

Environmental and demographic factors influence the spatial genetic structure of an intertidal barnacle in central-northern Chile

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Supplement. Development of microsatellite loci for the barnacle *Notochthamalus scabrosus*

One adult individual was sampled at Estación Costera de Investigaciones Marinas (ECIM), Las Cruces, central coast of Chile (33.30°S), and placed in ethanol 95%. Total genomic DNA was extracted from the whole organism using the modified method of salt/Proteinase K (Aljanabi & Martinez 1997). The lysis buffer was composed of 100 mM Tris-HCl pH 8.0, 1.4 M NaCl, 0.02 M EDTA pH 8.0, CTAB 2%, SDS 0.3% and PEG 1%. Tissue lysis was completed in a 2 mL Eppendorf® tube with 800 µL of lysis buffer and 5 µL of Proteinase K (20 mgmL⁻¹) at 65°C for 12 hours. Lysate was purified twice with 800 µL of chloroform:isoamyl alcohol (20:1). DNA was precipitated with isopropanol:ammonium acetate (10:1) and washed with ethanol 70%, and re-suspended in 25 µL of ultrapure. DNA was further purified with Wizard® Genomic DNA Purification kit (Promega) and quantified in a spectrophotometer NanoDrop® ND-1000 (ThermoScientific). Total DNA was sequenced by pyrosequencing on a GS Junior System (454 Life Sciences). Bioinformatics search of microsatellites was performed using MISA Perl script and primer design with the Primer3 tools. Only perfect motifs (di, tri and tetranucleotides) with the highest number of repeats and amplified fragment size between 100 and 300bp were selected. Designed primers were checked with AmplifX1.5 to avoid designing primers in poly X areas and to prevent the formation of dimers or loops. Primers amplification was evaluated and PCR conditions were optimized by genotyping seven individuals from seven different sites.

All primers had a fluorescent M13 tail at the 5' end of the forward primer (i.e. 5'-CAC GAC GTT GTA AAA CGA C-3'). PCR was performed in 10 µL total volume containing 2 µL DNA diluted at 15 ngµL⁻¹, 1 X buffer, 2.5 mM MgCl₂, 0.2 mM dNTPs, 0.35 U *Taq* DNA polymerase (GoTaqFlexi, Promega). The PCR mix contained also 1X BSA, 150 nM M13-tailed forward primer, 150 nM reverse primer, and 150 nM non-tailed forward primer. For all loci PCR cycling conditions consisted of an initial denaturation at 95°C for 5 min followed by 35 cycles: denaturation at 95°C for 1 min, annealing at specific temperature (Table S1) for 1 min, extension at 72°C for 1 min, and with a final extension at 72°C for 10 min.

Alleles were identified on the ABI Prism 3130xl (Applied Biosystems), with the size standard GeneScan 500 LIZ (AppliedBiosystems) and the allele size was determined with PeakScanner 1.0 (AppliedBiosystems). *Notochthamalus scabrosus* sequencing provided 25,161 reads with an average length of 359.26 bp and an average quality of 31.81 (Phred quality score). For the present study, 28 candidate loci from a total of 416 identified microsatellite loci, were selected for PCR optimization, of which seven loci gave a reliable amplification and polymorphic genotypes (Table S1).

Table S1. Characteristics of the microsatellites markers developed for *Notochthamalus scabrosus* (NS). TA is the annealing temperature, Size (bp) is the allele size range. Untailed sequences are provided.

Locus	Repeat motif	Primers sequences (5'-3')	TA (°C)	Size (bp)	GenBank ID
NS3	(GCCT) ₈	F: <u>CCACCAGCCTGTTATGAGGT</u> R: <u>AGATCAGAGCTCGGTTTTGG</u>	53	190-230	KX011326
NS5	(ACAG) ₈	F: <u>TCACTGGGGAAAGAAACTCG</u> R: <u>GTGCCGAGCCACAGAACC</u>	61	160-172	KX011327
NS7	(GTCT) ₁₅	F: <u>GCAAAAATATGCCACGATTC</u> R: <u>CTCCTACGGCAGAGGATACG</u>	60	143-287	KX011328
NS8	(GACA) ₁₀	F: <u>TTGGCATTGTTTTATCGCAA</u> R: <u>GCTGTAATAGCCGCGAGTTT</u>	53	192-220	KX011329
NS9	(AGAC) ₈	F: <u>GGGGAGGGGAGGAGTATGTA</u> R: <u>CCCTGTTTGTCTGACCGTCT</u>	53	254-310	KX011330
NS10	(CCG) ₈	F: <u>TGTCAGGTCACCAGATCGTC</u> R: <u>ATCGACACGGAAAGCTCCTT</u>	61	238-292	KX011331
NS18	(GCA) ₈	F: <u>ACGTGTTTGGTGGTTGGTTT</u> R: <u>AGGACAGGACTGGTTTGGTG</u>	53	178-220	KX011332

Table S2. Summary statistics of genetic diversity within *Notochthamalus scabrosus* by locus and site and overall, and per-site values of predictive variables of genetic diversity of *N. scabrosus*. Code sites as follow; CBaj: Carrizal Bajo, Apol: Apolillado, Temb: Temblador, Guan: Guanaqueros, PTal: Punta de Talca, LMol: Los Molles, Mont: Montemar, Cura: Curaumilla, ECIM: Estación Costera de Investigaciones Marinas, Pich: Pichilemu.

Locus	CBaj	Apol	Temb	Guan	PTal	LMol	Mont	Cura	ECIM	Pich	Total
Ns3											
Na	9	12	14	14	11	10	13	12	10	12	20
Pa	0	1	1	0	0	0	0	1	0	0	3
Ar	8.875	11.766	13.673	13.582	10.766	9.891	12.658	11.782	9.989	11.773	11.476
Gd	0.824	0.856	0.898	0.900	0.856	0.863	0.877	0.874	0.829	0.877	0.865
He	0.818	0.850	0.893	0.894	0.845	0.856	0.874	0.869	0.822	0.875	0.860
Ho	0.467**	0.500**	0.567**	0.533**	0.200**	0.500**	0.700**	0.600**	0.433**	0.767	0.527**
F _{IS}	0.434**	0.416**	0.369**	0.407**	0.766**	0.420**	0.202	0.314**	0.477**	0.126	0.393
An	0.187	0.183	0.166	0.184	0.345	0.186	0.086	0.138	0.207	0.051	0.173
Ns5											
Na	5	5	4	6	5	3	6	6	5	4	8
Pa	0	0	0	0	1	0	0	0	0	0	1
Ar	4.900	4.999	3.900	5.891	4.892	3.000	5.883	5.982	4.892	3.900	4.824
Gd	0.610	0.689	0.548	0.697	0.603	0.613	0.584	0.729	0.524	0.461	0.606
He	0.606	0.685	0.545	0.694	0.599	0.610	0.581	0.723	0.523	0.458	0.602
Ho	0.400**	0.433**	0.400**	0.500**	0.367**	0.400**	0.433**	0.367**	0.433	0.267**	0.400**
F _{IS}	0.344	0.371*	0.270	0.282	0.392	0.348	0.258	0.497**	0.173	0.421	0.336
An	0.123	0.143	0.089	0.108	0.140	0.125	0.088	0.201	0.053	0.126	0.126
Ns7											
Na	20	26	26	23	25	23	22	24	22	22	42
Pa	2	0	0	1	0	0	0	0	0	0	3
Ar	19.605	24.893	25.277	22.640	23.931	22.139	21.147	22.863	20.964	21.155	22.461
Gd	0.897	0.960	0.971	0.961	0.957	0.951	0.935	0.951	0.934	0.944	0.946
He	0.889	0.954	0.963	0.953	0.951	0.949	0.929	0.946	0.929	0.938	0.940
Ho	0.464**	0.621**	0.517**	0.536**	0.567**	0.800**	0.600**	0.667**	0.633**	0.633**	0.604**
F _{IS}	0.482**	0.353**	0.467**	0.442**	0.408**	0.159**	0.358**	0.299**	0.322**	0.329**	0.362
An	0.317	0.218	0.271	0.304	0.190	0.069	0.164	0.137	0.147	0.151	0.197
Ns8											
Na	11	12	14	13	14	12	12	13	9	11	21
Pa	1	1	1	0	0	0	0	2	0	0	5
Ar	10.691	11.675	13.481	12.673	13.498	11.682	11.782	12.849	8.883	10.790	11.800
Gd	0.864	0.869	0.876	0.871	0.876	0.863	0.887	0.881	0.840	0.876	0.870
He	0.863	0.868	0.872	0.868	0.872	0.859	0.885	0.880	0.838	0.873	0.868
Ho	0.833	0.800	0.600**	0.667**	0.633**	0.633**	0.767**	0.800	0.733**	0.700**	0.717**
F _{IS}	0.035	0.079	0.315**	0.235*	0.277**	0.266**	0.135	0.092	0.127	0.201	0.176
An	0.008	0.029	0.139	0.101	0.121	0.115	0.055	0.035	0.050	0.086	0.074
Ns9											
Na	6	7	6	6	6	5	8	6	9	8	15
Pa	0	0	0	1	2	0	0	0	1	0	4
Ar	5.700	6.920	5.931	5.792	5.964	4.900	7.866	5.899	8.851	7.858	6.568
Gd	0.648	0.629	0.571	0.511	0.604	0.660	0.544	0.668	0.544	0.655	0.603
He	0.644	0.626	0.567	0.508	0.598	0.656	0.540	0.663	0.541	0.650	0.599
Ho	0.400**	0.448	0.379**	0.333**	0.286**	0.433**	0.267**	0.367**	0.345**	0.414**	0.367**
F _{IS}	0.383	0.287	0.336	0.348	0.527**	0.344	0.510**	0.451**	0.367**	0.368**	0.392
An	0.143	0.186	0.199	0.111	0.312	0.129	0.172	0.173	0.207	0.212	0.184
Ns10											
Na	12	14	15	14	12	12	14	14	11	15	27
Pa	0	3	1	3	0	0	0	0	0	0	7
Ar	11.848	13.775	14.781	13.819	12.000	11.709	13.775	13.549	10.766	14.573	13.060
Gd	0.880	0.881	0.933	0.874	0.818	0.759	0.828	0.753	0.771	0.895	0.839
He	0.869	0.871	0.924	0.866	0.809	0.748	0.819	0.746	0.762	0.888	0.830
Ho	0.241**	0.276**	0.400**	0.464**	0.370**	0.172**	0.310**	0.367**	0.233**	0.500**	0.333**
F _{IS}	0.726**	0.687**	0.571**	0.469**	0.547**	0.773**	0.625**	0.513**	0.697**	0.441**	0.605
An	0.375	0.359	0.266	0.310	0.379	0.373	0.325	0.212	0.295	0.199	0.309
Ns18											
Na	16	15	16	13	15	16	13	13	14	12	25
Pa	0	1	0	0	0	1	1	0	1	0	4
Ar	15.391	14.475	15.390	12.790	14.641	15.381	12.582	12.581	13.599	11.956	13.879
Gd	0.921	0.906	0.913	0.909	0.907	0.902	0.889	0.868	0.920	0.882	0.902
He	0.919	0.904	0.912	0.907	0.904	0.902	0.889	0.868	0.918	0.884	0.901
Ho	0.833	0.767*	0.867*	0.800**	0.724**	0.900	0.900	0.833*	0.800**	0.967	0.839**
F _{IS}	0.095	0.154	0.050	0.120	0.202	0.002	-0.013	0.040	0.130	-0.096	0.068
An	0.037	0.065	0.016	0.049	0.150	0.000	0.000	0.011	0.054	0.000	0.038

Table S2 *continued*

Locus	CBaj	Apol	Temb	Guan	PTal	LMol	Mont	Cura	ECIM	Pich	Total
<i>Pooled 7 loci</i>											
Na	79	91	95	89	88	81	88	88	80	84	158
Pa	3	6	3	5	3	1	1	3	2	0	27
Ar	11.001	12.643	13.205	12.455	12.242	11.243	12.242	12.215	11.135	11.715	12.010
Gd	0.806	0.827	0.816	0.818	0.803	0.802	0.792	0.818	0.766	0.799	0.805
He	0.801	0.823	0.811	0.813	0.797	0.797	0.788	0.814	0.762	0.795	0.800
Ho	0.520**	0.549**	0.533**	0.548**	0.450**	0.548**	0.568**	0.571**	0.516**	0.607**	0.541
F _{IS}	0.355**	0.336**	0.347**	0.330**	0.440**	0.316**	0.283**	0.301**	0.326**	0.240**	0.327
An	0.170	0.169	0.164	0.167	0.234	0.142	0.127	0.129	0.145	0.118	0.156
<i>Predictive variables</i>											
CHLA1	0.1345	0.3949	0.4745	0.3282	0.0518	0.2129	0.3743	0.1802	0.4737	0.2192	
CHLA2	0.0803	0.4229	0.5458	0.1387	-0.0052	-0.2136	-0.2449	-0.2303	-0.5415	-0.2138	
SST1	0.2951	0.3265	0.3183	0.3284	0.3195	0.3343	0.3087	0.3090	0.3420	0.2749	
SST2	-0.4728	-0.2548	-0.2999	-0.3360	-0.0661	0.1254	0.2217	0.3617	0.2792	0.4802	
Rec	0.0121	0.1425	1.1945	0.0823	0.0004	0.0008	0.0236	0.0072	0.3475	0.0559	
Cov	0.7846	3.3714	22.524	31.235	0.4375	0.1084	3.0625	9.1915	7.9778	0.8241	
Lat	28.09	29.21	29.30	30.11	30.55	32.14	32.57	33.06	33.30	34.25	
Lon	71.16	71.48	71.19	71.28	71.40	71.30	71.32	71.44	71.37	72.02	

Na, observed number of alleles; Pa, number of private alleles; Ar, allelic richness; Gd, gene diversity; He, the expected heterozygosity; Ho, the observed heterozygosity; F_{IS}, the inbreeding coefficient; An, the null allele frequency. CHLA1, PC1 of chlorophyll *a* concentration; CHLA2, PC2 of Chlorophyll-*a* concentration; SST1, PC1 of sea surface temperature; SST2, PC2 of sea surface temperature; Rec, recruitment rate of *N. scabrosus*; Cov, adult cover of *N. scabrosus*; Lat, latitude; Lon, longitude. *Significant at the nominal level ($p < 0.05$); **Significant after FDR correction.

Table S3. Pearson correlations among variables used. Values of the correlation coefficient r below the diagonal and p -values above the diagonal. Values in bold indicates significant at the nominal level p -value < 0.05 .

	Ar	Gd	CHLA1	CHLA2	SST1	SST2	Cov	Rec	Lat	Lon
Ar	1	0.06	0.33	0.03	0.85	0.47	0.08	0.32	0.43	0.86
Gd	0.62	1	0.64	0.01	0.70	0.14	0.62	0.96	0.10	0.82
CHLA1	0.34	-0.17	1	0.64	0.23	0.91	0.04	0.00	0.96	0.66
CHLA2	0.70	0.78	0.17	1	0.99	0.01	0.43	0.37	0.01	0.43
SST1	0.07	-0.14	0.42	-0.00	1	0.57	0.58	0.89	0.70	0.10
SST2	-0.26	-0.50	-0.04	-0.78	-0.21	1	0.61	0.68	0.00	0.04
Cov	0.57	0.18	0.65	0.28	0.20	-0.19	1	0.01	0.75	0.78
Rec	0.35	-0.02	0.86	0.32	0.05	-0.15	0.75	1	0.97	0.97
Lat	-0.28	-0.55	0.02	-0.81	-0.14	0.98	-0.12	-0.10	1	0.05
Lon	-0.07	-0.08	-0.16	-0.28	-0.56	0.65	-0.21	0.02	0.62	1

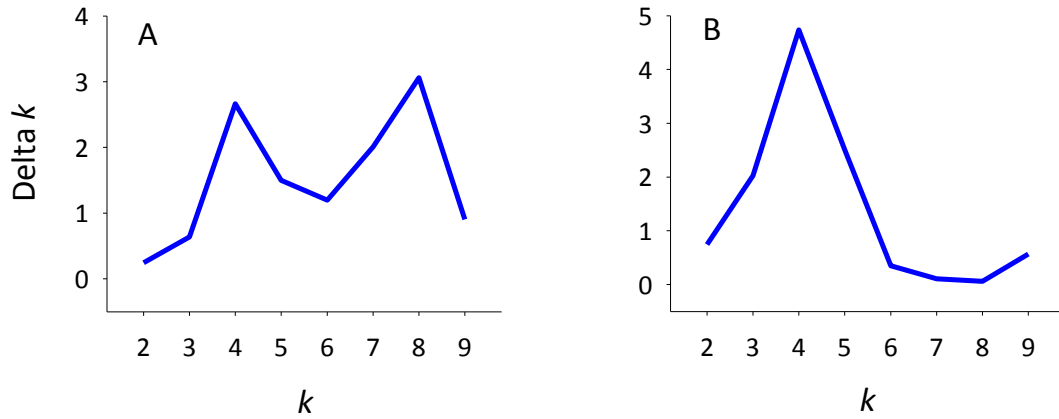


Figure S1. Delta k values (Δk , a measure of the rate of change in the STRUCTURE likelihood function) as a function of k , the number of putative populations. Graphs A and B show the results for the model without and with option that accounts for the null alleles, respectively.

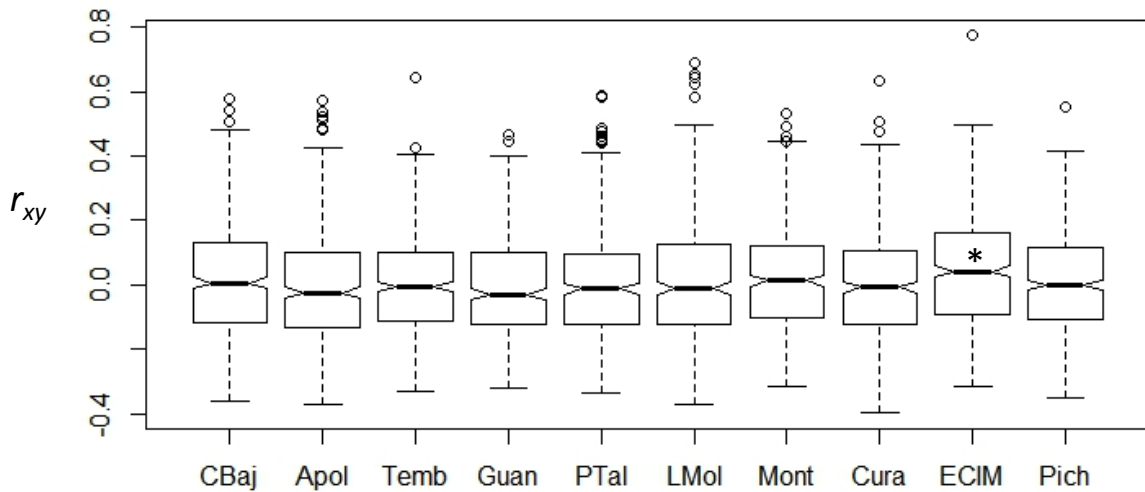


Figure S2. Average (+EE) of relatedness coefficient r_{xy} calculated following to Queller and Goodnight (1989) between all individuals of studied sites.*Significant at the nominal p -value < 0.05.

References

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