Biochemical Systematics and Ecology 65 (2016) 234–237

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Characterisation of eleven new polymorphic microsatellite markers for the coastal stingray *Dasyatis brevicaudata* (Dasyatidae Hutton 1875), and cross-amplification in seven dasyatid species

A. Le Port [a, b, c, d, *], E.J. Roycroft [b], V. Thakur [b], S.D. Lavery [a, b]

[a] Institute of Marine Science, Leigh Marine Laboratory, University of Auckland, PO Box 349 Warkworth 0941, New Zealand
[b] School of Biological Sciences, University of Auckland, Private Bag 92019 Auckland, New Zealand
[c] Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER), James Cook University, Townsville QLD 4811, Australia
[d] Centre for Sustainable Tropical Fisheries and Aquaculture, James Cook University, Townsville, QLD 4811, Australia

ARTICLE INFO

Article history:
Received 17 November 2015
Received in revised form 29 January 2016
Accepted 7 February 2016
Available online 5 March 2016

Keywords:
Coastal elasmobranch
Microsatellite
Conservation
Habitat degradation
Population genetics
PCR primers

ABSTRACT

Twelve microsatellite loci were developed for the short-tailed stingray *Dasyatis brevicaudata* using a next-generation sequencing approach, and their utility for population genetics studies was assessed. Eleven out of twelve loci were polymorphic (4–15 alleles) and amplified reliably, with observed heterozygosities between 0.32 and 0.86 (mean = 0.56). Successful cross-species amplification of many of these markers was achieved across seven other dasyatid species currently listed as "Data Deficient" by the IUCN, suggesting these microsatellites will be a useful resource for the investigation of ecological and conservation genetics in a range of species of conservation concern.

1. Introduction

A quarter of the world's sharks and rays are threatened with extinction, with ray species found to be at a higher risk than sharks (Dulvy et al., 2014). In particular, coastal ray populations are in decline due to the combined threats of fishing and habitat degradation (Dulvy et al., 2014), and their populations have been substantially declining. Despite this, effective conservation and management measures are hindered by the lack of basic information on ray life history and spatial ecology, with the majority of species listed as "Data Deficient" globally by the IUCN Red List.

Although the short-tailed stingray *Dasyatis brevicaudata* (Hutton 1875) is a common and ecologically important component of the coastal fish assemblage in the southern hemisphere and particularly in the southwest Pacific (Last and Stevens, 2009), there is a lack of information pertaining to the population structure and gene flow of this species. Assessing levels of genetic diversity and population structure is essential for establishing appropriate management and conservation actions. One of the main causes for the lack of genetic data is the absence of informative molecular markers for this and other *Dasyatid* species. Recent work using mitochondrial DNA in this species from New Zealand, Australia and South Africa

* Corresponding author. Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER), James Cook University, Townsville QLD 4811, Australia.
E-mail address: agnes.leport@gmail.com (A. Le Port).

http://dx.doi.org/10.1016/j.bse.2016.02.009
0305-1978/

has indicated strong population structure in the maternal line both regionally and around coastal New Zealand (Le Port and Lavery, 2012).

With growing evidence of differing levels of genetic structure between the sexes in sharks and rays (Chevolot et al., 2006; Portnoy et al., 2010; Karl et al., 2011; Veríssimo et al., 2012), the goal of this project was to generate a library of microsatellite markers for *D. brevicaudata* using next-generation sequencing technology (Roche 454 pyrosequencing) in order to investigate the potential for sex-biased dispersal in these stingrays, thereby refining population connectivity throughout its southern hemisphere distribution. In addition, cross-species transferability of these loci was assessed on 7 rays of the family Dasyatidae. In comparison to other fish species, there are relatively few microsatellite loci developed for ray species (Sellas et al., 2011; Kang et al., 2012), and Bhummakasikara et al. (2013) remains the only study to date focused on microsatellite development in a dasyatid species (*Himantura chaophraya*).

2. Materials and methods

DNA used for the generation of the microsatellite library was extracted from the fin clip of an individual sampled from New Zealand using a standard phenol/chloroform extraction procedure (Sambrook et al., 1989). Whole genome shotgun sequencing was performed using 500 ng of DNA on the Roche Genome Sequencer Junior Titanium series instrument utilizing the GS Junior Titanium Rapid Library Preparation Kit (Roche Applied Sciences, Indianapolis, USA) following the manufacturer's protocol at the Centre for Genomics, Proteomics and Metabolomics at the University of Auckland, New Zealand (part of New Zealand Genomics Ltd). The library quality was assessed by an Agilent 2100 Bioanalyzer using DNA HiSensitivity chips, and quantified using a 96-well plate fluorometer against a standard curve. The library was then processed through the GS Junior Titanium emulsion polymerase chain reaction (emPCR) kit, with $2 \times 10\ E^7$ molecules (2 molecules per bead $\times$ 10 million beads) of library DNA added to the emPCR reaction following the manufacturer's protocol. Sequencing was performed in a GS Junior Titanium PicoTiterPlate. The sequences were screened for potential microsatellite loci using QDD2.1 (beta) software on the Linux platform (Meglécz et al., 2010), under the default settings. QDD2.1 searches for perfect microsatellites with repeat units of 1–6 bp and is able to filter for unique sequences and discard those associated with duplications or mobile elements. Of the 163,149 reads generated, 31,512 contained putative microsatellite loci.

In total, 22 primer pairs were selected for amplification trials, and comprised 13 dinucleotide, four trinucleotide, four tetranucleotide and one pentanucleotide loci. Candidate primers were selected based on recommended criteria described in Meglécz et al. (2014). Amplified PCR fragments were labelled with fluorescent dyes using a universal fluorescent labelling method (Schuelke, 2000): each forward primer was synthesized with a 5′ CAG sequence tail (5′-CAGTCGGGCGTCATCA-3′), and used in conjunction with a fluorescently labelled CAG sequence primer. Initially, five *D. brevicaudata* samples from New Zealand were used to test the amplification of loci and evaluate polymorphic content. Loci that amplified consistently were then genotyped in additional *D. brevicaudata* individuals sampled from New Zealand, Australia and South Africa populations to confirm reliable amplification across the species ($n = 114$; New Zealand: 92, Australia: 12, South Africa: 10). Cross-species amplification was assessed on 1–2 individuals per species for 7 Dasyatidae species.

PCR reactions were carried out in volumes of 10 μL, containing 20 ng DNA template, 0.4 μM CAG primer labelled with a fluorophore (6FAM™, VIC®, NED™ or PET®; Applied Biosystems), 0.1 μM forward CAG-tailed primer, 0.4 μM reverse primer, 0.2 mM dNTP mix, 1.25 mM magnesium chloride ($MgCl_2$) and 0.125 U Platinum(R) Taq DNA Polymerase (Invitrogen, Life Technologies), with associated PCR buffer. Cycling conditions were: an initial denaturation at 95 °C for 1 min; followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s and extension at 72 °C for 60 s with a final extension of 72 °C for 5 min. Following PCR, amplicons were diluted 1:10, and pooled with amplicons labelled with the other fluorophores. Pooled PCR products were mixed with formaldehyde and a size standard (GeneScan™—600 LIZ™), and size-separated in a DNA sequencer (Applied Biosystems 3130×l). Microsatellite fragments were analysed and scored using GENEIOUS R7 (Biomatters) with Microsatellite plugin version 1.4.0. Control samples of known allele size were used on each run to ensure consistent allele calling.

The number of alleles per locus, observed (H_o) and unbiased expected heterozygosity (uH_e) were calculated using GeneAlEx v.6.5.1 (Peakall and Smouse, 2006). Deviations from Hardy–Weinberg equilibrium (HWE) across all samples were characterised and tested using the exact test in the software GENEPOP v.4.2 (Rousset, 2008). When the observed genotype frequencies deviated significantly from HWE ($p < 0.05$), the program MICRO-CHECKER v.2.2.3 (Van Oosterhout et al., 2004) was used to test the presence of null alleles.

3. Results and discussion

Out of the 22 primer pairs tested, 11 loci consistently amplified strong and clear PCR fragments across all samples, exhibited polymorphism and were assessed as useful for population genetics study (Table 1, GenBank accession numbers: KU587602–KU587613). Locus Dbr206 was excluded from further analyses as it amplified inconsistently and scoring was ambiguous. The number of alleles per locus (k) ranged from 4 to 15 ($7.18 \pm SD = 3.16$), and observed (H_o) and unbiased expected (uH_e) heterozygosities were relatively high (H_o ranged from 0.30 to 0.82 ($0.56 \pm SD = 0.14$) and H_E from 0.30 to 0.87 ($0.59 \pm SD = 0.15$). With the exception of Dbr078, none of the locus showed significant deviations from Hardy–Weinberg equilibrium and no significant linkage disequilibrium was detected for any of the 11 paired loci comparisons after Bonferroni correction. The minor deviation from Hardy–Weinberg equilibrium detected at locus Dbr078 in the form of heterozygote