Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0409.v1

Isolation and characterization of polymorphic microsatellite *loci* from the invasive worm *Branchiomma luctuosum* (Grube, 1870) (Annelida: Sabellidae)

Flávia Ariany Belato [a,b,c], Elisa Maria Costa-Paiva [c], Paulo Cesar Paiva [b], Anderson Vilasboa [a,*]

a. Laboratório de Genética Pesqueira e da Conservação, Universidade do Estado do Rio de Janeiro, RJ, Brazil
b. Laboratório de Polychaeta, Universidade Federal do Rio de Janeiro, RJ, Brazil
c. Laboratório de Biologia Evolutiva Teórica e Aplicada, Universidade Federal do Rio de Janeiro, RJ, Brazil

*Corresponding author: Laboratório de Genética Pesqueira e da Conservação, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil
E-mail: anderson.vasconcellos@uerj.br

Abstract

Introduction of exotic species in new areas through anthropic action is one of the major problems that can affect biodiversity. *Branchiomma luctuosum* is known for its highly invasive potential and the actual occurrence of species commonly associated with port activity areas is an extra evidence that this anthropogenic activity should not be underestimated. In order to develop suitable molecular markers for future studies on colonization routes and population dynamics of the invading individuals of *B. luctuosum*, nine highly polymorphic microsatellite *loci* were isolated and their polymorphism levels were evaluated. These *loci* showed a range of number of alleles per *locus* from five to ten and all *loci* had a high level of genetic diversity, and exhibited significant heterozygote deficiencies probably due to the presence of null alleles. Significant deviations from the Hardy-Weinberg equilibrium were detected at several *loci* and most of them were related to a heterozygous deficit. Heterozygous deficiency can be expected in this case due to the biology and history of this invasive species, in relation to its recent introduction in Brazilian coast and possible action of multiple introductory events.

Keywords: Bioinvasion; Molecular Markers; Fanworm; Biofouling

Acknowledgements

We thank Antonio Solé-Cava for the use of laboratory during microsatellite markers development and optimization. Fellowships for FAB and PCP were provided by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPERJ (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro, Process: E26/110.015/2011). EMCP received a fellowship from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

© 2018 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0409.v1

Introduction of exotic species in new areas through anthropic action is one of the major problems that can affect biodiversity. With the increasing maritime traffic, introduction of alien species has occurred more often, especially with ballast water and biofouling (Mastrototaro et al. 2015). Exotic organisms usually do not have natural predators in the new environment and can cause deep ecological, economic and health risk impacts. Originally described for the Red Sea, *B. luctuosum* is known for its highly invasive potential (El Haddad et al. 2008). Currently, *B. luctosum* is spread in Mediterranean Sea (Giangrande, 1989), eastern Iberian coast (El Haddad et al. 2008), Italian coast (Licciano and Giangrande, 2008), Atlantic Coast of Africa (Mastrototaro et al. 2015) and Brazil (Nogueira *et al*. (2006).

To develop suitable molecular markers for studies on colonization routes and population dynamics of *B. luctuosum*, nine polymorphic microsatellite *loci* were isolated and characterized in the present study. Twenty-five specimens of *B. luctuosum* were collected in Florianópolis (SC), South Brazil. Genomic DNA was extracted from the body wall using a slightly modified protocol from Hillis *et al*. 1990. Microsatellite libraries were developed following the methods of Jones *et al*. (2002). For genotyping, we employed the tailed primer method (Schuelke, 2000). PCR mix consisted of 1 U GoTaq (Promega), 0.20 mM dNTPs, 2.5 mM $MgCl_2$, 1 mM BSA, 0.5 µM of reverse primer, 0.25 mM of oligo marked with a dye (tail) primer, and 0.13 of mM forward primer, with a final volume of 15 µL per reaction containing 30 ng of DNA template. Cycling conditions were: 94°C, 3 min, 30 cycles at 94°C, 45 sec (s); 52°C–67°C, according to each primer, 45 s; 72°C, 45 s, 8 cycles at 94°C, 45 s; 53°C, 45 s; 72°C, 45 s and 72°C, 30 min. Samples were pooled with a size standard (GeneScan 500-LIZ), and genotyped using the automated platform ABI3500. The GeneMarker® software V 2.6.3 (SoftGenetics LLC) was used for genotyping score and allele sizing. The possibility of occurrence of genotyping errors was calculated using Micro-Checker V 2.2.3 (Van Oosterhout et al. 2004). Number of alleles, allele frequencies, observed and expected heterozygosities were evaluated using Fstat V 2.9.3 (Goudet, 2002). The 4.2 online version of GENEPOP (Raymond and Rousset, 1995) was used to test for linkage disequilibrium.

All nine *loci* had a high level of genetic diversity (Table 1) and exhibited significant heterozygote deficiencies probably due to the presence of null alleles, as suggested by Micro-Checker. Moreover, the occurrence of null alleles has been commonly reported for similar studies with annelids (Weinmayr et al. 2000; Pettengill et al. 2003; Du et al. 2007). Our results did not observed any significant linkage disequilibrium between any pair of *loci*. We observed heterozygous deficiency and it was expected considering the biology and history of this invasive species (recent introduction and the possibility of multiple introductory events). Therefore, the microsatellite markers developed here may be very useful for studies on population dynamics of this invasive species.

References

Du H, Han J, Lin K et al (2007) Characterization of 11 microsatellite *loci* derived from genomic sequences of polychaete *Capitella capitata* complex. Mol Ecol Notes 7(6): 1144-1146

Haddad M, Azzati RC, García-Carrascosa AM (2008) Branchiomma luctuosum (Polychaeta: Sabellidae): a non-indigenous species at Valencia Port (western Mediterranean Sea, Spain). Mar Biodivers Rec 1: e61

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0409.v1

Giangrande A (1989) Censimento dei policheti dei mari italiani: Sabellidae Malmgren, 1867. Atti Soc Toscana Sci Nat Pisa, Mem, Ser B 96: 153–189

Goudet J (2002) FSTAT v.2.9.3.2: a computer program to calculate F-statistics. J Hered 86: 485-486

Hillis DM, Larson A, Davis SK, Zimmer EA (1990) Nucleic acids III: sequencing. In: Moritz C et al (ed) Molecular systematics, 3rd edn. Sunderland, Massachusetts, pp 318-370

Jones KC, Levine KF, Banks JD (2002) Characterization of 11 polymorphic tetranucleotide microsatellites for forensic applications in California elk (*Cervus elaphus canadensis*). Mol Ecol Notes 2(4): 425-427

Licciano M, Giangrande A (2008) The genus Branchiomma (Polychaeta: Sabellidae) in the Mediterranean Sea, with the description of B. maerli n. sp. Sci Mar 72(2): 383-391

Mastrototaro F, Chimienti G, Matarrese A et al (2015) Growth and population dynamics of the non-indigenous species *Branchiomma luctuosum* Grube (Annelida, Sabellidae) in the Ionian Sea (Mediterranean Sea). Mar Ecol 36(3): 517-529

Nogueira JMM, Rossi MCS, Lopez E (2006) Intertidal species of *Branchiomma* Kolliker and *Pseudobranchiomma* Jones (Polychaeta: Sabellidae: Sabellinae) occurring on rocky shores along the state of São Paulo, southeastern Brazil. Zool Stud 45(4): 586

Pettengill JB, Hadfield MG, Schug MD, Wendt DE (2003) Characterization of six polymorphic microsatellites for the polychaete tubeworm *Hydroides elegans* and cross-species amplification in the congener *Hydroides hexagonus*. Mol Ecol Notes 3(3): 369-371

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): Population Genetics Software for exact tests and ecumenicism. J Hered 86(3): 248-249

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18(2): 233-234

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4: 535-538

Weinmayr G, Vautrin D, Solignac M (2000) Isolation and characterization of highly polymorphic microsatellites from the Polychaete *Pectinaria koreni*. Mar Biotechnol 2(1): 92-99

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0409.v1

Table I - Characteristics of nine polymorphic microsatellite markers developed for *B. luctuosum*. Locus name; Repeat motif; F: forward primer sequence; R: reverse primer sequence; N = number of samples; Na = Number of alleles; H(o) = Observed Heterozygosity; H(e) = Expected Heterozygosity.

Locus		Primer Sequence (5'-3')	Motif	Size range (bp)	N	Na	H(o)	H(e)
Bluc07	F	GACAATTCAAACTGCGACTGAC	GT(12)	284-298	22	6	0.636	0.771
	R	GTGTTTAGGGTTCTAGGGCAAA						
Bluc08	F	CAACTGCCATACAAAACTACACTGA	CA(8)	270-284	24	8	0.583	0.867
	R	AGGGACAGCCAGGGTTTG						
Bluc23	F	GGAGACAACATTCAAAAAGTGA	AC(15)	299-319	25	9	0.640	0.723
	R	GGATTCAAACTGACGACTCTG						
Bluc25a	F	AATTCAATGGCTAGGTCTATCC	GT(10)	197-207	25	6	0.760	0.809*
	R	GGCTGAGAAATACAGATTTTTG						
Bluc25b	F	AATTCAATGGCTAGGTCTATCC	GT(10)	348-356	25	5	0.000	0.650*
	R	GGCTGAGAAATACAGATTTTTG						
Bluc27	F	GTTCGTTTGTCCGTTATTCAG	TG(9)...TG(12)	232-240	22	5	0.909	0.754
	R	TATGGCTTCAGCATTAAATCTG						
Bluc32	F	TGTTGCTGCTGTTGTTGTTGTA	TTG(7)	393-411	20	5	0.300	0.755*
	R	CTGACTGACACCTGACACCTATG						
Bluc34	F	CCCAACTCAAGTAACCAGTCCT	TTG(6)	331-370	25	10	0.440	0.811*
	R	CGAAACAACACTAATTCAACGC						
Bluc36	F	TTCCTTGCTGGACACTGAGATA	GTT(4)	267-291	25	9	0.720	0.874*
	R	ACAATTAGGCCAGATGAGTGCT						

* Significant deviations from HWE