

Supplementary material

Novel multimarker comparisons address the genetic population structure of silvertip sharks (*Carcharhinus albimarginatus*)

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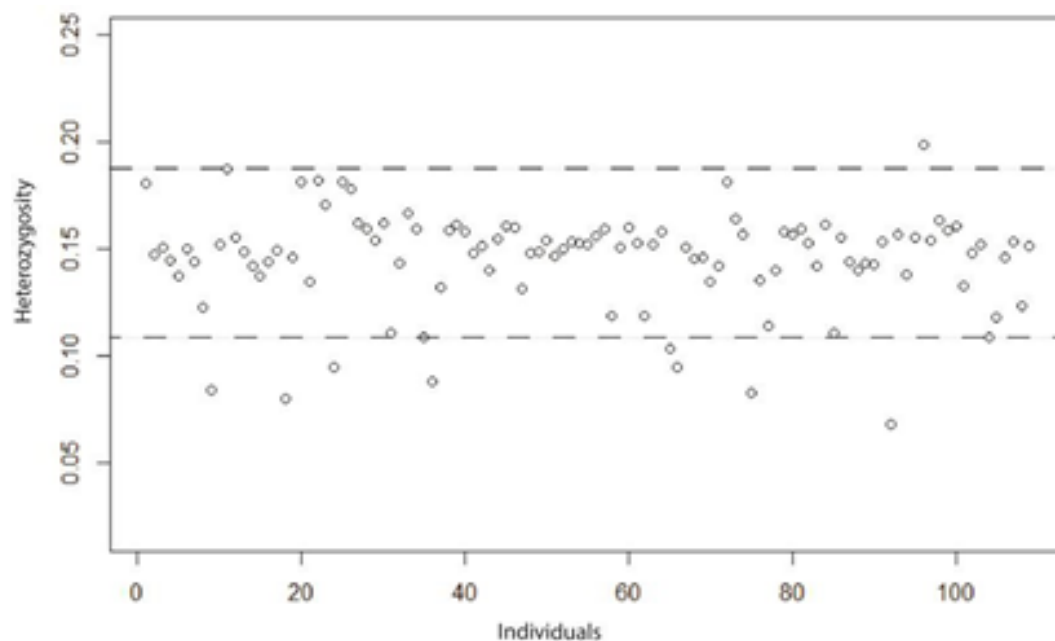


Fig. S1. Average heterozygosity of single nucleotide polymorphism (SNP) loci per individual during filtering process (not final SNP set of 6461 SNPs). Dashed lines represent cut off range (<0.11 and >0.18) in the SNP filtering process. Heterozygosity was filtered to remove potential individuals of poor DNA quality or sample contamination. Thresholds were selected to remove individuals outside average range for the SNP dataset (0.11-0.18).

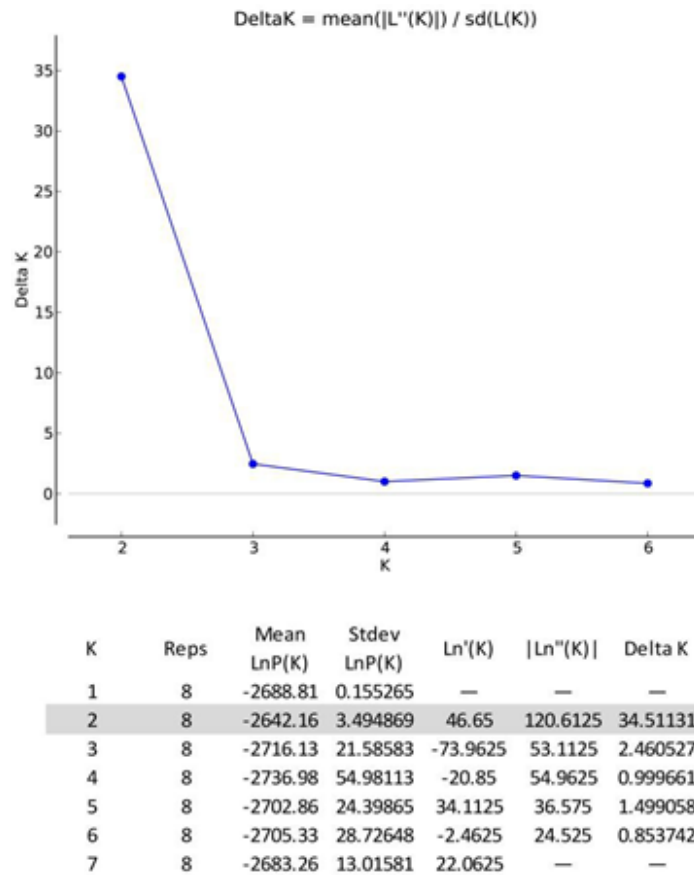


Fig. S2. Outputs from microsatellite STRUCTURE analysis showing Evanno’s Delta *K* value (above), a method based on the rate of change in log probability of data and Evanno table output for *K* = 1–7 (below).

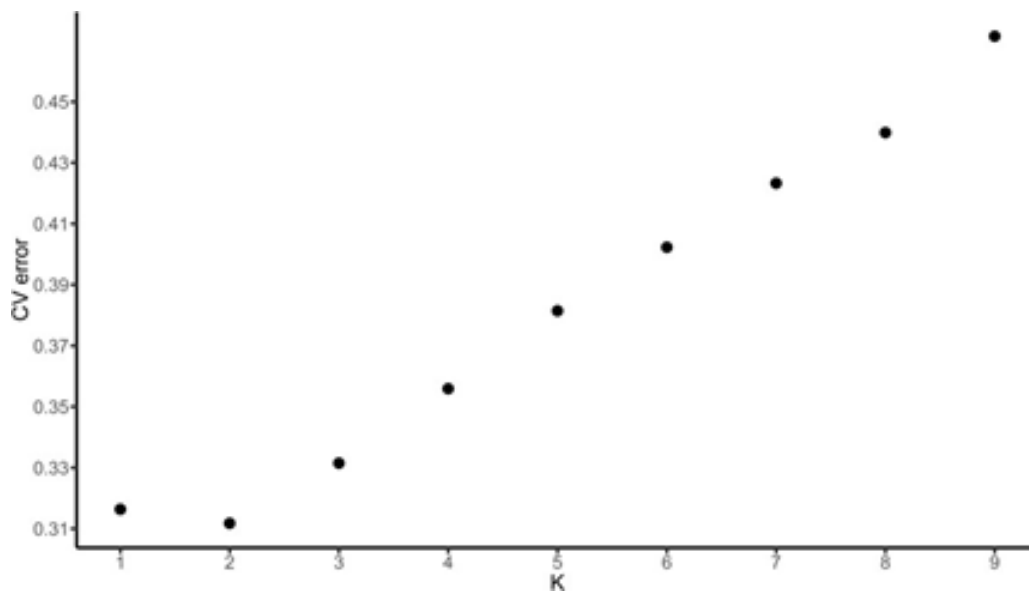


Fig. S3. Outputs from single nucleotide polymorphism (SNP) ADMIXTURE analysis showing CV error values for scenarios of *K* = 1–9. Note *K* = 2 has the lowest CV value.

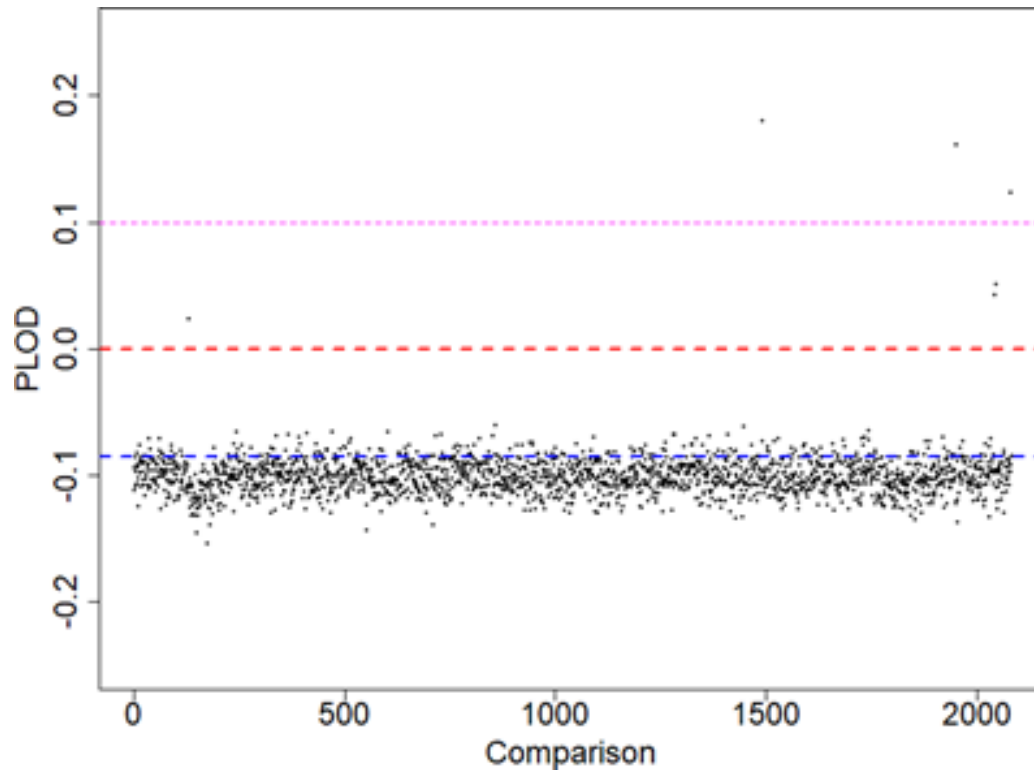


Fig. S4. Output from kin inference using method described in Hillary *et al* (2018). Each point is a comparison of single nucleotide polymorphism (SNP) genotypes of two individuals plotted against the PLOD score. The blue and magenta lines denote the expected values for unrelated pairs (UPs) and full-sibling pairs (FSPs) respectively. The red line is representative of the cut off, where anything above is estimated either FSP or POP.

Table S1. Novel microsatellite loci for *C. albimarginatus* isolated in this study

Annealing temperature (T_A), sample size (n), number of alleles (n_A) and polymorphic information content (PIC). Superscript numbers next a locus represents the multiplex reaction (1, 2, 3 or 4)

Locus	Primer sequence (5'-3')	Repeat motif	Size range (bp)	T_A (°C)	n_A	PIC	GenBank accession number
¹ ALS1	F: GGTTGGTCTCCAGAGTTGGG _[PET] R: GTCAACATGATGTGCCAGCG	(GA)	268–322	55	30	0.906	KY996371
¹ ALS2	F: AAGCAATGGACTGTGGCGAT _[VIC] R: GGCGAACTTCACATCTTGCC	(GA)	276–288	55	7	0.667	KY996372
⁴ ALS4	F: AGGCTGGATGTAGCAAGCAA _[VIC] R: TTACATCCCGGAGTGGACCA	(TG)	288–300	50	7	0.747	KY996382
¹ ALS6	F: GAAGCGATGAGGGAGGCC _[FAM] R: GGACAGTCCACCATTACCC	(TG)	284–294	55	6	0.689	KY996373
² ALS7	F: CGTAGGCTCGCTGACATCAT _[NED] R: TAGGTGCTTGAAGGCCACTG	(GA)	223–231	55	5	0.299	KY996376
³ ALS9	F: CAGCTCTCCCTCCACAATCG _[FAM] R: TTCCTTTCAATCGGAGGCC	(AG)	232–234	50	2	0.058	KY996379
² ALS11	F:GGGCTTCTTGACACTTTGTG _[FAM] R: GCAGTGCTTACCAACATGCC	(TG)	296–338	55	2	0.009	KY996377
⁴ ALS14	F: TTCTCTGTTCTGTTGGCCC _[FAM] R: TGAGCTATCCCAGTCCCTCC	(AC)	235–276	50	2	0.519	KY996381
² ALS23	F: TCATAGTGGGCAGGGATGGA _[VIC] R: TGGTTTGGCCTCAGCTCATT	(GA)	248–274	55	3	0.515	KY996375
³ ALS42	F: TGCCGTAAGTAGATCCCT _[NED] R: GGGAGCCAGGACCCAGATTA	(CCCT)	240–260	50	5	0.684	KY996378
³ ALS51	F: GCATCGAGGGATCATATTGACA _[PET] R: GACTTTGGTGCAGAGGGTCA	(AGG)	289–292	50	2	0.009	KY996380
¹ ALS52	F: CCAGTGCTTACTTTGTGCTGT _[NED] R: AGGAAGCCGTGAATGACAAA	(TTG)	237–267	55	8	0.467	KY996374

Table S2. Allele frequencies per location, per locus for 12 microsatellite loci

See Table S3 for sample sizes per locus per collection location

ALS 1	Seychelles	Papua New Guinea	East Australia
268	0.350	0.211	0.109
270	0.050	0.047	0.022
272	0.083	0.023	0.130
274	0.050	0.070	0.022
276	0.017	0.055	0.109
278	0.083	0.117	0.087
280	–	0.078	0.043
282	–	0.070	0.043
284	–	0.063	0.022
286	0.017	0.039	0.043
288	–	0.008	0.043
290	–	0.016	–
292	0.100	–	–
294	–	–	0.043
296	0.033	0.008	–
298	–	–	0.043
300	0.033	–	–
302	–	0.047	0.022
304	0.017	0.016	–
306	–	0.016	0.022
308	–	–	0.022
310	0.033	0.016	0.022
312	0.017	–	–
316	0.017	–	–
318	0.033	0.023	–
320	0.017	0.016	–
322	0.017	0.008	–
326	–	0.008	–
330	–	0.016	–
332	–	–	0.022
ALS 2			
276	–	0.086	0.174
278	0.600	0.281	0.283
280	0.017	0.117	0.065
282	0.050	0.086	–
284	0.300	0.367	0.370
286	–	0.023	0.043
288	–	0.008	0.022
ALS 4			
288	–	0.055	–
290	0.383	0.242	0.348
292	0.217	0.133	0.130
294	0.117	0.141	0.196
296	0.283	0.297	0.217
298	–	0.078	0.109
300	–	0.055	–
ALS 6			
284	–	0.047	–
286	0.117	0.133	0.130
288	0.083	0.086	0.109
290	0.417	0.320	0.370
292	–	0.047	0.065
294	0.350	0.336	0.283
ALS 7			
223	–	0.008	0.043

ALS 1	Seychelles	Papua New Guinea	East Australia
225	0.100	0.188	0.152
227	–	0.023	–
229	0.900	0.633	0.717
231	–	0.008	–
ALS 9			
232	0.967	0.961	0.978
234	–	0.039	0.022
ALS 11			
298	1.000	0.992	1.000
300	–	0.008	–
ALS 14			
263	0.967	0.969	0.978
265	–	0.031	0.022
ALS 23			
248	0.067	0.352	0.478
252	0.750	0.523	0.413
254	0.183	0.125	0.109
ALS 42			
240	–	0.031	0.087
244	0.367	0.336	0.348
248	0.317	0.266	0.261
252	0.250	0.219	0.261
260	0.033	0.148	0.043
ALS 51			
289	–	0.016	–
292	0.933	0.891	0.957
ALS 52			
237	0.083	0.008	0.022
240	–	0.008	–
243	0.733	0.625	0.391
246	0.083	0.180	0.196
249	–	0.070	0.065
252	–	0.008	–
255	0.017	–	0.022
258	0.050	0.008	–

Table S3. Summary statistics for microsatellite loci per population

The table describes the following parameters per microsatellite loci for each location; Number of alleles (n_A), observed (H_O) and expected (H_E) heterozygosity, inbreeding coefficient (F_{IS}), Hardy–Weinberg Equilibrium P -value (HWE_P). Bold HWE_P values are consider statistically significant ($P < 0.05$). Information not applicable due to a single allele at locus (NA)

Locations Parameter	ALS1	ALS2	ALS4	ALS6	ALS7	ALS9	ALS11	ALS14	ALS23	ALS42	ALS51	ALS52
Seychelles ($n = 30$)												
n	29	29	30	29	29	29	29	29	29	30	30	29
n_A	17	4	4	4	2	1	1	1	3	4	1	5
H_O	0.862	0.552	0.733	0.793	0.200	0.000	0.000	0.000	0.433	0.724	0.000	0.414
H_E	0.831	0.515	0.712	0.661	0.180	0.000	0.000	0.000	0.399	0.681	0.000	0.407
F_{IS}	-0.04	-0.07	-0.03	-0.20	-0.11	NA	NA	NA	-0.08	-0.06	NA	-0.02
HWE_P	0.684	0.139	0.894	0.230	1.000	1.000	1.000	1.000	0.826	0.572	1.000	0.659
Papua New Guinea ($n = 64$)												
n	62	62	64	62	55	64	64	64	64	64	58	58
n_A	22	7	7	6	5	2	2	2	3	5	2	7
H_O	0.855	0.694	0.828	0.710	0.345	0.078	0.016	0.063	0.641	0.656	0.000	0.466
H_E	0.904	0.741	0.804	0.739	0.409	0.075	0.016	0.061	0.587	0.746	0.034	0.479
F_{IS}	0.05	0.06	-0.03	0.04	0.16	-0.04	-0.01	-0.03	-0.09	0.12	1.00	0.03
HWE_P	0.045	0.129	0.062	0.182	0.433	1.000	1.000	1.000	0.818	0.068	0.009	0.800
East Australia ($n = 23$)												
n	20	22	23	22	21	23	20	23	23	23	21	16
n_A	18	6	5	5	3	2	1	2	3	5	1	5
H_O	0.900	0.818	0.783	0.818	0.143	0.043	0.000	0.043	0.522	0.696	0.000	0.438
H_E	0.916	0.723	0.765	0.727	0.353	0.043	0.000	0.043	0.589	0.733	0.000	0.594
F_{IS}	0.02	-0.13	-0.02	-0.13	0.59	-0.02	NA	-0.02	0.11	0.05	NA	0.26
HWE_P	0.180	0.217	0.199	0.936	0.004	1.000	1.000	1.000	0.385	0.006	1.000	0.311

Table S4. Filtering process for SNPs identified for *C. albimarginatus*

Call rate per single nucleotide polymorphism (SNP) has been filtered twice, initially at >0.7 (step 2), then finally at 1 (step 10). HWE, Hardy–Weinberg equilibrium

Filtering step	SNPs remaining	Individuals remaining
Initial SNPs	717 800	
Initial Individuals		$n = 146$
Remove linked SNPs	412 771	
Retain call rate per SNP (>0.7)	88 207	
Remove monomorphic SNPs	57 400	
Retain call rate per individual (>0.80)		$n = 109$
Remove monomorphic SNPs	56 171	
Remove SNPs with minor allele frequency (<0.02)	32 942	
Retain SNPs with heterozygosity per individual (between 0.11 and 0.18)		$n = 92$
Remove SNPs with HWE (<0.05)	29 549	
Remove monomorphic SNPs	29 212	
Retain call rate per SNP (=1.0)	6461	
Total	6461	$n = 92$

Table S5. Results of power analysis conducted in POWSIM for microsatellites and single nucleotide polymorphisms (SNPs)

t , time in generations; N_e , effective population size of subpopulations

12 microsatellites				6461 SNPs			
F_{ST}	t	N_e	Power	F_{ST}	t	N_e	Power
0.05	100	1000	1.000	0.05	100	1000	1
0.02	50	1000	1.000	0.02	50	1000	1
0.01	20	1000	0.985	0.01	20	1000	1
0.004	10	1000	0.669	0.004	10	1000	1
0.001	2	1000	0.102	0.001	2	1000	1
0.05	200	2000	1	0.05	200	2000	1
0.02	100	2000	1	0.02	100	2000	1
0.01	40	2000	0.989	0.01	40	2000	1
0.004	16	2000	0.513	0.004	16	2000	1
0.001	5	2000	0.129	0.001	5	2000	1

Table S6. Location data for known collection points of individuals used in this study

Unknown location, samples were collected on observer vessels and fin markets, some collection locations were unable to be reported. Although exact locations cannot be reported it is highly likely artisanal fishers have not collected sharks outside of Papua New Guinea's (PNG) exclusive economic zone (EEZ) (see Appleyard *et al.* 2018)

Sample ID	Latitude	Longitude	Reef, island or sea	Location
J2686	-18.743	147.26	Keeper	East Australia
J2622	-18.524	147.393	Glow	East Australia
R5557	-18.519	147.385	Glow	East Australia
J2633	-18.468	146.859	Rib	East Australia
J2669	-18.745	147.252	Keeper	East Australia
J2674	-18.632	147.016	Brewer	East Australia
J2633	-18.684	147.102	Lodestone	East Australia
J2630	-18.622	147.287	Helix	East Australia
J2636	-18.803	147.522	Wheeler	East Australia
R5561	-18.803	147.522	Wheeler	East Australia
J2614	-18.803	147.522	Wheeler	East Australia
J2627	-18.803	147.522	Wheeler	East Australia
R1632	-18.803	147.522	Wheeler	East Australia
R5590	-18.803	147.522	Wheeler	East Australia
J2618	-18.803	147.522	Wheeler	East Australia
J2649	-18.803	147.522	Wheeler	East Australia
R6298	-18.803	147.522	Wheeler	East Australia
R5582	-18.803	147.522	Wheeler	East Australia
J2617	-18.804	147.523	Wheeler	East Australia
J2623	-18.804	147.523	Wheeler	East Australia
R1656	-18.804	147.523	Wheeler	East Australia
J2648	-18.804	147.523	Wheeler	East Australia
J2816	-18.738	147.264	Keeper	East Australia
J2815	-18.622	147.292	Helix	East Australia
J2972	-18.513	147.39	Glow	East Australia
R6204	-18.513	147.39	Glow	East Australia
J3014	-18.53	147.386	Glow	East Australia
J3019	-18.624	147.29	Lodestone	East Australia
J3105	-18.622	147.303	Helix	East Australia
J3108	-18.699	147.091	Lodestone	East Australia
J3102	-18.759	147.257	Keeper	East Australia
J3123	-18.759	147.257	Keeper	East Australia
J3136	-18.7586	147.2574	Keeper	East Australia
C. albi 1	-4.7412	55.4297	Mahé	Seychelles
C. albi 2	-4.7412	55.4297	Mahé	Seychelles
C. albi 3	-4.7412	55.4297	Mahé	Seychelles
C. albi 4	-4.7412	55.4297	Mahé	Seychelles
C. albi 5	-4.7412	55.4297	Mahé	Seychelles
C. albi 6	-4.7412	55.4297	Mahé	Seychelles
C. albi 7	-4.7412	55.4297	Mahé	Seychelles
C. albi 8	-4.7412	55.4297	Mahé	Seychelles
C. albi 9	-4.7412	55.4297	Mahé	Seychelles
C. albi 10	-4.7412	55.4297	Mahé	Seychelles
C. albi 11	-4.7412	55.4297	Mahé	Seychelles
C. albi 12	-4.7412	55.4297	Mahé	Seychelles
C. albi 13	-4.7412	55.4297	Mahé	Seychelles
C. albi 14	-4.7412	55.4297	Mahé	Seychelles
C. albi 15	-4.7412	55.4297	Mahé	Seychelles
C. albi 16	-4.7412	55.4297	Mahé	Seychelles
C. albi 17	-4.7412	55.4297	Mahé	Seychelles

Sample ID	Latitude	Longitude	Reef, island or sea	Location
C. albi 18	-4.7412	55.4297	Mahé	Seychelles
C. albi 19	-4.7412	55.4297	Mahé	Seychelles
C. albi 20	-4.7412	55.4297	Mahé	Seychelles
C. albi 21	-4.7412	55.4297	Mahé	Seychelles
C. albi 22	-4.7412	55.4297	Mahé	Seychelles
C. albi 23	-4.7412	55.4297	Mahé	Seychelles
C. albi 24	-4.7412	55.4297	Mahé	Seychelles
C. albi 25	-4.7412	55.4297	Mahé	Seychelles
C. albi 26	-4.7412	55.4297	Mahé	Seychelles
C. albi 27	-4.7412	55.4297	Mahé	Seychelles
C. albi 28	-4.7412	55.4297	Mahé	Seychelles
C. albi 29	-4.7412	55.4297	Mahé	Seychelles
C. albi 30	-4.7412	55.4297	Mahé	Seychelles
C. albi 31	-4.7412	55.4297	Mahé	Seychelles
PNG010036	-2.2984	149.878	Bismarck Sea	PNG
PNG010074	-2.9506	146.77	Bismarck Sea	PNG
PNG010079	-2.847	146.67	Bismarck Sea	PNG
PNG010126	-2.234	150.862	Bismarck Sea	PNG
PNG050141	-2.8344	146.548	Bismarck Sea	PNG
PNG050034	-2.1376	149.821	Bismarck Sea	PNG
PNG050128	-1.3764	149.194	Bismarck Sea	PNG
PNG050137	-2.4199	146.166	Bismarck Sea	PNG
PNG050115	-1.8319	145.263	Bismarck Sea	PNG
PNG050122	-2.4862	146.225	Bismarck Sea	PNG
PNG050150	-2.8344	146.548	Bismarck Sea	PNG
PNG050151	-2.613	146.442	Bismarck Sea	PNG
PNG050210	-2.613	146.442	Bismarck Sea	PNG
PNG050213	-2.1542	150.084	Bismarck Sea	PNG
PNG050214	-1.8862	150.074	Bismarck Sea	PNG
PNG050217	-1.5151	149.438	Bismarck Sea	PNG
PNG050218	-1.3764	149.195	Bismarck Sea	PNG
PNG050219	-1.3764	149.195	Bismarck Sea	PNG
PNG060018	-2.3165	149.871	Bismarck Sea	PNG
PNG060019	-1.811	143.819	Bismarck Sea	PNG
PNG060058	-1.811	143.819	Bismarck Sea	PNG
PNG060060	-1.8351	144.112	Bismarck Sea	PNG
PNG060061	-3.1043	142.669	Bismarck Sea	PNG
PNG060062	-3.1043	142.669	Bismarck Sea	PNG
PNG070055	-3.1043	142.669	Bismarck Sea	PNG
PNG090521	-2.4268	145.996	Bismarck Sea	PNG
PNG010177	-11.741	154.099	Solomon Sea	PNG
PNG030100	-5.8309	154.429	Solomon Sea	PNG
PNG030170	-3.8053	153.265	Solomon Sea	PNG
PNG030200	-5.8309	153.265	Solomon Sea	PNG
PNG030239	-5.1475	154.355	Solomon Sea	PNG
PNG040323	-11.009	155.385	Solomon Sea	PNG
PNG040324	-11.009	155.385	Solomon Sea	PNG
PNG040336	-11.043	155.385	Solomon Sea	PNG
PNG040353	-11.037	155.337	Solomon Sea	PNG
PNG040354	-11.037	155.337	Solomon Sea	PNG
PNG040355	-11.037	155.337	Solomon Sea	PNG
PNG – total per region				<i>n</i>
Above Bismarck Archipelago				27
Below Bismarck Archipelago				10
Unknown location				45

Table S7. Accompanying metadata for individuals identified as either full siblings (FS) or parent–offspring pairs (POP) using the kinship inference method from Hillary *et al.* (2018)

Asterisk indicates likely incorrect total length measurement since *C. albimarginatus* pups are born ~72 cm (Smart *et al.* 2017a), therefore likely relationship (i.e. POP or FS) cannot be determined (?). Age estimates are based on total length (cm) and sex-specific growth curves calculated in Smart *et al.* (2017b)

Tag ID	Kin relationship	PLOD	Sex	Total length (cm)	Age (years)	Location	Latitude	Longitude	Date collected
J2648	POP	0.043	F	161	11–12	Wheeler Reef, Australia	–18.80432	147.52258	23-Apr-201
R1656			F	77	1–2	Wheeler Reef, Australia	–18.80432	147.52258	23-Apr-201
J2648	POP	0.051	F	161	11–12	Wheeler Reef, Australia	–18.80432	147.52258	23-Apr-201
R6298			M	80	1–2	Wheeler Reef, Australia	–18.80292	147.52162	22-Apr-201
R1656	FSP	0.124	F	77	1–2	Wheeler Reef, Australia	–18.80432	147.52258	23-Apr-201
R6298			M	80	1–2	Wheeler Reef, Australia	–18.80292	147.52162	22-Apr-201
50115	FSP	0.181	M	124	6–7	Manus Island, PNG	–2.61304	146.44194	20-May-201
50151			F	127	6–7	Manus Island, PNG	–2.61304	146.44194	31-May-201
10177	POP	0.024	F	218	16–18	Sudest Island, PNG	–11.74051	154.09861	12-Jun-201
10219			F	137	7–8	Sudest Island, PNG	–11.74051	154.09861	20-Jun-201
J2617	FSP/POP?	0.162	F	113	5–6	Wheeler Reef, Australia	–18.80432	147.52258	23-Apr-201
J2636			F	12.9	*	Wheeler Reef, Australia	–18.80292	147.52162	22-Apr-201

Supplementary methods

Microsatellite primer design, characterisation and optimisation

DNA was extracted using the Wizard SV Genomic DNA Purification system (Promega); tissue extractions were undertaken using SV minicolumns following modifications to the manufacturer’s instructions (i.e. overnight tissue digestion; reduction of supernatant for DNA elution and increased DNA elution time). A single sample of purified *C. albimarginatus* DNA (130 ng μL^{-1}), representing the Indo-Pacific region, was sent to the AGRF for library preparation and next generation sequencing on the Illumina MiSeq (Illumina) (2×250 -bp end reads) with base calling undertaken using Real Time Analysis (ver. 1.18.54). The Illumina bcl2fastq 2.17.1.14 pipeline was used to generate the sequence data, with the FASTAQ sequences stitched using PEAR assembler (Zhang *et al.*, 2014).

Shotgun sequencing resulted in 20 469 712 paired-end reads (10.23 Gb). Microsatellite detection of the sequenced sample was performed using QDD (ver. 3.1.2) (Megléc *et al.* 2010; <http://gsite.univ-provence.fr/gsite/Local/egee/dir/meglec/QDD.html>) and Primer (ver. 3; Rozen and Skaletsky 2000) was used to design primers for the detected microsatellites. Following QDD detection, 229 348 putative microsatellite loci were detected. The following filters were applied to further screen the loci (according to Megléc *et al.* 2010): (a) primer alignment score between 1-2.75, (b) minimum primer target distance between 80 and 147 base pairs (bp), (c) length of PCR product <305 bp, (d) pure microsatellites, (repeats >6), (e) no homopolymers, (f) no micro and nanosatellites in the flanking regions, (g) no compound

microsatellites, (*h*) a Primer3 penalty value of <3. Filtering resulted in 30 loci being selected for initial PCR optimization using unlabelled primers.

PCR amplification and optimisation was tested using DNA from eight *C. albimarginatus* individuals (from across different spatial locations). PCR amplifications conditions consisted of 1× GoTaq Colourless Master Mix (Promega), 1 μL of Bovine Serum Albumin (Promega), 0.2 μM of each individual F and R primer, and 0.8 ng μL⁻¹ DNA in a 25-μL reaction volume. Thermal cycling (in an Eppendorf Mastercycler, Eppendorf, Germany) consisted of initial denaturation at 94°C over 3 min, 35 cycles of 94°C for 1 min, T_A (as per Supplementary material, Table S1) × 30 s, 72°C for 1 min and a final extension of 72°C for 10 min. Amplification success was visualised on agarose gels containing SYBR Safe DNA gel stain (ThermoFisher Scientific, USA).

Twelve loci successfully amplified and forward primers for these loci were labelled with proprietary fluorophore dyes; 6-FAM, VIC, NED, PET (Applied Biosystems, Foster City, CA, USA). Loci were pooled into four PCR multiplex sets based on fragment size and fluorophore (Supplementary material, Table S1). Following PCR amplifications in each of the *C. albimarginatus* individuals (including labelled primers and as per PCR conditions above), GeneScan 500 LIZ size standard (ThermoFisher Scientific) and formamide were added to 3 μL of each PCR reaction and 20-μL sample volumes were run on an ABI 3130XL AutoDNA sequencer (ThermoFisher). Genotypes were scored and checked by eye using Geneious R8.1.4 Microsatellite plug-in program (Biomatters, Auckland, New Zealand).

The final 12 loci were characterised and found to be polymorphic among 117 individuals of *C. albimarginatus* from four locations within the Indo-Pacific Ocean. Primer details for these loci have been submitted to GenBank (accession numbers KY996371–KY996382). Microsatellite loci were then used for population genetic analysis on 123 individuals from three locations; Seychelles, PNG and Australia. Summary statistics from population genetic analysis including the number of alleles (n_A), observed (H_O) and expected (H_E) heterozygosities, inbreeding coefficient (F_{IS}), deviations from HWE (HWE_p) and presence of null alleles are presented in Table S3.

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