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Genetic structure of *Hediste diversicolor* (Polychaeta, Nereididae) from the northwestern Mediterranean as revealed by DNA inter-simple sequence repeat (ISSR) markers

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ABSTRACT: The polychaete *Hediste diversicolor* is characterised by a high degree of genetic structure among populations. The genetic differentiation has been related to the presence of cryptic species, corresponding to Atlantic and Mediterranean lineages whose divergence may have been driven by vicariance and/or selection. In the present study we used DNA inter-simple sequence repeat (ISSR) markers to validate the genetic structure in 9 populations from the northwestern Mediterranean. We also analysed patterns of genetic differentiation among Mediterranean and Atlantic samples to assess the occurrence of further cryptic species in the northwestern Mediterranean. We compared our results with previous mitochondrial DNA data to assess whether nuclear and mitochondrial genetic patterns were consistent. Analysis of ISSR banding patterns showed: (1) a geographical structuring within the northwestern Mediterranean, and (2) a sharp genetic differentiation between Atlantic and Mediterranean groups of populations, confirming the findings from previous mitochondrial DNA surveys. The pattern of genetic variability found in the northwestern Mediterranean is unlikely to reflect the occurrence of cryptic species in this area. On the other hand, our results are consistent with the occurrence of 2 cryptic species in the Mediterranean and Atlantic, whose genetic differentiation may have been driven by vicariance. Finally, the present study further highlights the usefulness and effectiveness of ISSR markers for monitoring genetic diversity in aquatic invertebrates.

KEY WORDS: *Hediste diversicolor* · ISSR markers · Genetic diversity · Cryptic species · Habitat fragmentation · Bayesian analysis

INTRODUCTION

The ragworm *Hediste diversicolor* (O.F. Müller 1776) (Polychaeta, Nereididae) is a suitable model for studying the effects of processes that may affect species' genetic structure. This polychaete occurs in naturally fragmented habitats, and it is an important member of brackish water ecosystems in which it plays a major role (Scaps 2002). Its dispersal capabilities are mainly limited by the sedentary life-style of the adults, the brooding behaviour of females, and the lack of pelagic larval stages (Scaps 2002). Studies carried out on heritable morphological traits and molecular markers (Scaps 2002, Maltagliati et al. 2006a and references therein) showed a high degree of population differentiation at both local and regional spatial scales. Along with the limited or absent inter-population gene flow due to species' life-history traits (Abbiati & Maltagliati 1996, Breton et al. 2003), different local selective pressures and stochastic pro-

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cesses coupled with habitat discontinuity (Virgilio & Abbiati 2004, 2006), as well as the presence of hydrographical barriers among estuaries (Fong & Garthwaite 1994), have been suggested to play important roles in shaping species' genetic structure.

Hediste diversicolor is likely to be a complex of cryptic species, which is quite common in estuarine and marine polychaetes (Audzijonyte et al. 2008, Virgilio et al. 2009 and references therein). Indeed, based upon allozyme and mitochondrial DNA data, Audzijonyte et al. (2008) pointed out that the 2 genetic types of *H. diversicolor* from the North and Baltic Seas (Röhner et al. 1997) were likely to be reproductively isolated and were referred to as species A and B. These authors also hypothesised a recent introduction of species B in the Baltic Sea by two or more colonisation events. Subsequently, a large-scale phylogeographical survey showed the occurrence of 3 main, deeply divergent mtDNA lineages with a nearly disjunct geographical distribution (Virgilio et al. 2009). Indeed, one lineage (phylogenetically related to species A haplotypes) was spread mainly across the Atlantic, and may represent a local gene pool. The remaining two were related to the different lineages found in species B, and were mainly recovered in the Mediterranean and the Black Sea, respectively (Virgilio et al. 2009). These results supported a recent introduction of species B in the North and Baltic Seas (Audzijonyte et al. 2008), suggesting that vicariance and, eventually, different environmental conditions among the basins, may have shaped the mtDNA pattern of differentiation (Virgilio et al. 2009).

In the present study we used inter-simple sequence repeat (ISSR) analysis (for details, see Zietkiewicz et al. 1994) to investigate the patterns of genetic structure in populations of *Hediste diversicolor*. ISSR molecular markers have been proven to be useful in studying inter- and intraspecific relationships (Casu et al. 2005, 2006, 2009, Maltagliati et al. 2005, 2006b, Varela et al. 2007, De Aranzamendi et al. 2008, Lai et al. 2008, Pannacciulli et al. 2009). Although ISSRs are dominant markers, simulation studies indicated that this kind of markers may be as efficient as codominant ones in estimating genetic diversity if an appropriate number of loci is used (Mariette et al. 2002).

Our aim was to assess the occurrence of genetic structure in populations of *Hediste diversicolor* from the northwestern Mediterranean, paying particular attention to the Sardinian-Corsican region and to the influence of potential barriers to dispersal, such as the north Tyrrhenian Sea. We also analysed patterns of genetic variation among Mediterranean and Atlantic samples to evaluate if any genetic structure found in the northwestern Mediterranean may reflect the occurrence of further cryptic speciation within this area. Concordant patterns of genetic structure as inferred from mitochondrial (Virgilio et al. 2009) and nuclear markers would point to differentiation driven by vicariance.

MATERIALS AND METHODS

Samples and DNA extraction

Individuals of *Hediste diversicolor* were collected from March to October 2002 at 12 sites, located in the western Mediterranean and on European Atlantic coasts (Fig. 1). For each locality, at least 30 individuals were sampled, labelled, and preserved in ab-

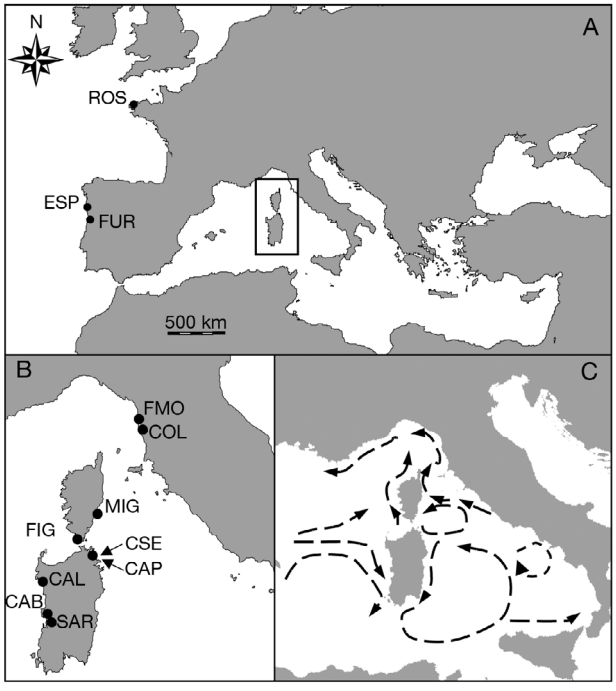

Fig. 1. *Hediste diversicolor.* (A) Geographical location of Atlantic and Mediterranean sampled populations. (B) Sampling locations in the northwestern Mediterranean. (C) Mean annual currents in the northwestern Mediterranean (modified from Millot 1999, Rio et al. 2007). CAL, Calich pond (40° 36′ 24″ N, 8° 18′ 28″ E); CAB, Cabras pond (39° 54′ 41″ N, 8° 30′ 57″ E); SAR, S'Ena Arrubia pond (39° 49′ 24″ N, 8° 34′ 36″ E); CAP, Cala Petralana (41° 11′ 10″ N, 9° 20′ 20″ E); CSE, Costa Serena (41° 11′ 32″ N, 9° 20′ 32″ E); FIG, Figari bay (41° 26′ 30″ N, 9° 07′ 21″ E); MIG, Migliacciaru (42° 59′ 35″ N, 9° 26′ 40″ E); FMO, Fiume Morto (43° 44′ 03″ N, 10° 17′ 02″ E); COL, Idrovora di Coltano (43° 38′ 29″ N, 10° 24′ 49″ E); FUR, Furadouro estuary (40° 51′ 16″ N, 8° 39′ 32″ W); ESP, Esposende lagoon (41 °32′ 29″ N, 8° 47′ 25″ W); ROS, Roscoff bay (48° 42′ 46″ N, 4° 00′ 03″ W)

solute ethanol at 4°C until genetic analyses were performed. Genomic DNA was extracted from 4 to 6 parapodia per individual using the QIAGEN® DNeasy Tissue kit (QIAGEN). Only specimens that were bright- or dark-green coloured were analysed to minimise the presence of individuals belonging to different age classes (Dales & Kennedy 1954). Once extracted, DNA was stored in solution at 4°C.

ISSR amplifications

A set of 22 primers (13 found on the web at www.biosci.ohio-state.edu/~awolfe/ISSR/protocols.ISSR.html, and 9 designed by ourselves) was preliminarily assayed on a limited number of individuals. This screening allowed the identification of 5 primers: UBC809: 5′-$(AG)_8$G-3′; UBC811: 5′-$(GA)_8$C-3′; UBC827: 5′-$(AC)_8$G-3′; SAS1: 5′-$(GTG)_4$GC-3′; and SAS3: 5′-$(GAG)_4$GC-3′ that produced scorable and reproducible bands (Table 1). The PCR reaction mixture (25 µl volume) contained 0.5 units of *Taq* DNA Polymerase (Pharmacia®), 1× reaction buffer (Pharmacia®), 2.5 mM $MgCl_2$, 0.2 µM primer, 200 µM of each dNTP (Roche®), and up to 30 ng of genomic DNA. PCR amplification was performed in an i-cycler Thermal Cycler (Biorad®) programmed for 1 cycle of 3 min at 94°C, 35 cycles of 40 s at 94°C, 45 s at 50°C, and 1 min and 40 s at 72°C. At the end of these cycles a post-treatment at 72°C for 5 min to complete partial amplification and a final cooling at 4°C were performed. For each primer, negative controls and 2 sample replicates were included in the amplifications to verify repeatability of results.

Electrophoresis and visualisation of amplification products

The PCR products were analysed by electrophoresis using a 2% agarose gel in 1× TAE buffer (0.04 M Tris-acetate, and 0.001 M EDTA). Gels were run at 80 V (4 V cm^{-1}) for 2.5 h and stained by soaking gel in a 1 µl per 10 ml ethidium bromide solution for 15 min. ISSR banding patterns were visualized using a photo-UV transilluminator system and recorded by digital photography. One hundred base pair ladders (DNA Molecular Weight Marker XIV; Roche®) were run for reference with each primer.

Table 1. *Hediste diversicolor*. Inter-simple sequence repeat (ISSR) primers used in this study. Primer sequence and number and size of bands scored

Primer	Sequence (5′-3′)	No. of bands scored	Range of band size (bp)
UBC 809	AGA GAG AGA GAG AGA GG	37	330–2500
UBC 811	GAG AGA GAG AGA GAG AC	23	500–2642
UBC 827	ACA CAC ACA CAC ACA CG	28	500–>2500
SAS 1	GTG GTG GTG GTG GC	25	230–1730
SAS 3	GAG GAG GAG GAG GC	29	350–2100

Statistical treatment of ISSR data

The number of bands, number and percentage of polymorphic loci at the 95% criterion (assuming each band represented a locus), and the Shannon index (*I*) (Lewontin 1972) were calculated using the software GENALEX 6.4 (Peakall & Smouse 2006). For each pair of populations a *t*-test on arcsine square root transformed values of *I* was performed to test significant differences among populations. Bonferroni's sequential correction for multiple tests was applied to adjust the significance level in order to avoid a Type I error (Hochberg 1988).

The genetic structure was investigated by means of an individual based approach, which may avoid the artefacts due to the assumption of predefined populations (Mank & Avise 2004). To this end the Bayesian model-based clustering algorithm implemented in the software STRUCTURE ver. 2.3.1 was used (Pritchard et al. 2000, Falush et al. 2007). This method assigns individuals to clusters according to the multilocus genotype, without prior knowledge of their geographical origin. The number of clusters (*K*) is chosen in advance, and the posterior probability of data, lnP(D), is estimated for each value of *K*. We applied both the no-admixture model with independent allelic frequencies and the admixture model with correlated allelic frequencies (Falush et al. 2003). For each value of *K*, ranging from 1 to the maximum number of populations plus 3, 10 independent runs were performed, each consisting of 100 000 iterations after a burn-in period of 100 000, in order to assess consistency of results across runs. To identify clusters of individuals that might reflect sharp discontinuities in gene frequencies (Garnier et al. 2004), we considered: (1) the average value of lnP(D), (2) the consistency of results across replicated runs for a given *K*, and (3) the method described in Evanno et al. (2005), which retrieves the highest hierarchical structure present in the data. This procedure was applied to: (1) a dataset containing only samples from the northwestern Mediterranean, and (2) the

former dataset plus the Atlantic samples. All runs were performed partly at the CBSU server, partly at Oslo University bioportal (Kumar et al. 2009).

The software CLUMPP ver. 1.1.2 (Jakobsson & Rosenberg 2007) was used to check the consistency of results across replicate runs. The average pairwise similarity (H) among replicate runs for a given K was estimated by a Greedy search algorithm with 10000 random input orders. Replicate runs with similarity values above 0.85, which corresponds to a similar population structure (Rosenberg et al. 2002), were then merged to obtain the average membership of all individuals to each cluster. The results were displayed by the software DISTRUCT ver. 1.1 (Rosenberg 2004).

The genetic structure was also investigated by hierarchical analysis of molecular variance (AMOVA) using the approach described in Excoffier et al. (1992) and implemented in the software package ARLEQUIN ver. 3.5.1.3 (Excoffier & Lischer 2010). The partitioning of genetic variation was analysed within and among populations and among groups of populations. We used 2 grouping schemes, one of which corresponded to the Mediterranean and Atlantic regions. A further grouping scheme took into account the results of model-based clustering analysis within the northwestern Mediterranean region. The significance of the Φ statistics parameters was assessed by a non-parametric permutation test with 16000 replicates.

Table 2. *Hediste diversicolor.* Estimates of within-population genetic diversity. Population abbreviations are given in Fig. 1. PLN: number of polymorphic loci; PLP: percentage of polymorphic loci; I: Shannon index. Values are mean ± SE

Populations	No. of ind.	No. of bands	Private bands	PLN	PLP	I
CAL	30	25	0	24	16.9	0.090 (0.018)
CAB	29	47	7	32	22.5	0.131 (0.019)
SAR	30	55	5	45	31.7	0.170 (0.021)
CAP	22	32	1	21	14.8	0.080 (0.015)
CSE	27	69	17	44	31	0.146 (0.016)
FIG	29	26	0	23	16.2	0.093 (0.018)
MIG	30	29	1	28	19.7	0.083 (0.016)
FMO	30	29	0	26	18.3	0.098 (0.018)
COL	30	35	0	30	21.1	0.096 (0.017)
FUR	26	46	2	37	26.1	0.109 (0.017)
ESP	30	54	6	51	35.9	0.155 (0.020)
ROS	30	54	4	44	31	0.165 (0.021)

RESULTS

A total of 142 DNA fragments, whose size ranged from 230 to 2642 base pairs (Table 1), was scored. All markers were polymorphic in at least one population, and almost all individuals displayed different ISSR banding patterns. For these presumptive loci the average percentage of polymorphism within populations was 23.8% and ranged between 14.8% (CAP, Sardinia) and 35.9% (ESP, Portugal) (Table 2). The former population was also characterised by the lowest values of the Shannon diversity index (I = 0.080 ± 0.015; mean ± SE), while the highest value of this parameter was found in the sample from the Sardinian population of SAR (I = 0.170 ± 0.021) (Table 2). Pairwise t-tests performed on the Shannon diversity index yielded 27 significant differences, but they dropped to 1 after the Bonferroni sequential correction (data not shown).

The abundance of private bands, namely bands observed in only one population (43 out 142, corresponding to 30.3%), represented the first signal of the high level of genetic divergence among populations (Table 2). Moreover, about half of the total fragments (70 out 142) were shared by Atlantic and Mediterranean population groups; the others were partitioned in 19 Atlantic-private and 53 Mediterranean-private bands.

Both the no-admixture model with independent allele frequencies and the admixture model with correlated allele frequencies yielded highly similar results; for both datasets, the logarithm of posterior probabilities, lnP(D), increased from K = 1 to a value after which the curve began to flatten until reaching a plateau. This value corresponded to K = 6 for dataset with northwestern Mediterranean samples only, and to K = 7 for the entire dataset.

The most consistent results across replicate runs were found in models considering 4 and 2 clusters for the smaller (samples from the northwestern Mediterranean) and the entire datasets, respectively. The average pairwise similarity of H = 0.99 was far beyond the threshold value of 0.85 for highly similar clustering results (Rosenberg et al. 2002) . All other simulations showed more than one clustering solution with average pairwise similarity values <0.85, with the exception of the simulation at K = 6 under the admixture model for the entire dataset (H = 0.98).

When we searched for the uppermost hierarchical structure present in the data, the ΔK statistics (Evanno et al. 2005) showed for both models a clear peak occurring at K = 4 in the northwestern Mediterranean, and K = 2 when Atlantic samples were included in the analysis. In the first dataset individuals were subdivided into clusters that roughly corre-

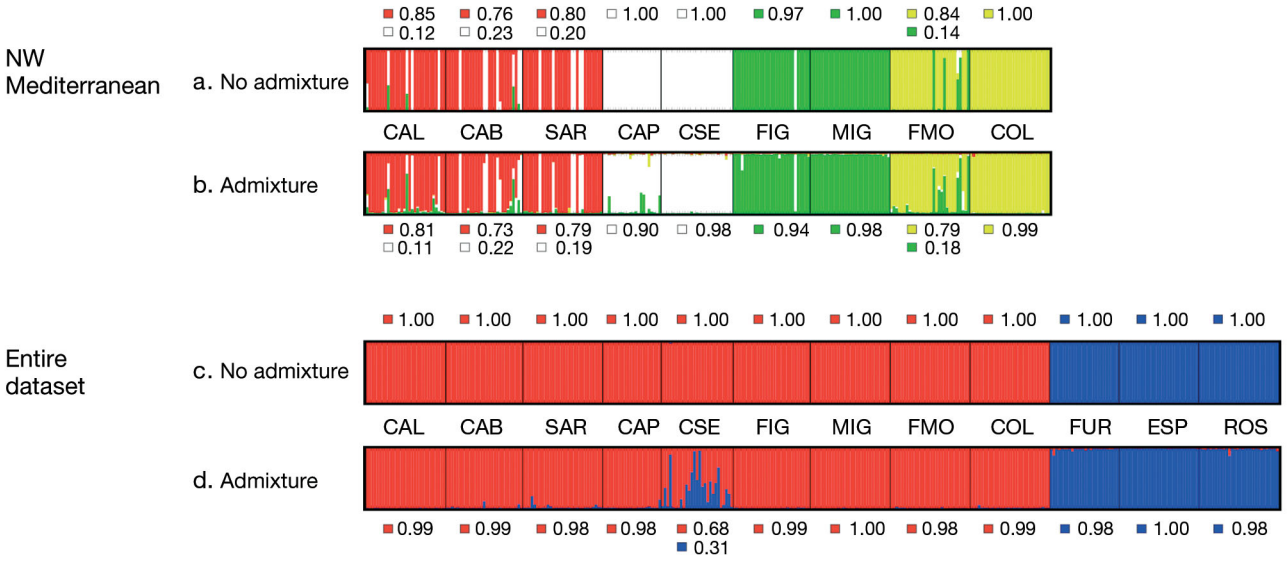

Fig. 2. *Hediste diversicolor*. Bayesian model-based clustering analysis: output of the simulations that displayed the most consistent results across replicate runs (similarity coefficient >0.85) and corresponded to the uppermost hierarchical structure retrieved by the Δ*K* statistics method (Evanno et al. 2005). For each bar chart, individuals are represented by vertical segmented coloured bars describing the proportion of individual genotype assigned to a given cluster; population coefficient memberships >0.10 for a given model are indicated above and below the bar charts. Each bar chart corresponds to no-admixture and admixture model Bayesian clustering results for the northwestern Mediterranean (a and b, respectively), and the entire dataset (c and d, respectively)

sponded to geographical groups (Fig. 2a,b). Indeed, different clusters were predominant in geographical areas corresponding to Tuscany, Corsica, and western and eastern Sardinia. The degree of genetic homogeneity varied across areas and populations; CAP and CSE (eastern Sardinia), FIG and MIG (Corsica), and COL (Tuscany) appeared to be genetically homogeneous, with more than 90% of their genome being assigned to a single cluster (Fig. 2a,b). On the other hand, the remaining populations were more genetically heterogeneous, as <90% of the genome was assigned to the prevailing cluster. More than 10% of the genetic makeup in western Sardinian populations (CAL, CAB, and SAR) was assigned to the same cluster as CAP and CSE, as well as a similar portion in FMO (Tuscany) that had the same genetic makeup as populations from Corsica (Fig. 2a,b). Using an individual membership coefficient threshold of $Q > 0.8$ to denote pure ancestry to a cluster (Vähä et al. 2007), some specimens showed admixed ancestry, but their number was different according to the model used. Five (2%) and 22 (9%) individuals were scored as being admixed by the no-admixture (Fig. 2a) and the admixture model (Fig. 2b), respectively.

When we applied the no-admixture model to the entire dataset, specimens were clustered into geographical groups corresponding to Mediterranean and Atlantic regions (Fig. 2c). Under the admixture model the results were very similar, with the exception of 17 out 257 individuals from the Mediterranean (Fig. 2d). Three specimens showed a pure 'Atlantic' genetic makeup. All the remaining individuals but 2 showed admixed ancestry in both clusters, with a prevailing 'Mediterranean' genetic makeup ($0.2 \leq Q \leq 0.8$). Nevertheless, at increasing values of *K*, these same individuals were re-allocated to clusters that grouped only individuals from the Mediterranean, and, more specifically, from Sardinia. In particular, at $K = 6$ all individuals from northeastern Sardinia were split into 2 genetic clusters, one of which was predominant in CAP, the other in CSE, though these populations are only 2 km apart (data not shown). The other clusters resembled the groups found when only western Mediterranean individuals were used, whereas the Atlantic specimens were grouped in a genetically homogeneous cluster.

Analysis of molecular variance (AMOVA) performed on the total dataset revealed a significant differentiation among populations ($\Phi_{ST} = 0.475$, $p < 0.001$), which accounted for almost half of the total genetic variation (Table 3). Moreover, when differences among groups were considered, a large portion of genetic differentiation, corresponding to about 20% of total variation ($\Phi_{CT} = 0.198$, $p < 0.01$) was due to differences between Atlantic and Mediterranean population groups (Table 3). A significant

Table 3. *Hediste diversicolor*. 2-level and 3-level analysis of molecular variance (AMOVA) performed on ISSR (inter-simple sequence repeat) genotypes for the entire data set. Groups for 3-level AMOVA were defined according to geographical regions (Mediterranean and Atlantic) and model-based clustering analyses. df: degrees of freedom; SS: sum of squares; var. comp.: variance component; % var: percentage of variation. $^{**}p < 0.01$, $^{***}p < 0.001$

Source of variation	df	SS	Var. comp.	% var	Φ-statistics
2-level AMOVA					
Among populations	11	1832.345	5.61380	47.46	$\Phi_{ST} = 0.475^{***}$
Within populations	331	2057.299	6.21540	52.54	
3-level AMOVA					
Atlantic, Mediterranean					
Among groups	1	478.112	2.65055	19.79	$\Phi_{CT} = 0.198^{**}$
Among populations within groups	10	1354.233	4.52670	33.80	$\Phi_{SC} = 0.266^{***}$
Within populations	331	2057.299	6.21540	46.41	$\Phi_{ST} = 0.424^{***}$
Atlantic, western Sardinia, eastern Sardinia, Corsica, Tuscany					
Among groups	4	1084.632	2.435	20.01	$\Phi_{CT} = 0.200^{***}$
Among populations within groups	7	747.714	3.516	28.90	$\Phi_{SC} = 0.361^{***}$
Within populations	331	2057.299	6.215	51.09	$\Phi_{ST} = 0.489^{***}$

genetic differentiation was found after populations were subdivided into groups according to STRUCTURE results (Table 3). However, the portion of variance explained by this alternative partitioning increased only very slightly ($\Phi_{CT} = 0.200$, $p < 0.001$).

DISCUSSION

The first aim of our study was the appraisal of spatial genetic structure in populations of *Hediste diversicolor* from the northwestern Mediterranean, with a focus on the potentially isolated Sardinian-Corsican region. Our results were consistent with previous studies on allozymes and mtDNA sequences (e.g. Abbiati & Maltagliati 1996, Röhner et al. 1997, Breton et al. 2003, Virgilio & Abbiati 2004, 2006, Audzijonyte et al. 2008, Virgilio et al. 2009) in showing a high degree of among-population genetic differentiation. In particular, ISSR genetic patterns matched the mtDNA results of Virgilio et al. (2009), as most of individuals from Tuscany were grouped into the same cluster and were genetically distinct from Sardinian-Corsican counterparts. In addition, a clear geographical structure within the latter region was evidenced (Fig. 2, Table 3), since most individuals were grouped according to the sampling area (Corsica, western and eastern Sardinia). This pattern may reflect the influence that the hydrographical regime, habitat specificity, and distribution exert on the genetic structure of nearshore species of invertebrates (e.g. Ayre et al. 2009, Kelly & Palumbi 2010). For instance, in the cyprinodontiform *Aphanius fasciatus*, which shares similar life history traits with *H. diversicolor*, species' genetic structure was consistent with genetic drift due to habitat isolation and limited gene flow through coastal waters (Maltagliati 1998, 1999). Similarly, isolation due to the natural habitat fragmentation of brackish-water systems may have enhanced genetic drift and local adaptation in *H. diversicolor* (Cognetti & Maltagliati 2000, Virgilio & Abbiati 2004, 2006). Furthermore, the pattern of genetic differentiation observed in the northwestern Mediterranean may have been influenced by the complex and seasonally variable hydrographical regime of this area, characterised by local and mesoscale currents, gyres, and many small energetic eddies (Millot 1999, Rio et al. 2007). Though *H. diversicolor* spends its entire life-cycle in brackish water systems, juveniles and/or adults can be flushed to the coastal waters by exceptional floods or rainfalls. Here, given the ability to survive in sea water, they may disperse by drifting or rafting. The genetic structure within the northwestern Mediterranean is unlikely to reflect the presence of further cryptic species, despite the isolation of the SardinianCorsican region. The inferred genetic clusters were not completely spatially disjunct; individuals with similar genetic makeup were found across different areas, and at least 5 of them shared ancestry in more than a cluster, thus suggesting the chance of hybridisation (Fig. 2c).

Accordingly, when we included samples from the Atlantic in the analysis the uppermost hierarchical structure in the data corresponded to $K = 2$, whereas we would have expected higher values if further cryptic species were present in the northwestern Mediterranean. Conversely, the genetic structure we uncovered matches the findings of

Virgilio et al. (2009), in which the mitochondrial haplotypes from western Mediterranean and northeastern Atlantic were grouped into distinct clades. Indeed, model-based clustering analysis showed a clear split between the Atlantic and the Mediterranean populations (Fig. 2c,d), which accounted for about 20% of total variation (Table 3). Mediterranean and Atlantic lineages are likely to be reproductively isolated, as evidenced by allozyme patterns in populations from the Baltic Sea, where the different mtDNA lineages occurred in sympatry (Audzijonyte et al. 2008). In this context, results from our ISSR survey may add a further line of evidence to the presence of cryptic species in *Hediste diversicolor*. Previous researches showed that these markers are able to discriminate with accuracy closely related species of polychaetes (Maltagliati et al. 2005), cyprinodontiform fishes (Maltagliati et al. 2006b), and proseriate flatworms (Casu et al. 2009). Interestingly, Bayesian clustering may be successful in species' delimitation (e.g. Casu et al. 2009), as genetic clusters conform to the metapopulation lineages species' definition (Shaffer & Thomson 2007). According to this criterion, the primary property needed for the identification of species' boundaries is the presence of sets of populations (lineages) that have severely restricted or no gene exchange (De Queiroz 2007). It could be argued that the 2 genetic clusters found in *H. diversicolor* are not separately evolving lineages, because some individuals from the northwestern Mediterranean shared, at least partly, the same genetic makeup as Atlantic ones under the admixture model (Fig. 2d). Nonetheless, this may be an artefact: in the presence of diverging populations due to isolation (e.g. reproductive isolation, habitat fragmentation) no-admixture models are more robust than admixture ones that have larger margins of error in the assignment of individuals to clusters (Francois & Durand 2010).

Finally, the consistent patterns revealed by ISSRs and mtDNA reinforce the hypothesis that vicariance may have driven the divergence between Atlantic and Mediterranean lineages as suggested by Virgilio et al. (2009). In fact, similar patterns of spatial genetic structure at both mitochondrial and nuclear molecular markers are likely only when genetic drift associated with vicariant events occurred (Knowles & Richards 2005).

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