Molecular Ecology Notes (2007) 7, 141–143 doi: 10.1111/j.1471-8286.2006.01556.x

PRIMER NOTE

Isolation and characterization of 13 polymorphic microsatellite loci for the Florida pompano, *Trachinotus carolinus*

SEIFU SEYOUM,* STEVEN H. DENISON† and MICHAEL D. TRINGALI*
*Florida Fish and Wildlife Research Institute, 100 Eighth Avenue SE, Saint Petersburg, FL 33701-5095, USA, †Collegium of Natural Sciences, Eckerd College, 4200 54th Avenue, South Saint Petersburg, FL 33711, USA

Abstract

Here we describe 13 polymorphic, dinucleotide microsatellite loci for *Trachinotus carolinus* (Florida pompano), isolated by using PIMA, a polymerase chain reaction-based technique. The number of alleles at each locus ranged from 3 to 29 (mean = 11.4) in 45 specimens collected from bay and nearshore waters around St Petersburg, Florida. Levels of expected and observed heterozygosities ranged from 0.15 to 0.94 (mean = 0.69) and from 0.16 to 0.98 (mean = 0.70), respectively. No significant departures from Hardy–Weinberg equilibrium expectations were observed. In exact tests for genotypic disequilibrium, there was no evidence of linkage for any pair of loci. The ability of these markers to cross-amplify in two congeneric *Trachinotus* species — *T. falcatus* (permit) and *T. goodei* (palometa) — was also assessed. The loci were well-resolved, highly polymorphic, and independently segregating in these taxa, also suggesting a general utility for intraspecific studies, species identification, and investigation of interspecific hybridization.

Keywords: cross-species amplification, Florida pompano, microsatellites, palometa, permit, *Trachinotus carolinus*, *Trachinotus falcatus*, *Trachinotus goodei*

Received 14 July 2006; revision accepted 7 August 2006

The Florida pompano, *Trachinotus carolinus*, is one of six Western Atlantic members of the carangid fish genus *Trachinotus*, which is composed of 20 species worldwide. Florida pompano occur from Massachusetts southward to Brazil, but are absent from waters of Bahamas and similar clear-water islands (Robins & Ray 1986; Smith 1997). They inhabit coastal waters, bays, and estuaries. During part of the year, Florida pompano occur in beach environments, where they are easily accessible to anglers who fish from shore or wade into shallow water. At other times, they form pelagic schools over offshore reef and rocky environments. The Florida pompano is the highest-priced marine food fish (for non-sushi grade products) harvested from the USA Exclusive Economic Zone. In Puerto Rico, the species is actively being developed for open-water net-pen aquaculture. In Florida, it ranked high on a prioritized list of candidate species for marine aquaculture (Anon. 1998). At present, the genetic stock structure of the species is unknown, although Florida managers have assessed Gulf of Mexico and Atlantic pompano separately because of different fishery dynamics. In the Gulf of Mexico, two discernable pulses of recruitment occur (late spring and late summer) and two morphological forms putatively exist. Thus, two or more distinct stocks within the Gulf of Mexico may be unknowingly grouped into a single assessment unit. Microsatellite markers have been used to investigate genetic stock structure, assign individuals to source populations, and reveal patterns of migration and recruitment in fishes (e.g. Gold *et al*. 2002; Berry *et al*. 2004; Castric & Bernatchez 2004). Here we report polymerase chain reaction (PCR) primers for 13 dinucleotide polymorphic microsatellite loci for the Florida pompano, and also report their ability to cross amplify in two closely related congeneric, coranging *Trachinotus* species — permit, *T. falcatus*, and palometa, *T. goodei*.

Microsatellite loci were isolated following the polymerase chain reaction (PCR)-based isolation of microsatellite arrays (PIMA) method of Lunt *et al*. (1999). Our modifications to

Correspondence: Michael D. Tringali, Fax: +01-727-823-0166; E-mail: mike.tringali@myfwc.com

© 2006 The Authors
Journal compilation © 2006 Blackwell Publishing Ltd

Table 1 Characterization of 13 microsatellite loci for 45 specimens of *Trachinotus carolinus*

Locus	Primer sequence (5′–3′) and fluorescent dye	Repeat motif	k	Allele size range	H_O	H_E	GenBank Accession no.
*Tca*01	F: AACAATTGTCTTCATACATGCAGTT-FAM R: GGTAGAAGAATCGTCCTGGTGT	$(GT)_{21}$	7	211–225	0.78	0.74	DQ375765
*Tca*04	F: TAAACTCATTTATGGGGCCTG-FAM R: GTGCCGTTCATTCACAGATAA	$(AC)_9$	3	173–175	0.16	0.15	DQ375766
*Tca*05	F: ATGGAGGAAAAGTTCAACAAGA-NED R: GTTCCAACAGAGTTTGTGACATTT	$(GT)_{14}$	21	157–283	0.77	0.87	DQ375767
*Tca*06	F: AAATATTAATGAGCTTTGAGACCAC-FAM R: AGAGGGAGCAAGATGGTCTCT	$(GT)_{20}$	21	209–283	0.89	0.88	DQ375768
*Tca*08	F: AAGAGAGGGGGCAGAATAAAA-FAM R: AAATAGCACTGTTTTCATCAATGTG	$(AC)_{15}$	5	140–152	0.61	0.61	DQ375769
*Tca*09	F: TGGAGGAGCAGCGTTATATTA-FAM R: ACATGCTGCAGGGACTAGAA	$(AC)_{22}$	9	105–147	0.61	0.63	DQ375770
*Tca*10	F: GCAAGAAACCACATTCGAC-NED R: ATAGGTCAGTTAAGTATGCCTGG	$(AC)_{26}$	11	137–163	0.89	0.85	DQ375771
*Tca*11	F: GGTGGATCAATAAAAACACCTC-NED R: AGCATTTCAACACAGCGTG	$(TG)_5/(TG)_{16}$	6	228–248	0.53	0.48	DQ375772
*Tca*13	F: CCTGCTCCTGTTGAACTCTG-NED R: AAATGTGAATGGGTACGGAG	$(TC)_{11}$	3	131–147	0.59	0.51	DQ375773
*Tca*15	F: ACCACCCACCAAACACAC-HEX R: AGAAGAGGGGATGACAGGAATAC	$(TC)_7(AC)_{31}/(CT)_{12}$	29	248–320	0.98	0.94	DQ375774
*Tca*18	F: CCAGCAGCTTCATCACTGATT-HEX R: AACTGCATAACCAGAACAGAAAGG	$(AC)_{22}$	17	101–149	0.93	0.91	DQ375775
*Tca*19	F: CACGGCCATGAATATGCATA-HEX R: AGCAGCGTTCCACTAATAGGG	$(CA)_{20}$	6	181–191	0.77	0.79	DQ375776
*Tca*22	F: AATCGCCGTCACACTCCCACG-FAM R: GCAGCAACTGCCGCTTCACG	$(CA)_{27}$	10	79–115	0.63	0.65	DQ375764

k, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.

this method have been previously reported (Seyoum *et al*. 2005). Briefly, nuclear DNA (nDNA) was purified from a single Florida pompano specimen and used to seed multiple random amplified polymorphic DNA (RAPD) PCRs. Each 50-µL RAPD-PCR contained 15-ng of nDNA, two randomly chosen primers from a set of 2010-mer RAPD primers (Operon Technologies) and *Taq* polymerase/buffer (Promega). The reaction profile was 94 °C for 2 min, 32 × (94 °C for 40 s, 55 °C for 40 s, 72 °C for 45 s) and 72 °C for 7 min. Purified PCR products (StratPrep, Stratagene) were cloned into plasmid T-vectors (Bluescript PBC KS-, Stratagene). Recombinant colonies were screened by performing PCR containing T3 and T7 vector primers and five repeat-specific primers [5′-$(AC)_{10}$-3′, 5′-$(AG)_{10}$-3′, 5′-$(ATT)_{12}$-3′, 5′-$(AGC)_8$-3′, and 5′-$(ACC)_8$-3′]. Colonies for which PCR products had ≥ two bands in 1.5% low-EEO agarose gels were selected for another round of PCR, this time with only vector primers. PCR products were cycle-sequenced from both directions; sequencing products were visualized on an Applied Biosystems ABI PRISM 3100-*Avant* Genetic Analyser. PCR primers were designed for 15 candidate loci by using OLIGOPERFECT DESIGNER (http://www.invitrogen.com/); forward primers were 5′-end-labelled with a fluorescent dye. Under the PCR profile described above, these markers were used to genotype 45 Florida pompano specimens collected from bay and nearshore waters of St Petersburg, Florida, and 45 specimens each of permit and palometa obtained from Puerto Rico. Fragments were visualized on an ABI 3130 genetic analyser and genotyped by using GENEMAPPER software version 3.7 (Applied Biosystems). For all fragment assays, we used a custom ROX-labelled size standard (fragment range; 78–424 bp), which was developed in-house following DeWoody *et al*. (2004). Thirteen loci were found to be polymorphic in Florida pompano (Table 1); 13 loci cross-amplified in permit and 9 were polymorphic; 11 loci cross-amplified in palometa and 10 were polymorphic (Table 2).

Number of alleles, observed and expected heterozygosities, conformance to Hardy–Weinberg equilibrium (HWE) expectations, and population genotypic disequilibrium were assessed using GENEPOP (http://wbiomed.curtin.edu.au/genepop/). For the Florida pompano, the average number of alleles per locus was 11.4, the mean observed heterozygosity was 0.70, and the mean expected heterozygosity was 0.69. For permit and palometa, the average number of alleles per locus was 8.2 and 3.9, the mean observed

© 2006 The Authors
Journal compilation © 2006 Blackwell Publishing Ltd

Table 2 Characterization of 13 microsatellite loci for 45 specimens each of *Trachinotus falcatus* and *T. goodei*

T. falcatus					*T. goodei*				
Locus	k	Allele size range	H_O	H_E	Locus	k	Allele size range	H_O	H_E
*Tca*01	4	195–231	0.37	0.34	*Tca*01	5	210–218	0.60	0.56
*Tca*04	1	170	—	—	*Tca*04	3	169–175	0.21	0.23
*Tca*05	20	186–240	0.88	0.87	*Tca*05†	—			
*Tca*06	36	245–305	1.00	0.96	*Tca*06	3	221–229	0.53	0.48
*Tca*08	1	132	—	—	*Tca*08*	2	136–142	0.08	0.46
*Tca*09	3	131–139	0.30	0.36	*Tca*09	4	139–145	0.23	0.21
*Tca*10	9	139–153	0.67	0.75	*Tca*10	3	133–137	0.07	0.11
*Tca*11	1	200	—	—	*Tca*11†	—			
*Tca*13*	16	149–197	0.47	0.84	*Tca*13	6	143–153	0.50	0.55
*Tca*15	2	218–220	0.33	0.44	*Tca*15	6	248–256	0.60	0.61
*Tca*18	5	97–105	0.48	0.59	*Tca*18	1	89	—	—
*Tca*19	7	193–203	0.78	0.78	*Tca*19	8	179–193	0.81	0.81
*Tca*22	1	77	—	—	*Tca*22	2	69–75	0.02	0.02

k, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.
*denotes significant departure from Hardy–Weinberg equilibrium $P < 0.05$.
†no amplification in PCR.

heterozygosity (for polymorphic loci) was 0.59 and 0.37, and the mean expected heterozygosity (for polymorphic loci) was 0.66 and 0.40, respectively. In Florida pompano, no significant departures from Hardy–Weinberg or genotypic equilibrium expectations were observed. However, highly significant departures from HWE expectations ($P < 0.0001$) were observed at one locus (*Tca*13) in permit and at one locus (*Tca*08) in palometa (Table 2). In both cases, the deviations were heterozygote deficits, possibly the result of null allelism.

The above markers provide the first known set of microsatellite DNA loci isolated for a member of *Trachinotus*. Given the high rate of successful cross-species amplification and wide variation in allele sizes among the congeneric species, the primers reported here provide potentially useful markers for intraspecific studies, genetic species identification, and investigations of introgressive hybridization for the genus.

Acknowledgements

We thank Kathy Guindon, Chris Powell, Liz Wallace, Jim McVey, and José A. Rivera for assistance with specimen collection. This work was supported financially by the State of Florida and the Federal Sport Fish Restoration Act, Department of the Interior, US Fish and Wildlife Service, Grant F-69.

References

Anonymous (1998) Developing Florida's marine food fish industry. Proceedings from the Marine Aquaculture Development Workshop, April 16–18, 1998. Florida Department of Agriculture and Consumer Services, Tallahassee, Florida.

Berry O, Tocher MD, Sarre SD (2004) Can assignment tests measure dispersal? *Molecular Ecology*, **13**, 551–561.

Castric V, Bernatchez L (2004) Individual assignment test reveals differential restriction to dispersal between two salmonids despite no increase of genetic difference with distance. *Molecular Ecology*, **13**, 1299–1312.

DeWoody JA, Schupp J, Kenefic L, Busch J, Murfitt L, Keim P (2004) A universal method for producing ROX-labeled size standards suitable for automated genotyping. *BioTechniques* **37**, 348–352.

Gold JR, Pak E, DeVries DA (2002) Population structure of king mackerel (*Scomberomorus cavalla*) around peninsular Florida, as revealed by microsatellite DNA. *Fishery Bulletin*, **100**, 491–509.

Lunt D, Hutchinson W, Carvalho G (1999) An efficient method or PCR-based isolation of microsatellite arrays (PIMA). *Molecular Ecology*, **8**, 891–894.

Robins CR, Ray GC (1986) *A Field Guide to Atlantic coast fishes of North America*. Houghton Mifflin Company, Boston.

Seyoum S, Tringali MD, Sullivan JG (2005) Isolation and characterization of 27 polymorphic microsatellite loci for the common snook, *Centropomus undecimalis*. *Molecular Ecology Notes*, **5**, 9242–9927.

Smith CL (1997) National Audubon Society field guide to tropical marine fishes of the Caribbean, the Gulf of Mexico, Florida, the Bahamas, and Bermuda. Alfred A. Knopf, New York.

© 2006 The Authors
Journal compilation © 2006 Blackwell Publishing Ltd