

KARYOTYPE STUDY IN THE SURF CLAM *MESODESMA DONACIUM*
LAMARCK, 1818 (BIVALVIA: VENEROIDA: MESODESMATIDAE)

ESTUDIO CARIOTIPICO EN LA MACHA MESODESMA DONACIUM
LAMARCK, 1818 (BIVALVIA: VENEROIDA: MESODESMATIDAE)

Gabriel Amar^{1*}, Claudio Palma Rojas², Elisabeth von Brand³
& Pedro Jara-Seguel⁴

¹Universidad Pedro de Valdivia, Facultad de Ciencias de la Salud, Cuatro Esquinas 060, La Serena, Chile.

²Universidad de La Serena, Departamento de Biología, Casilla 599, La Serena, Chile.

³Universidad Católica del Norte, Departamento de Biología Marina, Casilla 117, Coquimbo, Chile.

⁴Universidad Católica de Temuco, Escuela de Ciencias Ambientales, Casilla 15-D, Temuco, Chile.

*Corresponding author, e-mail: gabamar@gmail.com

ABSTRACT

Mesodesma donacium is a marine clam distributed from Sechura Bay (5°S) in northern Perú to Chiloé Island (43°S) in southern Chile. Due to the commercial importance of this species, their populations have been reduced in the later years. In this work the karyotype morphology of *M. donacium* is described for the first time, to be additional to taxonomical, ecological, morphological, and reproductive antecedents previously documented for the species. *M. donacium* shows a diploid karyotype $2n = 38$ with one metacentric, fifteen submetacentric and three subtelocentric chromosome pairs. The total haploid set length was 42.4 μm , and the mean chromosome size was 2.2 μm . *M. donacium* showed similitude in chromosome number with other species of the Veneroida order, but differences were found in morphology and size of the chromosomes. The cytogenetic relationships between *M. donacium* and other species of Veneroida is also discussed.

KEYWORDS: Veneroida, Mesodesmatidae, Cytogenetic relationship, chromosomes, karyotype morphology.

RESUMEN

Mesodesma donacium es un almeja distribuida desde Sechura Bay (5°S) en el Norte de Perú hasta la isla de Chiloé (43°S) en el sur de Chile. Debido a la importancia económica de esta especie, su población ha sido reducida en los últimos años. En este trabajo se describe por primera vez la morfología cariotípica de *M. donacium*, como antecedentes adicionales taxonómicos, ecológicos, morfológicos y reproductivos descritos previamente para la especie. *M. donacium* muestra un cariotipo diploide $2n=38$ con un par metacéntrico, quince submetacéntricos y tres pares cromosómicos subtelocéntricos. La longitud total del set haploide fue de 42,4 μm y el tamaño cromosómico medio fue de 2,2 μm . *M. donacium* mostró una similitud en el número cromosómico con otras especies del Orden Veneroida, sin embargo fueron encontradas diferencias en tamaño y morfología de los cromosomas. Se discuten las relaciones citogenéticas entre *M. donacium* y otras especies de Veneroida.

PALABRAS CLAVES: Veneroida, Mesodesmatidae, Relaciones citogenéticas, cromosomas, cariotipo.

INTRODUCTION

Mesodesmatidae is represented in Chile by *Mesodesma donacium* Lamarck 1818 and *Ervilia producta* Odhner 1922. *M. donacium* has an extensive latitudinal distribution from Sechura Bay in northern Perú to Chiloé Island in southern Chile (5°S-43°S) (Osorio & Bahamonde 1970), whereas *Ervilia producta* Odhner 1922 has restricted distribution in the coasts of the Juan Fernández archipelago (Osorio & Bahamonde 1970).

In Chile, *M. donacium* is the most known species of the family due to its commercial importance, but in the later years their populations have been reduced due to superextraction. At present, the biological information for *M. donacium* is scarce where only ecology, fishery, morphology, taxonomy, and reproduction have been the most studied field (Osorio & Bahamonde 1968; Osorio 1979; Brown & Guerra 1979; Peredo *et al.* 1987; Fuentes 1988; Ortiz & Stotz 1996, 2003; Joo & Dupré 2002). Besides, preliminary genetic studies in *M. donacium* have included isozyme analysis for five populations from northern, central and southern Chile (Urriola & von Brand 2003), but the gap on genome information is still prevalent for their populations. Genome data are crucial to elaborate programs trends to the conservation of those species of commercial importance whose populations have been reduced by super-extraction. Thus, the chromosome studies may be a basis to understand detail on genome organization in the clam *M. donacium*, such as has been previously documented for other bivalve species of the Veneroida order (see a revision in Méndez *et al.* 2001 and Thiriot-Quévèreux 2002).

In order to know the first cytogenetic antecedent for *M. donacium*, in this work the karyotype morphology is described and its cytogenetic relationship with other species of the order Veneroida is also discussed.

MATERIALS AND METHODS

Adult specimens of *Mesodesma donacium* were obtained from Tongoy and Coquimbo Bays (30°S), in Central Chile. Gametes were obtained by stripping the gonads by making superficial scalpel incisions near the foot and removing gametes by pipette. Samples from each individual were examined by light microscopy to determine the sex of gametes obtained, as the gonads do not demonstrate sexual dimorphism. Gametes from 3 to 5 mature females for *in vitro* fertilization were

suspended in 400 ml of filtered seawater at room temperature (~20°C). Spermatozoa from two to three mature males were separately collected into 100 ml of the same water above mentioned and were then mixed with the eggs. The fertilization and early development stages were monitored by light microscopy. When the embryos reached the 8 to 16 cell stage they were treated with 0.05% Colchicine in seawater diluted to 70% of its original salinity using distilled water for 90 min. For fixation and extraction of vitellum the embryos were treated with ethanol-chloroform-acetic acid (6:3:1 v/v) for 24 h, and then preserved in methanol-glacial acetic acid (3:1 v/v) until require.

Embryos staining were carried out using the Feulgen reaction as modified by Navarrete *et al.* (1983). Squash preparations were made of the embryos and coverslips were raised using freezing with CO₂. The embryos were then counterstained using 2% Giemsa in pH 6.8 phosphate buffer.

In microphotographs of ten metaphase plates the short arms (SA) and long arms (LA) were measured and the relative length of each chromosome-arm (expressed as percentage of the total haploid set length) was calculated. Besides, total haploid set length (THL in μm) and mean chromosome size (CZ in μm) were determined for *M. donacium*. The values for mean relative length for SA and LA (\pm confidence interval at 95%) were displayed in a Karyo-Idiogram (Spotorno 1985). The karyotype was constructed according to decreasing chromosome length and chromosome morphology on the basis of the categories proposed by Levan *et al.* (1964).

RESULTS

The gametes obtained by stripping were viable, and *in vitro* fertilization occurred with no adverse complications. A modal chromosome number $2n = 38$ was obtained for *M. donacium* when 100 metaphase plates were counted. Besides, four meiotic cells were observed which showed 19 bivalents (data not shown). The karyotype of *Mesodesma donacium* is composed of one metacentric, fifteen submetacentric and three subtelocentric chromosome pairs (Figs. 1 and 2). Secondary constrictions were not observed in the chromosomes. The pair 1 (LR = 7.59%) is the only metacentric chromosome within the karyotype. The total haploid set length for *M. donacium* was 42.2 μm and the mean chromosome size was 2.2 ± 0.53 μm . Quantitative karyotype data are shown in Table I.

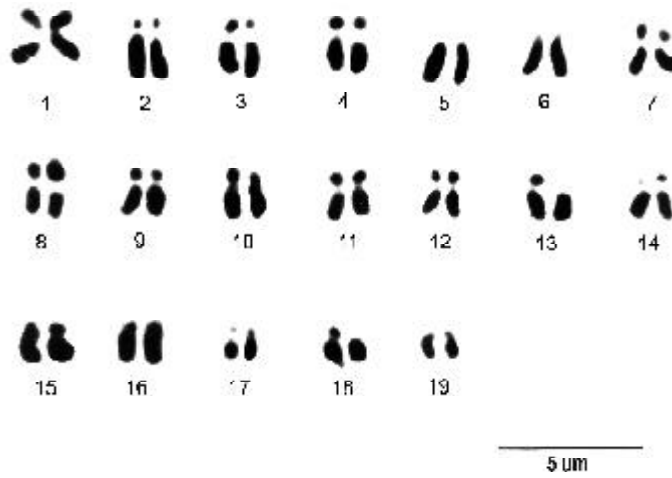


FIGURE 1: Karyotype of *Mesodesma donacium*, $2n = 38$ (1M + 15SM + 3 ST).

FIGURA 1: Cariotipo de *Mesodesma donacium*, $2n = 38$ (1M + 15SM + 3 ST).

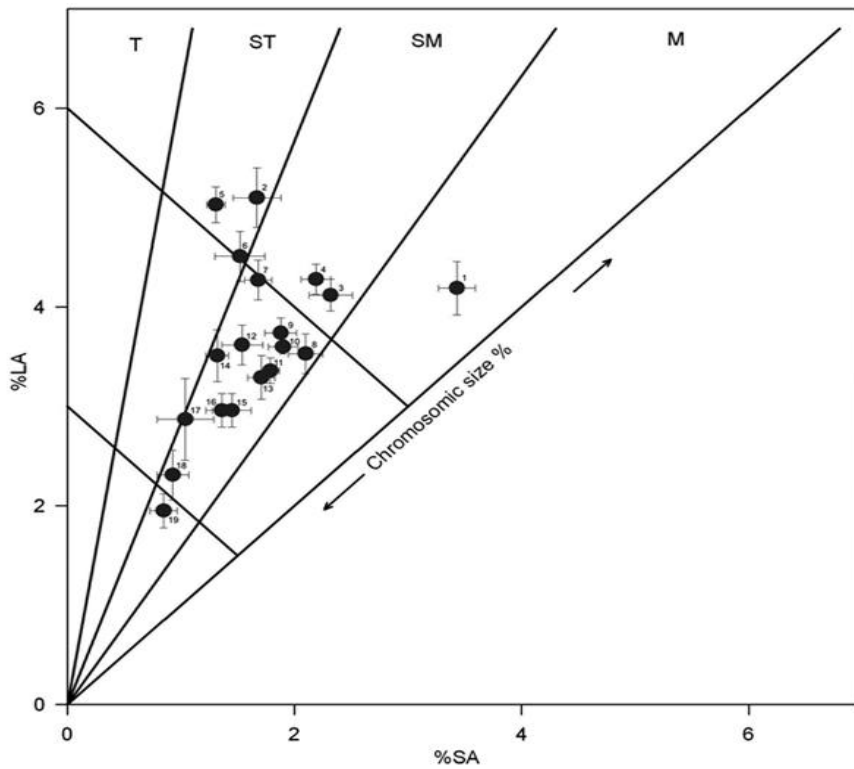


FIGURE 2: Karyo-Idiogram that represents of the relative length of the short arm (SA) and the long arm (LA) of each chromosome pairs of *Mesodesma donacium*, $n = 19$. M = metacentric, SM = submetacentric, ST = subtelocentric, T = telocentric. Spots represent centromere position and the bars represent 95% confidence intervals for SA and LA.

FIGURA 2: Cario-Idiograma que representa la longitud relativa del brazo corto (SA) y del brazo largo (LA) de cada par cromosómico en *Mesodesma donacium*, $n = 19$. M = metacéntrico, SM = submetacéntrico, ST = subtelocéntrico, T = telocéntrico. Puntos negros representan la posición del centrómero y las barras representan el 95% de intervalo confianza para SA y LA.

TABLE I: Chromosome measurements on *Mesodesma donacium*. SA, length of the short arm; LA, length of the long arm; C, total chromosome length; s.d., standard deviation; CI, 95% confidence interval.TABLA I: Medidas cromosómicas en *Mesodesma donacium*. SA, longitud del brazo corto; LA, longitud del brazo largo; C, longitud cromosómica total; s.d., desviación estándar; CI, 95% intervalo de confianza.

Chr. pair	Absolute arm lengths (μm)			Relative arm lengths (%)				Type
	SA \pm s.d.	LA \pm s.d.	C \pm s.d.	SA \pm s.d.	CI	LA \pm s.d.	CI	
1	1.42 \pm 0.29	1.73 \pm 0.37	3.15 \pm 0.64	3.40 \pm 0.24	0.16	4.19 \pm 0.42	0.27	M
2	0.75 \pm 0.19	2.07 \pm 0.56	2.82 \pm 0.60	1.67 \pm 0.32	0.21	5.10 \pm 0.46	0.30	ST
3	0.94 \pm 0.35	1.84 \pm 0.40	2.78 \pm 0.51	2.32 \pm 0.29	0.19	4.12 \pm 0.24	0.16	SM
4	0.89 \pm 0.39	1.85 \pm 0.44	2.74 \pm 0.56	2.19 \pm 0.20	0.13	4.28 \pm 0.23	0.15	SM
5	0.62 \pm 0.23	2.03 \pm 0.45	2.65 \pm 0.50	1.31 \pm 0.13	0.08	5.03 \pm 0.27	0.18	ST
6	0.64 \pm 0.23	1.89 \pm 0.40	2.53 \pm 0.46	1.52 \pm 0.33	0.22	4.51 \pm 0.39	0.25	ST
7	0.78 \pm 0.24	1.63 \pm 0.36	2.42 \pm 0.50	1.68 \pm 0.19	0.12	4.27 \pm 0.30	0.20	SM
8	0.88 \pm 0.36	1.55 \pm 0.24	2.43 \pm 0.44	2.10 \pm 0.23	0.15	3.53 \pm 0.31	0.20	SM
9	0.74 \pm 0.18	1.64 \pm 0.31	2.38 \pm 0.40	1.88 \pm 0.22	0.14	3.74 \pm 0.22	0.15	SM
10	0.68 \pm 0.20	1.63 \pm 0.40	2.31 \pm 0.38	1.90 \pm 0.20	0.13	3.60 \pm 0.10	0.07	SM
11	0.75 \pm 0.26	1.51 \pm 0.22	2.25 \pm 0.42	1.54 \pm 0.27	0.18	3.62 \pm 0.30	0.20	SM
12	0.78 \pm 0.12	1.46 \pm 0.34	2.24 \pm 0.43	1.79 \pm 0.11	0.08	3.36 \pm 0.17	0.13	SM
13	0.72 \pm 0.11	1.39 \pm 0.45	2.11 \pm 0.48	1.71 \pm 0.18	0.12	3.29 \pm 0.33	0.22	SM
14	0.58 \pm 0.13	1.40 \pm 0.45	1.98 \pm 0.45	1.32 \pm 0.16	0.10	3.51 \pm 0.39	0.26	SM
15	0.62 \pm 0.19	1.33 \pm 0.39	1.95 \pm 0.47	1.45 \pm 0.22	0.17	2.96 \pm 0.22	0.17	SM
16	0.53 \pm 0.14	1.36 \pm 0.25	1.90 \pm 0.31	1.36 \pm 0.16	0.14	2.96 \pm 0.20	0.17	SM
17	0.54 \pm 0.35	1.06 \pm 0.22	1.60 \pm 0.25	1.04 \pm 0.30	0.25	2.87 \pm 0.48	0.41	SM
18	0.40 \pm 0.13	0.92 \pm 0.22	1.32 \pm 0.33	0.93 \pm 0.21	0.14	2.31 \pm 0.38	0.25	SM
19	0.39 \pm 0.09	0.86 \pm 0.23	1.26 \pm 0.30	0.85 \pm 0.12	0.12	1.95 \pm 0.17	0.17	SM

DISCUSSION

Within the bivalve class, the most studied karyotype characters are $2n$ number, chromosome morphology and chromosome size. In this work, those three characters were determined in the karyotype of *M. donacium*.

The chromosome number of *M. donacium* counted here is coincident with the most frequent number $2n = 38$ so far documented for other 16 species of Veneroida (Méndez *et al.* 2001, Thiriot-Quévieux 2002, Jara-Seguel 2007). Besides, meiotic cells with 19 bivalents corroborate that $2n$ number. These data suggest conservatism in the chromosome number within the order Veneroida on the basis of the nine families world-wide studied.

The most actualized cytogenetic revisions so far documented for Veneroida have discussed the polymorphism in chromosome morphology and chromosome size present among the species. Following that tendency, the karyotype morphology described here for *M. donacium* is different to the

karyotypes described for other species of the order, where intra-specific variation has also been observed in several species of the families Cardiidae, Tellinidae and Veneridae (Méndez *et al.* 2001, Thiriot-Quévieux 2002). Furthermore, the total haploid set length (THL = 42.4 μm) and mean chromosome size (CZ = 2.2 μm) described here for *M. donacium* are lower to the values described for other species of the order with 38 chromosomes, such as *Donax trunculus* (THL = 114.28 μm , CZ = 6.01 μm) (Donacidae) (Cornet & Soulard 1990a), *Macoma balthica* (THL = 84.58 μm , CZ = 4.45 μm) (Tellinidae) and *Tellina tenuis* (THL = 104.97 μm , 5.24 μm) (Tellinidae) (Cornet & Soulard 1990b). Those differences in chromosome sizes observed among *M. donacium* and the Veneroida species mentioned above may be related with inter-specific variations in nuclear DNA content (2C-value). Nevertheless, although *D. trunculus* and *M. balthica* have longer chromosome size and high 2C-values of 3.19 pg and 4.6 pg, respectively (Méndez *et al.* 2001), the expected positive co-relation between both variable is not evident. At present, 2C-values

for 21 Veneroida species with 38 chromosomes have been described and vary in a wide range from 2.2 pg to 4.6 pg (Méndez *et al.* 2001, Thiriot-Quévieux 2002). In the future to much remain to be done on descriptive and molecular cytogenetics in Chilean taxa of Mesodesmatidae including *M. donacium* and *E. producta*. Besides, the nuclear DNA content is also necessary to estimate for both species.

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