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► To cite this version:

Mariana Padrón, Marine Milhes, Adline Delcamp, Erwan Guichoux, Katell Guizien. Development of a set of SNP markers for population genetics of the sea peach (*Halocynthia papillosa*). Conservation Genetics Resources, 2023, 10.1007/s12686-023-01313-7 . hal-04004739

HAL Id: hal-04004739

<https://hal.science/hal-04004739v1>

Submitted on 25 Feb 2023

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2 **Development of a set of SNP markers for population genetics of the sea peach**

3 **(*Halocynthia papillosa*)**

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12 **Abstract**

13 A set of single nucleotide polymorphism (SNP) markers was developed from the
14 transcriptome of the solitary ascidian *Halocynthia papillosa*. 1399 putative SNPs with
15 coverage larger than 100 reads were identified, and a selected set of 320 SNPs was tested
16 using a MassARRAY System on 95 samples from the NW Mediterranean. A total of 175
17 SNPs were successfully genotyped, polymorphic and in Hardy-Weinberg equilibrium over all
18 samples. The newly developed loci will be a valuable tool for population genetics studies of
19 this endemic ascidian species of the Mediterranean Sea.

20 **Keywords**

21 *Halocynthia papillosa*, Single nucleotide polymorphism, MassARRAY

23 **Introduction**

24 The solitary ascidian *Halocynthia papillosa* is an endemic species of the Mediterranean sea
25 (Pérès and Picard, 1964) and Eastern Atlantic from the Canary Islands to the Berlengas

26 Islands in Portugal (Ramos-Esplá, 1991). It is a common sessile mega-benthic species with
27 medium size of about 15 cm that distributes evenly with low population density (a few
28 individuals per meter square) on hard bottom between 10 to 100 m in the Mediterranean sea
29 (Antoniadou et al. 2006; Coppari et al. 2014). Tunicates have been extensively studied for
30 their tunique made of a unique animal cellulose (Helbert et al. 1998). In contrast, the ecology
31 of the species has driven less interest. The species is a filter-feeder with a broad natural diet
32 including detrital organic matter, heterotrophic bacteria, *Prochlorococcus* sp., *Synechococcus*
33 sp., protozoa and phytoplankton with a mean size range from 0.6 to 70 μm (Ribes et al.
34 1998). It is a simultaneous hermaphrodite with a spawning period extending over 2 to 3
35 months, between September and November (Becerro and Turon, 1992). Ecologically, it is
36 considered an indicator of good conditions and unperturbed rocky areas (Naranjo et al. 1996).
37 Low population density of *H. papillosa* has been correlated to high SCUBA diving frequency
38 (Luna-Pérez et al., 2010). The deleterious impact of SCUBA diving on *H. papillosa* was
39 further tested experimentally by applying treatments with various intensities of flapping, with
40 highest flapping intensity altering *H. papillosa* growth and abundance (Luna-Pérez et al.,
41 2011). Nevertheless, the species has been found on artificial reefs deployed over soft-bottom
42 substrates, showing the potential of the species to survive in turbid environments (Blouet et
43 al. 2022). In addition, the colonisation of artificial reefs located at a few kms from any rocky
44 substrate question the dispersal potential of this species, an essential mechanism for
45 population recovery in a sensitive species. The objective of the present study is to propose a
46 set of Single Nucleotide Polymorphism markers that will be useful to study *H. papillosa*
47 population genetics and connectivity.

48

49 **Material and Methods**

50 *Transcriptome sequencing*

51 The transcriptome of *H. papillosa* was sequenced from the total RNA of ten individuals
52 collected by SCUBA diving between 10 and 20 meters depth in Banyuls sur Mer, France.
53 Small fragments were immediately frozen in liquid nitrogen, and preserved at -80 °C. Total
54 RNA extractions were performed with Maxwell 16 LEV simplyRNA purification kits. RNA
55 concentrations and quality were analysed with an Agilent 2100 Bioanalyzer. RNA-seq
56 libraries were prepared according to Illumina's protocols using the Illumina TruSeq Stranded
57 mRNA sample prep kit, using 11 cycles of PCR to amplify libraries. Library quality was
58 assessed using a Fragment Analyzer. Libraries were quantified by qPCR using the Kapa
59 Library Quantification Kit. Sequencing was achieved on an Illumina HiSeq3000 using a
60 paired-end read length of 2×150 pb.

61

62 *Marker development and screening*

63 Sequence read quality was assessed using FastQC v0.10.1 (Andrews 2010). Trimmomatic
64 v0.32 was used to remove low quality reads with a Phred score below 20, as well as the
65 Illumina adapters (Bolger et al. 2014). FastQC was performed again to verify the integrity of
66 the remaining raw Illumina sequence reads. High-quality reads were then used for the de
67 novo transcriptome assembly, using Trinity with its default k-mer value of 25 (Grabherr et al.
68 2011). DiscoSnp ++ v2.2.10 (Uricaru et al. 2015) was used independently with kmer size of
69 61 and a minimal coverage per read 20 to call SNPs using the high-quality reads of the 10
70 individuals transcriptomes. A total of 28320 SNPs were found among which 1399 SNPs had
71 good coverage (> 100 reads) in each of the 10 individual's transcriptomes. A set of 320 SNPs
72 were randomly selected and submitted for assay design using the MassARRAY Assay
73 Designer version 4.0.0.2 (Agena Biosciences). The assay was performed on the genomic
74 DNA extracted from 95 samples of *H. papillosa* collected in 6 locations in the NW
75 Mediterranean (Table 1, 5 locations in Agde and 1 location in Saint-Cyprien), using the

76 DNeasy Blood and Tissue kits from QIAGEN. Allele calling was carried out with Typer
77 Viewer v.4.0.24.71 (Agena Biosciences). Monomorphic SNPs, loci with weak or ambiguous
78 signal (i.e., displaying more than three clusters of genotypes or unclear cluster delimitation)
79 and loci with too much missing data were all discarded. The genetic parameters, including
80 observed heterozygosity, expected heterozygosity, and the Hardy–Weinberg equilibrium
81 (HWE), were calculated using Genepop 4.7.5 (Rousset 2008).

82 **Table 1.-** Location and number of individuals (N) of *Halocynthia papillosa* used for SNP
 83 validation using MassArray
 84

Location	Site	Latitude (decimal degrees)	Longitude (decimal degrees)	Depth (m)	N
Cap d'Agde - natural rocks	A1	43,252556	3,519840	13	20
	A4	43,244578	3,519568	25	15
Cap d'Agde – artificial reef	AGD26	43,2386	3,4992	20	10
	AGD28	43,2521	3,5378	20	6
	AGD30	43,2543	3,4756	10	20
Saint Cyprien – artificial reef	CYP 54	42,6293	3,0720	30	20

85

86

87 **Results**

88 A total of 220 SNPs were successfully amplified across more than half of the samples. A
 89 subset of 188 loci were found to be polymorphic, resulting in a detectable rate of 85.4 %. The
 90 observed heterozygosity ranged from 0.011 to 0.989, while the expected heterozygosity
 91 varied from 0.011 to 0.503 (Supplementary Material). Among the 188 loci, 175 were found in
 92 HWE proportions over 95 samples ($p > 0.01$). These results provide a valuable resource for
 93 future population genetic studies of this endemic ascidian species in the Mediterranean Sea.

94

95 **Acknowledgements**

96 This work was funded by the Agence de l'Eau Rhône-Méditerranée-Corse under project
 97 ICONE - Impacts actuels et potentiels de la CONnectivité Ecologique ajoutée par les récifs
 98 artificiels sur la biodiversité fixée des substrats durs du Golfe du Lion (PI, K. Guizien, AAP
 99 2016). The authors gratefully acknowledge the helpful assistance during sampling of S.
 100 Blouet and the staff of the Aire Marine Protégée Agatoise. We also thank the scuba divers, JC
 101 Roca and B Hesse, for their technical assistance for field sampling in Banyuls sur Mer. We
 102 are grateful to the Bio2Mar core facility (<http://bio2mar.obs-banyuls.fr>) for providing access
 103 to instrumentation for RNA extractions. RNAseq was performed at the GeT-PlaGe core

104 facility, INRAE Toulouse. SNP genotyping was performed at the PGTB
105 (doi:10.15454/1.5572396583599417E12) with the help of Laure Dubois.

106

107 Author contributions: MP and KG designed the study. MM, AD, and EG, performed the
108 sequencing and genotyping. MP analyzed the data. KG wrote the main manuscript text. All
109 authors reviewed the manuscript.

110

111 Funding: This work was funded by the Agence de l'Eau Rhône-Méditerranée-Corse under
112 project ICONE - Impacts actuels et potentiels de la CONnectivité Ecologique ajoutée par les
113 récifs artificiels sur la biodiversité fixée des substrats durs du Golfe du Lion (PI, K. Guizien,
114 AAP 2016, grant numbers : 2017 7267 / 2017 7268 / 2018 0697).

115

116 Data availability: The RNA seq data will be released publicly through National Center for
117 Biotechnology Information portal (<https://submit.ncbi.nlm.nih.gov>).

118

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