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RESEARCH NOTE

Development and characterization of the first 16 microsatellites loci for *Panulirus pascuensis* (Decapoda: Palinuridae) from Easter Island using Next Generation Sequencing

Desarrollo y caracterización de 16 loci microsatélites para la langosta de Isla de Pascua *Panulirus pascuensis* (Decapoda: Palinuridae) mediante el uso de Secuenciación Masiva de ADN

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Abstract.- The spiny lobster *Panulirus pascuensis* stands out among the endemic species of Easter Island, due to its cultural and economic importance. A total of 16 microsatellite loci were characterized in 18 individuals, 9 of which were polymorphic. The mean number of alleles per locus was 3.44 (2-6) and the observed heterozygosity ranged from 0.11 to 0.93. None of the loci exhibited significant linkage disequilibrium or departures from HWE. These new microsatellites will be used to obtain information about migration, population structure and genetic diversity of *P. pascuensis* in order to improve the future sustainable management and conservation plans.

Key words: *Panulirus pascuensis*, Easter Island, microsatellite, genetic marker

INTRODUCTION

Remote island systems are characterized by having a unique fauna, since the high degree of isolation results in high endemism (DeMartini & Friedlander 2004). Given the particular characteristic of the remote islands, the study of their biota allows research on metapopulation dynamics, speciation processes and mechanisms underlying the maintenance of biodiversity (Hixon & Webster 2002, Almany *et al.* 2009, Friedlander *et al.* 2013). In the current context of global change and increased anthropogenic pressure which could have negative effects on the diversity and abundance of the biota, the study of these systems has acquired importance to anticipate possible effects, mainly due to their greater susceptibility to environmental changes (Hanski 1999, Bell 2008).

Easter Island or Rapa Nui is considered one of the most isolated inhabited islands in the world (Anderson 1995, Boyko 2003); it is located 4,130 km west of the Chilean coast and 2,415 km east of Ducie Island (Abbott & Santelices 1985). Its levels of species diversity are relatively low but include high endemism (Rehder 1980, Randall & Cea-Egaña 1984, Roberts *et al.* 2002), which could be explained by its high isolation level (Friedlander *et al.* 2013). One of the most emblematic marine species of Easter Island is the endemic spiny lobster *Panulirus pascuensis* Reed, 1954 (Order Decapoda, Family Palinuridae); its main distribution includes Easter Island and Salas y Gómez Island, but it has also been reported in Pitcairn Island and the austral islands of French Polynesia (Retamal 2004, Poupin 2008). This species represents an important fishery resource for the Rapa Nui population (Boyko 2003, Tapia 2010), and although its exploitation is mainly for subsistence or handicrafts (Castilla *et al.* 2014), historic data show a decrease in population sizes as well as in the average size of individuals (CORFO 1978, Castilla *et al.* 2014, Yáñez *et al.* 2014). There is not much information about the biology and ecology of this species, for example information about its ontogeny is lacking (Vereschaka 1990, 1995; Parin *et al.* 1997, Rivera & Mujica 2004). Moreover, molecular markers to study the migration patterns, population structure and the genetic diversity of this species have not yet been developed. Massively parallel Next Generation Sequencing (NGS) makes it possible to develop

Table 1. Primer sequences and characteristics for 16 microsatellite loci of *Panulirus pascuensis* from Easter Island. T_a= annealing temperature, N_a= number of alleles / Secuencia de los primers y características de los 16 loci microsatellites descritos para *Panulirus pascuensis* de Isla de Pascua. T_a= temperatura de alineamiento, N_a= número de alelos

Locus	GenBank accession no.	Sequence (5' → 3')	Repeat motif	T_a (°C)	N_a	Size range (bp)
LAN2	KX553880	F: GTTATTAGAAGTGTCACTTG R: AAAAATAAGTCCTAGGATAG	$(TATC)_7$	55	1	173
LAN3	KX553881	F: CCATCTAATGAGTATTTGTA R: GCTAATGTTCTAAATGTGTA	$(ATCT)_5$	54	2	164-172
LAN8	KX553882	F: AGTTAGACATGCTTAGTTTT R: AGTGACACTACATAAAATCA	$(CA)_{13}$	55	2	163-165
LAN12	KX553883	F: GTAATATGAGGATATGAGGT R: TAACATACAATTTACAGGTC	$(AC)_{11}$	55	1	242
LAN16	KX553884	F: GAAGATATATGCTATGTTTC R: GACTAATTACTAAGACTTGGT	$(AC)_{12}$	54	1	259
LAN17	KX553885	F: TCCTAAGACATGTGTACTAG R: ACACCTAACTCGTACTATCT	$(GCCA)_7$	55	4	322-346
LAN18	KX553886	F: AGATAGTTGCTTATACAGAA R: AAATCAGTCACACACACTAC	$(TCCC)_5$	55	2	338-342
LAN19	KX553887	F: ATTTGACTTATCTGAACTCT R: ATGCTATCTATCTATCTATCC	$(AGAT)_6$	55	1	348
LAN21	KX553888	F: ATAAAGTCAGAAGAAACAAC R: TAGATAGTATTAGTAGCTTGG	$(TCTA)_5$	55	1	360
LAN24	KX553889	F: AGAGATAGAGTGAGTGAGAA R: ATAATGTTGTCTATACCTCA	$(CA)_{16}$	55	4	357-367
LAN26	KX553890	F: AGTACTAAAATAAAGCTCAG R: GTGTTTGTACTGTTCTTGTG	$(AGGC)_5$	55	1	441
LAN28	KX553891	F: CTAATTCTAAGAGAGTTCACT R: GAGGATACCACTATTAATATC	$(GT)_{18}$	55	6	418-430
LAN33	KX553892	F: ACAGTAGTCACAGATTGTAA R. GAGATATAAGACAAGAACAGT	$(AC)_8$	55	1	440
LAN37	KX553893	F: ATATTGTTGTATATTCGAGC R: GATAGAAGAGAACACAGGAG	$(GT)_{13}$	55	2	147-149
LAN41	KX553894	F: GAAGAGTAGAAGTGGGATAT R: ATACATCATTGTTGTGAGTA	$(AC)_{16}$	55	4	229-239
LAN48	KX553895	F: ACAGTTCCTTTTAGTTAAAC R: GTCTCTTAACCTTGTAAATA	$(TG)_6$	55	5	357-377

microsatellite markers in non-model species (*e.g.*, Vega-Retter *et al.* 2016). The objective of this study was to identify and characterize microsatellite loci for *P. pascuensis*, in order to perform future genetic studies for the design and implementation of appropriate conservation and management plans in fishery.

MATERIALS AND METHODS

Eighteen adult individuals of *P. pascuensis* were collected in Easter Island (27°13'S, 109°22'W) between September 2013 and November 2015, and one pereiopod of each individual was stored in 95% ethanol. A small piece of muscle (approximately 1 mg) was used for DNA extraction using the salt-extraction protocol (Aljanabi & Martinez 1997). DNA concentrations were measured with a Nanodrop Spectrophotometer (Thermo Fisher)[1]. One individual was chosen for sequencing, and its quality was checked with the Bioanalyzer Agilent Model 2100. The library was built using the GS Rapid Library Preparation kit in OMICS-Solutions, Chile. In order to maximize sequencing, 4 different species were barcoded for the same run in a 454 GS Junior system (Roche); thus 1/4 of the reads were for *P. pascuensis*. After sequencing, repeated motifs (di and tetra) were searched for with MISA software and primers were designed using Primer3. Fifty loci (LAN1 to LAN50) were tested in 4 individuals with a 12 µl polymerase chain reaction containing 100 ng template DNA, 0.5 µl each primer (0.25 µM), 2.4 µl dNTP (100 µM dNTP) (Applied Biosystems), 0.5 µl $MgCl_2$ (2 mM), 1.3 µl 10x PCR buffer (0.96x), 0.12 µl Taq Polymerase (0.5 U) (Invitrogen) and 4.68 µl H_2O. Cycling conditions consisted of an initial denaturing step of 2 min at 95°C, followed by 35 cycles of 30 s at 95°C, 1 min at the

[1]Thermo Fisher Scientific Inc. <www.thermofisher.com>

annealing temperature, 1 min at 72°C and a final elongation step at 72°C for 3 min. Sixteen of the 50 loci showed reliable amplifications using agarose gel electrophoresis. To evaluate polymorphism in an automatic sequencer, the 18 individuals collected were analyzed for these 16 loci. PCR products were genotyped in the sequencing core at the Pontificia Universidad Católica, Chile, using the internal size standard LIZ 500 (Applied Biosystems) and with the reverse primers of each microsatellite locus marked with a fluorescent dye. Sequences were published in GenBank with accession numbers KX553880-KX553895 (Table 1). 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. Allele frequencies and parameter estimates were calculated using the GENETIX software (Belkhir *et al.* 1996-2004). Linkage disequilibrium was estimated for all pairs of loci, and deviations from Hardy-Weinberg expectations (HWE) were calculated using the permutation test associated with the F_{IS} calculation in the GENETIX software.

Results and discussion

Of the 16 characterized microsatellite loci, 7 showed a tetra- and 9 a dinucleotide motif. Nine out of 16 microsatellites were polymorphic (Table 1). There was no evidence of null alleles or stuttering errors in the polymorphic microsatellites. No significant deviations from HWE were detected and significant linkage disequilibrium was not detected among pairs of loci, indicating that the loci are probably not closely linked on chromosomes. These microsatellites showed allele sizes ranging from 147 bp (LAN37) to 441 (LAN26), and numbers of alleles from one (LAN2, LAN12, LAN16, LAN19, LAN21, LAN26, LAN33) to 6 (LAN28). The 9 polymorphic loci showed an average of 3.44 alleles per locus with observed heterozygosity ranging from 0.29 (LAN17) to 0.93 (LAN28) (Table 2). While 7 of the loci described in this work presented only one allele, it is important to consider that a small sample size was used from one island. It is probable that these loci may exhibit more than one allele in a more extensive sample including different islands.

Considering that direct approaches are difficult to perform in marine environments (Levin 2006, Gawarkiewicz *et al.* 2007), the molecular tools here characterized will be helpful to investigate the connectivity of the spiny lobster populations. Moreover, these microsatellite loci will allow information about the population structure and genetic diversity of this species, which together with the connectivity pattern is a fundamental information to build appropriate conservation and management plans of this important lobster species in Easter Island.

Table 2. Characteristics of the 9 polymorphic microsatellite loci of *Palinurus pascuensis*. N= number of analyzed individuals, N_a= number of alleles, H_o/H_e= observed and expected heterozygosity. F_{IS} individual F-statistic accounting for deviations in the observed number of heterozygotes. No significant departures from HWE were observed, tested using 5,000 permutations / Características de los 9 loci microsatélites polimórficos de *Palinurus pascuensis*. N= número de individuos analizados, N_a= número de alelos, H_o/H_e= heterocigosidad observada y esperada. El índice F_{IS} relaciona las heterocigosidades para determinar posibles desviaciones al Equilibrio Hardy-Weinberg (EHW). El análisis de permutaciones (5.000 permutaciones) no detectó desviaciones estadísticamente significativas al EHW

Locus	N	N_a	H_o/H_e	F_{IS}
LAN3	18	2	0.11/0.10	-0.03
LAN8	18	2	0.44/0.40	-0.08
LAN17	17	4	0.29/0.35	0.19
LAN18	17	2	0.35/0.46	0.26
LAN24	16	4	0.69/0.62	-0.08
LAN28	14	6	0.93/0.76	-0.19
LAN37	17	2	0.41/0.49	0.19
LAN41	17	4	0.35/0.46	0.25
LAN48	10	5	0.50/0.55	0.13

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