergence. The present results can nonetheless be discussed in the light of previously published results from allozymes and nuclear-DNA length polymorphisms.

4.1. Strong genetic structure among *Mytilus* spp. populations of the Southern Hemisphere

Three groups of samples can be recognized in the Southern Hemisphere: (i) samples exclusively composed of clade-N haplotypes, (ii) samples exclusively composed of clade-S haplotypes and (iii) samples that are polymorphic for both. We observed the first kind of samples in Chile, South Africa and Western Australia. These populations likely originate from recent introductions. The recent introduction of *M. galloprovincialis* mussels in South Africa has already been reported (Grant and Cherry, 1985; McDonald et al., 1991; Daguin and Borsa, 2000). Recently introduced *M. galloprovincialis* populations have also been reported in Eastern Asia (Wilkins et al., 1983; Lee and Morton, 1985; Daguin and Borsa, 2000), California (Mc Donald and Koehn, 1988; Daguin and Borsa,

2000) and Chile (Daguin and Borsa, 2000). Here, we report the probable introduction of mussels from the Northern Hemisphere to Western Australia. The phylogenetic position of haplotypes from sample AUS indicates that these mussels are once again *M. galloprovincialis*. However, the same sample appeared to be rather intermediate between Southern- (Tasmania and New Zealand) and Northern Hemisphere *M. galloprovincialis* at the nuclear locus *mac-1* (Daguin and Borsa, 2000). Further analyses will be needed to clarify the genetic composition of mussels from Western Australia. Samples of the second kind, exclusively composed of clade-S haplotypes, can be considered to belong to southern endemic populations. These samples comprised three genetic entities that reached reciprocal monophyly and corresponded to distinct regions: South America (MAU and PAT) and Kerguelen with haplotypes of the clade S1, New Zealand (DUN and GRS) with haplotypes of the clade S2 and Tasmania (HOB, SIM and CBL) with haplotypes of the clade S3 (Fig. 3B). In contrast to Hilbish et al. (2000) who sampled exclusively clade-N haplotypes in their small sample ($N = 5$) from the Kerguelen Islands, we did not sample any clade-N haplotypes in our Kerguelen sample ($N = 99$). Population structure was also observed to a lesser extent among the three clade-S1 samples (Fig. 2), as well as between HOB and the two other samples from Tasmania (Fig. 2). The level of population differentiation was not well correlated with geographic distance. For instance, genetic differences between Tasmania and New Zealand populations were stronger than between South America and Kerguelen, in spite of being geographically closer. One explanation relies on the geographic fragmentation of landmasses, together with present and past routes of major oceanic currents that go through the mid-latitudes of the Southern Hemisphere. For instance, although the Kerguelen archipelago is isolated by the polar front and the Antarctic circumpolar current, latitudinal displacements of the front may have connected the Kerguelen Islands to South America in the recent past (Hollyday and Read, 1998). Conversely, the eastern seaboard of Tasmania is affected by the East Australian Current, whose southern extremity pinches off warm-core anticlockwise eddies drifting south near the margin of the continental shelf through the colder waters of the Southern Ocean (Nilsson and Cresswell, 1981). Southeastern Tasmania may therefore be isolated by local oceanic circulation. Alternatively, barriers to mtDNA exchange may be genetic instead of geographic as it has previously been suggested for populations of the Northern Hemisphere (Quesada et al., 1995; Riginos et al., 2004). Finally, we observed a sample from New Zealand (WHB) in which clade-N haplotypes occur at low frequency within a population otherwise composed of clade-S haplotypes. Unfortunately, it is not possible with mtDNA haploid data to distinguish admixture (whereby northern migrants do not reproduce in panmixia with native Southern-Hemisphere mussels) from introgression (whereby northern alleles have invaded the genetic background of southern natives).

4.2. The isolation between mtDNA lineages of populations from the two hemispheres predates the divergence between *M. edulis* and *M. galloprovincialis* haplotypes

Hilbish et al. (2000) previously showed with the *16S* locus that haplotypes from endemic Southern-Hemisphere *Mytilus* spp. clustered together into a single clade. In their topology the southern clade was related to haplotypes sampled in Mediterranean *M. galloprovincialis*. With our new dataset for the *16S* locus, we obtained a slightly different although poorly supported topology, where the clade-S branched ancestrally to the clade N. We interpret the discrepancy between the *16S* tree of Hilbish et al. (2000) and ours by the lack of phylogenetic signal with this locus. Much better statistical support was obtained with the *COI* locus. The *COI* tree not only reveals the distinction between endemic southern and

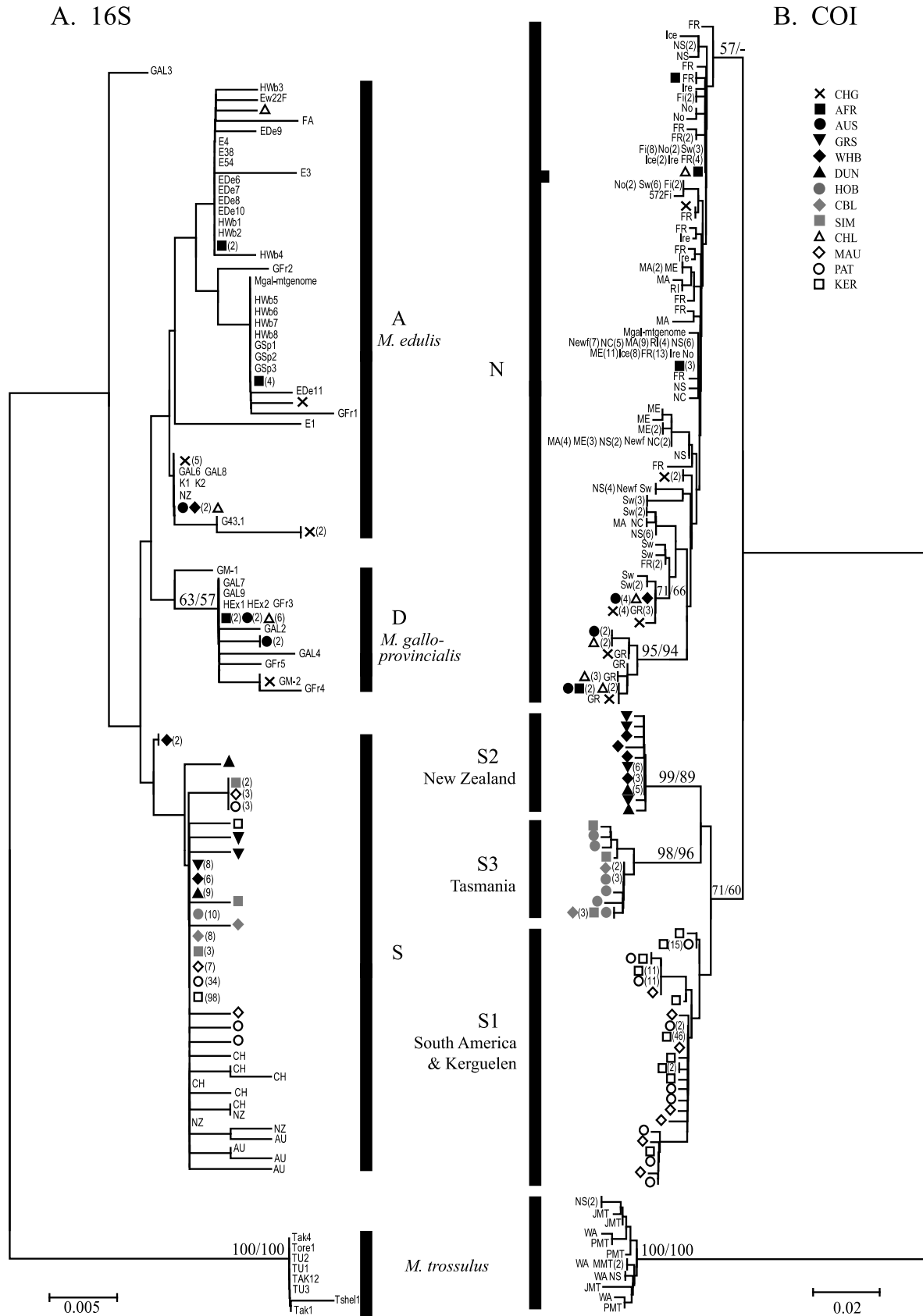


Fig. 3. Phylogenetic relationships of (A) 16S rDNA and (B) COI haplotypes in *Mytilus* spp. mussels inferred using NJ algorithm. Bootstrap scores larger than 50% are indicated on branches for NJ and ML, respectively. Tree-length scales given at the bottom of each tree. Note that the scale of 16S tree is four times larger than that of the COI tree. See Table 1 for site abbreviations.

Table 3

Estimation of relative divergence between *Mytilus* spp. samples from Northern and Southern Hemispheres

Comparison, Parameter	Average	Min.	Max.
$\theta_{\pi}/2$	0.0046	0.0018 (± 0.0005)	0.0076 (± 0.0015)
<i>M. trossulus</i> vs. any other sample			
d_{xy}	0.146	0.144 (± 0.012)	0.149 (± 0.013)
$d_{(1)}$	0.141	0.123	0.161
<i>M. edulis</i> [*] vs. any other sample			
d_{xy}	0.040	0.036 (± 0.002)	0.043 (± 0.004)
$d_{(2)}$	0.035	0.025	0.046
$d_{(1)}/d_{(2)}$	4.17	2.67	6.44

$\theta_{\pi}/2$ was calculated for each of the five samples (*M. trossulus*, *M. edulis* from Sweden and from Nova Scotia, PAT and KER). d_{xy} : average divergence; d : net nucleotide divergence. For each parameter, the average was calculated from all possible values, among which are the indicated extreme values (min., max.). Standard error is indicated in brackets.

* *M. edulis* from Sweden or Nova Scotia.

northern haplotypes but also indicates that this dichotomy pre-dates the divergence of typical *M. edulis* and *M. galloprovincialis* haplotypes. An attempt to date the divergence between populations of the two hemispheres led to an estimate of 0.84 MY (0.54–1.31 MY). This divergence dates from the Pleistocene (0.01–1.8 MY) as already inferred by Hilbish et al. (2000).

4.3. Can we reconcile mitochondrial and nuclear data into a single scenario?

From the genetic data on southern *Mytilus* spp. mussels provided by markers of various types, two conflicting scenarios of trans-equatorial migration can be inferred. The first scenario is supported by allozymes (McDonald et al., 1991) and two introns (*mac-1* and *EFbis*, Daguin, 2000; Borsa et al., 2007). It distinguishes South American and Kerguelen populations that share similarities with *M. edulis*, from Australasian populations (Australia, New Zealand, Tasmania) that share similarities with *M. galloprovincialis*. This pattern suggests two independent trans-equatorial migrations, one each from *M. edulis*-like and *M. galloprovincialis*-like populations that would have occurred after the separation of the two species. The second scenario comes from the most parsimonious interpretation of the mtDNA data and suggests a unique trans-equatorial migration, which would be older than the coalescence of all the northern haplotypes.

Although conflicting at first view, cyto-nuclear discordance may reveal interesting details about the evolution of species and may have important consequences for evolutionary inference if undetected (Sang and Zong, 2000; Funk and Omland, 2003). We here propose to consider how the scenario suggested by one genome (nuclear vs. organelle) can fit the results observed with the other one.

Under the first scenario, we need to explain why the divergence of the southern and northern haplotypes appears more ancient than the divergence between typical *M. edulis* and *M. galloprovincialis* haplotypes, and why the two trans-equatorial migrations did not result in a paraphyletic pattern of the southern and northern lineages. Pre-divergence coalescence can be invoked to explain that the southern clade branches deeply in the tree. The present mtDNA differences between *M. edulis* and *M. galloprovincialis* are not strong and one can hypothesize that they were less pronounced at the time of migrations. Post-migration lineage sorting would then have resulted in the successful retention of an old lineage in southern populations—i.e. a gene tree that does not match the population tree. However, this hypothesis of mismatched lineage sorting does not explain the reciprocal monophyly of the

clades S and N under the two-migration hypothesis. This is why one may invoke the effect of semi-permeable barriers to gene flow either in the northern or in the southern populations. The barrier to gene flow between *M. edulis* and *M. galloprovincialis* is semi-permeable (Skibinski et al., 1983; Bierne et al., 2002) and strong mitochondrial introgression has been documented (Quesada et al., 1998b). The flow of mtDNA genes is likely to have continued while the flow of some nuclear genes was already impeded. Alternatively, hybridization and introgression within the Southern Hemisphere would have erased the signature of one trans-equatorial migration on the mtDNA genome. Be they localised in the North or in the South, the presence of semi-permeable barriers leading to differential organelle and nuclear gene flow greatly helps in reconciling the data obtained with the two genomes under the two-migration scenario.

The second scenario consists of a single southward trans-equatorial migration early in the Pleistocene before the presumed speciation of *M. edulis* and *M. galloprovincialis*. Under this scenario the distinction, based on nuclear markers, of *M. edulis*-like and *M. galloprovincialis*-like mussels in the Southern Hemisphere would be merely virtual, constrained by human's wish to always assign new samples to reference populations assumed to represent the genetic composition of a given taxon (e.g. Hey, 2001a,b). In addition, the few nuclear markers used to date might suffer from an ascertainment bias as they have often been chosen to discriminate *M. edulis* and *M. galloprovincialis* populations in the Northern Hemisphere. However, it is difficult to rule out that the high stochasticity of the coalescence process on a limited number of loci did not result in some populations being apparently closely related to one of the references.

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