Revista de Biología Marina y Oceanografía
ISSN: 0717-3326
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Universidad de Valparaíso
Chile

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Revista de Biología Marina y Oceanografía, vol. 51, núm. 3, diciembre, 2016, pp. 695-698
Universidad de Valparaíso
Viña del Mar, Chile

Revista de Biología Marina y Oceanografía
Vol. 51, Nº3: 695-698, diciembre 2016
DOI 10.4067/S0718-19572016000300021

RESEARCH NOTE

Characterization of sixteen microsatellite loci from the marine gastropod *Monetaria caputdraconis* (Gastropoda: Cypraeidae) by next generation sequencing

Caracterización de dieciséis loci microsatélites en el gasterópodo marino *Monetaria caputdraconis* (Gastropoda: Cypraeidae) mediante el uso de secuenciación masiva de ADN

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Abstract.- Easter Island possess a unique marine biodiversity. *Monetaria caputdraconis* is important among these species due to its cultural and economic importance for the islanders. For this reason, a total of 16 microsatellite loci were characterized for this species. The mean number of alleles per locus in the 76 studied individuals was 11.25 (2-20 alleles per locus) and the observed heterozygosity ranged from 0.08 to 0.88. While none of the loci exhibited significant linkage disequilibrium, 9 showed departures from Hardy-Weinberg equilibrium. The heterozygote deficit observed in *M. caputdraconis* has been described for other marine invertebrates with planktonic larval development.

Key words: Easter Island, planktonic development, 454 method

INTRODUCTION

Oceanic islands sufficiently isolated from other habitats promote differentiation of organisms that achieve their colonization. Thus such environments provide natural laboratories for the study of the processes involved in the formation of new species and the origins of biodiversity (Emerson 2002). However, these systems are vulnerable and generally information about their flora and fauna is scarce.

Easter Island is considered the most remote inhabited island on the planet (Mieth & Bork 2005). World famous for its remarkable monolithic human figures, or moai, Easter Island is also recognized for its unique marine life (Glynn *et al*. 2003, Randall & Cea 2011). One of these species, *Monetaria caputdraconis* (Melvill, 1888) is a gastropod endemic to Easter Island and Salas and Gomez Island, which is called *pure* by the islanders. This species has internal fertilization; eggs are deposited in capsules attached to rocks and planktonic veliger larvae then develop after 7 days of incubation (Osorio *et al*. 1992). Local craftsmen exploit this species for commercial purposes (Seaver 1986), which has led to a significant decrease in its abundance (Rivera 2003). Moreover, considering the high anthropogenic perturbation to which the snail is exposed, its genetic diversity has probably been severely affected. However, molecular markers to evaluate this situation have not yet been developed. The massively parallel Next Generation Sequencing (NGS) has made possible the development of microsatellite markers in non-model species. For *M. caputdraconis*, these microsatellite loci could provide information on genetic diversity as well as the rate and direction of migration between islands, which could lead to improvements in terms of conservation and management plans. The objective of this study was to characterize polymorphic microsatellite loci for *M. caputdraconis*.

MATERIALS AND METHODS

Seventy-seven specimens of *M. caputdraconis* were collected in Easter Island (27°7'S; 109°22'W) in March, 2014 and stored in 95% ethanol. A small piece of foot tissue (approximately 1 mg) was used for DNA extraction with the Wizard Genomic DNA Purification Kit (Promega). DNA quantities were measured with a Nanodrop Spectrophotometer (Thermo Fisher). One individual was sequenced. After extraction, its DNA quality was checked with the Bioanalyzer Agilent Model 2100. The library was built using the GS Rapid Library Preparation kit in OMICS-Solutions[1]. In order to maximize sequencing,

[1]<http://omics-solutions.cl>

4 different species were barcoded for the same run in a 454 GS Junior system (Roche); thus 1/4 of the reads were for *M. caputdraconis*. After sequencing, repeated motifs (di and tetra) were searched for with MISA software[2] and primers were designed using Primer3 (Untergrasser *et al*. 2012). Fifty loci (Pure1 to Pure50) were tested in 4 individuals of *M. caputdraconis* with a 12 mL polymerase chain reaction containing 1.5 mL template DNA (100 ng), 0.5 mL each primer (0.25 mM), 2.4 mL dNTP (100 mM each dNTP) (Applied Biosystems), 0.5 mL $MgCl_2$ (2 mM), 1.3 mL 10x PCR buffer (0.96x), 0.12 mL Taq Polymerase (0.5 U) (Invitrogen) and 4.68 mL H_2O. Cycling conditions for all loci consisted of an initial denaturing step of 2 min at 95ºC, followed by 35 cycles of 30 s at 95ºC, 1 min at 55ºC, 1 min at 72ºC and a final elongation step at 72ºC for 3 min. Sixteen of these 50 loci showed reliable amplification using agarose gel electrophoresis.

Table 1. Primer sequences and characteristics for 16 microsatellite loci of *Monetaria caputdraconis* from Easter Island. Ta= annealing temperature, N= number of analyzed individuals, N_A= number of alleles, Ho/He= observed and expected heterozygosity; **P* < 0.01 for significant departures from HWE, tested using 3000 permutations in GENETIX (Belkhir *et al*. 1996) / Secuencias de los partidores y características de los 16 loci de microsatélites descritos para *Monetaria caputdraconis* de la Isla de Pascua. Ta= temperatura de alineamiento, N= número de individuos analizados, N_A= número de alelos, Ho/He= heterocigosidad observada y esperada; **P* < 0,01 para desviaciones significativas del equilibrio de Hardy-Weinberg, testeo realizado con 3000 permutaciones en GENETIX (Belkhir *et al.* 1996)

	Motif	Primer sequence (5'-3')	Ta (ºC)	GenBank accession no.	N	N_A	Size Range (bp)	Ho/He
Pure1	$(GTCT)_8$	F: CTTTGATAACAACAACTACT R: CTAGTACAGAAAAAGAGAGAC	55	KP793177	74	4	117-133	0.23/0.47*
Pure3	$(ATCC)_{11}$	F: TGTATATCTACTCGTATGTGT R: ATACACTTTTATAAGTCCAG	55	KP793178	76	6	131-151	0.55/0.57
Pure7	$(TAGA)_{14}$	F: CAGACAGATAGATAGATAGGT R: GTAGACACGTAGAACATATAA	55	KP793179	75	13	169-219	0.87/0.88
Pure15	$(ACAG)_8$	F: CTGAGGAAGTTCTAAGATA R: TCGATGTCTACTATATAGTCA	55	KP793180	76	9	270-302	0.76/0.74
Pure18	$(TG)_{17}$	F: ATATTGTACATACTGTGTGTG R: TATGATAGTTGTTACAGCTAC	55	KP793181	76	17	215-249	0.88/0.91
Pure19	$(AG)_{18}$	F: GTTTGTGAAAGATAGAGTTA R: GTGTAGTATTGCTTTCTATAG	55	KP793182	61	16	224-254	0.32/0.88*
Pure28	$(ACCT)_{15}$	F: CTAACATAGAGGAACTAAAC R: CATTCATGTGTATATAGGTAG	55	KP793183	66	19	310-382	0.65/0.88*
Pure31	$(AGAC)_{11}$	F: GTATGGACATTTAGATGAA R: CTCTCTCCATATTAATTTAC	55	KP793184	66	9	351-387	0.26/0.74*
Pure33	$(TG)_{14}$	F: GTTGTTCTTTTTACACACT R: ACTATACCTGAAACATAAAG	55	KP793185	76	17	329-367	0.86/0.90
Pure34	$(GA)_9$	F: ACTTGTACCTACATACATGTA R: ATATGACAAGCATAATCTAC	55	KP793186	46	2	342-346	0.13/0.19*
Pure42	$(GT)_{12}$	F: TATTGTATTTAGTTAGGTCC R: GAATGATACTCTGTTACAGTA	55	KP793187	67	10	351-369	0.73/0.75
Pure45	$(ATGT)_{24}$	F: AATGAAACTATTACTGACAG R: TATAAACCTGACTATGACTC	55	KP793188	53	17	410-478	0.23/0.90*
Pure46	$(AC)_{23}$	F: ATTTAACTAACACACTTCC R: AGACTAGTTCATAACCTATGT	55	KP793189	61	20	440-500	0.47/0.92*
Pure47	$(TC)_{12}$	F: ACTAGCAATGTCAATAATAG R: CACATTGTACACAAGTAGAT	55	KP793190	76	8	439-455	0.47/0.54
Pure48	$(GA)_{12}$	F: CTCTATTCTTGTTACATGAT R: TACGGTCTAATGTAAGTCT	55	KP793191	75	11	448-470	0.53/0.85*
Pure50	$(TA)_6$	F: CTGAGTAAGTTAACTAAGGA R: TGGAGAGTTAACTTAGACTA	55	KP793192	50	2	467-469	0.08/0.15*

[2]<http://pgrc.ipk-gatersleben.de/misa/>

Polymorphism was evaluated for the 77 individuals in an automatic sequencer. PCR products were genotyped in the sequencing core at the Pontificia Universidad Católica, Chile, using the internal size standard LIZ 500 (Applied Biosystems). Reverse primers of each microsatellite locus were marked with a fluorescent dye. Sequences were published in GenBank with accession numbers KP793177 to KP793192 (Table 1). Allele frequencies and parameter estimates were calculated using the GENETIX software (Belkhir *et al*. 1996). Linkage disequilibrium was estimated for all pairs of loci, and deviations from Hardy-Weinberg Equilibrium (HWE) were calculated using the permutation test associated with the F_{IS} calculation in the GENETIX software. The Micro-Checker software (Van Oosterhout *et al.* 2004) was used to detect potential genotyping errors and the presence of null alleles in the microsatellite data.

Results and discussion

Of the 16 polymorphic loci tested in 76 individuals of *M. caputdraconis*, 7 contained tetra- and 9 contained dinucleotide motifs. These microsatellites showed allele sizes ranging from 117 bp (Pure1) to 498 bp (Pure46), and number of alleles from 2 (Pure34 and Pure50) to 20 (Pure46) (Table 1), with an average of 11.25 alleles per locus. The observed heterozygosity ranged from 0.08 (Pure50) to 0.88 (Pure18). No linkage disequilibrium was detected between the loci; significant deviations from HWE were observed in 9 microsatellite loci (Table 1), but is important to note that the Micro-Checker software indicated that 8 of these loci may present null alleles. Descriptions of deviations from HWE are common in marine gastropods with planktonic larval development, such as *Crepidula fornicata* (Linnaeus, 1758) (Dupont & Viard 2003), *Haliotis kamtschatkana* (Jonas, 1845) (Miller *et al*. 2001) and *Littorina obtusata* (Linnaeus, 1758) (Kemppainen *et al.* 2009), among others. These departures have been explained by the presence of null alleles, the Wahlund effect or natural selection. However, empirical and theoretical evidence has suggested that non-random larval dispersal (Véliz *et al.* 2006) and high variance in reproductive success (Broquet *et al*. 2013) could explain this pattern.

Due to the characteristics of microsatellites (high mutation rate, co-dominant inheritance and selectively neutral) they are excellent markers to analyze the genetic structure of geographically separated populations (Selkoe & Toonen 2006). Taking into account that the 16 microsatellite loci described in this study showed moderately high levels of polymorphism, they will be a useful tool to estimate population genetic structure and connectivity between populations of *M. caputdraconis* in Easter Island and Salas y Gomez Island, separated by approximately 400 km. As pointed out by Cowen & Sponaugle (2009), the connectivity assessed with genetic markers provides a view of the connectivity taking place over many generations, thus it allows inferences about migration across longer periods of time instead of estimating connectivity of the last generation as with other methods such as mark-recapture and chemical signatures. The new information about the population structure and connectivity obtained with the microsatellites described here will improve future conservation efforts.

Acknowledgements

We thank R. Espejo and OMICS Solutions Chile for Next Generation Sequencing. This study was supported by the Chilean Millennium Initiative (NC120030) grant.

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Received 19 August 2015 and accepted 25 August 2016

Editor: Claudia Bustos D.