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Mar Biol (2012) 159:2797–2807
DOI 10.1007/s00227-012-2040-4

ORIGINAL PAPER

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Received: 4 April 2012 / Accepted: 2 August 2012 / Published online: 29 August 2012

Abstract Human-made structures are increasingly built in marine coastal habitats for a variety of purposes. Offshore oil and gas production platforms are among the largest examples. Yet, biological effects of these increasing density artificial substrata are under evaluated. The objective of our study is to investigate the possible role of offshore platforms in modifying the genetic composition of populations of natural rocky shores species. The serpulid *Pomatoceros triqueter* was used as a model, and genetic variation was assessed using a 419 bp fragment of the mtDNA COI gene in samples collected on eleven offshore gas platforms, on one coastal buoy on the sandy shore and in four sites located on natural rocky shores in the Adriatic Sea. Deep phylogenetic lineages were uncovered over all samples. Nucleotide diversity and mean number of pairwise differences among haplotypes were significantly smaller in offshore platform samples compared to rocky shores samples. No significant genetic structure was observed over all samples. We found direct evidence of lower genetic diversity on platforms confirming that, although artificial structures attract and support species typical of hard bottoms, they are not analogues of natural rocky habitats.

Communicated by T. Reusch.

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Introduction

Disturbances related to human activities (e.g. habitat loss and fragmentation, global environmental change, overexploitation and other effects due to fishing, pollution and tourism) are worldwide recognized as a major threat to marine ecosystems (Lotze et al. 2006; Worm et al. 2006; Airoldi and Beck 2007). Among recognized anthropogenic pressures, the impact of artificial structures introduction has received increased attention in the recent decades (see Bulleri and Chapman 2010 for a review).

Artificial hard structures are most often built in sandy areas that, as a result, generate unnatural changes in species composition, abundance and diversity of native soft-bottom assemblages (Connell 2001; Martin et al. 2005; Airoldi et al. 2005; Bulleri 2005; Moschella et al. 2005; Bulleri and Chapman 2010). Besides these modifications at the community and species level, artificial structures also play a role at the population level, providing new colonizing substrata (Page et al. 2006; Fauvelot et al. 2009). Indeed, genetic diversity on these artificial structures may be inclined to deep changes, as recently observed for the limpet *Patella caerulea* collected on breakwaters (Fauvelot et al. 2009). Since genetic diversity can affect the species productivity, population growth and stability, as well as inter-specific interaction within community, and ecosystem-level processes (Hughes et al. 2008), this

parameter is crucial for species survival in highly variable environments or those subject to rapid anthropogenic changes (Reusch et al. 2005). In addition, since all benthic organisms directly associated with artificial structures rely on a pelagic larval phase to disperse and colonize new habitats, artificial structures may enhance connectivity among differentially adapted populations, (i.e. artificial structures may serve as "stepping stones" for hard-bottom species) which may ultimately lead to negative effects of biogenic homogenization (see Olden et al. 2004 for a review).

Offshore oil and gas production platforms are among the largest artificial structures in the marine environment. They are associated with several environmental issues, related to their installation, daily shipping movements, extraction activities, maintenance and final decommissioning (Grant and Briggs 2002; Fabi et al. 2002; Page et al. 1999, 2006; Bomkamp et al. 2004; Terlizzi et al. 2008). The north Adriatic Sea is one of the major fields for extraction of natural gas in the Mediterranean basin (Teatini et al. 2000). Since 1950, more than 100 platforms (supplying over 70 % of the total Mediterranean gas production) have been built over an area of 6,400 km^2. These structures constitute most of the hard substrate in this sandy region and provide suitable habitat for the development of massive mussel (*Mytilus galloprovincialis*) beds, which are commercially exploited on permit from the petroleum companies (Fabi et al. 2002).

Despite the extent of these offshore activities in the North Adriatic Sea, few ecological impact studies have been carried out, and these have focussed on possible changes in the physiology, composition and structure of benthic and pelagic assemblages associated with the presence of offshore platforms (Relini et al. 1998; Crema et al. 2001; Ponti et al. 2002; Fabi et al. 2004; Consoli et al. 2007; Gorbi et al. 2008, 2009). However, so far, no study has investigated the genetic consequences of the platforms colonization on populations of natural rocky shores species. The goal of our study was to examine the potential consequences of the presence of offshore platforms on the genetic diversity and structure of natural rocky shore organisms. During a preliminary survey conducted in July 2005, benthic organisms have been collected and identified from three offshore platforms in order to select several target species as model organisms. The primary objectives of this survey were to identify the fauna that could be sampled on the legs of the offshore platforms, and from these, select the ones that could be sampled in sufficient numbers to conduct reliable analysis. Among macrobenthic organisms, we found a majority of mussels (*Mytilus galloprovincialis*), serpulids (*Pomatoceros triqueter* and in lower densities *Serpula vermicularis*), oysters (*Ostrea edulis* and *Crassostrea gigas*), and in lower densities barnacles, brittle stars and errant polychaetes (Nereididae). A detailed description of the platform fauna at Ravenna can be found in Relini et al. (1998) and in Ponti et al. (2002). Due to health and safety regulations, we were not authorized to sample benthic organisms from the platforms ourselves, and we had to rely on what was sampled by the professional divers harvesting mussels on the legs of platforms. We chose to discard *Mytilus galloprovincialis* as a target species to avoid a possible harvesting impact on genetic diversity, and thus focused our sampling effort and genetic analyses on the second most common species on the platforms, *Pomatoceros triqueter* (Linnaeus, 1767), a benthic worm typical of the fouling community (Crisp 1974).

The serpulid genus *Pomatoceros* are sessile, tubicolous and filter-feeders, occurring on a variety of substrata at depths ranging from low water intertidal into the shallow benthic, with a wide geographical distribution from cold temperate latitudes to the equator in the northern hemisphere (Crisp and Ekaratne 1984). *Pomatoceros* spp. have feeding, planktonic larvae (Kupriyanova 2003) and are capable of reproducing throughout the year (Castric-Fey 1984). The larval duration of *Pomatoceros triqueter* varies between 15 days and 4 weeks, depending on the temperature, as for its sister species *Pomatoceros lamarckii* (Castric-Fey 1984; Hayward and Ryland 1995; Cotter et al. 2003). *P. triqueter* and *P. lamarckii* (co-occurring in the Mediterranean Sea, though not encountered on the platforms, Ponti et al. 2002, personal observations) can be distinguished morphologically by differences in their operculum structure; in *P. triqueter*, the operculum is convex, cone-shaped and obliquely mounted on the peduncle, whereas in *P. lamarckii*, the operculum is concave, cup-shaped and mounted centrally on its stalk (Zibrowius 1968).

In this study, we investigated the genetic diversity and structure of *Pomatoceros triqueter* collected on eleven offshore gas platforms, on one costal buoy and in four sites located on natural rocky shores in the Adriatic Sea. We specifically tested (a) whether the genetic diversity of populations on platforms was reduced compared to populations on natural rocky shores, as could be expected from the recent founding of the platforms, or alternatively enhanced if the platform populations act as reservoir of genetic diversity and (b) for differences in the genetic structuring of populations between platform and natural shores.

Materials and methods

Sampling

The sampling was conducted in the Adriatic Sea, on natural rocky shores, and more intensively on gas platforms. Sampling was conducted between May and August 2006. Platforms have been selected according to their location (in order to cover the range of platforms inter-distances) and the

possibility to sample with vessels. Sampling was completed by the use of the vessels from the Cooperative Society 'La Romagnola' which conducted daily sampling on the platforms for mussels harvesting. In total, ten effective platforms have been sampled (Agostino-A, Amelia-D, Anemone-B, Angelina, Antonella, Armida, Cervia-K, Garibaldi-C, Porto Corsini Est-80bis and Porto Corsini C), as well as the wreck platform Paguro (Fig. 1). For each platform, mussel beds collected by the professional divers were brought to the laboratory for sorting, and *Pomatoceros triqueter* tubes were detached from mussel shells.

Along the west shoreline of the Adriatic Sea, natural hard-bottom habitats, represented by isolated rocky promontories, interrupt large sandy coasts extending between the Friuli-Venezia Giulia and Puglia regions (NE and SE of Italy, respectively). The largest of these rocky promontories are Sistiana/Miramare (Friuli-Venezia Giulia), Gabicce (Emilia-Romagna), Conero (Marche) and Gargano (Puglia). Sampling along the Adriatic coasts was conducted along the sea shore by SCUBA diving in Trieste (Miramare), Tegnue di Chioggia, Ancona (Conero), Brindisi, Taranto and in Zaglav (on the Croatian island of Dugi Otok) where *Pomatoceros triqueter* were found on rocks (Fig. 1), except in Tegnue di Chioggia where *P. triqueter* were found on buoys delimiting the marine protected area. In Ancona, only *Pomatoceros lamarckii* was encountered and sampled. In Brindisi, the sampling consists mostly of *P. lamarckii* so that only few *P. triqueter* could be analysed.

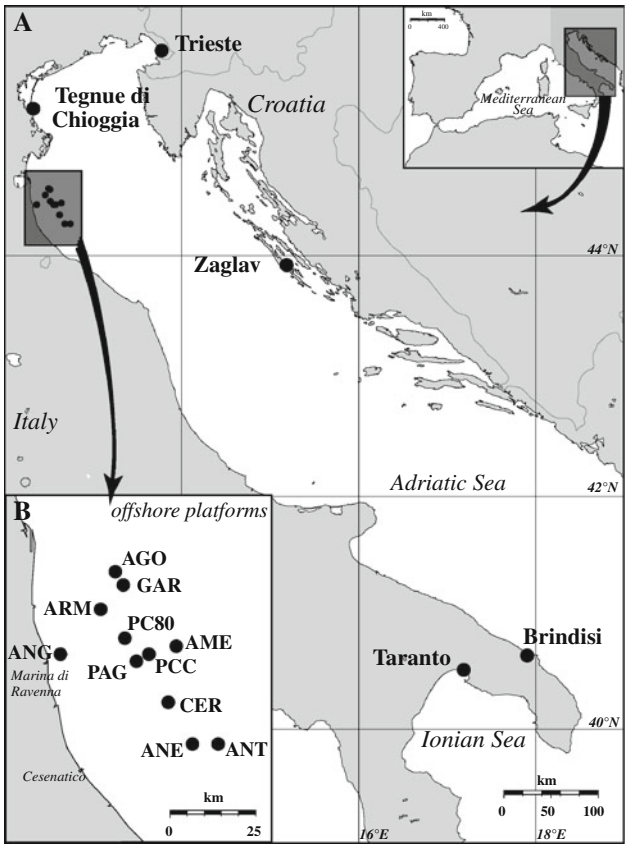

Fig. 1 Location of sampling sites (*A*) along the coasts of Italy (Tegnue di Chioggia, Trieste, Brindisi, Taranto) and Croatia (Zaglav on the island of Dugi Otok) and (*B*) from eleven offshore platforms off the coast of Ravenna (*AGO* Agostino-A, *AME* Amelia-D, *ANE* Anemone-B, *ANG* Angelina, *ANT* Antonella, *ARM* Armida, *CER* Cervia-K, *GAR* Garibaldi-C, *PC80* Porto Corsini Est-80bis, *PCC* Porto Corsini C, *PAG* Paguro)

Molecular analysis

Immediately after sampling, alive individual worms were placed at 4 °C for at least 3 h in order to facilitate the extraction of the worm body from its calcareous tube without damaging the body. Each collected worm was examined alive under binocular microscope for species identification and operculum shape notification. Each worm was then stored in individual Eppendorf tubes in 80 % EtOH and kept at −20 °C.

Genomic DNA was isolated from the caudal extremity of 11–29 individuals per site, using a cetyltrimethyl ammonium bromide (CTAB) protocol (Hillis and Moritz 1990). Specifically, each tissue sample was digested for 2 h at 65 °C in 400 µl of 2X CTAB solution containing 3 U of Proteinase K. Genomic DNA was extracted using Chloroform–Isoamyl Alcohol (24:1) and precipitated in 100 % EtOH.

Like all non-model organisms, there were no markers available in the literature for *Pomatoceros triqueter*. We choose to test several universal primers amplifying mtDNA genes due to the development cost of microsatellites. Three genes could successfully be amplified in *P. triqueter*: COI, 12S and 16S (details available on request). We chose to conduct further genetic analysis using COI since this marker happened to be useful in revealing the genetic structure in two polychaete tubeworms (Jolly et al. 2006). Furthermore, the COI gene is more polymorphic than the 16S and 12S genes in annelids, both conserved within species (Jolly et al. 2006; Halanych and Janosik 2006). A 489-bp fragment of the mitochondrial DNA cytochrome oxidase I gene was amplified using the primers PtCOI-F (5′-GCT TGA GCC GGA ACC TGT GG-3′) and PtCOI-R (5′-CCC CCA GCT AAT ACA GGA AC-3′), which were specifically designed for *P. triqueter* based on several sequences obtained using LCO1490 and HCO2198 (Folmer et al. 1994). Each 25 µl PCR amplification contained 1X PCR buffer, 2 mM $MgCl_2$, 0.08 mM of each dNTP, 3 µg of BSA (Bovine Serum Albumin), 0.2 µM of each PtCOI-F and PtCOI-R primer, 0.25 U of Taq polymerase and about 30 ng DNA. Amplified fragments were purified using the ExoSAP-IT kit (USB) following the

manufacturer recommendations and cycle sequenced in both directions using Big Dye Terminator sequencing kit (Applied Biosystems). Sequences were run on an ABI 310 Genetic Analyser (Applied Biosystems). Forward and reverse sequences were compared for about 10 % of the individuals in BioEdit (Hall 1999). Then, since forward and reverse sequences gave identical results, further sequences were obtained using only the forward primer (PtCOI-F). Individual sequences were then aligned by eye in GeneDoc (Nicholas and Nicholas 1997).

Data analysis

Haplotypes were identified, and their relative frequency within samples estimated using Arlequin version 3.11 (Excoffier et al. 2005). Genetic diversity within populations was estimated as haplotype diversity (Hd, Nei 1987), nucleotide diversity (π, Nei 1987) and mean number of pairwise nucleotide differences between individuals (k, Tajima 1983) using DnaSP 4.0 (Librado and Rozas 2009). Significant differences in genetic diversity (Hd, π and k) between natural versus artificial habitats were tested using a Wilcoxon test.

Phylogenetic relationships among haplotypes were constructed using Bayesian analyses implemented in MrBayes 3.1 (Huelsenbeck and Ronquist 2001) and employed a cold chain and three incrementally heated chains with $T = 0.1$. Starting trees for each chain were random, and the default values of MrBayes were chosen for all settings (including prior distributions). Each metropolis-coupled Markov chain Monte Carlo (MCMC) was run for 30 million generations, with trees sampled every 5,000 generations, and discarded the first 20 million generations (4,000 trees). By this time, the chains had converged to stable likelihood values <0.01. Posterior probabilities (PP) were used to assess clade supports. Analyses were run using the evolutionary model selected by the Akaike information criterion of MrModeltest (Nylander 2004). *Pomatoceros larmarkii* was used as outgroup: two specimens were sampled in Ancona (Marche, Italy, Adriatic Sea), and sequences were obtained using primers LCO1490 and HCO2198 as described above. Genetic divergences between supported monophyletic clades were estimated in Mega version 4 (Tamura et al. 2007) using the mean net nucleotide divergence (Nei and Li 1979), defined as d_{xy}–0.5 ($d_x + d_y$), that subtracts the average 'within-group' divergence from the observed 'between-group' estimate and p-distance model.

A minimum spanning network (MSN) displaying evolutionary relationships between mtCOI haplotypes was constructed in TCS version 1.13 (Clement et al. 2000) to infer the most parsimonious branch connections at the 95 % confidence level between haplotype pairs. The MSN was then redrawn by hand in Adobe® Illustrator® 9.0 (Adobe Systems Incorporated).

Data analyses of spatial genetic structure among populations were performed using Arlequin. Genetic divergences between pairwise samples were estimated using Φ-Statistics (Φ_{ST} based on haplotype frequencies and molecular divergence using pairwise differences). P values were obtained using a non-parametric permutation procedure with 10,000 permutations. In order to examine the partition of the genetic variance among samples based on the type of habitat or geographical locations, analyses of molecular variance (AMOVA) (Excoffier et al. 1992) were conducted in Arlequin.

Results

The amplification of a fragment of the mtDNA cytochrome oxidase I gene in a total of 350 individuals of *Pomatoceros triqueter* from 16 geographical samples resulted in fragments of 419 bp that could unambiguously be aligned and analysed. Among the 350 individuals sequenced, we identified 160 different haplotypes (GenBank accession numbers JX308620–JX308779), distinguished by 136 polymorphic sites and a total number of 162 mutations (i.e. some nucleotide positions carried more than two allelic states). Among the 16 sample sites, the number of haplotypes per sample ranged between 11 and 21 haplotypes. From the 160 observed haplotypes, only 21 were found in at least two individuals, and the 139 remaining haplotypes were singletons (i.e. haplotypes carried by only one individual, Table 1).

Over all the 16 samples, haplotype diversity ranged between 0.89 and 1, nucleotide diversity between 0.020 and 0.048 and mean number of pairwise differences between 9.05 and 20.16. Excluding TEG for the comparisons (as it was neither a platform or a rocky shore sample), all three genetic diversity estimates were significantly different among samples when comparing offshore platform against rocky shores samples (mean $Hd_{platforms} = 0.942 \pm 0.023$, mean $Hd_{natural} = 0.982 \pm 0.019$, $P = 0.006$; mean $\pi_{platforms} = 0.0245 \pm 0.002$, mean $\pi_{natural} = 0.0364 \pm 0.010$, $P = 0.045$; mean $k_{platforms} = 10.27 \pm 0.88$, mean $k_{natural} = 15.24 \pm 4.05$, $P = 0.045$, Fig. 2).

The Bayesian tree (Fig. 3) revealed two highly supported and reciprocally monophyletic clades (both with 100 % posterior probability). Clade 2 was only represented by two haplotypes that were found in Taranto (H125) and Trieste (H144). Clade 1 included a number of supported subclades (PP > 95 %) as well as paraphyletic haplotypes. The mean net divergence between the two main *P. triqueter* clades was 12.40 ± 1.46 % and 20.92 ± 1.73 % between *P. triqueter* and *P. lamarkii*.

The minimum spanning network (MSN) showing the most parsimonious branch connections at the 95 % level

Table 1 Spatial distribution of the *Pomatoceros triqueter* mtCOI haplotypes and genetic diversity per sample

	AGO	AME	ANE	ANG	ANT	ARM	CER	GAR	PAG	PC80	PCC	BRI	TAR	TEG	TRI	ZAG	Total
H1	5	4	3	5	3	6	2	4	4	5	1	1	2	6	2	5	58
H2	2	4	2	6	4	2	5	6	3	1	4		1	2		2	44
H3	2	3	4	1	2	1	3	3	2	2	3	1	2			2	31
H4	1	1	1	2		1	3	1	1	3		1	1		1		17
H5		1	1		1			1	1		2	1	1			4	13
H6			1		1	1		1	1		1						6
H7				1		2	1					1				1	6
H8				1	1				1						1		4
H9		1						2								1	4
H10		1				1			1					1			4
H11		1			1										1		3
H12							1		1					1			3
H13		1					1										2
H14			1		1												2
H15					1			1									2
H16											1	1					2
H17			1	1													2
H18		1									1						2
H19			1							1							2
H20					1							1					2
H21							1							1			2
H22-H28	7																7
H29-H37		9															9
H38-H49			12														12
H50-H57				8													8
H58-H66					9												9
H67-H75						9											9
H76-H82							7										7
H83-H88								6									6
H89-H96									8								8
H97-H104										8							8
H105-H117											13						13
H118-H121												4					4
H122-H130													9				9
H131-H137														7			7
H138-H146															9		9
H147-H160																14	14
N	17	27	27	25	25	23	24	25	23	20	26	11	16	18	14	29	350
H	11	19	21	15	19	16	15	14	17	13	20	11	14	12	13	20	160
Hd	0.912	0.957	0.972	0.913	0.967	0.933	0.938	0.917	0.96	0.926	0.969	1	0.983	0.895	0.989	0.956	0.945
π	0.023	0.026	0.024	0.026	0.027	0.022	0.025	0.024	0.026	0.021	0.026	0.028	0.04	0.022	0.048	0.029	0.027
k	9.69	10.93	10.04	11.04	11.31	9.05	10.64	9.96	10.71	8.61	11.03	11.71	16.94	9.111	20.17	12.15	11.34

Abbreviations of sampling sites as on Fig. 1; *BRI* Brindisi, *TAR* Taranto, *TEG* Tegnue di Chioggia, *TRI* Trieste and *ZAG* Zaglav, *N* number of individuals, *H* number of haplotypes, *Hd* haplotype diversity, π nucleotide diversity, *k* mean number of pairwise differences

also revealed the presence of two networks (Fig. 4) that could not be connected since haplotypes 125 and 144 were too divergent from the rest of the haplotypes (69 substitutions out of 419 bp). The bigger network showed a partition in two groups that were connected by haplotypes exclusively found in Taranto (H127, H128 and H129). Apart from these haplotypes, the two sub-networks were connected by a minimum of 13 mutation steps. The haplotypes found within the sub-networks were not specific of any geographical location, and all three sub-networks were

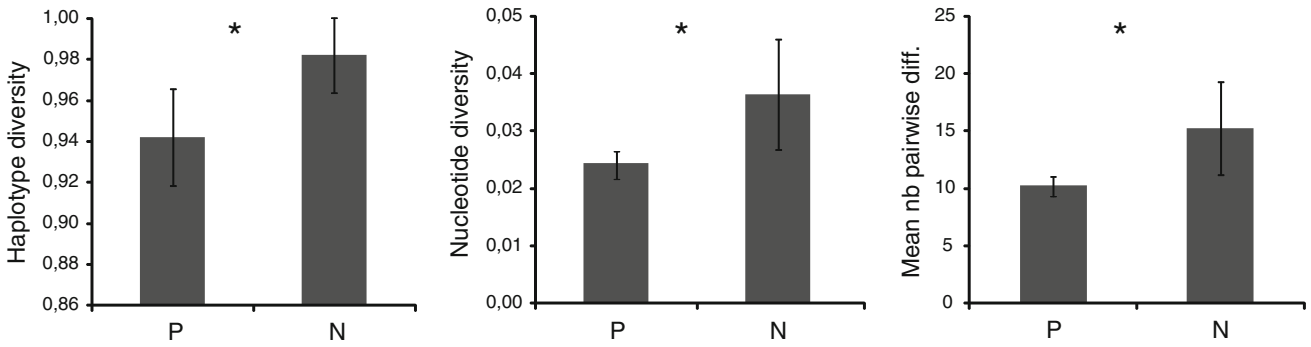

Fig. 2 Average haplotype diversity, nucleotide diversity and mean number of pairwise differences among mtCOI sequences of *Pomatoceros triqueter* over all platform samples (P) and natural rocky shore samples (N). *$P < 0.05$ (Wilcoxon test)

sympatric. The MSN revealed a star-like structure within each sub-networks, with central haplotypes shared among several samples and singletons diverging by only few substitutions from the central haplotypes.

No significant genetic structure was observed overall samples ($\Phi_{ST} = 0.0177$, $P = 0.073$), with 1.77 % of the total variance being found among the 16 samples. The pairwise Φ_{ST} estimates among samples ranged from 0 to 0.223 ($P = 0.004$) between Tegnue di Chioggia and Garibaldi platform, but none of the pairwise comparisons were significant after adjustment of the significance level $\alpha = 0.05$ according to the sequential Bonferroni correction (data not shown). The AMOVAs conducted revealed no significant partition of the variance when considering offshore platforms versus natural substrates or north versus south Adriatic samples ($P > 0.05$, data not shown).

Discussion

Deep mitochondrial phylogeny or evidence of cryptic species?

The genetic divergence observed between *Pomatoceros triqueter* and its closely related species *Pomatoceros lamarckii*, also present in the Adriatic Sea, was about 20 %. These two species are well differentiated on the basis of the operculum morphology (Zibrowius 1968), their karyotype (Dixon et al. 1998) and genetics (Ekaratne et al. 1982; Crisp and Ekaratne 1984). The genetic analyses conducted on a fragment of the mtDNA COI gene revealed the presence of two deep (12 % divergent) monophyletic clades in *P. triqueter* within the Adriatic Sea. No morphological differences were observed between the individuals belonging to the two deep monophyletic clades. Shape differences of the operculum were observed among all 350 individuals but fell in those described in the literature (Zibrowius 1968; Dixon et al. 1998) and were not related to specific clades. The sympatric occurrence of highly divergent mitochondrial clades has already been observed in other polychaete tubeworms in European seas, with similarly high net genetic divergences among clades (16.4 % divergence between two sympatric clades of *Pectinaria koreni* and 16 % divergence between two sympatric clades of *Owenia fusiformis*; Jolly et al. 2006). Therefore, we considered the two uncovered monophyletic clades as belonging to *Pomatoceros triqueter* and thus rejected the occurrence of a possible cryptic species in our data set.

The phylogenetic analysis also recovered divergent groups of haplotypes, which formed a well-supported clade (Bayesian analysis: PP = 100 %, mean of 12 % genetic divergence, two subnetworks in the MSN). The occurrence of divergent groups of sequences within *Pomatoceros triqueter* may be attributed to different glacial relicts, resulting from geographical isolation of *P. triqueter* populations during lowered sea levels followed by a recent colonization from different ancestral populations located in the Mediterranean Sea, as observed for numerous marine organisms (Lee 2000; Schroth et al. 2002; Goetze 2003; Peijnenburg et al. 2004). Investigating the phylogeographic patterns of *P. triqueter* at the European scale may permit identifying the locations from which relic haplotypes arose.

May the introduction of platforms enhanced genetic connectivity?

The absence of significant pairwise comparisons and significant structure among various groups of samples may have several explanations. The first one is that the mtCOI gene may not be the best molecular marker to reveal potential genetic structure among *Pomatoceros triqueter* samples due to a high number of singletons and low number of shared haplotypes. The way to test for this hypothesis would be to use additional polymorphic nuclear markers such as microsatellites loci in order to test for

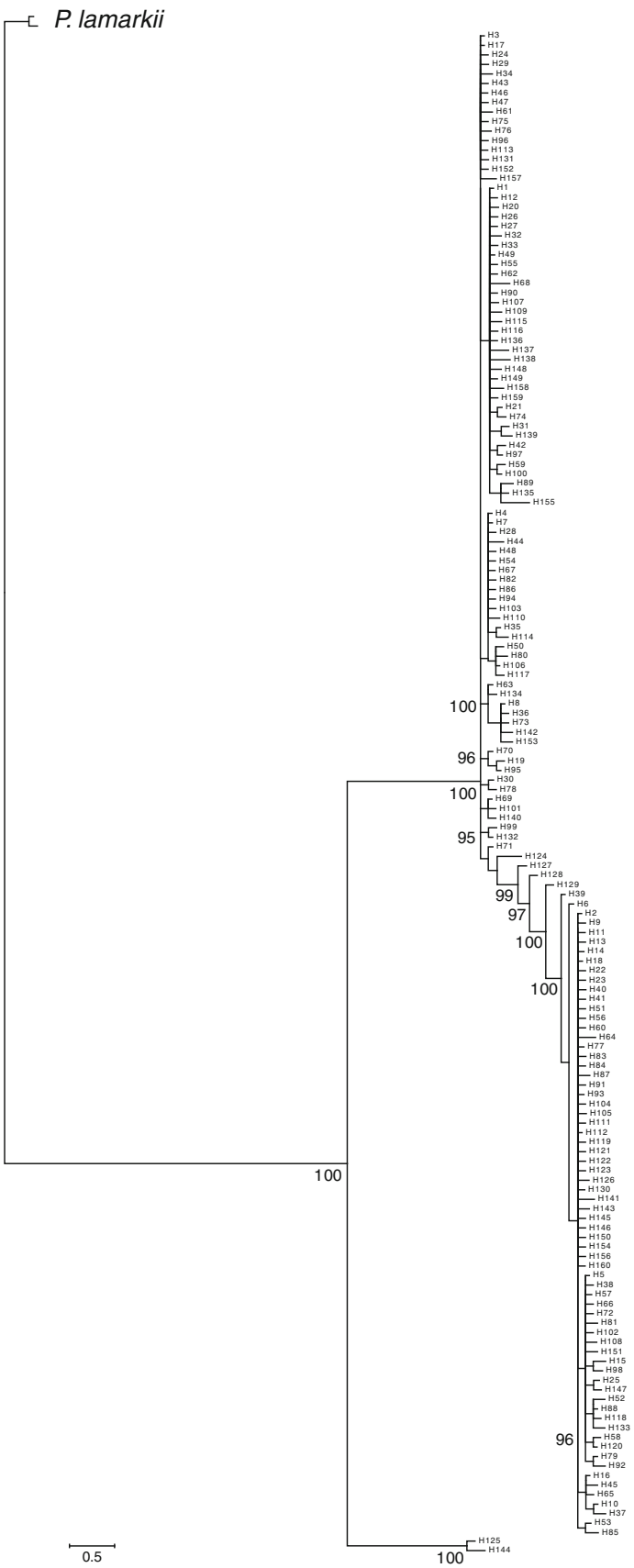

Fig. 3 Bayesian phylogeny of 160 mtCOI haplotypes of *Pomatoceros triqueter* rooted with *P. lamarkii*. Posterior probability ≥95 % is indicated. Haplotypes are coded according to Table 1

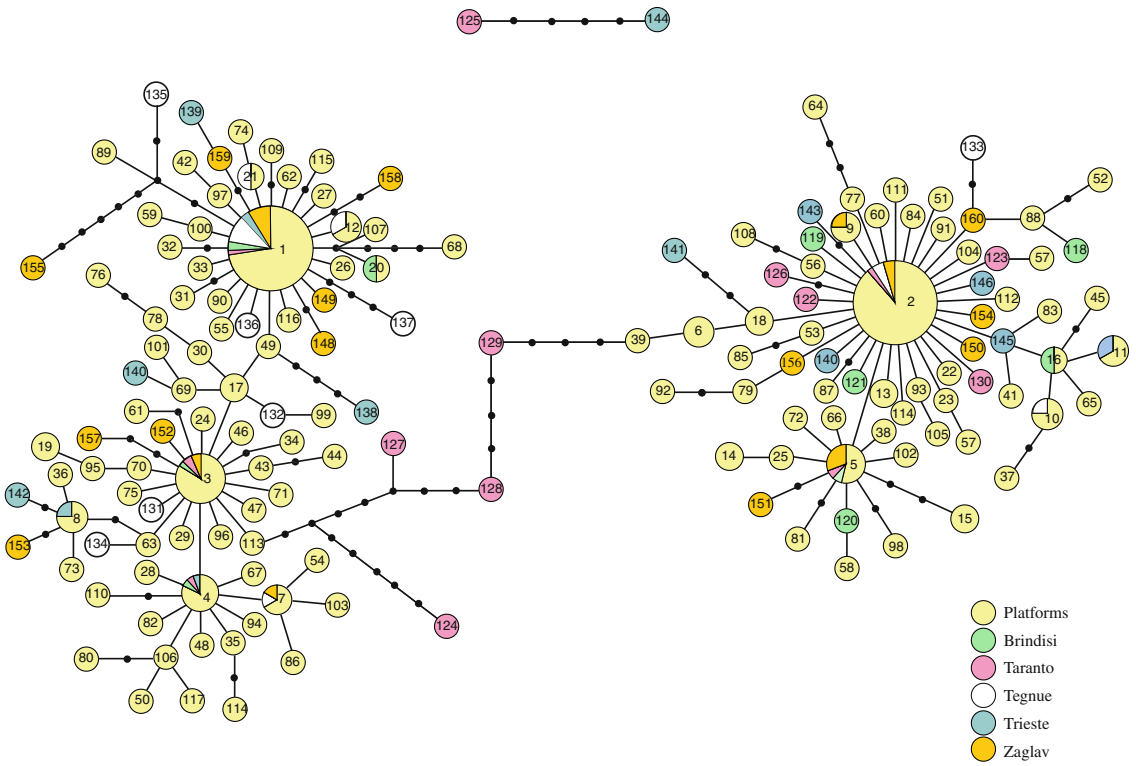

Fig. 4 Minimum spanning network illustrating the phylogenic relationships among the 160 mtCOI haplotypes of *Pomatoceros triqueter*. *Circles* represent haplotypes with their corresponding number as in Table 1, while *small black circles* represent missing haplotypes within the network (i.e. haplotypes not encountered within the samples). Each *line* linking *two circles* represents one base pair substitution along the 419-bp length-analysed sequence. The locations of the haplotypes are indicated with *color code* as in the legend (color figure online)

significant differences among samples. However, we believe that if there would have been a significant partition of the genetic variation, mtDNA COI haplotypes would not appear randomly spatially distributed.

A second explanation for the lack of significant structuring of the samples would be that *Pomatoceros triqueter* populations were genetically connected prior the introduction of the platforms. This could be the result of a rather long larval duration of *P. triqueter* (about 3 weeks), which would allow sufficient gene flow among samples to homogenize genetic structure within the Adriatic Sea. Similar patterns have been observed (using more fast evolving nuclear markers) in the same area for the limpet *Patella caerulea* (Fauvelot et al. 2009), a species inhabiting natural rocky shores as well as artificial substrates along the shore (e.g. breakwaters). Alternatively, the observed genetic structure at the mtCOI may depict the footprint of the recent colonization of the Adriatic Sea after the Last Glacial Maximum (LGM). Indeed, during the LGM (about 18,000 ya), the sea level was about 100 m below the actual mean water level, and most of the Adriatic Sea bed was dried (Dondi et al. 1985; Thiede 1978). The sea water invaded the Adriatic during the last 10,000 years, and the colonization by the marine flora and fauna is very recent. Genetic similarities in Adriatic samples of *P. triqueter* may reflect past founder effects linked with the colonization of the Adriatic Sea after the Pleistocene glaciation. Indeed, several studies have recently stressed the relevance of palaeoecological events in determining the genetic patterns in marine populations (e.g. Fauvelot et al. 2003; Wilson 2006; Imron et al. 2007; Virgilio et al. 2009). Interestingly, intermediate haplotypes between the two observed sub-networks are only located at the Taranto locality, suggesting that this part of Italy may have been a refuge for the Adriatic fauna during lowered sea levels.

A last explanation would be that platforms act as stepping stones for hard-bottom species, and thus the introduction of platforms has created corridors among former discrete natural populations. Because we did not have samples from natural rocky shores prior the introduction of all artificial structures nowadays found in the Adriatic Sea, it is impossible to conclude that artificial structures are responsible for the genetic connectivity currently observed for both *Pomatoceros triqueter* and *Patella caerulea* (Fauvelot et al. 2009). A possible way to test for genetic connectivity increase due to the platforms would be to conduct a study on *P. triqueter* genetic structure in an area where only discrete populations occur on natural shores

and where no artificial structures may connect them, in order to estimate the dispersal kernel of *P. triqueter* in none impacted environments. Unfortunately, such areas likely do not exist anymore (Airoldi and Beck 2007).

Do the platforms affect the genetic diversity of *P. triqueter*?

Significantly smaller genetic diversity was observed in *Pomatoceros triqueter* samples located on the platforms as compared to natural rocky shore samples. Lower genetic diversity levels on artificial urban structures as compared to natural rocky shores have already been observed in another invertebrate sampled in the same area, *Patella caerulea* (Fauvelot et al. 2009). It has been evidenced that artificial structures support assemblages that differ significantly in composition, structure, reproductive output, patterns of recruitment and population dynamics from assemblages on nearby natural rocky habitats (see Bulleri and Chapman 2010 for a review) suggesting important functional and ecological differences between artificial and natural habitats. At the population level, the propagule pressure (Lockwood et al. 2005) through small inoculum size (i.e. the number of viable settlers) creates a filter from the amount of genetic diversity found in surrounding natural populations, further causing genetic diversity to decrease while maintaining genetic homogeneity between artificial and natural populations (Roman and Darling 2007). Our results support this hypothesis.

Lower levels of haplotype diversity have also already been found on meiobenthic harpaticoids (Copepoda) populations living near platforms (<50 m) as compared to distant populations (>3 km) in the Gulf of Mexico (Street and Montagna 1996). In this study, the low genetic diversity in samples near platforms was likely attributed to high concentrations of potentially toxic substances (hydrocarbons and trace metals) in the sand content, but no alternative hypothesis was proposed. Reductions in genetic diversity of *Pomatoceros triqueter* populations on platforms could similarly be attributed to extraction activities (i.e. pollution, Gorbi et al. 2008), and/or periodical removal of mussel beds (for commercial mussel exploitation and cleaning of fouling) on which *P. triqueter* grow, creating local bottlenecks (England et al. 2003). Eventually, the lower genetic diversity on artificial substrata as compared to natural ones may not be a result of a single factor but could likely be the consequence of a combination of these various processes.

Conclusion

All together, our results suggest that the phylogeographic patterns observed for *Pomatoceros triqueter* in the Adriatic Sea based on mtCOI gene likely reflect the interaction between historical events (associated with Pleistocene sea level changes) and contemporary gene flow. The samples collected on platforms showed lower genetic diversity than those collected on the natural rocky shores. Our results contribute to the growing body of evidence showing that although artificial structures attract and support species typical of hard bottoms, they are not analogues of natural rocky habitats (see among others Bulleri 2005; Glasby and Connell 1999; Moreira et al. 2006; Moschella et al. 2005; Fauvelot et al. 2009). Our study calls for additional investigations related to the local functional and ecological processes that act on populations in a fragile marine ecosystem increasingly impacted by anthropogenic activities.

Acknowledgments This work was supported by the EU project EUMAR (European Marine Genetic Biodiversity—EVK3CT2001-00048) and by the AGIP funded research project BIOMARE (Project for the development of methodologies for monitoring the impact of off-shore structures in the northern Adriatic). We thank M. Pierpaoli for assistance in the lab. We are grateful to the Cooperative Society 'La Romagnola' (Marina di Ravenna) and to all the people that helped collecting samples.

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