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TECHNICAL NOTE

Isolation and characterization of 9 polymorphic microsatellite markers for the endangered boring giant clam (*Tridacna crocea*) and cross-priming testing in three other Tridacnid species

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Abstract We report the development and characterization of nine microsatellite markers from the *Tridacna crocea* genome. In a wild population of 20 individuals from Ulugan Bay in the Republic of the Philippines, we observed an average of 9 alleles per locus and expected heterozygosity ranging from 0.41 to 0.92. Cross-species priming was tested in three additional *Tridacna* species with moderate success. These markers will be used in studies of fine-scale population genetic structure and gene flow in *T. crocea.* The markers may also be useful in a forensic context by identifying source populations of giant clams collected illegally for the live aquarium trade, while protecting legal exports from aquaculture facilities.

Keywords Boring giant clam · *Tridacna crocea* · Microsatellites · *Symbiodinium*

Giant clams are an important part of coral reef habitats, providing reef stability and settlement substrate. Individuals are harvested for the aquarium trade, subsistence food, and for the shell trade (Copland and Lucas 1988) resulting in population decline due to overfishing. All species are listed in Appendix II of CITES (UNEP-WCMC 2007) and on the IUCN Red List of Threatened Species (Mollusk Specialist Group 1996). Restocking efforts are ongoing, especially in the Philippines (Gomez and Mingoa-Licuanan 2006). In order to provide genomic tools for inferring fine-scale population genetic structure and demographic parameters on recent time scales, we developed 9 new microsatellite markers for the boring giant clam (*Tridacna crocea*) and tested for cross-amplification in three congeners.

We isolated genomic DNA from fresh muscle tissue of one individual obtained legally from the aquarium trade using the QIAGEN DNeasy Tissue Kit and digested with a combination of AluI, XmnI, and RsaI. STR enrichment followed Glenn and Schable (2005) except for our choice of biotinylated oligonucleotide probes: $(AC)_{13}$ $(AAC)_6$, $(ACAT)_8$, $(ACTC)_6$, $(AGAT)_8$, and $(ATCC)_5$. Plasmids were cloned with PROMEGA pGEM-T Vector System I cloning kit according to the manufacturer's protocols and grown on LB agar plates containing ampicillin and X-gal. Positive clones were recovered in PCR reactions using T7 and M13R universal primers and sequenced using the BigDye Terminator method (ABI) on an ABI 3730. Sequences were edited in Sequencher 4.6 (Gene Codes) and short tandem repeats (STRs) were identified with the Simple Sequence Repeat Identification Tool (Temnykh et al. 2001). We designed primers in PRIMER3 (Rozen and Skaletsky 2000) and screened them using AUTODIMER (Vallone and Butler 2004).

We assessed allelic variation in 20 individuals from Ulugan Bay, Philippines. Cross-priming was tested on 8 individual *T. maxima* and *T. squamosa* and 3 individual *T. gigas.* Genomic DNA was extracted using 10% Chelex (Walsh et al. 1991). PCR amplification was in 10 µl reactions containing 1.0 µl DNA template, 0.25U Taq Gold polymerase (ABI), 1 µl 10 × PCR Buffer, 1 µl dNTPs

Electronic supplementary material The online version of this article (doi:10.1007/s12686-010-9249-7) contains supplementary material, which is available to authorized users.

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Table 1 Microsatellite loci and thermocycling parameters

Locus	Primer	Sequence	T_a (°C)	Special PCR conditions	PCR multiplex	STR Motif	Dye	Size range (bp)	N_a	H_E	H_O	HWE (*P*-value)
Tc004	Tc04 7F	TTT ACG AAT TAT GCA ACA GAC CTC	47.1	2 × MgCl2	1	$(TG)_{13}$	NED	134–141	3	0.447	0.400	0.232
	Tc04 162R	CCT ATG TTG TGG GCA CTG AA	47.1	2 × MgCl2								
Tc160	Tc160 196R	TGC TTT ATT GTC TTA ACT GCC ATT	47.1	2 × MgCl2	1	$(AGAT)_9$	PET	146–204	8	0.772	0.650	0.133
	Tc160 33F	CGG AAG GTT GGG TAG GTA GA	47.1	2 × MgCl2								
Tc040	Tc040 39F	GCA CGG TTC CAA GAA CAT TT	50		2	$(ATCT)_{10}$	NED	123–151	4	0.406	0.300	0.485
	Tc040 183R	AAT CGG AGC GGT TCA ATC TT	50									
Tc050	Tc050 56F	CAT CCA CCC ATC CAT CAA TC	50		2	$(AATC)_8$	6-FAM	106–180	9	0.858	0.650	0.010
	Tc050 195R	TCA GGA TGA ACC ACA GGT CA	50									
Tc157**	Tc157 48F	TGC TTG CAA AGC ACT AAA ACA	50		2	$(CA)_{12}$	PET	96–134	9	0.806	0.692	0.130
	Tc157 148R	GAT TGT GCT GCT GCT CTG TG	50									
Tc092	Tc092 140F	GCA ACC CAC TGA GTT CCC TA	50		3	$(TC)_{13}TTAA(AC)_{18}$	6-FAM	176–234	16	0.915	0.300	0.000*
	Tc092 356R	TGC AGA GCG ATA ACA TAC AGG	50									
Tc059	Tc059 8F	AGG TGA CTT GAA GGT TAA TGT TG	57.9		4	$(AAC)_{15}$	PET	110–140	10	0.867	0.800	0.713
	Tc059 138R	GGG TTT AAA ACA ACA CGG TGA	57.9									
Tc074	Tc074 21F	CCA AAA ACA GTC TTC CTT GAC A	57.9		4	$(AGTG)_{10}$	6-FAM	202–266	11	0.850	0.700	0.129
	Tc074 271R	AGA CTG CTC GCT GAC CTT TT	57.9									
Tc161	Tc161 104R	TGA AAA TAT GTT AAC CCT CAT CCT G	57.9		4	$(AC)_8$	NED	92–122	13	0.906	0.950	0.927
	Tc161 2F	TTT GCA GAC TGG TTT AGT CAA CA	57.9									

GenBank Accession numbers for clone sequences are HM187766–HM187774

T_a annealing temperature, N_a number of alleles, H_E and H_O expected and observed heterozygosities

** Locus Tc157 was only scoreable in 13 of 20 individuals

Table 2 Cross-priming success in 3 additional *Tridacna* species

Locus	*T. maxima* ($n = 8$)			*T. squamosa* ($n = 8$)			*T. gigas* ($n = 3$)		
	N_{indiv}	Size range (bp)	N_a	N_{indiv}	Size range (bp)	N_a	N_{indiv}	Size range (bp)	N_a
Tc004	1	145–149	2	3	141–143	2	0	NA	–
Tc160	1	150	1	0	NA	–	0	NA	–
Tc040	7	123–139	5	1	135	1	3	100	1
Tc050	7	86–210	7	8	174–194	5	3	92	1
Tc092	3	180–218	4	0	NA	–	2	84–224	2
Tc059	5	95–98	2	0	NA	–	0	NA	–
Tc074	5	226–238	3	5	230	1	0	NA	–
Tc157	0	NA		6	96–108	4	2	146	1
Tc161	5	76–90	2	6	90–94	2	0	NA	–

N_{indiv} number of individuals successfully genotyped, N_a number of alleles

(8 mM), 0.8 µl $MgCl_2$ (25 mM), and 0.5 µl of each primer (10 mM). For loci Tc004 and Tc160 the amount of $MgCl_2$ was doubled and water reduced to a final volume of 10 µl. Thermocycling parameters were: 1 × 94°C (10 min), 30× [30 s at 94°C, 30 s T_a °C, 40 s at 72°C], and 1 × 72°C (60 min). Annealing temperatures (T_a) for each locus are in Table 1.

Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were assessed in ARLEQUIN 3.11 (Excoffier et al. 2005). Locus Tc157 amplified well in all samples, but was only scoreable in some individuals due to multiple peaks. Locus Tc092 showed a significant departure from HWE after Bonferroni correction (Table 1). Linkage disequilibrium was only significant in 1 out of 36 pairwise comparisons at the $P < 0.01$ level (Tc074 vs. Tc092) indicating virtually no linkage among loci.

Since no species-specific PCR optimization was attempted, the results reported here represent a minimum rate of cross-species primer conservation. The test resulted in 5 loci amplifying in *T. maxima* (5 polymorphic); 4 loci amplifying in *T. squamosa* (3 polymorphic); and 4 loci amplifying in *T. gigas* (1 polymorphic) (Table 2). The *T. gigas* were from cultured stocks, therefore the lack of polymorphism is expected and may not represent diversity in natural populations.

A technical challenge to developing microsatellites in this species is targeting the host genome rather than that of symbiotic dinoflagellates (*Symbiodinium* spp.). Symbionts are restricted to mantle tissue in giant clams, therefore we extracted genomic DNA from muscle tissue for primer development. To verify that none of our primers amplify symbiont DNA, we tested them on 7 symbiont types using PCR conditions as described above (Table S1). Excepting Tc004, no significant amplification was achieved in the range of allele sizes reported for *T. crocea* and we are confident that the loci developed are specific to giant clam DNA. For Tc004, a 128 bp fragment was amplified in two isolates. This is a common allele in *T. crocea* (T.S. DeBoer unpublished data); therefore, caution should be employed when using this locus.

Population genetic studies using mtDNA show strong structure in *T. crocea* (DeBoer et al. 2008, Kochzius and Nuryanto 2008). Microsatellites are expected to be more variable, and one application where this would be useful is forensic identification of clams sold in the aquarium trade. It should be possible to identify source regions for traded clams, as well as distinguish wild from cultured stocks. This would assist efforts to stop illegal trade in these internationally protected species. These markers will also be useful for inferring modern genetic connectivity and gene flow among natural and restored populations.

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References

Copland JW, Lucas JS (1988) Giant clams in Asia and the Pacific. Australian Center for International Agricultural Research, Canberra

DeBoer TS, Subia MD, Ambariyanto Erdmann MV, Kovitvongsa K, Barber PH (2008) Phylogeography and limited genetic connectivity in the endangered boring giant clam across the Coral Triangle. Conserv Biol J 22:1255–1266

Excoffier L, Laval G, Schneider S (2005) Arlequin, version 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. Methods Enzymol 395:202–222

Gomez ED, Mingoa-Licuanan SS (2006) Achievements and lessons learned in restocking giant clams in the Philippines. Fish Res 80:46–52

Kochzius M, Nuryanto A (2008) Strong genetic structure in the boring giant clam *Tridacna crocea* across the Indo-Malay Archipelago: implications related to evolutionary processes and connectivity. Mol Ecol 17:3775–3787

Mollusk Specialist Group (1996) *Tridacna crocea*. In: IUCN 2006. 2006 IUCN Red List of Threatened Species

Rozen S, Skaletsky H (2000) Primer 3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp 365–386

Temnykh S, DeClerck G, Lukashova A et al (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential. Genome Res 11:1441–1452

UNEP-WCMC (2007) UNEP-WCMC Species Database: CITES-Listed Species

Vallone PM, Butler JM (2004) Autodimer: a screening tool for primer–dimer and hairpin structures. BioTechniques 37:226–231

Walsh PS, Metzger DA, Huiguchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10:506–513