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Research note

Cross amplification of microsatellite loci developed for *Atractosteus spatula* in *Atractosteus tropicus*

Amplificación de microsatélites desarrollados para *Atractosteus spatula* en *Atractosteus tropicus*

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Abstract. Due to recent population declines in tropical gar (*Atractosteus tropicus*), a greater understanding of its population structure is needed. A key step in gaining this understanding is the development of microsatellite loci for use in this species. For this purpose, 33 microsatellite loci from alligator gar (*A. spatula*) were screened in 52 individuals from a population in Zanjón del Chino, El Salvador. Twenty-five of these loci successfully amplified in this species, and 9 of those loci were polymorphic in this population. These loci should provide a useful tool for genotyping *A. tropicus*, both in studying existing wild populations and in monitoring genetic diversity in aquaculture.

Key words: aquaculture, conservation genetics, Lepisosteidae.

Resumen. Debido al reciente declive en las poblaciones del pejelagarto (*Atractosteus tropicus*), es necesario un mayor conocimiento de la estructura de sus poblaciones. En este sentido, el desarrollo de microsatélites para esta especie sería un paso fundamental. Con este propósito, se examinaron 33 microsatélites de *A. spatula* en 52 individuos de *A. tropicus* provenientes de una población del Zanjón del Chino, El Salvador. Veinticinco de estos loci amplificaron exitosamente y 9 de ellos fueron polimórficos. Estos loci pueden convertirse en una herramienta útil en el reconocimiento del genotipo de poblaciones naturales de *A. tropicus*, así como también para el monitoreo de la diversidad genética en proyectos de acuicultura.

Palabras clave: acuacultura, genética de la conservación, Lepisosteidae.

The tropical gar (*Atractosteus tropicus*) is a small, highly pigmented gar that reaches an average total length of about 60 cm (Mora-Jamett et al., 1997). The current range extends from southern Mexico to Costa Rica (Barrientos-Villalobos and Espinosa-de los Monteros, 2008). However, its distribution is not contiguous and populations are often isolated in drainages separated by hundreds of kilometers. Reported to be relatively abundant in Mexico, they are often fished for food and are considered to be one of the top 5 resources for commercial fishing (Aguilera et al., 2002).

Since the 1990's, annual captures of *A. tropicus* have declined in Mexico (Aguilera et al., 2002). The species is also now legally protected in Costa Rica (Ley Nº 30102-MINAE del 27 de noviembre, 2001), Nicaragua (Ley Nº 34, la Gaceta No. 92 del 16 mayo, 2008), and El Salvador (Acuerdo Nº 36, 5 June 2009). Part of this decline has been attributed to destruction of spawning grounds due to dam building and other anthropogenic factors (Mendoza et al., 2002). Aquaculture has been a popular solution for enhancing depleted stocks (Aguilera et al., 2002) since gar are well suited to aquaculture given their tolerance to low dissolved oxygen, high ammonia, high nitrites, and many diseases (Alfaro et al., 2008). Analyses of mitochondrial DNA (mtDNA) in this species have shown highly structured populations across Mexico and Central America (Barrientos-Villalobos and Espinosa-de los Monteros, 2008). Although this work has provided insight into *A. tropicus* population structure, further study

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is still warranted. Identifying a set of microsatellite nuclear markers is an important step in effective management and hatchery planning.

Fortunately, although microsatellite development can be an expensive and time-consuming process (Selkoe and Toonen, 2006), many studies have demonstrated that loci isolated for one species cross amplify in other species within the genus, or even within the same family (e.g., Holman et al., 2005). Moyer et al. (2009) developed a number of microsatellite loci for use with *A. spatula* (alligator gar) and determined that a subset of those loci successfully amplified microsatellite loci in 2 other species of gar, *Lepisosteus oculatus* and *L. osseus*. Here, we test these same loci and several loci not previously reported by Moyer et al. (2009) in *A. tropicus*.

Fifty-two *A. tropicus* were collected from within a 5 km area of the Zanjón del Chino drainage in Ahuachapan, El Salvador (13º45'10.0" N, 90º03'23.9" W). Fin clips were preserved in 95% ethanol. Tissue was digested with SDS and Proteinase K, and DNA was extracted through ethanol precipitation (Miller et al., 1988). DNA was amplified using PCR mix A and PCR conditions from Moyer et al. (2009). All loci were initially screened at an annealing temperature of 56º C, but other annealing temperatures were tested if the amplification conditions for a locus required further optimization. We screened a total of 33 loci. Twenty of these loci are the ones described in Moyer et al. (2009), and we also tested an additional 13 undescribed loci from that project (GenBank accesion numbers: EU625551, EU625555, EU625558, EU625561-EU625563, and JX183078-JX183084). A LICOR 4300 DNA analyzer was used to visualize the PCR product with a 50-350 bp size standard (LI-COR). Alleles were scored using GeneProfiler 4.05 (Scanalytics Inc.). All loci were first tested using 25 samples. Polymorphic loci were then tested on the remaining 27 samples to determine the size range for each locus and the extent of genetic diversity.

Each microsatellite locus was checked for null alleles in MicroChecker 1.0 (VanOosterhout et al., 2004) and in Cervus 3.0 (Kalinowski et al., 2007). Observed (H_O) and expected (H_E) heterozygosities and the polymorphism information content (PIC) for each locus were calculated in Cervus 3.0. Each locus was also checked for Hardy-Weinberg equilibrium and linkage disequilibrium in Genepop 4.0.10 (Raymond and Rousset, 1995) with statistical significance adjusted using a sequential Bonferroni correction (Rice, 1989).

Nine microsatellite loci were polymorphic in this population of *A. tropicus* (Table 1) after excluding those loci that showed stutter too severe for accurate genotyping. The number of alleles ranged from 2-4 per locus (average= 3), the observed heterozygosity ranged from 0.043-0.686 (average= 0.303), the expected heterozygosity ranged from 0.042-0.649 (average= 0.343), and the PIC ranged from 0.041-0.587 (average= 0.305). While these loci were not highly polymorphic, these genetic diversity measures fall within the range reported for the loci found to be polymorphic in *A. spatula*. Locus *Asp*021 deviated from Hardy-Weinberg equilibrium ($p< 0.001$) and showed a greater than expected number of homozygotes, which suggests the possibility of null alleles that were estimated to occur at a frequency of 0.3846. Although the preponderance of homozygotes may be an artifact of this particular population, the possibility of null alleles should be considered when using this locus. All other loci were at Hardy-Weinberg equilibrium and showed no evidence of null alleles or linkage.

The 3 polymorphic loci not previously described in Moyer et al. (2009) were *Asp*053 (F: 5'-TGGTGGGTTGTTCAGCCTAT-3', R: 5'-

Table 1. Characteristics of the 9 polymorphic microsatellite loci including the annealing temperature (T_a), size range of alleles (in base pairs), the number of individuals (N) amplified at each locus, the number of alleles (A) for each locus, the observed and expected heterozygosity (H_O/H_E), and the polymorphism information content (PIC)

Locus	T_a (°C)	*Size range (bp)*	*N*	*A*	H_0	H_E	*PIC*	*GenBank accession*
*Asp*007	56	152-161	51	4	0.686	0.652	0.587	EU625547
*Asp*021	56	182-197	48	4	0.250	0.561	0.458	EU625568
*Asp*053	53	236-242	48	2	0.125	0.118	0.110	JX183080
*Asp*066	56	247-271	51	3	0.549	0.656	0.574	EU625554
*Asp*072	56	171-174	47	2	0.043	0.042	0.041	EU625555
*Asp*084	56	202-222	50	3	0.280	0.265	0.240	EU625556
*Asp*095	56	194-202	49	4	0.224	0.208	0.196	EU625557
*Asp*159	56	253-271	48	4	0.292	0.321	0.288	EU625560
*Asp*168	56	242-248	50	2	0.280	0.298	0.252	EU625561

TCCTTAGCAGGATCAATGTGC-3'), *Asp*072 (F: 5'-TGTATATTGGTGCCCCGTTT-3', R: 5'-AACTGGTCGCTCAGAGGAAA-3'), and *Asp*168 (F: 5'-TGCCATTACAGAAAGCCAGA-3', R: 5'-AACGCAGCTTTTGCCATATC-3'). Sixteen of the Moyer et al. loci were monomorphic in this sample, but they may yet be useful in other populations of *A. tropicus*. These loci were *Asp*010, *Asp*012, *Asp*016, *Asp*029, *Asp035*, *Asp*040, *Asp*046, *Asp*046b, *Asp*054, *Asp*057, *Asp*096, *Asp*108, *Asp*109, *Asp*116, *Asp*122, and *Asp*324. The loci that did not successfully amplify in *A. tropicus* were *Asp*004, *Asp*019, *Asp*023, *Asp*031, *Asp*058, *Asp*302, *Asp*339, and *Asp*341.

In total, 25 microsatellite loci were successfully amplified in *A. tropicus*. Nine of these loci are known to be polymorphic, and the remaining 16 also have the potential to be useful molecular markers across a wider range of *A. tropicus* populations. Hopefully, these tools will enable a greater understanding of *A. tropicus* population structure and be useful in broodstock management.

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