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TECHNICAL NOTE

Polymorphic microsatellite loci from Brazilian and Hooded slipper lobsters (*Scyllarides brasiliensis* and *S. deceptor*), and cross-amplification in other scyllarids

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Abstract We isolated and characterized the first polymorphic microsatellite for the Brazilian and Hooded slipper lobsters species (*Scyllarides brasiliensis* and *S. deceptor*). Thirteen polymorphic loci (2–31 alleles/locus, $H_o = 0.056–0.975$, $H_e = 0.155–0.958$), were characterized in *S. brasiliensis* (N = 40). Twelve polymorphic loci (3–22 alleles/locus, $H_o = 0.333–0.900$, $H_e = 0.337–0.940$), were characterized in *S. deceptor* (N = 30) from different localities on the Brazilian coast. These loci were also tested in four scyllarid lobsters, *Scyllarides aequinoctialis*, *S. delfosi*, *Scyllarus depressus* and *Parribacus antarcticus*.

Keywords SSR markers · Population structure · Scyllaridae

Slipper lobsters (Scyllaridae) were generally categorized as of minor economic importance in comparison to clawed (Nephropidae) or spiny lobsters (Palinuridae), and this has resulted in inadequate management regulations for slipper lobsters fisheries (Lavalli and Spanier 2007). However, in response to overfishing of spiny lobsters over the last 10 years, fishing pressure over slipper lobsters has increased (Groeneveld et al. 2006; Phillips and Melville-Smith 2006), leading to a significant decrease in their relative abundance where they have been the target of a local fishery (Duarte et al. 2010; Spanier and Lavalli 2007). Among all scyllarid lobsters from the Western Atlantic, the Brazilian and Hooded slipper lobsters (*Scyllarides brasiliensis* and *S. deceptor*) are the main species caught, with *S. brasiliensis* being captured mostly in northeast Brazil (Pernambuco and Alagoas States) and *S. deceptor* in south and southeast Brazil (Rio de Janeiro to Santa Catarina States) (Duarte et al. 2010; Santos and Freitas 2002). We present new highly polymorphic microsatellite loci to help population genetics studies that will be useful for their management and conservation.

Microsatellite loci for *S. brasiliensis* and *S. deceptor* were isolated from enriched genomic libraries (Bloor et al. 2001). Genomic DNA was obtained from muscle tissue by a salt extraction (Miller et al. 1988). For each species, a pool of high-quality genomic DNA (10 µg) was digested with *Sau*IIIA and ligated to phosphorylated double-stranded linkers. Fragments (400–1,000 bp) were hybridized with biotinylated $(CA)_{12}$ and $(CAA)_8$ probes, and isolated using streptavidin-coated magnetic beads. The DNA containing microsatellites was amplified by PCR with the forward linker oligo as a primer. Enriched fragments were cloned using pGEM-T vectors and One Shot TOP10 competent cells. The presence of microsatellite inserts in the recombinant clones was confirmed by double banded PCR products after amplification using the forward linker oligo and (nonbiotinylated) microsatellite oligos as primers. Seventy-six positive clones, for each species, were amplified using M13 universal primers and subsequently sequenced in both directions in an ABI3500 sequencer. Sequences were edited with *SeqMan* II 4.0 (DNAstar Inc.).

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Table 1 Microsatellite loci developed for *Scyllarides brasiliensis* and *S. deceptor*

Locus/GenBank	Repeat motif	Primer sequence (5′–3′)	*S. brasiliensis*		*S. deceptor*	
			T_a^a	$MgCl_2^b$	T_a^a	$MgCl_2^b$
Sbra05	$(AC)_{10}$	F: TGAATGGGTATCTGGCGTAA	60	2.5	60	2.5
KC893319		R: CTTGGGTGGTAGGTATGGCT				
Sbra06	$(AC)_{13}$	F: CATTGATAAAGGGCACACAT	59	3.0	54	2.5
KC893320		R: ATTGGGCAGGTGTGTATATG				
Sbra10	$(AC)_5$	F: CTCCACTCAACACACAACCAA	60	2.5	–	–
KC893321		R: GTGATTCCGAGGACTTGCAT				
Sbra11	$(TG)_7(AG)_{11}(TG)_{10}$	F: CCAGAACTAACGGCCTTCT	60	2.5	60	2.5
KC893322		R: ATGTAACGGTGGGAGGTAA				
Sbra13	$(TG)_5C(GT)_6$	F: ACTAGATTGGTGGGTCGCA	60	2.5	60	2.5
KC893323		R: TGATTGCAGAGCATGTAGGC				
Sdec02	$(CA)_9$	F: ATGTAACTCCGGGCAAGA	60	3.0	60	3.0
KC893324		R: CATCTTGGCTTAATTGACG				
Sdec03	$(GT)_{11}$	F: TAGACACGACTGGAGGATCTTG	–	–	60	2.5
KC893325		R: GTGTAAAACTCTCGCCCTGTAA				
Sdec06	$(CA)_{10}CG(CA)_8$	F: TGTCCAAACACTACACGCAT	–	–	60	2.5
KC893326		R: CTTCACATCCTTTCCGACAC				
Sdec07	$(AC)_{11}$	F: TGACATTCACACTTTCACCCA	60	2.5	60	2.5
KC893327		R: GCATGTTTGTTGCAGCTTGT				
Sdec08	$(CA)_{13}$	F: AGACACGCACACACCTACA	–	–	60	3.0
KC893328		R: GAAAGTACCTCTGACATGCG				
Sdec10	$(TG)_8$	F: GTGAGTGATTGTGTGAGTGTG	60	2.5	60	2.5
KC893329		R: CGATAGAGCTTCACGAATATG				
Sdec14	$(TG)_{20}$	F: TCACAGATAACACCATCTTGCC	56	3.0	60	2.5
KC893330		R: TGTATGACAGAAGCGTGAGGTT				
Sdec17	$(CAA)_4$	F: CACAACATCACCGAGACACTTA	60	2.5	60	2.5
KC893331		R: GCTACACTCTTGTTCCTTGTCG				
Sdec20	$(TG)_{11}$	F: CGCTCACCGTACATCTGGTA	56	2.5	60	3.0
KC893332		R: AATCCAAACACACACAGGCA				
Sdec21	$(TG)_{10}$	F: CAGCCTAAGGCAGGGTTAAA	60	2.5	60	2.5
KC893333		R: CGTTTATCTCGGGGTTCTTG				
Sdec23	$(GT)_{10}$	F: CACTATGCCAACCTTTCGGT	60[c]	3.0	58	3.0
KC893334		R: AACGCTGGTAGGTAGGCTGA				

[a] Annealing temperature of the PCR reaction in °C

[b] Concentration of $MgCl_2$ in mM

[c] Addition of the labeled M13 primer during the final cycles

Of the 76 clones sequenced for each species, 23 and 26 distinct microsatellite loci were identified for *S. brasiliensis* and *S. deceptor*, respectively. From those, 14 primer pairs for *S. brasiliensis* (Sbra) and 23 for *S. deceptor* (Sdec) were designed using *WebSat* (Martins et al. 2009). The tailed primer method was used (Schuelke 2000). All 37 primer pairs were tested in both species. PCR consisted of 1 U GoTaq, 0.2 mM of each dNTP, 2.5 mM or 3.0 mM $MgCl_2$, 5 µg BSA, 0.13 µM of forward tailed primer, 0.26 µM of labeled M13 primer (with 6-FAM, VIC, NED or PET), and 0.5 µM of reverse primer, in 15 µL reactions with approximately 30 ng of DNA template. Cycling conditions were: 94 °C, 5 min, 30× [93 °C, 45; T_a (between 54 and 64 °C), 45 s; 72 °C, 45 s], 8× [93 °C, 45 s; 53 °C, 45 s, 72 °C, 45 s], 72 °C, 30 min. Due to the presence of unspecific products in Sdec23 in *S. brasiliensis*, re-amplification of this locus was done interrupting the cycling for the addition of the labeled M13 primer during the final cycles (de Arruda et al. 2010). In total, sixteen primer pairs were optimized, of which thirteen primers amplified successfully for *S. brasiliensis* and fifteen for *S. deceptor* (Table 1).

Table 2 Characterization of microsatellite loci developed for *Scyllarides brasiliensis* and *S. deceptor* and cross-amplification in other scyllarids

Locus	*S. brasiliensis*						*S. deceptor*					
	N	N_a	Size range (bp)	H_o–H_e	P_{HWE}	Null freq.	N	N_a	Size range (bp)	H_o–H_e	P_{HWE}	Null freq.
Sbra05	40	6	222–236	0.725–0.739	0.320	0.017	20	1	212	–	–	–
Sbra06	38	13	127–157	0.553–0.878	0.001*	0.168	30	1	137	–	–	–
Sbra10	40	6	212–224	0.525–0.726	0.110	0.112	–	–	–	–	–	–
Sbra11	40	31	139–211	0.975–0.958	1.000	0	30	22	149–203	0.833–0.940	0.047	0.047
Sbra13	39	3	196–200	0.179–0.229	0.042	0.038	30	6	184–202	0.900–0.737	0.319	0
Sdec02	40	2	126–128	0.275–0.453	0.029	0.119	30	6	131–143	0.400–0.791	0.001*	0.213
Sdec03	–	–	–	–	–	–	30	7	150–164	0.733–0.765	0.115	0.011
Sdec06	–	–	–	–	–	–	30	10	191–221	0.833–0.868	0.629	0.011
Sdec07	40	19	162–206	0.625–0.917	0.001**	0.147	30	11	163–195	0.733–0.757	0.463	0.006
Sdec08	–	–	–	–	–	–	30	10	162–196	0.833–0.818	0.976	0
Sdec10	36	2	159–161	0.056–0.155	0.009	0.084	30	1	159	–	–	–
Sdec14	40	3	236–248	0.300–0.265	1.000	0	30	13	258–290	0.833–0.823	0.541	0
Sdec17	39	2	335–338	0.179–0.166	1.000	0	30	3	335–341	0.333–0.337	1.000	0
Sdec20	40	13	281–307	0.800–0.843	0.115	0.018	30	5	289–299	0.567–0.629	0.316	0.032
Sdec21	40	5	217–233	0.650–0.569	0.689	0	30	7	219–235	0.567–0.649	0.169	0.043
Sdec23	35	3	168–172	0.057–0.255	0.001**	0.155	30	5	158–174	0.833–0.692	0.186	0

Locus	*S. aequinoctialis*			*S. delfosi*			*Scyllarus depressus*			*Parribacus antarticus*		
	T_a	N_a	Size range (bp)	T_a	N_a	Size range (bp)	T_a	N_a	Size range (bp)	T_a	N_a	Size range (bp)
Sbra05	58	2	224–226	58	3	212–228	–	–	–	–	–	–
Sbra06	–	–	–	54	1	157	–	–	–	–	–	–
Sbra10	60	2	228–258	60	2	224–258	56	1	298	–	–	–
Sbra11	60	2	143–151	60	1	149	–	–	–	–	–	–
Sbra13	60	2	190–192	60	2	202–210	–	–	–	56	3	192–212
Sdec02	60	1	139	60	4	139–145	–	–	–	–	–	–
Sdec03	60	4	150–172	60	3	122–152	54	1	130	–	–	–
Sdec06	58	3	173–195	58	2	199–205	–	–	–	–	–	–
Sdec07	60	1	175	60	1	175	56	4	169–207	56	2	173–187
Sdec08	–	–	–	–	–	–	–	–	–	–	–	–
Sdec10	60	2	157–173	60	2	161–163	–	–	–	–	–	–
Sdec14	60	4	244–282	60	3	248–282	–	–	–	–	–	–
Sdec17	60	3	343–357	60	1	343	50	1	337	50	1	349
Sdec20	–	–	–	–	–	–	–	–	–	–	–	–
Sdec21	54	2	207–225	60	1	235	–	–	–	50	2	271–353
Sdec23	60	2	162–164	60	2	174–176	50	1	194	50	1	128

N, number of genotyped individuals; N_a, number of alleles observed; *size range (bp)*, size range of alleles in base pairs; H_o, observed heterozygosity; H_e, expected heterozygosity; P_{HWE}, *P* value of the HWE test; *null freq*, null allele frequency; T_a, annealing temperature of the PCR reaction in °C. Amplification failure is indicated by a minus

* Locus is not in HWE ($P < 0.05$). ** These heterozygote deficiencies were likely due to Wahlund effect, see text for details

Polymorphism levels were evaluated in *S. brasiliensis* (Bahia: N = 20; Espírito Santo: N = 20) and *S. deceptor* (Rio de Janeiro: N = 20; Santa Catarina: N = 10). PCR products were pooled with GS500-LIZ and separated in an ABI3500 sequencer. Allele size calling was performed with the program *GeneMarker* 2.2.0 (SoftGenetics), and the allelic binning was done with *Autobin* 0.9 (Salin 2010).

All loci were polymorphic for *S. brasiliensis*, and twelve were polymorphic for *S. deceptor*. (Table 2). Summary statistics were calculated using the program *Genepop* 4.0 (Raymond and Rousset 1995). No linkage disequilibrium was detected between any loci pair in either species. Significant departures ($P < 0.05$ after sequential Bonferroni correction—Rice 1989) from Hardy–Weinberg equilibrium

were found in three loci (Sbra06, Sdec07 and Sdec23) of *S. brasiliensis* and one locus (Sdec02) of *S. deceptor*. To verify if the deviations were due to Wahlund effect, deviations from expected HWE were tested separately for each locality. With this approach, only the loci Sbra06 in *S. brasiliensis* and Sdec02 in *S. deceptor* continued to show a clear heterozygote deficiency. The deficiencies were possibly caused by the presence of null alleles, whose estimated frequencies, using *Micro-Checker* 2.2.3 (van Oosterhout et al. 2004), were higher than 11 %. No evidence of scoring errors due to stuttering or large-allele dropout was found.

Cross-amplification was tested in four scyllarid lobsters, *Scyllarides aequinoctialis*, *S. delfosi*, *Scyllarus depressus* and *Parribacus antarcticus* (Table 2). Two individuals of each species were analyzed using a Qiaxcel System (Qiagen) with high resolution capillaries. Cross-amplification in species from different genera was very poor, with only five loci amplifying in *S. depressus* and *P. antarcticus*. Contrastingly, amplification in congeneric species was very efficient, with 13 loci amplifying in *Scyllarides aequinoctialis* and 14 loci in *S. delfosi*.

The microsatellite markers developed have wide applicability in studies on *Scyllarides* lobsters population structure and may provide valuable information for effective management and monitoring of these species.

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