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High genetic distance between marine bivalves of the genus *Mesodesma* inhabiting the Atlantic and Pacific coasts of South America

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Abstract

Molluscs of the genus *Mesodesma* are marine bivalves inhabiting the middle littoral on sandy beaches of temperate and subtropical systems, where they usually are the dominant organisms. This genus is represented on South America's Atlantic coast by *Mesodesma mactroides*, and by *Mesodesma donacium* on the Pacific coast. Samples of these species from Brazil and Chile were compared genetically by electrophoresis at 26 isozyme loci. Out of 17 monomorphic loci, 14 were fixed for different alleles. Nine polymorphic loci were found; where 44 alleles were detected, 35 of these were distinct between both species. The statistical analysis showed a Nei's genetic distance of 1.90. The high genetic distance observed here corroborates the controversy about mesodesmatid systematic problems.

Keywords: Bivalves; Mesodesmatidae; *Mesodesma mactroides*; *Mesodesma donacium*; South America; High genetic distance; Isozyme; Electrophoresis

1. Introduction

The family Mesodesmatidae, also called Amphidesmatidae in Australia and New Zealand, is represented by few genera and species of marine bivalves. These organisms inhabit the middle littoral on sandy beaches of temperate and subtropical systems, where they usually are the dominant organisms. The distribution of *Mesodesma donacium* is restricted in the West coast of South America from 43°S (Isla de Chiloe, Chile) to 5°S (Bahia de Sechura, Peru), with this species endemic of the Chile–Peru Malacological Zoogeographic Province [27], while *Mesodesma mactroides* is distributed in the East coast of South America from 41°S (Bahia de San Blas, Argentina) to 23°S (São Paulo, Brazil). These bivalves have supported high value commercial fisheries, *M. donacium* in central Chile (40–42°S) [23] and *M. mactroides* in Uruguay [7] and Argentina [2,21].

According to Lamy [12] the genus *Mesodesma* contains seven subgenera: *Mesodesma*, *Taria*, *Paphies*, *Atactodea*, *Donacilla*, *Davila* and *Anapella*. The species that inhabits the subtropical and temperate sand beaches of South American Atlantic coast is *M. (Taria) mactroides* [4]. This is the only species of the subgenus distributed out of Australia and New Zealand, where the most interesting cases of Mesodesmatidae speciation have been noted [6]. The other South American species, *M. (Mesodesma) donacium*, is more closely related to the counterparts from North American Atlantic coast of the same subgenus [21]. The following subgenera have a wider distribution than *Mesodesma* and *Taria*: *Paphies* in New Zealand, *Donacilla* in Mediterranean Sea, Black Sea, Atlantic European and Pacific Boreal, *Atactodea* in both Indic and Pacific oriental oceans, *Davila* in Philippines and Australia, and *Anapella* in south of Australia and Tasmania.

Beu and De Rooij-Schuiling [3] suggested a new classification for mesodesmatids, with *Paphies* as the main genus, with five subgenera: *Paphies* = *Taria*, spe-

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cies mainly from New Zealand; *Mesodesma*, as the only American species; *Amesodesma* from Indonesia, Philippines and Southeast Australia; *Atactodea* from Southeast Australia and West Indo-Pacific; *Regterenia* from South Africa. The proposal of a new classification indicates taxonomic controversies for mesodesmatids, which have been classified on the basis of morphological characters only.

Methodologies of classification based on genetic and molecular techniques, such as isozyme electrophoresis, have been very important in resolving taxonomic and systematic problems. The identification of one or more loci (genes) with variants (alleles) has brought out considerable value in both taxonomic context and population genetics research [30]. Despite the rise of new methodologies to genetic analysis based on molecular biology, isozyme electrophoresis still has been successfully used to solve questions in invertebrate systematics [11,14,19,22,29] and also in gene flow and populational structure studies [5,10,17,18].

The aim of this work was to compare genetically the two species representing the Mesodesmatidae family in South America, based on allele frequency data obtained by isozyme electrophoresis.

2. Material and methods

Fifteen specimens of *M. mactroides* and *M. donacium* (Fig. 1) were collected in the middle littoral of the Cassino beach (Rio Grande, RS-Brazil; 32°S) and San Antonio (Valparaíso, Chile; 33°S), respectively, during June 1991 (Fig. 2). In the laboratory, pieces of foot muscle and hepatopancreas were sampled from each individual and immediately stored at −80°C until electrophoretic analysis. The procedure for tissue homogenization and electrophoretic conditions were according to Marins et al. [15]. The protocols to detect the isozyme systems were those recommended by Harris and Hopkinson [8] and Levy [13]. The zymogram analysis followed the same pattern in all experiments, considering alleles with the same electrophoretic mobility, under the same conditions, as products from the same locus or from the same structural gene. The nomenclature of the loci follow those proposed by Shaklee et al. [24], while the allele denomination was according to Allendorf and Utter [1], using *M. mactroides* data as standard. Nei's [20] unbiased genetic distance (D) and unbiased genetic identity (I), allele frequencies and the usual genetic variability analysis (mean number of allele per locus, percentage of polymorphic loci and mean heterozygosity) were calculated by BIOSYS-1 computer program [26].

3. Results

For the 17 isozyme systems studied (Table 1), we observed 26 loci with 75 alleles, which were analysed and genetically interpreted (Table 2). Nine loci were considered polymorphic (at the 99% level) showing a total of 44 alleles, from which seven were shared by both species and 37 were different. Out of 17 monomorphic loci, 14 were fixed for different alleles and only three shared their alleles, resulting in a total of 31 alleles.

The statistical analysis based on allele frequency data showed a Nei's genetic distance of 1.90 and a genetic identity of 0.15. The genetic variability measures of Brazilian and Chilean samples are shown in Table 3.

4. Discussion

The two *Mesodesma* species (*M. mactroides* and *M. donacium*) analysed here inhabit dissipative beaches on both sides of South America, and they have similar morphological and adaptation aspects [16]. However, the genetic data here obtained indicate very high genetic differences among them, although the genetic variability analysis showed no significant differences between the two analysed species. Mean heterozygosity (observed and expected), mean number of alleles per locus and percentage of polymorphic loci were very similar between Brazilian and Chilean samples. The first important difference observed between both *Mesodesma* species was that they shared only 10 (13.3%) of the 75 identified alleles. Second, the low observed value of I (0.15), according to Thorpe [28], would correspond to genetic differences between different genera. In the same way, we observed a high genetic distance between both species, where D was 1.90.

Discrepancies between morphological and genetic affinities had been observed in bivalves. Passamonti et al. [22] analysed genetic relationships among four species of Tapetinae (Bivalvia, Veneridae): *Tapes philippinarun*, *Tapes decussatus*, *Venerupis aurea* and *Paphia undulata*. They found that *T. philippinarun* proved the most dissimilar to the congeneric *T. decussatus*, with a D value of 1.69. The authors suggested that the phyletic relationships of Tapetinae, commonly based on morphological grounds, should also be traced on additional and newer methodologies. Skibinski et al. [25] investigated systematic relationships in the marine mussels *Modiolus modiolus*, *Mytilus edulis* and *Mytilus galloprovincialis*. The *Modiolus–Mytilus* lineage represents an evolutionary transition from an endobyssate to epibyssate lifestyle, which has involved several modifications in the shell and umbone's position, and other changes, all of which are believed to be specializations for more powerful anchorage to the substrate. The

authors reported a low *I* value of about 0.21 and a high *D* value of about 1.55 between the lineages. However, the genetic data here obtained from comparing two species of the same genus (*M. mactroides* and *M. donacium*) showed higher genetic differences than those observed for *Modiolus–Mytilus* lineage which, according paleontological evidences, puts the time of divergence of *Modiolus* and *Mytilus* at between 200 and 300 million years ago.

The degree of genetic divergence between species is probably related to the time since reproductive isolation happened and their evolution in different environments. The ancestral stock of Mesodesmatidae, according to paleontological data, seems to have originated in Australasia, and gradually invaded South Africa, New Zealand, the Antarctic and, in successive migrations, South America. Von Ihering [9] observed the presence of the genus *Mesodesma* (*Mesodesma attenuata*) in Oamaru formation (New Zealand Tertiary), while that genus is absent in fossil records from Patagonia Tertiary deposits. This observation led von Ihering to consider that this genus appeared in South America in

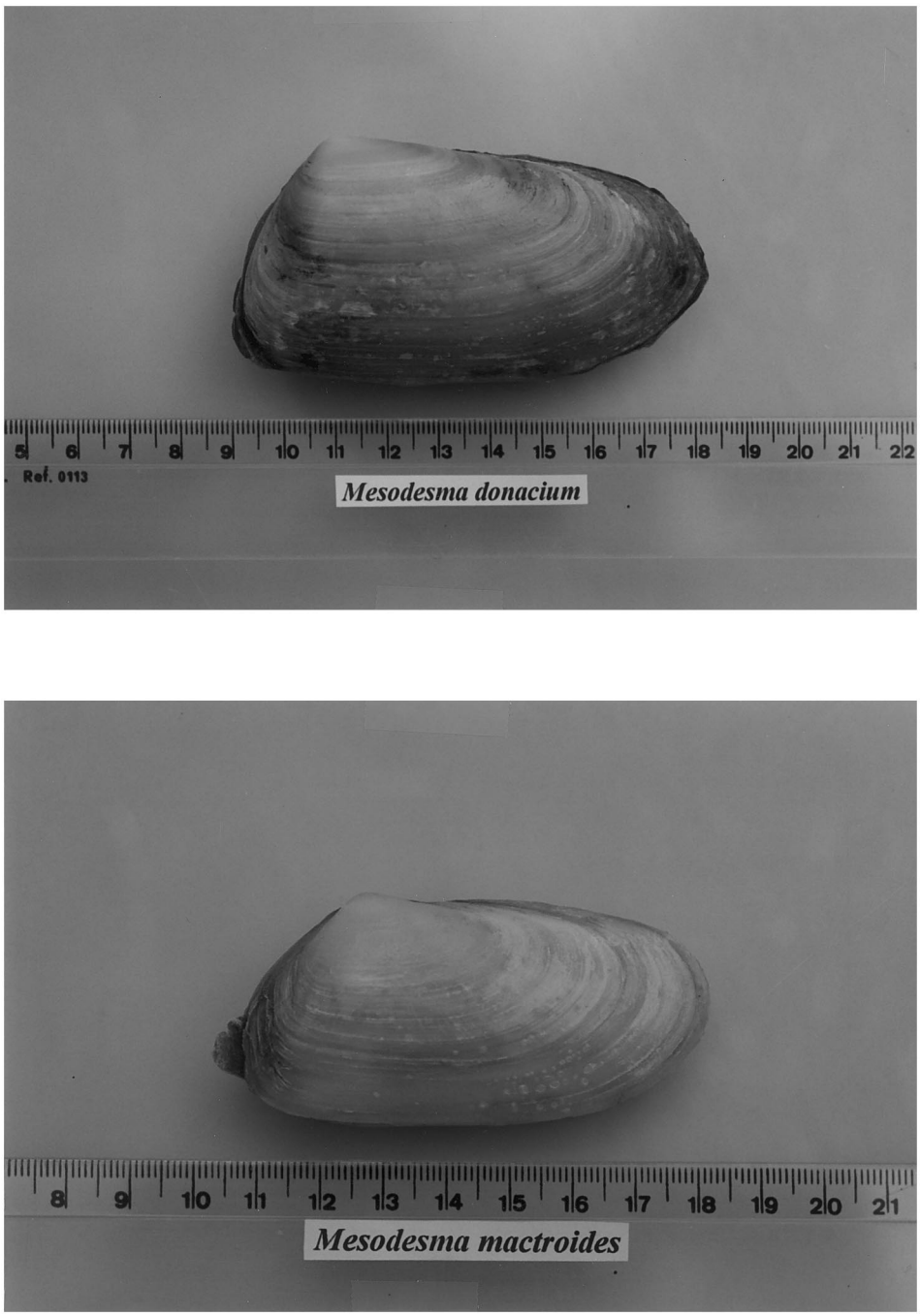

Fig. 1. The two marine bivalves representing the genus *Mesodesma* in South America, *M. mactroides* and *M. donacium*.

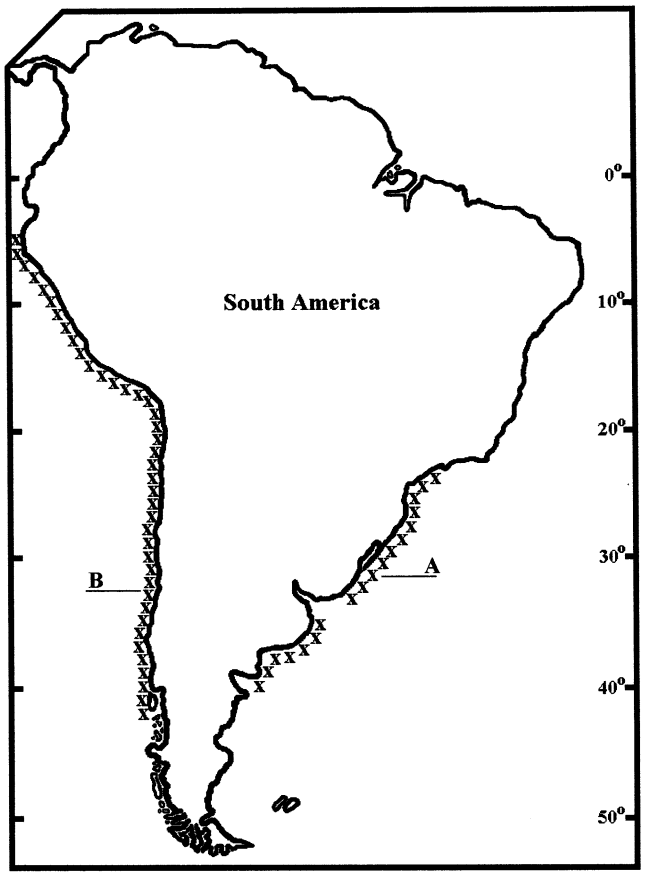

Fig. 2. Distribution of *M. mactroides* (East coast) and *M. donacium* (West coast) in South America. (A) *M. mactroides* sample point (Cassino Beach, Rio Grande, Brazil); (B) *M. donacium* sample point (San Antonio, Valparaiso, Chile).

the late Pliocene or possibly in the early Pleistocene, accompanying a considerable migration of Antarctic molluscs which colonized towards the north, modifying deeply the fauna of the Argentinean and Chilean coasts. This dispersion of the Antarctic marine fauna, according to von Ihering, was caused probably by decreasing temperatures towards the end of the Tertiary and early Quaternary periods, influencing molluscs habitats. In some cases, the molluscs adapted to the new environmental conditions and, in other cases, migrated north. Still, the dispersion of *Mesodesma* through the Patagonian coast occurred during the Pleistocene and has only recently encompassed the Brazilian littoral, delayed by a zoogeographic barrier, the mouth of Rio de La Plata, which could explain the small northern distribution of *M. mactroides*.

If the speciation process occurred together with the migration process, the reproductive isolation among *Mesodesma* species should have happened less than 12 million years ago (late Pliocene) and no earlier than 1 million years ago (early Pleistocene). On the contrary, if the speciation process occurred before the migration, the reproductive isolation should have happened more than 12 million years ago. Thorpe [28] showed that genetic distances obtained by Nei's coefficient present a clear correlation with the taxonomic divergence and with the reproductive isolating time of compared *taxa*, where a D value of 1.0 indicates a divergence time of 5 million years. The molecular clock hypothesis predicts that amino acid substitutions in protein molecules is an approximately regular, but stochastic, process and that consequently the number of substitutions occurring between homologous proteins may be related to evolutionary time. Thus, the genetic distance between *M. mactroides* and *M. donacium* calculated here ($D = 1.90$) would correspond to a reproductive isolating time of 9.5 million years. However, Yang et al. [31] hold that a D value of 1.0 is equivalent to 18 million years. In that case, the D value found by us would indicate a divergence time of 34 million years, supporting that the speciation process occurred before migration. In order to resolve that question, it would be necessary to use an internal calibration involving some well-defined biogeographic event or, at least, a calibration for some phylogenetically close species. Unfortunately, none of those possibilities were available to us. At any rate, we think that the genetic data reported here are an important first step in order to address the question about the systematic and the speciation process of the genus *Mesodesma* in South America. It is necessary to compare our data with other subgenera members and members of related genera in order to find shared characters (also using morphological data) which could be used to support the separation in different genera.

Table 1
Analysed enzymes comparing *M. mactroides* and *M. donacium*[a]

Enzyme		E.C.	Tissue
Acid phosphatase	*ACP*	3.1.3.2	H
Aldolase	*FBALD*	4.1.2.13	F
Alkaline phosphatase	*ALP*	3.1.3.1	H
Aspartate aminotransferase	*AAT*	2.6.1.1	F
Creatine kinase	*CK*	2.7.3.2	F
Esterases	*EST*	3.1.1.–	H
Glucose-6-phosphate isomerase	*GPI*	5.3.1.9	F
Glycerol-3-phosphate dehydrogenase	*G3PDH*	1.1.1.8	H
Isocitrate dehydrogenase	*IDHP*	1.1.1.42	H
Lactate dehydrogenase	*LDH*	1.1.1.27	F
Leucine aminopeptidase	*LAP*	3.4.11.–	H
Malate dehydrogenase	*MDH*	1.1.1.37	F
Malic enzyme	*MEP*	1.1.1.40	H
Phosphoglucomutase	*PGM*	2.7.5.1	F
6-Phosphogluconate dehydrogenase	*PGDH*	1.1.1.44	F
Superoxide dismutase	*SOD*	1.15.1.1	F
Xantine dehydrogenase	*XDH*	1.2.1.37	H

[a] E.C., Enzyme Commission number. F, foot muscle; H, hepatopancreas.

Table 2
Polymorphic and monomorphic *loci* observed in *M. mactroides* and *M. donacium*[a]

Polymorphic loci				Monomorphic loci			
Locus/allele		*M. mactroides* (*N* = 15)	*M. donacium* (*N* = 15)	Locus/allele		*M. mactroides* (*N* = 15)	*M. donacium* (*N* = 15)
*EST-1**				*AAT-1**			
	103	0.167	0.000	→	*100*	1.000	1.000
	102	0.000	0.100				
	100	0.600	0.000	*AAT-2**			
	98	0.000	0.900	→	*100*	1.000	1.000
	97	0.233	0.000				
				*ACP**			
*EST-2**					*100*	1.000	.000
→	*105*	0.100	0.133		*98*	0.000	1.000
→	*100*	0.833	0.067				
	98	0.000	0.633	*ALP**			
	97	0.000	0.100		*100*	1.000	.000
	95	0.033	0.000		*98*	0.000	1.000
→	*94*	0.033	0.067				
				*CK**			
*EST-3**					*100*	1.000	0.000
	104	0.000	0.233		*98*	0.000	1.000
	102	0.033	0.000				
→	*100*	0.967	0.467	*EST-5**			
	98	0.000	0.300		*100*	1.000	0.000
					98	0.000	1.000
*EST-4**							
	102	0.000	0.100	*FBALD**			
	100	0.833	0.000		*100*	1.000	0.000
	98	0.000	0.900		*98*	0.000	1.000
	97	0.133	0.000				
	95	0.033	0.000	*G3PDH**			
					102	0.000	1.000
*GPI**					*100*	1.000	0.000
	100	0.967	0.000				
	98	0.000	0.967	*LDH-1**			
	96	0.033	0.000		*102*	0.000	1.000
	94	0.000	0.033		*100*	1.000	0.000
*IDHP**				*LDH-2**			
	105	0.000	0.967		*100*	1.000	0.000
	103	0.033	0.000		*98*	0.000	1.000
→	*100*	0.967	0.033				
				*MDH-1**			
					102	0.000	1.000
*LAP-1**					*100*	1.000	0.000
	103	0.067	0.000				
→	*102*	0.033	0.033	*MDH-2**			
	100	0.867	0.000		*100*	1.000	0.000
	99	0.000	0.933		*98*	0.000	1.000
→	*98*	0.033	0.033				
				*MEP-1**			
					100	1.000	0.000
*PGDH**					*98*	0.000	1.000
	122	0.000	0.033				
	120	0.000	0.800	*MEP-2**			
	118	0.000	0.033		*102*	0.000	1.000
	116	0.000	0.067		*100*	1.000	0.000
	114	0.000	0.067				
	100	0.967	0.000	*SOD-1**			
	95	0.033	0.000		*102*	0.000	1.000
					100	1.000	0.000
*PGM**				*SOD-2**			
	103	0.067	0.000		*100*	1.000	0.000
	102	0.000	0.100		*98*	0.000	1.000
	100	0.833	0.000				
	98	0.000	0.900	*XDH-1**			
	97	0.100	0.000	→	*100*	1.000	1.000

[a] *N*, number of analysed individuals. Shared alleles are indicated by arrows (→).

Table 3
Genetic variability analysis of *M. mactroides* (Brazil) and *M. donacium* (Chile)[a]

	Mean number of alleles per locus	% Polymorphic loci ($P_{0,99}$)[b]	Mean heterozygosity	
			Observed	Expected
M. mactroides	1.6 (0.2)	34.6	0.072 (0.030)	0.077 (0.029)
M. donacium	1.7 (0.2)	34.6	0.087 (0.031)	0.093 (0.035)

[a] Standard errors are between parentheses.
[b] $P_{0,99}$, a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99.

It seems to us that it would be necessary to perform a deeper revision on mesodesmatid systematics, incorporating the genetic information provided by isozyme electrophoresis and, specially, by modern techniques of DNA analysis.

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