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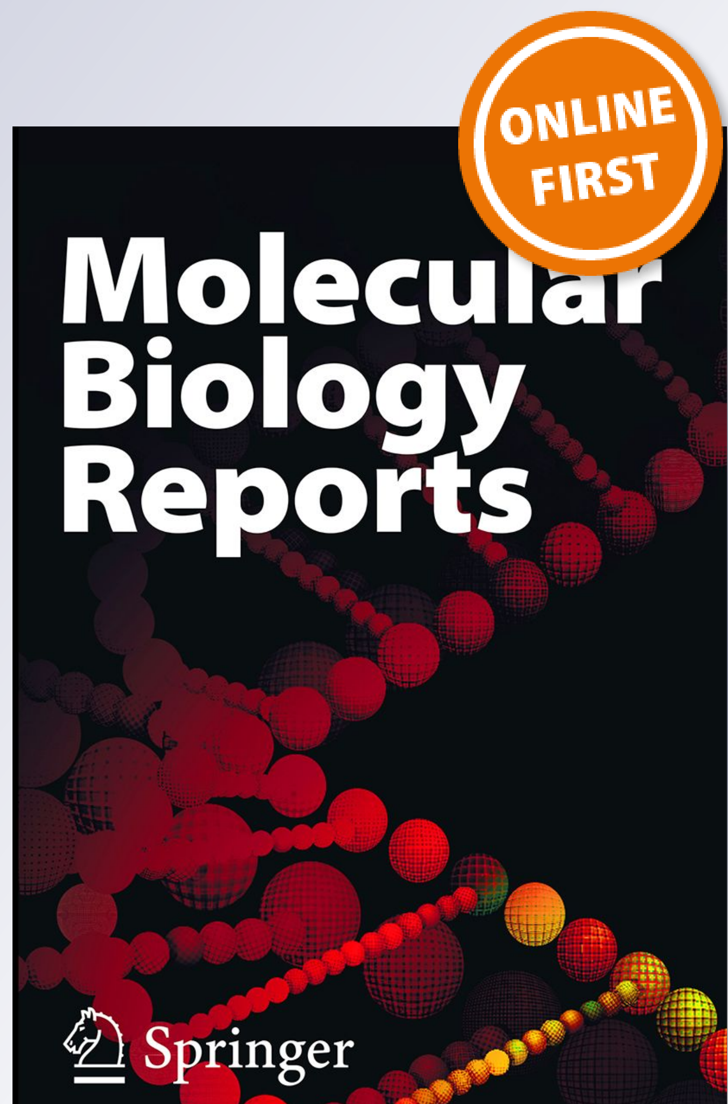

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SHORT COMMUNICATION

Isolation and characterization of 14 tetranucleotide microsatellite loci for the cannonball jellyfish (*Stomolophus* sp.) by next generation sequencing

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Abstract The Cannonball jellyfish (*Stomolophus* sp.) is a species of jellyfish with high relevance in artisanal fishing. Studies of their populations do not extend beyond the morphological descriptions knowing that presents a great morphological variability. However, there are no genetic studies to determine the number of independent populations, so microsatellite markers become a suitable option. Since there are no species-specific microsatellite loci, in this paper, 14 new microsatellite loci are characterized. Microsatellite loci were isolated de novo through next generation sequencing, by two runs on Illumina MiSeq. A total of 506,771,269 base pair were obtained, from which 142,616 were microsatellite loci, and 1546 of them could design primers. We tested 14 primer pairs on 32 individuals from Bahía de La Paz, Gulf of California. We observed low genetic variation among loci (mean number of alleles per locus = 4.33, mean observed heterozygosity 0.381, mean expected heterozygosity 0.501). These loci are the first ones described for the species and will be helpful to carry out genetic diversity and population genetics studies.

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Keywords Gulf of California · Microsatellite primers · Next generation sequencing · *Stomolophus* sp. · Stomolophidae · Cannonball jellyfish

Introduction

The cannonball jellyfish, *Stomolophus* sp., is a meroplanktonic scyphomedusae of the family Stomolophidae that inhabits the coastal waters of America [1], from North Carolina to Brazil in the Atlantic Ocean, and the Gulf of California, México to Ecuador in the Pacific [1, 2]. Like other species of jellyfish, the fishing interest in the cannonball jellyfish has expanded worldwide [3], becoming a relevant resource and one of the most exploited jellyfish species [4]. This fishery is characterized by an opportunistic exploitation conditioned for the unpredictability occurrence of blooms [5, 6]. Besides the increasing interest in the fisheries, there are no studies on genetic variability for cannonball jellyfish, which are crucial for management and conservation of the species.

Microsatellites are genetic markers widely used to estimate genetic diversity, population structure, and parameters with ecological and conservation interest such as migration rates, effective population size or genetic bottlenecks [7]. Traditionally, the development of microsatellites was a time-consuming process that could not easily be replicated [8]. The advent of next generation sequencing (NGS) technologies increased the capacity for affordable rapid sequencing of non-model species, such as cannonball jellyfish [9].

Here, we characterize 14 microsatellite loci obtained by NGS from cannonball jellyfish *Stomolophus* sp., which will be useful to assess genetic population diversity and structure.

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Materials and methods

DNA extraction

We used two genomic DNA extraction protocols: (a) an oral arm tissue sample from Las Guásimas (24°48′33″N; 111°56′26″W—Gulf of California) was extracted using EZNA DNA purification kit (Omega Bio-Tek, Norcross, GA), to create the genomic library useful for DNA sequencing and microsatellite isolation, and (b) 32 tissue samples from Bahía de La Paz (24°11′37″N; 110°28′48″W—Gulf of California) were extracted using $NaCl_2$ precipitation protocol [10], to microsatellite genotyping.

DNA sequencing and microsatellite isolation

Illumina TruSeq (Illumina, San Diego, CA) compatible DNA libraries were constructed by shearing the jellyfish DNA from a single individual from Las Guásimas on a Bioruptor UCD-300 sonication device (Diagenode, Denville, NJ) then using Kapa BioSciences (Woburn, MA) library preparation kits with custom adapters and indexes [11], checked for quality, normalized, pooled and run an Illumina (San Diego, CA) MiSeq v2 500 cycle kit at the Georgia Genomics Facility (University of Georgia) to produce paired-end 250 base reads. Sequences were screened for 2–6 bp microsatellite motifs with ≥5 tandem repeats using the software MSATCOMMANDER 1.03 [12]. Primers were designed using the PRIMER3 [13] functions of MSATCOMMANDER with the following specifications: (1) melting temperatures 50–70 °C with a maximum 2 °C difference between paired primers, (2) PCR product between 90 and 320 bp long, (3) GC content >40%, (4) primers length between 17 and 27 nucleotides and (5) primers self-complementarities and quality criteria with default parameters. We decided to work with tetranucleotides that are considered to be more effective and reliable because of minimal PCR stutter [14]. We designed primers pairs for 30 loci; forward primers were marked with four fluorophores: VIC®, 6 FAM™, PET®, and NED™.

Microsatellite genotyping

PCR was performed in a 10 µL volume reaction containing 40 ng of DNA, 1× PCR buffer (20 mM Tris–HCl, pH 8.4, 50 mM KCl), 0.3 mM of each dNTP, 0.3 µM of each primer, 1.5–2.5 mM $MgCl_2$, and 0.3 U *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA). Thermocycles conditions were 94 °C × 5 min as initial denaturation followed by 35 cycles at 94 °C × 30 s, 45 s at the locus-specific annealing temperature (Table 1), an extension at 72 °C × 30 s, and a final extension at 72 °C × 20 min. using a MyCycler thermocycler (BIORAD Laboratories, Hercules, CA, USA). PCR products were run on capillary electrophoresis with ABI PRISM® 310 Genetic Analyzer (Applied Biosystems®) and then genotypified with the fragment analysis software GeneMapper® Software Version 4.1 (Applied Biosystems®). Allele sizes were determined using the GeneScan™ 600 LIZ® Size Standard v2.0 (Applied Biosystems®).

Genetic analyses

Genetic diversity was evaluated from 32 individuals from Bahía de La Paz at 14 loci. Estimations of number of alleles per locus (N_A), observed (H_O) and expected (H_E) heterozygosity were obtained using GENALEX (Peakall and Smouse 2012). Frequencies of null alleles were estimated using FRENNA [15]. To assess if null alleles presence was due to large allele dropout, scoring errors due stuttering we used MICROCHECKER 2.2.3 [16]. Linkage disequilibrium and deviations from Hardy–Weinberg equilibrium (HWE) was tested using GENEPOP 4.0.10 [17]. The significance level of each analysis was considered after Sequential Bonferroni corrections [18].

Results and discussion

A total of 506,771,269 bp, from which 142,616 were microsatellite loci, were generated in two runs of the MiSeq (Illumina) genetic sequencer. Loci with primers (1546 loci) were represented in following proportions: dinucleotides 69% (1072), trinucleotides 20% (320), tetranucleotides 6% (103), pentanucleotides 3% (35), and hexanucleotides 2% (16).

Of the 30 selected loci, 14 of them presented PCR products on the expected allelic range. The optimal combination of annealing temperature and magnesium chloride concentration used to genotype individuals are detailed on Table 1.

From the 14 analyzed loci two are monomorphic (Smel-06 and Smel-18), and 12 polymorphic. N_A ranged from 2 to 8 (Smel-26 and Smel-29 respectively) with a mean of 4.33 (Table 2). H_O among loci ranged from 0.032 to 0.769 (Smel-25 and Smel-02 respectively, mean $H_O = 0.381$) and H_E ranged from 0.092 to 0.801 (Smel-15 and Smel-29 respectively, mean $H_O = 0.501$). No significant evidence for linkage disequilibrium existed in any comparisons by location or by locus after Bonferroni correction ($p < 0.001$). According, with Chapuis and Estoup [15], we find three negligible null alleles (Smel-02, Smel-05, and Smel-26), seven are moderate (Smel-08, Smel-09, Smel-10, Smel-13, Smel-15, Smel-27 and Smel-29) and two high (Smel-25 and Smel-30), with no evidence for scoring errors due to stuttering and large allele dropout. Four loci showed statistically significant deviations from HWE (Smel-10,

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Table 1 Characterization of 14 microsatellite loci for *Stomolophus* sp.

Locus	Primer sequence (5′–3′)	Repeat motif	Color	Ta [$MgCl_2$]
Smel-02	F: TCCACCTGCTCCGATTACAC R: TGAGATTTGCTGTTTCACGC	$AAAC_{(5)}$	VIC®	56 [2.5]
Smel-05	F: CCACCCTTGACCTGCATTAAC R: CCATCCTCTTCAATGACGGC	$AAGC_{(5)}$	6 FAM™	56 [1.5]
Smel-06	F: CGATCATTACTGGAGCGTCG R: CCAAGCACAGCAGTTGATCC	$AAAT_{(5)}$	$VIC_{®}$	70 [1.5]
Smel-08	F: GCTACCCTTGTCTCTAATTCGC R: CGTTCTTTATTGACACCCGC	$ACAT_{(7)}$	PET®	56 [2.5]
Smel-09	F: AATGCAAGTTGTCGATGGGC R: GATGTACAGCTGTGAGAACCC	$AAGC_{(13)}$	NED™	68 [2.5]
Smel-10	F: CCTCGTGTTCTCTTCAACGG R: GTCAGCGTCATTTCGTAGCC	$AACT_{(6)}$	VIC®	68 [2.0]
Smel-13	F: CACGCTGACACAAGAGAAGC R: AAATTTGCTTGGTTGCCTGC	$AAGC_{(7)}$	NED™	64 [2.5]
Smel-15	F: GCGGACGAGTTGTAACATGG R: TTTGGAGAGCGACATTGTCC	$AAAC_{(5)}$	6 FAM™	58 [1.5]
Smel-18	F: CTTCCAGGGCTGAAATTGGG R: CTTCTTCATGTCTGGCCACG	$AATC_{(10)}$	PET®	58 [2.5]
Smel-25	F: AGCTACATAGGACCACTCGC R: TCTCTGGGAAGACGAACTGC	$AACC_{(5)}$	NED™	60 [2.0]
Smel-26	F: GACCTACCTGCCAGTCTAGC R: GGTTTCCTTCTTGACGAGCC	$AATT_{(6)}$	6 FAM™	60 [2.0]
Smel-27	F: CTGCCTCCCTCCTCATCATG R: AGCAACATAGGTCCACTCGC	$ACCT_{(5)}$	PET®	64 [1.5]
Smel-29	F: CTCCAGGCCCAACTTAGATG R: TCCGCCCATTCTCAACAAATC	$AAAC_{(8)}$	VIC®	64 [1.5]
Smel-30	F: TCCTAGTACGACTTCCAAAGGG R: GTGTTGGAGGAAGTGATGCC	$AAAC_{(6)}$	6 FAM™	60 [1.5]

PCR amplification conditions as annealing temperature in °C (T_a) and magnesium chloride concentration in mM [$MgCl_2$]. Color of the fluorescent label: VIC®, 6 FAM™, PET®, and NED™

Table 2 Genetic diversity for 14 microsatellite loci of *Stomolophus* sp.

Locus	N	N_A	Ar	H_O	H_E	HWE	F_N
Smel-02	26	4	96–116	0.769	0.572	ns	0
Smel-05	32	4	152–168	0.563	0.463	ns	0
Smel-06	32	1	155				
Smel-08	32	4	151–199	0.156	0.249	ns	0.11
Smel-09	31	5	136–164	0.484	0.527	ns	0.06
Smel-10	32	7	253–293	0.500	0.745	*	0.14
Smel-13	23	6	134–190	0.391	0.480	ns	0.06
Smel-15	31	2	265–269	0.032	0.092	ns	0.11
Smel-18	32	1	155				
Smel-25	29	4	327–358	0.103	0.457	*	0.25
Smel-26	31	2	285–289	0.419	0.498	ns	0.05
Smel-27	32	3	397–413	0.438	0.597	ns	0.12
Smel-29	31	8	324–352	0.581	0.801	*	0.13
Smel-30	15	3	381–397	0.133	0.640	*	0.28

Individuals genotyped (N), number of alleles observed (N_A), allelic range (A*r*), observed and expected heterozygosities (H_O and H_E, respectively), statistical significance of deviation from Hardy–Weinberg equilibrium (HWE) and null allele frequency (F_N)

*Significance level after Sequential Bonferroni correction ($p < 0.003$). H_O and H_E, HWE deviations and FN were not estimated for Smel-06 and Smel-18 because they are monomorphic

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Smel-25, Smel-29 and Smel-30), which were detected after Bonferroni correction ($p < 0.003$). Null alleles could be the explanation of such deviations. Smel-06 and Smel-18 were excluded from the analyses due its monomorphic nature.

These markers could be adequate to species identification, amplify DNA of close related species is need to test this application. These 14 microsatellite loci are the first developed for this non-model organism and they are useful in studies such as population genetics, phylogeography, ecology, conservation, and management of this and related species.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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