

DNA content, karyotypes, and chromosomal location of 18S-5.8S-28S ribosomal loci in some species of bivalve molluscs from the Pacific Canadian coast

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Abstract

The DNA content of 10 species of bivalve molluscs from British Columbia coast was determined by image analysis, and the karyotypes of the horse clam *Tressus capax*, the bent-nose macoma *Macoma nasuta*, and the nuttall's mahogany clam *Nuttallia nuttallii* are described here for the first time. We also have analyzed the location of rDNA loci using a 28S-5.8S-18S probe in four of these species: *Mytilus californianus*, *M. trossulus*, *Macoma nasuta* and *N. nuttallii*. Results obtained report new data about cytogenetic characteristics of bivalve molluscs.

Keywords: Mollusca; bivalves; C-value; karyotype; ribosomal loci.

Introduction

The Mollusca are the second most abundant phylum of animals that inhabit the Earth. Belonging to this phylum, bivalves are represented by approximately 15 000 different species distributed throughout aquatic habitats. Karyotypes from only 200 species have been determined, and chromosome banding techniques have been applied in a scarce number of bivalves. These techniques were found to be very useful in the analysis of the distribution and composition of heterochromatin, and for localizing the nucleolar organizer regions (NORs) in mussels (Insua et al. 1994; Martínez-Lage et al. 1994, 1995, 1997a; Pasantes et al. 1996) and oysters (Insua and Thiriot-Quievreux 1993; Ladrón de Guevara et al. 1994; Li and Havenhand 1997). Other techniques such as fluorochrome staining were only employed in a very scarce number of mussels species (Martínez-Lage et al. 1994, 1995; Martínez-Expósito et al. 1997). Recently, in situ hybridization has been employed to localize the 18S-28S ribosomal genes in some species of Mytilidae (Insua and Méndez 1998; Torreiro et al. 1999) and the 5S ribosomal genes in one species of scallops and one of cockles (Insua et al. 1998, 1999).

Cytogenetics on bivalves from Pacific coasts are restricted to (1) the description of the chromosome number in different species of mussels, oysters, and clams (Menzel and Menzel 1965; Ahmed and Sparks 1967; Longwell et al. 1967; Menzel 1968), (2) the description of chromosome number and chromosome morphology of the mussels *Mytilus californianus* and *M. edulis* ecotype *M. trossulus* (Ahmed and

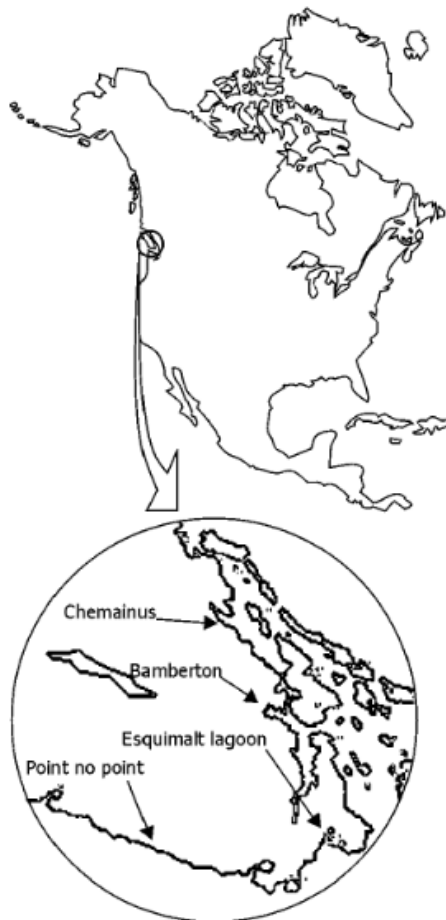
Sparks 1970), (3) the determination of the DNA content in a great number of mollusc species (Hinegardner 1974), and (4) the analysis of allozymic characters in the oyster *C. virginica* (Singh and Zouros 1978) and mussels belonging to genus *Mytilus* (McDonald and Koehn 1988; Sarver and Foltz 1993). More recent studies examined the karyotypes and chromosomal location of Ag-NORs in *M. californianus* and *M. trossulus* (Martínez-Lage et al. 1997a), the distribution of the telomeric sequence AATGGG (Guo and Allen 1997), satellite DNA sequences in the Pacific oyster *Crassostrea gigas* (Clabby et al. 1996), and the existence of polyploidy in a natural population of the mussel *M. trossulus* (González-Tizón et al. 2000). Phylogenetic molecular studies have been carried out in some species of the *M. edulis* complex (Heath et al. 1995; Kenchington et al. 1995; Heath et al. 1996; Heath and Hilbish 1998).

In this study we provide new data about some cytogenetic characteristics of 10 bivalve species from Pacific Canadian coasts. Data obtained come from the quantification of DNA content, the elaboration of karyotypes, and the location of rDNA genes by fluorescence in situ hybridization (FISH).

Material and methods

Samples collection

Figure 1 shows the localities on Vancouver Island (British Columbia, Canada) where the samples were collected: *Chlamys hastata* and *Hinnites giganteus* from Chemainus; *Macoma nasuta*, *Nuttallia nuttallii* (= *Sanguinolaria nuttallii*), *Tressus capax*, *Protothaca staminea*, *Crassostrea gigas*, and *Tapes philippinarum* (= *Tapes japonica*, = *Ruditapes philippinarum*) from Bamberton beach; *Mytilus californianus* from Point no Point, and *Mytilus trossulus* from Esquimalt Lagoon.



Once in the laboratory, animals were placed in tanks with filtered sea water and fed continuously on a suspension of *Isochrysis sp.*, and *Tetraselmis sp.* microalgae for 10–15 days. Metaphase obtention was made by treatment with colchicine solution (0.005%) for 6–8 h. Then, gills were dissected, treated twice with 0.56% KCl solution (15 min) and fixed in ethanol – glacial acetic acid (3:1). Fixed cells were dissociated in 45% acetic acid with water solution and dropped onto heated slides at 43°C. Metaphases were stained with 4% Giemsa in phosphate buffer pH 6.8 and photographed with a Nikon Optiphot microscope.

Figure 1. Geographic locations on Vancouver Island, Canada, where bivalve species were collected.

DNA content

For DNA measurements, metaphase plates were stained by Feulgen method (Feulgen and Rossenbuck 1924) as follows: treatment with 5M HCl, 30 min at room temperature and washed in distilled water; staining with Schiff's reagent, 80 min, followed by three washes (10 min each) in sulphurous water. Finally, slides were air-dried in a dark place until needed.

Five individuals from each one of the species and 40 nuclei from each individual were measured. Measurements of DNA content were done microdensitometrically using the GENIAS program v.4.0, included in the software image analysis system Magiscan (Applied Imaging, England). This software captures a black and white image from the microscope and it analyzes the different structures apparent in the image. The initial step of the analysis consists of splitting this image and depicting it as a binary image, i.e., assigning to each pixel (basic unity of image) a value of 1 if the pixel is black, or a value of 0 if the pixel is white. This function allowed the computer to identify the structures for measurement. The user may modulate this function to the computer such as to identify only the nuclei stained with Feulgen (structures to measure). Furthermore, the GENIAS program calculates the optic density (OD), according to the formula $OD = \log_{10}(1/T) = -\log_{10}T$; T = intensity of transmitted light \times intensity of incident light⁻¹. From this estimation, the computer integrates the values of OD obtained for each one of the pixels and it calculates the IOD ($IOD = \Sigma OD$). Observation of these values is carried out with a microscope Microphot FXA, operating at a wavelength of 560 nm, equipped with a Nikon apochromatic 100 \times objective. This microscope also employs a Bosch camera, which is connected to the image analysis system.

As standard we used the bivalve species *Donax trunculus*. This species has a value of IOD (B_{Donax}) that represents a DNA content of 3.19 ± 0.06 pg (represented by A), and was previously determined by Martínez-Lage et al. (1997b). The value of IOD obtained for each of the species analyzed (represented by B_{sp}) were transformed to picograms according to the formula $C_{sp} = A \times B_{sp} / B_{Donax}$, where C_{sp} represents the picograms of the corresponding species.

Chromosome analysis

Chromosome measurements were made using the GENIAS program 4.0, included in Magiscan. Total chromosome length was measured in 10 metaphases of gill tissue from each one of the species. The mean value of the length of the chromosome arms and the mean value for their total chromosome length were calculated for each one of the chromosome pairs. The relative length ($100 \times$ chromosome length \times total haploid length⁻¹), the centromeric index ($100 \times$ length of short arm \times total chromosome length⁻¹), the mean value and the standard error (standard deviation \times number of individuals⁻¹)^{1/2} of the relative lengths, and the centromeric index were also calculated. Chromosome nomenclature follows Levan et al. (1964).

Fluorescence in situ hybridization

The DNA probe used was pDm 238 from *Drosophila melanogaster* containing the repeat unit 18S-5.8S-28S rDNA (Roiha et al. 1981) labelled with digoxigenin-11-dUTP using a Nick Translation Kit (Boehringer Mannheim). Chromosomal DNA was denatured by immersing the slides in DNAase-free RNAase (100 μ g/mL in $2 \times$ SSC) for 1h at 37°C, followed by incubation in pepsin (10% in 100 mM HCl), post-fixed in formaldehyde (1% in PBS 50 mM) for 10 min, washed in $2 \times$ SSC for 10 min and, finally, dehydrated in graded ethanol series and air-dried.

The hybridization solution (consisted of 50% formamide in $2 \times$ SSC, 10% dextran sulphate, 0.33% SDS, 10 μ g salmon sperm DNA, and 100 ng of labelled DNA probe) was denatured for 15 min at 75°C. Then, 30 μ L of this hybridization solution was applied to each slide under a sealed coverslip. Hybridization was performed in a slide-PCR (MJ Research, MJ 100) as follows: 7 min at 75°C, 2 min at

55°C, 30 s at 50°C, 1 min at 45°C, 2 min at 42°C, 5 min at 40°C, 5 min at 38°C, 5 min at 37°C, and, finally, overnight at 37°C in a moist chamber.

Post-hybridization washes were carried out for 5 min at 42°C in 2× SSC, 10 min in 20% formamide and 0.2× SSC, 5 min in 0.1× SSC, 5 min in 2× SSC and, finally, 5 min in 0.1 M Tris, 0.15 M NaCl, 0.05% Tween-20. After blocking the slides for 30 min in 0.1 M Tris-HCl, 0.15 M NaCl, and 0.5% Boehringer Mannheim blocking reagent, probe hybridization sites were detected by immunocytochemical incubations in mouse anti-digoxigenin, rabbit anti-mouse-FITC (fluorescein isothiocyanate) and goat anti-rabbit-FITC.

Chromosomes were counterstained with propidium iodide (50 ng/mL anti-fade) and visualized and photographed using a Nikon Microphot AFX microscope equipped with the appropriate filters. The film used was Kodak Ektachrome 400 ASA.

Results and discussion

DNA content

The DNA content, or C-value, is a species-specific genetic characteristic, defined as the amount of DNA per haploid nucleus. DNA content has been determined in several groups of animal and plant species (see Cavalier-Smith 1978 for review). In bivalves, the most extensive analysis was made by Hinegardner (1974), who determined the DNA content in 55 species belonging to 22 families. Cavalier-Smith (1978), in a revision of all previous data, pointed out that the DNA content in bivalves ranges from 0.43–5.4 pg. Recently, Ieyama et al. (1994) determined the DNA content in 10 bivalve species from Japanese coasts and Rodríguez-Juiz et al. (1996) determined it for 21 bivalve species from the northwest of the Iberian Peninsula.

Our results are shown in Table 1. Five of the species analyzed belong to the subclass Pteriomorpha and the other five to the subclass Heterodonta. The values of DNA content showed in the present work are included in the range described by Cavalier-Smith (1978) for mollusc species, and vary from 1.82 pg for the oyster species *C. gigas* to 3.94 pg for the clam *Tapes philippinarum*. It supposes an approximately two-fold difference in the mean value for DNA content among these two species. It could be observed that values for DNA content in Heterodonta species show homogeneity in mean genome size; i.e., values range from 3.27 pg (the lowest value) for *T. capax* to 3.94 pg (the highest value) for *T. philippinarum*. But, there was significant heterogeneity in mean genome size of species belonging to Pteriomorpha; values vary from 1.82 pg (the lowest) for *C. gigas* to 3.28 pg (the highest) for *C. hastata*; while *H. giganteus* has a DNA content of 2.57 pg, *M. californianus* has 3.21, and *M. trossulus* 3.02.

Table 1 also shows the diploid chromosome number of the species analyzed (unknown in *H. giganteus*). The more frequent chromosome number in Heterodonta species results as $2n = 38$ (*M. nasuta*, *N. nuttallii*, *P. staminea*, and *T. philippinarum*). Only *T. capax* has 34 chromosomes. Pteriomorpha species show more heterogeneity in the chromosome number than Heterodonta, similar to the case of DNA content. So, Mytilidae species have 28 chromosomes, *C. gigas* shows 20 chromosomes, and *C. hastata* possess 38 chromosomes. Analyzing both cytogenetic characteristics, It is obvious that species belonging to the same family (Veneridae, Mytilidae, and Pectinidae) show differences in DNA content, despite the uniformity in chromosome number ($2n = 28$ for Mytilidae and $2n = 38$ for Veneridae). These differences also appear between species belonging to same genera (*M. californianus* and *M. trossulus*).

Table 1. DNA content of species analyzed.

Species	Family	pg DNA	2n
Subclass Heterodonta			
<i>Tressus capax</i>	Mactridae	3.27 ± 0.09	34
<i>Macoma nasuta</i>	Tellinidae	3.90 ± 0.09	38
<i>Nuttallia nuttallii</i>	Psammobiidae	3.48 ± 0.08	38
<i>Protothaca staminea</i>	Veneridae	3.50 ± 0.09	38 ^a
<i>Tapes philippinarum</i>	Veneridae	3.94 ± 0.12	38 ^b
Subclass Pteriomorphia			
<i>Mytilus californianus</i>	Mytilidae	3.21 ± 0.07	28 ^c
<i>Mytilus trossulus</i>	Mytilidae	3.02 ± 0.05	28 ^c
<i>Crassostrea gigas</i>	Ostreidae	1.82 ± 0.04	20 ^d
<i>Chlamys hastata</i>	Pectinidae	3.28 ± 0.10	38 ^a
<i>Hinnites giganteus</i>	Pectinidae	2.57 ± 0.12	Unknown

Note: 2n, diploid chromosome number; ^aUnpublished data; ^bIeyama (1985); ^cMartínez-Lage et al. (1997a); ^dAhmed and sparks (1967)

On the other hand, one hypothesis offered to explain the evolutionary significance of genome size is that generalized species (Psammobiidae, Veneridae, and Mytilidae) tend to possess a high DNA amount, while the specialized ones (Mactridae, Tellinidae, and Ostreidae) possess low DNA content (Hinegardner 1974). This assumption could be applied for some of the species analyzed here (e.g., *C. gigas* or *T. philippinarum*), but it can not be assumed for species such as *C. hastata* or *T. capax*. Subsequently, the relationship between specialization and low DNA content (or generalization and high DNA content) cannot be considered a universal rule, and, in this sense, some cases of evolution through increased DNA have been reported (John and Miklos 1988).

We think that the variation in genome size is caused by changes in the DNA involving repetitive and non repetitive sequences. The genetic mechanisms implied could be gene loci duplications, changes in number and size of repetitive sequences, and structural and (or) chromosome rearrangements.

Chromosome number and karyotypes

Chromosome analysis and karyotypes of the species *T. capax*, *M. nasuta*, and *N. nuttallii* are described for the first time. The diploid chromosome number in the species *T. capax* (family Mactridae) was calculated to be $2n = 34$ (Fig. 2a). Table 2 displays relative length and centromeric index values. Relative length varies from 7.41–4.35, and karyotype (Fig. 3a) is composed of 10 metacentric chromosome pairs (Nos. 1, 2, 6, 7, 8, 11, 12, 14, 15, and 16), and seven submetacentric pairs (Nos. 3, 4, 5, 9, 10, 13, and 17). Neither subtelocentric nor telocentric chromosomes have been observed.

Tressus capax shows a chromosome number different from other species belonging to the same family. So, *Labiosa plicatela* from Virginia coasts (U.S.A.), analyzed by Menzel (1968), and *Spisula solidissima* (Ropes 1972) possess a chromosome diploid complement of 36, whereas three different species of *Mactra* clams (Ieyama 1982; Corni and Trentini 1987), *Spisula subtruncata* (Corni and Trentini 1987), and *Mulinia lateralis* (Wada et al. 1990), from Delaware and Virginia, show 38 chromosomes. A chromosome number of 34 is not usual in bivalves, so only three species, belonging to the family Myoidea, show this chromosome number: *Mya arenaria* (Allen et al. 1982), *Barnea truncata*,

and *Cyrtopleura costata* (Menzel 1968). However, the only species of Macridae in which the karyotypes were described are *Mactra chinensis* (Wada and Komaru 1993) and *M. lateralis* (Wada et al. 1990), and both have 38 chromosomes. The karyotype of *M. chinensis* does not have metacentric chromosomes, while the entire chromosome complement of *M. lateralis* consists of subtelocentric and telocentric chromosomes. The karyotype of *T. capax* is very different from both of these species. These differences could be the consequence of chromosome rearrangements along evolution of these species, such as Robertsonian fusions, although a more exhaustive and detailed study must be carried out to confirm this possibility.

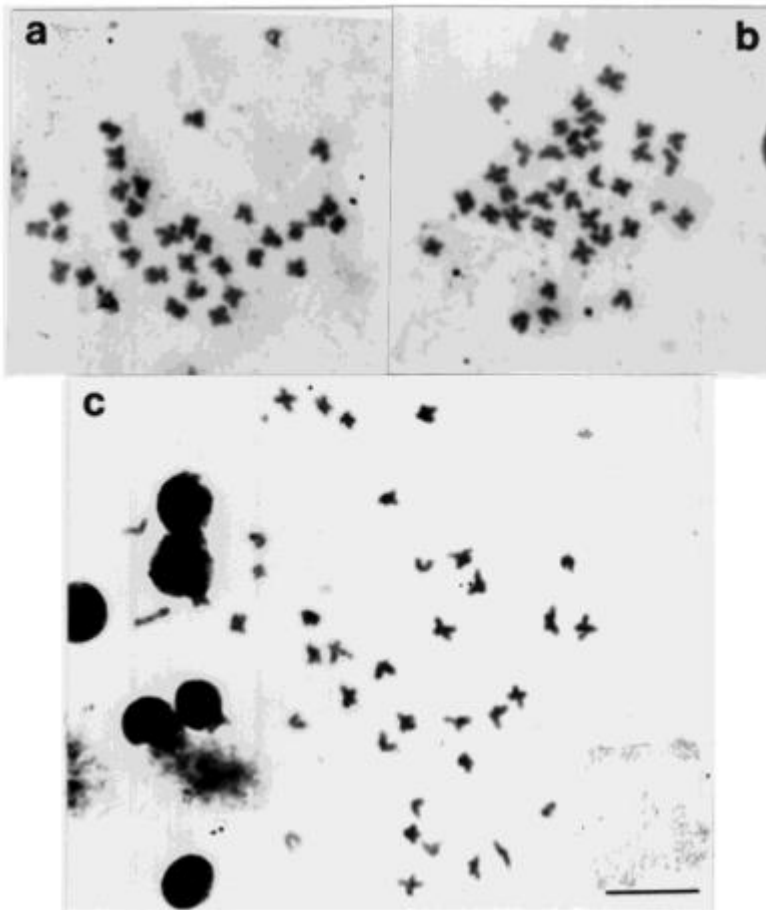


Figure 2. Giemsa metaphases of: (a) *Tressus capax*, (b) *Macoma nasuta*, and (c) *Nuttallia nuttallii*.

Chromosome number in *M. nasuta* is 38. As can be observed in Table 2, relative length values vary from 6.67 – 3.13. Karyotype (Fig. 3b) consists of eight metacentric pairs (Nos. 1, 3, 4, 5, 8, 12, 16, and 17), four submetacentric pairs (Nos. 6, 7, 10, and 14), two submetacentric–subtelocentric pairs (Nos. 2 and 11), one subtelocentric–submetacentric pair (No. 9), and four subtelocentric pairs (Nos. 13, 15, 18, and 19).

Macoma nasuta shows the same chromosome number as other species belonging to the family Tellinidae, but the karyotype is different from those described for

M. balthica and *Tellina tenuis* from different European populations (Cornet and Soulard 1990a; Wolowicz and Thiriou-Quievreux 1997). Therefore, the number of metacentric pairs in *M. nasuta* is smaller than in *M. balthica*, although metacentric chromosomes of the Canadian species are larger than those from the European ones.

Finally, the clam *N. nuttallii*, with a diploid chromosome number of 38, shows a relative length that varies from 7.15 – 3.48 (Table 2). Karyotype (Fig. 3c) is composed of seven pairs of metacentric chromosomes (Nos. 1, 3, 5, 8, 10, 15, and 16), 11 pairs of submetacentric (Nos. 2, 4, 6, 7, 9, 11, 13, 14, 17, 18, and 19) and one pair of submetacentric–subtelocentric chromosomes (No. 12).

To date, there is no reference about karyological data in species belonging to the family Psammobiidae. Thus, *N. nuttallii* analyzed in this work is the first one in which the chromosome number and karyotype is described. Chromosome number of *N. nuttallii* is coincident with the modal number of the species belonging to the superfamily Tellinaceae, and karyotype is similar to those described in other different European species belonging to this superfamily (Cornet and Soulard 1987; Cornet and Soulard 1990b).

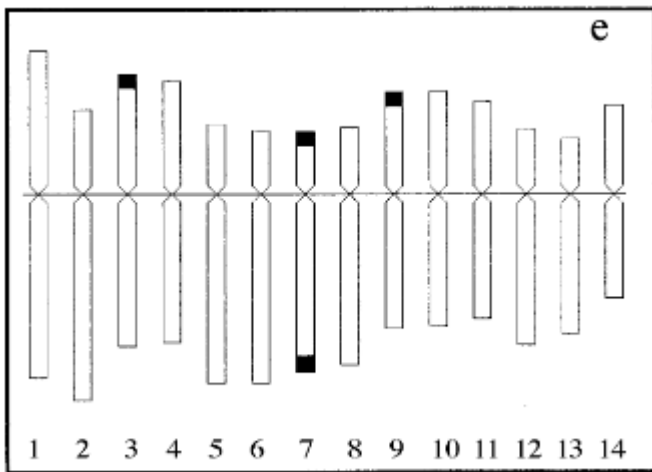
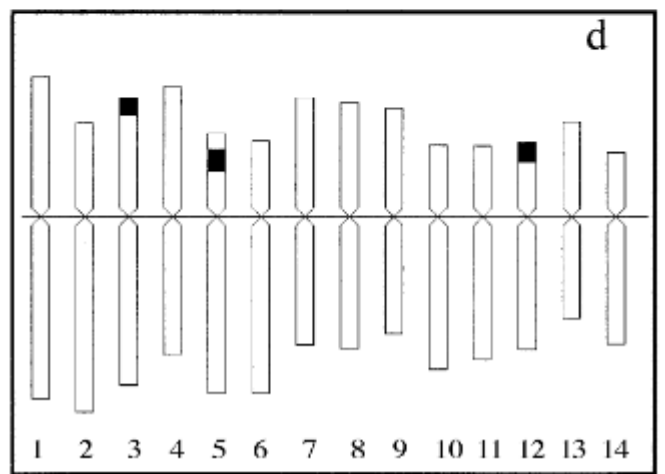
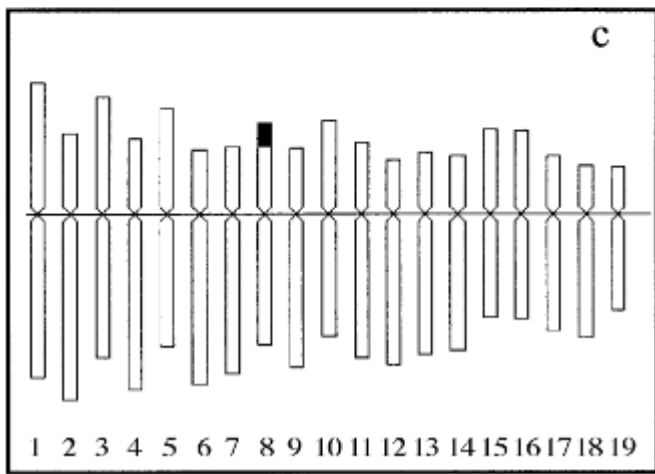
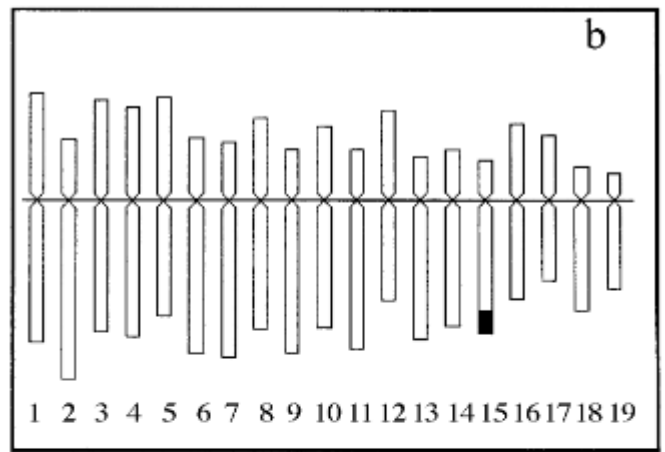
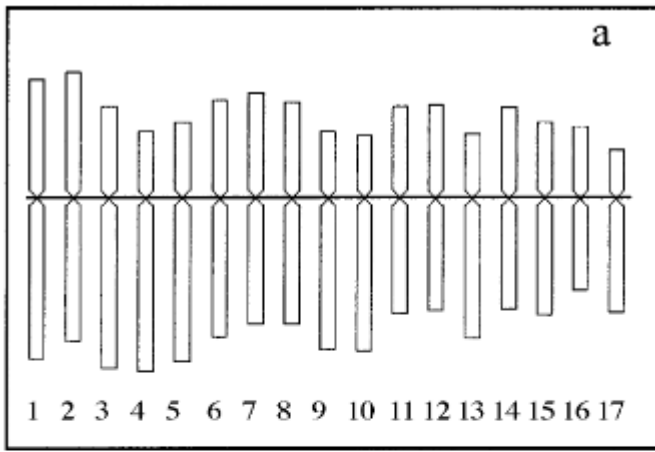


Figure 3. Idiograms of: (a) *Tressus capax*, (b) *Macoma nasuta*, (c) *Nuttallia nuttallii*, (d) *Mytilus californianus*, and (e) *M. trossulus*. (b–d) Show the location of 18S–5.8S–28S ribosomal loci. Bar = 5 μ m.

Table 2. Chromosome measurements and karyotype of *Tressus capax*, *Macoma nasuta*, and *Nuttallia nuttallii*.

<i>n</i>	<i>Tressus capax</i>			<i>Macoma nasuta</i>			<i>Nuttallia nuttallii</i>		
	R.L.	C.I.	Class	R.L.	C.I.	Class	R.L.	C.I.	Class
1	7.41 ± 0.03	42.31 ± 1.19	m	6.67 ± 0.03	42.87 ± 0.52	m	7.15 ± 0.30	44.21 ± 0.74	m
2	7.16 ± 0.01	46.86 ± 0.80	m	6.45 ± 0.25	25.01 ± 0.55	sm/ st	6.45 ± 0.11	30.21 ± 1.71	sm
3	7.02 ± 0.03	34.84 ± 0.27	sm	6.24 ± 0.10	43.23 ± 0.16	m	6.31 ± 0.14	44.72 ± 1.06	m
4	6.42 ± 0.09	31.76 ± 1.10	sm	6.15 ± 0.13	40.31 ± 0.13	m	6.04 ± 0.08	29.59 ± 0.75	sm
5	6.40 ± 0.09	27.71 ± 1.57	sm	5.90 ± 0.05	46.92 ± 0.92	m	5.75 ± 0.12	44.11 ± 0.82	m
6	6.30 ± 0.07	41.47 ± 0.75	m	5.80 ± 0.04	28.70 ± 0.11	sm	5.70 ± 0.06	27.43 ± 1.06	sm
7	6.14 ± 0.07	45.41 ± 0.90	m	5.78 ± 0.04	26.67 ± 0.25	sm	5.51 ± 0.08	29.52 ± 1.70	sm
8	5.91 ± 0.06	43.33 ± 0.93	m	5.67 ± 0.09	39.01 ± 0.22	m	5.36 ± 0.18	40.64 ± 0.33	m
9	5.86 ± 0.07	30.66 ± 1.05	sm	5.50 ± 0.06	24.88 ± 0.67	st/ sm	5.33 ± 0.06	30.00 ± 1.76	sm
10	5.73 ± 0.05	28.74 ± 1.02	sm	5.39 ± 0.20	36.51 ± 0.16	sm	5.22 ± 0.13	43.32 ± 0.73	m
11	5.52 ± 0.04	44.40 ± 1.03	m	5.36 ± 0.06	25.30 ± 0.44	sm/ st	5.22 ± 0.06	32.78 ± 1.11	sm
12	5.49 ± 0.03	44.92 ± 1.06	m	5.11 ± 0.03	47.30 ± 0.73	m	4.98 ± 0.08	26.03 ± 1.54	sm/ st
13	5.46 ± 0.12	31.34 ± 1.14	sm	4.91 ± 0.03	23.86 ± 0.62	st	4.91 ± 0.09	29.92 ± 1.60	sm
14	5.36 ± 0.07	44.78 ± 0.79	m	4.77 ± 0.05	28.56 ± 0.59	sm	4.72 ± 0.08	29.70 ± 0.97	sm
15	5.11 ± 0.14	39.30 ± 0.41	m	4.68 ± 0.07	23.16 ± 1.07	st	4.56 ± 0.18	45.03 ± 0.83	m
16	4.36 ± 0.22	43.61 ± 0.75	m	4.67 ± 0.07	43.59 ± 0.40	m	4.54 ± 0.12	43.89 ± 1.06	m
17	4.35 ± 0.18	29.69 ± 1.08	sm	3.91 ± 0.07	44.93 ± 0.39	m	4.24 ± 0.14	32.92 ± 2.38	sm
18	-	-	-	3.87 ± 0.17	23.42 ± 0.95	st	4.17 ± 0.15	27.65 ± 2.11	sm
19	-	-	-	3.13 ± 0.47	23.85 ± 0.47	st	3.48 ± 0.19	32.73 ± 2.42	sm

Note: *n*, chromosome number; **R.L.**, relative length; **C.I.**, centromeric index; **Class.**, classification; **m**, metacentric; **sm**, submetacentric; **st**, subtelocentric

Location of ribosomal rDNA loci

Despite of the use of FISH in other taxa (Szostak and Wu 1980; Coen et al. 1982; de Lucchini et al. 1993), there are few studies in bivalve molluscs. This technique has been applied to locate satellite (Clabby et al. 1996) and telomeric (Guo and Allen 1997) DNA sequences in *Crassostrea gigas* from American coasts, and ribosomal loci of some species of bivalves from European coasts (Insua and Méndez 1998). In this work we have analyzed the location of 18S-5.8S-28S ribosomal loci by FISH in four species: *M. californianus*, *M. trossulus*, *M. nasuta*, and *N. nuttallii*.

In the mussel *M. californianus*, the number of fluorescent signals varied from four to six per cell. Ribosomal loci appear located on the short arms of three chromosome pairs: on the telomeric region of pairs 3 and 12, meta and submetacentric, respectively, and in the subterminal position of the chromosome submetacentric pair 5 (Fig. 4a). These locations are coincident with the positions of Ag-NORs analyzed by Martínez-Lage et al. (1997a) in this same species; however, the fluorescent signal on chromosome 5 appeared weakly labelled or, in some cases, could not be observed. We think that it could be attributed to the existence of a low number of rDNA copies on this chromosome, or possibly a consequence of the condensation of metaphase chromosomes. Surely, the analysis of a high number of individuals allows us to observe it.

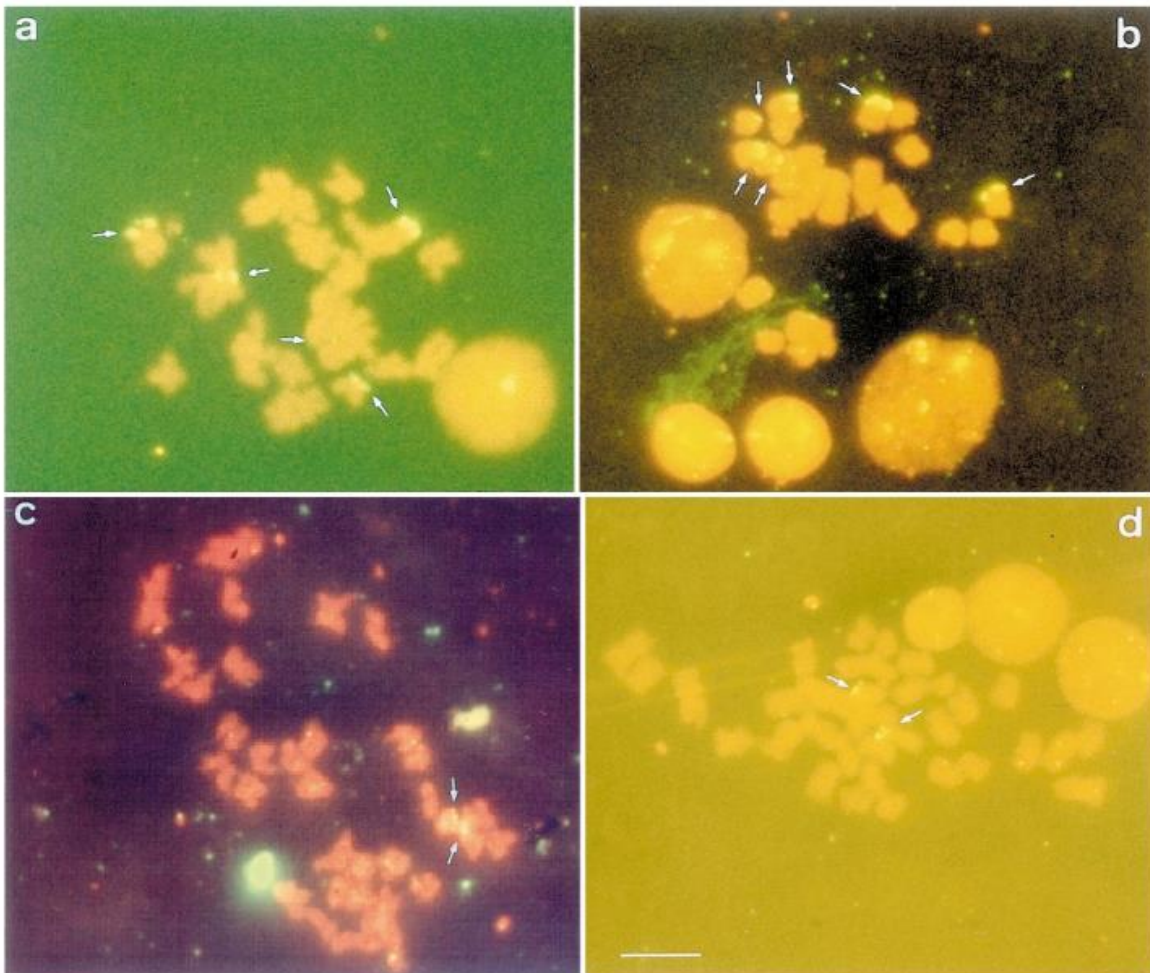


Figure 4. FISH with 18S-5.8S-28S rDNA probe in metaphase chromosomes of: (a) *Mytilus californianus*, (b) *M. trossulus*, (c) *Macoma nasuta*, and (d) *Nuttallia nuttallii*.

In *M. trossulus*, hybridization signals were spread on the telomeres of the short arm on chromosome metacentric pairs 3 and 9 and on the telomere of the short arm of chromosome submetacentric 7 (Fig. 4b).

These observations are coincident with the positions of Ag-NORs in this species (MartínezLage et al. 1997a), and we have not observed, either by FISH or silver staining, the simultaneous appearance of the signals on both chromosomal arms. Furthermore, such as happened with Ag-NORs; the signals on chromosome 7 always appear very slightly labelled. Again, this fact could be a consequence of the existence of a small number of copies in this chromosomal region. Such results are not unusual, considering that in eukaryotes, the number of chromosomal loci and the number of genes at each locus vary among species, among populations, and among individuals. In a great number of species the existence of intra-individual and inter-individual variability have been described for rDNA loci (Nardi et al. 1978; Mukai et al. 1991; Garrido-Ramos et al. 1995).

FISH resulted in labelling of two chromosomes of *M. nasuta* and *N. nuttallii* at telomeric positions. In *M. nasuta*, the two hybridization signals spread over the long arm on chromosome submetacentric pair No. 15 (Fig. 4c). *N. nuttallii* showed the hybridization signals over the short arm on chromosome metacentric pair No. 8 (Fig. 4d). Both species showed intra-individual variability; so, some of the metaphases showed both homologous chromosomes labelled in comparable intensity, while others showed the homologous unequally labelled. Again, it could be attributed to variation in the number of ribosomal loci.

Although scarce data exist about FISH in the bivalves, the comparison between our results and those described by other authors in other species and populations seem to suggest that these organisms have a tendency to locate the rDNA loci at the telomeric level.

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