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MICROSATELLITE LETTERS

Isolation and characterisation of twelve polymorphic microsatellite markers for *Xestospongia* spp. and their use for confirming species identity

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Abstract Barrel sponges are one of the largest and most conspicuous members of the coral reef fauna across the Indo-Pacific that are under threat from habitat degradation. Twelve novel microsatellite markers were developed for *Xestospongia testudinaria* from 454 sequence data and scored across 47 individual barrel sponges collected from the Sampela reef in the Wakatobi Marine National Park, Indonesia. All loci except one was polymorphic with the number of alleles per locus ranging from 5 to 24. Observed heterozygosity ranged from 0.08 to 0.77 and F_{IS} values ranged from −1.61 to 0.77, with the majority of loci being in Hardy–Weinberg equilibrium (HWE). We then tested our markers on 28 likely *Xestospongia bergquistia* specimens from the same reef. Observed heterozygosity ranged from 0.23 to 0.593 and F_{IS} values ranged from −0.078 to 0.373; all but two loci were in HWE. Furthermore, we confirmed the differentiation of these two species by Principle Coordinate Analysis and Analysis of Molecular Variance. These markers will be useful for a range of future fine-scale population genetics studies for these two important reef species.

Keywords Xestospongia · Sponge · Coral reef · Microsatellite · Barrel sponge · Indo-Pacific

Barrel sponges (*Xestospongia* spp.) represent some of the largest and most conspicuous organisms on coral reefs, but despite their size and likely importance to reef functioning through their filtering ability, little is known about their ecology. Here we describe the development of twelve novel microsatellite markers for specimens morphologically consistent with the original description of *Xestospongia testudinaria* (Fromont 1991) and assess the utility of these markers in likely specimens of the related species *Xestospongia bergquistia*. Furthermore, we conduct a preliminary analysis to determine if these markers support the division of these two species.

Genomic DNA was extracted from preserved *X. testudinaria* tissue samples (taken from Sampela 5°29′03″S, 123°45′11E) and primers developed from a 1/8 run on a Roche 454 GS Junior platform. Primers were screened using a GeneAmp 2700 (Life Technologies) thermocycler, and twelve loci were found to amplify consistently (see ESM Table 1) and were arranged into two multiplex PCR panels. For full details of the methods see the ESM.

Basic intra-population diversity indices (ESM Table 1) were calculated for 47 *X. testudinaria* collected and genotyped from a 250 m section of the Sampela reef (DNA extracted as above). One locus *X. testudinaria* was monomorphic (Xesto1). MICROCHECKER 2.2.3 found evidence

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for the presence of null alleles at two loci (Xesto6 and Xesto9). The selection detection workbench LOSITAN (Antao et al. 2008) identified Xesto10 as being under selection. FSTAT 2.9.3 was used to determine inbreeding coefficients for each locus, which ranged from −1.61 to 0.77 and were significantly different to zero for five loci. Observed heterozygosity was calculated by Arlequin 3.5.1.2 (Excoffier et al. 2005) and values ranged from 0.08 to 0.77 (ESM Table 2). GENEPOP 4.1.0 (Raymond and Rousset 1993) detected no evidence of significant linkage disequilibrium among any pairs of loci, but there were some significant deviations from Hardy–Weinberg equilibrium (HWE) following standard Bonferroni corrections (6 loci); Markov-chain parameters were 10,000 dememorization steps, 1,000 batches and 10,000 iterations per batch. FreeNA (Chapuis and Estoup 2007) found low levels (<8 %) of null allele frequencies across all loci.

We then genotyped 28 likely *X. bergquistia* specimens from the same 250 m of Sampela reef for the twelve loci (same PCR conditions). Inbreeding coefficients ranged from −0.078 to 0.373 and were only significantly different to zero for two loci (ESM Table 2). Observed heterozygosity ranged from 0.23 to 0.593. We detected no evidence of significant linkage disequilibrium among any pairs of loci, but five loci showed a significant deviation from HWE.

We then visualised the genetic relationships between our samples (excluding Xesto6, Xesto9 and Xesto10 due to null alleles and evidence for selection) to confirm the consistency of the taxonomic division between *X. testudinaria* and our likely *X. bergquistia* samples. A Principle Coordinate Analysis (PCoA) with GENEALEX 6.3 using the pairwise matrices of Nei's genetic distance found evidence for two genetic groups consistent with the species delineation (ESM Figure 1). A hierarchal analysis of molecular variance (AMOVA, n = 10,000 permutations) was conducted to determine the proportion of genetic variation that could be attributed to differences between the two groups using GENEALEX v 6.3. AMOVA showed that 51 % of the variation ($P < 0.001$) could be attributed to among group differences (ESM Table 3), with a PhiST value of 0.51 ($P = 0.01$). Based on these results, we propose that our samples are consistent with the division between *X. testudinaria* and *X. bergquistia*.

Our microsatellite markers are only the second panel developed for tropical sponges and nine of our markers will support a range of fine-scale connectivity studies of barrel sponges.

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