Isolation and characterisation of 16 microsatellite loci from a widespread tropical hydrozoan, Lytocarpia brevirostris (Busk, 1852)

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Abstract We isolated and characterized 16 polymorphic microsatellite loci for *Lytocarpia brevirostris* (Aglaopheniidae), a hydrozoan common in the tropical Indo-Pacific region. Four to 34 alleles per locus were detected at the Indo-Pacific scale. At the population level, observed (H_o) and expected (H_e) heterozygosities ranged across 0.100–0.625 and 0.097–0.597, respectively. Three markers showed significant deviation from Hardy–Weinberg equilibrium, all of them presenting null alleles. Linkage disequilibrium was detected in three pairs of loci among 120. These primers provide powerful tools for studying population genetic diversity and the implication of life cycle strategies on population differentiation in tropical hydrozoans. This will be valuable for the conservation of coral reefs biodiversity and the design of marine protected areas.

Data Accessibility Accessibility numbers in GenBank are indicated in Table 1.

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Text

Hydrozoans are one of the most diverse marine suspension feeders (Bouillon et al. 2006). Their various reproductive modes and life cycles are expected to affect population connectivity levels and diversification patterns. However, these aspects have been rarely explored, population genetics tools for these organisms being scarce. Here we describe the isolation of 16 microsatellite markers for *Lytocarpia brevirostris*, a typical and common tropical Aglaopheniidae, one of the most specious families in tropical reefs.

Total genomic DNA of eight individuals from Reunion Island was isolated using DNeasy Blood and Tissue kit (Qiagen^{TM}) following manufacturer's protocol and sent to GenoScreen, Lille, France (www.genoscreen.fr). One μg was used for the development of microsatellites libraries through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries as described in Malausa et al. (2011). A total of 22,708 reads were obtained, of which 4,196 contained microsatellite motifs. Obtained sequences were analysed with QDD (Meglécz et al. 2010). Finally, 16 microsatellite markers were selected and characterized for *L. brevirostris* (criteria: PCR product >100 bp and number of repeats ≥6).

Genotyping of these 16 markers was conducted on 42 individuals from a Reunion Island population and on four populations (n = 170) from Juan de Nova Island (Eparses Islands, Mozambique Channel) and one population (n = 63) from New Caledonia. Microsatellite loci developed in this study were directly fluorochrome labelled.

Table 1 Microsatellite loci developed for *Lytocarpia brevirostris* and their primers sequences (F: forward, R: reverse)

Name	Primer sequence (5′–3′)	GenBank accession number	Repeat motif	Size (bp)	N_a	N	H_O	H_E	F_{IS}	r
Lb01	F: TGTGTTTGTATGATGTTCAGTCCA	KM675771	$(GTTT)_5$	105–121	7	42	0.357	0.359	0.006	−0.007
	R: AAAGATGAAGAGTCCATAACATGAA								NS	No
Lb02	F: CAAGGTGAGTCCCAGATTTGA	KM675772	$(AC)_8$	133–166	21	41	0.341	0.538	0.369	0.162
	R: TCCAATGATGTTTCCCCATT								*	Yes
Lb03	F: GCAACTGTATTTGCATCCTTTG	KM675773	$(ACA)_8$	145–172	9	41	0.366	0.479	0.239	0.101
	R: ATGTAATAATCACAACTGAAAACAGA								NS	No
Lb04	F: AACAAGCGCAAAATAATTGTTAG	KM675774	$(TC)_7$	147–173	11	42	0.476	0.597	0.205	0.096
	R: AGTGAATGGGATACCGCAAG								NS	No
Lb05	F: TTGTTTTGTTGTAGTCTGTCCTG	KM675775	$(AG)_8$	203–243	16	41	0.293	0.256	−0.144	−0.157
	R: GTTCTGTTTCAGGCCTCAGC								NS	No
Lb06	F: TTGCTGTCTAAAGATAGGAACTTCG	KM675776	$(ACA)_6$	217–291	20	40	0.525	0.556	0.056 NS	0.013 No
	R: TGAAACCGTTTGATTGTAAGATG									
Lb07	F: ATCCACATCGGACAGTGAGTC	KM675777	$(TTG)_6$	254–272	7	40	0.625	0.564	−0.109	−0.070
	R: TTCAAGTACCAGTGCAACAGC								NS	No
Lb08	F: AACCAAGGAATATCGTCAACAGA	KM675778	$(GGA)_7$	291–333	15	41	0.512	0.583	0.123	0.059
	R: AAAACATTAAGCTACCAACGTTTTG								NS	No
Lb09	F: TCATTTGGTATGTAATGCTCTTTTG	KM675779	$(AAG)_8$	157–169	4	39	0.231	0.232	0.004	0.026
	R: CAGTTATTAATGAGAAGAGCGCAG								NS	No
Lb10	F: AAGACCCACATTCCGCTTTA	KM675780	$(GTT)_6$	154–187	20	41	0.244	0.259	0.059	0.013
	R: GGTGATTCTGGTTACAAAGCA								NS	No
Lb11	F: GCCGGTAAGGAGGAATAGGT	KM675781	$(AAG)_7$	176–188	7	40	0.425	0.367	−0.161	−0.100
	R: TTGAACATAAAAGGGACAAACAAA								NS	No
Lb12	F: TAACGGCATATGCGTCATTC	KM675782	$(CA)_6$	203–219	5	40	0.100	0.097	−0.030	−0.051
	R: TTCGCGTCTTAACAGGGTGT								NS	No
Lb13	F: TTGAAATTAATGCACTTAAGGAAA	KM675783	$(CAA)_6$	236–283	22	39	0.103	0.236	0.569	0.186
	R: CACTTTGTTATACTCGGGCG								***	Yes
Lb14	F: ACAGCATGTTTTGTTGAATGATTT	KM675784	$(AG)_6$	259–291	25	42	0.286	0.358	0.204	0.074
	R: TCCCATCATTTGTTCACTATACA								NS	No
Lb15	F: TTGAAGACAGCGTATGACGTTT	KM675785	$(TC)_7$	294–338	34	40	0.375	0.429	0.128	0.096
	R: AACACCCTAACCCATCACCA								NS	No
Lb16	F: GATCCTTCAACCAAGAATATCATTT	KM675786	$(AC)_7$	300–319	19	40	0.125	0.208	0.404	0.134
	R: GCTTGGCGGTAGCAAACTCT								**	Yes

Accession numbers in GenBank are indicated. Diversity indices are issued from Arlequin v3.5.1.2

N_a number of alleles per locus, N number of genotyped individuals, H_O observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient

Under the F_{IS} are indicated the significance of the P values for deviation to Hardy–Weinberg equilibrium, *NS* non significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Null allele frequencies (r) are issued from MicroChecker v2.2.3 (Van Oosterhout et al. 2004), and presence or absence is indicated below (yes or no). Annealing temperature $T_a = 55$ °C

Each amplification reaction was performed in a total volume of 10 µL: 5 µL of MasterMix Applied 2× (Applied Biosystems), 2.5 µL of demineralised water, 0.25 µL of each primer (10 µM) and 2 µL of genomic DNA (10 ng/µL). The thermocycling program was as follows: 94 °C for 5 min + 7 × (94 °C for 30 s, 62 °C [−1 °C at each cycle] for 30 s, 72 °C for 30 s) + 35 × (94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s) + 72 °C for 5 min. PCR products were genotyped using an ABI 3730 genetic analyzer (Applied Biosystems) and allelic sizes were determined using GeneMapper v4.0 (Applied Biosystems).

Allele size span and number of alleles per locus are indicated in Table 1; however population statistics were calculated on the Reunion Island population only. Diversity indices, Hardy–Weinberg equilibrium and linkage disequilibrium were assessed using Arlequin v3.5.1.2 (Excoffier

et al. 2005). All loci were polymorphic, the number of alleles for the tested population ranged from 2 to 9, observed heterozygosity ranged from 0.100 to 0.625 and expected heterozygosity from 0.097 to 0.597 (Table 1).

We performed tests of linkage disequilibrium between all pairs of loci using Genepop v4.2: 26 tests were significant ($\alpha = 0.05$) but only three remained significant after FDR correction for multiple testing (Lb03 $\times$ Lb07; Lb03 $\times$ Lb15; Lb02 $\times$ Lb08).

Three loci (Lb02, Lb13, Lb16) were found to deviate from Hardy–Weinberg equilibrium: all of them showed significant heterozygotes deficit (F_{IS} ranged from 0.369 to 0.569). This was probably due to the presence of null alleles as assessed using MicroChecker v2.2.3 (van Oosterhout et al. 2004; Table 1).

The development of these markers will be very useful in studying hydrozoan population genetics, which remain poorly documented. Moreover little is known on the existence of cryptic species and on the level of endemism in these organisms. Thus, assessing the genetic diversity of these species and studying their population structure is crucial in describing coral reefs biodiversity and their conservation.

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References

Bouillon J, Gravili C, Pagès F, Boero F (2006) An introduction to Hydrozoa. Mémoires du Muséum national d'Histoire naturelle (1936), vol 194. Muséum national d'Histoire naturelle, Paris, p 591

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Malausa T, Gilles A, Meglecz E, Blanquart H, Duthoy S, Costedoat C, Dubut V, Pech N, Castagnone-Sereno P, Delye C, Feau N, Frey P, Gauthier P, Guillemaud T, Hazard L, Le Corre V, Lung-Escarmant B, Male PJG, Ferreira S, Martin JF (2011) High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. Mol Ecol Res 11:638–644

Meglécz E, Costedoat C, Dubut V, Gilles A, Malausa T, Pech N, Martin JF (2010) QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. Bioinformatics 26:403–404

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538.