

Characterization of fourteen microsatellite loci in the endemic and threatened totoaba (*Totoaba macdonaldi*) from the Gulf of California

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Abstract The totoaba, *Totoaba macdonaldi*, is endemic to the Gulf of California and is listed as threatened on the IUCN red list. In preparation for a study of the population genetic structure of this species 14 microsatellite loci were developed, all found to be moderately or highly polymorphic. Observed heterozygosity ranged from 0.23 to 1.00 (average 0.67), with the number of alleles ranging from three to 23 in 26 individuals. Only one locus was found to have statistically significant deviations from Hardy–Weinberg expectations -*Tmac43* which exhibited a heterozygote deficit due possibly to null alleles. No statistically significant genetic disequilibrium was observed following Bonferroni correction. These microsatellite loci appear suitable for examining population structure, kinship assessment, and other applications.

Keywords *Totoaba macdonaldi* · Gulf of California · Sciaenidae · Microsatellites · Genomic library

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Species with small population size inhabiting restricted geographic areas or fragmented habitats are often at risk of extinction. One important obstacle to the development of conservation strategies for those species is a lack of basic information concerning their biology, population ecology, and population genetics. Without this knowledge formulation of recommendations for the protection or management of a species is difficult or futile. This is the case with the totoaba (*Totoaba macdonaldi* Gilbert 1891), the largest sciaenid fish, which reaches a length of 2 m and a weight of 135 kg (Román-Rodríguez and Hammann 1997). This species is endemic to the Gulf of California, where it exhibits ontogenetic migration with juveniles inhabiting estuaries and adults occupying offshore waters (Cisneros-Mata et al. 1997). Declines in population estimates have prompted the Mexican Government to employ progressively more stringent management measures, culminating in the designation of the totoaba's estuarine habitat as a Biosphere Preserve in 1993 and listing of the species as endangered by IUCN (Baillie and Groombridge 1996).

Biological studies describing the life history of totoaba (Rosales-Juárez and Ramírez-González 1987; Cisneros-Mata et al. 1995) were conducted in the early 1990s. However, current population assessments and delineations of population structure are lacking, generating controversy concerning the management of this species. Current proposals include reopening the recreational fishery and establishing an artificial enhancement program for this species, raising concerns about the possible effects these management options might have on the genetic integrity of totoaba (Allendorf and Luikart 2007).

Here we report the isolation and characterization of fourteen polymorphic microsatellite loci for *T. macdonaldi*. These loci were intended to serve as a tool useful for

Table 1 Summary data for microsatellites developed for *Tototaba macdonaldi*, including the repeat motif and primer sequence, annealing temperature (T_a), size range of the alleles, number of individuals assayed (n), number of alleles observed (N_a), observed and expected heterozygosities (H_O and H_E , respectively), and statistical significance (P_{HW}) of deviation from Hardy-Weinberg equilibrium

Locus	GenBank accession no.	Repeat motif	Primer sequence (5'-3')	T_a (°C)	Size range (bp)	n	N_a	H_O	H_E	P_{HW}
<i>Tmac03</i>	HM130045	(CA) ₉	F: GAGTTTGAGGACTGAATCACTA R: ATGGCTACCAAGTAGGAAGA	60	158–162	26	3	0.46	0.38	NS
<i>Tmac04</i>	HM130046	(CA) ₁₅	F: CTGTGTGGCTCTGTCA R: ACTGAGCGGAAATGAAAA	56	168–184	26	9	0.77	0.77	NS
<i>Tmac05</i>	HM130047	(GA) ₁₄	F: ATTTCTCTGCTGGTGGT R: TCCATGCTGTAGAAATATGG	60	150–156	26	4	0.50	0.50	NS
<i>Tmac06^a</i>	HM130048	(GT) ₁₇	F: ATTAAGAAGAGTGCAGGAAC R: TGTGCTTTTGTATGTGTTTGT	59	144–180	26	14	0.81	0.92	NS
<i>Tmac07^a</i>	HM130048	(GT) ₈ GC(GT) ₈	F: AAGAAATTGAAAAAAGTCTGAG R: AGAGAGGCTGCTTGAATGA	59	146–152	26	3	0.54	0.48	NS
<i>Tmac08</i>	HM130049	(GT) ₁₀ GC(GT) ₅	F: GTAAAGCTGCCTTCATCGTA R: CCTCAAAACAATGTTCAAAA	60	162–174	22	6	0.64	0.73	NS
<i>Tmac10</i>	HM130050	(CA) ₂ A(CA) ₁₄	F: CATA CGGAGAAAGAAACCGAT R: GGTTGTTGTAATAACAATG	59	200–214	26	6	0.54	0.71	NS
<i>Tmac25</i>	HM134218	(GT) ₂₂	F: CACCAGTAAATTTATGGTTAGAACA R: GGGACTGCTGTTTCTGAT	54	142–176	20	15	0.90	0.91	NS
<i>Tmac43</i>	EU784692	(TG) ₅ CG(TG) ₉	F: GTAGCAGCATGTGTCCTGT R: GGAGGAGTATTGACGTGAGACC	58	156–168	26	3	0.23	0.33	*
<i>Tmac44</i>	EU784693	(CA) ₁₆	F: ACAGAATGAGGGCCAGAGG R: GCCACAAAGACACAATGCAG	58	196–216	23	6	0.65	0.58	NS
<i>Tmac51</i>	EU784694	(TG) ₁₂ TT(TG)	F: GTTTTGCCTCTGCACACCTC R: TTCTGCCAAGATGACAGCAC	60	124–162	26	17	1.0	0.93	NS
<i>Tmac55</i>	EU784695	(GAT) ₂ (GT) ₁₂	F: TGCAAAAGCAGAAGAGAGGGTG R: TGAGCCCCGTTTTGATGATCT	60	166–186	24	9	0.71	0.74	NS
<i>Tmac56</i>	EU784696	CA(C) ₆ (A) ₅ (CA) ₁₂ GA	F: CCTCCACCTCCACCTTTAT R GCGTGTTCGCTCTTTGTAAAC	58	192–212	26	7	0.69	0.79	NS
<i>Tmac74</i>	HM134219	(AG) ₁₈ GG(AG) ₁₇	F: ATCGATTTTCATCAACAGGT R: GTCTTTCTCTCTGCGTTTCT	58	116–168	23	23	1.00	0.96	NS

* Significant after sequential Bonferroni correction ($P < 0.0035$)

^a *Tmac6* and *Tmac7* are derived from the same sequence

estimation of genetic diversity and population structure in this species.

Microsatellite DNA markers were developed using Glenn and Schable's (2005) protocols. Total genomic DNA was isolated from 20 to 50 mg of ethanol preserved skin tissue collected from an individual collected in Bahía de San Luis Gonzaga (30°02'00"N; 114°29'10"W) during 2006. A microsatellite-enriched partial DNA library was developed using the following mix of biotinylated oligos (Sigma-Genosys, The Woodlands, Texas): AC₁₂ AG₁₂, AAT₈, ATG₈, GGAT₆, ATCC₆, AACC₆, and AAGC₆. Final clone sequencing techniques employed a direct sequencing technique whereby clones that were putatively positive for microsatellite DNA inserts were individually picked from solid culture media and added to 20 µL of distilled water, lysed at 95°C for 5 min, chilled, and then centrifuged at 4°C, 4,000 RPM for 30 min. Exo-SAP It (USB Corp., Cleveland, OH) was used to clean PCR products prior to sequencing on a CEQTM 8000 DNA Analyzer (Beckman-Coulter, Inc., Fullerton, CA) using both forward and reverse M13 primers (Promega Corp., Madison, WI). Clones were isolated, amplified, purified, and sequenced. New primers were designed using Primer3 software (Rozen and Skaletsky 2000). PCR was carried out in 15 µL volume using a iCycler thermocycler (Bio Rad, Hercules, CA). Reactions contained <50 ng genomic DNA, 1× PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl), 0.2 mM of each dNTP, 0.2 µM of each primer, 1.5 µM of MgCl₂ and 0.5 U *Taq* DNA polymerase (Invitrogen, Carlsbad, CA). Cycling conditions included an initial denaturation at 94°C 5 min, followed by 30 cycles at 94°C 30 s, 30 s at locus-specific annealing temperature (Table 1) and extension at 72°C 30 s, followed by a final extension at 72°C 5 min. PCR products were run in 6% polyacrylamide gels with 7.5 M urea and visualized by silver staining. Allele sizes were determined using a 10-bp ladder (Invitrogen, Carlsbad, CA).

We characterized polymorphism of each marker using a sample of 26 totoabas collected from the upper Gulf of California, Mexico. We first checked for potential presence of null alleles using MICRO-CHEKER v2.2.3 (Van Oosterhout et al. 2004). The number of alleles per locus (N_a), expected (H_e) and observed (H_o) heterozygosity were obtained using GENALEX v6 (Peakall and Smouse 2006). Deviations from Hardy-Weinberg equilibrium, and linkage disequilibrium between markers were tested using GENEPOP version 3.4 (Raymond and Rousset 1995).

Locus designation, GenBank accession numbers, repeat motifs, PCR product sizes, number of alleles observed, observed and expected heterozygosity, and primer sequences for the 14 microsatellite markers are listed in Table 1. The

number of alleles per locus ranged from 3 (*Tmac03*, *Tmac07*, *Tmac43*) to 23 (*Tmac74*). Observed heterozygosity among the loci ranged from 0.23 (*Tmac43*) to 1 (*Tmac51* and *Tmac74*) with a mean of 0.67 and expected heterozygosity ranged from 0.33 (*Tmac43*) to 0.96 (*Tmac74*) with a mean of 0.69. No statistically significant deviations from Hardy-Weinberg expectations, except for locus *Tmac43* which demonstrated a heterozygote deficit due to null alleles, no significant genetic disequilibrium was observed following Bonferroni correction ($P = 0.00078$).

These 14 microsatellite loci are the first developed for *T. macdonaldi*. They will be useful in studies related to population genetics, ecology, conservation and fisheries management in that species.

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