Genome sizes and karyotypes in the razor clams *Ensis arcuatus* (Jeffreys, 1865) and *E. siliqua* (Linnaeus, 1758)

Juan Fernández-Tajes[a], Andrés Martínez-Lage[a], Ruth Freire[a] Alejandro Guerra[b] Josefina Méndez[a] and Ana M. González-Tizón[a*]

[a] Departamento de Biología Celular y Molecular, Facultade de Ciencias, Universidade da Coruña, 15071 La Coruña, Spain.

[b] Centro de Investigaciones Marinas, Xunta de Galicia, Pontevedra, Spain.

Cahiers de Biologie Marine, volume 49**,** issue 1, pages 79-85, march 2008
Received 11october 2007, accepted in revised form 27 november 2007, first published march 2008

How to cite:

Genome sizes and karyotypes in the razor clams *Ensis arcuatus* (Jeffreys, 1865) and *E. siliqua* (Linnaeus, 1758). J. Fernández-Tajes, A. Martínez-Lage, R. Freire, A. Guerra, J. Méndez and A. M. González-Tizón, *Cah. Biol. Mar.* 2008, **49**(1): 79-85. DOI: 10.21411/CBM.A.829E218F

Abstract

The razor clams *Ensis arcuatus* and *E. siliqua* show a diploid DNA content of 3.85 ± 0.049 pg and 4.00 ± 0.050 pg, respectively. Both have a diploid chromosome number of 38 although their karyotypes show remarkable differences. The karyotype of *E. arcuatus* consists of 4 metacentric, 1 metacentric-submetacentric, 7 submetacentric and 7 telocentric chromosome pairs, whereas that of *E. siliqua* possesses 3 metacentric, 7 submetacentric and 9 telocentric pairs. *In situ* hybridization using an 18S-5.8S-28S rDNA probe located this ribosomal locus on one chromosome pair for both species. Results demonstrate that large differences exist between them, probably caused by chromosome rearrangements along evolution of these two species, and increase the number of studies on bivalve cytogenetics.

Keywords: bivalves; razor clams; karyotype; ribosomal locus; FISH (fluorescence *in situ* hybridization)

Introduction

Ensis razor clams are highly specialized and successful bivalve species that inhabit fine sand, silt or mud bottoms. These animals belong to subclass Heterondonta, order Veneroida, family Pharidae, which includes 50-57 living species (Von Cosel, 1990) distributed in eight genera: *Ensis, Phaxas, Cultellus, Pharus, Siliqua, Sinonovacula, Pharella* and *Orbicularia* (Von Cosel, 1990). *Ensis* lives mainly in warm to cold-temperate waters, with only three know tropical species (Von Cosel, 1990). In Europe, seven *Ensis* species are described: *E. arcuatus* (Jeffreys, 1865), *E. siliqua* (Linnaeus, 1758), *E. ensis* (Linnaeus, 1758), *E. magnus* (Schumacher, 1817), *E. phaxoides* (Van Urk, 1964), *E. sicula* (Van Urk, 1964) and *E. minor* (Chenu, 1843).

Ensis species are not under such a great commercial exploitation, although in the European market the importation rates reached a quote of 550 million euros in 2004, 47% of which belongs to importation from Spain. Two of the most commercially important species are *Ensis arcuatus* (Jeffreys, 1865) and *E. siliqua* (Linnaeus, 1758), which distribute along Iberian Peninsula.

No cytogenetic study was made on these closely related species, although there are some references on chromosome data of taxa close to Pharidae. So, chromosome number has been reported in three species

belonging to family Tellinidae: *Macoma balthica* and *Tellina tenuis* (Cornet & Soulard, 1990) and *Macoma nassuta* (González-Tizón et al., 2000), two species belonging to family Solenidae: *Solen constrictus* (Wang et al, 1998) and *S. marginatus* (Fernández-Tajes et al., 2003) and one belonging to family Psammobiidae: *Nuttallia nuttallii* (González-Tizón et al., 2000). A considerable number of cytogenetic studies have been published in other species included in order Veneroida, basically on chromosome number, while those using conventional banding or fluorescence *in situ* hybridization (FISH) are scarce. In this sense, the vertebrate telomeric repeat (TTAGGG) and major ribosomal RNA have been assigned to different clam species (Insua et al., 1999; González-Tizón et al., 2000; Wang & Guo, 2001 & 2007; Martínez et al., 2002; Plohl et al., 2002; Fernández-Tajes et al., 2003; Hurtado & Pasantes, 2005). Very recently, molecular studies appeared in *E. siliqua* using random amplified DNA markers (Fernández-Tajes et al., 2007) and microsatellites (Varela et al., 2007).

Comparative cytogenetic analyses constitute valuable information to investigate systematic, phylogenetic and evolutionary processes of species, as well as to provide insights in the intraspecific variability and possible mechanisms of speciation. The central aim of this work was to obtain, describe and analyze the chromosome complement of *Ensis arcuatus* and *E. siliqua* and to locate the major ribosomal locus by means of fluorescent *in situ* hybridization, to gain information on cytogenetics of bivalve species.

Material and methods

Individuals of *E. arcuatus* were collected from Rodas de Cíes (42º 13' N, 8º 54' W) and those of *E. siliqua* from Sardiñeiro (42º 55' N, 9º 15' W) both on the northwest coast of Spain. In the laboratory, animals were fed with a suspension of *Isochrisis galbana* and *Tetraselmis sp*. microalgae for 10-15 days. Specimens were identified according to morphological characters (Van Urk, 1964).

Chromosome preparation, determination of DNA content, and fluorescence *in situ* hybridization were performed as described by Fernández-Tajes et al., (2003). For DNA measurements, Feulgen-stained nuclei from five individuals from each species and 40 nuclei from each individual were used. For karyotyping, 12 metaphases from 12 individuals from each species were analyzed. For *in situ* hybridization we analyzed 10 metaphases from eight individuals of *E. arcuatus* and ten individuals of *E. siliqua*.

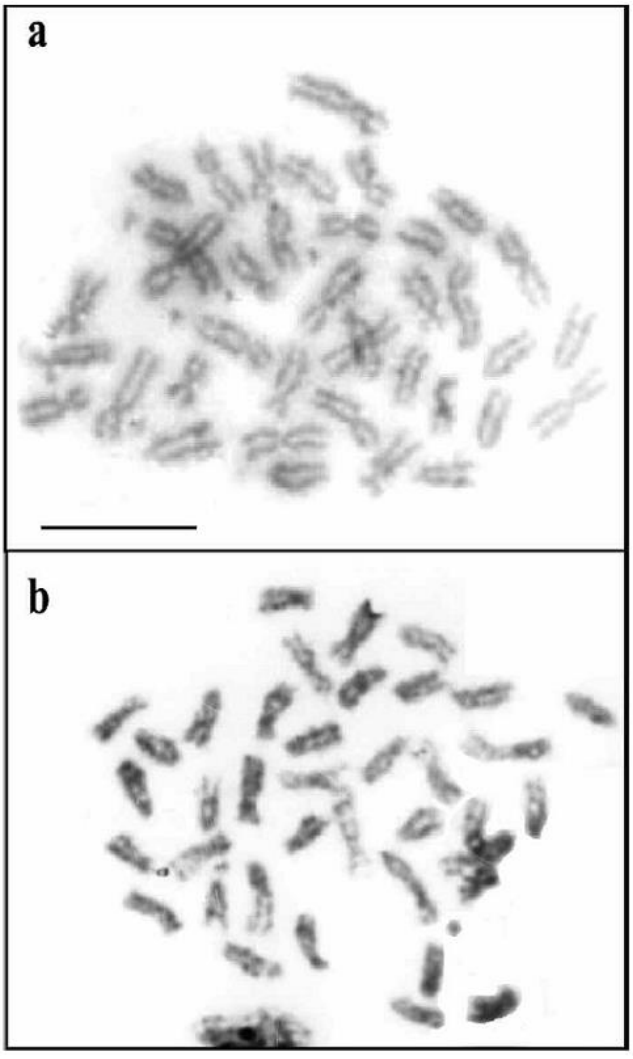

Figure 1. Metaphases of (a) Ensis arcuatus and (b) *E. siliqua*.

Bar = 10 μ m.

Results

DNA content and karyotypes

Analysis of Feulgen-stained nuclei revealed similar C-values for both species: *E. arcuatus* showed a diploid

DNA content of 3.85 ± 0.049 pg and that of *E. siliqua* was 4.00 ± 0.050 pg.

The diploid chromosome number for each species is 38 (Fig. 1a, b). In *E. arcuatus*, relative length varies from 3.82 to 7.15 (Table 1) and the karyotype consists of 4 metacentric chromosome pairs, 1 metacentric-submetacentric, 7 submetacentric and 7 telocentric (Fig. 2). In *E. siliqua*, relative length varies from 3.71 to 7.07 (Table 1) and the karyotype consists of 3 metacentric chromosome pairs, 7 submetacentric and 9 telocentric (Fig. 3).

Fluorescence in situ hybridization (FISH)

Fluorescence in situ hybridization using an 18S-5.8S-28S rDNA probe revealed the major ribosomal locus at the sub-centromeric region of one submetacentric chromosome pair in *E. arcuatus* (Fig. 4a). In *E. siliqua* this locus is located on one submetacentric pair at subtelomeric region (Fig. 4b), showing the FISH signals different intensity between the homologous chromosomes.

Table 1. Chromosome measurements and classification.

	E. arcuatus			***E. siliqua***		
C.N.	**Rel. Lgth.**	**Cen. Ind.**	**Class.**	**Rel. Lgth.**	**Cen. Ind.**	**Class.**
1	7.15 ± 0.52	32.04 ± 0.77	sm	7.07 ± 1.10	34.71 ± 1.10	sm
2	6.87 ± 0.85	38.48 ± 2.03	m-sm	6.53 ± 1.02	45.52 ± 0.46	m
3	6.63 ± 0.19	43.21 ± 1.02	m	6.49 ± 1.03	35.74 ± 1.15	sm
4	6.60 ± 0.27	32.77 ± 1.26	sm	6.46 ± 1.02	33.05 ± 0.70	sm
5	5.96 ± 0.17	33.52 ± 1.40	sm	5.93 ± 0.93	45.51 ± 0.67	m
6	5.82 ± 0.14	32.76 ± 0.80	sm	5.75 ± 0.89	32.75 ± 0.73	sm
7	5.77 ± 0.21	45.73 ± 0.61	m	5.51 ± 0.91	33.47 ± 1.16	sm
8	5.20 ± 0.14	0.37 ± 0.12	t	5.47 ± 0.85	0.45 ± 0.30	t
9	5.16 ± 0.10	30.14 ± 1.53	sm	5.16 ± 0.80	0.34 ± 0.27	t
10	5.02 ± 0.24	44.13 ± 0.97	m	5.14 ± 0.81	30.03 ± 0.64	sm
11	4.99 ± 0.14	30.82 ± 0.81	sm	5.02 ± 0.79	26.52 ± 0.95	sm
12	4.95 ± 0.11	35.24 ± 0.64	sm	4.99 ± 0.77	0.27 ± 0.10	t
13	4.86 ± 0.14	0.04 ± 0.08	t	4.82 ± 0.75	0.23 ± 0.08	t
14	4.69 ± 0.17	42.14 ± 1.41	m	4.65 ± 0.72	0.12 ± 0.07	t
15	4.64 ± 0.15	0.77 ± 0.34	t	4.53 ± 0.70	0.56 ± 0.33	t
16	4.41 ± 0.13	0.22 ± 0.14	t	4.50 ± 0.70	44.62 ± 1.13	m
17	4.24 ± 0.14	0.21 ± 0.17	t	4.28 ± 0.66	0.28 ± 0.19	t
18	4.14 ± 0.13	0.52 ± 0.28	t	4.00 ± 0.62	0.40 ± 0.14	t
19	3.82 ± 0.12	0.23 ± 0.11	t	3.71 ± 0.58	0.17 ± 0.08	t

C.N.: Chromosome number; **Rel. Lgth.**: Relative length; **Cen. Ind.**: Centromeric Index; **Class.**: classification; **m**: metacentric; **sm**: sub-metacentric; **t**: telocentric.

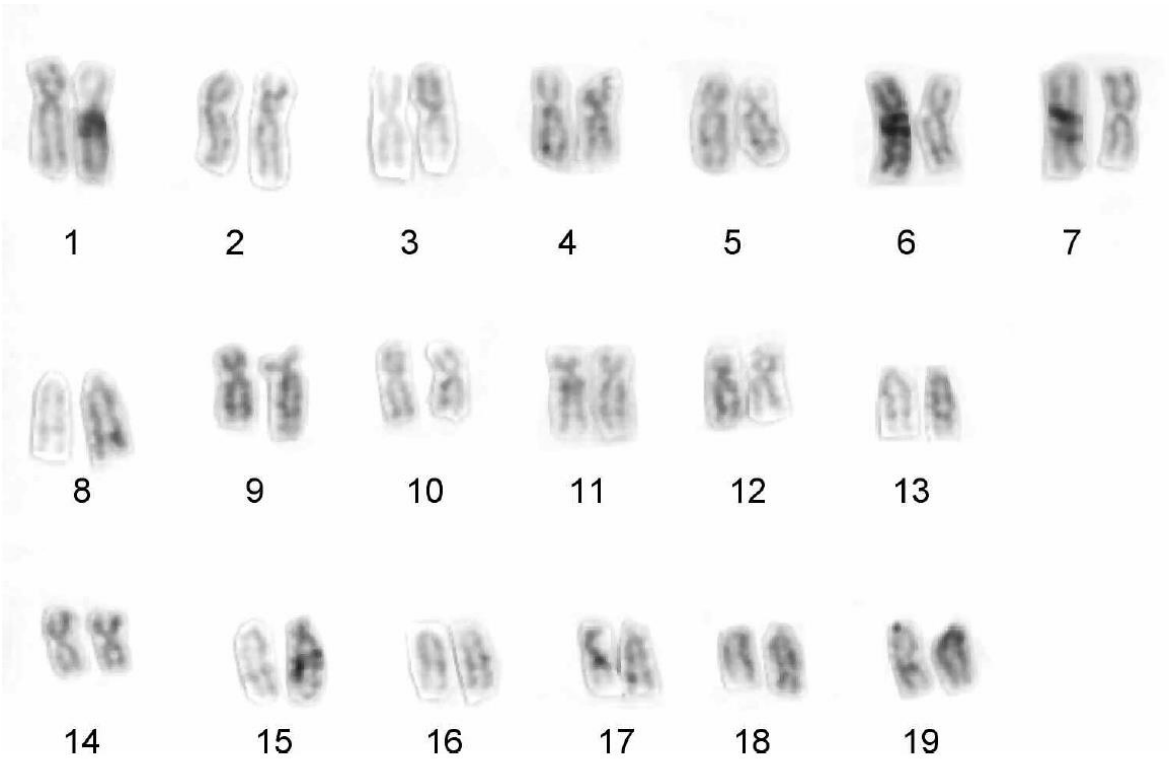

Figure 2. Karyotype of *Ensis arcuatus* (2n =38) with seven telocentric chromosome pairs (#8, 13, 15, 16, 17, 18 and 19)

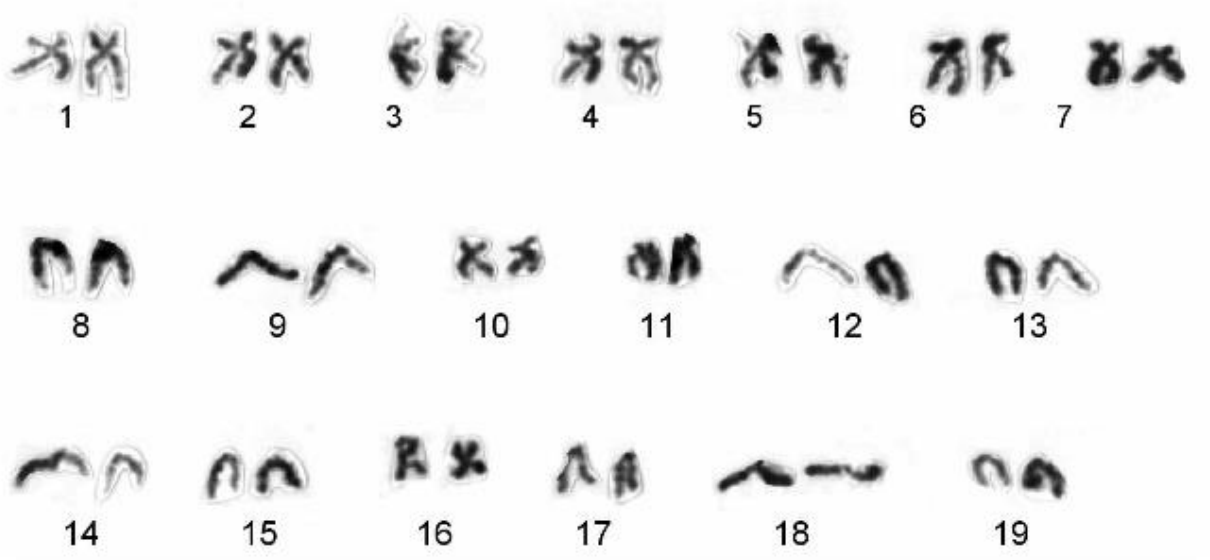

Figure 3. Karyotype of *Ensis siliqua* (2n = 38) with nine telocentric chromosome pairs (# 8, 9, 12, 13, 14, 15, 17, 18 and 19).

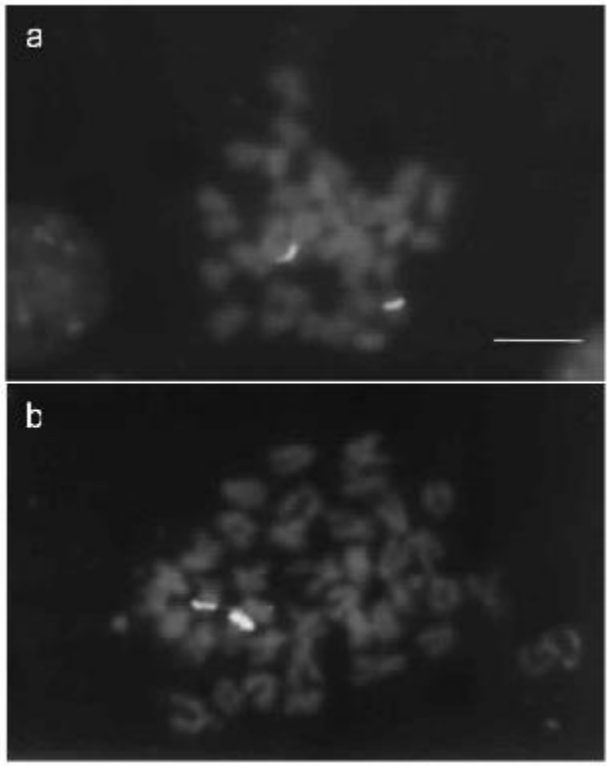

Figure 4. FISH with an 18S-5.8S-28S ribosomal probe in (a) *Ensis arcuatus* and (b) *Ensis siliqua* . Bar = 10 μ m.

Discussion

The C-values obtained for *E. arcuatus* and *E. siliqua* are included in the range obtained for other Heterodonta species analysed (Hinegardner, 1974; Cavalier-Smith, 1978; Rodríguez-Juiz et al., 1996; González-Tizón et al., 2000), and they are very similar to those described for some bivalves belonging to families close to Pharidae (González-Tizón et al., 2000; Fernández-Tajes et al., 2003), and higher than those reported by Hinegardner (1974) in the American *Ensis directus* (3.00 pg). The differences observed in DNA content among congeneric species can be due to the different methodologies used by the authors or to quantitative variations in the amounts of repetitive DNA/heterochromatin (Redi et al., 2001). In this sense, differences in the quantity, position and properties of heterochromatin among related species are very common in animals and plants (Sumner, 2003, p. 200) and, furthermore, a low correlation exists between nuclear DNA content and chromosome number (Méndez et al., 2001). So, for example, the DNA content of *Ostrea edulis* (with a diploid chromosome number of 20) is 2.33 pg, almost identical to that of *Spisula solida* (2.32 pg) with 2n = 36 chromosomes.

This study provides the first report about cytogenetics on *E. arcuatus* and *E .siliqua*. Both species have the same diploid chromosome number 2n = 38, which is the most frequent in most clam species analyzed until now. Their karyotypes are very different and allow us to clearly identify one species from another and to suggest the occurrence of chromosome rearrangements during the divergence of these species. Karyotypes display a high number of telocentric chromosomes, while the rest of Veneroida species studied show more biarmed than telocentric chromosomes (Nakamura, 1985; Thiriot-Quievreux, 1994 & 2002; Wang & Guo, 2007). In the case of superfamily Solenidae (which includes genus *Ensis* and *Solen*) cytogenetic studies are very scarce; only information about the karyotypes of *Solen constrictus*, with 18 biarmed chromosomes and one subtelocentric pair (Wang et al., 1998), *S. grandis* with 13 metacentric, three submetacentric, one subtelocentric and two telocentric pairs (Zhenxing et al., 2003), and *S. marginatus* with 11 metacentric-submetacentric, six subtelocentric and two telocentric chromosome pairs (Fernández-Tajes et al., 2003) are available. As the number of razor clam species studies is still small, it is very difficult to observe clear trends in karyotype evolution in these species. But, according to Surget-Groba et al. (2001), who pointed out that karyotypes with a high number of metacentric or submetacentric chromosomes are considered as more apomorphic than those with a high number of telocentrics, we could suppose that family Pharidae is more plesiomorphic than the rest of venerids studied until now. This is supported by the existence of only one ribosomal locus, as pointed out by Amemiya & Gold (1990). However, the 18S-5.8S-28S locus in *E. arcuatus* and *E. siliqua* (and *S. marginatus*, too) maps at subcentromeric and subtelomeric region, respectively. This condition is unusual in other bivalve species studied, as these clusters are usually located at telomeric, and more infrequently, at subtelomeric regions (Insua et al., 1998, 1999 & 2001; Insua & Méndez, 1998; Torreiro et al., 1999; Zhang et al., 1999; González-Tizón et al., 2000; Vitturi et al., 2000; Xu et al., 2001; Martínez et al., 2002; Cross et al., 2003), with the exception of *S. marginatus* (Fernández-Tajes et al., 2003), which show these clusters at subcentromeric and subtelomeric locations, *Hinnites distortus*,

with centromeric or pericentromeric signals (López-Piñón et al., 2005), and *Mercenaria mercenaria* (Wang & Guo, 2007), with one signal near the centromere and other at the telomere.

In contrast, the existence of one locus for major rRNA genes in both *Ensis* species resulted to be, *a priori*, considered as more plesiomorphic than the existence of more than one of this locus. Other species with 2n = 38 also showed one locus for rRNA, like *Cerastoderma edule*, *Donax trunculus*, *Macoma nasuta* and *Nutallia nutalli* (Insua et al., 1999; González-Tizón et al., 2000; Martínez et al., 2002), while *S. marginatus* (Fernández-Tajes et al., 2003) and *M. mercenaria* (Wang & Guo, 2007) showed two ribosomal loci. To explain this variation in the number of major ribosomal loci, Wang & Guo (2007) related the presence of two loci with the hypothesis of genome duplication during the evolution of bivalves, so that the existence of only one locus per genome would be consequence of the loss of one of the rRNA locus through chromosome rearrangements. In bivalves, the number of chromosome pairs carrying rRNA genes varies from one to four (Thiriot-Quiévreux, 2002), so it suggests that rearrangements involving loss and gains of rRNA loci are common during bivalve evolution. Maybe, in razor clams, chromosome rearrangements could originate the loss of one of the clusters or, alternatively, the fusion of both clusters leading to one. But, instead of all these findings, more cytogenetic studies on bivalve species need to be performed in order to accurately investigate chromosome evolution.

In *E. siliqua*, the intensity of FISH signals was different. This fact is frequently observed between homologous chromosomes or, even, between two chromatids of the same chromosome (Xu et al., 2001; Wang & Guo, 2004), and it is often explained by random and local differences in chromosome denaturating, FISH conditions or as consequence of the metaphase chromosome condensation. So, in *M. californianus* and *M. trossulus* mussels (González-Tizón et al., 2000) and in *M. mercenaria* Clam (Wang & Guo, 2007) some of the ribosomal loci appear weakly labeled after FISH. These results are not surprising because rRNA genes consist of highly repeated sequences arranged in tandem, and then the differences observed in fluorescence intensity can be due to variation in copy number of the target loci (Ito et al., 2000). In eukaryotes, the existence of intra- and interindividual variability for the rDNA loci has been described for different species (Nardi et al., 1978; Mukai et al., 1991; Garrido-Ramos et al., 1995; Shishido et al., 2000).

In conclusion, this work describes for the first time the cytogenetics of *Ensis arcuatus* and *E. siliqua* and the results contribute to the number of bivalve species analyzed today.

Acknowledgements

This work was funded by a Xunta de Galicia grant PGIDT99 MAR10302. We are grateful to Dr. S. Darriba for kindly providing information on the identification and morphological analysis of the species studied, and to Ms. A. Eiroa for revising the English style and grammar. We thank anonymous reviewers for constructive and valuable comments.

References

Amemiya C.T. & Gold J. R. 1990. Cytogenetic studies in North American minnows (Cyprinidae). XVII. Chromosomal NOR phenotypes of 12 species, with comments on cytosystematic relationship among 50 species. *Hereditas*, **112**: 231-247.

Cavalier -Smith T. 1978. Nuclear volume control by nucleo -skeletal DNA, selection for cell volume and cell growth rate and the solution of the C-value paradox. *Journal of Cell Science*, **34**: 247-278.

Cornet M. & Soulard C. 1990. Karyometric analysis of two species of the family Tellinidae: *Macoma balthica* (L.) and *Tellina tenuis* Da Costa (Mollusca, Bivalvia, Tellinacea). *Cytobios*, **64**: 93-101.

Cross I.,Vega L. & RebordinosL. 2003. Nuclear organizing regions in *Crassostrea angulata*: chromosomal location and polymorphism. *Genetica*, **119**: 65-74.

Fernández-Tajes J., González-Tizón A., Martínez-Lage A. & Méndez J. 2003. Cytogenetics in the razor clam *Solen marginatus* (Mollusca: Bivalvia: Solenidae*). Cytogenetic and Genome Research*, **101**: 43-46.

Fernández-Tajes J., Gaspar M., Martínez-Patiño D., McDonough N., Roberts D., González-Tizón A., Martínez-Lage A. & Méndez J. 2007. Genetic variation of the razor clam *Ensis siliqua* (Jeffreys, 1957) along the European coast based on based on random amplified polymorphic DNA markers. *Aquaculture Research*, **38**: 1205-1212.

Garrido-Ramos M.A., Jamilena M., Lozano R., Ruíz-Rejón C. & Ruíz-Rejón M. 1995. The *Eco*RI centromeric satellite DNA of the Sparidae family (Pisces, Perciformes) contains a sequence motive common to other vertebrate centromeric satellite DNAs. *Cytogenetics and Cell Genetics*, **71**: 345-351.

González-Tizón A., Martínez-Lage A., Rego I., Ausio J. & Méndez J. 2000. DNA content, karyotype and chromosomal location of 18S-5.8S-28S ribosomal locus in some species of bivalve mollusc from the Pacific Canadian coast. *Genome*, **43**: 1065-1072.

Hinegardner R. 1974. Cellular DNA content of the Mollusca. *Comparative Biochemistry and Physiology*, **47**: 447-460.

Hurtado N.S. & Pasantes JJ. 2005. Surface spreading of synaptonemal complexes in the clam *Dosinia exoleta* (Mollusca, Bivalvia). *Chromosome Research*, **13**: 575-580.

Insua A., Freire R., Ríos J. & Méndez J. 2001. The 5S rDNA of mussels *Mytilus galloprovincialis* and *M. edulis*: sequence variation and chromosomal location. *Chromosome Research*, **9**: 495-505.

Insua A., Freire R. & Méndez J. 1999. The 5S rDNA of the bivalve *Cerastoderma edule*: nucleotide sequence of the repeat unit and chromosomal location relative to 18S-28S rDNA. *Genetics selection evolution*, **31**: 509-518.

Insua A., López-Piñón M.J. & Méndez J. 1998. Characterization of *Aequipecten opercularis* (Bivalvia: Pectinidae) chromosomes by different staining techniques and fluorescent *in situ* hybridization. *Genes and Genetic Systems*, **73**: 193-200.

Insua A. & Méndez J. 1998. Physical mapping and activity of ribosomal RNA genes in mussel *Mytilus galloprovincialis. Hereditas*, **12**: 189-194.

Ito M., Ohmido N., Akiyama Y., Fukui K. & Koba T. 2000. Characterization of spinach chromosomes by condensation patterns and physical mapping of 5S and 45S rDNA by FISH. *Journal of the American Society Horticultural Science*, **125**: 59-62.

López-Piñón M.J., Insua A. & Méndez J. 2005. Chromosome analysis and mapping of ribosomal genes by one-and two-color fluorescent *in situ* hybridization in *Hinnites distortus* (Bivalvia: Pectinidae). *Journal of Heredity*, **96**: 52-58.

Martínez A., Mariñas L., González-Tizón A. & Méndez J. 2002. Cytogenetic characterization of *Donax trunculus* (Bivalvia: Donacidae) by means of karyotyping, fluorochrome banding and fluorescent *in situ* hybridization. *Journal of Molluscan Studies*, **68**: 393-396.

Méndez J., Insua A. & López-Piñón M.J. 2001. Caracterización citogenética en moluscos bivalvos. In: *Los moluscos bivalvos: aspectos citogenéticos, moleculares y aplicados*. (J. Méndez ed.), pp. 15-48. Universidade da Coruña. Servicio de publicacións.

Mukai Y., Endo T.R. & Gill B.S. 1991. Physical mapping of the 18S-28S rRNA multigene family in common wheat: Identification of a new locus. *Chromosoma*, **100**: 71-78.

Nakamura H.K. 1985. A review of molluscan cytogenetic information based on the CISMOCH-Computerized Index System for molluscan chromosomes. Bivalvia, Polyplacophora and Cephalopoda. *Japanese Journal of Malacology*, **44**: 193-225.

Nardi I., de Lucchini S., Barsacchi-Pilone G. & Andronico F. 1978. Chromosomal location of the ribosomal RNA genes in *Triturus vulgaris meridionalis* (Amphibia, Urodela). *Chromosoma*, **70**: 91-99.

Plohl M., Prats E., Martínez-Lage A., González-Tizón A., Méndez J. & Cornudella L. 2002. Telomeric localization of the vertebrate-type hexamer repeat, (TTAGGG)n, in the wedgeshell clam *Donax trunculus* and other marine invertebrate genomes. *Journal of Biological Chemistry*, **277**, 19839-19846.

Redi C.A., Garagna S., Zacharias H., Zuccotti M. & Capanna E. 2001. The other chromatin. *Chromosoma*, **110**: 136-147.

Rodríguez-Juiz A.M., Torrado M. & Méndez J. 1996. Genomesize variation in bivalve molluscs determined by flow cytometry. *Marine Biology*, **126**: 489-497.

Shishido R., Sano Y. & Fukui K. 2000. Ribosomal DNAs: an exception to the conservation of gene order in rice genomes. *Molecular and general Genetics*, **263**: 586-591.

Sumner A.T. 2003.Chromosomes. The karyotype and evolution. In *Chromosomes: Organization and Function* (A.T. Sumner, ed.), pp. 195-205. Blackwell Science Ltd.: United Kingdom.

Surget-Groba Y., Heulin B., Guillaume C.P., Thorpe R.S., Kupriyanova L., Vogrin N., Maslak R., Mazzotti S., Venczel M., Ghira I., Odierna G., Leontyeva O., Monney J.C. & Smith N. 2001. Intraspecific phylogeography of *Lacerta vivipara* and the evolution of viviparity. *Molecular Phylogenetics and Evolution*, **18**: 449-459.

Thiriot-Quievreux C. 1994. Advances in cytogenetics of aquatic organisms. In: *Genetics and Evolution of Aquatic Organisms*. (Beaumont ed.), pp. 369-388. AR. Chapman and Hall: London.

Thiriot-Quievreux C. 2002. Review of the literature on bivalve cytogenetics in the last ten years. *Cahiers de Biologie Marine*, **43**: 17-26.

Torreiro A., Martínez-Expósito M.J., Trucco M.I. & Pasantes JJ. 1999. Cytogenetics in *Brachidontes rodriguezi* d'Orb (Bivalvia, Mytilidae). *Chromosome Research*, **7**: 49-55.

Varela M.A., González-Tizón, A., Francisco-Candeira M. & Martínez-Lage A. 2007. Isolation and characterization of polymorphic microsatellite locus in the razor clam *Ensis siliqua. Molecular Ecology Notes*, **7**: 221-222.

Van Urk R.M. 1964. The genus *Ensis* in Europe. *Basteria*, **28**:13-44.

Vitturi R., Gianguzza P., Colomba M.S. & Riggio S. 2000. Cytogenetic characterization of *Brachidontes pharaonis* (Fisher P., 1870): karyotype, banding and fluorescent *in situ* hybridization (FISH) (Mollusca: Bivalvia: Mytilidae). *Ophelia,* **52**: 213-220.

Von Cosel, R. 1990. An Introduction to the razor shells (Bivalvia: Solenacea). In: *The Bivalvia Proceedings of a Memorial Symposium in Honour of Sir Charles Maurice Yongue,* (B. Morton, ed), pp. 283-305. Hong Kong University Press: Hong Kong.

Wang J., Zhao X., Zhou L. & Xiang J. 1998. Karyotypes of three species of marine bivalve molluscs. *Acta Oceanica Sinica,* **20**: 102-107.

Wang Y. & Guo X. 2001. Chromosomal mapping of the vertebrate telomeric sequence (TTAGGG)n in four bivalve molluscs by fluorescence *in situ* hybridization. *Journal of Shellfish Research*, **20**: 1187-1190.

Wang Y. & Guo X. 2004. Chromosomal rearrangement in pectinidae revealed by rRNA Loci and implications in bivalve evolution. *Biological Bulletin*, **207**: 247-256.

Wang Y. & Guo X. 2007. Chromosomal mapping of major ribosomal rRNA genes in the hard clam (*Mercenaria mercenaria*) using fluorescence *in situ* hybridization. *Marine Biology*, **150**: 1183-1189.

Xu Z., Guo X.M., Gaffney P.M. & Pierce J.C. 2001. Chromosomal location of the major ribosomal RNA genes in *Crassostrea virginica* and *Crassostrea gigas. Veliger*, **44**: 79-83.

Zhang Q., Yu G., Cooper R.K. & Tiersch T.R. 1999. Chromosomal location by fluorescence *in situ* hybridization of the 28S ribosomal RNA gene of the Eastern oyster. *Journal of Shellfish Research*, **18**: 431-435.

Zhenxing S., Yanqun S., Shengchao G., Yan Q. & Aiguo Y. 2003. Karyotypes of three species of the marine Veneroida molluscs. *Acta Oceanica Sinica,* **22**: 671-678.

* hakuna@udc.es