PRIMER NOTE

Development of microsatellite markers for the Mediterranean gorgonian coral *Corallium rubrum*

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Abstract

*Corallium rubrum*, an endemic Mediterranean gorgonian coral, has undergone an intensive exploitation leading to the extinction of local commercial banks and changes in the structure and dynamics of coastal populations. Management and conservation of this species requires a better understanding of the genetic structuring and connectivity among populations. With this aim, seven microsatellite loci have been isolated. All loci were polymorphic with allele numbers ranging from five to 26 and observed heterozygosity ranging from 0.18 to 0.68. Significant deviations from Hardy–Weinberg expected genotype frequencies due to heterozygote deficiency were detected at all loci.

Keywords: *Corallium rubrum*, gorgonian coral, Mediterranean Sea, microsatellites, population genetics

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The red coral *Corallium rubrum* is a gorgonian coral endemic of the Mediterranean Sea and neighbouring African and Portuguese Atlantic coasts. The species dwells on rocky bottoms between 10 and 200 m in depth, it has internal fertilization and brooding planula larvae. Planulae are released during summer and are assumed to disperse only over short distances (Carpine & Grasshoff 1975).

Red coral has been harvested since ancient time and most commercial banks have been overexploited. Changes in population size structure and extinction of local commercial banks has been widely reported (Santangelo & Abbiati 2001). In recent years major efforts have been devoted to the conservation of this species, and since 1992 red coral has been listed among the species of community interest of the European Union Habitat Directive (92/43/EEC, Appendix V).

Conservative management of red coral populations requires a better understanding of the biology of the species, especially of the life history traits (e.g. dispersal range of larvae and recruitment patterns). Nonsynchronous spawning and difficulties in tracking of larvae in field prevents direct observations and measurements of larval dispersal ability. Genetic markers could provide useful tools to quantify effective larval dispersal and capability of the species to naturally restore overexploited populations. Previous studies on population genetics using allozyme markers revealed high genetic structuring of red coral populations at small spatial scales with low levels of genetic variability (Abbiati et al. 1993). The use of highly variable markers with greater resolving power, such as microsatellites, could provide more insight on the reproductive strategies and genetic structuring of this species over a range of spatial scales.

Red coral colonies used in this study were collected at Calafuria (Tyrrhenian Sea) and preserved in 80% ethanol at 4 °C. Total genomic DNA was extracted from individual colonies (two to four polyps) according to the standard cetyltrimethyl ammonium bromide (CTAB) extraction procedure. The enriched library technique used for microsatellite isolation has been based on the fast isolation by AFLP of sequences containing repeats (FIASCO) method (Zane et al. 2002). Genomic DNA was simultaneously restricted with *MseI* (New England Biolabs) and ligated to *MseI* adaptors (*MseAdU* 5′-GACGATGAGTCCTGAG-3′ and *MseAdD* 5′-TACTCAGGACTCAT-3′). DNA fragments were amplified by polymerase chain reaction (PCR) using *MseI* primers (*MseIA* 5′-GATGAGTCCTGAGTAAA-3′, *MseIC* 5′-GATGAGTCCTGAGTAAC-3′, *MseIG* 5′-GATGAGTCCTGAGTAAG-3′, *MseIT* 5′-GATGAGTCCTGAGTAAT-3′) and hybridized with a biotynilated probe (AC)$_{12}$ for 15 min at room temperature. DNA fragments
hybridized with the probe were selectively captured by streptavidin-coated beads (Roche) and separated by a magnetic field. DNA was eluted from the bead-probes with TE 1X buffer at 95 °C for 5 min, ethanol precipitated, re-amplified with MseI adaptor-specific primers and cloned using the TOPO TA cloning kit (Invitrogen) following the manufacturer's protocol. Three hundred and two positive clones were amplified by PCR with M13 forward–reverse primers (Table 1) and the ROX HD500 (Applied Biosystems) as internal size standards. Besides the expected products, one locus (COR15) provided a second set of polymorphic PCR product. Overall, seven clones reliably amplified a PCR product: three dinucleotide repeats, two trinucleotide and two tetranucleotide repeats. All founded loci are perfect.

Developed microsatellite loci were tested for allelic polymorphism on 50 red coral colonies. Between five and 26 alleles were detected per locus. The average expected heterozygosity ranged from 0.67 to 0.94. No linkage disequilibrium was detected among loci using GENEPOP 3.1c (Raymond & Rousset 1995) and after Bonferroni correction. All loci showed significant deviation from Hardy–Weinberg (HW) expected genotype frequencies and a heterozygote deficit. According to Brookfield test (1996), discrepancy between observed and expected heterozygosities at loci COR15b, COR46 and COR58 could be explained by the occurrence of null alleles. However, previous studies based on allozymes performed on the same population (Abbiati et al. 1996), discrepancy between observed and expected heterozygosities at loci COR15b, COR46 and COR58 could be explained by the occurrence of null alleles. However, previous studies based on allozymes performed on the same population (Abbiati et al. 1996) suggested that within a single collection specimens belonging to different gene pools may occur (Wahlund effect), explaining the high number of HW equilibrium departures.

Described microsatellite markers are currently used in analysing patterns of genetic structuring and gene flow among western Mediterranean red coral populations over a wide range of spatial scales, from few metres to thousands kilometres. These studies could provide a relevant input for management and conservation of the species.

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**Table 1** Details of seven microsatellite loci for *Corallium rubrum*

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat sequence</th>
<th>Primer sequence</th>
<th>MgCl₂ (mM)</th>
<th>Tₐ (°C)</th>
<th>Cycles</th>
<th>Allele size range (bp)</th>
<th>No. of alleles</th>
<th>n</th>
<th>Hₑ/Hₒ</th>
<th>GenBank Accession no.</th>
</tr>
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<tbody>
<tr>
<td>COR9</td>
<td>(CA)₁₃</td>
<td>F: HEX-TGCTGATGCCTTTAAGTCTG R: AGCGATCATCCTTCTGCTT</td>
<td>1.5</td>
<td>55</td>
<td>30</td>
<td>175–219</td>
<td>16</td>
<td>50</td>
<td>0.89/0.21***</td>
<td>AY726758</td>
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<td>COR15</td>
<td>(AC)₇</td>
<td>F: TAMRA-CTTCCACACCTACACAAGC R: GCAGATACCTGCTCACG</td>
<td>1.5</td>
<td>57</td>
<td>35</td>
<td>222–258</td>
<td>5</td>
<td>50</td>
<td>0.67/0.68***</td>
<td>AY726759</td>
</tr>
<tr>
<td>COR15b</td>
<td>(CAGA)₃₀</td>
<td>F: TAMRA-CTTCCACACCTACACAAGC R: GCAGATACCTGCTCACG</td>
<td>1.5</td>
<td>57</td>
<td>35</td>
<td>346–382</td>
<td>10</td>
<td>50</td>
<td>0.86/0.24***</td>
<td>AY726763</td>
</tr>
<tr>
<td>COR16</td>
<td>(CAA)₁₉</td>
<td>F: HEX-TGCTGACAGCGATACAAGA R: CATGAAACCGCAACAAGT</td>
<td>1.5</td>
<td>54</td>
<td>30</td>
<td>256–536</td>
<td>26</td>
<td>50</td>
<td>0.94/0.27***</td>
<td>AY726760</td>
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<tr>
<td>COR46</td>
<td>(GTT)₁₅</td>
<td>F: HEX-TGATCTCTCACGCTGTT R: TGATCAGGAACCTTTGGCAGT</td>
<td>1.5</td>
<td>56</td>
<td>28</td>
<td>182–220</td>
<td>10</td>
<td>50</td>
<td>0.78/0.18***</td>
<td>AY726761</td>
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<tr>
<td>COR48</td>
<td>(GTT)₂₀</td>
<td>F: 6FAM-CTGCTCACTGCTGTT R: GCAGTCAAGGAGCATCCAG</td>
<td>1</td>
<td>58</td>
<td>28</td>
<td>178–202</td>
<td>8</td>
<td>50</td>
<td>0.73/0.27***</td>
<td>AY726762</td>
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<tr>
<td>CORS5</td>
<td>(TGG)₂₂</td>
<td>F: TAMRA-GGACCCTCTATAATTTGCGTTA R: GGTAGCTTTGAGTTTTGA</td>
<td>2.5</td>
<td>57</td>
<td>30</td>
<td>189–297</td>
<td>19</td>
<td>50</td>
<td>0.89/0.50***</td>
<td>AY726764</td>
</tr>
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</table>

Tₐ: Annealing temperature; n, number of individual analysed; Hₒ, observed heterozygosity; Hₑ, expected heterozygosity. ***p < 0.001.
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References


