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# 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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**Abstract**

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

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

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

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

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

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

PCR's were carried out in 15 μL volumes containing approximately 15 ng/μL template DNA, 0.35 μM reverse primer, 0.17 μM forward primer (with either an M13A or M13B sequence attached) (see Table 1), and 0.17 μM of either a M13A or M13B tail labelled primer, 0.45 U *Taq* polymerase (ROCHE), 1X reaction buffer (10 mMTris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3, ROCHE) and 0.3 mM dNTPs (Promega). Reactions were conducted using a GeneAmp 9700 PCR system (PE Applied Biosystems), with touchdown cycling parameters consisting of an initial denaturation step at 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 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 extension step at 72°C for 5 min. PCR products were screened with a 3730 DNA Analyzer (Applied Biosystems), co-run with a size standard, Genescan LIZ 600 (Applied Biosystems). Genotypes were scored manually using GeneMarker v. 1.92 (SoftGenetics Inc.).

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

The 12 loci were also tested on 7 other labrid species, using the same conditions as described above for *C. rubescens*. In general, the loci worked very well with the cogeners *C. schoenleinii* and *C. cyanodus*, for which, respectively, 8 and 9 loci were amplified with 100% success (Table 2). At least one polymorphic locus (and usually more) was amplified with 100% success from each of the 7 species (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 | *A* | Size range (bp) | $H_O$ | $H_E$ | *n* | *HW* |
|---|---|---|---|---|---|---|---|---|
| *CruA1* | F: [B]TAAACGAGCAGACTCAGACC | $(AC)_8T(CA)_6$ | 4 | 170-180 | 0.68 | 0.62 | 103 | 4/4 |
| (HM754266) | R: GCACACATCTATCTTCCATACC | | (3-4) | | (0.55-0.77) | (0.59-0.64) | (18- 30) | |
| *CruA2* | F: [B]TTCGCTCTGGACTAAGATGC | $(CA)_{13}G(CA)_{11}$ | 16 | 149-191 | 0.70 | 0.73 | 101 | 4/4 |
| (HM754266) | R: AGGAGGACGGGATTATTCC | | (9-12) | | (0.63-0.82) | (0.66-0.80) | (17-30) | |
| *CruA115* | F: [B]GAGGATTATCACCCCTGCAA | $(AC)_{15}$ | 24 | 198-264 | 0.89 | 0.89 | 110 | 4/4 |
| (HM754268) | R: CCCTCTCTGCGTCTTGTTTC | | (14-19) | | (0.85-0.92) | (0.82-0.92) | (26-30) | |
| *CruD7-2* | F: [B]TGAAGCGAAGGTGCATACTG | $(CA)_{16}$ | 10 | 238-268 | 0.68 | 0.72 | 115 | 4/4 |
| (HM754269) | R: CCAGAGGGTCAGAGCACAA | | (7-9) | | (0.63-0.76) | (0.69-0.74) | (26-30) | |
| *CruD1* | F: [A]CTCCAAATGAGGAGGGAACA | $(TATC)_{16}$ | 12 | 142-186 | 0.86 | 0.83 | 111 | 4/4 |
| (HM754270) | R: AGGCAGGGCGATAAGTGTTA | | (8-10) | | (0.73-0.92) | (0.80-0.84) | (26-30) | |
| *CruD2* | F: [B]TGTCAGGCTTGTATGACATTTG | $(TAGA)_{12}(GA)_{13}$ | 16 | 207-315 | 0.82 | 0.84 | 122 | 4/4 |
| (HM754271) | R: GGCACACCTTTGTGTGAAAG | | (7-14) | | (0.77-0.92) | (0.77-0.85) | (26-37) | |
| *CruD112* | F: [B]GTGGTGCACAGTCATTCCAG | $(TAGA)_{12}$ | 10 | 234-270 | 0.82 | 0.82 | 122 | 4/4 |
| (HM754272) | R: TGTGCCGGTCACTGAAGTC | | (8-9) | | 0.75-0.93 | (0.80-0.82) | (26-40) | |
| *CruD118* | F: [B]CTACCACGGCCATCAGAAGT | $(AGAT)_8\ldots(GATA)_3$ | 8 | 243-293 | 0.51 | 0.53 | 106 | 4/4 |
| (HM754273) | R: TGTAAACACGGGTTTTGTGC | | (4-6) | | (0.32-0.66) | (0.43-0.61) | (26-30) | |
| *CruD124* | F: [A]CGCTGTGATTGCTCTCATTC | $(ATAG)_6\ldots(ATAG)_8$ | 15 | 191-271 | 0.86 | 0.88 | 122 | 4/4 |
| (HM754274) | R: GGGATGGTCTCACTGGTTGT | | (9-13) | | (0.79-0.96) | (0.82-0.88) | (26-38) | |
| *CruC10* | F: [B]TACGCTCCAGCCTGACTTCT | $(TCTG)_6$ | 7 | 228-252 | 0.66 | 0.65 | 115 | 4/4 |
| (HM754275) | R: GCCCGGTGAGGTTGTTAGAT | | (5-7) | | (0.62-0.69) | (0.62-0.67) | (26-31) | |
| *CruC111* | F: [B]TTCCGGACATTCTGTGACAA | $(CAGA)_6$ | 5 | 191-207 | 0.40 | 0.58 | 108 | 3/4 |
| (HM754276) | R: CAGTTCGGAGGGAACAAGTC | | (3-5) | | (0.30-0.52) | (0.48-0.68) | (26-29) | |
| *CruC120-4* | F: [B]GGCAACACTGAGGCCTTACA | $(TCTG)_6$ | 2 | 261-265 | 0.18 | 0.19 | 115 | 4/4 |
| (HM754277) | R: TCGATCACCATGACAACCAC | | (2) | | (0.10-0.27) | (0.09-0.28) | (26-31) | |
| *CruD11* | F: [B]TTAAAGGGCTCTGGGCTTCT | $(TAGA)_{10}$ | 19 | 227-445 | 0.68 | 0.92 | 19 | 0/1 |
| (HM754278) | R: ACAATTTCCCGGTTTTGGAT | | | | | | | |
| *CruD12* | F: [B]GCACAAGTCACAAGGGTCAA | $(TATC)_{33}$ | 27 | 254-404 | 0.95 | 0.96 | 27 | 1/1 |
| (HM754279) | R: GGTGGAAAAGTAAGAAAGAGTTGG | | | | | | | |

**Table 1 continued.**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *CruD102* (HM754280) | F: [A]CCTCTTGTGTTGGCAACCT R: TTTTCATTTAGGCAGAAGTTGG | $(TAGA)_{21}(TGGA)_9(TAGA)_3$ | 13 | 179-267 | 0.56 | 0.84 | 18 | 0/1 |
| *CruD108* (HM754281) | F: [A]AGTGTGGTGACAGGCAGTTG R: TTGGTCACAAAAGCATCAGAA | $(TATC)_{15}$ | 15 | 182-276 | 0.96 | 0.89 | 28 | 1/1 |
| *CruD109* (HM754282) | F: [B]GATTGAGAGACACAAGAATATAGGG R: CTCCTCAGATCCTCCGACTG | $(TAGA)_7\ldots(TAGA)_9\ldots(TAGA)_{13}\ldots(TAGA)_{12}$ | 23 | 163-409 | 0.56 | 0.91 | 25 | 0/1 |
| *CruD110* (HM754283) | F: [A]GTGCCACGTTTGCAATACAC R: TTTGTCAAGGATAATGCGTTCTT | $(TAGA)_{13}$ | 26 | 202-312 | 0.88 | 0.94 | 26 | 0/1 |
| *CruD115* (HM754284) | F: [B]AGGCTGCTTAAATGCCAAAA R: GCAGACACTTTCCCAGGACT | $(TATC)_{11}\ldots(TATC)_{11}\ldots(TATC)_{11\ldots}(TATC)_{16}\ldots(TATC)_8\ldots(TATC)_{11}$ | 28 | 149-389 | 0.93 | 0.94 | 27 | 1/1 |
| *CruD117* (HM754285) | F: [A]TTTGACACATGGGAAAGTGTG R: CATGCATTCTGGGAGAGTGA | $(TATC)_{20}$ | 28 | 198-344 | 0.93 | 0.95 | 28 | 1/1 |
| *CruD123* (HM754286) | F: [B]CGTGGACGTAGACCATCACC R: AGCCTCAATTTATGTCCACCT | $(TAGA)_{28}$ | 24 | 206-302 | 0.96 | 0.94 | 28 | 1/1 |
| *CruC12* (HM754287) | F: [B]CGTACGGTCATGTGCAAAGT R: CGGATCACTCCCCAAATCTA | $(TCTG)_3(CCTG)(TCTG)_4$ | 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

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

**Table 2 Continued**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *CruD112* | | | | | | | |
| $n_{amp}/n$ | 5/6§ | 3/4√ | 3/5⌘ | 2/7⌘ | 3/7⌘ | 3/5⌘ | 3/6⌘ |
| Size | 234-250 | 242 | 242-246 | 242-250 | 242-250 | 242-250 | 218-246 |
| *A* | 4 | 1 | 2 | 2 | 3 | 2 | 4 |
| *CruD118* | | | | | | | |
| $n_{amp}/n$ | 5/6§ | 4/4§ | 1/5 ⌘ | 7/7§ | 6/7§ | 5/5§ | 6/6§ |
| Size | 251-255 | 251-255 | 251-255 | 251-255 | 251-255 | 251-255 | 251-255 |
| *A* | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| *CruD124* | | | | | | | |
| $n_{amp}/n$ | 6/6√ | 4/4√ | 5/5√ | 7/7 √ | 6/7√ | 4/5⌘ | 2/6⌘ |
| Size | 269-328 | 188-212 | 210 | 190 | 234-258 | 190-236 | 228-242 |
| *A* | 10 | 3 | 1 | 1 | 4 | 3 | 2 |
| *CruC10* | | | | | | | |
| $n_{amp}/n$ | 6/6⌘ | 4/4⌘ | 4/5§ | 5/7⌘ | 6/7⌘ | 5/5§ | 6/6⌘ |
| Size | 223 | 223-252 | 232-240 | 232-252 | 232-240 | 232-240 | 232-252 |
| *A* | 1 | 5 | 2 | 4 | 2 | 2 | 3 |
| *CruC111* | | | | | | | |
| $n_{amp}/n$ | 4/6⌘ | 2/4⌘ | 3 /5⌘ | 2/7⌘ | 3 /7⌘ | 5/5⌘ | 2/6⌘ |
| Size | 181-195 | 191-203 | 191-195 | 195 | 191-195 | 191-195 | 195-199 |
| *A* | 3 | 4 | 2 | 1 | 2 | 2 | 2 |
| *CruC120-4* | | | | | | | |
| $n_{amp}/n$ | 6/6√ | 4/4√ | 5/5√ | 7/7√ | 7/7√ | 5/5§ | 6/6√ |
| Size | 262-286 | 255-263 | 261-265 | 264 | 261-265 | 261-265 | 261-265 |
| *A* | 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; √ clear, strong fragments, § clear but weak fragments, ⌘ 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 Kazancıoglu et al. (2009).