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PRIMARY RESEARCH PAPER

Using a phylogeographic approach to investigate the diversity and determine the distributional range of an isopod (Crustacea: Peracarida), *Stenosoma nadejda* (Rezig, 1989) in the Atlantic-Mediterranean region

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Abstract The genus *Stenosoma* comprises 13 species in the NE Atlantic, Mediterranean and Black Sea. Recent studies have improved knowledge on the distributional patterns of the genus, reducing species ranges and allowing the identification of cryptic species. Lacking a free-swimming life-stage, *Stenosoma* typically display small- to medium-sized ranges, with the exception of *S. nadejda* which occurs in the Atlantic-Mediterranean region. In this study, we build upon previous work and examine phylogeographic patterns of *S. nadejda* throughout its entire range to assess levels of genetic differentiation and evidence of cryptic species. While Elongation Factor 1α gene sequences revealed an almost absence of genetic and geographic structure across the entire distribution of *S. nadejda*, the Cytochrome c oxidase subunit I gene sequences revealed a phylogenetic split defining two Mediterranean clades. Our results confirm the wide distribution of *S. nadejda* in the Atlantic/Mediterranean region and show that the distribution of the two Mediterranean clades does not match the east–west break in genetic continuity typically displayed by many species. This pattern is probably a consequence of sporadic long-distance dispersal by rafting. Conversely, an almost absence of shared haplotypes at the local level, conforms to the recognized poor ability of peracarids for autonomous dispersal.

Keywords Cytochrome c oxidase subunit I · Elongation factor 1 α · Rafting · Peracarid · Dispersal

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Introduction

The Superorder Peracarida is one of the richest groups of marine arthropods, comprising roughly one third (~21,000) of the known crustacean species. One of the most striking traits of peracarids, unique among crustaceans, is the presence of a brood pouch from where adult-like juveniles emerge. With the exception of the Mysida, for which position within Peracarida is debatable (Poore, 2005; Spears et al., 2005; Jenner et al., 2009), the larger peracarida Orders (Cumacea,

Tanaidacea, Amphipoda and Isopoda) encompass mostly benthic species which are relatively small-sized. Consequently, for strictly shallow water or coastal species the lack of a planktonic life-stage clearly limits their ability to disperse through all but very short distances. In these species rafting on seaweed or flotsam is thought to be the primary mechanism of long-range dispersal (Thiel & Haye, 2006). One of the strongest evidence of the importance of rafting as a driver for the dispersion of marine organisms comes precisely from the wide, sometimes disjunct, distributions of some species that otherwise lack any means of autonomous long-range dispersal (Thiel & Gutow, 2005a). Examples abound in peracarids, but recently the usage of molecular techniques has challenged the taxonomic status of many such species, showing that they often consist of localized and highly divergent genetic lineages forming complexes of cryptic species (Hurtado et al., 2010; Markow & Pfeiler, 2010; Baratti et al., 2011; Varela & Haye, 2012; Cabezas et al., 2013a, b, 2014; Larsen et al., 2014, Copilaş-Ciocianu & Petrusek, 2015). Notwithstanding, cases of species with large distributional ranges have also been confirmed by means of molecular evidence (Nikula et al., 2010; Haye et al., 2012), although they fall short of the increasing number of widely ranging *taxa* prompting for a taxonomic revision. Does this difference arise from a true sporadic nature of rafting as a long-range dispersal mechanism in peracarids?

There is clearly a shortage of data to answer unequivocally the previous question. Most of the studies mentioned focused on single or few species within a genus, and are by no means representative of the great diversity of forms and life-history traits found on peracarids. For example, the family Idoteidae is world-wide distributed, comprising roughly 180 shallow-water species (Poore, 2001) from which a single one is putatively cosmopolitan: the obligatory rafter *Idotea metallica* Fabricius, 1793 (Brusca, 1984). All other species have distributions that range from tens to thousands of kilometres and, despite being reported as common rafters (14 out of 38 isopod species, Thiel & Gutow, 2005b), there is but a single phylogeographic analysis of a widely distributed idoteid—*Idotea balthica* (Pallas, 1772)—which confirmed its amphi-Atlantic distribution, but did not include a substantial part of its range (Wares & Cunningham, 2001).

In this work, we explore the genetic diversity of *Stenosoma nadejda* (Rezig, 1989), a common idoteid that can be found in the intertidal and shallow-waters of the Atlantic-Mediterranean region, living among algae or sea-grasses. The phylogeny of the genus was recently assessed, showing that its evolution is strongly linked with the geological and climatic history of the Atlantic-Mediterranean region (Xavier et al., 2012b). This and other works have also contributed to redefine the ranges of most *Stenosoma* species, particularly by demonstrating that those once thought to occur in the whole region were actually restricted to either the Atlantic or the Mediterranean basins or to smaller areas within them (Santos et al., 2011; Xavier, 2011; Xavier et al., 2012a, b). Hence, *S. nadejda* remains as the only species of the genus with a large distribution, occurring in the Atlantic, from central Portugal down to Morocco, and throughout the entire Mediterranean (Xavier, 2011).

Initially taken as a presumptive Mediterranean invader in the Portuguese coast, *S. nadejda* was shown to occur naturally in the Atlantic coast of Iberia (Xavier et al., 2009), and contrarily to what was assumed, is likely to have an Atlantic origin (Xavier et al., 2012b). A phylogeographic survey focused in the Alboran Sea resulted in the identification of a new species, *Stenosoma stephenseni* Santos and Xavier 2011, sympatric with *S. nadejda* and from which it can be distinguished by a remarkable genetic divergence, but rather subtle morphological differences (Xavier et al., 2011). In this work, we extend the previous mtDNA (based on the cytochrome c oxidase subunit I—COI) phylogeographic analysis eastwards up to the Aegean Sea, and provide additional data on the variation of a nuclear marker (exon1 of the elongation factor—EF1α), to investigate whether *S. nadejda* "hides" further cryptic species or if it has dispersed farther into the eastern Mediterranean.

Materials and methods

Individuals were captured by submerging and washing collected algae in fresh water and were subsequently preserved in 96% ethanol. Specimens were identified according to a recent key (Santos et al., 2011). A total of 264 individuals were used for the present study. The dataset consisted of 94 newly collected individuals

from 14 Mediterranean localities east of the Almeria-Oran Front (AOF), which was complemented with 170 individuals collected at 18 locations from the Atlantic, the Alboran Sea, Algeria and west Tunisia and used in previous works (see Table 1; Fig. 1; Xavier et al., 2009, 2011).

DNA extraction and amplification of the COI were done for all the newly sampled specimens ($n = 94$) using the methodology previously described (Xavier et al., 2009). As previous works did not include nuclear DNA data, a portion of the nuclear gene Elongation factor 1α (EF1α) was amplified for 95 specimens selected from the 264 available, representative of the entire known distribution of *S. nadejda*. Primers used to amplify the EF1α were EF1AF- 5′ GAYTTCATYAAGAAACATG 3′ and EF1AR- 5′ GAAWGTCTCYACGCACATGGG 3′; these primer sequences were available at the Crandall lab primer database (http://crandalllab.byu.edu/PrimerDatabase.aspx, however, this database is no longer online. PCR conditions were as follows: initial 4 min denaturation at 94°C, followed by 30 cycles of 45 s at 94°C, 45 s at 55 ∘C and 1 min at 72°C. Final extension was achieved at 72°C for 12 min. PCR reactions were done in a volume of 20 µl with a magnesium concentration of 3 Mm. Platinum Taq (Invitrogen, Carlsbad, CA, USA) was used for all PCR amplifications. PCR products were all sequenced in both directions by a commercial company (High-Throughput Genomics Unit—HTGU, Department of Genome Sciences of the University of Washington).

Estimates of genetic diversity and tests of recombination

Sequences were checked and edited using CodonCode Aligner (CodonCode, Dedham, MA, USA). The software PHASE v.2.1.1 (Stephens et al., 2001; Stephens & Donnelly, 2003; Stephens & Scheet, 2005) was used to resolve EF1α haplotypes. Command line and input files for this software were generated by running SeqPHASE (Flot, 2010). Only haplotypes with probabilities higher than 90% were included in the analysis. Sequences were aligned using ClustalW (Thompson et al., 1994) as implemented in BioEdit (Hall, 1999). COI sequences were uploaded in DNASP (Librado & Rozas, 2009) and translated to aminoacids to obtain the number of synonymous and non-synonymous substitutions, and to search for premature stop codons, which are indicative of the presence of pseudogenes.

Measures of haplotype diversity (Hd) and nucleotide diversity (π) were estimated for COI for 28 localities using Arlequin v3.11 (Excoffier et al., 2005). Localities with less than four individuals or which displayed no polymorphisms were excluded from these analyses. The Maximum Chi-square test of Maynard Smith (Maynard Smith, 1992) and GeneConv (Sawyer, 1989), both available in the software package Recombination Detection Program version 3.44 (Martin et al., 2010), were used to test for recombination in the EF1α dataset.

Estimates of population structure and isolation by distance

Population structure was investigated for the COI and EF1α genes separately using two methods. One is implemented in the software BAPS 5 (Corander et al., 2004). This method uses Bayesian statistics to test mixture and to define clusters of populations, requiring no a priori knowledge about the genetic structure of populations (Corander et al., 2004). The optimal number of clusters in BAPS 5 was selected according to the best output log(ml) value and the posterior probabilities associated with each tested number of cluster (K). Values of K were set to vary between 1 and 20. Similarly, analysis of population structure using the concatenated (nuclear and mitochondrial) dataset was also conducted using BAPS.

The second method is based on the comparison of pairwise Fsts estimated with Arlequin v3.11 (Excoffier et al., 2005) (excluding localities with less than four individuals). For the COI dataset, Fst estimations using were made for the localities east of the Almeria-Oran oceanographic front using Arlequin v3.11 (Excoffier et al., 2005). Significance of pairwise Fst values was tested by performing 300 permutations of haplotypes between locations or clusters, under the null hypothesis of no differentiation. Due to the low level of population structure found for the EF1α gene, isolation by distance (IBD) was only tested for the mtDNA dataset of Mediterranean locations. IBD was tested using a non-parametric Mantel test as incorporated in the IBDWS v. 3.15 (Jensen et al., 2005), using Fst genetic and geographical distances (calculated as the approximate linear distance along shoreline) between population pairs, and 30,000 randomizations.

Table 1 Estimates of genetic diversity for COI gene of *S. nadejda* for sampled localities and population clusters defined by BAPS

Localities/BAPS clusters	Country	*N*	*H*	Hd	π	Haplotype codes	Accession numbers
Rabat*	Morocco	14	11	0.96	0.0087	Hap 1–11	JF915252-JF915254, JF915256-JF915261, JF915274-JF915278
Assilah*	Morocco	13	13	1	0.0061	Hap 12–24	JF915255, JF915262-JF915273
Cala Iris*	Morocco	10	6	0.80	0.0068	Hap 25–30	JF915207-JF915209, JF915211-JF915216, JF915219
Al Hoceima*	Morocco	10	9	0.98	0.0052	Hap 31–39	JF915198-JF915206, JF915219
Cap Mazari*	Morocco	2	2	–	–	Hap 25, 29	JF915217, JF915210
Baleal*	Portugal	12	4	0.45	0.0011	Hap 40–43	FJ905060, FJ905063,FJ905067
Oliveirinha*	Portugal	13	9	0.92	0.0043	Hap 40, 44–51	FJ905069, FJ905078-FJ905080, FJ905095
Porto de Mós*	Portugal	9	4	0.58	0.0021	Hap 52–55	FJ905096, FJ905074,FJ905075
Olhos d'Água*	Portugal	12	8	0.85	0.0033	Hap 55–62	FJ905061-FJ905062, FJ905070-FJ905073, FJ905076-FJ905077
Chipiona*	Spain	15	13	0.97	0.0044	Hap 63–75	FJ905053-FJ905059, FJ905089-FJ905094
Marbella*	Spain	14	13	0.99	0.0053	Hap 76–88	FJ905048-FJ905052, FJ905081-FJ905088
Alboran Island*	Spain	3	3	–	–	Hap 89–91	JF915220-JF915222
Peñiscola*	Spain	4	4	1.00	0.0070	Hap 92–95	JF915279-JF915282
Cabo de Gata*	Spain	6	4	0.80	0.0022	Hap 96–99	JF915283-JF915288
Villajoyosa	Spain	2	1	–	–	Hap 100	KT998456-KT998457
Banuyls-sur-Mer	France	5	3	0.70	0.0035	Hap 101–103	KT998545-KT998549
Sestri Levante	Italy	1	1	–	–	Hap 104	KT998514
Vernazza	Italy	1	1	–	–	Hap 105	KT998513
Santa Marinella	Italy	15	8	0.85	0.0140	Hap 106–113	KT998516-KT998530
Capo Colonna	Italy	14	6	0.85	0.0050	Hap 114–118	KT998531-KT998544
Giovinazzo	Italy	1	1	–	–	Hap 120	KT998515
Villanueva Monteleones	Italy	14	4	0.58	0.0015	Hap 121–124	KT998482-KT998495
Cap Mannu	Italy	3	3	–	–	Hap 125–127	KT998473-KT998475
Tighremt*	Algeria	2	2	–	–	Hap 128–129	JF915234-JF915240
Tigzirt*	Algeria	11	7	0.82	0.0025	Hap 129–135	JF915229-JF915233, JF915235-JF915239, JF915241
Cap Serrat*	Tunisia	6	3	0.60	0.0012	Hap 136–138	JF915246-JF915251
Bizerte*	Tunisia	14	5	0.76	0.0040	Hap 138–142	JF915223-JF915228, JF915242-JF915245
Cap Bon	Tunisia	15	8	0.79	0.0031	Hap 143–150	KT998458-KT998472
Nabeul	Tunisia	6	2	0.53	0.0028	Hap 149–150	KT998476-KT998481
Molivos	Greece	15	5	0.56	0.0011	Hap 151–155	KT998496-KT998510
Anaxos	Greece	1	1	–	–	Hap 152	KT998512
Skala Sykamineas	Greece	1	1	–	–	Hap 156	KT998511

Table 1 continued

Localities/BAPS clusters	Country	*N*	*H*	Hd	π	Haplotype codes	Accession numbers
North African Atlantic/Cluster I*	Morocco	27	24	0.99	0.0104	Hap 1–24	
Southwest Iberia + North Alboran/Cluster II*	Portugal	81	53	0.95	0.0080	Hap 40–88	
	Spain					Hap 96–99	
South Alboran/Cluster III*	Morocco	22	15	0.95	0.0091	Hap 25–39	
Mediterranean Cluster IV	Spain,	67	37	0.96	0.0095	Hap 89–95	
	Italy, Algeria, west Tunisia					Hap 100	
						Hap 107–120	
						Hap128–142	
Mediterranean Cluster V	France, Italy + Sardinia, east Tunisia	45	20	0.91	0.0085	Hap 101–105	
						Hap 121–127	
						Hap 143–150	
Mediterranean Cluster VI	Italy	22	7	0.71	0.0049	Hap 151–156	
	Greece					Hap 106	

Data published previously (Xavier et al., 2009; 2011) is marked with asterisk. Estimates of H and π were done only for sites with more than four individuals

N number of individuals, *H* observed number of haplotypes, *Hd* haplotype diversity, π nucleotide diversity

Uncorrected pairwise p-distances were calculated for all individuals based on COI to acess minimum and maximum genetic divergence distance. Additionally uncorrected p-distances were also conducted between clusters defined by BAPS. Genetic distances were calculated using MEGA5 software (Tamura et al., 2011).

Phylogenetic reconstruction

The phylogenetic tree built for the COI included all unique haplotypes from newly collected individuals, plus an additional set of randomly chosen unique haplotypes representative of the three major clades of *S. nadejda* found west of Almeria-Oran Front (AOF). The latter included six individuals from Atlantic Morocco (Genbank accession numbers JF915252-3, JF915255, JF915262 and JF915273-4) and 19 individuals from southwest Iberia and the Alboran basin (Genbank accession numbers FJ905064-65, JF915286, JF915288, FJ905079, FJ905077, FJ905074, FJ905070, FJ905067-68, FJ905053-54, FJ905048-49, JF915198-JF915200, JF915207, JF915211) (Xavier et al., 2009, 2011). Additionally, two COI sequences, one from *Stenosoma capito* (Rathke, 1837) and another from *Stenosoma lancifer* (Miers, 1881) were used as outgroups (Genbank accession numbers FJ905097 and FJ905098, respectively) (Xavier et al., 2009). The software jModelTest (Posada, 2008) was used to determine the adequate model of sequence evolution using the AIC criteria, which in this case was GTR+I+Γ (−ln 2892).

The methods used for tree reconstruction were Maximum Likelihood (ML) with the software package PhyML (Guindon & Gascuel, 2003), using 1000 bootstraps to estimate branch support, and Bayesian Inference (BI) using MRBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). For the BI, two independent runs were performed using 15×10^6 generations. Parameters were sampled every 100 generations with the heating parameter set to 0.1. Majority-rule consensus trees were estimated by combining results from duplicated analyses and a burnin of 10%. Adequate burnin was checked with the software Tracer v1.5 (Rambaut & Drummond, 2007). Haplotype networks reconstructions for the entire

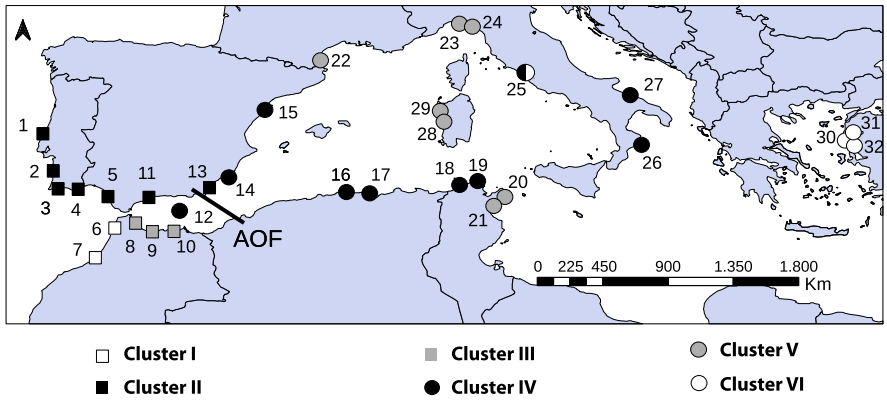

Fig. 1 Geographical location of sampling sites and groups defined by BAPS for the COI dataset. The black line represents the Almeria-Oran Front (AOF). Sampling sites are: *1* Baleal; *2* Oliveirinha; *3* Porto de Mós; *4* Olhos d'Água; *5* Chipiona; *6* Rabat; *7* Assilah; *8* Cap Mazari; *9* Calla Iris; *10* Al Hoceima; *11* Marbella; *12* Alboran Island; *13* Cabo da Gata; *14* Villajoyosa; *15* Peñiscola; *16* Tighremt; *17* Tigzirt; *18* Cap Serrat; *19* Bizerte; *20* Cap Bon; *21* Nabeul; *22* Banuyls-sur-Mer; *23* Sestri Levante; *24* Vernazza; *25* Santa Marinella; *26* Capo Colonna; *27* Giovanazzo; *28* Cap Mannu; *29* Villanueva Monteleones; *30* Skala Sykamineas; *31* Molivos; *32* Anaxos. Clusters retrieved with BAPS are coded with combinations of squares or circles and different colours (*white*, *grey* and *black*)

EF1α dataset and for the COI dataset including only Mediterranean individuals (collected east of the Almeria-Oran Front) were conducted using TCS (Clement et al., 2000) and output file was run through tcsBU (Santos et al., 2015).

Results

All sequences were translated to aminoacids and no stop codons were detected. The COI dataset alignment was 568 bp long, and included 156 haplotypes. A total of 42 haplotypes were found in the 94 individuals collected specifically for this work. The latter comprised 60 variable sites, of which 42 were parsimony informative. The amplified segment of EF1α comprised an intronic region with considerable length polymorphism that hindered the haplotype phase estimates. Therefore, this region was excluded from the final EF1α alignment that remained 687 bp long, with 23 haplotypes in a total of 95 individuals. EF1α haplotypes comprised 18 variable sites, from which 16 were parsimony informative. Haplotype diversity was generally high for COI (>0.8) with only a few exceptions: Baleal, Porto de Mós, Banuyls-sur-Mer, Villanueva Monteleones, Cap Serrat, Nabeul and Molivos. The highest values of nucleotide diversity were found at Santa Marinella. Diversity was also considerably high within the population clusters defined by BAPS, with lowest values (Hd = 0.71 and $\pi = 0.0049$) encountered in Cluster III (Table 1). Additionally haplotype sharing between localities was rare. On the contrary, for the EF1 α haplotype sharing was high (Table 2).

Finally, for the the EF1 α, no recombination events were detected at the significance level of $p = 0.05$.

Population structure and isolation by distance

For the COI dataset, uncorrected p-distance varied between 0 and 3.2% if only Mediterranean locations were analysed.

BAPS defined six population clusters with log(ml) of −3920 and with 100% posterior probability. The Atlantic-Alboran region was divided in three population clusters: Cluster I-, including populations from Atlantic North Africa; Cluster II,-including populations from Southwest Iberia (including Northern Alboran) and Cluster III, including Moroccan population from South Alboran. Within the Mediterranean, east of the Almeria-Oran front (AOF), another three clusters were also defined: cluster IV, including most of the southwestern sites but extending into the Adriatic Sea; cluster V, including most sites from the northwest and a few from the southeast; and cluster VI, including eastern locations (Greece) but also present in the northwest, at Santa Marinella, Italy (see Fig. 1).

For the EF1α dataset, BAPS defined three clusters with log(ml) of −730 and 99% posterior probability.

Table 2 Estimates of genetic diversity for EF1α gene of *S. nadejda*

Localities	Country	*N*	*H*	Haplotype codes	Assession numbers
Assilah	Morocco	3	1	Hap 1	KT998550-KT998552
Rabat	Morocco	6	2	Hap 1–2	KT998628-KT998633
Al Hoceima	Morocco	2	3	Hap 3–5	KT998598-KT998599
Calla Iris	Morocco	3	3	Hap 3, 5, 6	KT998602-KT998604
Baleal	Portugal	7	2	Hap 1, 7	KT998558-KT998564
Oliveirinha	Portugal	3	2	Hap 1, 7	KT998617-KT998619
Porto de Mós	Portugal	1	1	Hap 1	KT998624
Chipiona	Spain	4	4	Hap 1, 3, 7 ,8	KT998587-KT998590
Marbella	Spain	6	3	Hap 3, 7, 8	KT998605-KT998610
Cabo de Gata	Spain	1	1	Hap 8	KT998586
Villajoyosa	Spain	2	1	Hap 7	KT998638-KT998639
Peñiscola	Spain	4	1	Hap 7	KT998620- KT998623
Tigzirt	Algeria	1	2	Hap 3, 7	KT998636
Cap Serrat	Tunisia	2	3	Hap 1, 3, 23	KT998600-KT998601
Bizerte	Tunisia	5	7	Hap 3, 9, 10–14	KT998565-KT998569
Cap Bon	Tunisia	10	3	Hap 1, 3, 15	KT998570-KT998579
Nabeul	Tunisia	3	6	Hap1, 3,10, 15, 16, 23	KT998625-KT998627
Banuyls-sur-Mer	France	5	3	Hap 7, 17, 18	KT998553-KT998557
Vernazza	Italy	1	1	Hap 19	KT998637
Sestri Levante	Italy	1	1	Hap 19	KT998634
Santa Marinella	Italy	7	3	Hap 3, 19, 20	KT998591-KT998597
Capo Colonna	Italy	6	4	Hap 3, 19, 21, 22	KT998580-KT998585
Capo Mannu	Italy	1	1	Hap 18	KT998635
Villanueva Monteleones	Italy	5	1	Hap 18	KT998640-KT998644
Molivos	Greece	6	1	Hap 3	KT998611-KT998616

Estimates of H and π were done only for sites with more than four individuals

N number of individuals, *H* observed number of haplotypes, *Hd* haplotype diversity, π nucleotide diversity

Cluster I contains most of the individuals from Atlantic locations and some from the northern and southern Mediterranean. Cluster II contains most of the individuals from the northern Mediterranean as well as a few from the Atlantic-Alboran region and the southern Mediterranean. Cluster III only comprises individuals from the southern Mediterranean (Fig. 2).

Results obtained with BAPS for the concatenated datasets revealed three population clusters, with a log(ml) value was -5480 and 100% posterior probability: (1) North African Atlantic cluster, (2) Iberian Atlantic plus Alboran Sea cluster and (3) a Mediterranean cluster (Fig. 3). These population clusters agree with the major clades defined by the phylogenetic analysis of the COI dataset (see below).

A pattern of isolation by distance was rejected for the COI dataset ($r^2 = 1.47E{-}02, P > 0.05$). For COI, high levels of differentiation were observed between all Mediterranean localities (Table 3). Although for EF1α, levels of differentiation were lower than those of COI, most pairwise Fst values were significant at $\alpha = 0.05$ (Table 4). Uncorrected p-distances between the clusters defined by BAPS for the COI dataset are summarized in Table 5. The highest genetic distance values were found between Atlantic North African cluster and all the others (3.5–4.9%). P-distance between Atlantic and Mediterranean localites was also high (3–4.1%). .

Phylogenetic analysis

Phylogenetic reconstruction of the COI dataset (Fig. 4) showed three groups: (1) comprising all individuals from North African Atlantic (Group A), (2) a monophyletic group comprising individuals from Atlantic Iberia plus Alboran Sea and finally (Group B) and (3) a group containing all individuals from the Mediterranean (Group C). The three main groups recovered by the phylogenetic analyses correspond to

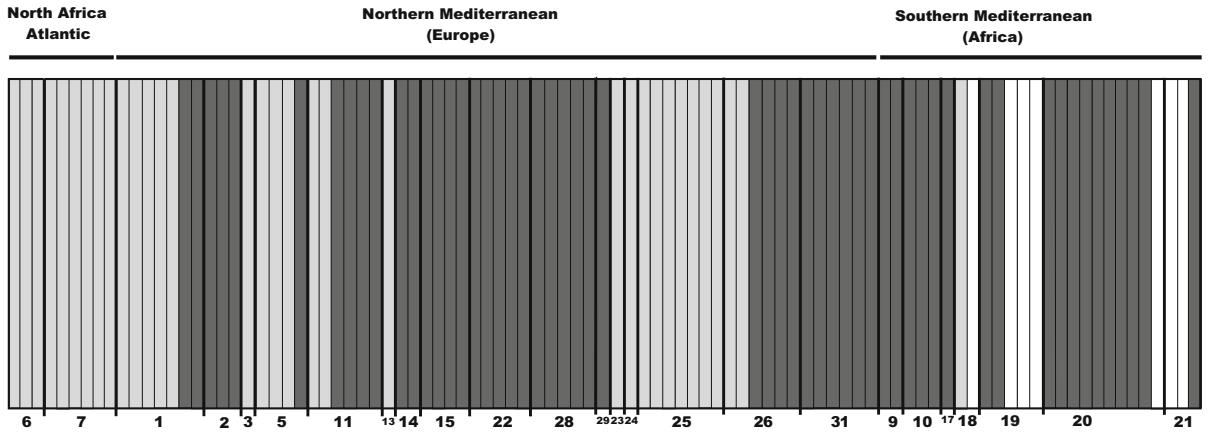

Fig. 2 Results from the best partition with BAPS analysis for the EF1α gene. Localities were coded with numbers as indicated in Fig. 1 and different clusters are coded by colours: Cluster I—*light grey*; Cluster II—*dark grey*; Cluster III–*white*

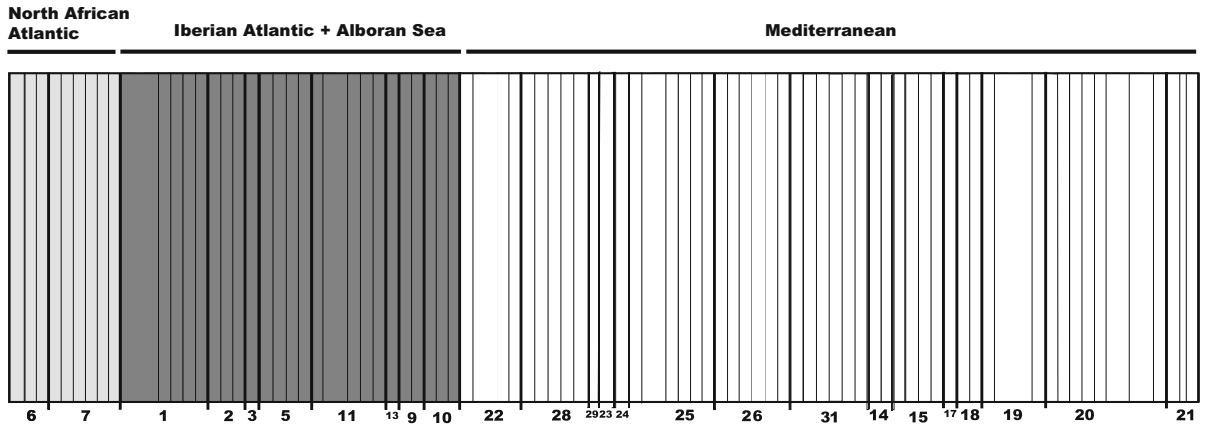

Fig. 3 Results from the best partition with BAPS analysis for the concatenated dataset (COI + EF1α). Localities were coded with numbers as indicated in Fig. 1

the population clusters defined by BAPS using the concatenated dataset (Fig. 3). The Mediterranean group is divided in two clades, both being present in the western and the eastern basins. The three Mediterranean population clusters obtained by BAPS based on COI are well evident in the haplotype network (Fig. S1 of supplementary material), as are the high numbers of private haplotypes. On the contrary, the network of EF1α haplotype revealed three central haplotypes that are frequent and widespread, and from which several tip haplotypes diverge mostly by a single mutation (Fig S2 of supplementary material).

Discussion

The present analyses of DNA sequence variation reveals no evidence for the existence of cryptic speciation between geographically segregated lineages, thus confirming the large geographical distribution of *Stenosoma nadejda*. Whereas nuclear DNA data showed no evidence for genetic structure, mtDNA data revealed two levels of intraspecific phylogeographic structure in the Mediterranean: local and regional. At the local level, an almost absence of shared haplotypes is in line with the recognized poor ability of peracarids for autonomous dispersal (Thiel & Gutow, 2005a) and with previously published results on this and other species of the same genus (Xavier et al., 2009, 2011; Xavier, 2011). At the

Table 3 Pairwise FS_T between localities for the COI dataset

	Cbo	Biz	CSe	Tiz	Nab	VLe	Mol	SMr	CCo	BSM
Biz	**0.826**									
CSe	**0.879**	0.364								
Tiz	**0.872**	0.600	**0.766**							
Nab	0.275	**0.807**	**0.901**	**0.877**						
VLe	**0.801**	**0.840**	**0.937**	**0.909**	**0.832**					
Mol	**0.857**	**0.879**	**0.953**	**0.924**	**0.885**	**0.922**				
SMr	0.599	0.340	0.370	0.353	0.500	0.544	0.563			
CCo	**0.857**	0.672	**0.746**	0.676	**0.844**	**0.870**	**0.878**	0.461		
BSM	**0.776**	**0.789**	**0.881**	**0.873**	**0.763**	**0.767**	**0.887**	0.487	**0.839**	
Pen	**0.842**	0.578	**0.722**	0.363	**0.817**	**0.884**	**0.889**	0.298	0.640	**0.800**

Pairwise F_{ST} values between sites from Mediterranean localities, located east of the Almeria-Oran front, based on 568 bp of mtDNA COI. Location names were coded as following: Biz-Bizerte; CSe-Cap Serrat; Tiz-Tigzirt; Nab-Nabeul; VLe-Villanueva Monteleones; Mol-Molivos; SMr-Santa Marinella; CCo-Capo Colonna; BSM-Banuyls-sur-Mer; CBo-Cap Bon: Pen-Peñiscola. Values highlighted in light grey represent intermediate differentiation between localities, whereas values highlighted in dark grey represent high levels of differentiation between localities. Values in bold represent extreme differentiation between localities. All F_{ST} values were significant, with P values <0.05. Data published previously (Xavier et al., 2011) is marked with asterisk

regional level, the existence of two distinct but overlapping clades within the Mediterranean region suggests a time of allopatry in the past history of *S. nadejda* but brings to light an unusual pattern of geographic distribution of genetic variability inside the Mediterranean Sea: instead of the east–west break in genetic continuity typically displayed by many Mediterranean species (Rolland et al., 2007; Arnaud-Haond et al., 2007; Calvo et al., 2009; Sá- Pinto et al., 2012), both mtDNA clades of *S. nadejda* are present in the western and the eastern Mediterranean basins.

Phylogeography of *Stenosoma nadejda* throughout its range

Contrary to what could be expected taking into account the poor autonomous dispersal ability of peracarids and the accumulated data on *S. nadejda*'s mtDNA phylogeography (Xavier et al., 2009, 2011), the analyses of the nuclear marker EF1α revealed an almost absence of phylogeographic structure from the Atlantic to the eastern Mediterranean. While the clustering analysis revealed three groups of individuals, as depicted in Fig. 2, these are admixed across regions. Sharing of EF1α variants across large areas of the distribution is also evident from the haplotype network (Fig. S2 of supplementary material), which also reveals a shallow phylogenetic structure, as haplotypes are closely related differing mostly by a single mutation.

Similar patterns of genetic homogeneity of the nuclear marker EF1α had already been observed for two other species of genus *Stenosoma*, in the northeast Atlantic. Yet, for *S. acuminatum* Leach, 1814 and *S. lancifer* (Miers, 1881) the pattern of genetic variation of EF1α was matched by the one of the mtDNA marker COI. Absence of genetic structure, significant deviations from neutrality and star-like haplotype networks have been observed for both COI and EF1α for *S. lancifer* as well as for the COI of *S. acuminatum*, these results being attributed to a recent colonization and demographic expansion (Xavier et al., 2012a). In

Table 4 Pairwise F_{ST} values between localities from the entire distribution of *Stenosoma nadejda*, based on 687 bp of nuclear EF1α gene

	BSM	Bal	Biz	CBo	CCo	Chi	SMr	Mar	Mol	Pen	Rab
Bal	0.639										
Biz	0.352	0.384									
Cbo	0.484	0.521	0.109								
Cco	0.420	0.391	0.094	*0.059*							
Chi	0.529	*-0.06*	0.247	0.389	0.244						
Smr	0.766	0.603	0.563	**0.717**	0.575	0.546					
Mar	0.235	0.289	0.250	0.340	0.246	*0.128*	0.581				
Mol	0.633	0.719	0.163	*0.010*	*0.078*	**0.7606**	**0.832**	0.398			
Pen	*0.102*	0.807	0.476	0.670	0.608	**0.735**	**0.866**	0.335	**1.000**		
Rab	0.667	0.220	0.431	0.586	0.466	*0.185*	0.574	0.406	**0.709**	**0.785**	
Vle	**0.729**	**0.847**	0.512	0.688	0.636	**0.792**	**0.877**	0.632	**1.000**	**1.000**	**0.803**

Location names were coded as following: BSM-Banuyls-sur-Mer; Bal- Baleal; Biz-Bizerte; CBo- Cap Bon; CCo-Capo Colonna; Chi-Chipiona; SMr- Santa Marinella Mar-Marbella; Mol-Molivos; Pen-Peñiscola; Rab-Rabat; VLe-Villanueva Monteleones; Values which are not highlighted depict low levels of differentiation and values in italics indicate non-significant F_{ST} values between locality pairs. Values highlighted in light grey represent intermediate differentiation between localities, whereas values highlighted in dark grey represent high levels of differentiation between localities. Values in bold represent extreme differentiation between localities

Table 5 Uncorrected p-distance between the six population clusters of *Stenosoma nadejda* obtained with BAPS for the COI gene

	Cluster I*	Cluster II*	Cluster III*	Cluster IV	Cluster V
Cluster II*	4.9				
Cluster III*	4.8	1.7			
Cluster IV	3.5	3.7	3.5		
Cluster V	3.8	3.4	3.0	2.2	
Cluster VI	3.6	4.1	3.6	2.4	1.6

Labelling of the clusters is the same used in Figs. 1 and 4. Data published in (Xavier et al., 2009, 2011) is marked with asterisk

the present study, however, there are no strong signs of selection or demographic expansion and the phylogeographic pattern of COI is highly structured (Figs. 1, 4, S1).

The COI phylogeographic pattern observed in the present work for locations to the east of the AOF are in line with those previously obtained from the Atlantic and Alboran regions (Xavier et al., 2009, 2011) with almost no haplotype sharing between sampled localities and a marked genetic structure, with several distinct haplogroups. The estimates of genetic differentiation between locations are also similar to what was previously found, suggesting low population connectivity at the local scale. However, the

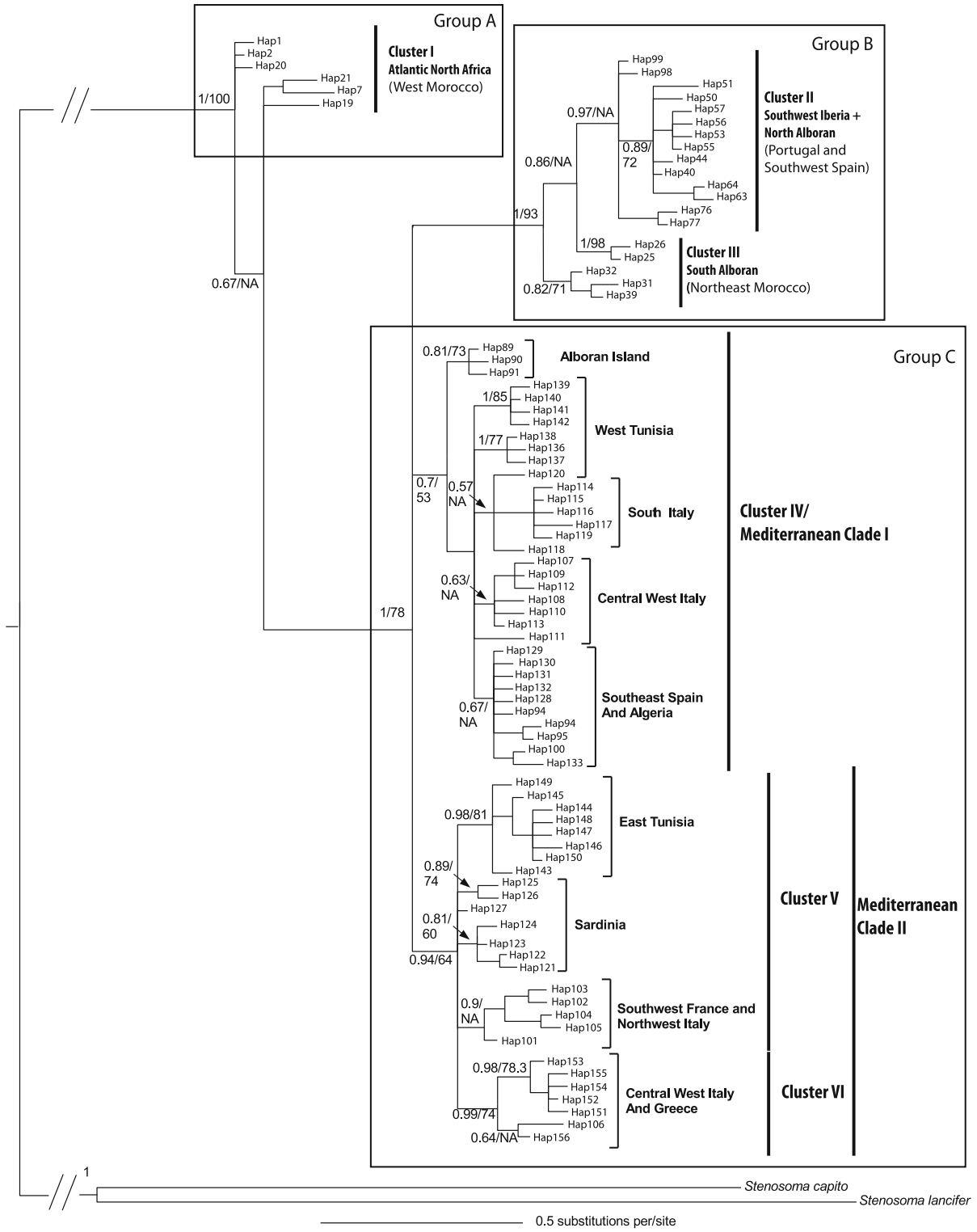

Fig. 4 Bayesian consensus tree for the mtDNA COI gene. Node values correspond to Bayesian posterior probabilities and bootstrap support, respectively. Clusters defined by BAPS are also identified (see Fig. 1 for details)

divergence found between Mediterranean specimens using uncorrected p-distances is inferior to the divergence found between *Stenosoma* species for the same gene fragment (less than 5–18%). Hence, despite the high structure of haplotype distribution, these results combined with the evidence from the EF1α data conform with the hypothesis of a single species that ranges from the Atlantic to the eastern Mediterranean.

Phylogeographic patterns suggest allopatry followed by long-distance dispersal

One of the most interesting results from the present work is the unusual geographic distribution of the two Mediterranean mtDNA clades (Figs. 4). While at the local scale connectivity seems to be very restricted, the two main Mediterranean clades were found to be present in both the western and the eastern basins. The pattern could be explained by a fairly long time of historical allopatry in two regions. The dating of the *Stenosoma* phylogenetic tree (Xavier et al., 2012b), which included Mediterranean samples of *S. nadejda*, suggests that diversification within this species was likely caused by isolation during the Pleistocene glaciations. If this is the case, and taking into account present mtDNA data, it is possible that there were two southern Mediterranean glacial refugia, as a west–east genetic break is present in the African coast (Figs. 1, 4). After glaciations, the spread of both clades across these two basins did not follow a coastal stepwise path, and must have been attained by sporadic long-distance dispersal through rafting.

The putative capacity of *S. nadejda* for long-distance dispersal through rafting seems to be unique within the genus, which comprises other 12 species inhabiting the same area. The two "true" Atlantic *Stenosoma* species (*S. acuminatum* and *S. lancifer*) range from southern UK to Morocco (~4000 km along the coast line) but do not enter the Mediterranean, while most of the remaining species are restricted to smaller areas, such as the Gulf of Cadiz, the Alboran Sea, Algeria or Tunisia (Xavier, 2011). The only exception is, for now, *S. capito* which has a similar distributional range (from the Black Sea into the Atlantic). However, molecular and morphological evidence suggests that *S. capito* is actually a complex of two species whose ranges do not even overlap: one inhabits the Black Sea and the eastern Mediterranean basin, whereas the other extends from the western Mediterranean basin into the Atlantic, reaching northern Morocco and southern Portugal (Xavier et al., 2012b).

Anecdotal evidence suggests that the striking difference between the distributional range of *S. nadejda* and those of the remaining species of the genus is likely related with its more efficient use of rafting for long-distance dispersal. In the southern Portuguese coast, where it coexists with *S. lancifer* and *S. accuminatum*, *S. nadejda* is by far the most common species found on low-shore intertidal algae. In this region, it is frequent to observe many individuals attached to drifting algae, especially on the invasive species *Asparagopsis armata* Harvey 1855 which often forms floating masses of considerable size. Experimental work has demonstrated that some idoteids, such as *I. balthica* and *I. granulosa*, can actively swim to colonise floating material such as detached seaweeds (Clarkin et al., 2012). Interestingly, *I. balthica* is the only idoteid for which molecular evidence confirms a large (amphi-Atlantic) distribution (Wares & Cunningham, 2001), although some degree of intraspecific differentiation exists between both sides of the Atlantic (Wares, 2001).

It remains to be known the degree to which *S. nadejda* is adapted to rafting conditions, since specific traits, such as feeding strategies, metabolic rates, or even the capacity to reproduce "en route", ultimately determine the success of long-distance dispersal (Gutow et al., 2006). However, the low levels of genetic connectivity found at the local scale suggest that rafting is not frequent in this species.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This work did not involve endangered or protected species, and no specific permissions were required to collect them. Most specimens were obtained through regular exchange with other institutions/co-workers.

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