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Short communication

Isolation and characterization of sixteen microsatellite loci for the rudderfish *Kyphosus elegans* (Centrarchiformes: Kyphosidae) from Easter Island, discovered with Next Generation Sequencing

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Introduction

The rudderfish of the genus *Kyphosus* inhabits rocky environments and coral reefs in warm waters worldwide (Sakai and Nakabo, 2014). In the Pacific, *Kyphosus elegans* (Peters, 1869), formerly *Pimelepterus sandwicensis* Sauvage (1880) [please see Knudsen and Clements (2013) for revision), have an antitropical distribution in the central and western Pacific (Randall and Cea, 2010;]. On Easter Island, *K. elegans* is an herbivorous species that feeds on macroalgae, such as *Lobophora variegata* and *Sargassum* and often occur in schools

Table 1
Primer sequences and characteristics for sixteen microsatellite loci for *Kyphosus elegans* from Easter Island, including annealing temperature (*T*m), number of analyzed individuals (*N*) and number of alleles (*N*A)

Locus	Primer sequence (5′-3′)	Repeat motif	Tm (°C)	GenBank accession no	n	Ho/He	N_A	Size range (bp)
Kyphosus 2	F: GTTACTGTTGAGTATTGAGA R: ACATATGGACATAAGTAACA	$(CA)_{20}$	55	KP064203	37	0.92/0.89	13	158–196
Kyphosus 9	F: GAGTAAGACACCTACTGTATT R: CTTAGTACAGAGTCTTTTGTT	$(TAGA)_{14}$	55	KP064204	36	0.90/0.86	15	233–297
Kyphosus 12	F: GTACTATTCTTATTGAGACGT R: GATTTGGTGAAATCTAATCT	$(TAGA)_{13}$	55	KP064205	35	0.71/0.83	10	230–270
Kyphosus 13	F: CTTAACTGTCATTACCATTT R: AGAGAACAGACTGTACACAC	$(TG)_{18}$	55	KP064206	35	0.63/0.83*	10	225–243
Kyphosus 18	F: GTATACCTTCTTCTGTTGTC R: CTTCTTATCTAGATTTGACTC	$(ACAG)_9$	55	KP064207	37	0.16/0.25*	6	320–348
Kyphosus 24	F: CTGTATAGTGCTTTCTAGTT R: GTCTGTCAGATTCAGTATTC	$(GT)_{20}$	54	KP064208	37	0.76/0.81	9	288–392
Kyphosus 26	F: ACTAACACTGAATACACAAG R: CATTCTGTCTTTAGATAGATC	$(TG)_{18}$	55	KP064209	35	0.89/0.86	13	406–440
Kyphosus 33	F: GAGCACAAATATATACACAC R: ACAAACTTCTGTGTATTTAG	$(CAAT)_{11}$	55	KP064210	37	0.86/0.82	6	138–158
Kyphosus 34	F: GAGACAGATTATATTTGAGTC R: TAATCACTTCCTATTAGAGA	$(ATAC)_{11}$	55	KP064211	34	0.47/0.44	3	265–273
Kyphosus 38	F: CATGTACAGTACTTCTATGAA R: CAGACTTACTCTCTCTCTTAC	$(GT)_{12}$	55	KP064212	37	0.76/0.66	5	135–151
Kyphosus 39	F: CCACAACATGAGTAATATAT R: GTGTAATTACTTGTCACAAT	$(AC)_{15}$	55	KP064213	33	0.51/0.76*	11	155–177
Kyphosus 40	F: AGAGAAAGATAGTGTGTATTC R: CTCTTTCTCTTCTATCTCTC	$(TG)_{13}$	55	KP064214	34	0.76/0.81	9	162–184
Kyphosus 44	F: TCTACAGAGATACAGTAGGAT R: GTAGCATTTAATGTGTGTAT	$(GT)_{11}$	55	KP064215	37	0.78/0.81	10	239–259
Kyphosus 45	F: ATCAAGAACACAGTACTTCT R: AAACACTACGAATACATATC	$(TG)_{13}$	55	KP064216	36	0.56/0.57	4	249–257
Kyphosus 51	F: ATACAAAGTCCTTACTTGAG R: TACTGAAATTACTTGTACCT	$(AG)_{17}$	55	KP064217	37	0.59/0.60	6	331–345
Kyphosus 54	F: ACTTCAGTTACAGTATGAAA R: TTTAACTAGATACTGTGTGC	$(TG)_{13}$	55	KP064218	32	0.72/0.74	8	372–388

*P < 0.01 for significant departures from HWE, tested after 3000 permutations in Genetix (Belkhir et al., 1996).

(Randall and Cea, 2010); it is also the most abundant species among herbivore fish, thus becoming important in local fisheries (Friedlander et al., 2013). The cultural importance of these fish can be observed, for instance, in the fact that the inhabitants of this Island have given them 11 different common names, based on their size and color (Disalvo et al., 1988). This difference in color was also noticed for this species in the Gulf of Panama (Topp, 1970). In order to improve current population management and conservation plans, this study aimed to describe sixteen polymorphic microsatellite loci for *Kyphosus elegans*.

Materials and methods

A specimen was collected on Easter Island (27° 7′ S; 109° 22′ W) in February 2014. A fin clip was stored in 95% ethanol, and total genomic DNA was extracted with the Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI). DNA quality and quantity was checked using a Bioanalyzer Agilent Model 2100 and the library was constructed using the GS Rapid library Preparation kit in OMICS-Solutions (http://omics-solutions.cl). In order to maximize sequencing, four different species were barcoded for the same run in a 454 GS Junior system (Roche, Penzberg, Germany), thus 1/4 of the lectures were for *K. elegans*. After sequencing, repeated motifs were sought as described by Zeng et al. (2013). Sixteen out of fifty primer pairs tested in our laboratory showed reliable amplifications in the agarose gel electrophoresis. To evaluate polymorphism in an automatic sequencer, reverse primers of each locus was marked with a fluorescent dye.

Polymerase chain reaction (PCR) amplification mixtures (12 μl) contained 100 ng template DNA, 0.25 μM of each primer, 100 μM of each dNTP (Applied Biosystems, Foster City, CA), 2 mM $MgCl_2$, 1.25 μl 10x PCR buffer and 0.5 U Taq Polymerase (Invitrogen, Carlsbad, CA). 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 specific temperature, 1 min at 72°C, and a final elongation step at 72°C for 3 min. PCR products were genotyped in the sequencing core at Pontificia Universidad Católica de Chile (PUC), using the internal size standard LIZ 500 (Applied Biosystems). Sequences were published in GenBank with the accession numbers: KP064203 to KP064218 (Table 1).

Results and discussion

About 17 727 952 bases were sequenced, yielding 42 025 sequences with an average read length of 421 bp. Of the 3460 fragments showing repeat motifs, 16 were used for the analysis of polymorphism.

For these 16 microsatellites, we observed between three (locus 34) and 15 alleles (locus 9) per locus for the 37 individuals analyzed (Table 1). Observed heterozygosity (H_O) ranged from 0.16 (Locus 18) to 0.92 (Locus 9) and significant deviations from HWE were observed in only three out of 16 loci (loci 13, 18 and 39). Furthermore, significant linkage disequilibrium was not detected among pairs of the studied loci (GENETIX; Belkhir et al., 1996), indicating that the loci are probably not closely linked on chromosomes. Consequently, the loci may be considered as independent markers. These microsatellites will allow to determine population structure, gene diversity and migration rate of *K. elegans* in Easter Island and Salas y Gómez Island, information that will be useful to devise plans for genetic conservation and management of this species.

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