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PERMANENT GENETIC RESOURCES

Isolation and characterization of 13 polymorphic nuclear microsatellite primers for the widespread Indo-Pacific three-spot damselfish, *Dascyllus trimaculatus*, and closely related *D. auripinnis*

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Abstract

A set of 13 simple sequence repeat markers was developed from *D. trimaculatus* genomic DNA, tested for *D. auripinnis* and characterized using 40 individuals per species. All the loci were polymorphic with a number of alleles ranging from three to 30. Observed heterozygosities varied from 0.23 to 0.89 for *D. trimaculatus* and from 0.11 to 0.85 for *D. auripinnis*. Early results show that these will be powerful markers for the study of ecological and evolutionary mechanism in this coral reef fish species complex.

Keywords: *Dascyllus auripinnis*, *Dascyllus trimaculatus*, microsatellites, speciation

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The damselfish genus *Dascyllus* comprises 10 species divided in three complexes (Godwin 1995; Bernardi & Crane 1999; McCafferty *et al*. 2002). One of them, the *trimaculatus* complex (Bernardi *et al*. 2002), comprises four described species, namely: *D. trimaculatus*, *D. albisella*, *D. strasburgi*, and the recently described *D. auripinnis* (Randall & Randall 2001). In this study, we isolated 13 microsatellites from *D. trimaculatus*, and tested cross-amplification in the closely related *D. auripinnis*. Both species are reef-associated and occur in parapatry in the central Pacific (Randall & Randall 2001). *D. trimaculatus* is widely distributed from the coast of Eastern Africa and Red Sea to the Central Pacific, whereas *D. auripinnis* is restricted to the Line and Phoenix islands. Nuclear microsatellite markers are powerful genetic markers because of their high level of variability (Zane *et al*. 2002). In this note, we describe 13 highly polymorphic microsatellite loci that cross-amplified *D. trimaculatus* and *D. auripinnis*, thus providing valuable tools to address questions of population structure, ecological inferences, and connectivity.

Fin tissues were collected from fish sampled by hand net or spear, and were stored in 90% ethanol. Genomic libraries enriched for microsatellite motifs were constructed by Genetic Identification Services (GIS, http://www.genetic-id-services.com). Libraries were built using a sample containing100 μg of genomic DNA extracted from muscle tissue from one individual *D. trimaculatus* following a standard phenol–chloroform procedure (Sambrook *et al*. 1989). Libraries were enriched for CA, CATC, TACA, and TAGA motifs. GIS sequenced 70 microsatellite-containing clones using universal M-13 primers.

We tested 13 of these microsatellites, which were determined to have flanking sequences of length sufficient for primer design using Designer PCR version 1.03 (Research Genetics, Inc.). The reaction volumes for the polymerase chain reaction (PCR) was 13 μL containing: 12.25 μL of 1.1 PCR Mastermix (Thermo Scientific), 0.625 μL of both 20 mM primers forward and reverse and approximately 2 ng of DNA template. Using a fluorescently labelled forward primer and an unlabelled reverse primer (Table 1),

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Table 1 Characterization of microsatellite loci isolated from *Dascyllus trimaculatus*. Columns correspond to: microsatellite name (Locus), (F) forward and (R) reverse primer sequence, optimal annealing temperature (T_a), repeat motif (Repeat), and amplification size of original clone (Amp.). The next two sets of three columns pertain to *D. trimaculatus* and *D. auripinnis*, respectively. They correspond to: number of alleles per locus (N_a), amplifications range (Range size), and (H_O) observed and (H_E) expected heterozygosities. Asterisks (*) indicate loci departing from Hardy–Weinberg equilibrium. GenBank Accession nos are EU781542 to EU781554.

					D. trimaculatus			*D. auripinnis*		
Locus	Primer (5′– 3′)	T_a (°C)	Repeat	Amp.	N_a	Range size (bp)	H_O (H_E)	N_a	Range size (bp)	H_O (H_E)
DTR_A7	F: AAGTGCTGAATAAGAGATGAGG R: CTCCTGTTACCTGTTTTGTTCT	57.0	$(CA)_{11}$	246	6	238–245	0.49 (0.55)	4	237–245	0.64 (0.65)
DTR_A101	F: CTGGGTTGTGGTTCAACATTAG R: ATAGGGTTGCTCCTCTGAAGAC	56.8	$(CA)_{27}$	150	17	113–187	0.67 (0.73)	22	113–179	0.72* (0.92)
DTR_A103	F: GCTTACTGTAGCAAAGCACATG R: ATCCTTACAACTGTCCGTTGAG	57.3	$(CA)_{14}$	199	21	193–247	0.78* (0.81)	22	195–237	0.85 (0.87)
DTR_A105	F: AGGGTAGATAACGTCTGATTTG R: TCAGGTTTCTGAGTCTGACAG	56.4	$(CA)_{27}$	194	15	178–198	0.76 (0.81)	13	176–200	0.83 (0.87)
DTR_A111	F: CACGAGTGCTGTGTTTGAG R: ACCACTACATGCCGTTAGATC	56.8	$(CA)_{27}$	289	8	283–292	0.63 (0.75)	7	283–292	0.78 (0.74)
DTR_A114	F: GGATGGATGTGTAATGGTAATG R: GCTGAATGATGTTTATCACGAG	57.1	$(TC)_{19}(CA)_{22}$	217	16	189–220	0.63* (0.87)	20	187–248	0.82 (0.92)
DTR_A115	F: CATTTACTGATGCTGTGATCTG R: GAGCCATCTTTGTCTTCACTC	56.3	$(CA)_{29}$	166	10	122–160	0.73 (0.81)	15	122–174	0.8 (0.87)
DTR_A120	F: GCATAAATGGCTACACCTGAAC R: ACTTTGGTCCAGCAGAGTAGTG	57.6	$(CA)_{15}$	210	14	200–240	0.7 (0.82)	19	200–268	0.68* (0.82)
DTR_B103	F: CAGAACCAGAACGATTTCTAAT R: CGTGTGCCAATATCATATAGAG	57.1	$(CATC)_{15}$	272	30	266–380	0.89 (0.94)	22	253–372	0.78* (0.92)
DTR_B105	F: AGAAAGTCAAAGGAACCATCTG R: TGGGCTTACTCTTCAAGATAGC	57.5	$(CATC)_{11}$	236	7	234–246	0.57 (0.6)	10	226–256	0.81 (0.83)
DTR_B109	F: TAGGCAGTGAAGCCATTGTATT R: AGCCAGAGACACAACATGAAAG	58.0	$(CATC)_{9}$	244	13	238–344	0.4 (0.39)	14	224–332	0.79 (0.71)
DTR_B113	F: CCAGGTGGAGCTAGAAATATG R: GGAAACTTCCTTTGGAGGTAT	57.6	$(ACCC)_3(CATC)_9$	294	3	255–290	0.23 (0.22)	7	252–290	0.68 (0.65)
DTR_C12	F: TGAGTTATGGTGCGGACATAG R: CAGCCATGTACTGGTGAAGAT	56.3	$(TACA)_8(ACAG)_6$	130	17	165–248	0.59* (0.9)	6	177–224	0.11* (0.35)

35 PCR cycles were run in an Applied Biosystems GeneAmp PCR 9700 system, at denaturation and extension temperatures of 94 and 72 °C, respectively. Annealing temperatures are described in Table 1. The time of each stage was 30 s for denaturation, 40 s for annealing and 30 s for extension.

Forty individuals per species were scored using GeneMapper 3.7 (Applied Biosystems). Calculation of expected and observed heterozygosity values as well as test of linkage disequilibrium and deviation from Hardy–Weinberg (HW) equilibrium were performed using Arlequin (Excoffier *et al*. 2005). Results are summarized in Table 1. All loci amplified and were polymorphic for both species with a number of alleles ranging from three to 22. No evidence of linkage disequilibrium was found. Expected heterozygosity values ranged from 0.22 to 0.94 and from 0.35 to 92 for *D. trimaculatus* and *D. auripinnis*, respectively. For *D. trimaculatus*, three loci displayed a significant heterozygote deficit (A114, A103, C12) and for *D. auripinnis*, four loci deviated from HW equilibrium (A101, A120, B103, C12). Only one locus (C12) deviated from HW equilibrium in both species. Hardy–Weinberg disequilibrium can be driven by population substructure, inbreeding or technical cause. Micro–Checker (Van Oosterhout *et al*. 2006) analysis suggested that the presence of null alleles may explain the observed heterozygote deficit. However, because strong population structure across the distribution range of both species was previously described based on mitochondrial DNA sequences (Bernardi *et al*. 2002), a Wahlund effect may be causing that deficit. Our results therefore indicate that this new set of microsatellite markers will prove useful in parentage, population structure, and connectivity analyses.

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