

The following supplement accompanies the article

Complex genetic structure revealed in the circum-Antarctic broadcast spawning sea urchin *Sterechinus neumayeri*

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Table S1. Details of primers and PCR protocols¹ used for the amplification of the COI and 16S gene regions in *Sterechinus neumayeri*.

Gene region	Primers	Thermocycler protocol	Primer reference
COI	LCO11490ERC	ACACTATATTTGATTTTGG	Lee et al. (2004)
	HCOINERCH	CGACTACGTAGTATGTGTCA	
16S	16Sar	CGCCTGTTTATCAAAAACAT	Palumbi (1996)
	16Sbr	CCGGTCTGAACTCAGATCACGT	

¹All PCRs were in a total volume of 20 µL comprising 10 -100 ng of template DNA, 3.75 mM MgCl₂, 2 µL PCR Buffer (Bioline BioTaq Red PCR buffer), 1 µL of 10xBSA, 0.25 mM each dNTP, 0.25 mM each primer and 0.5 units of BioTaq Red DNA polymerase. PCR products were visualised on a 1% agarose gel containing 0.15 µL Goldview (Guangzhou Geneshun Biotech Ltd.). Qiagen QIAquick PCR Product Purification Kits were used to purify PCR products according to the manufacturer's instructions.

Lee YH, Song M, Lee S, Leon R, Godoy SO, Canete I (2004) Molecular phylogeny and divergence time of the Antarctic sea urchin *Sterechinus neumayeri* in relation to the South American sea urchins. Antarctic Science 16 29–36

Palumbi SR (1996) Nucleic acids II: the polymerase chain reaction. In: Hillis DM MC, Mable BK (ed) Molecular Systematics. Sinauer & Associates Inc., Sunderland, Massachusetts: 205–247

Table S2. Microsatellite loci for the Antarctic echinoid *Sterechinus neumayeri*.

Locus	Primer sequence	repeat motif	No of alleles	Dye *	Size range of alleles		Thermo-cycler protocols
Stenum 03	TTCGTATGATAAAGAATGGACAGC	(AG)14	12	D3	102-124	Multiplex 1	15 min 95°C 30s at 94°C, 90s at 58°C, 60s at 72°C
Stenum 08	TAAATCGTGCACCAGCTTTG TGCATACACAAGACATGAGTGG	(TAA)8	8	D4	175-205		
Stenum 11	GAATGCTCGATCGCCTTATC CTTATGAGCGCATAGCATGG	(GAT)12	7	D2	157-178		Repeated 29 times 72°C for 5 min
Stenum 06	ACGGTTAACAGGGTTAATCATC TTATATTGCGCGAACTGCAC	(ATT)11	13	D4	140-194	Multiplex 2	15 min 95°C 30s at 94°C, 90s at 56°C, 60s at 72°C
Stenum 13	TCTGTTTAGAACATAAGCATGAAG G	(ATGA)7	6	D2	78-131		
Stenum 18	CTACATCCATGCACCATTTG CATTGTTGGCCACTTGATTATG	(GAT)12	8	D3	197-139		Repeated 29 times 72°C for 5 min
Stenum 04	TCTTCTAAAATCACGTGGGAAG AGGCCTACAATTTGCCTCTG	(AG)15	19	D4	100-142	Multiplex 3	15 min 95°C 30s at 94°C, 90s at 58°C, 60s at 72°C
Stenum 15	AATAAGTGGTGCTGATCACAAAG TCTGTCCTACATTCCTACTATCCAG	(ATTG)9	6	D2	80-100		
Stenum 22	TTGCAAACTCGCTATGTGC CAAGCATGTACTTTCAGTCAGTCA	(TAC)12	14	D3	116-221		72°C for 5 min
Stenum 19	G	(AGC)11	8	D3	114-141	Multiplex 4	15 min 95°C 30s at 94°C, 90s at 58°C, 60s at 72°C
Stenum 21	GCTACTCCATCAGGCTCTTCC CCTAGCTAAGACCCCATTTATTTCC	(ACC)10	9	D2	100-124		
	AATGCAGGTACAGGGCAGTC TCCTCACCTCTTTCCACCAC						Repeated 29 times 72°C for 5 min
	TGTTCTACCCTTGCCTACCC CATCCCTGGGTCACCTGTAG						

*forward primer labelled in all reactions

Table S3a. Genetic diversity in the Antarctic echinoid *Sterechinus neumayeri* adults from the Vestfold Hills region. Allelic richness (A_R), Observed heterozygosity (H_O) and Expected heterozygosity (H_E).

	Old Wallow 1			Old Wallow 2			Boyd Island 1			Ellis Fjord 1			Ellis Fjord 2			Ellis Fjord 3		
Locus	H_O	H_E	A_R	H_O	H_E	A_R	H_O	H_E	A_R	H_O	H_E	A_R	H_O	H_E	A_R	H_O	H_E	A_R
Stenum 03	0.767	0.764	2.897	0.743	0.747	2.832	0.714	0.781	2.994	0.667	0.720	2.721	0.778	0.772	2.945	0.591	0.748	2.831
Stenum 08	0.483	0.539	2.136	0.771	0.621	2.328	0.643	0.589	2.229	0.643	0.550	2.193	0.679	0.589	2.229	0.625	0.587	2.287
Stenum 11	0.138	0.130	1.265	0.086	0.083	1.169	0.250	0.230	1.488	0.300	0.296	1.623	0.111	0.107	1.222	0.125	0.157	1.328
Stenum 06	0.346	0.587	2.366	0.429	0.825	3.157	0.435	0.828	3.177	0.304	0.797	3.07	0.500	0.766	2.931	0.150	0.698	2.651
Stenum 13	0.517	0.566	2.153	0.500	0.466	1.933	0.500	0.533	2.027	0.690	0.617	2.306	0.571	0.541	2.051	0.609	0.593	2.228
Stenum 18	0.067	0.320	1.6	0.129	0.479	2.022	0.296	0.590	2.317	0.154	0.322	1.647	0.214	0.383	1.733	0.273	0.518	2.035
Stenum 04	0.800	0.851	3.278	0.706	0.874	3.376	0.714	0.824	3.155	0.567	0.827	3.168	0.714	0.848	3.264	0.500	0.776	2.988
Stenum 15	0.733	0.516	1.946	0.600	0.587	2.192	0.667	0.575	2.179	0.667	0.580	2.174	0.679	0.580	2.174	0.542	0.536	2.026
Stenum 22	0.200	0.343	1.718	0.206	0.338	1.735	0.192	0.338	1.733	0.172	0.335	1.726	0.346	0.432	1.912	0.375	0.353	1.741
Stenum 19	0.133	0.583	2.233	0.156	0.618	2.364	0.111	0.601	2.259	0.182	0.597	2.318	0.125	0.652	2.504	0.364	0.701	2.615
Stenum 21	0.333	0.370	1.79	0.229	0.234	1.488	0.250	0.437	1.94	0.533	0.544	2.23	0.429	0.450	2.01	0.333	0.470	2.037
	Trigwell Island 1			Trigwell Island 2			Trigwell Island 3			Zappit Point 1			Zappit Point 2			Zappit Point 3		
	H_O	H_E	A_R	H_O	H_E	A_R	H_O	H_E	A_R	H_O	H_E	A_R	H_O	H_E	A_R	H_O	H_E	A_R
Stenum 03	0.643	0.663	2.576	0.533	0.706	2.666	0.596	0.737	2.788	0.657	0.769	2.933	0.697	0.702	2.688	0.656	0.681	2.63
Stenum 08	0.714	0.558	2.125	0.357	0.562	2.161	0.447	0.577	2.197	0.559	0.599	2.281	0.588	0.604	2.255	0.441	0.597	2.233
Stenum 11	0.143	0.136	1.282	0.200	0.188	1.397	0.106	0.102	1.209	0.143	0.162	1.338	0.118	0.112	1.227	0.088	0.113	1.233
Stenum 06	0.560	0.815	3.107	0.417	0.705	2.667	0.314	0.826	3.143	0.414	0.814	3.109	0.321	0.847	3.249	0.267	0.767	2.902
Stenum 13	0.536	0.493	1.943	0.500	0.473	1.927	0.591	0.567	2.164	0.515	0.555	2.08	0.394	0.548	2.118	0.618	0.596	2.238
Stenum 18	0.286	0.503	1.959	0.321	0.503	1.985	0.136	0.447	1.931	0.258	0.536	2.102	0.250	0.483	1.988	0.212	0.327	1.647
Stenum 04	0.684	0.814	3.161	0.741	0.826	3.181	0.723	0.847	3.232	0.829	0.871	3.365	0.727	0.843	3.224	0.765	0.830	3.182
Stenum 15	0.714	0.517	1.951	0.552	0.574	2.149	0.596	0.604	2.253	0.794	0.593	2.224	0.676	0.600	2.237	0.765	0.575	2.15
Stenum 22	0.222	0.353	1.761	0.500	0.498	2.09	0.250	0.359	1.781	0.242	0.468	2.027	0.394	0.473	2.055	0.375	0.549	2.23
Stenum 19	0.150	0.664	2.505	0.130	0.640	2.389	0.200	0.523	2.117	0.200	0.601	2.255	0.250	0.625	2.37	0.129	0.587	2.285
Stenum 21	0.321	0.328	1.656	0.467	0.437	1.944	0.304	0.292	1.619	0.400	0.391	1.848	0.353	0.399	1.863	0.412	0.402	1.88

Table S3b. Genetic diversity in the Antarctic echinoid *Sterechinus neumayeri* adults from the Windmill Islands region. Allelic richness (A_R), Observed heterozygosity (H_O) and Expected heterozygosity (H_E).

Locus	Browning Peninsula 1			Browning Peninsula 2			Browning Peninsula 1			Sparkes Bay 1			Sparkes Bay 2		
	H_O	H_E	A_R	H_O	H_E	A_R	H_O	H_E	A_R	H_O	H_E	A_R	H_O	H_E	A_R
Stenum 03	0.565	0.695	2.659	0.629	0.701	2.666	0.656	0.692	2.631	0.677	0.779	2.961	0.667	0.739	2.783
Stenum 08	0.692	0.655	2.456	0.457	0.495	2	0.576	0.506	2.08	0.531	0.545	2.146	0.618	0.555	2.17
Stenum 11	0.182	0.170	1.357	0.088	0.085	1.174	0.219	0.268	1.525	0.129	0.121	1.239	0.121	0.116	1.237
Stenum 06	0.450	0.808	3.095	0.394	0.742	2.777	0.393	0.730	2.758	0.393	0.771	2.923	0.400	0.790	2.989
Stenum 13	0.538	0.613	2.307	0.629	0.564	2.128	0.531	0.590	2.201	0.625	0.634	2.341	0.417	0.486	1.953
Stenum 18	0.217	0.328	1.663	0.147	0.363	1.733	0.214	0.415	1.829	0.290	0.435	1.904	0.212	0.245	1.502
Stenum 04	0.615	0.877	3.419	0.571	0.856	3.299	0.688	0.874	3.383	0.621	0.853	3.286	0.722	0.879	3.399
Stenum 15	0.538	0.521	2.024	0.529	0.513	1.961	0.516	0.505	1.927	0.500	0.541	2.065	0.500	0.542	2.037
Stenum 22	0.269	0.419	1.914	0.212	0.415	1.921	0.226	0.387	1.84	0.194	0.377	1.786	0.364	0.468	2.031
Stenum 19	0.217	0.509	2.131	0.111	0.639	2.413	0.179	0.684	2.575	0.174	0.578	2.232	0.217	0.673	2.553
Stenum 21	0.292	0.329	1.708	0.200	0.210	1.433	0.500	0.414	1.888	0.219	0.306	1.652	0.344	0.379	1.833

Fig. S1. Results from STRUCTURE analysis of the Antarctic echinoid *Sterechinus neumayeri* showing A) Estimated membership coefficient (Q) for individuals for each of two possible populations, B). Change of log likelihood values (Delta K) against possible number of populations (K) f and C) Mean log likelihood (Ln) consisting of a given number of populations (K).

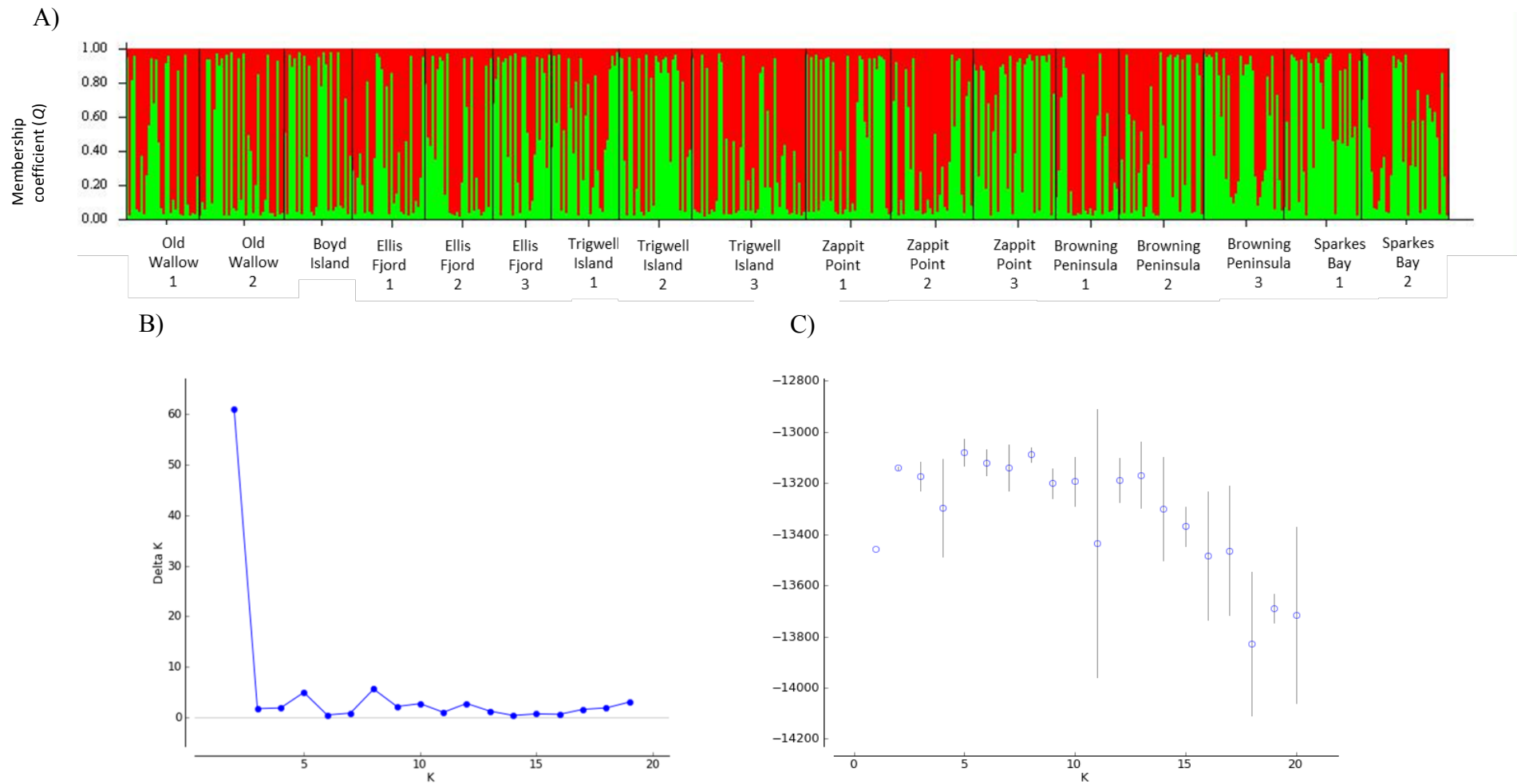


Table S4. Fixation index (F_{IS}) values for populations of the Antarctic echinoid *Sterechinus neumayeri* the Windmill Islands and Vestfold Hills regions. Significance denoted as * at the 0.05 level, ** at the 0.01 level and *** at the 0.001 level. Locations in the Vestfold Hills region are; Old Wallow (OW), Boyd Island (BO), Ellis Fjord (EL), Trigwell Island (TR), Zappit Point (ZP). Locations in the Windmill Islands region are Browning Peninsula (BP) and Sparkes Bay (SB).

	Vestfold Hills Region											Windmill Islands Region					
	OW1	OW2	BO1	EL1	EL2	EL3	TR1	TR2	TR3	ZP1	ZP2	ZP3	BP1	BP2	BP3	SB1	SB2
Stenum 03	-0.050	-0.011	0.066	0.055	-0.007	0.185	-0.001	0.194	0.135	0.132	0.008	0.023	0.146	0.041	-0.052	0.111	0.064
Stenum 08	0.077	-0.265	-0.115	-0.184	-0.152	-0.133	-0.342	0.322	0.176	0.053***	0.026	0.246	-0.118**	0.027	-0.208	0.012***	-0.144
Stenum 11	-0.064	-0.036	-0.089	-0.020	-0.038	0.161***	-0.056	-0.073	-0.045	0.078	-0.088	0.195***	-0.074	-0.062	0.067	-0.091	-0.051
Stenum 06	0.375	0.540***	0.545***	0.608**	0.347***	0.785***	0.290	0.393*	0.620***	0.492***	0.685	0.687*	0.483***	0.496*	0.496***	0.490	0.616***
Stenum 13	-0.008	-0.101	-0.023	-0.146	-0.055	-0.027	-0.108	-0.073	-0.041	0.071	0.257	-0.059***	0.101	-0.136	0.076	0.015	0.093
Stenum 18	0.783*	0.725***	0.473*	0.519***	0.441	0.473	0.419***	0.349	0.695***	0.519	0.470	0.347	0.331	0.592	0.478**	0.333	0.119
Stenum 04	0.074	0.192*	0.151***	0.337	0.175*	0.356	0.188	0.103	0.146**	0.048**	0.137	0.109	0.298**	0.351	0.227***	0.290	0.178*
Stenum 15	-0.402	-0.023	-0.133	-0.126	-0.145	-0.011	-0.146	0.038	0.013***	-0.339	-0.128	-0.296	-0.033**	-0.005	0.010	0.106	0.077
Stenum 22	0.413	0.391***	0.428***	0.482***	0.191	-0.061	0.327	-0.004	0.304***	0.482***	0.167**	0.302***	0.357	0.486***	0.413***	0.483*	0.224**
Stenum 19	0.756***	0.747***	0.873***	0.689	0.788***	0.578***	0.774***	0.796	0.606***	0.750**	0.592***	0.770**	0.562***	0.807***	0.733***	0.675***	0.677***
Stenum21	0.082	0.024	0.423	0.011	0.015	0.276	0.019	-0.069	-0.050***	-0.035	0.110***	-0.042	0.102	0.036	-0.217	0.275*	0.094**

Table S5 Pairwise population differentiation among populations of the Antarctic echinoid *Sterechinus neumayeri*. Locations in the Vestfold Hills region are; Old Wallow (OW), Boyd Island (BO), Ellis Fjord (EL), Trigwell Island (TR), Zappit Point (ZP). Locations in the Windmill Islands region are Browning Peninsula (BP) and Sparkes Bay (SB). Josts' bias corrected D (D_{EST}) upper diagonal, F_{ST} lower diagonal. Negative values converted to 0. Significance at $p < 0.05$ after Bonferroni correction is denoted by *.

	OW1	OW2	BO1	EL1	EL2	EL3	TR1	TR2	TR3	ZP1	ZP2	ZP3	BP1	BP2	BP3	SB1	SB2
OW1		0.039*	0.026*	0.005	0.004	0.007	0.019	0.005	0.017	0.015	0.036*	0.010	0.026*	0.005	0.020	0.026*	0.026*
OW2	0.006		0.007	0.023	0.018	0.054*	0.022	0.027*	0.002	0.020	0.009	0.026*	0.021	0.011	0.053*	0.037	0.040*
BO1	0.000	0.005		0.006	0.010	0.014	0.000	0.012	0.003	0.000	0.008	0.009	0.027	0.006	0.050*	0.027	0.047*
EL1	0.003	0.005	0.000		0.000	0.000	0.008	0.005	0.003	0.006	0.015	0.007	0.019	0.004	0.015	0.025	0.025*
EL2	0.000	0.007	0.000	0.002		0.000	0.010	0.000	0.005	0.002	0.007	0.000	0.016	0.013	0.034*	0.038	0.041*
EL3	0.005	0.015*	0.000	0.003	0.004		0.008	0.000	0.017	0.000	0.022*	0.002	0.028*	0.018	0.021	0.026	0.041*
TR1	0.000	0.000	0.000	0.001	0.000	0.008		0.000	0.003	0.000	0.004	0.006	0.021	0.016	0.038*	0.039	0.044*
TR2	0.009	0.011*	0.003	0.009	0.003	0.007	0.000		0.011	0.002	0.019*	0.001	0.022	0.018	0.031*	0.034	0.038*
TR3	0.011*	0.001	0.004	0.008	0.014*	0.018*	0.013*	0.02*		0.000	0.001	0.003	0.000	0.002	0.029*	0.02	0.028*
ZP1	0.002	0.003	0.000	0.004	0.007	0.005	0.000	0.014*	0.008		0.000	0.000	0.003	0.004	0.019	0.007	0.03*
ZP2	0.003	0.000	0.000	0.005	0.000	0.004	0.000	0.008	0.007	0.002		0.002	0.000	0.022	0.048*	0.044*	0.048*
ZP3	0.014*	0.026*	0.002	0.016*	0.008	0.024*	0.003	0.019*	0.03*	0.013*	0.013*		0.011	0.004	0.035*	0.026	0.042*
BP1	0.008	0.015*	0.005	0.010	0.008	0.022*	0.009	0.022*	0.018*	0.016*	0.004	0.013*		0.023	0.031*	0.037*	0.051*
BP2	0.003	0.005	0.000	0.003	0.006	0.02*	0.000	0.016*	0.015*	0.005	0.014*	0.013*	0.02*		0.020	0.012	0.030*
BP3	0.008	0.02*	0.006	0.003	0.018	0.012	0.010	0.02*	0.027*	0.009	0.022*	0.03*	0.028*	0.012*		0.000	0.010
SB1	0.012*	0.018*	0.000	0.007	0.014*	0.014*	0.006	0.019*	0.027*	0.009	0.022*	0.014*	0.023*	0.002	0.001		0.012
SB2	0.021*	0.031*	0.023*	0.017*	0.016*	0.029*	0.017*	0.019*	0.041*	0.036*	0.026*	0.021*	0.021*	0.022*	0.022*	0.013*	

Table S6. Genetic diversity in larvae of the Antarctic echinoid *Sterechinus neumayeri* collected from three sites in the Vestfold Hills region. H_o = observed heterozygosity, H_E = expected heterozygosity, A_R = allelic richness

	<u>Hawker Island</u>			<u>Kazak Island 1</u>			<u>Kazak Island 2</u>		
	H_o	H_E	A_R	H_o	H_E	A_R	H_o	H_E	A_R
Stenum									
03	0.833	0.795	3.109	0.750	0.750	3.214	1.000	0.500	2
Stenum									
08	0.750	0.497	1.984	0.833	0.625	2.434	0.400	0.320	1.667
Stenum									
11	0.182	0.165	1.338	0.200	0.180	1.4	0.333	0.278	1.667
Stenum									
06	0.583	0.628	2.427	0.400	0.740	3.062	0.000	0.375	1.786
Stenum									
13	0.667	0.517	2.018	0.400	0.580	2.305	0.500	0.656	2.643
Stenum									
18	0.417	0.413	1.773	0.400	0.320	1.667	0.250	0.469	1.929
Stenum									
04	0.500	0.792	3.101	0.857	0.867	3.615	0.800	0.820	3.467
Stenum									
15	0.538	0.536	2.039	0.714	0.561	2.181	0.400	0.540	2.229
Stenum									
22	0.385	0.435	1.951	0.143	0.133	1.286	0.200	0.460	2.067
Stenum									
19	0.154	0.695	2.695	0.286	0.643	2.546	0.000	0.480	1.924
Stenum									
21	0.250	0.351	1.748	0.500	0.403	1.909	0.400	0.480	2.2

Table S7. Results of the hierarchical Analysis of Molecular Variance for adults of the Antarctic echinoid *Sterechinus neumayeri*.

	F-statistic	var. component	% variance	p-value
Among regions	0.005	0.035	5	0.169
Among locations within regions	0	0	0	0.470
Among sites within locations (Within sites)	0.008	0.052	7	0.001
	0.099	0.652	88	

Table S8. Results from assignment of individual larvae to adult populations larvae of the Antarctic echinoid *Sterechinus neumayeri* from the Vestfold Hills.

Larval Group	Likely source population											unassigned	
	OW1	OW2	BO1	EL1	EL2	EL3	TR1	TR2	TR3	ZP1	ZP2		ZP3
Hawker Island			1	3			1			1			7
Kazak Island 1				1			2			1			3
Kazak Island 2			1	1						1	1		2

Fig. S2. Results testing for differences in relatedness of adults and larvae of the Antarctic echinoid *Sterechinus neumayeri* from the Vestfold Hills. A) Comparison of the relatedness between larvae within groups compared with relatedness of adults within sites B) comparison of the relatedness of juveniles within groups compared to the relatedness of juveniles from different groups.

