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## Development and characterization of 11 novel microsatellite loci for the roundscale spearfish *Tetrapturus georgii* and their cross-species amplification among other istiophorid species

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Eleven novel polymorphic microsatellite loci were developed and characterized for the recently validated roundscale spearfish *Tetrapturus georgii*. Characterization of these markers, based on 35 roundscale spearfish from the western North Atlantic, revealed two to 21 alleles per locus with an average expected heterozygosity ( $H_E$ ) of 0.09–0.94, and all loci conformed to Hardy–Weinberg expectations. Cross-amplification of these 11 loci against all other eight known istiophorid species indicates promising prospects for the utility of these markers for istiophorids in general.

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Key words: Atlantic; billfish; Istiophoridae; population genetics; primer sequence.

There is increasing concern about the population status of many billfish (Istiophoridae) species as they are heavily fished in pelagic fisheries either as targets or by-catch, and their management is made complex due to the international nature of their fisheries (Collette *et al.*, 2011a). Adding to this management complexity is that one billfish, the roundscale spearfish *Tetrapturus georgii* Lowe 1841, was only recently validated as a legitimate species (Shivji *et al.*, 2006). The lack of historical recognition of *T. georgii* is due to its strong morphological similarity to the sympatric white marlin *Kajikia albida* (Poey 1860), itself a severely overfished and IUCN Red List vulnerable species (Collette *et al.*, 2011a, b). To make matters worse, it is now clear that *T. georgii* has also frequently been misidentified as the longbill spearfish *Tetrapturus pfluegeri* Robins & de Sylva 1963, another very similar looking billfish species (unpubl. data).

Given its recent validation, almost nothing is known about the biology and population structure of *T. georgii*. The species is currently listed as data deficient on the IUCN Red List (Collette *et al.*, 2011b). It is suspected to be a solitary species capable

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TABLE I. Characterization of 11 microsatellite loci in *Tetrapturus georgii* in western North Atlantic Ocean waters, including GenBank accession number, primer sequences, M13 dye label, repeat motif, annealing temperature ( $T_A$ ), number alleles observed ( $A$ ), allele size range, observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosities, and Hardy–Weinberg equilibrium values (HWE)

Locus	GenBank	Primer sequence (5'–3') forward (F) and reverse (R)	Primer 5' label	Repeat motif	$T_A$ (°C)	$A$ (size range (bp))	$H_O$	$H_E$	HWE
<i>tge23</i>	JQ755290	*F: GGAAATGGGTGGATGAAATG R: CTACATGGCTTGCTGGCAIT	6-FAM	(TGGA) <sub>3</sub> TAGATGGG(TGGA) <sub>15</sub>	58	3 (223–231)	0.43	0.49	0.19
<i>tge54</i>	JQ755291	*F: GCTCTAGCGCCCTACTTCAG R: TCTGCAGCCTGTCTGCTAA	VIC	(AC) <sub>20</sub>	58	12 (202–232)	0.83	0.86	0.26
<i>tge76</i>	JQ755292	*F: GTGGGAAGTTGGCTTGTTCAT R: TAAACGGGCTTGTTCACAT	NED	(TG) <sub>10</sub>	58	3 (103–109)	0.09	0.08	1.00
<i>tge79</i>	JQ755293	*F: CTGTCTCCGGCAACTGATTT R: CGGTTTCACAGAGAACTCAGG	6-FAM	(TACA) <sub>6</sub>	58	14 (129–197)	0.86	0.82	0.66
<i>tge105</i>	JQ755294	*F: GCGCTGGTTGTAGAGGTGT R: AGTCCACCTGTGTGTATGTG	PET	(AC) <sub>12</sub>	60	11 (193–227)	0.69	0.73	0.43
<i>tge119</i>	JQ755295	*F: CCCAGGCAAGAACTAAGAG R: TGATTTTCAGCATGGTGCTC	6-FAM	(TG) <sub>16</sub>	60	20 (187–229)	0.57	0.49	0.53
<i>tge121</i>	JQ755296	*F: TTGCAATTAATTAAGAAGAAGCAG R: TGGTTTGAACCTGCAGCAGAG	VIC	(TATG) <sub>10</sub>	60	2 (229–233)	0.86	0.89	0.48
<i>tge135</i>	JQ755297	*F: TTTTCACTCCGAGTGTCCAGC R: CACTCTGGAGGAATGTCCGATT	NED	(ACAT) <sub>16</sub>	60	21 (177–277)	0.83	0.94	0.13
<i>tge139</i>	JQ755298	*F: ACCGGAACAATAGACATTTGG R: ATTGATGATGGACTGTGCTGA	PET	(TATG) <sub>7</sub>	60	5 (111–127)	0.77	0.65	0.72
<i>tge144</i>	JQ755299	*F: TCTFACCCTTAATGGTGTCTACCC R: ACCTGTGGAAACCTGAGTG	PET	(ATGG) <sub>9</sub>	60	17 (114–186)	0.83	0.83	0.31
<i>tge151</i>	JQ755300	*F: CCATTAGGCTTAACGAACCAA R: TCGATAACACTGTGTATGGGCTA	VIC	(GT) <sub>16</sub>	60	8 (191–205)	0.60	0.67	0.11

\*Primer 5' end labelled with M13 tail (5'-TGTA AACGACGGCCAGT-3') (Schuelke, 2000).

TABLE II. Results of cross-amplification testing of 11 microsatellite loci from *Tetrapturus georgii*. Cross-amplification was performed across eight species of Istiophoridae using a single individual per species

Species	Locus										
	<i>tge23</i>	<i>tge54</i>	<i>tge76</i>	<i>tge79</i>	<i>tge105</i>	<i>tge119</i>	<i>tge121</i>	<i>tge135</i>	<i>tge139</i>	<i>tge144</i>	<i>tge151</i>
White marlin <i>Kajikia albida</i>	-	-	+	-	+	+	+	+	+	-	+
Striped marlin <i>Kajikia audax</i>	+	+	+	+	+	+	+	+	+	+	+
Blue marlin <i>Makaira nigricans</i>	+	+	+	+	+	+	+	+	+	+	+
Black marlin <i>Makaira indica</i>	+	+	+	+	+	+	+	+	-	+	+
Longbill spearfish <i>Tetrapturus pfluegeri</i>	+	+	+	+	+	+	+	+	-	+	+
Mediterranean spearfish <i>Tetrapturus belone</i>	+	+	+	+	+	+	+	+	+	+	+
Shortbill spearfish <i>Tetrapturus angustirostris</i>	+	+	+	+	+	+	+	+	+	-	+
Sailfish <i>Istiophorus platypterus</i>	+	+	+	+	+	+	+	+	+	+	+

+, positive amplification; -, an absence of clean amplification of the species at the particular locus.

of extensive migrations (Arocha & Ortiz, 2006), and probably plays an ecosystem role as an apex predator like other istiophorid species. Of concern from a conservation and management perspective is that *T. georgii* may have been subject to similar levels of overexploitation as *K. albida* (Beerkircher *et al.*, 2009). Understanding the life history, population structure, evolutionary history and fishery dynamics of *T. georgii* is therefore of great importance for conservation and management purposes.

The development and characterization of 11 microsatellite loci markers for assisting with these efforts is reported here. The cross-species amplification performance of these markers on eight other istiophorid species of conservation and management interest is also presented. Tissue samples (fin clips) were collected from 35 *T. georgii* individuals captured in western North Atlantic Ocean pelagic fisheries. The samples were stored in 95% ethanol until DNA extraction. Genomic DNA (gDNA) was extracted from 25 mg of tissue using the DNeasy Kit (QIAGEN; www.qiagen.com) following the manufacturer's protocol.

Microsatellite marker development was performed utilizing Glenn & Schable's (2005) enrichment protocol. gDNA from a single *T. georgii* was digested using BstUI and XmnI, followed by ligation of SuperSNX24 linkers to the ends of the digested gDNA fragments to act as priming sites for subsequent polymerase chain reactions (PCR). Biotinylated tetranucleotide probes [(AAAT)<sub>8</sub>, (AACT)<sub>8</sub>, (AAGT)<sub>8</sub>, (ACAT)<sub>8</sub> and (AGAT)<sub>8</sub>] were hybridized to the gDNA fragments. The biotinylated probe-gDNA complex was then added to magnetic beads coated with streptavidin (Dynabeads M-280: Invitrogen; www.invitrogen.com) and washed twice with ×2 sodium chloride, sodium citrate (SSC), 0.1% sodium dodecyl sulphate (SDS) and four times with 1×SSC, 0.1% SDS at 52° C. For the final two washes, the solution was incubated at 52° C for 1 min. Enriched fragments were removed from the biotinylated probes by denaturation at 95° C and subsequently precipitated using 95% ethanol and 3 M sodium acetate. To increase the amount of enriched fragments, a recovery PCR was performed in a 25 µl reaction containing 1× PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.5 mM MgCl<sub>2</sub>, 0.16 mM of each deoxynucleotide triphosphate (dNTP), 10× bovine serum albumin (BSA), 0.52 µM of the Super-SNX24 forward primer, 1U *Taq* DNA polymerase (QIAGEN), and *c.* 25 ng enriched gDNA fragments. Thermal cycling was performed in a Bio-Rad DYAD (Bio-Rad; www.bio-rad.com) as follows: 95° C for 2 min, followed by 25 cycles of 95° C for 20 s, 60° C for 20 s and 72° C for 90 s and a final elongation step of 72° C for 30 min. The resulting PCR fragments were cloned using the TOPO-TA Cloning kit following the manufacturer's protocol (Invitrogen). Bacterial colonies containing a vector with gDNA were used as a template for subsequent PCR in a 25 µl reaction containing 1× PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.5 mM MgCl<sub>2</sub>, 0.12 mM of each dNTP, 10× BSA, 0.25 µM of the M13 primers and 1U *Taq* DNA polymerase. Thermal cycling was as follows: an initial denaturing step of 95° C for 7 min, followed by 35 cycles of 95° C for 20 s, 50° C for 20 s and 72° C for 90 s. PCR products were subsequently cleaned using MultiScreen-PCR Filter plates following the manufacturer's protocol (Millipore; www.millipore.com), and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems; www.appliedbiosystems.com). Sequencing reactions were precipitated with ethanol and 125 mM EDTA and run on a 3730 DNA Analyzer (Applied Biosystems). Primers flanking core microsatellite repeats were developed using Primer3

(Rozen & Skaletsky, 2000). A total of 74 primer pairs were tested to determine amplification success and relative levels of polymorphism. Of these primer pairs, 11 amplified *T. georgii* consistently and showed evidence of polymorphism. Characterization of these loci was subsequently performed on 35 individuals.

All microsatellite PCRs were conducted in a total reaction volume of 25  $\mu$ l and contained 1  $\mu$ l of unquantified genomic DNA, 1 $\times$  PCR buffer, 0.2 mM of each dNTP, 0.33 mM MgCl<sub>2</sub>, 0.4  $\mu$ M of the fluorescently labelled universal M13 primer (5'-TGAAAACGACGGCCAGT-3') and the species-specific reverse primer, 0.16  $\mu$ M of the species-specific forward primer with a 5'-M13 tail (Schuelke, 2000) and 0.5U of HotStar *Taq* DNA Polymerase (QIAGEN). PCR was performed in a Mastercycler Gradient (Eppendorf; www.eppendorf.com) thermal cycler as follows: an initial denaturing step of 95° C for 15 min, followed by 35 cycles of 94° C for 1 min, 1 min at the primer annealing temperature (Table I), 72° C for 1 min, followed by a final extension of 20 min at 72° C. Amplification products were resolved on an AB3130 genetic analyser (Applied Biosystems) and scored using LIZ 600 as the internal allele size standard and the software GeneMapper 3.7 (Applied Biosystems).

Expected and observed heterozygosities and departures from Hardy–Weinberg (HWE) and linkage equilibrium (LE) were assessed using the software Genepop v4.0 on the web (Raymond & Rousset, 1995; Rousset, 2008). Significance was estimated using Fisher's exact test (1000 dememorizations, 100 batches and 1000 iterations) and Markov Chain Monte-Carlo iterations. The frequency of null alleles was estimated using the programme FreeNA (Chapuis & Estoup, 2007). A summary of the microsatellite loci is presented in Table I. Expected heterozygosities varied considerably across loci (0.09–0.94); however, no evidence of departures from HWE and LE were found and the frequency of null alleles was negligible (*e.g.*, <5.0%). Cross-amplification success of these 11 loci was tested on eight istiophorid species. High levels of successful amplification were found across species (Table II).

The microsatellite loci described here will be useful for a variety of population structure and demographic history studies of the little understood *T. georgii*. They also show potential for use in similar studies of other istiophorid species, all of whom are exploited in multinational fisheries.

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## References

- Beerkircher, L., Arocha, F., Barse, A., Prince, E., Restrepo, V., Serafy, J. & Shivji, M. (2009). Effects of species misidentification on population assessment of overfished white marlin *Tetrapturus albidus* and roundscale spearfish *T. georgii*. *Endangered Species Research* **9**, 81–90.
- Chapuis, M.-P. & Estoup, A. (2007). Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* **24**, 621–631.
- Collette, B. B., Carpenter, K. E., Polidoro, B. A., Juan-Jordá, M. J., Boustany, A., Die, D. J., Elfes, C., Fox, W., Graves, J., Harrison, L. R., McManus, R., Minte-Vera, C. V., Nelson, R., Restrepo, V., Schratwieser, J., Sun, C.-L., Amorim, A., Brick Peres, M.,

- Canales, C., Cardenas, G., Chang, S.-K., Chiang, W.-C., de Oliveira Leite, N. Jr, Harwell, H., Lessa, R., Fredou, F. L., Oxenford, H. A., Serra, R., Shao, K.-T., Sumaila, R., Wang, S.-P., Watson, R. & Yáñez, E. (2011a). High value and long life – double jeopardy for tunas and billfishes. *Science* **333**, 291–292.
- Glenn, T. C. & Schable, N. A. (2005). Isolating microsatellite DNA loci. *Methods in Enzymology* **395**, 202–222.
- Raymond, M. & Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248–249.
- Rousset, F. (2008). Genepop '007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* **8**, 103–106.
- Rozen, S. & Skaletsky, H. J. (2000). Primer3 on the WWW for general users and for biologist programmers. In *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (Krawetz, S. & Misener, S., eds), pp. 365–386. Totowa, NJ: Humana Press.
- Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* **18**, 233–234.
- Shivji, M. S., Magnussen, J. E., Beerkircher, L. R., Hinteregger, G., Lee, D. W., Serafy, J. E. & Prince, E. D. (2006). Validity, identification, and distribution of the roundscale spearfish, *Tetrapturus georgii*, (Teleostei: Istiophoridae): morphological and molecular evidence. *Bulletin of Marine Science* **79**, 483–491.

### Electronic References

- Arocha, F. & Ortiz, M. (2006). *Roundscale spearfish*. *ICCAT Field Manual*. Chapter 2.1.8.4. Available at <http://www.iccat.es/en/ICCATManual.asp> (last accessed 18 August, 2012).
- Collette, B., Amorim, A. F., Bizsel, K., Boustany, A., Carpenter, K. E., de Oliveira Leite, N. Jr, Die, D., Fox, W., Fredou, F. L., Graves, J., Viera Hazin, F. H., Hinton, M., Juan Jordá, M., Masuti, E., Minte Vera, C., Miyabe, N., Nelson, R., Oxenford, H., Restrepo, V., Schratwieser, J., Teixeira Lessa, R. P. & Pires Ferreira Travassos, P. E. (2011b). *Kajikia albida*. *IUCN Red List of Threatened Species*. Version 2011.2. Available at [www.iucnredlist.org](http://www.iucnredlist.org) (last accessed 26 January, 2012).