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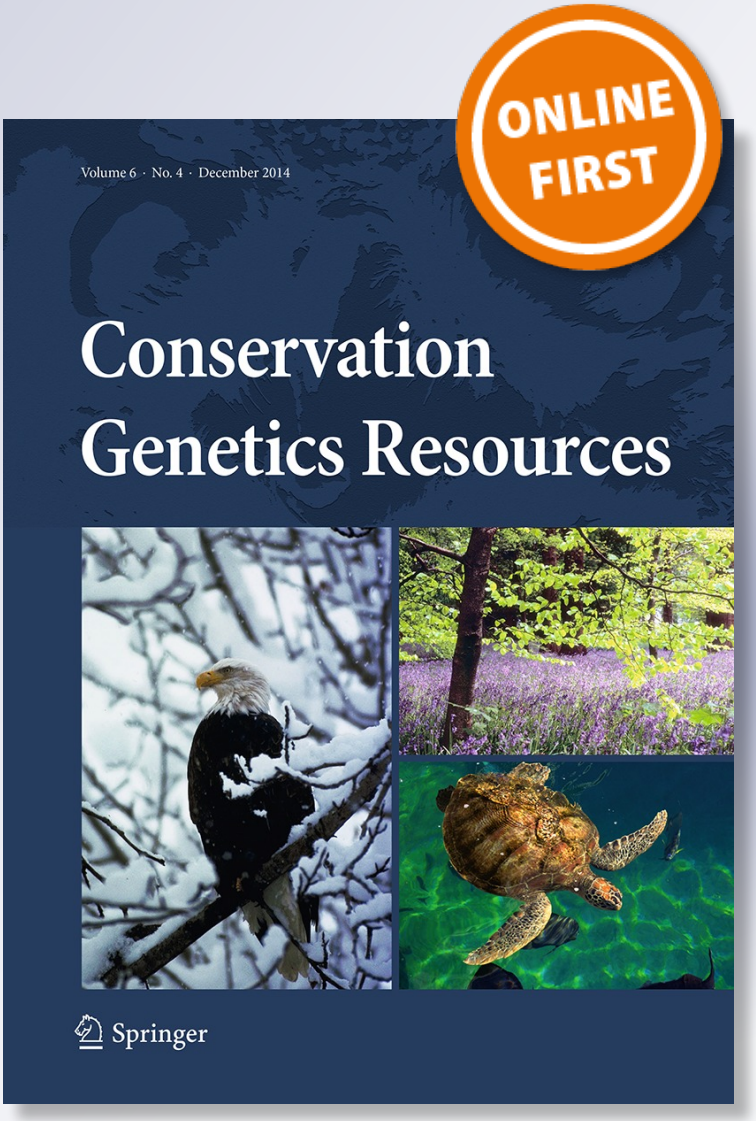


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MICROSATELLITE LETTERS

# Isolation and characterization of 12 microsatellite loci to study connectivity in the yellow jawfish *Opistognathus aurifrons*

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**Abstract** Marine Protected Areas (MPAs) are conservation strategies to preserve the degradation of marine ecosystems by allowing species to naturally recover. Central to MPA design is the assumption of connectivity in marine populations over hundreds of kilometers, but only in a few handful of species the scale of connectivity has been estimated. To facilitate the study of connectivity of reef fishes, we newly developed 12 microsatellite loci for the yellow jawfish *Opistognathus aurifrons*. We tested all microsatellite loci in eight Caribbean populations with various degrees of divergence. We found between 9 and 26 alleles per locus with polymorphism that ranged from 0.652 to 0.976. All loci were in Hardy–Weinberg equilibrium, except loci *1588* and *7983*. The described markers provide the most sensitive tools yet available to study connectivity at the finest spatial scale and evaluate if current networks of Caribbean MPAs maximize the potential for the recovery of reef fish populations.

**Keywords** Pyrosequencing · Marine Protected Areas · Coral reef · Conservation · Caribbean

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Tropical marine ecosystems have degraded in the last decades as a result of human activities. This trend is marked in coastal habitats such as coral reefs, where populations of reef-dwelling organisms have collapsed and species commonly seen in the 1970s are rarely observed today. To mitigate such degradation, Marine Protected Areas (MPAs) have been implemented, and when properly designed can achieve the long-term conservation of marine populations. To generate networks of successful MPAs, populations must be biologically connected to the extent that the realized dispersal of managed species largely offsets the geographical distance between any two adjacent MPAs. As such, larval dispersal determines the degree of connectivity among marine populations, providing information on the ideal reserve size to achieve self-recruitment and the minimum spacing among reserves to maintain connectivity (Mora et al. 2006).

Traditionally, use of MPAs has been based on the premise that marine populations are open, with populations connected over hundreds of kilometers given the long larval planktonic duration (>30 days) of many marine species. However, recent studies suggest that for some reef species, connectivity is more common at the scale of tens of kilometers and populations segregated by habitats may even surprisingly have connectivity reduced to within hundreds of meters (Prada and Hellberg 2014). In light of these new studies, understanding the geographical scale at which marine populations are connected is critical to adequately design successful MPAs. Population genetic studies provide a powerful indirect approach to measure connectivity among populations (Jones et al. 1999). To adequately quantify the scale of connectivity across networks of MPAs in the Caribbean, we developed and tested 12 microsatellite loci for the common reef dweller, the yellow jawfish, *Opistognathus aurifrons*.

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**Table 1** Characterization of 12 microsatellite loci in 42 *Opistognathus aurifrons* individuals

| Name | Code | Repeat motif | Primer sequence (5′–3′) | size (bp) | Ta (°C) | ($N_A$) | ($H_O$) | ($H_E$) | *P*HW |
|---|---|---|---|---|---|---|---|---|---|
| HLUJHHW01BHFL5 | 785 | AGC | F: CAGAGGCTTGCCTTGAAGTG<br>R: CGTTCGCTGCAGGTCATAC | 186 | 59 and 53 | 20 | 0.905 | 0.934 | 0.313 |
| HLUJHHW01CE13G | 1588 | AGC | F: GAAAGAGAAGTTGCCGCCTC<br>R: CTCCCATTCCTGACTCACCC | 264 | 59 and 53 | 14 | 0.929 | 0.776 | 0.004* |
| HLUJHHW01BM47D | 1438 | AAAG | F: CTTAGTTGGGATTGCGTGGC<br>R: GGCCTCAGGAATTTCATCGC | 224 | 59 and 53 | 14 | 0.929 | 0.884 | 0.254 |
| HLUJHHW01CDWXY | 8462 | CCG | F: TGTTGTTGTTTCTCCGGCAC<br>R: CTATTCTTTGGACACCGCGG | 248 | 59 and 53 | 12 | 0.738 | 0.863 | 0.02 |
| HLUJHHW01CP5G8 | 325 | AAT | F: TTGGGTTGCAACTCTGTGTG<br>R: TGCCATCTGTGTCCATTGTG | 203 | 59 and 53 | 23 | 0.952 | 0.932 | 0.441 |
| HLUJHHW01DLIPU | 170 | AGAT | F: TGACATCCACCACTGACAGG<br>R: TATCGGCTGGTCCTTTCTGC | 260 | 59 and 53 | 26 | 0.976 | 0.949 | 0.385 |
| HLUJHHW01EM6DJ | 516 | AAGAT | F: TCTCCTCAGCCACCAAGAAG<br>R: GGGTATCAGCACTGTTGTCC | 150 | 59 and 53 | 14 | 0.967 | 0.887 | 0.005 |
| HLUJHHW01CL23R | 150 | ACTG | F: GCGGCACACCTCTATTAAGC<br>R: TCACGCAAACAGATGATAGCC | 296 | 59 and 53 | 14 | 0.976 | 0.897 | 0.052 |
| HLUJHHW01DN5ZM | 246 | AAG | F: GCAGCACGATCGAGAAACTG<br>R: CAGATGGCCTCGTCAAACAC | 289 | 56 and 53 | 12 | 0.976 | 0.889 | 0.048 |
| HLUJHHW01EZ6IS | 8537 | CCG | F: CTGAAACTTCCCAACCAGCC<br>R: CCTCGATGCTGCTTGATGTG | 287 | 59 and 53 | 24 | 0.857 | 0.927 | 0.069 |
| HLUJHHW01D9S09 | 1358 | ACT | F: ACTCGACCCATGTTTCATCATC<br>R: ACATCCACAGTTGTCACTTGC | 247 | 59 and 53 | 25 | 0.976 | 0.945 | 0.335 |
| HLUJHHW01C84TE | 7983 | CCG | F: CGGTATAGTGTGGGAGGGTC<br>R: AAACTGGGATTGATGCGTGG | 295 | 59 and 53 | 9 | 0.652 | 0.891 | 0.001* |

*Ta* annealing temperature (°C), $N_A$ number of alleles, $H_O$ observed heterozygosity, $H_E$ expected heterozygosity

* Deviation from Hardy–Weinberg equilibrium ($P > 0.0045$) after Bonferroni's correction, *P*HW: Hardy–Weinberg probability test

To isolate the microsatellite loci, we sampled 42 individuals (seven each from MPAs in Curaçao, Puerto Rico, Mona Island and Dominican Republic). To expedite microsatellite isolation, we pyrosequenced one diploid individual from Puerto Rico at the Duke Genome Facility. Briefly, after DNA extraction, we sonicated gDNA and prepared libraries following standard 454 chemistry protocols and quality control steps. We sequenced the library at a depth of 0.2× given a genome size of 1 GB for *O. aurifrons*. To find microsat-repeats with regions to develop primers, we biased our microsatellite search towards pairs of primers with melting temperatures around 59 °C, 2 % paired primer divergence and a primer size between 18 and 30 bp. After our initial filter, we then gave preference to microsatellites with >6 repeats and unique primers.

We extracted over 10,071 reads with microsatellite repeats. We randomly selected 48 sequences to synthesize primers and test them using at least three fish per population. Out of the 48, 16 worked consistently across populations. To facilitate genotyping, we added an M13-tail to the forward primer of each primer pair and followed a two-round PCR approach (Schuelke 2000). In the first PCR the targeted region that contains the microsatellite is amplified and in the second step the fluorescently labeled M13-tail is incorporated. After adding the M13-tail, 12 out of the 16 loci amplified consistently. Fragment scoring was performed in Geneious 7.1.7 (Kearse et al. 2012).

Allelic diversity ranged from 0.652 in locus *7983* to 0.976 in loci *170, 150, 246,* and *1358.* We found all loci under Hardy–Weinberg equilibrium, except loci *1588* and *7983* (Table 1). All microsatellites amplified well across populations without null alleles. The consistent amplifications along with the high levels of variation across these 12 microsatellites provide a powerful tool to evaluate the scale of connectivity among jawfish populations across MPAs and test whether such populations are connected and sustainable in the long-term.

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