Research note

Isolation and characterization of microsatellite loci in the Charal de Xochimilco *Chirostoma humboldtianum*

Aislamiento y caracterización de loci de microsatelites en el charal de Xochimilco *Chirostoma humboldtianum*

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Abstract. Microsatellite loci were isolated and characterized for the endemic fish *Chirostoma humboldtianum* using an enrichment procedure. Eight polymorphic microsatellites were genotyped for 32 - 48 individuals from Tepuxtepec Dam, Michoacán. The number of alleles per locus ranged from 3 to 11 and the average observed and expected heterozygosities were 0.61 and 0.63, respectively. All loci deviated significantly from Hardy-Weinberg expectations, which might be related to small population sizes associated to human disturbances and habitat loss. These are the first loci described for the species and the genus and could be useful in studies of population genetics, conservation and management of the species.

Key words: Atherinopsidae, Hardy-Weinberg, null alleles, enrichment.

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out using 15 µl reactions containing 50 to 100 ng DNA, 10 pmol each primer, 0.5 U Taq DNA polymerase (Promega), 200 µM of each dNTP, 2.5 mM MgCl₂ and 1X PCR buffer (15 mM MgCl₂, 200 mMTris-HCl, pH 8.5,75 mM (NH₄)₂SO₄). PCR amplifications were performed in a BIO-RAD thermocycler as follows: 95°C C for 5 min or 10 min, followed by 30 cycles of 15 sec at 94°C, 45 sec at 50°C to 60°C depending on the locus and 15 sec at 72°C C, with a final extension at 70°C C for 5 min. The PCR products were analyzed by capillary electrophoresis in the automatic sequencer ABI Prism 3100 Avant at Laboratorio Divisional de Biología Molecular (LDBM) of the UAM-Iztapalapa. Allele sizes were determined using LIZ-500 as size standard (Applied Biosystems) and GeneMarker 2.4.0 software.

Polymorphism for each microsatellite loci was characterized by screening a sample of 32 to 48 individuals of *C. humboldtianum* from Tepuxtepec Dam, Michoacán (19°59’42” N, 100°13’33” W). The total number of alleles per locus, observed (H₀) and expected (Hₑ) heterozygosities were calculated. All loci were tested for Hardy-Weinberg equilibrium (HWE), and all pairwise combinations of loci were tested for linkage disequilibrium (LD). All these parameters and tests were computed in Arlequin version 3.5.1.2 (Excoffier and Lischer, 2010). Microchecker version 2.2.3 (Van Oosterhout et al., 2004) was used to estimate the frequency of null alleles in the microsatellites.

From the 12 amplified microsatellite loci, 4, Chum422, 443, 490 and 363 were monomorphic in all the individuals analyzed. The number of alleles per locus in the remaining 8 loci ranged from 3 (Chum21) to 10 (Chum496). Expected and observed heterozygosities ranged from 0.44 to 0.78 and 0.34 to 0.89, respectively (Table 1). No LD was detected between each pair of loci. All loci exhibited HWE departure after the sequential Bonferroni correction (α = 0.05, k = 8). Micro-Checker (Van Oosterhout et al., 2004) suggested that this phenomenon might be due to the presence of null alleles that were present in 3 of the 8 loci (Chum496, Chum450 and Chum358). However, the HWE departures were still observed after the genotyping correction using FreeNA. Deviation from HWE may be outcome of heterozygote deficiency found in some loci (Chum387, Chum411, Chum450, Chum358), which in turn might be caused by endogamy as has been suggested in other species of the genus (Barriga-Sosa et al., 2004). On the other hand, an excess of heterozygotes were detected in the remaining 4 loci. Heterozygosity excess has been

Table 1. Primer sequences and microsatellite repeat motifs for 8 microsatellite loci on Chirostoma humboldtianum. Annealing temperature (Ta), number of individuals (n), number of alleles (Nₐ), observed and expected heterozygosities (H₀ and Hₑ respectively), p value for test of Hardy-Weinberg equilibrium (PₑHW), expected null allele frequency (Null) and GenBank accession numbers

<table>
<thead>
<tr>
<th>Locus and GenBank accession</th>
<th>Primer sequences (5'-3’)</th>
<th>Repeat motif</th>
<th>Ta (°C)</th>
<th>Allele size (pb)</th>
<th>n</th>
<th>Nₐ</th>
<th>H₀</th>
<th>Hₑ</th>
<th>PₑHW</th>
<th>Null</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chum48 KF016982</td>
<td>F:GCTAGCAGAAGCCATCTAAGTC</td>
<td>(CA)₉</td>
<td>50</td>
<td>132-170</td>
<td>46</td>
<td>4</td>
<td>0.522</td>
<td>0.484</td>
<td>0.018*</td>
<td>-0.0289</td>
</tr>
<tr>
<td>Chum78</td>
<td>F:TCCCTCCTGCTGCTTCTCAC</td>
<td>(CT)₄₉(A)₉</td>
<td>52</td>
<td>220-252</td>
<td>40</td>
<td>7</td>
<td>0.800</td>
<td>0.731</td>
<td>0.000*</td>
<td>-0.0452</td>
</tr>
<tr>
<td>Chum387</td>
<td>F:AAGCTGCTGGACATTCTGC</td>
<td>(T₉₅(A)₁₂(C)₂₃</td>
<td>55</td>
<td>245-310</td>
<td>43</td>
<td>7</td>
<td>0.535</td>
<td>0.719</td>
<td>0.000*</td>
<td>-0.0106</td>
</tr>
<tr>
<td>Chum496</td>
<td>F:TTGACACAGCTAAGCAGT</td>
<td>(A)₄₁(T)₁₇</td>
<td>57</td>
<td>134-169</td>
<td>42</td>
<td>10</td>
<td>0.786</td>
<td>0.778</td>
<td>0.000*</td>
<td>0.2658</td>
</tr>
<tr>
<td>Chum21</td>
<td>F:CCATGCGTACGGTGGGAC</td>
<td>(GA)₁₂GGGC(GA)₁₀</td>
<td>58</td>
<td>78-106</td>
<td>46</td>
<td>3</td>
<td>0.891</td>
<td>0.625</td>
<td>0.003*</td>
<td>-0.1686</td>
</tr>
<tr>
<td>Chum411</td>
<td>F:GGTGGTCCTGGCTTCCTGCCG</td>
<td>(CT)₂₁(T)₈₉(GA)₁₁</td>
<td>50</td>
<td>383-424</td>
<td>32</td>
<td>4</td>
<td>0.344</td>
<td>0.552</td>
<td>0.008*</td>
<td>-0.1055</td>
</tr>
<tr>
<td>Chum450</td>
<td>F:AGGACAGTACCGAGCCAG</td>
<td>(G)₁₀</td>
<td>60</td>
<td>229-250</td>
<td>48</td>
<td>9</td>
<td>0.604</td>
<td>0.712</td>
<td>0.000*</td>
<td>0.1357</td>
</tr>
<tr>
<td>Chum358</td>
<td>F:TCCTCGTCTGTTGTTTGG</td>
<td>(AC)₁₁</td>
<td>60</td>
<td>234-250</td>
<td>48</td>
<td>7</td>
<td>0.396</td>
<td>0.439</td>
<td>0.020*</td>
<td>0.0614</td>
</tr>
</tbody>
</table>

*p < 0.05 after sequential Bonferroni corrections*
associated with population bottleneck events (Cornuet and Luikart, 1996), and has also been a phenomenon suggested in other freshwater fish species inhabiting central Mexico (Domínguez-Domínguez et al., 2008). The bottleneck event could be associated to disturbances and habitat loss caused by human activities, which in turn might give result to small population sizes causing deviations from HWE. The TPM under Wilcoxon test did not show significant recent bottleneck (heterozygosity excess) \((p=0.187)\). Likewise, decrease in observed heterozygosity suggests nonrandom mating and genetic drift (Loew et al., 2005), due that genetic drift causes random fixation and loss of alleles within population. In contrast, heterozygote excess appears to be due to the presence of hybrids within the sample (Pitchar et al., 2007) as has been earlier suggested for species of the genus (Barriga-Sosa et al., 2001). Translocations among populations and hybridization have been reported for this and others species of the genus (Alaye, 1996; Barriga-Sosa et al., 2001), however, this issues needs to be punctually addressed in further studies. These 8 microsatellite loci are the first developed for *C. humboldtianum* and will be useful in investigating population genetics, conservation, and management of this and closely related species.

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**Literature cited**


