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PERMANENT GENETIC RESOURCES NOTE

Isolation and characterization of 16 microsatellite loci in the humbug damselfish, *Dascyllus aruanus* (family Pomacentridae)

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Abstract

Here we report on 16 microsatellite loci designed for the damselfish *Dascyllus aruanus*. All loci were tested on 98 individuals and were polymorphic (seven to 35 alleles). Expected heterozygosity ranged from 0.705 to 0.942. Six loci showed Hardy-Weinberg disequilibrium due to the occurrence of null alleles. Cross-species amplifications conducted within the genus *Dascyllus* (*D. carneus*, *D. strasburgi*, *D. trimaculatus*) lead to polymorphic fragments in 32 out of 48 tests. These 16 loci will enable future research into the behavioural ecology and population ecology of *Dascyllus aruanus* throughout the Indo-Pacific.

Keywords: coral reef fish, *Dascyllus aruanus*, microsatellites, parentage analyses, Pomacentridae, population structure

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Dascyllus aruanus Linnaeus 1758 is an obligate coral-dwelling Pomacentridae, distributed throughout Indo-Pacific reefs from French Polynesia to Mozambique. It is found exclusively in lagoon habitats (Allen 1991) where it lives in branching coral colonies in hierarchical groups of 10 to 80 individuals (Holbrook *et al*. 2000; Cole 2002). Within each group, there is a size-based dominance hierarchy (Forrester 1991); the largest individual is male and smaller individuals tend to be females or nonbreeders (Cole 2002). The fishes are protogynous hermaphrodites (Cole 2002); if the dominant male of the group disappears, a large female from the group or another nearby group changes sex and takes his place (Coates 1982). *Dascyllus aruanus* has been the subject of investigation by behavioural ecologists, population ecologists and geneticists, and reproductive biologists for over 30 years and the development of highly polymorphic microsatellite markers informing about individual relatedness will provide significant additional insights on the evolutionary ecology of *D. aruanus*.

Between 10 μg and 20 μg of genomic DNA was isolated from a single individual fin clip preserved in 95% EtOH using the DNeasy Kit (QIAGEN). Microsatellite enrichment was achieved using double stranded SNX linkers and streptavidin-coated magnetic spheres as in Hamilton *et al*. (1999), with minor modifications described in Smith-Keune *et al*. (2006). Enrichment amplicons were cloned using a pMOS Blue Blunt-ended cloning kit (Amersham Biosciences) and white colonies were picked into 96-well plates containing 50 μL water. Microsatellite insert containing colonies were detected through polymerase chain reaction (PCR) using universal T7 and U19 primers (Smith-Keune *et al*. 2006), and DOT blotting on positively charged nylon membranes (Hamilton *et al*. 1999).

In total, 164 PCR-amplified fragments from 336 positive colonies were randomly selected and sequenced in both directions using a BigDye Terminator sequencing kit and an ABI 3730 XL Genetic Analyser (Applied Biosystems). From these, 51 primer pairs were designed using Primer 3

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Table 1 Primer sequences, repeat motif, annealing temperature (T_a), multiplex PCR primer mix number used for each primer pair (mix), and variability measures resulting from the amplification of 98 individuals in *Dascyllus aruanus* at 16 microsatellite loci

Locus	GenBank Accession no.	Primer sequences	Motif	T_a (°C)	Mix	Dye	N_A	H_O	H_E	F_{IS}
Da304	FJ225427	F: ATCCTTCGCTGAGAGGCATA R: ACAGCACCTGCAGAGATGG	$(CA)_{15}$	63	1	D2	14	0.806	0.805	0.004
Da314	FJ225428	F: ACCTGAAACATGCCCGTAAG R: TTGTATCCAAAGCATTTATTTATGTGA	$(CTGT)_{11}$	63	1	D4	14	0.279	0.814	0.660*
Da331	FJ225429	F: GACAAGATACACGATTAAAATTCTGC R: AGCAAATGGCTTCTTGGATG	$(CAT)_9$	63	1	D3	7	0.755	0.705	–0.066
Da360	FJ225430	F: TCTCGCTTTTCCTCCAACTC R: TCTGCTGAGGAGTGTGCATC	$(CA)_{13}$	57	2	D2	13	0.816	0.770	–0.055
Da371	FJ225431	F: CAAACAATTGAACAAGTACACCAAG R: GCATGTGTTGTATGTCCAGTGA	$(TACA)_5(CA)_{17}$	63	1	D3	8	0.389	0.713	0.459*
Da408	FJ225432	F: GGCAACAGTTGTTCCACCTT R: GAAGAGATTGCTTCGGCAAG	$(CA)_{16}$	63	1	D2	19	0.429	0.812	0.476*
Da432	FJ225433	F: TTTATGCATGGCAGTATGCAG R: CCTTCCCAAACACACACACA	$(TC)_{22}(TG)_{23}$	60	3	D4	15	0.742	0.863	0.145*
Da479	FJ225434	F: TCACGCTCTCACTTTTTGTCTT R: CTGATTAGGACCAATGGAAAGG	$(CA)_{16}CG(CA)_4$	60	3	D3	14	0.546	0.831	0.347*
Da494	FJ225435	F: ATCTCCACTGCTCTCCCTCA R: GCTCCCTCAAGGACTGTGAC	$(CA)_{23}$	57	2	D4	29	0.857	0.883	0.035
Da523	FJ225436	F: CCTGCAGGAAAGATTGATCC R: TGACCACCTCTGAACAATGG	$(GT)_{26}$	57	2	D2	21	0.806	0.853	0.061
Da542	FJ225437	F: TGGGGAGTGGGTATGGTAAA R: TCAACGTTCCTGGTCTGTGA	$(GT)_{22}$	57	2	D3	25	0.949	0.934	–0.011
Da565	FJ225438	F: GGAAAGCAGATTGGTTTGACA R: CAAAGACAAACATGGCCAAA	$(TG)_{31}$	57	2	D3	13	0.755	0.768	0.022
Da589	FJ225439	F: TAAAGCCCTTTCGAGCACTG R: GCTCAGCTTCAGTGGAAAGC	$(CT)_{28}$	63	1	D3	30	0.949	0.940	–0.005
Da590	FJ225440	F: GGCTGCACTGTTTCCAAGAG R: GGTGACTCACTGACATTTTTGC	$(GT)_{21}$	60	3	D3	30	0.908	0.913	0.01
Da593	FJ225441	F: TTCAACAGGCAGAGGAGTGA R: CACAAACGCCCTTGTTTCAT	$(TG)_{31}$	60	3	D2	21	0.878	0.848	–0.029
Da603	FJ225442	F: TGAACTGGCTGACAAAGCAT R: TTTTGTAATCCAAGCTTCTTTGG	$(CTGT)_{17}$	60	3	D4	35	0.473	0.942	0.503*

N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, Wrights' fixation index; *significant heterozygote deficiency.

(http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and tested at various annealing temperatures using PCRs of 10 μL containing 1 × PCR buffer (HT Biotechnology), 0.5 mM $MgCl_2$, 0.12 mM of each dNTP, 2 μg of BSA, 0.03 μM of each primer, 0.5 U *Taq* Polymerase (Eppendorf) and approximately 3 ng DNA. PCRs were conducted in an Eppendorf Thermocycler (Eppendorf): 3-min denaturation at 94 °C, 40 cycles of 30 s at 94 °C, 30 s at various annealing temperatures (55 to 65 °C) and 30 s at 72 °C, and a final extension of 5 min at 72 °C. PCR products producing a single band on 2% agarose gels were further run on Spreadex gels (Elchrom Scientific) for higher resolution, and based on the presence of polymorphism, 18 loci were selected. PCRs were conducted as described above, with the forward primers labelled with Beckman Coulter dyes D2, D3 or D4 (Table 1). Amplified fragments were separated on a Beckman Coulter CEQ 8000 Genetic Analysis System, with a 400-bp internal size standard (Beckman Coulter). Of these 18 loci, 16 produced interpretable and repeatable patterns and were subsequently pooled into three multiplex PCR mixes (of six, five and five loci respectively, Table 1) and amplified with the QIAGEN Multiplex PCR kit (QIAGEN) with annealing temperatures given in Table 1.

Variability was tested in 98 individuals collected within the lagoon of Moorea, French Polynesia. Genotypes and allele sizes were scored using Beckman Coulter CEQ 8000 Genetic Analysis System associated software. Number of alleles and heterozygosity per locus were estimated using Genetix (Belkhir *et al*. 2002). Tests for gametic disequilibrium and departure from Hardy–Weinberg proportions were performed using exact tests implemented in GenePop version 3.4 (Raymond & Rousset 1995). The presence of

Table 2 Allele size range (in base pairs) of generated fragments resulting from cross-species amplifications at 16 microsatellite loci

Locus	*Dascyllus aruanus* (98)	*Dascyllus carneus* (4)	*Dascyllus strasburgi* (8)	*Dascyllus trimaculatus* (3)
Da304	111–161	111–141	129–139	113–137
Da314	144–180	—	—	—
Da331	109–130	112–130	m	118–127
Da360	210–250	206–292	216–262	212–238
Da371	147–161	150–174	164–176	152–178
Da408	180–228	180–244	192–212	182–228
Da432	186–216	—	—	—
Da479	85–129	m	108–144	99–129
Da494	146–222	138–216	172–216	152–186
Da523	120–172	124–136	136–150	130–154
Da542	184–260	163–221	173–187	175–191
Da565	143–179	—	—	—
Da589	167–259	—	—	—
Da590	147–225	132–174	150–212	150–168
Da593	147–197	141–163	149–171	145–171
Da603	168–392	321–333	—	m

Numbers in parenthesis represent the number of individuals for which the amplifications were conducted; –, failure; m, locus monomorphic.

null alleles was detected using Micro-Checker (van Oosterhout *et al*. 2004).

Between seven and 35 alleles per locus were observed in *D. aruanus*, with expected heterozygosity values ranging from 0.705 to 0.942 (Table 1). No significant gametic disequilibrium was found for any pair of loci ($P > 0.01$). Significant heterozygote deficiencies were found in six loci (Da314, Da371, Da408, Da432, Da479 and Da603; all $P < 0.003$, the threshold following Bonferroni standard correction). Null alleles were detected for these loci using Micro-Checker software which identified a general homozygote excess for most allele class sizes (combined probability for all classes $P < 0.001$). This was experimentally confirmed by the consistent non-amplification of several individuals despite repeated PCR attempts and successful amplification of unaffected loci in the same individuals.

Cross-species amplifications were conducted on *Dascyllus carneus* collected in Madagascar (four individuals), *Dascyllus strasburgi* from the Marquesas Islands (eight individuals) and *Dascyllus trimaculatus* from Madagascar (three individuals), and were successful in 32 out of 48 tests (Table 2). Da314, Da432, Da565 and Da589 are *D. aruanus* specific markers since they did not amplify any fragments for any of the other species. The number of putative useful polymorphic loci is 11 for *D. carneus* and *D. trimaculatus*, and 10 for *D. strasburgi*.

The 16 useful polymorphic loci for *D. aruanus* are currently being used (i) to investigate connectivity of *D. aruanus* populations within different reef systems at various spatial scales throughout the Pacific Ocean, and (ii) to investigate relatedness and reproductive skew within groups at small spatial scales in Moorea, French Polynesia.

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