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**Michelle J. Gardner · Jennifer A. Chaplin · Kristine M. Shaw**

Development and characterisation of novel microsatellite loci for the baldchin groper (*Choerodon rubescens*) and cross-amplification in seven other labrid species.

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41 **Abstract**

42

43 We describe the development and characterisation of novel microsatellite loci for the baldchin groper,  
44 *Choerodon rubescens*. The purpose was to identify loci that can be used to resolve uncertainties about  
45 the population (stock) structure of this fish species, which is endemic to a narrow region of the west  
46 coast of Australia and showing evidence of overfishing in some locations. Of 22 loci characterised, 12  
47 appear to be ideally suited for population-level analyses. Utilising data obtained from four sampling  
48 locations across the distribution of *C. rubescens*, the total number of alleles observed at each of the 12  
49 loci ranged from 2 to 24, while the overall values of expected heterozygosity ranged from 0.19 to 0.89.  
50 Cross-amplification of the 12 loci in 7 other labrid species was often successful, especially in  
51 congeners.

52

53 **Keywords** *Choerodon* · Labridae · microsatellite marker · population structure

54

55 The baldchin groper, *Choerodon rubescens*, is a large, long-lived labrid endemic to a region of  
56 the western continental shelf of Australia between Coral Bay (*ca* 23°S) and Geographe Bay (*ca* 34°S)  
57 (Fairclough 2005). This species is prized by recreational boat-fishers and spear-fishers and, due to its  
58 life history characteristics, is vulnerable to the effects of over-fishing (Nardi et al. 2006). Concern  
59 over the sustainability of the stock(s) of this species is growing and overfishing may already be  
60 occurring in some locations (Wise et al. 2007). There is also a more general concern about the  
61 ecosystem-level impacts of declining numbers of higher-level predators, like *C. rubescens*, in reef  
62 habitats (see DeMartini et al. 2008).

63 Ideally, the processes that are put in place to manage the fisheries for *C. rubescens* should  
64 operate at a spatial scale(s) relevant to the population (stock) structure of this species; as yet this  
65 structure is unknown. This paper describes the development, characterisation and testing of novel  
66 microsatellite markers for *C. rubescens*. These markers were developed primarily for resolving  
67 uncertainties about the population structure of this species, however, their ability to cross-amplify loci  
68 in 7 other labrid species was also assessed. Very few studies have published microsatellite markers for  
69 labrids (see Guillemaud et al. 2000; Poortvliet et al. 2009), despite the diversity and abundance of this  
70 fish family (see Westneat and Alfaro 2005).

71 Genetic Identification Services (GIS, <http://www.geneticidentificationservices.com>)  
72 constructed microsatellite-enriched partial genomic libraries for *C. rubescens*, following the methods  
73 described by Jones et al. (2002). GIS was supplied with approximately 30 µg of high molecular weight  
74 DNA extracted from muscle tissue from a *C. rubescens* obtained from waters off Lancelin (115°15'E,  
75 30°55'S). The DNA was extracted using a MasterPure™ DNA Purification Kit following the  
76 manufacturer's protocol for soft tissue, except that tissue and reagent quantities were significantly  
77 increased and a 5% sodium dodecyl sulfate (SDS) extraction buffer was substituted for the tissue and  
78 cell lysis solution. Four libraries, enriched for CA, ATG, TAGA, and CAGA motifs, were constructed  
79 and 36 recombinant clones from each library were randomly selected for sequencing, of which 34, 29,  
80 34 and 30 contained microsatellites, respectively (GenBank accession numbers HM754266-392).

81 Primer pairs were designed for a total of 36 loci using PRIMER 3 software (Rozen and  
82 Skaletsky 2000). PCR products were fluorescently labelled using the three-primer PCR method of  
83 Schuelke (2000). Depending on the locus, 2 different M13 sequences – either M13A-  
84 CACGACGTTGTAAAACGAC or M13B- GAGTTTTCCCAGTCACGAC – and, depending on the  
85 assay, three different dyes, namely FAM (GeneWorks), NED and VIC (Applied Biosystems), were  
86 used.

87 PCR's were carried out in 15 µL volumes containing approximately 15 ng/µL template DNA,  
88 0.35 µM reverse primer, 0.17 µM forward primer (with either an M13A or M13B sequence attached)  
89 (see Table 1), and 0.17 µM of either a M13A or M13B tail labelled primer, 0.45 U *Taq* polymerase  
90 (ROCHE), 1X reaction buffer (10 mM Tris-HCl, 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, pH 8.3, ROCHE) and  
91 0.3 mM dNTPs (Promega). Reactions were conducted using a GeneAmp 9700 PCR system (PE  
92 Applied Biosystems), with touchdown cycling parameters consisting of an initial denaturation step at  
93 94°C for 2 min followed by 24 cycles of 94°C for 30 s, 62°C (decreased 0.5°C per cycle) for 30 s and  
94 72°C for 60 s; and 25 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 60 s, followed by a final  
95 extension step at 72°C for 5 min. PCR products were screened with a 3730 DNA Analyzer (Applied  
96 Biosystems), co-run with a size standard, Genescan LIZ 600 (Applied Biosystems). Genotypes were  
97 scored manually using GeneMarker v. 1.92 (SoftGenetics Inc.).

98 Twenty-two of the 36 loci evaluated consistently produced readable banding patterns when  
99 tested on 14 individuals of *C. rubescens* from the Abrolhos Islands (*ca* 113°30'-114°10'E, 28°10'-  
100 29°00'S). These 22 loci were then tested on a sample of 28 individuals from northern parts of the  
101 Abrolhos Islands. Twenty-one of the 22 loci were polymorphic, 17 conformed with Hardy-Weinberg  
102 equilibrium expectations after Bonferroni adjustments (Rice 1989; Table 1), and the genotypes at all  
103 but one pair (*CruD102* and *CruD108*) were independent of each other, as assessed using exact tests  
104 implemented by Genepop v. 4.0 (Raymond and Rousset 1995). Twelve loci were selected for further  
105 analysis based upon genetic and technical criteria and tested on 3 samples of *C. rubescens* from across  
106 the species' geographic range. The patterns of variation for all but one of the additional sample-loci  
107 combinations were also consistent with Hardy-Weinberg equilibrium expectations (see Table 1), with  
108 no evidence of linkage disequilibrium. The total number of alleles at each of the 12 loci ranged from 2  
109 to 24, while expected heterozygosity ranged from 0.19 to 0.89 (Table 1).

110 The 12 loci were also tested on 7 other labrid species, using the same conditions as described  
111 above for *C. rubescens*. In general, the loci worked very well with the congeners *C. schoenleinii* and *C.*  
112 *cyanodus*, for which, respectively, 8 and 9 loci were amplified with 100% success (Table 2). At least  
113 one polymorphic locus (and usually more) was amplified with 100% success from each of the 7 species  
114 (see Table 2).

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**Table 1** Characteristics of 22 microsatellite loci developed for *Choerodon rubescens*

Locus (Genbank Accession No.)	Primer sequence	Repeat motif	<i>A</i>	Size range (bp)	<i>H<sub>O</sub></i>	<i>H<sub>E</sub></i>	<i>n</i>	<i>HW</i>
<i>CruA1</i> (HM754266)	F: <sup>B</sup> TAAACGAGCAGACTCAGACC R: GCACACATCTATCTTCCATACC	(AC) <sub>8</sub> T(CA) <sub>6</sub>	4 (3-4)	170-180	0.68 (0.55-0.77)	0.62 (0.59-0.64)	103 (18-30)	4/4
<i>CruA2</i> (HM754266)	F: <sup>B</sup> TTCGCTCTGGACTAAGATGC R: AGGAGGACGGGATTATTCC	(CA) <sub>13</sub> G(CA) <sub>11</sub>	16 (9-12)	149-191	0.70 (0.63-0.82)	0.73 (0.66-0.80)	101 (17-30)	4/4
<i>CruA115</i> (HM754268)	F: <sup>B</sup> GAGGATTATCACCCCTGCAA R: CCCTCTCTGCGTCTTGTTC	(AC) <sub>15</sub>	24 (14-19)	198-264	0.89 (0.85-0.92)	0.89 (0.82-0.92)	110 (26-30)	4/4
<i>CruD7-2</i> (HM754269)	F: <sup>B</sup> TGAAGCGAAGGTGCATACTG R: CCAGAGGTCAGAGCACAA	(CA) <sub>16</sub>	10 (7-9)	238-268	0.68 (0.63-0.76)	0.72 (0.69-0.74)	115 (26-30)	4/4
<i>CruD1</i> (HM754270)	F: <sup>A</sup> CTCCAAATGAGGAGGGAACA R: AGGCAGGCGATAAGTGTTA	(TATC) <sub>16</sub>	12 (8-10)	142-186	0.86 (0.73-0.92)	0.83 (0.80-0.84)	111 (26-30)	4/4
<i>CruD2</i> (HM754271)	F: <sup>B</sup> TGTCAGGCTTGATGACATTTG R: GGCACACCTTTGTGTGAAAG	(TAGA) <sub>12</sub> (GA) <sub>13</sub>	16 (7-14)	207-315	0.82 (0.77-0.92)	0.84 (0.77-0.85)	122 (26-37)	4/4
<i>CruD112</i> (HM754272)	F: <sup>B</sup> GTGGTGCACAGTCATTCCAG R: TGTGCCGGTCACTGAAGTC	(TAGA) <sub>12</sub>	10 (8-9)	234-270	0.82 (0.75-0.93)	0.82 (0.80-0.82)	122 (26-40)	4/4
<i>CruD118</i> (HM754273)	F: <sup>B</sup> CTACCACGGCCATCAGAAGT R: TGTAACACGGGTTTTGTGC	(AGAT) <sub>8</sub> ...(GATA) <sub>3</sub>	8 (4-6)	243-293	0.51 (0.32-0.66)	0.53 (0.43-0.61)	106 (26-30)	4/4
<i>CruD124</i> (HM754274)	F: <sup>A</sup> CGCTGTGATTGCTCTCATTC R: GGGATGGTCTCACTGGTTGT	(ATAG) <sub>6</sub> ...(ATAG) <sub>8</sub>	15 (9-13)	191-271	0.86 (0.79-0.96)	0.88 (0.82-0.88)	122 (26-38)	4/4
<i>CruC10</i> (HM754275)	F: <sup>B</sup> TACGCTCCAGCCTGACTTCT R: GCCCGGTGAGGTTGTTAGAT	(TCTG) <sub>6</sub>	7 (5-7)	228-252	0.66 (0.62-0.69)	0.65 (0.62-0.67)	115 (26-31)	4/4
<i>CruC111</i> (HM754276)	F: <sup>B</sup> TCCGGACATTCTGTGACAA R: CAGTTCGGAGGGAACAAGTC	(CAGA) <sub>6</sub>	5 (3-5)	191-207	0.40 (0.30-0.52)	0.58 (0.48-0.68)	108 (26-29)	3/4
<i>CruC120-4</i> (HM754277)	F: <sup>B</sup> GGCAACTGAGGCCTTACA R: TCGATCACCATGACAACCAC	(TCTG) <sub>6</sub>	2 (2)	261-265	0.18 (0.10-0.27)	0.19 (0.09-0.28)	115 (26-31)	4/4
<i>CruD11</i> (HM754278)	F: <sup>B</sup> TTAAAGGGCTCTGGGCTTCT R: ACAATTTCCCGGTTTTGGAT	(TAGA) <sub>10</sub>	19	227-445	0.68	0.92	19	0/1
<i>CruD12</i> (HM754279)	F: <sup>B</sup> GCACAAGTCACAAGGGTCAA R: GGTGAAAAGTAAGAAAGAGTTGG	(TATC) <sub>33</sub>	27	254-404	0.95	0.96	27	1/1

**Table 1 continued.**

<i>CruD102</i> (HM754280)	F: <sup>A</sup> CCTCTTGTTGGCAACCT R: TTTTCATTTAGGCAGAAGTTGG	(TAGA) <sub>21</sub> (TGGA) <sub>9</sub> (TAGA) <sub>3</sub>	13	179-267	0.56	0.84	18	0/1
<i>CruD108</i> (HM754281)	F: <sup>A</sup> AGTGTGGTGACAGGCAGTTG R: TTGGTCACAAAAGCATCAGAA	(TATC) <sub>15</sub>	15	182-276	0.96	0.89	28	1/1
<i>CruD109</i> (HM754282)	F: R: CTCCTCAGATCCTCCGACTG	(TAGA) <sub>7</sub> ...(TAGA) <sub>9</sub> ...(TAGA) <sub>13</sub> ...(TAGA) <sub>12</sub>	23	163-409	0.56	0.91	25	0/1
<i>CruD110</i> (HM754283)	F: <sup>A</sup> GTGCCACGTTTGAATACAC R: TTTGTCAAGGATAATGCGTTCTT	(TAGA) <sub>13</sub>	26	202-312	0.88	0.94	26	0/1
<i>CruD115</i> (HM754284)	F: <sup>B</sup> AGGCTGCTTAAATGCCAAAA R: GCAGACACTTCCCAAGACT	(TATC) <sub>11</sub> ...(TATC) <sub>11</sub> ...(TATC) <sub>11</sub> ...(TATC) <sub>16</sub> ...(TATC) <sub>8</sub> ...(TATC) <sub>11</sub>	28	149-389	0.93	0.94	27	1/1
<i>CruD117</i> (HM754285)	F: <sup>A</sup> TTTGACACATGGGAAAGTGTG R: CATGCATTCTGGGAGAGTGA	(TATC) <sub>20</sub>	28	198-344	0.93	0.95	28	1/1
<i>CruD123</i> (HM754286)	F: <sup>B</sup> CGTGGACGTAGACCATCACC R: AGCCTCAATTTATGTCCACCT	(TAGA) <sub>28</sub>	24	206-302	0.96	0.94	28	1/1
<i>CruC12</i> (HM754287)	F: <sup>B</sup> CGTACGGTCATGTGCAAAGT R: CGGATCACTCCCCAAATCTA	(TCTG) <sub>3</sub> (CCTG) (TCTG) <sub>4</sub>	1	205	0	0	30	na

A and B, respectively, indicate either a M13A or M13B tail was attached to the 5' end of the forward primer; number of individuals assayed (*n*); number of alleles observed (*A*); average observed ( $H_o$ ) and expected heterozygosity ( $H_e$ ). All 22 loci were tested on a sample from the northern Abrolhos Islands; the first twelve loci were also tested on samples from the northern (Monkey Rock, *ca* 113°07'E-26°05'S), southern (Two Rocks, *ca* 115°20'E-31°30'S) and central (southern Abrolhos Islands) parts of the species' geographic range. Information for these loci is presented as the value for all assayed individuals (total sample), with the range in values for individual samples in parentheses. *HW* indicates the number of samples with genotype frequencies that conformed to Hardy-Weinberg equilibrium expectations after sequential Bonferroni correction ( $P < 0.001$ ), na indicates not tested due a lack of polymorphism.



**Table 2** Cross-amplification of 12 microsatellite loci designed for *Choerodon rubescens* in 7 other species of Labridae

Species	Blackspotted tuskfish ( <i>Choerodon schoenleinii</i> )	Blue tuskfish ( <i>Choerodon cyanodus</i> )	Weed whiting ( <i>Siphonognatus attenuatus</i> )	Foxfish ( <i>Bodianus frenchii</i> )	Blue groper ( <i>Achoerodus gouldii</i> )	Maori wrasse ( <i>Ophthalmolepis lineolatus</i> )	Brown spotted wrasse ( <i>Notolabrus parilus</i> )
<i>CruA1</i>							
<i>n<sub>amp</sub>/n</i>	6/6 <sup>v</sup>	4/4 <sup>v</sup>	5/5 <sup>v</sup>	2/7 <sup>**</sup>	3/7 <sup>**</sup>	4/5 <sup>s</sup>	4/6 <sup>**</sup>
<i>Size</i>	161-175	172-173	170-180	170-180	170-180	170-180	170-180
<i>A</i>	3	2	2	2	2	2	2
<i>CruA2</i>							
<i>n<sub>amp</sub>/n</i>	6/6*	4/4 <sup>v</sup>	3/5*	2/7 <sup>**</sup>	4/7 <sup>**</sup>	3/5*	4/6*
<i>Size</i>	161-193	186-218	157-161	157	157-161	157-163	157-161
<i>A</i>	7	4	3	1	3	4	3
<i>CruA115</i>							
<i>n<sub>amp</sub>/n</i>	6/6 <sup>v</sup>	4/4 <sup>v</sup>	3/5*	2/7 <sup>**</sup>	3/7 <sup>**</sup>	3/5 <sup>**</sup>	4/6 <sup>**</sup>
<i>Size</i>	214-254	197-305 <sup>v</sup>	206-226	198-208	216-225	198-226	196-226
<i>A</i>	7	8	3	2	4	5	5
<i>CruD7-2</i>							
<i>n<sub>amp</sub>/n</i>	6/6 <sup>v</sup>	4/4 <sup>v</sup>	5/5 <sup>v</sup>	5/7 <sup>**</sup>	5/7 <sup>**</sup>	3/5 <sup>**</sup>	3/6 <sup>**</sup>
<i>Size</i>	269-291	219-221	239-255	239-255	220-255	239-255	239-255
<i>A</i>	6	2	2	3	6	2	3
<i>CruD1</i>							
<i>n<sub>amp</sub>/n</i>	4/6 <sup>**</sup>	4/4 <sup>s</sup>	2/5 <sup>**</sup>	3/7 <sup>**</sup>	5/7 <sup>**</sup>	4/5 <sup>v</sup>	3/6 <sup>**</sup>
<i>Size</i>	150-170	162-172	154-170	158-162	154-172	154-166	154-170
<i>A</i>	4	3	4	2	7	3	3
<i>CruD2</i>							
<i>n<sub>amp</sub>/n</i>	6/6 <sup>v</sup>	1/4 <sup>**</sup>	na	na	na	1/5 <sup>**</sup>	1/6 <sup>**</sup>
<i>Size</i>	297-347	305-325				262-264	259-263
<i>A</i>	7	2				2	2

**Table 2 Continued**

<i>CruD112</i>								
<i>n<sub>amp</sub>/n</i>	5/6 <sup>§</sup>	3/4 <sup>√</sup>	3/5 <sup>⊗</sup>	2/7 <sup>⊗</sup>	3/7 <sup>⊗</sup>	3/5 <sup>⊗</sup>	3/6 <sup>⊗</sup>	
Size	234-250	242	242-246	242-250	242-250	242-250	218-246	
<i>A</i>	4	1	2	2	3	2	4	
<i>CruD118</i>								
<i>n<sub>amp</sub>/n</i>	5/6 <sup>§</sup>	4/4 <sup>§</sup>	1/5 <sup>⊗</sup>	7/7 <sup>§</sup>	6/7 <sup>§</sup>	5/5 <sup>§</sup>	6/6 <sup>§</sup>	
Size	251-255	251-255	251-255	251-255	251-255	251-255	251-255	
<i>A</i>	2	2	2	2	2	2	2	
<i>CruD124</i>								
<i>n<sub>amp</sub>/n</i>	6/6 <sup>√</sup>	4/4 <sup>√</sup>	5/5 <sup>√</sup>	7/7 <sup>√</sup>	6/7 <sup>√</sup>	4/5 <sup>⊗</sup>	2/6 <sup>⊗</sup>	
Size	269-328	188-212	210	190	234-258	190-236	228-242	
<i>A</i>	10	3	1	1	4	3	2	
<i>CruC10</i>								
<i>n<sub>amp</sub>/n</i>	6/6 <sup>⊗</sup>	4/4 <sup>⊗</sup>	4/5 <sup>§</sup>	5/7 <sup>⊗</sup>	6/7 <sup>⊗</sup>	5/5 <sup>§</sup>	6/6 <sup>⊗</sup>	
Size	223	223-252	232-240	232-252	232-240	232-240	232-252	
<i>A</i>	1	5	2	4	2	2	3	
<i>CruC111</i>								
<i>n<sub>amp</sub>/n</i>	4/6 <sup>⊗</sup>	2/4 <sup>⊗</sup>	3/5 <sup>⊗</sup>	2/7 <sup>⊗</sup>	3/7 <sup>⊗</sup>	5/5 <sup>⊗</sup>	2/6 <sup>⊗</sup>	
Size	181-195	191-203	191-195	195	191-195	191-195	195-199	
<i>A</i>	3	4	2	1	2	2	2	
<i>CruC120-4</i>								
<i>n<sub>amp</sub>/n</i>	6/6 <sup>√</sup>	4/4 <sup>√</sup>	5/5 <sup>√</sup>	7/7 <sup>√</sup>	7/7 <sup>√</sup>	5/5 <sup>§</sup>	6/6 <sup>√</sup>	
Size	262-286	255-263	261-265	264	261-265	261-265	261-265	
<i>A</i>	6	3	2	1	3	2	2	

number of individuals successfully genotyped ( $n_{amp}$ ) and the total number of individuals assayed ( $n$ ); the overall quality of the fragments in the chromatograms, where the symbols indicate the following:  $\sqrt$  clear, strong fragments,  $\S$  clear but weak fragments,  $\otimes$  inconsistent amplification among individuals, \* a significant amount of stutter and/or mis-priming; and the number ( $A$ ) and size range in base pairs (bp) of the observed alleles. na indicates that the locus was not successfully amplified from any individuals of the species. Species are ordered from most to least closely related to *C. rubescens* following Kazancioglu et al. (2009).