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TECHNICAL NOTE

# Identification of seventeen microsatellite loci for conservation genetic studies of the endemic wrasse *Coris bulbifrons*

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**Abstract** Coral reefs around the world are in decline, in part due to various anthropogenic factors, including fishing pressure. *Coris bulbifrons* is a large wrasse endemic to only four oceanic locations off Australia's east coast: Middleton Reef, Elizabeth Reef, Lord Howe Island and Norfolk Island. The species is listed as vulnerable by the IUCN due to the potential threat of overfishing. Although these remote locations, some within Marine protected Areas, experience limited fishing pressure, populations may quickly decline with minimal fishing effort as seen in the overfishing of other large wrasses. We developed primers for 17 microsatellite loci to examine gene flow, population genetic structure, and genetic diversity within and among these four locations. Observed heterozygosities ranged 0.126–0.752 in 37 individuals from Lord Howe Island indicating that these loci will be useful in *C. bulbifrons* population genetic studies.

**Keywords** Coral reef fish · Isolated islands · Genetic diversity · Lord Howe Island

Coral reefs around the world are in decline from factors such as coastal development, pollution, and global climate change; however, fisheries are responsible for the most direct influence on these and other marine ecosystems

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**Table 1** Details for 17 *C. bulbifrons* microsatellite loci developed from 454 shotgun sequences

| Locus | Repeat motif | Primer sequence | Size range (bp) | N | $N_a$ | $H_o$ | $H_e$ | pHWE | PIC | Genbank accession no. |
|---|---|---|---|---|---|---|---|---|---|---|
| *C. bulbifrons* | | | | | | | | | | |
| Cb1 [1A] | $(AATCAG)_7$ | [FAM]TCCAAAGAAGCTGGGGTTATTACTTGGCAGATAAAGGCGAT | 73–103 | 36 | 7 | 0.639 | 0.668 | 0.985 | 0.619 | JX952210 |
| Cb2 [2A] | $(AGGGTT)_5$ | [FAM]GGGTTAAAATGGGACAAGGGGGTTTGGGTTAGGGTTAGGG | 87–95 | 35 | 2 | 0.286 | 0.245 | 0.477 | 0.215 | JX952211 |
| Cb4 [1B] | $(GGAGA)_9$ | [FAM]ATTTTGCAAAGTGTGGTCCCCTCTTCTGTTGCTCCCTGCT | 200–210 | 34 | 3 | 0.118 | 0.164 | 0.000 | 0.157 | JX952212 |
| Cb5 [4A] | $(CTCAG)_8$ | [FAM]ACAGCCCAGAGAAAACAGGAGTTGTGGAAAAACACCCACC | 160–175 | 34 | 4 | 0.412 | 0.529 | 0.000 | 0.429 | JX952213 |
| Cb6 [1C] | $(AGGAG)_5$ | [NED]GAGGAGAGGAGAGGAGGAGGGCTGATTGAACGGACCAGAT | 78–118[a] | 26 | 8 | 0.308 | 0.752 | 0.000 | 0.721 | JX952214 |
| Cb7 [4B] | $(CTAT)_{11}$ | [NED]TAAGAGGTGTGCTGCGTTTG CTGACAGGGCAGCATTTGTA | 150–182 | 29 | 5 | 0.448 | 0.487 | 0.002 | 0.431 | JX952215 |
| Cb8 [2B] | $(TGTC)_{10}$ | [NED]TCATTTCCTTCCCCTGTCTGTGAGACTAAAGCAGCGAGCA | 124–140 | 35 | 5 | 0.486 | 0.442 | 0.477 | 0.413 | JX952216 |
| C10 [3A] | $(AACA)_6$ | [FAM]GAGCTGAAGGAAACGCAAACAGCCTCTGGGAACATGAGAA | 132–158 | 33 | 6 | 0.606 | 0.679 | 0.000 | 0.623 | JX952217 |
| Cb11 [2C] | $(ATGA)_6$ | [VIC]GCCTCAGAGAAACAATTGGCCCTCCATCCTCTTTCATCCA | 109–117 | 36 | 3 | 0.250 | 0.335 | 0.477 | 0.288 | JX952218 |
| Cb12 [1D] | $(AGGG)_6$ | [VIC]GGATGGAGAAAAGAGGGGAGCAAGAGGTGTGAGCGACAAA | 87–95 | 36 | 2 | 0.139 | 0.219 | 0.053 | 0.195 | JX952219 |
| Cb13 [4C] | $(AGG)_{13}$ | [VIC]CTCAACGCATGAACTCCTGATCCCCTGCTCGTCTAAGTTG | 140–156[a] | 34 | 5 | 0.382 | 0.505 | 0.002 | 0.470 | JX952220 |
| Cb15 [3B] | $(ATG)_{12}$ | [NED]TGTAACAGCTTCATCAGGCGGCTGACCCTCTCACACCATT | 125–134 | 32 | 4 | 0.406 | 0.436 | 0.160 | 0.409 | JX952221 |
| Cb18 [1E] | $(AC)_{17}$ | [NED]GGGCCATCAAAACACTCTGTTGAGCAGAGTGGGGAGTTCT | 190–202 | 31 | 3 | 0.387 | 0.447 | 0.916 | 0.360 | JX952222 |
| Cb23 [3C] | $(TG)_{10}$ | [VIC]CCGTCACCCAAACTTTCACTGGAACCTCCCTTCAAACACA | 105–121 | 36 | 9 | 0.778 | 0.699 | 0.000 | 0.658 | JX952223 |
| Cb26 [5A] | $(ATTC)_7$ | [FAM]CCTCTTCCTGCTTTTGGTGATTGGTATATGTGGCAGGCAA | 216–228 | 30 | 3 | 0.133 | 0.126 | 0.985 | 0.121 | JX952224 |
| Cb30 [5B] | $(CA)_9$ | [NED]TGTCTTGTCAGAGCCCACAGACACGCGTTGCTACAGACAC | 184–188 | 34 | 3 | 0.118 | 0.138 | 0.002 | 0.132 | JX952225 |
| Cb36 [1F] | $(TC)_9$ | [VIC]CATTCCTGAACCACAGCTCAGTCTTCAAAGGTGACCGAGC | 270–274 | 31 | 3 | 0.613 | 0.534 | 0.916 | 0.434 | JX952226 |

$T_A$, annealing temperature; *N,* sample size; $N_A$, number of alleles; $H_O$, observed heterozygosity; $H_E$, expected heterozygosity; *p* HWE, Hardy–Weinberg equilibrium significance value at $p < 0.05$ after FDR correction; PIC, polymorphic information content

[a] Locus may have null alleles

(Jackson et al. 2001). Terrestrial endemics on remote islands are well known for their vulnerability to over exploitation (Whittaker 1998); however, much less is known of the vulnerability of their marine counterparts. Some remote locations and islands are rare examples of coral reefs with limited fishing pressures (Friedlander and DeMartini 2002). These same locations are also hotspots for coral reef endemicity (Jones et al. 2002).

The doubleheader wrasse, *Coris bulbifrons,* is endemic to four locations in the South–West Pacific Ocean: Middleton Reef (MR), Elizabeth Reef (ER), Lord Howe Island (LHI) and Norfolk island (NI; Randall 1976). It is abundant in sheltered habitats within the Marine Protected Areas (MPAs) at MR, ER and LHI (Choat et al. 2006a; Hobbs and Feary 2007; Hobbs et al. 2009). On the other hand, NI is a peripheral location of *C. bulbifrons*, has the lowest densities of all four locations and is not protected by any MPAs. Although *C. bulbifrons* is abundant at three of the four locations, it is listed as vulnerable by the IUCN (Choat and Pollard 2010) since populations may quickly decline with minimal fishing effort as seen in the overfishing of other large wrasses (Choat et al. 2006b).

This study describes the development of 17 polymorphic microsatellite markers for *C. bulbifrons* using 454 shotgun pyrosequencing on a Roche GS-FLX (Australain Genome Research Facility, AGRF, Brisbane, Australia). Genomic DNA was extracted using a Qiagen Gentra Puregene (Qiagen, Doncaster, Australia) extraction protocol and was RNAse treated. The DNA was shotgun sequenced on 12.5 % of a Roche GS-FLX (Australian Genome Research Facility, AGRF, Brisbane, Australia) following Gardner et al. (2011).

The resulting sequences (totalling 103,719 reads) were screened for di, tri, tetra, penta, and hexanucleotide microsatellite loci with six or more repeats using the default settings of QDD1 (Meglécz et al. 2009). This process identified 8,878 microsatellite loci (within 8.56 % of sequences obtained); PCR primers were successfully designed for 1,110 (1.07 %) of loci found. Of these, directly labelled forward primers (FAM, NED or VIC) were synthesised for 24 loci deemed the best candidates for performance and polymorphism. Loci were initially tested for amplification success and specificity in eight individuals using a Type-it microsatellite PCR kit (Qiagen, Doncaster, Australia). Individual amplifications were performed in 10 μL reactions, containing Type-it Multiplex PCR Master Mix, 20–50 ng DNA template, and 0.2 μM each primer (forward and reverse). All primers were tested and optimised using a C1000 Thermal Cycler, Bio-Rad, Australia (see Table 1) with an initial denaturation of 94 °C for 3 min followed by 28 cycles of 94 °C for 40 s, 58 °C for 40 s and 72 °C for 40 s followed by 30 min at 72 °C. PCR products were column purified using an Ammonium Acetate protocol. Genotypes were run on a 3730XL DNA Analyzer (Applied Biosystems, Saudi Arabia) at the King Abdullah University of Science and Technology (KAUST, Saudi Arabia) with a 550 bp size standard and scored using GeneMarker (SoftGenetics, USA). Primer pairs for 17 loci reliably amplified products of the expected size, with no additional products and were polymorphic, representing four dimer, two trimer, six tetramer, three pentamer and two hexamer simple sequence repeat (SSR) loci. Loci were pooled for PCR multiplex reactions consisting of at least three loci per multiplex (Table 1), using the same optimised conditions as above, in 37 *C. bulbifrons* individuals from LHI. Characteristics of the 17 loci are summarised in Table 1. GENALEX 6 (Peakall and Smouse 2006) was used to examine the number of alleles, observed and expected heterozygosities and conformation to Hardy–Weinberg Equilibrium (HWE). CERVUS 3.0 (Kalinowski et al. 2007) was used to calculate polymorphic information content (PIC) for each locus. GENEPOP 4.0.10 (Rousset 2008) and MICROCHECKER 2.2.3 (Van Oosterhout et al. 2004) were used to test linkage dis equilibrium and the presence of null-alleles, respectively.

Nine of the 17 loci were in HWE and no linkage disequilibrium between any locus pairs (zero out of 272) were detected after FDR correction (Benjamini and Hochberg 1995). In addition, null alleles were suggested for only two loci (Cb6 and Cb13) due to homozygote excess. The *C. bulbifrons* markers displayed medium–high allelic richness (mean $N_a = 4.412 \pm 2.063$, range 2–9) and medium–high levels of expected heterozygosity ($H_e = 0.436 \pm 0.203$, range 0.126–0.752). The polymorphic information content (PIC) for the combined microsatellite loci was 0.393 indicating moderate discrimination between individuals, making the loci useful for studies of connectivity and population genetic structure in this taxon. The newly developed primers reported here will provide a useful tool to examine the gene flow, population genetic structure and genetic diversity in *C. bulbifrons*.

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