PRIMER NOTE
Development and characterization of microsatellite markers for the Amazonian blackwing hatchetfish, *Carnegiella marthae* (Teleostei, Gasteropelecidae)

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Abstract

The blackwing hatchetfish, *Carnegiella marthae*, is a small characin species distributed in forest streams of the Negro and upper Orinoco River basins in Amazonia. Freshwater hatchetfish are popular in the aquarium trade and represent an economic resource for the riverine people from middle Rio Negro, in Brazil. We isolated and characterized seven microsatellite DNA loci for the blackwing hatchetfish. Number of alleles and heterozygosity per locus in a sample of 30 fish ranged from three to 17 and from 0.19 to 0.87, respectively. These microsatellite loci provide powerful markers for studies on taxonomy, management and phylogeographic history of Amazonian hatchetfish.

Keywords: Amazon rainforest, *Carnegiella marthae*, conservation genetics, Gasteropelecidae, microsatellites, phylogeography

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The rivers of northern South America, especially those draining the Amazonia rainforest, contain an exceptional diversity of freshwater fish. This diversity is largely understudied both from an ecological and from a biogeographic perspective. We are generating large DNA data sets using microsatellites, mitochondrial DNA and intron DNA markers to investigate population history in four codistributed fish groups from central Amazonia (e.g. Beheregaray et al. 2004a, b; Beheregaray et al. 2005). The present study adds to this effort by describing a set of microsatellite DNA markers for the fourth study species of our comparative study, the blackwing hatchetfish, *Carnegiella marthae* (Teleostei, Gasteropelecidae). This peculiarly shaped and small fish is found in forest streams throughout the Amazon basin and upper Orinoco (Géry 1977; Weitzman & Palmer 2003). Blackwing hatchetfish are popular in the aquarium trade and represent a valuable resource for ornamental fishermen from middle Rio Negro, in Brazil (Chao et al. 2001). We expect that the microsatellite markers described here will prove useful for studies on taxonomy, phylogeography and conservation management of Amazonian hatchetfish.

Blackwing hatchetfish microsatellite loci were isolated using a modified enrichment technique (Fischer & Bachmann 1998). Genomic DNA was digested with *Rsa*I and *Hae*III and fragments ligated to two oligo adaptors (Edwards et al. 1996). Two biotinylated oligo probes (dGA<sub>10</sub> and dGT<sub>10</sub>) were hybridized to the digested DNA and separated using streptavidin magnetic particles (Promega). Polymerase chain reactions (PCRs) were performed on the microsatellite-enriched eluate using one of the oligo adaptors as a primer. The enriched library was purified using a gene clean kit (Qbiogene), ligated into pCR 2.1-TOPO vector (Invitrogen) and transformed into TOP10 cells (Invitrogen). The plasmid DNA was purified and 38 putative positive clones were sequenced on an ABI 377 automated DNA sequencer (PE Applied Biosystems) using dye terminator chemistry. Primers flanking eight dinucleotide microsatellite loci were designed using primer 3 (Rozen & Skaletsky 1997).

We assessed allelic and genotypic variation at these eight microsatellite loci by PCR using a 10-µL radiolabelled reaction containing ~50–100 ng of template DNA, 12 pmol of each primer, 0.5 U of *Taq* DNA polymerase (Promega),
Table 1 Primer sequences and characteristics of seven blackwing hatchetfish (*Carnegiella marthae*) microsatellite loci. Number of alleles (*N*) and expected (*H*) heterozygosities are based on a sample of 30 individuals. *T* is the annealing temperature(s) used in PCRs.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer sequences (5′−3′)</th>
<th>Repeat structure</th>
<th><em>T</em> (°C)</th>
<th><em>N</em></th>
<th>Size range (bp)</th>
<th><em>H</em>E</th>
<th><em>H</em>I</th>
<th>GenBank Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cm3</td>
<td>CCTCAGGTTGAGTCTGAATTTAAGGG</td>
<td>(CA)21</td>
<td>63−55</td>
<td>8</td>
<td>241−277</td>
<td>0.57/0.47</td>
<td></td>
<td>DQ297667</td>
</tr>
<tr>
<td>Cm4</td>
<td>CGCGCTGCTGAGTCTGAGCTTCC</td>
<td>(CA)27</td>
<td>63−55</td>
<td>4</td>
<td>210−228</td>
<td>0.29/0.45</td>
<td></td>
<td>DQ297668</td>
</tr>
<tr>
<td>Cm6</td>
<td>AGCTGTCTGAGGCAATTGTTG</td>
<td>(GA)27</td>
<td>55−47</td>
<td>17</td>
<td>240−316</td>
<td>0.80/0.80</td>
<td></td>
<td>DQ297666</td>
</tr>
<tr>
<td>Cm8</td>
<td>CAGAAGCGCTGATAGGCTGAC</td>
<td>(CT)29</td>
<td>55−47</td>
<td>16</td>
<td>106−146</td>
<td>0.87/0.87</td>
<td></td>
<td>DQ297670</td>
</tr>
<tr>
<td>Cm10</td>
<td>CACCACCTCACACATAGG</td>
<td>(CT)14</td>
<td>60</td>
<td>15</td>
<td>274−322</td>
<td>0.80/0.84</td>
<td></td>
<td>DQ297671</td>
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<tr>
<td>Cm20</td>
<td>CACCTATTTAGAGGCTGAC</td>
<td>(CA)3</td>
<td>63−55</td>
<td>3</td>
<td>123−169</td>
<td>0.20/0.19</td>
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<td>Cm23</td>
<td>TGTTACACCAGTTGTGTTG</td>
<td>(GT)20</td>
<td>55−47</td>
<td>5</td>
<td>160−172</td>
<td>0.27/0.39</td>
<td></td>
<td>DQ297673</td>
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</tbody>
</table>

References


Acknowledgements

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