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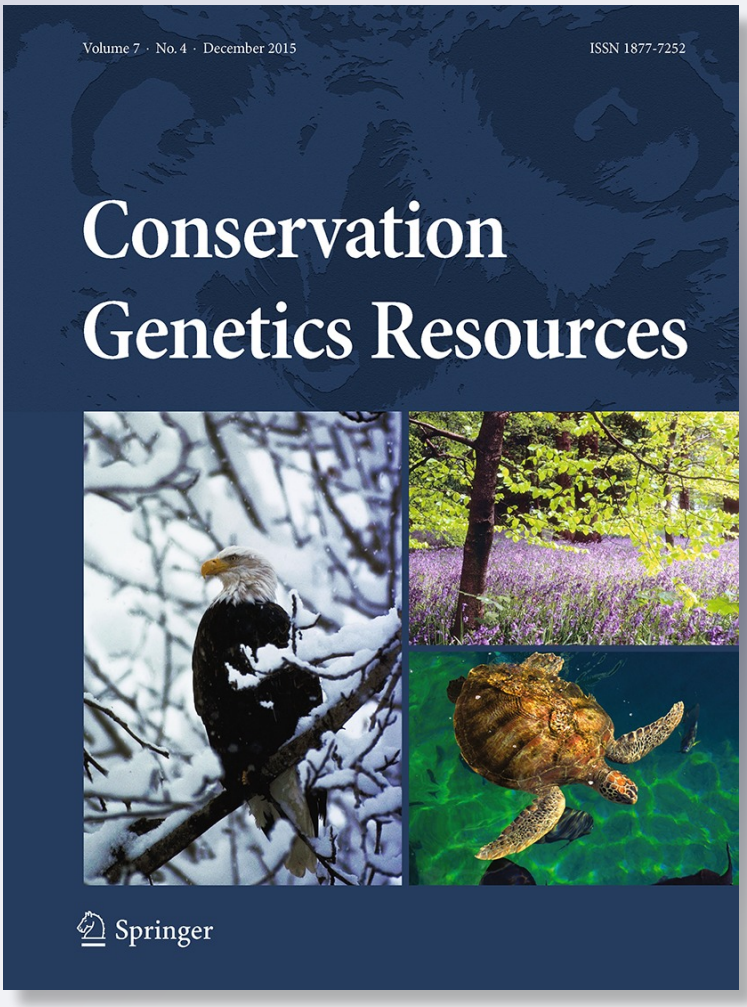

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MICROSATELLITE LETTERS

Development of 10 microsatellite markers for the Atlanto-Mediterranean sponge *Petrosia ficiformis*

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Abstract Due to anthropogenic disturbances, the common sponge *Petrosia ficiformis* has suffered from severe disease outbreaks coincidental with episodic raises in seawater temperature. Here, we report on the optimization of ten microsatellites that can be used to estimate population connectivity and structure. This information will be critical for further conservation efforts across *P. ficiformis'* Atlanto-Mediterranean distribution. Microsatellites were isolated by genomic pyrosequencing and tested in two populations from the Mediterranean and Eastern Atlantic, each with 27 and 16 individuals, respectively. The allele number per locus ranged from 4 to 12, observed heterozygosity from 0.399 to 0.686, and expected heterozygosity from 0.421 to 0.715. No linkage disequilibrium between pairs of loci was detected.

Keywords Porifera · Conservation genetics · Pyrosequencing · Disease outbreak

Sergi Taboada and Ana Riesgo have equally contributed to this work.

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Introduction

Sponges are abundant organisms in marine benthic environments that play crucial ecological roles in most habitats where they occur (Bell 2008). As sessile filter-feeders that rely on their larvae for dispersion, sponges are particularly susceptible to anthropogenic environmental disturbances. In the Mediterranean, reports of disease outbreaks and mass mortality events of some sponge populations have been increasingly prevalent in recent years; in particular, the common sublittoral Atlanto-Mediterranean sponge *Petrosia ficiformis* is reported to suffer of partial tissue necrosis, presumably after episodes of abnormally high seawater temperatures (Cerrano et al. 2001).

Here we report on the isolation and amplification of ten microsatellite markers for further estimation of connectivity and structure among populations of *P. ficiformis* to assess its degree of vulnerability across its Atlanto-Mediterranenan distribution.

Materials and methods

P. ficiformis was collected by SCUBA-diving in Blanes, NW Mediterranean (41°40′20.52″N 2°48′12.87″E; 15 m), preserved in absolute EtOH and stored at −20° C. Tissue was dissociated prior to DNA extraction and bacterial symbionts removed by sequential centrifugation (Freeman et al. 2013). Genomic DNA was extracted using DNeasy Tissue and Blood extraction kit (QIAGEN) to a final concentration of 2 µg. Pyrosequencing was performed on a Roche Life Science 454 GS-FLX System at the Scientific and Technological Centers, University of Barcelona (CCiT-UB). Resulting 394,841 sequences were searched for microsatellites with at least eight repeats and enough

Table 1 *T* temperature, *N* sample size, *Na* number of alleles per locus, H_e expected heterozygosity, H_O observed heterozygosity, F_{IS} inbreeding coefficient, *G–W* Garza–Williamson index

Optimization details					Blanes (Mediterranean)					São Miguel Island (Atlantic)				
Locus	Fluor.	Repeat motif	T	Size range	N	Na	Ho/He	F_{IS}	G–W	N	Na	Ho/He	F_{IS}	G–W
1PETRO	FAM –	(TA)*14	56–59	232–292	24	9	**0.583/0.720**	0.189**	0.113	16	3	0.625/0.486	−0.285	0.454
4PETRO	FAM –	(AG)*12	52–59	429–532	27	7	**0.630/0.636**	0.010***	0.067	14	2	**0.143/0.408**	0.650*	0.428
7PETRO	FAM –	(ATG)*14	58–60	188–240	26	11	0.846/0.734	−0.153	0.207	14	3	**0.929/0.589**	−0.576*	0.076
11PETRO	FAM –	(ATT)*13	56–60	101–141	27	10	**0.630/0.761**	0.172	0.135	15	3	0.333/0.384	0.133	0.23
15PETRO	VIC –	(ACCT)*6	55–65	385–485	23	7	**0.609/0.713**	0.146*	0.269	7	3	0.286/0.357	0.200	0.032
17PETRO	FAM –	(AC)*5GTA(AC)*13	52–58	371–390	27	10	**0.667/0.802**	0.169**	0.454	11	5	**0.091/0.731**	0.876***	0.172
18PETRO	FAM –	(AG)*12	58–60	414–432	26	4	0.577/0.550	−0.050	0.21	14	2	0.143/0.133	−0.077	0.285
19PETRO	FAM –	(TG)*12	56–59	168–204	27	5	**0.926/0.594**	−0.559*	0.135	16	3	1.000/0.529	−0.889**	0.23
25PETRO	PET –	(TAA)*17	58	190–233	23	9	**0.478/0.790**	0.395***	0.191	16	5	**0.438/0.596**	0.266*	0.076
30PETRO	FAM –	(TACA)*16	58–60	190–251	24	12	**0.917/0.856**	−0.071***	0.088	16	1	0.000/0.000	N/A	0.666
Total							0.686/0.715	0.025	0.187 ± 0.106			0.399/0.421	0.033	0.286 ± 0.188

* ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$). Significant deviation from HWE after Narum correction ($P < 0.05$). Locus showing significant deviation from expected heterozygosity in bold letters. *N/A* not applicable

priming regions with Phobos (http://www.rub.de/spezzoo/cm/cm_phobos.htm). A total of 18,501 sequences contained microsatellites (23.2 % dinucleotide, 2.0 % trinucleotide, 71.3 % tetranucleotide, 3.5 % pentanucleotide). Thirty primer sets were designed with the software PRIMER 3 for 14 di-, 12 tri-, and 4 tetra-nucleotide loci.

Amplification success and microsatellite polymorphism were tested in 2 populations: 26 individuals from a population of the NW Mediterranean (Blanes, BL) collected in 2006–2012; and 17 individuals from a population of the Eastern Atlantic [São Miguel Island (SM)] collected in 2006 (see Xavier 2009). Total DNA was extracted and amplified using the REDExtract-N-Amp Tissue PCR Kit (Sigma Aldrich), labeling forward primers with a fluorescent dye (Table 1), and with the following PCR conditions: 94 °C/3 min-(94 °C/30 s; 52–65 °C/35 s; 72 °C/20 s) × 30–35 cycles; 72 °C/5 min. Amplification products were analyzed on an Applied Biosystems 3730xl Genetic Analyzer at the CCiT-UB and alleles checked using the internal size standard GeneScan 600LIZ and PEAKSCANNER v1.0 (Applied Biosystems).

Linkage disequilibrium, number of alleles per loci and population, observed heterozygosity (Ho), expected heterozygosity (He), and Tajima's D index were calculated with GenAlEx (http://biology.anu.edu.au/GenAlEx/Welcome.html) and ARLEQUIN vs 3.5.1.2 (http://cmpg.unibe.ch/software/arlequin35/). Genetic diversity [Garza–Williamson (G–W) index], inbreeding coefficients (F_{IS}), and departure from Hardy–Weinberg Equilibrium (HWE) were calculated using ARLEQUIN and GenPop (http://genepop.curtin.edu.au/). Narum ($P < 0.05$) corrections of the P values for multiple tests were applied.

Results and discussion

Ten polymorphic microsatellites were optimized for *P. ficiformis* (Suppl. Mat.) with no evidence of linkage disequilibrium across all pairwise comparisons. Locus 30PETRO was monomorphic for SM but highly polymorphic in BL. Failed amplifications due to null alleles were detected in locus 15PETRO affecting 13 individuals of both populations. Overall populations were in HWE after Narum corrections, but seven markers for BL and five for SM showed HW disequilibrium, potentially inbreeding due to the low larval dispersal (Maldonado and Riesgo 2009). Eight loci presented heterozygosity deficit. Average gene diversity over ten loci using Tajima index was 0.676 ± 0.380 in BL, and 0.415 ± 0.279 in SM (Table 1). Genetic G–W index indicated that all markers showed bottleneck events in both populations (Table 1), although more populations should be sequenced for further confirmation. Further genotyping of these markers in additional *P. ficiformis* populations will shed light on the spatial distribution of genetic diversity and help assessing the recovery potential of diseased populations, which will be crucial for developing efficient management strategies for the conservation of *P. ficiformis*.

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