

**Table S1.** Reef Manta rays (*Mobula alfredi*) sampling information in New Caledonia (NC) and East Australia.

Lab ID	Manta ID	Date	Location	Longitude	Latitude	Sex	Colour morph
T1	CD-MA-0034	12/09/2018	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T2	CD-MA-0029	12/09/2018	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T3	CD-MA-0031	12/09/2018	Touho, NC	Undisclosed	Undisclosed	Female	Black
T4	CD-MA-0307	11/09/2018	Touho, NC	Undisclosed	Undisclosed	Female	Black
T5	CD-MA-0128	12/09/2018	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T6	CD-MA-0049	13/09/2018	Touho, NC	Undisclosed	Undisclosed	Female	Black
T7	CD-MA-0039	13/09/2018	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T8	CD-MA-0044	30/10/2018	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T9	CD-MA-0051	30/10/2018	Touho, NC	Undisclosed	Undisclosed	Female	Black
T10	CD-MA-0317	13/03/2019	Touho, NC	Undisclosed	Undisclosed	Female	Black
T11	CD-MA-0217	12/03/2019	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T12	CD-MA-0033	28/11/2018	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T13	CD-MA-0026	28/11/2018	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T14	CD-MA-0067	30/11/2018	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T15	CD-MA-0042	28/05/2019	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T16	CD-MA-0207	28/05/2019	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T18	CD-MA-0046	28/05/2019	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T19	CD-MA-0065	29/05/2019	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T20	CD-MA-0052	30/05/2019	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T21	CD-MA-0088	30/05/2019	Touho, NC	Undisclosed	Undisclosed	Male	Black
T22	CD-MA-0030	30/05/2019	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T23	CD-MA-0060	30/05/2019	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T24	Unknown	29/11/2018	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
P1	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Unknown	Unknown
P2	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Unknown	Unknown
P3	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Male	Unknown
P4	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Male	Unknown
P5	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Unknown	Unknown
P6	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Female	Unknown
P7	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Female	Unknown
P8	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Female	Unknown
B1	CD-MA-0078	25/10/2018	Noumea, NC	166.26	-22.29	Female	Black
B2	Unknown	25/10/2018	Noumea, NC	166.26	-22.29	Female	Black

B3	Unknown	17/04/2019	Noumea, NC	166.26	-22.29	Female	Black
B4	CD-MA-0140	17/04/2019	Noumea, NC	166.26	-22.29	Male	Black
B5	CD-MA-0076	17/04/2019	Noumea, NC	166.26	-22.29	Female	Chevron
B6	CD-MA-0153	13/05/2019	Noumea, NC	166.26	-22.29	Male	Chevron
B7	CD-MA-0018	22/05/2019	Noumea, NC	166.26	-22.29	Male	Black
B8	CD-MA-0147	13/05/2019	Noumea, NC	166.26	-22.29	Female	Chevron
B9	CD-MA-0011	13/05/2019	Noumea, NC	166.26	-22.29	Female	Black
B10	CD-MA-0183	13/05/2019	Noumea, NC	166.26	-22.29	Male	Black
B11	Unknown	13/05/2019	Noumea, NC	166.26	-22.29	Male	Chevron
B12	CD-MA-0036	13/05/2019	Noumea, NC	166.26	-22.29	Male	Black
B13	CD-MA-0313	20/06/2019	Noumea, NC	166.26	-22.29	Female	Black
B14	CD-MA-0009	20/06/2019	Noumea, NC	166.26	-22.29	Female	Chevron
B15	CD-MA-0230	9/07/2019	Noumea, NC	166.26	-22.29	Male	Chevron
B16	CD-MA-0328	9/07/2019	Noumea, NC	166.26	-22.29	Female	Black
B17	CD-MA-0330	9/07/2019	Noumea, NC	166.26	-22.29	Male	Chevron
B18	CD-MA-0006	9/07/2019	Noumea, NC	166.26	-22.29	Female	Black
B19	CD-MA-0100	10/07/2019	Noumea, NC	166.26	-22.29	Female	Black
B20	CD-MA-0134	10/07/2019	Noumea, NC	166.26	-22.29	Female	Black
B21	CD-MA-0184	10/07/2019	Noumea, NC	166.26	-22.29	Female	Black
B22	CD-MA-0083	10/07/2019	Noumea, NC	166.26	-22.29	Female	Black
B23	CD-MA-0079	10/07/2019	Noumea, NC	166.26	-22.29	Female	Black
B24	CD-MA-0329	10/07/2019	Noumea, NC	166.26	-22.29	Male	Black
O1	CD-MA-0319	1/05/2019	PS, Ouvea, NC	166.39	-20.72	Female	Black
O2	CD-MA-0311	4/05/2019	PS, Ouvea, NC	166.39	-20.72	Female	Black
O3	CD-MA-0320	5/05/2019	PN, Ouvea, NC	166.44	-20.45	Female	Chevron
O4	CD-MA-0321	5/05/2019	PN, Ouvea, NC	166.44	-20.45	Female	Chevron
O5	CD-MA-0075	5/05/2019	PN, Ouvea, NC	166.44	-20.45	Female	Black
O6	CD-MA-0024	5/05/2019	PN, Ouvea, NC	166.44	-20.45	Male	Black
O7	CD-MA-0322	5/05/2019	PN, Ouvea, NC	166.44	-20.45	Female	Chevron
O8	CD-MA-0323	6/05/2019	PS, Ouvea, NC	166.39	-20.72	Female	Chevron
O9	CD-MA-0023	6/05/2019	PS, Ouvea, NC	166.39	-20.72	Female	Chevron
O10	CD-MA-0325	6/05/2019	PS, Ouvea, NC	166.39	-20.72	Male	Black
O11	CD-MA-0270	9/05/2019	PS, Ouvea, NC	166.39	-20.72	Female	Chevron
O12	CD-MA-0215	9/05/2019	PS, Ouvea, NC	166.39	-20.72	Female	Black
O13	CD-MA-0120	9/05/2019	PS, Ouvea, NC	166.39	-20.72	Male	Chevron
O14	CD-MA-0122	29/07/2019	PN, Ouvea, NC	166.44	-20.45	Female	Black
O15	CD-MA-0333	29/07/2019	PN, Ouvea, NC	166.44	-20.45	Female	Chevron
O16	CD-MA-0224	31/07/2019	PN, Ouvea, NC	166.44	-20.45	Male	Black
O17	CD-MA-0335	31/07/2019	PN, Ouvea, NC	166.44	-20.45	Male	Chevron

O18	CD-MA-0334	31/07/2019	PN, Ouvea, NC	166.44	-20.45	Female	Chevron
MA01	Unknown	13/01/2016	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA08	Unknown	15/01/2016	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA10	Unknown	15/01/2016	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA13	Unknown	21/01/2016	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA16	Unknown	23/01/2016	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA43	Unknown	9/06/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA49	Unknown	9/06/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA50	Unknown	10/06/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA53	Unknown	10/06/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA54	Unknown	10/06/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA58	Unknown	13/09/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA72	Unknown	16/09/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA78	Unknown	16/09/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA79	Unknown	17/09/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA136	Unknown	9/01/2017	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA138	Unknown	9/01/2017	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA147	Unknown	12/01/2017	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA150	Unknown	14/01/2017	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA152	Unknown	26/01/2017	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA158	Unknown	27/01/2017	NSI, Australia	142.72	-24.1	Unknown	Unknown

NC: New Caledonia, PS: Pleiades Sud, PN: Pleiades Nord, NSI: North Stradbroke Island, LEI: Lady Elliot Island.

**Table S2.** Alpha-scores simulated for testing hypotheses of structure for reef manta rays (*Mobula alfredi*) for East Australia and New Caledonia (regional dataset) using 3619 neutral SNPs.

Australia	New Caledonia	Mean	SD	#PCs	#DAs	Method for #PCs
0.62	0.03	0.33	0.31	30	10	N/3
1	0	0.50	0.70	1	10	Simulated

N/3: indicates the highest-recommended-number method.

Simulated: indicates the optimized-simulation-based method.

**Table S3.** Alpha-scores simulated for testing hypotheses of structure New Caledonian reef manta rays (*Mobula alfredi*) (local dataset) using 2676 SNPs.

Noumea	Ouvea	Touho	Mean	SD	#PCs	#DAs	Method for #PCs
0.36	0.28	0.21	0.28	0.10	21	10	N/3
0.31	0.49	0.14	0.25	0.14	9	10	Simulated

N/3: indicates the highest-recommended-number method.

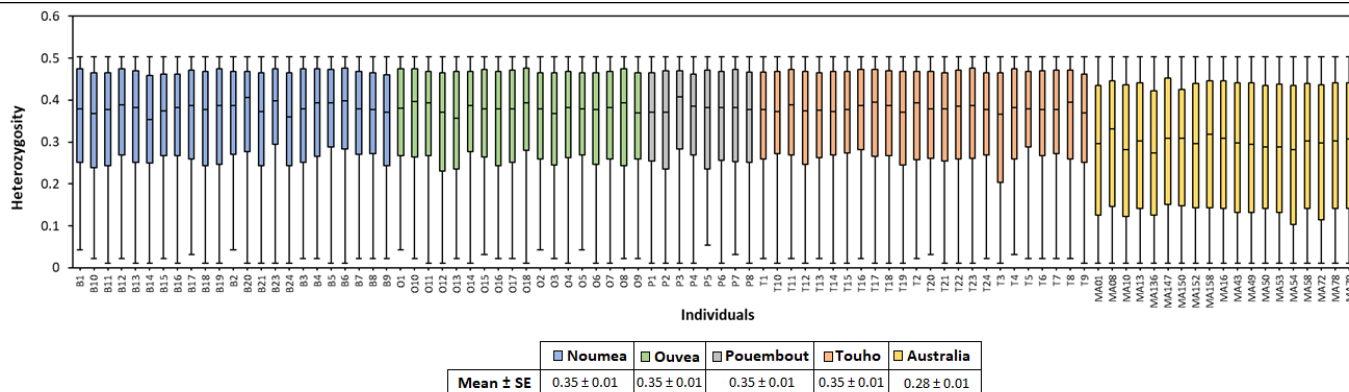
Simulated: indicates the optimized-simulation-based method.

**Table S4.** Confusion matrix showing predicted assignments for all individuals of reef manta rays (*Mobula alfredi*) of New Caledonia (local dataset) using 2676 SNPs the local based on DAPC using 2702 SNPs.

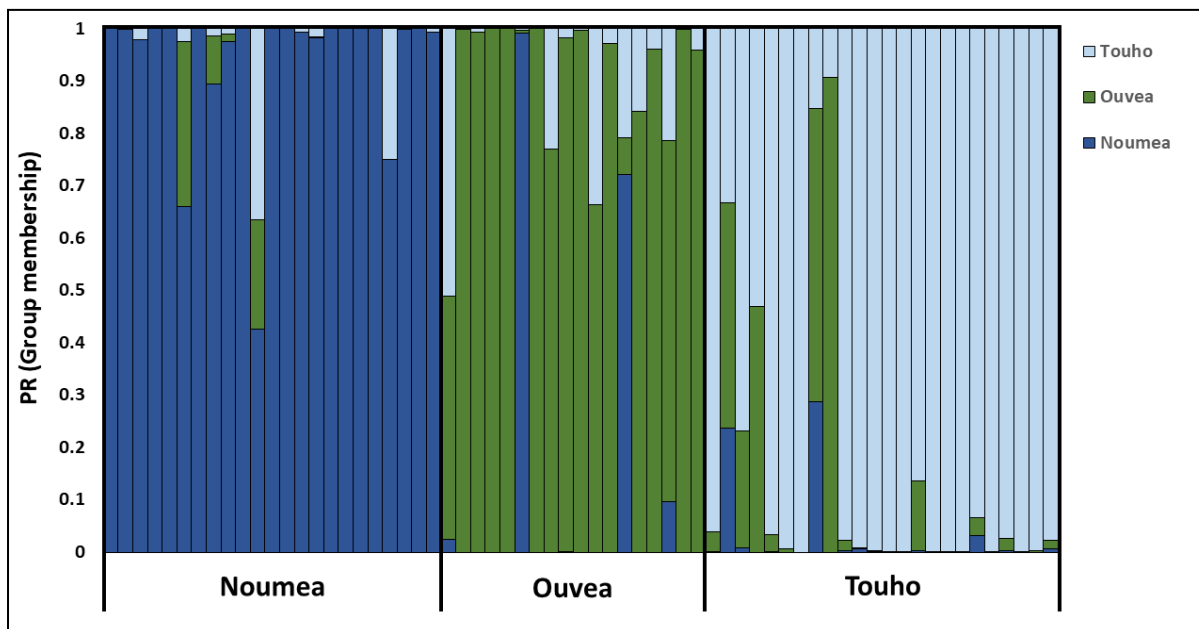
		Assigned Cluster			Prior	Posterior
		Noumea	Ouvea	Touho		
Group of origin	Noumea	23	0	0	0.3538	0.9411 ± 0.1384
	Ouvea	2	15	1	0.2769	0.7979 ± 0.3071
	Touho	0	3	21	0.3692	0.8543 ± 0.2723
<b>Overall</b>					0.3382	0.8694

**Table S5.** Reef manta rays (*Mobula alfredi*) individuals average membership probability from the DAPC for each population from New Caledonia and East Australia (regional dataset) and within New Caledonia (local dataset) using 3619 and 2676 neutral SNPs.

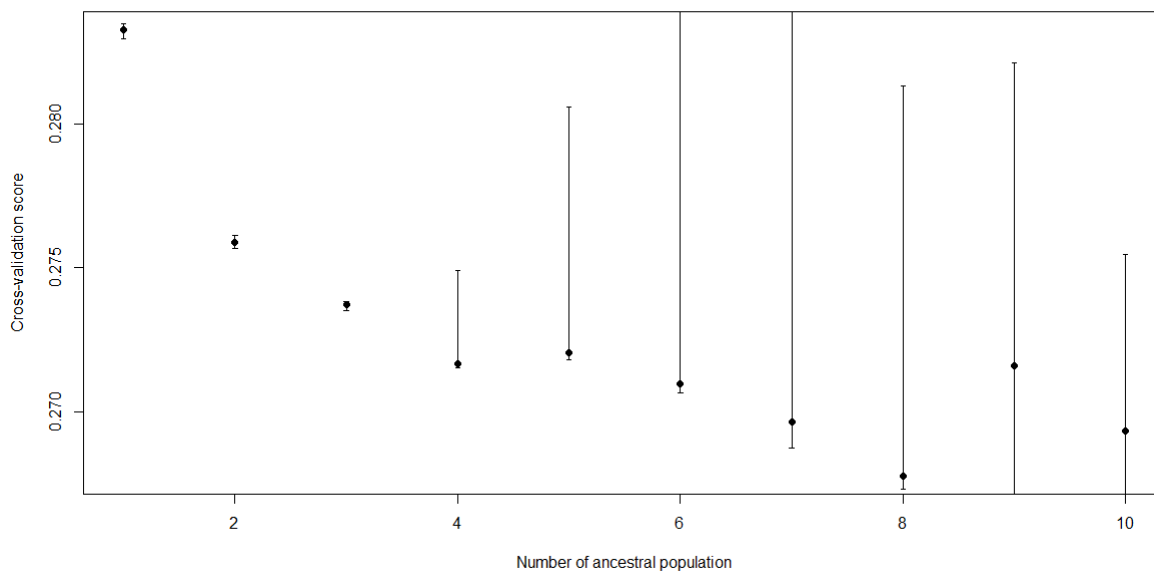
Local dataset		Assigned Cluster		
		Noumea	Ouvea	Touho
Group of origin	Noumea	0.9412 ± 0.1384	0.0276 ± 0.0764	0.0312 ± 0.003
	Ouvea	0.1020 ± 0.2713	0.7979 ± 0.3071	0.1001 ± 0.1425
	Touho	0.0246 ± 0.0724	0.1211 ± 0.2293	0.8543 ± 0.2723
Regional dataset		New Caledonia	Australia	
Group of origin	New Caledonia	1	0	
	Australia	0	1	



**Figure S1.** Boxplot showing mean and 95% CIs for of individual manta ray heterozygosity, with inset showing mean and standard error (SE) by sampling site. Based on East Australia (regional dataset) using 3619 SNPs (before applying MAF > 0.05 filter).



**Figure S2.** Probability of group membership of each individual of reef manta rays (*Mobula alfredi*) within the four sampling locations from New Caledonia (local dataset) using 2676 neutral SNPs.



**Figure S3.** Cross-validation score each K values from 1 to 10, with a maximum number of 100 iterations per run (20 repetitions) and a tolerance value of  $10^{-7}$  for reef manta rays (*Mobula alfredi*) from East Australia and New Caledonia (regional dataset) using 3676 neutral SNPs.