

**Supplementary material**

**Elucidation of fine-scale genetic structure of sandfish (*Holothuria scabra*) populations in Papua New Guinea and northern Australia**

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**Table S1. Summary of microsatellite marker suites, associated primers, relevant polymerase chain reaction (PCR) conditions, final primer concentrations and fluorescent labels utilised in the present study**

Markers with an asterisk were dropped from the analysis. Original source of primer sequences is Fitch *et al.* (2012)

Locus	Repeat motif	Marker suite	Final primer concentration (μM)	Observed allele size range (bp)	Primer sequence (5'-3') and fluorescent tag used
Lca1*	(GT) <sub>8</sub>	M2	0.15	76–113	F: <sup>NED</sup> CTTAGTCTGGTACGGTTGTCC R: GTTACTAAGCAAGTGTACAAAC
Lca4	(GT) <sub>11</sub>	M4	0.2	110–133	F: <sup>FAM</sup> AGAGCATGTATGTATCATCGAAACC R: AAACGGAACGGAACAAGCC
Lca17	(AC) <sub>11</sub>	M4	0.2	182–206	F: <sup>PET</sup> AGATTCATTTGGGAACCTTGGC R: AGGGTTGATGTAAGCTGCG
Lca20	(AC) <sub>12</sub>	M2	0.15	184–230	F: <sup>FAM</sup> TGCGTGTGGTGATTTTGAC R: ACCATTCTACAGCTCGTCCC
Lca24	(AC) <sub>18</sub>	M4	0.2	193–225	F: <sup>VIC</sup> TCCTTCGTCGCAGCATGAC R: TTCTTGTATTCCTTTGCAGGC
Lca28*	(GAT) <sub>15</sub>	M5	0.3	190–381	F: <sup>PET</sup> TTCTGGTCTCGACTGGCAC R: TCAGTATCGGCTCCACAGG
Lca31*	(ATGT) <sub>11</sub>	M5	0.3	163–185	F: <sup>VET</sup> TGTAGGTAGGTAGGTAGGTATGTATG R: CAGCAGTGGGTTTGGACAC
Lca40	(ATGT) <sub>13</sub>	M3	0.35	235–351	F: <sup>PET</sup> GCATTGATCATGTGGAATTTGCG R: CACCATAGACCTGGCTTGC
Lca44	(AAAC) <sub>9</sub>	M5	0.3	165–202	F: <sup>FAM</sup> GACGGTACGTCACCAGAGG R: TTCTTCGTCTTTTGGCGGG
Lca48	(GT) <sub>8</sub>	M4	0.15	145–167	F: <sup>VIC</sup> ACAATGCGGACGACAATGG R: ATCGTGTTTACAAGCGGGC
Lca49	(ACAG) <sub>8</sub>	M2	0.2	157–241	F: <sup>PET</sup> TGAGCACGGTGTATTGTCC R: TGATGTGAGCCACTGCG
Lca54	(CTGT) <sub>8</sub>	M3	0.2	186–222	F: <sup>FAM</sup> AGACAGTTGTGGGAAGGGC R: TGGATGGAATAACAATAGGTGTCC
Lca59	(AC) <sub>8</sub>	M3	0.25	234–262	F: <sup>FAM</sup> AGAGCACACGTATCCCCAC R: GGGGCAGGATAGAGCACATAG
Lca42*	(AC) <sub>18</sub>	M5	0.25	188–211	F: <sup>NED</sup> TCCTTCGTCGCAGCATGAC R: TTCTTGTATTCCTTTGCAGGC
Lca62*	(AT) <sub>8</sub>	M2	0.2	245–255	F: <sup>VIC</sup> AGCTAGCAGGAAAGAAGAAAG R: AGAGGCGGATGCTCTTACC

**Table S2. Population by locus Oosterhout estimate of null alleles (Van Oosterhout *et al.* 2004)**

BE, Belifu; SI, Sivasat; UN, Ungakum; BA, Bangatang; LN, Limanak North; MB, Milne Bay; KB, Kimbe Bay; LS, Limanak South; MR, Manus Island Rambutyo; ML, Manus Island Loi; DW, Darwin (Australia); CY, Cape York (Australia)

Locus	BE	SI	UN	BA	LN	MB	KB	LS	MR	ML	DW	CY
Lca20A	-0.0045	0.0659	0.1229	0.0485	0.0202	0.0515	0.0204	-0.0351	0.0624	0.0757	0.0827	-0.0038
Lca49A	0.2536	0.1626	0.1114	0.0967	0.0108	0.117	0.0571	0.0532	0.0672	0.0781	0.1164	0.2438
Lca54A	0.0781	0.068	0.0591	0.1096	0.1285	0.0539	0.0617	0.1003	0.095	0.0658	-0.1526	0.0749
Lca59A	0.0948	0.1606	0.0998	0.1542	0.1457	0.0869	0.0956	0.0725	0.1037	0.1203	0.0167	0.1098
Lca40A	0.0871	0.007	0.1106	0.0641	0.1347	0.0876	0.0213	0.1162	0.0278	0.0674	0.0649	0.0845
Lca4A	0.1442	0.2001	0.2015	0.4028	0.2545	0.2709	0.3011	0.2273	0.2148	0.2949	0.3438	0.137
Lca48A	0.2615	0.1329	0.1735	0.1947	0.3336	0.1743	0.3684	0.2281	0.2075	0.1743	0.0149	0.2707
Lca24A	0.0372	0.0452	0.0028	0.084	0.0178	-0.0706	-0.0577	0.0636	-0.095	0.0702	0.006	0.0427
Lca17A	0.1764	0.3192	0.2035	0.3199	0.2762	0.2154	0.1377	0.3368	0.2174	0.1678	0.4178	0.1788
Lca44A	-0.0511	0.0054	-0.036	-0.0052	0.0083	-0.0813	0.097	0.0605	0.0771	0.0502	0.0299	-0.0614

**Table S3. Genetic-diversity summary statistics of sandfish (*Holothuria scabra*) populations, showing number of alleles ( $N_a$ ), allelic richness ( $A_r$ ), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ) and inbreeding coefficient ( $F_{IS}$ )**

Departures from Hardy–Weinberg expectations of heterozygosity (HWE) is indicated: sig, significant,  $P < 0.05$ ; n.s., not significant. Underlined text indicates a different microsatellite locus

Parameter	Belifu	Sivasat	Ungakum	Bangatan	Liminak North	Milne Bay	Kimbe Bay	Liminak South	Rambutyo	Loi	Darwin	Cape York	Global ( $\pm$ s.e.)
$n$	30	45	29	15	39	19	29	37	50	50	32	38	375
<u>Lca17</u>													
$N_a$	5	5	4	6	7	3	7	5	6	5	7	4	5.3 $\pm$ 0.4
$A_r$	3.6	3.7	3.2	5.6	4.8	2.9	4.4	4.1	4.2	3.7	5.4	4.0	4.1 $\pm$ 0.2
$H_o$	0.30	0.17	0.29	0.21	0.26	0.05	0.31	0.10	0.36	0.35	0.12	0.11	0.22 $\pm$ 0.03
$H_e$	0.50	0.57	0.51	0.68	0.66	0.32	0.52	0.54	0.61	0.55	0.74	0.74	0.58 $\pm$ 0.03
$F_{IS}$	0.41	0.71	0.44	0.70	0.60	0.84	0.41	0.82	0.42	0.38	0.85	0.85	0.62 $\pm$ 0.05
HWE	n.s.	sig	sig	n.s.	sig	sig	sig	sig	sig	sig	sig	sig	
<u>Lca20</u>													
$N_a$	13	15	14	15	16	14	18	17	15	18	16	8	14.9 $\pm$ 0.7
$A_r$	10.7	10.3	10.5	12.4	11.3	10.7	11.1	11.8	10.2	11.5	11.4	5.9	10.7 $\pm$ 0.5
$H_o$	0.90	0.77	0.72	0.87	0.87	0.83	0.86	0.95	0.80	0.77	0.78	0.44	0.80 $\pm$ 0.04
$H_e$	0.90	0.89	0.90	0.88	0.91	0.88	0.89	0.91	0.89	0.91	0.90	0.79	0.89 $\pm$ 0.01
$F_{IS}$	0.01	0.14	0.22	0.05	0.05	0.08	0.05	-0.02	0.12	0.17	0.15	0.46	0.12 $\pm$ 0.04
HWE	n.s.	n.s.	sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	sig	
<u>Lca24</u>													
$N_a$	7	12	7	5	10	8	8	9	9	10	8	9	8.5 $\pm$ 0.5
$A_r$	5.3	7.4	5.1	4.4	7.7	6.6	6.8	6.9	5.7	6.1	5.6	5.72	6.1 $\pm$ 0.3
$H_o$	0.46	0.59	0.48	0.29	0.75	0.63	0.74	0.61	0.63	0.47	0.63	0.63	0.58 $\pm$ 0.04
$H_e$	0.48	0.63	0.47	0.37	0.74	0.61	0.69	0.67	0.56	0.55	0.60	0.61	0.58 $\pm$ 0.03
$F_{IS}$	0.05	0.08	-0.00	0.26	0.00	0.00	-0.05	0.10	-0.12	0.16	-0.03	-0.01	0.04 $\pm$ 0.03
HWE	n.s.	sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	sig	n.s.	n.s.	
<u>Lca4</u>													
$N_a$	12	12	7	6	10	8	12	13	10	10	7	12	9.9 $\pm$ 0.7
$A_r$	9.2	8.5	5.7	6.0	8.0	6.6	9.9	8.8	6.8	7.6	6.3	7.52	7.6 $\pm$ 0.4
$H_o$	0.63	0.50	0.45	0.09	0.38	0.28	0.33	0.44	0.43	0.33	0.18	0.60	0.39 $\pm$ 0.04
$H_e$	0.87	0.86	0.77	0.73	0.84	0.63	0.88	0.81	0.81	0.82	0.78	0.80	0.80 $\pm$ 0.02
$F_{IS}$	0.30	0.43	0.43	0.89	0.57	0.58	0.63	0.47	0.48	0.61	0.78	0.27	0.54 $\pm$ 0.05
HWE	sig	sig	sig	sig	sig	sig	sig	n.s.	sig	sig	sig	n.s.	

Parameter	Belfu	Sivasat	Ungakum	Bangatan	Liminak North	Milne Bay	Kimbe Bay	Liminak South	Rambutyo	Loi	Darwin	Cape York	Global (±s.e.)
<u>Lca40</u>													
Na	22	28	27	15	26	18	22	25	26	28	25	26	24.0 ± 1.1
Ar	13.2	14.5	16.3	13.4	13.6	13.9	13.6	14.2	13.7	15.0	14.5	14.79	14.2 ± 0.2
Ho	0.77	0.93	0.70	0.86	0.68	0.71	0.89	0.72	0.88	0.82	0.78	0.86	0.80 ± 0.02
He	0.92	0.94	0.95	0.92	0.93	0.92	0.93	0.94	0.93	0.95	0.94	0.95	0.94 ± 0.00
F <sub>is</sub>	0.18	0.03	0.28	0.10	0.28	0.26	0.06	0.24	0.07	0.15	0.18	0.11	0.16 ± 0.02
HWE	n.s.	n.s.	n.s.	n.s.	sig	n.s.	n.s.	n.s.	n.s.	sig	sig	sig	
<u>Lca44</u>													
Na	8	11	7	7	10	7	7	10	9	7	7	9	8.3 ± 0.4
Ar	5.5	6.5	5.4	6.3	6.2	5.8	6.2	6.2	6.2	5.4	5.9	6.4	6.0 ± 0.1
Ho	0.80	0.76	0.74	0.64	0.72	0.76	0.64	0.64	0.67	0.68	0.78	0.81	0.72 ± 0.02
He	0.72	0.76	0.70	0.72	0.74	0.69	0.78	0.76	0.76	0.74	0.80	0.81	0.75 ± 0.01
F <sub>is</sub>	-0.09	0.01	-0.04	0.15	0.04	-0.08	0.19	0.17	0.12	0.10	0.04	0.01	0.05 ± 0.03
HWE	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	sig	n.s.	
<u>Lca48</u>													
Na	8	7	9	5	3	5	4	11	9	7	5	5	6.5 ± 0.7
Ar	5.6	5.0	7.5	4.8	2.7	4.8	3.7	6.8	4.3	4.7	4.7	3.6	4.9 ± 0.4
Ho	0.30	0.44	0.50	0.33	0.12	0.41	0.11	0.38	0.28	0.36	0.69	0.29	0.35 ± 0.04
He	0.72	0.64	0.77	0.54	0.51	0.69	0.59	0.69	0.53	0.60	0.70	0.56	0.63 ± 0.02
F <sub>is</sub>	0.59	0.33	0.38	0.42	0.78	0.43	0.83	0.46	0.48	0.41	0.04	0.50	0.47 ± 0.06
HWE	n.s.	sig	n.s.	n.s.	sig	n.s.	sig	sig	sig	sig	n.s.	sig	
<u>Lca49</u>													
Na	13	15	12	11	17	10	11	14	15	20	17	12	13.9 ± 0.8
Ar	9.2	10.0	8.6	10.4	10.0	8.8	8.4	9.2	10.2	11.3	11.6	8.6	9.7 ± 0.3
Ho	0.43	0.57	0.63	0.71	0.85	0.63	0.71	0.75	0.78	0.72	0.69	0.69	0.68 ± 0.03
He	0.85	0.88	0.82	0.89	0.88	0.85	0.83	0.86	0.88	0.90	0.90	0.83	0.86 ± 0.01
F <sub>is</sub>	0.51	0.36	0.25	0.23	0.05	0.30	0.15	0.14	0.12	0.20	0.25	0.18	0.23 ± 0.03
HWE	sig	sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
<u>Lca54</u>													
Na	8	7	7	7	7	6	10	8	7	7	4	5	6.9 ± 0.4
Ar	6.8	5.2	5.4	6.2	5.4	5.7	7.4	5.6	5.5	5.8	2.6	4.3	5.5 ± 0.3
Ho	0.70	0.63	0.68	0.60	0.56	0.68	0.72	0.61	0.65	0.69	0.26	0.50	0.61 ± 0.03
He	0.82	0.76	0.76	0.78	0.77	0.79	0.82	0.76	0.79	0.80	0.23	0.69	0.73 ± 0.04
F <sub>is</sub>	0.17	0.19	0.13	0.26	0.28	0.16	0.14	0.21	0.18	0.14	-0.10	0.29	0.17 ± 0.03
HWE	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	sig	n.s.	n.s.	n.s.	n.s.	n.s.	

Parameter	Belifu	Sivasat	Ungakum	Bangatan	Liminak North	Milne Bay	Kimbe Bay	Liminak South	Rambutyo	Loi	Darwin	Cape York	Global (±s.e.)
<b>Lca59</b>													
<i>Na</i>	11	11	12	8	10	9	10	9	8	12	13	8	10.1 ± 0.5
<i>Ar</i>	7.9	7.1	8.0	7.4	6.9	7.9	6.2	6.3	5.7	6.5	9.3	6.2	7.1 ± 0.3
<i>Ho</i>	0.60	0.55	0.64	0.46	0.54	0.59	0.64	0.65	0.57	0.55	0.81	0.72	0.61 ± 0.03
<i>He</i>	0.79	0.79	0.79	0.79	0.77	0.80	0.74	0.77	0.76	0.74	0.84	0.74	0.78 ± 0.01
<i>F<sub>IS</sub></i>	0.26	0.32	0.20	0.45	0.31	0.30	0.15	0.17	0.25	0.27	0.05	0.04	0.23 ± 0.03
HWE	n.s.	n.s.	n.s.	n.s.	sig	n.s.	sig	n.s.	n.s.	n.s.	n.s.	n.s.	
<b>Pop. mean (±s.e.)</b>													
<i>Na</i>	10.7 ± 1.5	12.3 ± 2.0	10.6 ± 2.1	8.5 ± 1.2	11.6 ± 2.1	8.8 ± 1.4	10.9 ± 1.7	12.1 ± 1.8	11.4 ± 1.9	12.4 ± 2.3	10.9 ± 2.1	9.8 ± 2.0	10.8 ± 0.6
<i>Ar</i>	7.7 ± 0.9	7.8 ± 1.0	7.6 ± 1.2	7.7 ± 1.0	7.7 ± 1.0	7.4 ± 1.0	7.8 ± 1.0	8.0 ± 1.0	7.3 ± 1.0	7.8 ± 1.2	7.7 ± 1.2	6.7 ± 1.0	7.6 ± 0.3
<i>Ho</i>	0.59 ± 0.07	0.59 ± 0.07	0.58 ± 0.05	0.51 ± 0.09	0.57 ± 0.08	0.56 ± 0.08	0.60 ± 0.08	0.59 ± 0.07	0.60 ± 0.06	0.57 ± 0.06	0.57 ± 0.09	0.57 ± 0.07	0.57 ± 0.03
<i>He</i>	0.75 ± 0.05	0.77 ± 0.04	0.74 ± 0.05	0.73 ± 0.05	0.77 ± 0.04	0.72 ± 0.06	0.77 ± 0.04	0.77 ± 0.04	0.75 ± 0.04	0.76 ± 0.05	0.74 ± 0.07	0.75 ± 0.04	0.75 ± 0.02
<i>F<sub>IS</sub></i>	0.24 ± 0.07	0.26 ± 0.06	0.23 ± 0.05	0.35 ± 0.08	0.30 ± 0.08	0.29 ± 0.08	0.26 ± 0.08	0.28 ± 0.07	0.21 ± 0.06	0.26 ± 0.05	0.22 ± 0.10	0.27 ± 0.08	0.26 ± 0.04

## References

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