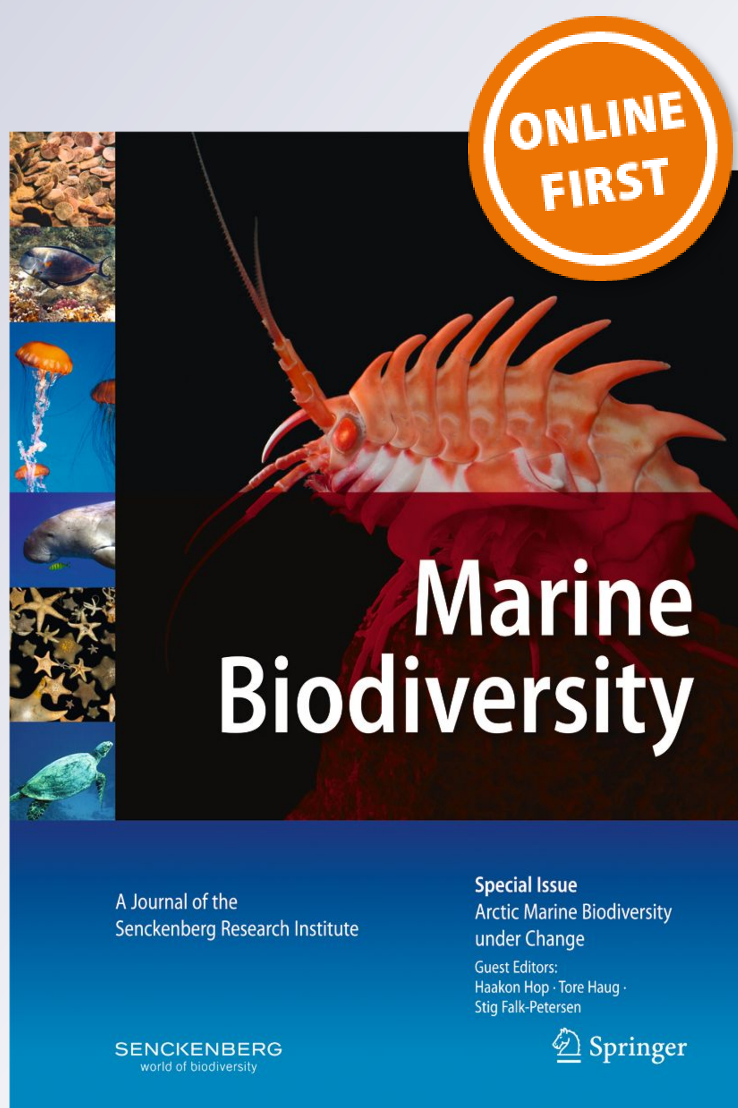
Novel polymorphic microsatellite markers for population genetics of the endangered Caribbean star coral, Montastraea faveolata

Sarah W. Davies, Muneeb Rahman, Eli Meyer, Elizabeth A. Green, Emmanual Buschiazzo, Mónica Medina & Mikhail V. Matz

Marine Biodiversity

ISSN 1867-1616

Mar Biodiv
DOI 10.1007/s12526-012-0133-4



Springer

Your article is protected by copyright and all rights are held exclusively by Senckenberg Gesellschaft für Naturforschung and Springer-Verlag Berlin Heidelberg. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your work, please use the accepted author's version for posting to your own website or your institution's repository. You may further deposit the accepted author's version on a funder's repository at a funder's request, provided it is not made publicly available until 12 months after publication.

Springer

Author's personal copy

Mar Biodiv
DOI 10.1007/s12526-012-0133-4

SENCKENBERG

SHORT COMMUNICATION

Novel polymorphic microsatellite markers for population genetics of the endangered Caribbean star coral, *Montastraea faveolata*

Sarah W. Davies · Muneeb Rahman · Eli Meyer · Elizabeth A. Green · Emmanual Buschiazzo · Mónica Medina · Mikhail V. Matz

Received: 29 May 2012 / Revised: 9 October 2012 / Accepted: 15 October 2012
© Senckenberg Gesellschaft für Naturforschung and Springer-Verlag Berlin Heidelberg 2012

Abstract *Montastraea faveolata* is a reef-building Caribbean coral that is currently listed as endangered across its range. A better understanding of the population genetic structure, genetic diversity and connectivity is needed to make sound conservation plans for this species. Here, we describe nine novel polymorphic microsatellite loci mined from currently available sequence data. Loci were screened in two widely separated populations (n=21 individuals per population) from the Flower Garden Banks (northern Gulf of Mexico) and Curaçao (Netherland Antilles, southern Caribbean). Allelic diversity ranged from 3 to 16 and observed heterozygosities ranged from 0.095 to 0.905. For all loci but one, the Hardy–Weinberg equilibrium hypothesis was not rejected within each population. These loci failed to amplify symbiont DNA isolated from pure *Symbiodinium* cultures, confirming their coral-specific origin. We also describe a multiplexing protocol for these markers reducing the costs and time required for future genetic studies. Finally, all markers were tested in the two sister species, *M. franksi* and *M. annularis,* and successful amplification and polymorphism were confirmed. The marker panel reported here, in combination with previously published markers for the same species complex, will facilitate coral reef connectivity research for this ecologically important genus, *Montastraea*, across the Caribbean.

Keywords *Montastraea annularis* species complex · Scleractinia · Coral reef · Multiplex · Simple sequence repeats · SSR · Conservation · Connectivity

S. W. Davies (✉) · M. Rahman · M. V. Matz
Integrative Biology Section, The University of Texas at Austin,
1 University Station C0930,
Austin, TX 78712, USA
e-mail: daviessw@gmail.com

E. Meyer
Department of Zoology, Oregon State University,
Cordley Hall 3029,
Corvallis, OR 97331, USA

E. A. Green · E. Buschiazzo · M. Medina
School of Natural Sciences, University of California, Merced,
5200 North Lake Road,
Merced, CA 95343, USA

Introduction

Coral reefs are the most biologically diverse marine ecosystems; however, they are at risk due to global climate change and other anthropogenic stressors (Hoegh-Guldberg et al. 2007). Coral population declines are particularly pronounced in the Caribbean, and the persistence of many species is becoming increasingly uncertain (Gardner et al. 2003; Mumby et al. 2007). Understanding genetic structure and gene flow among coral populations is essential to optimize the scale of conservation efforts and identify the reefs that serve as propagule sources or stepping-stones, and therefore are of the highest conservation priority (Cowen et al. 2006). Studying coral dispersal and gene flow is challenging, since the dispersive stage of a coral is microscopic and planktonic and therefore cannot be directly tracked (Jones et al. 2009). Consequently, patterns of coral connectivity for most coral species remain poorly understood, despite the importance of connectivity in shaping ecological processes and informing conservation decisions (Cowen et al. 2006). Even in the light of recent advances in next-generation sequencing techniques, microsatellites are still some of the most practical tools for

Published online: 31 October 2012

Author's personal copy

studying connectivity in marine populations on both evolutionary and ecological timescales (Ellegren 2004).

Caribbean corals in the genus *Montastraea* are slow growing (Gladfelter et al. 1978) and their decline across the Caribbean (Edmunds and Elahi 2007) is therefore particularly alarming, prompting these species to be red-listed as endangered (IUCN 2011). *Montastraea*-dominated reefs have consistently been associated with high species richness and species abundances, and are therefore considered the greatest contributor to Caribbean reef ecosystem function (Mumby et al. 2008). *Montastraea* reef health also has high socioeconomic value since it greatly affects fishing and tourism (Edwards et al. 2010). Previous research has examined *Montastraea* population structure in the Caribbean (e.g., Baums et al. 2010; Goodbody-Gringley et al. 2012; Foster et al. 2012); however, these studies have only used a limited number of microsatellite loci (seven, Severance et al. 2004). Due to inconsistent allele scoring, only 4–6 of these initially described loci have been adopted for subsequent use (Severance and Karl 2006; Foster et al. 2007, 2012; Baums et al. 2010; Levitan et al. 2011).

Population genetic studies of Caribbean *Montastraea* species would greatly benefit from additional microsatellite markers (Levitan et al. 2011). The aim of this study was to increase the informative loci available to better elucidate the genetic structure of *Montastraea faveolata* and demonstrate transferability to other closely related *Montastraea* species. Here, we describe nine novel microsatellites examined in 21 *M. faveolata* individuals from the Flower Garden Banks (FGB), of which all were polymorphic. We also describe a robust multiplexing protocol (eight loci) that reduces the costs and time spent genotyping, and this protocol was also verified in 21 individuals from Curaçao. This panel of nine loci will be a useful tool for population genetics, clone detection, and parentage analysis in *M. faveolata* and for the entire species complex.

Materials and methods

Isolation and characterization of microsatellite loci

Microsatellites were mined from publicly available *M. faveolata* EST data (n=33,206 sequences) in the SymBioSys database (http://sequoia.ucmerced.edu/SymBioSys/). ESTs were compared against NCBI's core UniVec database (ftp.ncbi.nih.gov/pub/UniVec/) using VecScreen (NCBI toolkit), and trimmed to exclude regions matching vector sequences (score ≥100). Next, trimmed ESTs were scanned for candidate microsatellite targets using RepeatMasker (www.repeatmasker.org). We first identified sequences bearing repeats ≥50 bp in length, with ≤20 % deviation from perfect repeat structure (n=544). Sequences lacking non-repetitive flanking regions for primer design were excluded, leaving n=309. To identify unique microsatellite loci, repetitive sequences were assembled with CAP3 (Huang and Madan 1999), identifying n=173 candidates. To further screen for redundancy, candidates were grouped by repeat type (e.g., ATG), and each group screened using BLASTN (bit-score ≥40), identifying 59 non-redundant loci. Flanking primers were designed for 53 of these using Primer3 (primer3.sourceforge.net) with GC content between 45 and 55 % and amplicon length between 100 and 500 bp. Twelve were tested in this study, nine of which are presented here. Two were found to be monomorphic in the FGB population and one primer set appeared to amplify a duplicated region (more than two alleles observed for all individuals tested).

Sample collection and DNA extraction

In August 2011, tissue samples were collected from 21 *M. faveolata* colonies from the East Flower Garden Banks, Gulf of Mexico. Colonies were at least 10 m apart to avoid sampling the same clonal individual. Tissue was stored in 96 % ethanol and DNA was later extracted by immersing tissue in digest buffer (100 mM NaCl, 10 mM Tris-Cl pH 8.0, 25 mM EDTA pH 8.0, 0.5 % SDS, 0.1 $\mathrm{mg\,ml^{-1}}$ Proteinase K, and 1 $\mathrm{\mu g\,ml^{-1}}$ RNaseA) for 1 h at 42 °C followed by a standard phenol–chloroform extraction protocol (Chomczynski and Sacchi 2006).

In September 2011, coral cores from 21 *M. faveolata* colonies spaced at least 5 m apart were collected from Curaçao. Coral cores were flash-frozen in a dry shipper and DNA was isolated using a modified MoBio Power Plant Bead protocol. (PowerPlant DNA Isolation Kit Cat#13200-100; MO BIO Laboratories, Carlsbad, CA). Approximately 50 mg of coral powder was homogenized with Power Plant Bead Solution and 10 U/μL of Ready-Lyse Lysozyme, and these samples were incubated for 10 min at room temperature on a rotator. Following the addition of 20 mg/mL of Proteinase K, samples were incubated for an additional 60–90 min at 65 °C. Two-sized zirconia/silica beads (400 mg each of 0.1 mm and 0.5 mm) were added and homogenized for 30 s using a Mini-BeadBeater-16 (BioSpec Product Cat#607) and purified as in the MoBio Power Plant Bead Protocol.

PCR amplification

Each 10 μl of polymerase chain reaction (PCR) mixture contained 10 ng of DNA template, 0.1 μM fluorescently-labeled forward primer, 0.1 μM reverse primer, 0.2 mM dNTP, 1 μl 10X *ExTaq* buffer, 0.025 U *ExTaq* Polymerase (Takara Biotechnology) and 0.0125 U *Pfu* Polymerase (Agilent Technologies). Amplification was performed using a DNA Engine Tetrad2 Thermal Cycler (Bio-Rad, Hercules, CA, USA). Cycling began at 94 °C for 5 min, followed by

Author's personal copy

Table 1 Summary of nine polymorphic *Montastraea faveolata* SSR markers, their transferability to *M. franksi* and *M. annularis*, and their multiplexing groups for 21 individuals from the Flower Garden Banks (FGB)

Locus (Repeat)	Primer Sequence[a] 5'-3'	Observed (bp)	*n*	n_a	Ho	He	Accession no.	P_{ID}	HWE	*M. franksi*[b]	*M. annularis*[b]
M_fav3[A] $(ATG)_{25}$	F: NED-cccagttctcaaatagcgga R: tacaagtcgagttccccacc	161–218	21	7	0.333	0.300	GW267284	5.4E-01	1.000	100	100
M_fav4[B] $(TTTG)_{17}$	F: FAM-attagggtcgaggtcaaggg R: caagcccgaataaagcata	365–417	21	11	0.381	0.735	GW254471	1.1E-01	0.008	100	80
M_fav5[A] $(CGA)_{17}$	F: FAM-aatgcatttccctgctatcg R: tgcacgaccctgtacaacat	340–394	21	16	0.905	0.887	GW257722	3.1E-02	0.917	80	80
M_fav6[B] $(CA)_{33}$	F: HEX-ttcgcacgcgtacttaacag R: caaggatggctaaggatgga	401–445	21	8	0.632	0.647	GW255333	4.8E-01	0.031	80	100
M_fav7[A] $(CAT)_{24}$	F: HEX-gccgaatccgtcttttgata R: gctgaggttgctcttgctgt	465–519	21	14	0.905	0.859	GW248775	3.4E-02	0.485	100	100
M_fav8[C] $(CAA)_{38}$	F: NED-gaccccagttcaactcctca R: tcctgtccctcttgtatgcc	289–355	21	12	0.524	0.838	GW250389	4.6E-02	0.163	100	40
M_fav9[B] $(CAAT)_{21}$	F: NED-attcgtgacaaaacgagggtc R: aattgaccgaaaagtgaccg	278–322	21	9	0.762	0.771	GW249713	5.3E-02	0.986	100	100
M_fav29 $(CAT)_{16}$	F: NED- agttagcgatgcagttggct R: tcgttgccattgtcagttgt	417–477	19	10	0.789	0.812	GW275092	6.1E-02	0.001*	80	40
M_fav30[C] $(TTTTG)_8$	F: NED- aagaaacgggccgatagagt R: cggtagcatattgccgtttt	227–247	21	4	0.286	0.389	GW274811	3.9E-01	0.500	100	100

n number of individuals, n_a number of alleles, *Ho* observed heterozygosity, *He* expected heterozygosity

HWE Hardy–Weinberg Equilibrium *P* values, P_{ID} is the probability of identity for each locus

A, *B*, *C* Multiplex primer groups A (testing also included FAM-labeled maMS8 from Severence et al. 2004), B and C

*Deviates significantly from Hardy–Weinberg Equilibrium (HWE) after Bonferroni correction (α=0.0055)

[a] *FAM*, *HEX* or *NED* at the 5' –end of the primer indicate FAM, HEX or NED-labeled fluorescent primer

[b] SSR transferability percentage out of five individuals tested

Author's personal copy

35 cycles of 94 °C for 40 s, 60 °C for 60 s, and 72 °C for 60 s and then a 10 min extension period at 72 °C. Amplicons were resolved on agarose gels to verify amplification and molecular weights were analyzed using the ABI 3130XL capillary sequencer with a ROX-labeled size standard. To confirm coral-specific origin of loci, all primer pairs were tested on two strains of *Symbiodinium* (B184 and D206) found in *Montastraea* species, with the *Symbiodinium*-specific internal transcribed spacer (ITS1) of the ribosomal RNA gene as a positive control, and no amplification was observed except in the ITS1 control.

Data analysis

Genotypes were called using GeneMarker 1.70 (SoftGenetics). Observed (H_o) and expected (H_e) heterozygosities, number of alleles (N_a), probability of identity for each locus (P_{ID}), and Hardy–Weinberg equilibrium (HWE) were calculated in GenAlEx version 6.4 (Peakall and Smouse 2006). GENEPOP v.1.2 (Raymond and Rousset 1995) was used to test for linkage disequilibrium (LD) between loci using 1,000 dememorizations with 100 batches (1,000 iterations per batch). Microchecker (Van Oosterhout et al. 2004) was used to detect null alleles within the sampling populations and allele scoring error rates were estimated by re-genotyping 10 individuals and calculating the proportion of variant calls divided by the total consensus alleles (Selkoe and Toonen 2006). Loci were checked for cross-amplification in samples of *M. franksi* (n=5) and *M. annularis* (n=5) also collected from the FGB.

Optimizing multiplex PCR

Microsatellite multiplexing was benchmarked by testing several loci in a multiplex PCR reaction and comparing the genotyping results with individual reactions. Overlap between amplicon sizes was minimized irrespective of the tag color to avoid interference of fluorescence in other channels. Only loci with amplicons at least 40 bp apart were assigned the same fluorescent tag to avoid allele call confusion. Ten nanograms of template were used in each 20-μl multiplex PCR reaction containing 0.1 μM fluorescently-labeled forward primer and 0.1 μM reverse primer for each locus in the group. All other components of the mixture were identical to the single reactions described above. To achieve representation of all loci, PCR cycling was modified by increasing the annealing time. PCR conditions began at 94 °C for 5 min, followed by 35 cycles of 94 °C for 40 s, 60 °C for 2 min, and 72 °C for 60 s and then a 10-min extension period at 72 °C. One previously described SSR locus was included in the multiplex protocol (FAM-labeled maMS8; Severance et al. 2004) for family A. One locus (Mfav_29) described in the present study was not included in the final multiplex groups as it deviated significantly from the Hardy–Weinberg equilibrium (HWE, Table I).

Results and discussion

Connectivity studies of Caribbean *Montastraea* that have inferred genetic structure from available microsatellite loci (Severence et al. 2004) have found little to no genetic structure throughout the Caribbean basin. Severence and Karl (2006) found *M. faveolata* populations to be well mixed across large geographical expanses (Puerto Rico, Mexico, Florida), and Baums et al. (2010) also demonstrated no genetic subdivision along the Florida reef tract and between Florida and Mexico for *M. faveolata*. To date, the most extensive analysis of *Montastraea* genetic structure across the Caribbean (*M. annularis* from 26 sites) has also demonstrated high levels of admixture throughout the basin with some evidence of structure between the East and West

Table 2 Summary of eight polymorphic *Montastraea faveolata* SSR markers and their multiplexing groups for 21 individuals from Curaçao

Locus (Repeat)	Observed (bp)	n	n_a	Ho	He	P_{ID}	HWE
M_fav3[A] $(ATG)_{25}$	145–218	21	6	0.238	0.261	5.5E-01	0.001*
M_fav4[B] $(TTTG)_{17}$	375–407	21	8	0.524	0.819	5.5E-02	0.154
M_fav5[A] $(CGA)_{17}$	337–397	21	15	0.762	0.871	2.8E-02	0.026
M_fav6[B] $(CA)_{33}$	489–425	21	5	0.333	0.658	1.7E-01	0.007
M_fav7[A] $(CAT)_{24}$	429–498	21	14	0.905	0.856	3.5E-01	1.000
M_fav8[C] $(CAA)_{38}$	299–341	21	13	0.762	0.900	1.8E-02	0.006
M_fav9[B] $(CAAT)_{21}$	260–300	21	9	0.762	0.771	6.0E-02	0.257
M_fav30[C] $(TTTTG)_{8}$	238–248	21	3	0.095	0.094	8.3E-01	0.997

n number of individuals, n_a number of alleles, *Ho* observed heterozygosity

He expected heterozygosity, *HWE* Hardy-Weinberg Equilibrium P-values P_{ID} is the probability of identity for each locus

A, *B*, *C* Multiplex primer groups A, B and C

*Deviates significantly from Hardy–Weinberg Equilibrium (HWE) after Bonferroni correction (α=0.006)

Author's personal copy

Caribbean basin (Foster et al. 2012). Overall, these studies have found little population differentiation between Caribbean locations; however, the small number of informative loci (n ≤7) may have constrained the ability to detect genetic structure between regions.

Of the nine novel polymorphic microsatellite loci for *Montastraea faveolata*, five are trinucleotide repeats, one dinucleotide, two tetranucleotide and one pentanuclotide. A total of 91 alleles (4–16 alleles per locus) were detected across these loci in the 21 colonies sampled from the FGB (Table 1). Observed (H_o) and expected (H_e) heterozygosities ranged from 0.286–0.905 and 0.300–0.887, respectively. One locus (M_fav29) deviated from HWE after Bonferroni correction (p=0.0055), while three loci (M_fav4, M_fav8, and M_fav30) showed large, but non-significant, differences between H_o and H_e. Probability of identities (P_{ID}) were low (≤1.1E−01) for all loci and significant LD was detected for one pair of loci: M_fav7 and M_fav8 (p<0.0001). Evidence for null alleles was present for three loci (Mfav_4, Mfav_5, and Mfav8), however error rates in allele calling were also low (<5 %) for all loci tested.

To ensure that these loci were useful markers across the species range, an additional 21 *M. faveolata* colonies from Curaçao were genotyped using the multiplexing protocol described above (eight loci). A total of 73 alleles (3–15 alleles per locus) were detected and all loci were found to be polymorphic for this population (Table 2). Observed (H_o) and expected (H_e) heterozygosities ranged from 0.095to 0.905 and 0.094 to 0.900, respectively. One locus (M_fav3) deviated from HWE after Bonferroni correction (p=0.006) and two loci (M_fav6 and M_fav8) demonstrated marginal significance. Overall, these data suggest that the loci described here will likely be useful for genetic studies across the range of *Montastraea faveolata.*

In the cross-species amplification analysis with *M. franksi* and *M. annularis* from the FGB, all but two loci (M_fav8 and M_fav29) were amplified in all specimens (n= 5) with ≥80 % success, indicating effective transferability to the *Montastraea* complex (Table 1). These cross-amplified loci were also capillary-sequenced and found to be polymorphic in both species.

Conclusion

The nine novel microsatellite markers described here more than doubles the number of markers available for the ecologically important *Montastraea* species complex in the Caribbean. Over the past three decades, populations of *Montastraea* corals have been decreasing rapidly across their entire Caribbean range (Gardner et al. 2003; Mumby et al. 2007). The availability of additional markers along with the streamlined multiplexing protocol will aid future conservation genetics research by improving the power of detecting genetic structure, recent migrants, parentage analysis, and clone identification of not only *M. faveolata* but also the *Montastraea* species complex throughout their entire distribution range.

Acknowledgments The study was supported by the National Science Foundation grant DEB-1054766 to M.V.M., startup grant from the University of Texas at Austin's Ecology, Evolution and Behavior graduate program to S.W.D., and NSF grants IOS 0644438 and IOS 0926906 to M.M. Ship time and fieldwork at the Flower Garden Banks was funded by the Flower Garden Banks National Marine Sanctuary (FGBNMS) under permit #FGBNMS-2009-005-A1. We also thank the reviewers for their thoughtful comments on the manuscript.

References

Baums IM, Johnson ME, Devlin-Durante MK, Miller MW (2010) Host population genetic structure and zooxanthellae diversity of two reef-building coral species along the Florida Reef Tract and wider Caribbean. Coral Reefs 29:835–842

Chomczynski P, Sacchi N (2006) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. Nat Protoc 1:581–585

Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. Science 311:522–527

Edmunds P, Elahi R (2007) The demographics of a 15-year decline in cover of the Caribbean reef coral *Montastraea annularis*. Ecol Monogr 77:3–18

Edwards HJ, Elliott IA, Pressey RL, Mumby PJ (2010) Incorporating ontogenetic dispersal, ecological processes and conservation zoning into reserve design. Biol Conserv 143:457–470

Ellegren H (2004) Microsatellites: simple sequences with complex evolution. Nat Rev Genet 5:435–445

Foster NL, Baums IB, Mumby PJ (2007) Sexual vs. asexual reproduction in an ecosystem engineer: the massive coral *Montastraea annularis*. J Anim Ecol 76:384–391

Foster NL, Paris CB, Kool JT, Baums IB, Stevens JR, Sanchez JA, Bastidas C, Agudelo C, Bush P, Day O, Ferrari R, Gonzalez P, Gore S, Guppy R, McCartney MA, McCoy C, Mendes J, Srinivasan A, Steiner S, Vermeij MJA, Weil E, Mumby PJ (2012) Connectivity of Caribbean coral populations: complementary insights from empirical and modelled gene flow. Mol Ecol 21:1143–1157

Gardner T, Cote I, Gill J, Watkinson A (2003) Long-term region-wide declines in Caribbean corals. Science 301:958–960

Gladfelter E, Monahan R, Gladfelter W (1978) Growth-Rates of 5 Reef-Building Corals in the Northeastern Caribbean. Bull Mar Sci 28:728–734

Goodbody-Gringley G, Woollacott RM, Giribet G (2012) Population structure and connectivity in the Atlantic scleractinian coral *Montastraea cavernosa* (Linnaeus, 1767). Mar Ecol 33:32–48

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742

Huang XQ, Madan A (1999) CAP3: A DNA sequence assembly program. Genome Res 9:868–877

IUCN (2011) IUCN Red List of Threatened Species. Version 2011.1. IUCN, Switzerland

Author's personal copy

Jones G, Almany G, Russ G, Sale P, Steneck R, van Oppen M, Willis B (2009) Larval retention and connectivity among populations of corals and reef fishes: history, advances and challenges. Coral Reefs 28:307–325

Levitan DR, Fogarty ND, Jara J, Lotterhos KE, Knowlton N (2011) Genetic, spatial, and temporal components of precise spawning synchrony in reef building corals of the *Montastraea annularis* species complex. Evolution 65:1254–1270

Mumby PJ, Hastings A, Edwards HJ (2007) Thresholds and the resilience of Caribbean coral reefs. Nature 450:98–101

Mumby PJ, Broad K, Brumbaugh DR, Dahlgren CP, Harborne AR, Hastings A, Holmes KE, Kappel CV, Micheli F, Sanchirico JN (2008) Coral reef habitats as surrogates of species, ecological functions and ecosystem services. Conserv Biol 22:941–951

Peakall R, Smouse P (2006) Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Raymond M, Rousset F (1995) Genepop (version 1.2), population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Selkoe KA, Toonen R (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol Lett 9:615–629

Severance EG, Karl SA (2006) Contrasting population genetic structures of sympatric, mass-spawning Caribbean corals. Mar Biol 150:57–68

Severance EG, Szmant AM, Karl S (2004) Microsatellite loci isolated from the Caribbean coral, *Montastraea annularis*. Mol Ecol Notes 4:74–76

Van Oosterhout C, Hutchinson WF, Will D, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538