Title

Microsatellite loci for the blue swimming crab (Callinectes bellicosus) (Crustacea: Portunidae) from the Gulf of California, Mexico

Journal

Conservation Genetics Resources, 2(1)

ISSN

1877-7260

Authors

Munguia-Vega, A.
Torre, J.
Castillo-Lopez, A.
et al.

Publication Date

2010-12-01

DOI

10.1007/s12686-010-9192-7

Peer reviewed

Conservation Genet Resour (2010) 2:135–137
DOI 10.1007/s12686-010-9192-7

TECHNICAL NOTE

Microsatellite loci for the blue swimming crab (*Callinectes bellicosus*) (Crustacea: Portunidae) from the Gulf of California, Mexico

A. Munguia-Vega · J. Torre · A. Castillo-Lopez · T. Pfister · R. Cudney-Bueno

Received: 21 January 2010 / Accepted: 30 January 2010 / Published online: 14 February 2010

Abstract Twelve microsatellite markers (six di-nucleotides, four tri-nucleotides and two tetra-nucleotides) were isolated and characterized for *Callinectes bellicosus*, a commercial crustacean species from the Gulf of California, Mexico. One locus was monomorphic and 11 loci were polymorphic in 32 individual samples from a single location. Overall polymorphic loci, the number of alleles per locus ranged from 2 to 24 (average 10.0), the observed heterozygosity ranged from 0.094 to 0.969 (average 0.603), and the expected heterozygosity varied from 0.089 to 0.935 (average 0.597). One locus deviated significantly from Hardy–Weinberg equilibrium due to an excess of heterozygotes, while another locus showed evidence for the presence of a null allele. No evidence of linkage disequilibrium was found among pair of loci. These markers will be helpful to estimate the level of genetic connectivity over a small spatial and temporal scale in order to identify stocks for the management of this small-scale fishery in the Gulf of California.

Keywords Gulf of California · Fisheries · *Callinectes bellicosus* · Blue swimming crab · Connectivity · Microsatellites

A. Munguia-Vega (✉)
Conservation Genetics Laboratory, School of Natural Resources and the Environment, BioSciences East 317, The University of Arizona, Tucson, AZ 85721, USA
e-mail: airdrian@email.arizona.edu

J. Torre
Comunidad y Biodiversidad A.C., Blvd. Agua Marina 297 entre jaiba y tiburón, Col. Delicias, 85420 Guaymas, Sonora, Mexico

A. Castillo-Lopez · T. Pfister · R. Cudney-Bueno
School of Natural Resources and the Environment, BioSciences East 325D, The University of Arizona, 1311 E 4th st., Tucson, AZ 85721, USA

A. Castillo-Lopez
Centro Intercultural de Estudios de Desiertos y Oceanos, A.C., Eficio Agustin Cortes s/n, Fraccionamiento Las Conchas, 83550 Puerto Peñasco, Sonora, Mexico

R. Cudney-Bueno
Institute of Marine Sciences, Long Marine Laboratories, University of California Santa Cruz, 100 Schaffer Road, Santa Cruz, CA 95060, USA

The blue swimming crab, *Callinectes bellicosus*, is a decapod crustacean with a geographic distribution from San Diego (California) to Oaxaca (Mexico), including the Gulf of California (Hendrickx 1995). The species reaches up to 17 cm of carapace lenght and 0.5 kg of fresh weight, and is relatively abundant in shallow coastal lagoons up to 55 m in depth with sandy and muddy bottoms, and eelgrass (*Zostera marina*) and mangrove zones.

The blue crab fishery, one of the main fisheries in the US (Rosenfield 1998), has experienced significant fluctuations due to overexploitation and habitat deterioration (Engel and Thayer 1998; Jordan 1998). This has produced a growing demand for crab products from other countries. Crab exports (*Callinectes* sp. and *Portunus* sp.) have increased mainly from Asia and Latin America to the US (Oesterling 1998). In 1995, México was first in exports from Latin America, and second in the world, with 1,500 t of crabmeat valued at US $16 million. In Mexico, the crab fishery started in the Gulf of Mexico, and developed in the Gulf of California on a larger scale in the mid 1980s (Gonzalez-Ramirez et al. 1996; Molina-Ocampo 2000). In order to accomplish a better management of *C. bellicosus*, which constitutes 95% of the capture in the Gulf of California, it is important to identify the spatial distribution of distinct stocks. We isolated 12 microsatellite loci to

estimate the level of genetic connectivity over a small spatial and temporal scale relevant for management of this commercial fishery.

Genomic DNA was extracted from five individuals using the DNeasy Blood & Tissue Kit (Qiagen). These DNAs were pooled and used to create microsatellite-enriched libraries following a modified version of Glenn and Schable (2005). Approximately 5 μg of genomic DNA was digested with *Rsa*I (NEB), and the resulting fragments ligated to SuperSNX-24 linkers. Linker-ligated fragments ranging 300–1,200 bp were recovered by the polymerase chain reaction (PCR), using the SuperSNX-24 forward primer and Platinum high-fidelity Taq DNA polymerase (Invitrogen). Linker-ligated fragments were then hybridized to 5′-biotinylated microsatellite oligonucleotide probes $(GT)_{15}$, $(CT)_{15}$, $(GTAT)_{10}$ and $(GTCT)_{10}$ (1 μM each). Enriched fragments were isolated with streptavidin-coated magnetic beads (Dynal, Invitrogen). Polymerase chain reactions (PCR) were carried out as described above to recover fragments containing potential microsatellites. Libraries were ligated into the vector PCR4-TOPO (Invitrogen), transformed into TOP10 chemically competent *Escherichia coli* cells (Invitrogen) and plated on Invitrogen imMedia^{TM} Amp agar. One-hundred and ninety-six clones were amplified by PCR using M13 primers under standard PCR conditions, visualized on 2% agarose gels and suitably sized inserts (>500 and <1,500 bp) were selected for sequencing in both directions on an Applied Biosystems 3730XL DNA Analyzer using the BigDye Terminator Cycle Sequencing Kit. One-hundred and sixty-three (83.1%) of the clones had recognizable microsatellite sequences, but from those only 40 represented unique clones and had adequate flanking regions to design primers using PRIMER3 (Rozen and Skaletsky 2000). The universal M13 primer was added at the 5′ end of the forward primers to allow fluorescent labeling of the amplicons using M13 labeled oligonucleotides (Schuelke 2000). Reverse primers were designed with a "pig-tail" at

Table 1 Twelve microsatellite loci cloned from *Callinectes bellicosus*, including: locus name, GenBank accession number, repeat motif found in the cloned allele, primer sequences, clone size, size range of observed allelic variation, number of alleles (N_A), and observed (H_O) and expected (H_E) heterozygosities

Locus genbank	Repeat mofif	Primer sequences (5′–3′)	Clone size (bp)	Size range (bp)	N_A	H_O	H_E
Cabe28	$(GT)_{36}$	F: GAATGTGAGAAGCAGTGATGC	265	255–273	4	0.710	0.575
GU338216		R: GAGACGGAGGGAGGAAGAAG					
Cabe30	$(CA)_{19}$	F: AACGCAGCAGGATAATACCG	179	157–169	6	0.387	0.384
GU338217		R: CATTTCAGCTTGCGTGAGTG					
Cabe31B	$(CCA)_7$	F: CGTGCAGATCAAGGGTGAAT	135	132	1	0.0	–
GU338218		R: ACGGGCAGGTTGTAGTGGTA					
Cabe44	$(TAG)_{32}$	F: TGGAACAGTAGTAGGTAGTAGCTGTA	197	171–213	14	0.733	0.893
GU338219		R: GGTGTCATCATTACTATTATCAATATT					
Cabe45B	$(CA)_9$	F: CAGCAGCAGCGGTAGTAGTG	178	176–178	2	0.094	0.089
GU338220		R: GCTAGCCTTCGTTCACAAGC					
Cabe55	$(GT)_{11}$	F: GATTCTCATCATCTATATGTATGTATG	194	196–198	2	0.097	0.092
GU338221		R: TCCACCTCAAGGGACTCA					
Cabe78	$(GA)_{33}$	F: TCCTCTTTCCTCTCTTCACGA	182	149–205	21	0.903	0.935
GU338222		R: AGTAACGCTACCACGCCAAC					
Cabe80B	$(GTT)_{18}$	F: TGGTTGGGAAATGTGATCTG	179	156–222	17	0.696	0.896
GU338223		R: TGAGGGTGAATAATTTACAGAAAAGA					
Cabe2	$(GTAT)_9$	F: GAGTCTCTTTCTCTCTCTGTGTGTATG	263	191–203	5	0.406	0.470
GU338224	$(GT)_3$ $(GTAT)_{16}$	R: TGATAGAAGGTCGAAATTCAAATG					
Cabe73	$(GCCT)_3$	F: TGGCAAGATACTTCATGATTCC	266	239–305	13	0.767	0.781
GU338225	$(GTCT)_{10}$	R: CTGGCTAAGGGCAACAAAAA					
Cabe81	$(CAA)_5$	F: CTACCCTTCCTCCACCCATC	128	123–135	3	0.969	0.514*
GU338226		R: AGGTCTAACGGCCTCTTGCT					
Cabe90	$(CA)_{14}$	F: AGGCAGACGGCTAGACTGAC	217	250–308	24	0.875	0.933
GU338227		R: TGAGCGTGACCAGAGTGAAG					

* Locus that deviated significantly from HWE after Bonferroni correction (adjusted critical $P < 0.0041$)

the 5′ end to reduce variability in adenylation of amplification products (Brownstein et al. 1996).

PCRs were performed in 15 µl volumes containing ~50 ng genomic DNA, 1× PCR buffer, 0.2 mM each dNTP, 0.02 µM of the unlabeled M13-tailed forward primer, 0.2 µM of the reverse and 0.2 µM of the fluorescently-labeled M13 primers, 1.5 mM $MgCl_2$ (except loci *Cabe28* and *Cabe55* for which 3 mM $MgCl_2$ were used), 0.5 U taq DNA polymerase (Invitrogen), and 0.2% BSA. All loci, except locus *Cabe28* and *Cabe55*, were amplified with a touchdown protocol that included an initial denaturation at 94°C for 5 min, 15 cycles at 94°C for 30 s, annealing at 65–50°C for 30 s (1°C decrease in each cycle), 72°C for 30 s, followed by 40 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 30 s and a final extension of 72°C for 5 min. Loci *Cabe28* and *Cabe55* were amplified by an initial denaturation at 94°C for 5 min, followed by 40 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 30 s and a final extension of 72°C for 5 min. Microsatellite genotyping was performed on an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems). Allele sizes were estimated by GENOTYPER 3.7 (Applied Biosystems) and classified into bins with FLEXIBIN (Amos et al. 2007). Data were analyzed using GENALEX 6.2 (Peakall and Smouse 2006) to calculate observed and expected heterozygosities, deviations from Hardy–Weinberg equilibrium (HWE) and probability of identity. Tests for linkage disequilibrium (LD) between pairs of loci were conducted in FSTAT (Goudet 1995). MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) was used to check for scoring errors and null alleles. Adjusted P values were obtained using a sequential Bonferroni test for multiple comparisons (Rice 1989). The experiment-wise error rate was predefined at 0.05.

Twelve loci successfully amplified and were scored on 32 individuals collected from San Jorge island, Sonora, in the Gulf of California, Mexico (Table 1). One locus (*Cabe31B*) was monomorphic. Overall polymorphic loci, the number of alleles per locus ranged from 2 to 24 (average 10.0). Observed heterozygosity ranged from 0.094 to 0.969 (average 0.603), and expected heterozygosity varied from 0.089 to 0.935 (average 0.597). A significant deviation from HWE was detected only at locus *Cabe81* using a Chi-square test, due to an excess of heterozygotes. The distribution of homozygote-size classes suggested the presence of a null allele at locus *Cabe80B* with an estimated frequency of 0.111 (Van Oosterhout et al. 2004). No significant linkage disequilibrium was detected among the tests for each pair of loci (all adjusted P values > 0.0007). The probability of identity calculated combining all loci was 1.65×10^{-11}.

Acknowledgments We acknowledge the financial support provided from The David and Lucile Packard Foundation and the Wallace Research Foundation. AMV and ACL received graduate scholarships from Consejo Nacional de Ciencia y Tecnologia (CONACYT). We thank CEDO's staff for helping collecting samples. This is a scientific contribution of the PANGAS project: www.pangas.arizona.edu.

References

Amos W, Hoffman JI, Frodsham A, Zhang L, Best S, Hill AVS (2007) Automated binning of microsatellite alleles: problems and solutions. Mol Ecol Notes 7:10–14

Brownstein MJ, Carpten D, Smith JR (1996) Modulation of non-templated nucleotide addition by Taq DNA polymerase: primer modifications that facilitate genotyping. Biotechnolgy 20:1004–1010

Engel DW, Thayer GW (1998) Effects of habitat alternation on blue crabs. J Shellfish Res 17:579–585

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

Gonzalez-Ramirez P, Garcia-Borbon JA, Loreto-Campos PA (1996) Pesqueria de jaiba. In: Casas-Valdez M, Ponce-Diaz G (eds) Estudio del Potencial Pesquero y Acuicola de Baja California Sur, Volumen I, Mexico, pp 207–225

Goudet J (1995) FSTAT (Version 2.9.3.2): a computer program to calculate F-statistics. J Hered 86:485–486

Hendrickx ME (1995) Cangrejos. In: Fischer W, Krupp F, Schneider W, Sommer C, Carpenter KE, Niem VH (eds) Guía FAO para la identificación de especies para los fines de la pesca: Pacífico centro oriental, Volumen I. FAO, Roma, pp 565–636

Jordan S (1998) Introduction. J Shellfish Res 17:369–370

Molina-Ocampo RE (2000) La pesquería de jaiba (Callinectes bellicosus) en el estado de Sonora, Mexico. In: Sustentabilidad y Pesca Responsable en Mexico: Evaluacion y Manejo, 1999–2000. SEMARNAP-INP, Mexico, DF, pp 329–348

Oesterling MJ (1998) "Blue" crab resources in other countries: implications for the U.S. industry. J Shellfish Res 17:368–375

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Rosenfield A (1998) Preface. J Shellfish Res 17:367

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, New Jersey, pp 365–386

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotech 18:233–234

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538