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Population structure of a deep-water squaloid shark, the Portuguese dogfish (*Centroscymnus coelolepis*)

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The genetic population structure of the deep-water squaloid *Centroscymnus coelolepis* (the Portuguese dogfish) in the eastern Atlantic was investigated using eight polymorphic nuclear microsatellite markers and a 496-bp fragment of the mitochondrial DNA control region (mtDNA CR). Samples (20–50 individuals per location) were collected off Ireland, Portugal, Madeira, Mauritania, South Africa, and the Azores (Mid-Atlantic Ridge). High levels of genetic diversity were found at the nuclear microsatellite loci (mean $A = 17.3$; overall $H_o = 0.77$), although there was low diversity at the mtDNA CR ($h = 0.65$, $\pi = 0.0018$). Genetic diversity for the Portuguese dogfish was homogeneously distributed among sampling locations, resulting in low and non-significant indices of genetic differentiation (e.g. $F_{ST} = -0.0013$ to 0.0096, $\Phi_{ST} = -0.017$ to 0.033; $p > 0.5$), consistent with the absence of population structure within the area sampled. The results indicate that *C. coelolepis* has a high dispersal potential within the eastern Atlantic, and its apparent spatial segregation by size and maturity stage suggest large-scale migration associated with the reproductive cycle.

Keywords: deep-water, eastern Atlantic population structure, Portuguese dogfish, squaloid shark.

Introduction

The squaloids (order Squaliformes) are the second most diverse group of sharks, mostly benthopelagic in habit, and preferentially living in cold, bathyal waters (Musick *et al.*, 2004). Squaloid taxa have conservative life-history features such as late sexual maturation, potentially long lifespans, and low lifetime fecundities (see Kyne and Simpfendorfer, 2010, for more details). Although such features are generally shared by all elasmobranch taxa, they are particularly pronounced in squaloid sharks and even more so in those occupying deep water, rendering them particularly vulnerable to population depletion and overexploitation (Kyne and Simpfendorfer, 2010). Other than their generally conservative life histories, we know little about the biology and ecology of most squaloids, particularly those living in deep water, and almost nothing about the patterns of species distribution and population structure.

In the absence of studies on the population structure of deep-water squaloids, important clues about the factors contributing to such structuring may be drawn from shallow-water elasmobranch taxa. Recent studies suggest that vagility, environmental conditions, and life strategy exert the strongest influences in shaping population structure in shallow-water elasmobranchs. Highly mobile species such as the shortfin mako (*Isurus oxyrinchus*) exhibit genetic homogeneity over large spatial scales (Schrey and Heist, 2003), whereas less vagile species with more restricted geographic ranges, such as the zebra shark (*Stegostoma fasciatum*), show comparatively greater genetic differentiation across similar spatial scales (Dudgeon *et al.*, 2008). Regardless of a species' vagility, genetic differentiation has been found among populations separated by unsuitable environmental conditions. For instance, warm equatorial waters coincide with the areas of genetic break in temperate sharks, such as the spiny dogfish (*Squalus acanthias*; Veríssimo *et al.*, 2010), whereas open-ocean expanses separate populations of the coastal lemon shark (*Negaprion brevirostris*; Schultz *et al.*, 2008). Life-history strategy also influences the population structure of elasmobranchs. Discrete population units may be found along areas of continuous habitat in taxa where females exhibit philopatry to nursery areas, such as in the blacktip shark (*Carcharhinus limbatus*; Keeney *et al.*, 2003).

This work is the first approach to the study of the patterns of population structure in deep-water squaloids, using the Portuguese dogfish (*Centroscymnus coelolepis*) as a case study. The Portuguese dogfish is a medium-sized squaloid (<1.5 m) with an extensive geographic distribution including the Atlantic, southern Indian, and western Pacific oceans (Compagno *et al.*, 2005). In the eastern Atlantic, the species inhabits continental slope waters from west of the British Isles to South Africa (SA), including the Mediterranean, as well as off Madeira (MAD) and the Canary Islands, and along the Mid-Atlantic Ridge (MAR) from Iceland to (at least) the Azores (Compagno *et al.*, 2005; Menezes *et al.*, 2009). Its bathymetric range extends from middle to lower bathyal depths (500–1700 m), but may vary with region (Yano and Tanaka, 1988; Clarke *et al.*, 2001). Like most elasmobranch taxa, the Portuguese dogfish exhibits bathymetric segregation according to size, sex, and maturity stage. Immature fish are usually found deeper, mature males at intermediate depth, and mature females occupying the uppermost limit of the depth range (Yano and Tanaka, 1988; Girard and DuBuit, 1999; Clarke *et al.*, 2001).

The Portuguese dogfish reproduces through yolk-sac viviparity, attains sexual maturity at a large size, usually 85–90 cm long in males (maximum length 118 cm) and 99–102 cm in females (maximum length 122 cm), and has low mean fecundities of 10–14 pups per litter (Girard and DuBuit, 1999; Clarke *et al.*, 2001; Veríssimo *et al.*, 2003; Figueiredo *et al.*, 2008). The reproductive cycle in *C. coelolepis*, as in many of its deep-water relatives, does not appear to be synchronous among mature males and females (Girard and DuBuit, 1999; Clarke *et al.*, 2001; Veríssimo *et al.*, 2003; Figueiredo *et al.*, 2008). Moreover, females undergo a resting stage between consecutive gestation periods, as indicated by the absence of ripe oocytes at parturition (Veríssimo *et al.*, 2003). Pups are born at a length of ~30 cm after a gestation period of 2 years or more (Girard and DuBuit, 1999; Clarke *et al.*, 2001; Veríssimo *et al.*, 2003; Figueiredo *et al.*, 2008). No mating or nursery grounds have yet been reported for the species.

The Portuguese dogfish is taken commercially in the eastern North Atlantic, and until recently, it was caught regularly in mixed trawl and longline fisheries (ICES, 2009). Large-scale commercial exploitation off the European continental slope began for the species in the early 1990s and peaked in 2003 (10 876 t), decreasing thereafter (ICES, 2009). In 2005, the stock abundance of this and other species (e.g. *Centrophorus squamosus*) was determined to be depleted and below any candidate limit reference point, so a zero catch of deep-water sharks was recommended (ICES, 2009). Despite the state of the resources, management efforts have been developed without a good understanding of stock structure. In cases where the managed stock does not correspond to the true biological stock, e.g. where the managed stock includes several biological stocks or excludes part of the biological stock, the effectiveness of management plans may be compromised. Our study provides data that will help elucidate the intraspecific patterns of the population structure of *C. coelolepis* for future inclusion in fishery management. Moreover, the findings may provide insights into potential structuring factors of populations of other deep-water sharks.

Material and methods

Portuguese dogfish sample collections were obtained from six locations in the eastern Atlantic Ocean (Table 1)—off Ireland (IRE), mainland Portugal (POR), Mauritania (MAU), South Africa (SA), Madeira (MAD) and the mid-Atlantic ridge (MAR) north of the Azores—including most of the species' range in the region. Females were more abundant than males in all samples except SA (Table 1). The mean total length per collection was between 95.0 and 108.6 cm (i.e. adults or maturing fish), except MAU, where the mean total length was 62.3 cm (i.e. small juveniles; Table 1). The former collections (IRE, POR, MAD, SA, and MAR) are hereafter designated as adult collections and the latter (MAU) as the juvenile collection. Samples consisted of muscle tissue or finclips and were preserved in DMSO buffer saturated with NaCl (Seutin *et al.*, 1991). Total genomic DNA (gDNA) was extracted from each individual sample using the Qiagen DNeasy Tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

All fish were genotyped for a total of eight nuclear microsatellite loci (Table 2). Four new microsatellite loci (*CcoeGT25*, *CcoeGT55*, *Ccoe61*, and *Ccoe75*) were developed *de novo*, as described in Veríssimo *et al.* (in press). Two microsatellite loci, *SacaGA11* and *Saca3853*, were obtained as described in Veríssimo *et al.* (2010). Locus *Saca7551* (GenBank Accession no. EG027551) was obtained from the publicly available expressed sequence tag library for *S. acanthias* (GenBank dbEST ID 47209646). Finally, one locus (*DF J451*) was obtained from McCauley *et al.* (2004). All loci were chosen after testing for consistent amplification and conformation of genotypic distributions to the expectations of the Hardy–Weinberg equilibrium (HWE). Annealing temperatures and polymerase chain reaction (PCR) conditions were optimized for each microsatellite locus (see Table 2 and below for details). Microsatellite genotyping was conducted via PCR amplification in 5-μl reactions containing 5–15 ng of gDNA, 0.0375 μM forward primer with an added T3 tail, 0.15 μM reverse primer, 0.1 μM fluorescently labelled T3 primer (e.g. NED, PET, VIC, or 6FAM), 0.025 units *Taq* DNA polymerase (Qiagen), 0.2 mM of each DNTP, 1.5 mg $MgCl_2$, 1× *Taq* buffer (Qiagen), 1.0 μg of Bovine Serum Albumine (BSA), and autoclaved mili-Q water. PCR conditions consisted of an initial denaturation of 3 min at 94°C, followed by 35–45 cycles of 1 min at 94°C, 35–60 s at the corresponding annealing temperature, 35–60 s at 72°C, and a final extension step for 7 min at 72°C. The products were run on an ABI Prism 3130*xl* (Applied Biosystems, Warrington, UK). Genotypes were scored manually with the software GeneMarker version 1.3 (Softgenetics, LLC, State College, PA, USA) and the presence of null alleles or scoring errors was tested for each locus using Micro-Checker (van Oosterhout *et al.*, 2004). Conformation of genotypic distributions to HWE expectations for each locus within each sample collection and tests of linkage disequilibrium between each pair of loci within and among all collections were calculated in Genepop version 4.0 (Raymound and Rousset, 1995; Rousset, 2008).

Nucleotide sequences of the mitochondrial DNA (mtDNA) control region (CR) were obtained via PCR amplification using

Table 1. Genetic diversity indices for the Portuguese dogfish (*C. coelolepis*), integrated over all microsatellite loci and all mtDNA CR haplotypes from each sampling location.

	Nuclear microsatellites					Mitochondrial CR sequences						
Sampling location	***n***	H_o	F_{is}	***A***	R_s	***n***	***H***	***h***	π	***k***	**F/M**	**TL (cm)**
Ireland (IRE)	45	0.77	0.006	11.3 (6)	8.2	38	8 (2)	0.64	0.0019	0.926	14.0	97.4
Portugal (POR)	41	0.77	−0.032	11.4 (7)	8.1	42	7	0.57	0.0014	0.677	47.0	108.6
Madeira (MAD)	17	0.76	−0.017	8.0 (6)	7.9	15	6 (3)	0.74	0.0020	1.009	2.0	101.6
Azores (MAR)	40	0.76	0.026	11.0 (1)	8.1	35	9 (3)	0.74	0.0022	1.106	2.3	103.7
Mauritania (MAU)	46	0.78	−0.041	12.0 (12)	8.5	40	7	0.63	0.0017	0.826	1.4	62.3
South Africa (SA)	22	0.77	−0.008	8.9 (3)	7.9	22	6	0.69	0.0019	0.922	0.2	95.0

n, number of samples; H_o, observed mean heterozygosity; F_{is}, inbreeding coefficient; *A*, mean number of alleles (number of unique alleles); R_s, mean allelic richness; *H*, number of haplotypes (number of unique haplotypes); *h*, haplotype diversity; π, nucleotide diversity; *k*, mean number of nucleotide differences between haplotypes; F/M, females/males; TL, mean total length.

Table 2. Nuclear microsatellite loci of the Portuguese dogfish (*C. coelolepis*), with the respective repeat motif, annealing temperature (T_a), overall expected heterozygosity (H_e), total number of alleles scored per locus (A), and GenBank accession no.

Marker name	Repeat motif	T_a (°C)	H_e	A	GenBank accession no.
Ccoe25	$[TG]_7$	56	0.61	3	HQ462567
Ccoe55	$[CA]_{35}$	56	0.95	46	HQ462568
Ccoe61	$[TG]_{24}$	56	0.87	26	HQ462569
Ccoe75	$[TG]_{10}$	62	0.89	26	HQ462570
Saca7551	$[AG]_{14}$	58	0.71	7	EG027551
DFJ451	$[AC]_{10}$	55	0.67	6	AY584503
SacaGA11	$[TG]_9$ C [TG]	50	0.76	18	GU553360
Saca3853	$[TC]_9$ GC [$TC]_2$	50	0.69	6	ES883853
Overall			0.77	17.3	

the forward primer Pro-L 5′-AGGGRAAGGAGGGTCAAACT-3′ (Keeney *et al.*, 2003) and the reverse primer 5′-GGAGGATCTGTAAATCTTGAGACAG-3′ (developed *de novo*). PCR master mixes of 25-μl reactions included 10–20 ng of gDNA, 1 μM of each primer, 200 μM of each dNTP, 1× *Taq* buffer with 1.5 mM $MgCl_2$ (Qiagen), 0.025 units of *Taq* polymerase, 5 μg of BSA, and autoclaved mili-Q water. PCR conditions consisted of an initial denaturation of 5 min at 94°C, followed by 35 cycles of 1 min at 94°C, 35 s at 58°C, and 1 min at 72°C, and a final extension step for 7 min at 72°C. The amplicons were cleaned with the QIAquick PCR Purification kit (Qiagen) according to the manufacturer's protocol. The reverse strands of the amplicons were sequenced using the ABI Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) and run on an ABI Prism 3130*xl* genetic analyser (Applied Biosystems). The resulting DNA sequences were imported into Sequencher version 4.8 (Gene Codes Corp., Ann Arbor, MI, USA) and checked for quality and accuracy in nucleotide base assignment. All individual sequences were aligned in MacVector version 8.0 (MacVector, Inc., CA, USA) using the ClustalW multiple alignment algorithm (Thompson *et al.*, 1994). Haplotypes were confirmed by sequencing the forward strand of one individual per unique haplotype.

Indices of genetic diversity at the nuclear microsatellite loci were estimated in FSTAT version 2.9.3.2 (Goudet, 2002), including number of alleles per locus (A) and per sampling location, inbreeding coefficient (F_{is}), observed heterozygosity (H_o), and allelic richness (R_s). mtDNA CR diversity indices were calculated in DnaSP version 5 (Librado and Rozas, 2009) including number of haplotypes (H), haplotype diversity (h), nucleotide diversity (π), and the average number of nucleotide differences among sequences within each sampling location (k) and over all sequences. A visual representation of the genetic variability at the nuclear microsatellite loci within and among sampling locations was constructed using a factorial correspondence analysis (FCA) as implemented in Genetix version 4.05.2 (Belkhir *et al.*, 2004). In this analysis, individual genotypes are plotted in an n-factorial dimensional space according to their allelic composition over all loci, whereby individuals with similar genetic compositions are plotted closer together. Maximum parsimony mtDNA CR haplotype networks (Polzin and Daneschmand, 2003) were constructed using the median-joining algorithm (Bandelt *et al.*, 1999) with default parameters using Network 4.5.1.0 software (fluxus-engineering.com).

Levels of among-population genetic differentiation based on nuclear microsatellite data and mtDNA sequence data were estimated by the Weir and Cockerham's F_{ST} analogue (θ) in FSTAT and by Φ_{ST} values based on the mean number of pairwise differences among sequences in Arlequin version 3.11 (Excoffier *et al.*, 2005), respectively. Statistical significance of either estimate was assessed through 10 000 permutations of the dataset. The statistical power of the F_{ST} tests (i.e. rejection of the null hypothesis H_0 of genetic homogeneity among two subpopulations when it is false) and the α level (i.e. rejection of H_0 when it is true) were estimated with the POWSIM software (Ryman and Palm, 2006) using a sampling scheme of four subpopulations with 45 individuals each, plus two subpopulations with 20 individuals. The analyses were conducted using 10 000 dememorizations, 100 batches, and 1000 iterations per batch. An analysis of molecular variance (AMOVA) was conducted in Arlequin with both the nuclear and the mtDNA datasets, using 10 000 permutations of the data.

An isolation-by-distance (IBD) analysis was conducted with the program ISOLDE as implemented in Genepop version 4.0 (using the Mantel option) to test the null hypothesis of independence between genetic distances and sampling locations (p calculated using 1000 iterations). To this purpose, multilocus Cavalli–Sforza and Edwards' genetic distances were calculated in Genetix based on the microsatellite data, and uncorrected mean p-distances were calculated in Arlequin based on the mtDNA CR sequence data. Geographic distances were calculated as the shortest straight-line distance between sampling locations.

Past reduction in effective population size was investigated with nuclear microsatellites using the M-ratio test of Garza and Williamson (2001), and comparing it with the critical value (M_c) based on seven loci, $\theta = 10$, and the conservative parameters of 90% one-step mutations and a mean size of non-one-step mutations of 3.5 (Garza and Williamson, 2001). In addition, the occurrence of bottlenecks was investigated by the excess heterozygosity test implemented in Bottleneck version 1.2.02 (Piry *et al.*, 1999), using the Wilcoxon test under the null hypothesis of no significant heterozygosity excess (Piry *et al.*, 1999). The two methods were used for consistency purposes and to infer the relative timing and severity of detected bottleneck events (Williamson-Natesan, 2005). Changes in population size were investigated with Arlequin using the mtDNA CR sequence data and the mismatch distribution of pairwise nucleotide differences among sequences (10 000 permutations; Schneider and Excoffier, 1999). Deviations from selective neutrality for the mitochondrial CR were also tested with Tajima's D (Tajima, 1989) and Fu's F_S statistics (Fu, 1997; 1000 permutations, $\alpha = 0.05$) based on an infinite-site model without recombination.

Results

Genetic diversity

Multilocus microsatellite genotypes were obtained for 211 Portuguese dogfish, of which 20 had data missing at one locus. Genotypic distributions conformed to HWE expectations for all locus/population combinations and there was no evidence of linkage disequilibrium among loci (data not shown). Moreover, no null alleles were detected at any of the microsatellite loci. Gene diversity and total number of alleles per locus varied between 0.61 and 0.95 (mean = 0.77) and between 3 and 46 (mean = 17.3), respectively (Table 2). The levels of genetic diversity at the microsatellite loci were similar among sampling locations (Table 1), with mean observed heterozygosity varying

Table 3. Haplotype list for the Portuguese dogfish (*C. coelolepis*) showing the variable nucleotide positions ($n = 12$) and respective polymorphisms in the 496-bp fragment of the mtDNA CR.

	Variable nucleotide positions												GenBank accession no.
Haplotype	32	120	204	269	275	300	308	313	328	474	480	484	
H1	T	A	A	G	T	G	C	A	G	C	T	G	HQ664432
H2	.	.	.	.	.	.	.	.	A	.	.	.	HG664433
H3	.	.	.	.	.	C	.	.	.	.	.	.	HG664434
H4	.	.	.	.	A	.	.	.	A	.	.	.	HG664435
H5	.	G	.	.	.	.	.	.	A	.	.	.	HG664436
H6	.	.	.	.	.	.	.	.	A	.	C	.	HG664437
H7	.	G	.	.	.	.	.	.	.	.	.	.	HG664438
H8	.	.	.	.	.	.	.	G	A	.	.	.	HG664439
H9	.	.	.	.	.	.	.	G	A	.	C	.	HG664440
H10	.	.	.	–	.	.	.	.	.	.	.	.	HG664441
H11	.	.	.	A	.	.	.	.	A	.	.	.	HG664442
H12	.	.	.	A	.	.	.	.	.	.	.	.	HG664443
H13	.	.	.	.	.	.	T	.	A	.	.	.	HG664444
H14	G	.	.	.	.	.	.	.	.	.	.	.	HG664445
H15	.	.	.	.	.	.	.	.	.	.	.	A	HG664446
H16	.	.	G	.	.	.	.	.	.	.	.	.	HG664447
H17	.	.	.	.	.	.	.	.	.	A	.	.	HG664448
H18	.	.	.	.	.	.	.	.	.	.	C	.	HG664449

between 0.76 (MAD and MAR) and 0.78 (MAU), and mean allelic richness between 7.9 (MAD) and 8.5 (MAU).

Nucleotide sequences of a 496-bp fragment of the mtDNA CR were obtained for 192 of the Portuguese dogfish sampled, resulting in 18 unique haplotypes (GenBank accession no. HQ664432–HQ664449). There were 12 polymorphic positions corresponding to eight transitions, four transversions, and one deletion (Table 3). The mean nucleotide and haplotype diversities across all sequences were 0.0018 and 0.65, respectively, and the mean number of nucleotide differences between all sequences was 0.872. The number of haplotypes found in each sampling location was small, ranging from a minimum of six in MAD and SA to a maximum of nine in MAR, and the haplotype diversities were low, varying between 0.57 (POR) and 0.74 (MAD and MAR; Table 1). Ten of the 18 haplotypes (56%) were shared by two or more sampling locations (Table 4), comprising some 96% of the fish sequenced. Singleton haplotypes were found in IRE ($n = 2$), MAR ($n = 3$), and MAD ($n = 3$; Table 4).

Table 4. mtDNA CR haplotype frequencies found per sampling location of the Portuguese dogfish (*C. coelolepis*).

Haplotype	IRE	POR	MAD	MAR	MAU	SA	Total
H1	22	26	7	15	21	11	102
H2	7	10	4	10	13	6	50
H3	–	–	–	1	–	–	1
H4	–	–	–	1	–	–	1
H5	–	–	–	1	–	–	1
H6	2	1	–	1	–	1	5
H7	3	2	–	3	2	–	10
H8	–	–	–	2	1	1	4
H9	1	–	–	1	1	–	3
H10	1	–	–	–	–	–	1
H11	1	–	–	–	–	–	1
H12	1	1	1	–	–	–	3
H13	–	–	1	–	–	–	1
H14	–	–	1	–	–	–	1
H15	–	–	1	–	–	–	1
H16	–	–	–	–	1	2	3
H17	–	1	–	–	1	–	2
H18	–	1	–	–	–	1	2
Total	38	42	15	35	40	22	192

Genetic differentiation and population structure

Individual multilocus microsatellite genotypes were used in an FCA to provide a visual representation of the underlying structure in the data matrix. The resulting analysis indicated that eigenvectors 1–3 explained 70% of the inertia and showed largely overlapping distributions of multilocus genotypes among collections. This overlap was particularly evident among individuals from IRE, MAR, MAU, and SA (Figure 1), whereas those from POR and MAD were spread over different quadrants of the bi-dimensional space. Overall, the FCA indicated great similarity among the individual multilocus microsatellite genotypes sampled from different locations. When comparing adult/maturing vs. juvenile fish genotypes (80-cm total length cut-off), the latter showed a more restricted distribution in space than the former, but there was still a broad overlap of juvenile genotypes with those of adult/maturing fish (data not shown).

Pairwise F_{ST} tests indicated no significant genetic differentiation among sampling locations (−0.0013 to 0.0096) after Bonferroni correction (Table 5). However, most pairwise F_{ST} values including the MAD collection were significant ($p < 0.05$, except for MAR) before Bonferroni correction. The power analysis for detecting genetic differentiation using the nuclear microsatellite loci indicated that a pairwise F_{ST} of 0.01 could be detected in 100% of the runs, whereas an F_{ST} 0.002 could only be detected in 66% of the runs. F_{ST} values <0.02 could not be detected in most runs (data not shown). The α-value (type I error) was 0.05. In terms of the pairwise Φ_{ST} tests, there was no indication of genetic differentiation among sampling locations (−0.017 to 0.033; $p > 0.05$). The null hypothesis of global panmixia considering only the adult fish collections (i.e. excluding MAU) was tested by AMOVA and was not rejected with either the nuclear microsatellite data or the mtDNA sequence data ($F_{ST} = 0.00038$ and $\Phi_{ST} = 0.00153$; $p > 0.5$). This result did not change when MAU

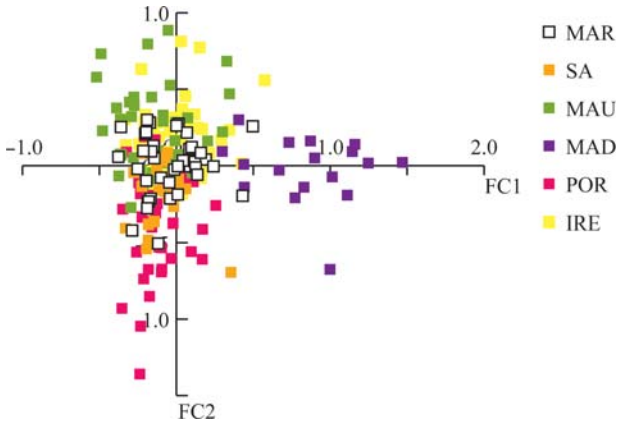

Figure 1. Factorial component analysis of multilocus microsatellite genotypes of the Portuguese dogfish (*C. coelolepis*), according to sampling collection (see Table 1 for abbreviations). Factorial component 1 (FC1) is the *x*-axis (30.4% inertia) and factorial component 2 (FC2) is the *y*-axis (20.7% inertia).

Table 5. Levels of genetic divergence among populations of *C. coelolepis*, with pairwise microsatellite F_{ST} below the diagonal and pairwise mtDNA CR Φ_{ST} above the diagonal.

	Φ_{ST}					
F_{ST}	**IRE**	**POR**	**MAD**	**MAU**	**SA**	**MAR**
IRE		−0.017	−0.016	−0.005	−0.009	0.012
POR	−0.001		−0.012	−0.002	0.000	0.033
MAD	0.000	0.002		−0.016	−0.017	0.007
MAU	0.000	0.000	0.010		−0.022	0.003
SA	0.000	−0.001	0.000	−0.001		0.000
MAR	0.000	0.000	−0.001	−0.010	−0.004	

No *p*-value was significant after Bonferroni correction.

was included in the analysis ($F_{ST} = 0.00086$ and $\Phi_{ST} = -0.00259$; $p > 0.5$). There was no correlation between multilocus Cavalli–Sforza and Edwards genetic distances and geographic location, and the null hypothesis of independence between the two variables was not rejected ($a = 0.0194$, $b = 1.8 \times 10^{-4}$; $p > 0.5$). Similarly, no evidence of IBD was apparent in the mtDNA sequence data ($a = 0.920$, $b = -1.6 \times 10^{-3}$; $p > 0.5$).

The haplotype network of the mtDNA CR fragment had a star-shaped conformation with two common, central haplotypes differing by only one mutation step and found in 53 and 23% of the fish, respectively. Multiple, less frequent haplotypes (<6%, or <10 fish) differed from one of the central haplotypes by a single mutation step (except haplotype 9, two mutation steps from the closest central haplotype; Figure 2). Overall, haplotypes differed by 1–4 mutation steps. In terms of haplotype distribution, no discernible geographic pattern was evident from the network. The two central haplotypes were found at all sampling locations and made up the majority of the fish at each site (from 72 to 86%). The less-frequent, more-derived haplotypes were found in only 1–2 fish per site, and were shared among locations throughout the sampling area, i.e. were not restricted to any particular geographic region (Figure 2).

Demographic analyses

All samples were included in one group, which was used in all demographic analyses described below. The occurrence of past

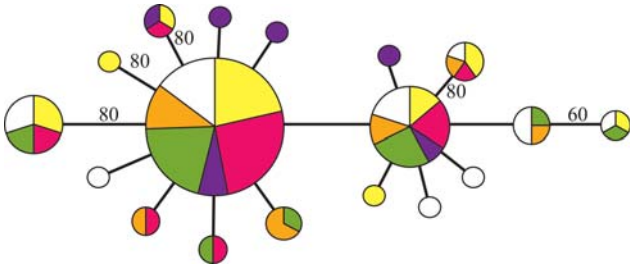

Figure 2. Maximum parsimony haplotype network of the mtDNA CR of the Portuguese dogfish (*C. coelolepis*). Haplotypes ($n = 18$) are represented by circles with sizes proportional to absolute frequency in the total sample. All connections correspond to one nucleotide substitution between haplotypes. Support values of connections were 100% except where noted. Colour code: yellow, Ireland; dark pink, Portugal; white, the Azores; purple, Madeira; green, Mauritania; orange, South Africa.

population size reductions was rejected by the two tests applied to the nuclear microsatellite data. The Wilcoxon test did not detect excess heterozygosity ($p > 0.05$, irrespective of mutation model), and the *M*-ratio test indicated a mean observed *M* of 0.93 (variance 0.26), which was larger than the critical value of *M* under conservative parameters ($M_c \approx 0.675$). Alternatively, the results from the mtDNA-based population expansion analyses were rather ambiguous. Evidence of past population growth was suggested by negative Tajima's *D* (−1.40, $p > 0.05$) and Fu's F_S statistics (−14.60, $p < 0.01$), although only the latter, most-sensitive test (Ramos-Onsins and Rozas, 2002) was significant. In contrast, the frequency distribution of pairwise differences among sequences did not conform to a model of sudden population expansion (Harpending's raggedness = 0.11, $p < 0.01$).

Discussion

We found no evidence of genetic population structure among collections of the Portuguese dogfish from the eastern Atlantic and the MAR north of the Azores. The genetic diversity found at the nuclear microsatellite loci and at the mtDNA CR was homogeneously distributed among sampling locations, resulting in low levels of genetic differentiation. Also, there was no indication of increased genetic divergence with geographic distance in our dataset. The observed pattern of genetic diversity is consistent with the absence of genetic population structure in the Portuguese dogfish in the above area. Moreover, our demographic analyses showed no indication of past reduction in population size, and the signal of population growth was ambiguous. These results suggest that the population of the Portuguese dogfish in the eastern Atlantic has historically been relatively stable in size.

Our ability to detect the signal of genetic divergence over the noise may have been compromised by random sampling errors associated with marker variability or sample size, or by non-random sampling errors attributable to an incomplete representation of the entire population (Waples, 1998). Indeed, the low levels of genetic diversity found in the mtDNA CR of *C. coelolepis*, although not unexpected in elasmobranch taxa (Martin and Palumbi, 1993; Martin, 1999), may have reduced the power to detect genetic differentiation among locations. Similarly, the genetic diversity at the nuclear microsatellite loci resulted in limited power to detect genetic differentiation levels as low as those calculated with our dataset. On the other hand, there was

the potential for an upward bias in the F_{ST} values resulting from small sample sizes (e.g. MAD) and unequal composition of sample collections, e.g. dominated by juveniles or maturing/adult fish (Waples, 1998). Nevertheless, all pairwise F_{ST} values were consistently low (in fact, most were <0.0005; Table 5), suggesting little genetic differentiation among locations. These results were in agreement with the FCA and the AMOVA. All analyses are consistent in suggesting a lack of geographic partitioning of the genetic variability surveyed at the mtDNA CR and at the nuclear microsatellite loci in *C. coelolepis*.

The genetic homogeneity found among Portuguese dogfish collections separated by thousands of kilometres implies gene flow among the sampled locations either currently or in the recent past. Because long-distance dispersal and gene flow in elasmobranchs strictly depend on active swimming of the individuals (Musick *et al.*, 2004), a considerable number of migrant fish per generation must be exchanged among populations to account for the low levels of genetic differentiation found among Portuguese dogfish sample collections. This observation strongly suggests that *C. coelolepis* has high dispersal potential, and there appear to be no major barriers to dispersal within the eastern Atlantic. However, the generation times of squaloid sharks are often on the order of decades (Kyne and Simpfendorfer, 2010). In the Portuguese dogfish, it may be close to 20 years, as estimated for its similar-sized relative *Centroselachus crepidater* (Irvine *et al.*, 2006). Therefore, long-distance dispersal in *C. coelolepis* may take place over many years. Also, because vagility and body length appear to be intimately related in elasmobranchs (Musick *et al.*, 2004), it may be reasonable to expect that other deep-water squaloids of similar or larger size than *C. coelolepis* may be equally capable of long-distance movements within the span of a generation.

Life-history strategy is also an important factor shaping the overall pattern of population structure in elasmobranchs. Some important insights into the life strategy of *C. coelolepis* have been provided by earlier studies, but of particular relevance to our work is the maturity stage composition of the Portuguese dogfish collected at different locations (Table 6). In general, all stages of the reproductive cycle were found in any given area, although with different relative frequencies. Mature females predominated over immature females in catches from all areas surveyed, although the latter had slightly higher frequencies off POR and off the British Isles. Females in the first stages of the reproductive cycle, i.e. before ovulation (resting/maturing, or with ripe oocytes), constituted the vast majority of the mature females caught on the MAR and off MAD, but roughly half of those caught off western Europe. Pregnant females accounted for 34–51% of the mature females off POR, but were less frequent along the MAR and west of the British Isles (<26%). Spent females (post-partum) were reported only off POR and west of the British Isles. Interestingly, mature males dominated over immature/maturing males in areas where most of the females were in early reproductive stages (i.e. MAR and MAD), the opposite being true where pregnant females were more common (e.g. off POR). In terms of the distribution of small, juvenile Portuguese dogfish (mostly <70 cm), several studies have reported their absence in catches from deep demersal longlines and trawls operating off the British Isles and POR (Girard and DuBuit, 1999; Clarke *et al.*, 2001; Veríssimo *et al.*, 2003). In contrast, 88% of the *C. coelolepis* sampled off the Mauritanian continental slope with demersal trawls were juveniles (L. Fernandéz, pers. comm.), suggesting that the area may be a nursery for *C. coelolepis*.

The above data suggest that the Portuguese dogfish may be spatially segregated according to size and maturity stage and that mature females may undergo large-scale migration within the eastern North Atlantic associated with the reproductive cycle. For instance, mating may take place along the MAR and west of the British Isles, with pregnant females potentially moving along the continental slope off western Europe south to MAU to give birth. After parturition, spent females may move back to the feeding areas and undergo a resting period before the subsequent breeding cycle. The absence of small juveniles in catches from the western European continental slope and northern MAR (where adults dominate) may indicate recruitment to these areas only when individuals are nearing sexual maturation (ca. 80 cm). If

Table 6. Maturity stage composition (% of total sample) of male and female Portuguese dogfish (*C. coelolepis*).

	Males			Females							
Sampling location	TL (cm)	IM/Mat	Adult	TL (cm)	IM	Mat/Rest	Ripe	Preg	Spent	Depth (m)	Reference
Off British Isles	70–116	36	64	90–121	28	55[a]		14	31	500–2 000	Clarke *et al.* (2001)[b], $n = 644$
Off Portugal	68–100	85	15	82–122	26	33	25	34	7	800–1 600	Figueiredo *et al.* (2008), $n = 2\,177$
	68–100	85	15	76–120	15	25	18	51	6	700–1 500	Veríssimo *et al.* (2003), $n = 956$
Off Madeira (Seine Seamount)	85–99	11	99	92–115	10	68	16	16	–	180–2 100	G. Menezes, pers. comm., $n = 33$
Off MAR (north of the Azores)	80–100	21	79	80–120		20	77	3	–	840–3 400	C. Cotton, pers. comm., $n = 63$
	85–98		100	85–119	9	70	5	25	–	740–2 000	G. Menezes, pers. comm., $n = 39$

TL, total length range; IM, immature; Mat, maturing; Rest, resting; Preg, pregnant.
[a]Includes mature, resting, and ripe females.
[b]Only 57% of individuals were from longlines, and the remainder were from deep-water trawls (M. Clarke, pers. comm.).

that is true, the above life-history strategy of the Portuguese dogfish would be consistent with the absence of population structure within the eastern North Atlantic. Nevertheless, the low levels of genetic divergence between the SA collection and those to the north may reflect connectivity between the two areas of the eastern Atlantic.

The pattern of spatial segregation described above for *C. coelolepis* needs further confirmation because the catch composition reported by the different studies was not obtained in a standardized fashion. Nevertheless, the bias associated with interstudy comparisons was reduced by considering only data from deep-water longlines (except off western IRE; Table 6), which are more efficient at catching deep-water elasmobranchs and are also less size-selective for *C. coelolepis* than demersal trawls (Clarke *et al.*, 2005). Moreover, only those studies where fishing occurred throughout the whole bathymetric range of the species were compared (Table 6) to avoid bias associated with the bathymetric segregation by size, sex, and maturity stage reported for *C. coelolepis* (Girard and DuBuit, 1999; Clarke *et al.*, 2001). Future studies should aim at providing a more detailed view of the life strategy of the species, by extending the area surveyed and by standardizing catch compositions.

Spatial segregation according to size, sex, and maturity stage has been previously reported for other elasmobranch taxa. The existence of spatially discrete nursery areas, with juveniles away from adult habitats, has been found for most of the carcharhinid and sphyrnid sharks studied to date (Grubbs, 2010). Also, spatial segregation by sex and maturity stage has been described for the blue shark (*Prionace glauca*; Nakano and Seki, 2003) and the sandbar shark (*Carcharhinus plumbeus*; Grubbs, 2010). The complex population structures and migration patterns in these epipelagic oceanic sharks have been associated with the challenges of finding food, mates, and nursery areas (i.e. of increased productivity) in the vast and rather unproductive epipelagic marine environment (Stevens, 2010). This particular life strategy may help in maximizing resource partitioning, so reducing intraspecific competition in taxa found in habitats with limited food availability. Similarly, deep-water squaloids may benefit from adopting such a strategy in the large and food-limited deep-sea environment.

Independent evidence of spatial segregation with maturity stage in other deep-water squaloids has been suggested by several authors. For instance, few gestating females of the great lanternshark (*Etmopterus princeps*) were reported off Iceland (Jakobsdóttir, 2001), and pregnant and ripe females were dominant along the MAR (Cotton, 2010). Pregnant female leafscale gulper sharks (*C. squamosus*) were also rarely caught along the western European continental slope (Girard and DuBuit, 1999; Clarke *et al.*, 2001; Figueiredo *et al.*, 2008), but made up 34% of the females sampled off MAD (Severino *et al.*, 2009). In contrast, all *Centrophorus* cf. *niaukang* caught in the western North Atlantic were adult females, and most were pregnant (Cotton, 2010). Clearly, more data are needed to confirm these trends, but the circumstantial evidence above strongly suggests that intraspecific spatial segregation in deep-water squaloid shark populations by sex and maturity stage may be found in taxa other than the Portuguese dogfish. Consequently, the spatial distribution of a given population unit may include a considerably large geographic area.

In the light of our results, future fishery management plans for the Portuguese dogfish need to consider the genetic stock unit to include the whole eastern Atlantic and the northern section of the MAR. However, it should be kept in mind that the rates of migration between neighbouring areas may not be sufficient to ensure short-term recovery in abundance in the event of localized depletion, although they may be enough to homogenize the gene pool between those areas (Carvalho and Hauser, 1994). Also, as a result of the apparent spatial segregation by size and maturity stage of *C. coelolepis,* fishing operations conducted in different regions of the eastern Atlantic are likely to target different components of the population, e.g. gestating females or small juveniles. This practice can have repercussions not only locally, but over the entire population, so the distribution of fishing effort needs to be managed accordingly.

To summarize, we found no evidence of genetic differentiation among sample collections of the Portuguese dogfish (*C. coelolepis*), including an area extending from west of the British Isles to SA, including the northern section of the MAR. This result was consistent between nuclear microsatellite and mtDNA CR datasets. The results therefore suggest that there is no genetic population structure of the Portuguese dogfish in the area studied.

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