Cytogenetic characterisation of the razor shells *Ensis directus* (Conrad, 1843) and *E. minor* (Chenu, 1843) (Mollusca: Bivalvia)

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Abstract

The European razor shell *Ensis minor* (Chenu 1843) and the American *E. directus* (Conrad 1843) have a diploid chromosome number of 38 and remarkable differences in their karyotypes: *E. minor* has four metacentric, one metacentric–submetacentric, five submetacentric, one subtelocentric and eight telocentric chromosome pairs, whereas *E. directus* has three metacentric, two metacentric–submetacentric, six submetacentric, six subtelocentric and two telocentric pairs. Fluorescent in situ hybridisation (FISH) using a major ribosomal DNA probe located the major ribosomal genes on one submetacentric chromosome pair in both species; FISH with a 5S ribosomal DNA (5S rDNA) probe rendered one chromosomal (weak) signal for *E. minor* and no signal for *E. directus*, supporting a more dispersed organisation of 5S rDNA compared to the major ribosomal genes. The vertebrate telomeric sequence $(TTAGGG)_n$ was located on both ends of each chromosome, and no interstitial signals were detected. In this work, a comparative karyological analysis was also performed between the four *Ensis* species analysed revealing that the three European species studied so far, namely *E. minor*, *E. siliqua* (Linné 1758) and *E. magnus* Schumacher 1817 show more similarities among them than compared to the American species *E. directus*. In addition, clear karyotype differences were found between the morphologically similar species *E. minor* and *E. siliqua*.

Keywords: Razor shells; Karyotype; FISH; 18S-5.8S-28S rDNA; 5S rDNA; Telomeric sequence

Introduction

The genus *Ensis* Schumacher 1817 (Mollusca: Bivalvia: Pharidae) is composed of about 12 extant species that live on fine sand, silt or mud bottoms off the European, African and American coasts. In Europe, four species are native, *E. ensis* (Linné 1768), *E. magnus* Schumacher 1817 [syn. *E. arcuatus* (Jeffreys 1865)], *E. minor* (Chenu 1843) and *E. siliqua* (Linné 1758) and one introduced, *E. directus* (Conrad 1843) [syn. *E. americanus* (Gould 1870)]. This species was introduced to the German Bight at the end of the 1970 s from Atlantic North America, probably through ballast water (Cosel et al. 1982). In European coastal waters, the distribution areas of the different *Ensis* species are mostly overlapping and there are few areas occupied by only one species (Cosel 2009) though *E. directus* prefers brackish waters. The European *E. minor* has often been confused with its homonym *E. minor* Dall 1899 (which is native to the SE United States), although they constitute different species. Additional taxonomic confusion is due to the fact that both European *E. minor* and *E. siliqua* are very similar in terms of shell morphology. Cosel (2009) states "*E. minor* was frequently treated under the name *E. siliqua* or as a subspecies of that taxon; however, along the Atlantic coast the two species occur sympatrically with only very few possible intergrades which occasionally were found at the south coast of Brittany (Pl. 8f–k) and also a few at Ile de Ré, Charente Maritime. They are looking superficially like *E. minor* but mostly have the rounded posterior cross-section of *E. siliqua* (Pl. 8e). Only molecular research will elucidate this".

The two species studied in this work are distributed as follows: *E. minor* from British North Sea coast from the east coast of Scotland to southern England, The Netherlands, and the Channel; European Atlantic coast from the NW part of Wales southward to North Morocco and throughout the Mediterranean. The introduced *E. directus* is now well established in Europe and occurs from Denmark to France, England and Sweden (Cosel 2009).

Whereas recent molecular analyses have been performed in a number of *Ensis* (Varela et al. 2007, 2009; Vierna et al. 2009, 2010, 2011), there is only one report about karyotypes of these species (specifically only in *E. magnus* and *E. siliqua* by Fernández-Tajes et al. 2008). It is worthwhile to mention that there are more than 20,000 different molluscan species worldwide distributed in aquatic habitats, and only about 200 species have been cytogenetically studied (i.e. karyotyped). This is due to the fact that cytogenetic studies of molluscs are usually complicated because the difficulties derived of the very low mitotic index in adult tissues and the problems to gain higher mitotic indexes. Recent articles (since year 2000) dealing about cytogenetic characterisation of commercial marine molluscs have been performed in different species of mussels (Vitturi et al. 2000; Martínez-Lage et al. 2002; Iqbal et al. 2008; Pérez-García et al. 2010, 2011), clams (González-Tizón et al. 2000; Martínez et al. 2002; Plohl et al. 2002; Hurtado and Pasantes 2005; Leitao et al. 2006; Wang and Guo 2007, 2008), razor shells (Fernández-Tajes et al. 2003, 2008), oysters (Xu et al. 2001; Cross et al. 2005; Wang et al. 2005a, b; Huang et al. 2007a, b; Zhang et al. 2007), cockles (Leitao et al. 2006), and pectinids (Pauls and Afonso 2000: López-Piñón et al. 2005; Odierna et al. 2006; Huang et al. 2007a, b; Zhang et al. 2008).

Cytogenetic analyses are important as they provide information about the number and morphology of chromosomes, the differential distribution of euchromatin-heterochromatin regions, the occurrence of chromosomal re-arrangements along evolution, phylogenetic relatedness between taxa, etc., and they help to clarify species status, which is extremely important in conservation biology. Therefore, they can be applicable in aquaculture as cytogenetic techniques, mainly FISH, are a significant part of genomic research to facilitate the construction of linkage maps, which are useful to identify loci of interest in economic marine species.

In the present study, we describe the chromosome number and morphology, and the location of the major ribosomal loci (18S-ITS1-5.8S-ITS2-28S) and the telomeric sequences in *E. minor* and *E. directus*. The location of 5S ribosomal DNA (5S rDNA) in *E. minor* is also provided. In addition, we perform a

comparative karyological analysis among *E. minor*, *E. directus*, *E. magnus* and *E. siliqua* in order to infer phylogenetic relationships based on karyotype differences and to clarify *E. minor–E. siliqua* taxonomic status.

Materials and methods

Biological material

Specimens of *E. minor* were collected from La Capte (43°02′N, 6°09′E) and Bandol (43°08′N, 5°46′E) (both localities on the Eastern Gulf of Lion, France), and those of *E. directus* were caught in Vester Vedsted (55°17′N, 8°38′E) (Denmark). In the laboratory, animals were fed with a suspension of *Isochrysis galbana* and *Tetraselmis* sp. microalgae for 10–15 days. Specimens were identified according to Cosel (2009).

Chromosome preparation

Metaphases were obtained from gill tissue of adult specimens after treatment with colchicine solution (0.005 %) for 6–8 h. Gills were dissected and treated twice with 0.56 % KCl solution for 15 min. After fixation in ethanol–glacial acetic acid (3:1), cells were dissociated in 45 % acetic acid and dropped onto slides heated at 43 °C. Metaphases were stained with 4 % Giemsa in phosphate buffer pH 6.8 and photographed using a Nikon Microphot-FXA microscope equipped with a NIS-Elements D 3.10 software and a digital camera DS-Qi1Mc.

Karyotyping

Chromosome measurements were performed using the Leica Chantal image analysis software system described in González-Tizón et al. (2000). Chromosome measurements were carried out in 12 metaphases from 12 individuals in both species. Mean value of the length of the chromosome arms and the mean value for their total chromosome lengths were calculated for each of the chromosome pairs. The relative length (100 × chromosome length/total haploid length), the centromeric index (100 × length of short arm/total chromosome length), the mean value and the standard error (standard deviation/number of individuals)$^{1/2}$ of the relative lengths and centromeric indices were also calculated. Karyotypes were arranged by decreasing size and classified according to the centromeric index, following the nomenclature of Levan et al. (1964).

Fluorescent in situ hybridisation (FISH)

Chromosomal location of rDNA loci was performed by FISH as described in González-Tizón et al. (2000). The DNA probe pDM 238 from *Drosophila melanogaster* (Roiha et al. 1981), containing the repeat unit 18S-5.8S-28S rDNA, was labelled by nick translation with digoxigenin-11-dUTP (Roche) for chromosome mapping of major ribosomal genes. The 5S rDNA probe was obtained by PCR using the primers 5S-Univ-F (5′-ACCGGTGTTTTCAACGTGAT) and 5S-Univ-R (5′-CGTCCGATCACCGAAGTTAA) designed by Vierna et al. (2009). These primers had opposite orientations and were separated by 3 bp. The probe was labelled by PCR with digoxigenin-11-dUTP (Roche).

Telomeric FISH was carried out as in Plohl et al. (2002) with the $(TTAGGG)_{22}$ probe labelled with digoxigenin by a standard PCR procedure. Slides were counterstained with propidium iodide (50 ng/mL antifade) and visualised and photographed using a Nikon Microphot-FXA microscope equipped with a NIS-Elements D 3.10 software and a digital camera DS-Qi1Mc.

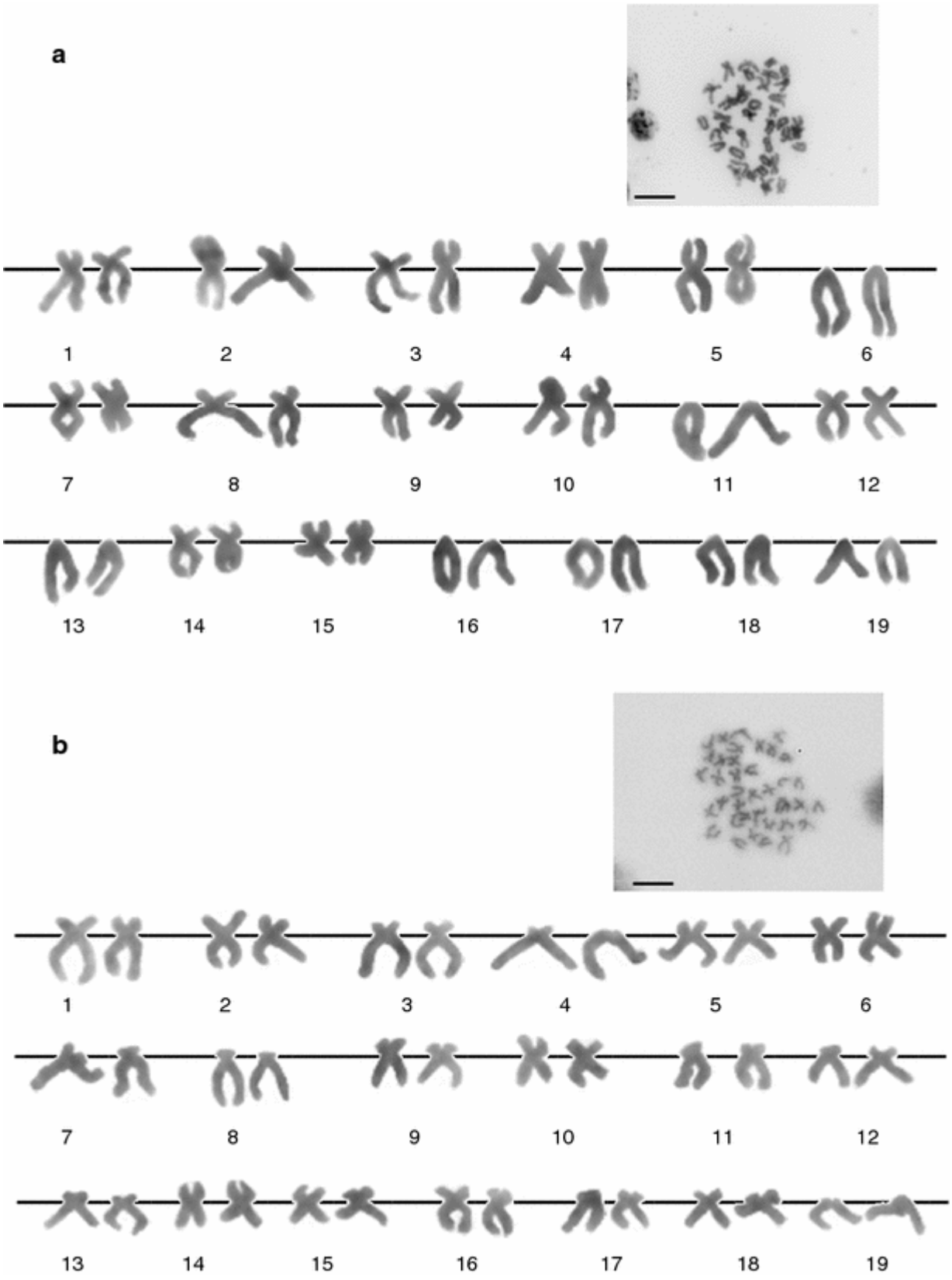

Fig. 1. Karyotypes of **a** *Ensis minor* and **b** *E. directus* (both 2*n* = 38 chromosomes). *Bar* 10 μm

Results

Analysis of 38 metaphases from 15 specimens of *E. minor* and 42 metaphases from 12 specimens of *E. directus* revealed a diploid chromosome number for each species of 2*n* = 38 (Fig. 1a, b). For karyotyping, ten well-spread metaphases were paired on the basis of chromosome size and centromere position and used for chromosome measurements and classification. In *E. minor*, relative length varied from 3.40 to 6.99, and in *E. directus*, it ranged from 3.46 to 6.93 (Table 1). The karyotype of *E. minor* consisted of four metacentric chromosome pairs, one metacentric–submetacentric, five submetacentric, one subtelocentric and eight telocentric (Figs. 1a, 3a). The species *E. directus* had three metacentric, two metacentric–submetacentric, six submetacentric, six subtelocentric and two telocentric chromosome pairs (Figs. 1b, 3b).

In both species, FISH using a 18S-ITS1-5.8S-ITS2-28S probe revealed the location of a major ribosomal locus on the short arm of one submetacentric chromosome pair at an interstitial position (Figs. 2a, b, 3a, b). Telomeric signals appeared at the end of all chromosomes on both *Ensis* species (Fig. 2c, d), and a signal for the 5S rDNA gene family was located at a subtelomeric region of one medium-size telocentric chromosome pair in *E. minor* (Fig. 2e), whereas *E. directus* did not show fluorescent signal after 5S rDNA hybridisation. In our previous studies (Vierna et al. 2009, 2011), PCR amplifications of 5S rDNA in *E. directus* generated multiple fragments, with sizes ranging between 406 and 739 bp, corresponding to 5S rDNA variants that differed in the length of the nontranscribed spacer region (NTS). Additional PCR products were identified as dimer and trimer sequences formed by two and three contiguous monomers, respectively. The species *E. minor* was not studied in these reports, but differences in 5S rDNA organisation are not to be expected among *E. minor* and the other European species analysed (i.e. *E. ensis*, *E. siliqua* and *E. magnus*).

Table 1. Chromosome measurements and classification.

Ensis minor				*Ensis directus*			
	RL	CI	Class		RL	CI	Class
1	6.99 ± 0.06	32.73 ± 0.61	sm	1	6.93 ± 0.03	26.18 ± 0.79	sm
2	6.61 ± 0.03	35.52 ± 0.76	sm	2	6.56 ± 0.07	41.09 ± 0.37	m
3	6.36 ± 0.03	33.74 ± 0.26	sm	3	6.24 ± 0.06	29.43 ± 0.41	sm
4	6.24 ± 0.04	31.36 ± 0.52	sm	4	6.16 ± 0.04	22.53 ± 0.57	st
5	6.05 ± 0.03	39.88 ± 0.56	m	5	5.99 ± 0.11	33.80 ± 0.60	sm
6	5.88 ± 0.08	0.11 ± 0.01	t	6	5.74 ± 0.05	37.85 ± 0.37	m–sm
7	5.75 ± 0.03	37.95 ± 0.65	m–sm	7	5.66 ± 0.07	33.28 ± 0.79	sm
8	5.39 ± 0.05	0.12 ± 0.01	t	8	5.60 ± 0.04	8.16 ± 0.35	t
9	5.29 ± 0.06	39.61 ± 0.63	m	9	5.31 ± 0.03	22.58 ± 0.84	st
10	5.22 ± 0.05	33.46 ± 0.51	sm	10	5.25 ± 0.06	38.84 ± 0.30	m
11	5.11 ± 0.05	0.12 ± 0.01	t	11	5.17 ± 0.04	22.56 ± 0.47	st
12	4.87 ± 0.08	17.75 ± 1.48	st	12	5.02 ± 0.04	21.66 ± 0.71	st
13	4.84 ± 0.04	2.65 ± 0.84	t	13	4.87 ± 0.04	38.06 ± 0.72	m–sm
14	4.78 ± 0.07	38.53 ± 0.58	m	14	4.66 ± 0.06	38.56 ± 0.58	m
15	4.60 ± 0.08	39.98 ± 0.62	m	15	4.55 ± 0.05	30.12 ± 1.24	sm
16	4.53 ± 0.03	0.14 ± 0.01	t	16	4.41 ± 0.04	23.33 ± 0.96	st
17	4.23 ± 0.04	0.15 ± 0.01	t	17	4.22 ± 0.03	14.38 ± 0.95	st
18	3.87 ± 0.04	0.16 ± 0.01	t	18	4.20 ± 0.04	27.23 ± 0.66	sm
19	3.40 ± 0.05	1.46 ± 0.41	t	19	3.46 ± 0.06	7.67 ± 0.34	t

RL relative length, *CI* centromeric index, *Class* classification, *m* metacentric, *sm* submetacentric, *st* subtelocentric, *t* telocentric

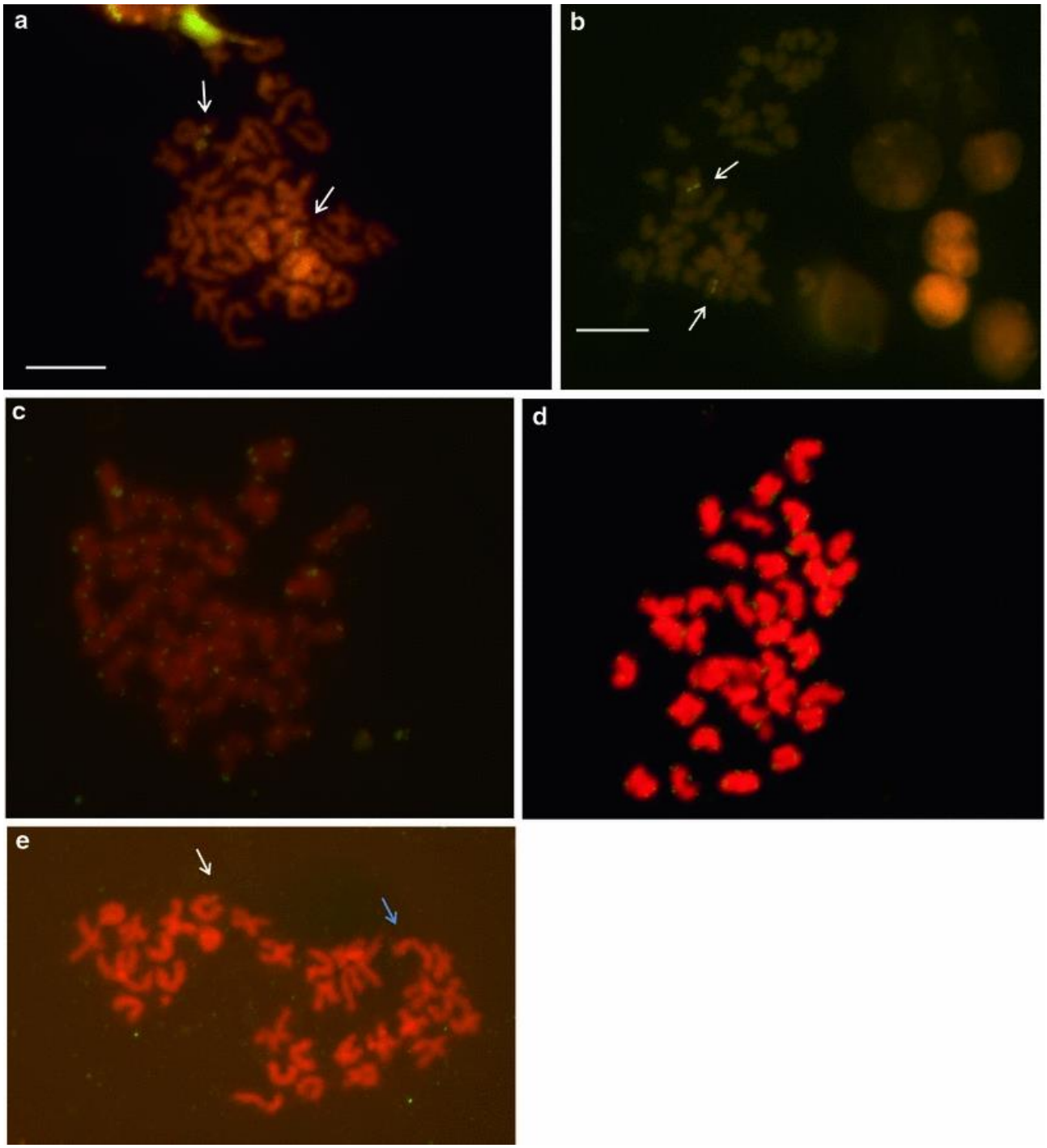

Fig. 2. Metaphases of *Ensis minor* after **a** FISH with an 18S-ITS1-5.8S-ITS2-28S ribosomal DNA probe, **c** FISH with a telomeric probe, **e** FISH with a 5S ribosomal DNA probe. Metaphases of *E. directus* after **b** FISH with an 18S-ITS1-5.8S-ITS2-28S ribosomal DNA probe, **d** FISH with a telomeric probe. *Bar* 10 μm

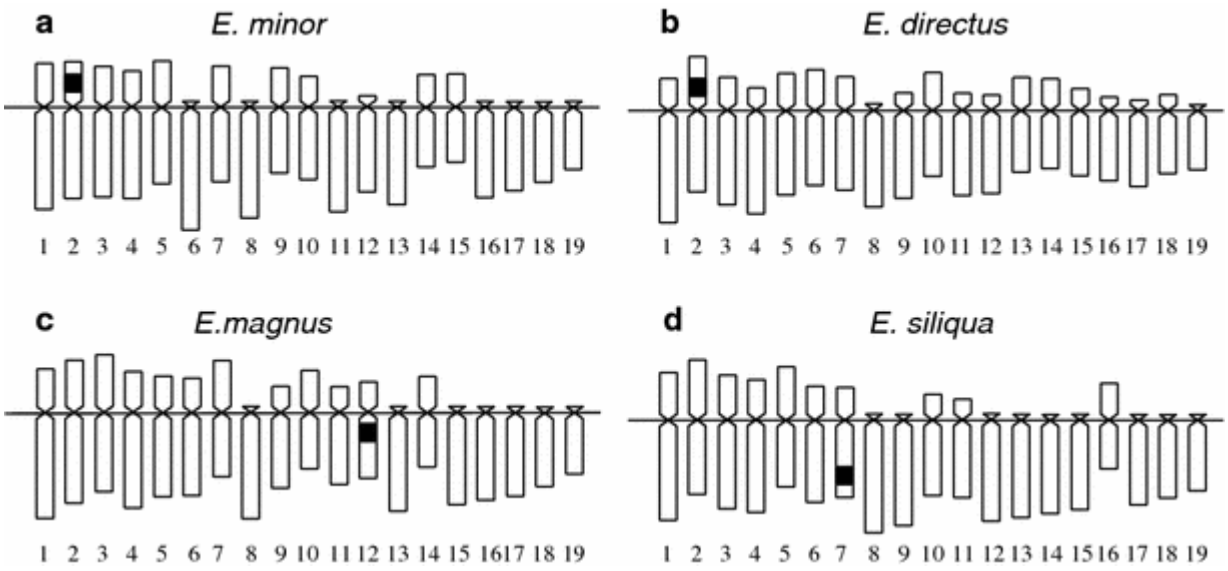

Fig. 3. Idiograms of **a** *Ensis minor*, **b** *E. directus*, **c** *E. magnus* and **d** *E. siliqua*. *Blackrefill* represents the location of the 18S-ITS1-5.8S-ITS2-28S ribosomal loci. **c**, **d** Data taken from Fernández-Tajes et al. (2008)

Discussion

This study reveals that *E. minor* and *E. directus* have a diploid chromosome number of 38 chromosomes, which is coincident with those previously reported on their congenerics *E. magnus* and *E. siliqua* (Fernández-Tajes et al. 2008), and with the majority of the karyotypes studied within the Heterodonta bivalves. However, there are some exceptions as *Kidderia minuta* (Thiriot-Quievreux et al. 1988a), *Lasaea australis* (Thiriot-Quievreux 1992), *Spaherium corneum* (Petkeviciute et al. 2006) and *Cyclinia sinensis* (Wang et al. 2001), all of them with $2n = 36$ chromosomes, *Cyclocardia astartoides* (Thiriot-Quievreux et al. 1990) with $2n = 30$, and some *Lasaea* species with different levels of polyploidy (Thiriot-Quievreux et al. 1988b, 1989; Ó Foighil and Thiriot-Quievreux 1999).

The karyotypes of *E. minor* and *E. directus* have telocentric pairs, which is not very usual in Heterodonta. To our knowledge, only the karyotypes of 29 Heterodonta species reported so far belonging to 12 different families have telocentric chromosomes (Table 2). The number of telocentric pairs varies from one in *Donax trunculus* (Martínez et al. 2002), *Lasaea colmani* (Ó Foighil and Thiriot-Quievreux 1999), *Scrobicularia plana* (Cornet and Soulard 1989), *Sinonovacula constricta* (Wang et al. 1998), and *Ruditapes aureus* (= *Venerupis aurea*) (Borsa and Thiriot-Quievreux 1990; Carrilho et al. 2011) to 19 (all the complement) in *Mulinia lateralis* (Wang and Guo 2008).

The comparative analysis of *Ensis* karyotypes shows that the diploid chromosome number is the same for the four species and that the number of telocentrics is higher in the European ones than in the American species (nine pairs in *E. siliqua*, eight in *E. minor* and seven in *E. magnus*, whereas *E. directus* has only two telocentric pairs). In the four species, the number of metacentric and submetacentric pairs is similar: 11 in *E. directus* and *E. magnus* and 10 in *E. minor* and *E. siliqua*, being the major differences in the number of subtelocentrics: one pair in *E. minor* and *E.magnus* and six pairs in *E. directus*, whereas *E. siliqua* has no subtelocentrics. These differences lead to karyotypes with numbers of chromosome arms of: 72 in *E. directus*, 62 in *E. magnus*, 60 in *E. minor* and 58 in *E. siliqua*, this representing a major divide between the American species (*E. directus*) and the European species (*E. magnus*, *E. siliqua* and *E. minor*). According to White (1978) who pointed out that karyotypes with higher proportion of metacentrics are generally

considered as more primitive (plesiomorphic) and show relative more chromosome stability than karyotypes with few metacentric chromosomes (apomorphic), the results obtained for the *Ensis* species suggest that *E. directus* is the species with the most ancestral karyotype, whereas the karyotypes of the native European species may be more recent. Differences in the number of bi-armed and mono-armed chromosomes lead us to think that structural re-arrangements (involving loss of chromosomal arms and emergence of telocentrics, fusion of telocentric chromosomes and reciprocal or robertsonian translocations, or inversions) could have occurred during the evolution of *Ensis* species, as suggested Wang and Guo (2004) for Pectinidae species and Wang and Guo (2008) for *M. lateralis*.

These results confirm *E. siliqua* and *E. minor* as separate species, clarifying their taxonomic status, and may be very useful in future programmes on aquaculture and conservation of these species.

Table 2. List of Heterodonta species with telocentric chromosomes.

Taxa an diploid chromosome number	No. of telocentric pairs	Authors	No. of 18S–28S loci (FISH)
Family CARDIIDAE			
Cerastoderma edule (2*n* = 38)	3	Insua and Thiriot-Quievreux (1992), Insua et al. (1999)	1
Family CARDITIDAE			
Cyclocardia astartoides (2*n* = 30)	10	Thiriot-Quievreux et al. (1990)	
Family CYAMIIDAE			
Kidderia bisulcata (2*n* = 38)	5	Thiriot-Quievreux et al. (1988a)	
Kidderia minuta (2*n* = 36)	5	Thiriot-Quievreux et al. (1988a)	
Family DONACIDAE			
Donax trunculus (2*n* = 38)	1	Martínez et al. (2002)	1
Family LASAEIDAE			
Lasaea australis (2*n* = 36)	5	Thiriot-Quievreux (1992)	
Lasaea colmani (2*n* = 40)	1	Ó Foighil and Thiriot-Quievreux (1999)	
Lasaea consanguinea (2*n* = 100–120)[a]	4	Thiriot-Quievreux et al. (1988b)	
Lasaea rubra (*n* = 63–340)[a]	9	Thiriot-Quievreux et al. (1989)	
Family MACTRIDAE			
Mulinia lateralis (2*n* = 38)	19	Wang and Guo (2008)	2
Family PHARIDAE			
Ensis directus (2*n* = 38)	2	Present work	1
Ensis magnus (*E. arcuatus*) (2*n* = 38)	7	Fernández-Tajes et al. (2008)	1
Ensis minor (2*n* = 38)	8	Present work	1
Ensis siliqua (2*n* = 38)	9	Fernández-Tajes et al. (2008)	1
Family SEMELIIDAE			
Scrobicularia plana (2*n* = 38)	1	Cornet and Soulard (1989)	
Family SOLECURTIDAE			
Sinonovacula constricta (2*n* = 38)	1	Wang et al. (1998)	
Family SOLENIDAE			
Solen grandis (2*n* = 38)[b]	2	Sun et al. (2003)	
Solen linearis (2*n* = 38)[b]	2	Chen et al. (2008)	
Solen marginatus (2*n* = 38)	2	Fernández-Tajes et al. (2003)	2
Family TEREDINIDIAE			
Teredo utriculus (2*n* = 38)	14	Vitturi et al. (1983)	

Taxa an diploid chromosome number	No. of telocentric pairs	Authors	No. of 18S–28S loci (FISH)
Family VENERIDAE			
Chamelea gallina (2*n* = 38)	4	Corni and Trentini (1986)	
Circe scripta (2*n* = 38)	3	Ebied and Aly (2004)	
Cyclina sinensis (2*n* = 36)[b]	11	Wang et al. (2001)	
Meretrix meretrix (2*n* = 38)[b]	3	Wu et al. (2002)	
Ruditapes aureus (*V. aurea*) (2*n* = 38)	1	Borsa and Thiriot-Quievreux (1990), Carrilho et al. (2011)	1
Ruditapes decussatus (2*n* = 38)	5	Ebied and Aly (2004)	
Tapes rhomboides (2*n* = 38)	4	Carrilho et al. (2011)	1
Venerupis rhomboides (2*n* = 38)	3	Insua and Thiriot-Quievreux (1992)	
Venus verrucosa (2*n* = 38)	4	Ebied and Aly (2004)	

[a] Different levels of polyploidy
[b] In Chinese; only abstract in English

Concerning the major ribosomal loci, these have been mapped using FISH in 10 Heterodonta species: *Cerastoderma edule* (Insua et al. 1999), *Donax trunculus* (Martínez et al. 2002), *Solen marginatus* (Fernández-Tajes et al. 2003), *Dosinia exoleta* (Hurtado and Pasantes 2005), *Mercenaria mercenaria* (Wang and Guo 2007), *E. magnus* and *E. siliqua* (Fernández-Tajes et al. 2008) (Fig. 3c, d), *M. lateralis* (Wang and Guo 2008) and *Tapes rhomboides* and *V. aurea* (Carrilho et al. 2011). All of them showed one major ribosomal locus, except *S. marginatus* (Fernández-Tajes et al. 2003) and *M. lateralis* (Wang and Guo 2008), which had two loci. The four *Ensis* species showed only one chromosomal interstitial signal on one submetacentric pair (Fig. 3). In *E. minor* and *E. directus*, the fluorescent signal appeared interstitially located on the p arm of chromosome number 2, whereas in *E. magnus* and *E. siliqua*, the signal is on q arm of chromosome number 12 and 7, respectively. These differences in location of major ribosomal genes could be explained by translocations, as suggested Wang and Guo (2004) for pectinids. In *E. minor* and *E. directus*, FISH signals were stronger on one of the homologous chromosomes than on the other, which, as pointed out by Xu et al. (2001) is probably due to random variation in FISH or the differences (loss or gain) in the number of rDNA repeats.

The FISH experiments using 5S rDNA probes were only obtained until now in three species belonging to the subclass Heterodonta, the cockle *C. edule* (Insua et al. 1999), which revealed nine hybridisation signals, and the clams *T. rhomboides* and *V. aurea* (Carrilho et al. 2011) with one signal. In this present work, we reported the occurrence of at least one 5S rDNA array containing a sufficient number of repeats to yield a (weak) fluorescent signal in *E. minor*. Even though in bivalve species, it is usual that some of the metaphases analysed do not yield any FISH signal (as it has previously been described by Wang et al. (2005a, b) in an oyster), the absence of a 5S rDNA signal, compared to the clear signal obtained with the major ribosomal genes probe, may well be explained by differences in the genomic organisation of these gene families. Thus, if 5S rDNA is much more dispersed in the genome of *Ensis* razor shells, compared to the major ribosomal genes (as suggested by Vierna et al. 2010), then FISH using that probe should produce a so weak signal which may probably be invisible. Differences in the genomic organisation between both species may be explained by the phylogenetic distance between American and European species, as revealed by the karyotypes (this study), shell morphology (Cosel 2009) and the ITS1-5.8S-ITS2 region (Vierna et al. 2010). The 5S rDNA multigene family is formed by a 5S rRNA coding region (corresponding to 120 nucleotides of the mature RNA) and a variable in length NTS. The 5S rDNA is characterised by a flexible organisation, as it has been found in clusters composed of similar or divergent tandemly arranged repeats (differences mainly occur in the NTS) and in clusters of 5S rDNA repeats tandemly linked to other multigene families. A

dispersed organisation of 5S rDNA has also been reported, and some species were found to have more than one type of organisation within the genome (Vierna et al. 2011 and references therein). The ITS1-5.8-ITS2 and 5S rDNA regions have been studied in terms of evolutionary genetics in some *Ensis* species (Vierna et al. 2009, 2010, 2011). Vierna et al. (2010) concluded that the long-term evolution of these multigene families could be reconciled under a mixed process of concerted evolution, birth-and-death evolution and purifying selection, despite the different levels of intragenomic divergence detected (much higher within the 5S rDNA region). These authors suggested that these differences may be the consequence of a differential genomic organisation of the multigene families, that is, one or few 18S-ITS1-5.8-ITS2-28S loci containing several repeats and many 5S rDNA loci containing less repeats. Even though no conclusive data is available, our study supports their hypothesis, since the different intensities of FISH signals recorded may be explained by these differences in genomic organisation: we may have obtained weak (or none) 5S rDNA FISH signals because the repeats of this multigene family may be very dispersed within the genomes of *Ensis* species.

Finally, the hybridisation of the vertebrate telomere probe to termini of *E. minor* and *E. directus* chromosomes indicates that the vertebrate $(TTAGGG)_{22}$ sequence is present within the genomes of *E. minor* and *E. directus*, as was previously detected for the Heterodonta species *D. trunculus* (Plohl et al. 2002), *M. mercenaria* and *M. lateralis* (Wang and Guo 2001), *D. exoleta* (Hurtado and Pasantes 2005) and *T. rhomboides* and *V. aurea* (Carrilho et al. 2011).

In conclusion, this study provides new information on bivalve karyotypes, reveals important differences between American and European *Ensis* at the chromosome level, confirms *E. minor* and *E. siliqua* as separate species and supports a more dispersed organisation of the 5S rDNA compared to the major ribosomal genes.

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