Biochemical Systematics and Ecology 66 (2016) 109–113

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

Novel polymorphic microsatellite loci for a new target species, the sea cucumber *Holothuria mammata*

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ARTICLE INFO

Article history:
Received 7 February 2016
Accepted 12 March 2016
Available online 25 March 2016

Keywords:
Overexploitation
Sea cucumbers
Population genetics
Fisheries management

ABSTRACT

With traditional finfish fisheries declining and pushing transition to new invertebrates target species, sea cucumbers have been heavily explored, suffering global over-exploitation and worldwide depletion of their stocks.

Nowadays, holothurians from the Mediterranean Sea and NE Atlantic Ocean are being exported to Asian markets. The scarce knowledge about their biology, population dynamics, ecology and genetics, is promoting defective management of their fisheries.

We report the development of 9 novel polymorphic microsatellites markers for *Holothuria mammata* and characterized them by testing in three different sample locations. All nine microsatellites revealed high polymorphism and diversity, with high number of alleles, ranged from 11 to 22 and expected heterozygosity, between 0.52 and 0.92. Significant genetic differentiation was found between populations.

These microsatellites are providing valuable information which could be applied to fisheries management including, identification of stocks, assessment of their genetic diversity, estimation of gene flow and monitoring the fishery effects on exploited populations.

1. Introduction

The recent decline of several traditional finfish fisheries (Hutchings and Baum, 2005; Pauly et al., 2005; Eldridge et al., 2009), has forced the search for new fishing grounds and eventually, the transition from finfish to invertebrates as target groups, resulting in over pressure on most of non-finfish resources (Pauly, 1998).

Sea cucumbers, also known on fisheries markets as “beche-de-mer,” “trepang” or “haisom”, have been for centuries an Asiatic feeding product (Chen, 2003), but during the last 6 decades sea cucumber fisheries have grown exponentially not only in Asia but worldwide (Anderson et al., 2011).

With high commercial value (FAO, 2014) and fragile life histories (*e.g.* slow growth rate, late age at maturity (Uthicke et al., 2004)), sea cucumbers stocks have experienced unsustainable pressure from fisheries, causing nearly 60% of overexploitation in just a few decades (Purcell et al., 2013).

Due to the decline of Indo-Pacific fisheries coupled with increasing demand by Asian markets, the fisheries of sea cucumbers have been expanding worldwide (Purcell et al., 2013), including European species (Aydin, 2008; Sicuro and Levine, 2011). In Turkey, harvests and exportation to Asian market rapidly increased from 20 tonnes of sea cucumber in 2002 (Aydin,

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http://dx.doi.org/10.1016/j.bse.2016.03.012
0305-1978/

2008) to 550 tonnes in 2012 (González-Wangüemert et al., 2014), comprising species such as *Holothuria polii* (Delle-Chaije 1823), *Holothuria mammata* (Grube 1840) and *Holothuria tubulosa* (Gmelin 1970).

Recent studies have demonstrated that heavy fisheries reduce the genetic diversity of the populations (Pinsky and Palumbi, 2014; González-Wangüemert et al., 2015), reinforcing the need to develop molecular tools for detecting and measuring its magnitude (Allendorf et al., 2014).

We describe for the first time, the development and characterization of 9 polymorphic microsatellites for *H. mammata*, a recent exploited species in Mediterranean and North-Eastern Atlantic.

These molecular markers can be used to assess demographic and genetic parameters of stocks. This knowledge will improve the fishery and aquaculture management. For instance, paternity and inbreeding tests can optimize aquaculture production (Allendorf et al., 2013), while assessment of population genetic structure increases the effectiveness in the delineation of stocks (Gharbi and Said, 2011) and allows the preservation of the most vulnerable populations (ICES, 2014).

2. Materials and methods

Total genomic DNA was extracted from muscle tissues according to the procedure of Sambrook et al. (1989), with minor modifications. The development of the *H. mammata* specific primers for microsatellites was performed from 15 samples belonging to different locations.

Size selected fragments from genomic DNA were enriched for SSR content by using magnetic streptavidin beads and biotin-labelled CT and GT repeat oligonucleotides. The SSR enriched library was analysed on a Roche 454 platform using the GS FLX titanium reagents.

A total 7′082 reads had an average length of 159 base pairs. Of these, 435 contained a microsatellite insert with a tetra- or a trinucleotide of at least 6 repeat units or a dinucleotide of at least 10 repeat units. Suitable primer design was possible in 138 reads, of which 52 were tested for polymorphism. Finally 9 microsatellites were chosen according to feasibility and genetic diversity criteria (Table 1).

To optimize, characterize and determine the applicability of these microsatellites to population genetics studies, we tested them on 60 individuals from three different locations in the Atlantic and Mediterranean Sea, covering the edges of *H. mammata* geographical distribution (Peniche, Girona and Kusadasi; Fig. 1). The PCRs were performed according the conditions described in Table 2. The amplified samples were analysed with an ABI 3730 automated sequencer using forward primers labelled with FAM (SIGMA), VIC (Applied Biosystems), NED (Applied Biosystems) and PET (Applied Biosystems) to allow multiplexing.

Allele scoring was carried out using STRand v2.4.59. The number of alleles per locus (A), the observed (H_O) and expected (H_E) heterozygosity and the heterozygote deficiency (F_{IS}) were calculated using the software Genetix v.4.05.2 (Belkhir et al., 1996). Arlequin v. 3.5 (Schneider et al., 2000; Excoffier and Lischer, 2010) was used for assessing the genetic differentiation. The probability of “null alleles”, “stuttering”, and “large allele dropout” was evaluated using Micro-Checker v.2.2.3 (Van Oosterhout et al., 2004).

3. Results and discussion

Considering all populations, the nine microsatellite markers (GenBank accession numbers: KU904231–KU904239) showed 150 alleles and their polymorphism ranged from 11 alleles in Hm57/Hm15 to 22 alleles in Hm87/Hm74 (Table 1). The

Table 1
Dye, primers sequences, type of repeats, annealing temperature (T_a), number of alleles (A) and the size range, for *Holothuria mammata* microsatellites.

Locus	Dye	Primer sequences 5′→ 3′		Repeat type	T_a (°C)	A	Size range (bp)
Hm08	FAM	F	CGATGTTGAGCCATGACCAC	(GT) 15	56	18	63–105
		R	CGCTACTTGCGAGATGTCTAC				
Hm57	FAM	F	GGGACCAAAAAGCAAAACAAAAC	(AAAC)7	56	11	136–172
		R	GCCCAATCAAGTCGAAACCC				
Hm87	NED	F	ATGCTTAGCTGGCTTGTGTG	(TG)15	56	22	89–143
		R	CCTTCTTTGGCCATTAAGATGC				
Hm43	NED	F	CGGTGCATGCCCAGTTTG	(AC)12	56	16	83–115
		R	GCCACGCCTATTACTTTCCC				
Hm93	FAM	F	AGAACAGAGAGTTGGTTGTAAGC	(TG)13	56	14	130–166
		R	AGCAGTCACTCTAGAATCTCC				
Hm18	PET	F	CTGAGCAGCAACCTAATGCC	(TG)13	56	20	97–145
		R	ACGCAACAAATTTACACGGAAG				
Hm15	NED	F	CCATTGTTTAGGTCCTCGG	(CA)13	56	11	185–217
		R	GATGGCCCACTGGTAGAGAG				
Hm74	PET	F	ATACACACCCTCACCCACAC	(CA)13	56	22	71–149
		R	AATGTCCTCCTCCACGTAGC				
Hm03	VIC	F	TCTTTTAAGTGGCATTGTGTCC	(AC) 12	56	16	104–142
		R	TACCTTCTGCTCCTGACCTG				