

Isolation and characterization of 22 microsatellite loci from two coral species: Acropora muricata (Linnaeus, 1758) (Scleractinia, Acroporidae) and Porites lutea Milne-Edwards & Haime, 1851 (Scleractinia, Poritidae)

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Pauline Gélin, Clément Rougeux, Vincent Mehn, Mireille M. M. Guillaume, J. Henrich Bruggemann, et al.. Isolation and characterization of 22 microsatellite loci from two coral species: Acropora muricata (Linnaeus, 1758) (Scleractinia, Acroporidae) and Porites lutea Milne-Edwards & Haime, 1851 (Scleractinia, Poritidae): Microsatellite records. Conservation Genetics Resources, 2015, 7 (4), pp.917-944. 10.1007/s12686-015-0493-8 . hal-01254248

HAL Id: hal-01254248 https://hal.science/hal-01254248

Submitted on 4 May 2016

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Isolation and characterization of 22 microsatellite loci from two coral species: *Acropora muricata* (Linnaeus, 1758) (Scleractinia, Acroporidae) and *Porites lutea* Milne-Edwards & Haime, 1851 (Scleractinia, Poritidae)

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Total genomic DNA of eight colonies for each of the two species, *Acropora muricata* and *Porites lutea*, sampled on the west coast of Reunion Island, was isolated using DNeasy Blood & Tissue kit (Qiagen[™]) following the manufacturer's instructions and sent to GenoScreen, Lille, France (www.genoscreen.fr). We did not perform any separation of coral and zooxanthellae tissues, thus we extracted both DNA simultaneously; markers specificity was checked *a posteriori*. One µg was used for the development of microsatellite libraries through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries as described in Malausa et al. (2011). A total of 6840 and 5824 sequences containing microsatellite motifs were identified using the software QDD (Meglecz et al. 2010) and 532 and 529 primer pairs recognized for *A. muricata* and *P. lutea*, respectively. For each of the two species, 96 primer pairs were selected for amplification test were performed on 4 individuals and revealed on agarose gel (2%). The 52 and 40 primer pairs amplified for *A. muricata* and *P. lutea* respectively, were tested for polymorphism on 8 individuals for each species on an ABI 3730 XL (Gentyane Plateforme, INRA,

Clermont-Ferrand, France). To assess their specificity (coral or zooxanthellae), the 35 remaining markers for *A. muricata* and the 25 ones for *P. lutea* were then tested on DNA extracted from zooxanthellae cultures isolated from the 8 colonies used to build the library. Finally, 11 coral-specific microsatellite loci were isolated from each of the two species (from Am01 to Am11 for *A. muricata* and from Pl01 to Pl11 for *P. lutea*).

Characterization of the new developed loci was performed by genotyping two populations from the west coast of Reunion Island: one in Saint-Leu (21°10'51.14"S, 55°17'10.34"E) for *Acropora muricata* (n = 46) and one in Etang-Salé (21°16'13.31"S, 55°19'56.48"E) for *Porites lutea* (n = 48). PCR amplifications were performed in 10 μ L containing 5 μ L of MasterMix 2x (Applied Biosystems), 2.5 μ L of ultra-pure water, 0.25 μ L of each primer (10 μ M) and 2 μ L of DNA (10 ng/ μ L). The thermocycling program was: 94°C for 5 min + 7 x (94°C for 30 s, 62°C [-1°C at each cycle] for 30 s, 72°C for 30 s) + 30 x (94°C for 30 s, 55°C for 30 s, 72°C for 30 s) + 72°C for 5 min.

Diversity indices, Hardy-Weinberg equilibrium and linkage disequilibrium were assessed using Arlequin v 3.5.1.2. No linkage disequilibrium was found after FDR corrections.

The development of these loci will be very useful in studying the genetic diversity and gene flow between the populations of the staghorn coral *A. muricata* and the massive coral *P. lutea*, both remaining poorly documented.

Acknowledgments

The authors want to thank Vianney Denis and Lionel Bigot for assistance in collecting samples and stimulating discussions and master students Gaëlle Perazio, Laura Benestan, Juliette Fremery and Christophe Demichelis for their help in this study. Acknowledgments also go to the Gentyane Plateforme team (INRA, Clermont-Ferrand, France) and Mayalen Zubia for assisting us in zooxanthellae culturing.

Accession Numbers

Primer sequences are deposited on GenBank with accession numbers from KP298675 to KP298685 for *Porites lutea* and from KP298686 to KP298696 for *Acropora muricata*

Electronic Supplementary Material

The microsatellite sequences are available as electronic supplementary material (ESM_1.pdf)

Compliance with Ethical Standards

Research was conducted with permission of the regional authorities of marine affairs (decisions 53/DMSOI/2011 and 15-2013/DEAL/SEB/UBIO) and the marine park authorities of Reunion Island (RNMR).

The authors declare that they have no conflict of interest.

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