

Table S1: An overview of the key fishing pressures and relevant domestic regulations for wedgefishes in the focal regions.

Country	Main fishing pressure	Domestic regulations in place
South Africa	Shark nets, commercial and recreational fishing	<ul style="list-style-type: none"> • National Environmental Management: Biodiversity Act, 2004 (Government of South Africa 2004): illegal to catch, possess, sell, or transport wedgefish without a permit • Marine Living Resources Act, 1998 (Government of South Africa 1998): fishing restrictions, size limits, fishing permits, research and monitoring • Signatory to Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and Convention on Biological Diversity (CBD)
Mozambique	Artisanal and commercial fishing, especially in southern Mozambique	<ul style="list-style-type: none"> • Mozambican Fisheries Law no. 7 of 2019 (Government of Mozambique 2019): illegal to catch, possess, transport, or sell wedgefish • Signatory to CITES and CBD
Australia	Fishing effort is relatively low, but mainly commercial, indigenous and recreational fishing	<ul style="list-style-type: none"> • Commonwealth Environment Protection and Biodiversity Conservation Act, 1999 (Commonwealth of Australia 1999): "conservation dependent", illegal to kill, capture, or harm wedgefish without a permit • Fisheries Management Act, 1994 (Commonwealth of Australia 1994) • Marine Parks Act, 2007 (Government of South Australia 2007) • Other state and territory fisheries laws • Signatory to CITES and CBD
Madagascar	Artisanal and commercial fishing	<ul style="list-style-type: none"> • Fisheries and Aquaculture Act, 1993 (Government of Madagascar 1993): illegal to capture, sell, or transport wedgefish without a permit • Signatory to CITES and CBD
Réunion Island	The shark control program, recreational and commercial fishing	<ul style="list-style-type: none"> • Prefectural Order for the Protection of Marine Fauna and Flora of Réunion Island, 2009, order no. 1124 (Prefecture of Réunion Island 2009): illegal to capture, kill, possess, sell and transport wedgefish. The law also mandates the release of any wedgefish caught accidentally and prohibits the destruction of their habitats • Signatory to CITES and CBD (indirectly through membership to the European Union)
Seychelles	Artisanal and commercial fishing	<ul style="list-style-type: none"> • Marine Protected Areas Regulations, Regulation no. 5 of 2014 (Government of Seychelles 2014): illegal to take, sell and possess wedgefish in marine protected areas • Fisheries Conservation and Management Act no. 18 of 1998 (Government of Seychelles 1998): establishes closed seasons and closed areas for fishing, implements size limits for catches • Signatory to CITES and CBD
Tanzania	Artisanal fishing and commercial prawn trawling	<ul style="list-style-type: none"> • Fisheries Act no. 22 of 2003 (Government of Tanzania 2003): regulation of fishing gear, fishing methods, establishment of closed areas for fishing and restrictions on capture and sale of wedgefish • Marine Parks and Reserves Act no. 29 of 1994 (Government of Tanzania 1994): illegal to fish, hunt or collect wedgefish within the boundaries of marine parks and reserves, unless authorized by the Director of the Tanzania National Parks Authority • Signatory to CITES and CBD

Table S2: Microsatellite markers development (J. Rumbelow unpubl. data) and PCR-amplification conditions.

Development: methods	<p>Low-coverage whole-genome sequencing was performed for a <i>Rhynchobatus djiddensis</i> specimen from Sodwana Bay, KwaZulu-Natal, South Africa, and <i>Rhynchobatus australiae</i> from Unguja, Zanzibar, Tanzania on an Ion Torrent S5™ System by the Central Analytical Facility at Stellenbosch University, South Africa. All sequencing reads were quality filtered using Torrent Suite™ Software. Contigs were constructed using SPAdes v. 3.10.1 (Bankevich et al. 2012) and QUASt v4 (Gurevich et al. 2013) was used to evaluate the <i>de novo</i> genome assemblies. Detection of microsatellites (with a minimum of two repeats) was conducted in Perl 5 v22.1 using MISA (MIcroSATellite identification tool), and primers (one pair per microsatellite) were designed with Primer3 and assessed using Exonerate v2.2 and iPCress (In silico PCR Experiment Simulation System). The iPCress sequence outputs were selected for a Basic Local Alignment Search Tool (BLASTn) search on the NCBI (Benson et al. 2012) to highlight hits with known microsatellites of other elasmobranchs. Microsatellites were prioritised for selection based on repeat length, repeat composition, repeat variation between species and prior identification within elasmobranch species. Final primers were designed using Geneious Prime (Kearse et al. 2012) and PCR was performed. Markers that amplified successfully were fluorescently labelled using one of the following dyes: FAM, VIC, PET, or NED. Labelled primers were then used in the optimization of two multiplex panels (M1 and M2) using the Qiagen® Multiplex PCR Kit.</p>
Development: results	<p>The <i>R. australiae</i> SPAdes assembly had the highest number of contigs (129 297) and largest N50 (16 937) according to the QUASt assessment statistics, thus it was used for microsatellite loci identification. A total of 7202 candidate microsatellite loci consisting of compound repeats and repeats of 1 to 6 bp were identified. The 4664 compound and single bp repeat microsatellites were excluded and the remaining 2538 p2 - p6 microsatellite loci consisted of 2070 dinucleotides. After in silico PCR validation with the designed primers, 151 microsatellites were analysed in Geneious Prime of which 31 were selected based on repeat length, size differences between species and/ or BLASTn hits. 21 primer pairs underwent PCR validation, whereafter 9 labelled primer pairs amplified reliably and underwent characterisation.</p>
Protocol 1: Rhyn9 and Rhyn13; Rhyn17 and Rhyn20; Rhyn10, Rhyn19 and Rhyn27	<p>Each 15-μl PCR reaction consisted of 7.5 μl of Qiagen Multiplex Mix (containing HotStarTaq DNA Polymerase, PCR Buffer, 3 mM MgCl₂, and 200 μM of each dNTP), 0.375 μl of forward (1 pmol μl⁻¹) and 0.375 μl reverse primer (1 pmol μl⁻¹), and 1 μl of template DNA (50 ng μl⁻¹).</p>
Protocol 2: Rhyn2 and Rhyn11	<p>Same as Protocol 1 except 0.55 μl of forward (1 pmol μl⁻¹) and 0.55 μl reverse primer (1 pmol μl⁻¹).</p>
Thermocycling conditions	<p>Initial denaturation at 95°C for 10 min, 9 cycles of touchdown PCR at 95°C for 30 sec, annealing starting at 65°C and decreasing by 1°C per cycle to 57°C for 90 sec, elongation at 72°C for 45 sec, followed by 23 cycles at 95°C for 30 sec, annealing at 57°C for 90 sec, elongation at 72°C for 45 sec and final extension at 72°C for 35 min.</p>

Table S3: Molecular specimen identification results based on *COI* and *ND2* for *Rhynchobatus djiddensis* and *R. australiae*, with sampling location and GenBank record matches (accession number, percentage similarity). *CR* results for Australian samples were also included. N/A = bad sequence quality/ did not PCR- amplify/ not included.

Sample name	Location	Morphological and molecular ID	<i>COI</i> accession and match (%)	<i>ND2</i> accession and match (%)	<i>CR</i> accession and match (%)
RY1	Australia	<i>R. australiae</i>	<i>R. cf. laevis</i> : KF899689.1 (99.46), <i>R. australiae</i> : MG792126.1 (97.85)	<i>R. cf. laevis</i> : JQ518926.1 (100), <i>R. palpebratus</i> : JQ518925.1 (99.79), <i>R. australiae</i> : NC_030254.1 (95.52)	<i>R. australiae</i> : NC_030254.1 (97.45)
RY2	Australia	<i>R. australiae</i>	<i>R. cf. laevis</i> : KF899689.1 (99.28), <i>R. australiae</i> : MG792126.1 (97.50)	N/A	<i>R. australiae</i> : NC_030254.1 (97.45)
RY3	Australia	<i>R. australiae</i>	<i>R. cf. laevis</i> : KF899689.1 (99.51), <i>R. australiae</i> : MF508696.1 (97.87)	<i>R. cf. laevis</i> : JQ518926.1 (100), <i>R. palpebratus</i> : JQ518925.1 (99.79), <i>R. australiae</i> : NC_030254.1 (95.52)	<i>R. australiae</i> : NC_030254.1 (97.66)
RY4	Australia	<i>R. australiae</i>	<i>R. cf. laevis</i> : KF899689.1 (99.51), <i>R. australiae</i> : MF508696.1 (97.86)	<i>R. cf. laevis</i> : JQ518926.1 (100), <i>R. palpebratus</i> : JQ518925.1 (99.79), <i>R. australiae</i> : NC_030254.1 (95.52)	<i>R. australiae</i> : NC_030254.1 (96.82)
RY5	Australia	<i>R. australiae</i>	<i>R. cf. laevis</i> : KF899689.1 (99.28), <i>R. australiae</i> : MG792126.1 (97.50)	N/A	N/A

RY8	Australia	<i>R. australiae</i>	<i>R. cf. laevis</i> : KF899689.1 (99.51), <i>R. australiae</i> : MF508696.1 (97.87)	<i>R. cf. laevis</i> : JQ518926.1 (100), <i>R. palpebratus</i> : JQ518925.1 (99.79), <i>R. australiae</i> : NC_030254.1 (95.52)	<i>R. australiae</i> : NC_030254.1 (97.45)
RY10	Australia	<i>R. australiae</i>	<i>R. cf. laevis</i> : KF899689.1 (99.34), <i>R. australiae</i> : MF508696.1 (97.70)	<i>R. cf. laevis</i> : JQ518926.1 (100), <i>R. palpebratus</i> : JQ518925.1 (99.79), <i>R. australiae</i> : NC_030254.1 (95.52)	<i>R. australiae</i> : NC_030254.1 (97.45)
RY12	Australia	<i>R. australiae</i>	<i>R. cf. laevis</i> : KF899689.1 (99.51), <i>R. australiae</i> : MF508696.1 (97.87)	N/A	<i>R. australiae</i> : NC_030254.1 (97.45)
RY14	Australia	<i>R. australiae</i>	<i>R. cf. laevis</i> : KF899689.1 (99.51), <i>R. australiae</i> : MF508696.1 (97.87)	<i>R. cf. laevis</i> : JQ518926.1 (100), <i>R. palpebratus</i> : JQ518925.1 (99.79), <i>R. australiae</i> : NC_030254.1 (95.52)	<i>R. australiae</i> : NC_030254.1 (97.45)
RD1	Madagascar	<i>R. australiae</i>	MF508696.1 (99.46)	NC_030254.1 (100), JQ519023.1 (99.36)	N/A
RD2	Madagascar	<i>R. australiae</i>	MF508696.1 (99.46)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RD4	Madagascar	<i>R. australiae</i>	MF508696.1 (99.46)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RD6	Madagascar	<i>R. australiae</i>	OQ385076.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RD7	Madagascar	<i>R. australiae</i>	MF508696.1 (99.46)	NC_030254.1 (100), JQ519023.1 (99.36)	N/A
RD8	Madagascar	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A

RD9	Madagascar	<i>R. australiae</i>	MF508696.1 (99.46)	NC_030254.1 (100), JQ519023.1 (99.36)	N/A
RD10	Madagascar	<i>R. australiae</i>	MF508696.1 (99.46)	NC_030254.1 (100), JQ519023.1 (99.36)	N/A
RD11	Madagascar	<i>R. australiae</i>	MF508696.1 (99.46)	NC_030254.1 (100), JQ519023.1 (99.36)	N/A
RD12	Madagascar	<i>R. australiae</i>	MF508696.1 (99.64)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RD13	Madagascar	<i>R. australiae</i>	MF508696.1 (99.46)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RD14	Madagascar	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RD15	Madagascar	<i>R. australiae</i>	MF508696.1 (99.46)	NC_030254.1 (100), JQ519023.1 (99.36)	N/A
RD17	Madagascar	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
11.6	Réunion Island	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
11.98	Réunion Island	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
CR2-61	Réunion Island	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
CR2-62	Réunion Island	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
CR2-68	Réunion Island	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
CR2-76	Réunion Island	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
CR2-96B	Réunion Island	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
Nativ11	Réunion Island	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
PR2P-05	Réunion Island	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
PR2P-41	Réunion Island	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA1	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA2	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA6	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA7	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA8	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA9	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA11	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA12	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A

RA13	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA14	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA15	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA17	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA18	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA19	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA20	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA22	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA23	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA24	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA27	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA29	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA30	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA31	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA32	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA33	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA34	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA35	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA36	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA37	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA38	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA39	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A

RA40	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA41	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA44	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA45	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA47	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA48	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA49	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA50	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA51	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA52	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA53	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA54	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA55	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
RA56	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
RA57	Seychelles	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
FID6764	Tanzania	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
FID6846	Tanzania	<i>R. australiae</i>	MF508696.1 (99.46)	N/A	N/A
FID6874	Tanzania	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
FID6920	Tanzania	<i>R. australiae</i>	MF508696.1 (99.46)	NC_030254.1 (100), JQ519023.1 (99.36)	N/A
FID7035	Tanzania	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
FID7245	Tanzania	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
FID7269	Tanzania	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
FID7291	Tanzania	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
FID7344	Tanzania	<i>R. australiae</i>	MF508696.1 (99.46)	NC_030254.1 (100), JQ519023.1 (99.36)	N/A
FID7347	Tanzania	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
FID7489	Tanzania	<i>R. australiae</i>	MF508696.1 (99.46)	N/A	N/A

FID7503	Tanzania	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
FID7507	Tanzania	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
FID7562	Tanzania	<i>R. australiae</i>	MF508696.1 (99.46)	N/A	N/A
FID7604	Tanzania	<i>R. australiae</i>	MF508696.1 (99.46)	NC_030254.1 (100), JQ519023.1 (99.36)	N/A
FID7610	Tanzania	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
FID7731	Tanzania	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
FID7745	Tanzania	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (100), JQ519023.1 (99.15)	N/A
MB60	Tanzania	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
MB80	Tanzania	<i>R. australiae</i>	KU255184.1 (100)	N/A	N/A
MB86	Tanzania	<i>R. australiae</i>	MF508696.1 (99.46)	N/A	N/A
RD18	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	NC_066688.1 (99.79)	N/A
RD19	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD21	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD22	Durban	<i>R. djiddensis</i>	NC_066688.1 (99.64), JF494384.1 (99.64)	N/A	N/A
RD25	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD26	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD28	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD33	Durban	<i>R. djiddensis</i>	NC_066688.1 (99.82), JF494384.1 (99.82)	N/A	N/A
RD35	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD39	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD40	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD41	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD42	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD43	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD44	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD45	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD46	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A

RD48	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD49	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD50	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD51	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD52	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD53	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD54	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD56	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD58	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD59	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
RD60	Durban	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
SALS_041	Sodwana Bay	<i>R. djiddensis</i>	NC_066688.1 (100)	NC_066688.1 (99.79)	N/A
SALS_043	Sodwana Bay	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
SALS_044	Sodwana Bay	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
SALS_050	Sodwana Bay	<i>R. djiddensis</i>	NC_066688.1 (100)	NC_066688.1 (100)	N/A
SALS_058	Sodwana Bay	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
SALS_089	Sodwana Bay	<i>R. djiddensis</i>	NC_066688.1 (100)	NC_066688.1 (99.79)	N/A
SALS_154	Sodwana Bay	<i>R. djiddensis</i>	NC_066688.1 (100)	NC_066688.1 (99.79)	N/A
SALS_162	Sodwana Bay	<i>R. djiddensis</i>	NC_066688.1 (100)	NC_066688.1 (99.57)	N/A
SALS_CU52	Sodwana Bay	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
001-111033_7	Mozambique	<i>R. djiddensis</i>	NC_066688.1 (99.82), JF494384.1 (99.82)	NC_066688.1 (99.79)	N/A
001-142048	Mozambique	<i>R. djiddensis</i>	NC_066688.1 (100)	NC_066688.1 (99.79)	N/A
007-113922_0	Mozambique	<i>R. djiddensis</i>	NC_066688.1 (99.82), JF494384.1 (99.82)	N/A	N/A
MAP-01	Mozambique	<i>R. djiddensis</i>	NC_066688.1 (100)	N/A	N/A
SP7	Mozambique	<i>R. djiddensis</i>	NC_066688.1 (100)	NC_066688.1 (99.36)	N/A
SP8	Mozambique	<i>R. australiae</i>	KU255184.1 (100)	ON065567.1 (99.35)	N/A
SP9	Mozambique	<i>R. djiddensis</i>	NC_066688.1 (100)	NC_066688.1 (99.79)	N/A
SP10	Mozambique	<i>R. djiddensis</i>	NC_066688.1 (100)	NC_066688.1 (99.79)	N/A

*The *COI* and *ND2* reference sequences for *R. laevis* are from a single individual sourced from a fish market in India (Johri & Dinsdale 2020), but the source and methodology used for the morphological identification remain unverifiable. Additionally, *R. laevis* is currently not recognised as a valid species in Australia. When considered alongside the morphological and molecular data, the consensus identification for the Australian samples is thus *R. australiae*.

Table S4: Genetic diversity indices for *Rhynchobatus djiddensis* and *R. australiae* based on 559-bp and 472-bp alignments of *COI* and *CR*, respectively (n – sample size; H – number of haplotypes; h – haplotype diversity; π – nucleotide diversity; SA – South Africa; SWIO – Southwest Indian Ocean).

Sampling location	<i>COI</i>				<i>CR</i>			
	n	H	h	π	n	H	h	π
<i>R. djiddensis</i>	44	4	0.133	0.00032	32	2	0.063	0.00013
SA – Durban	28	3	0.140	0.00037	19	2	0.105	0.00022
SA – Sodwana Bay	9	1	--	--	6	1	--	--
Mozambique	7	2	0.286	0.00051	7	1	--	--
<i>R. australiae</i>	99	4	0.414	0.00553	95	14	0.481	0.00684
Australia	9	2	0.389	0.00139	8	3	0.464	0.00212
Madagascar	14	2	0.088	0.00266	12	3	0.621	0.00460
Réunion Island	10	1	--	--	8	2	0.250	0.00213
Seychelles	45	1	--	--	46	4	0.128	0.00102
Tanzania	21	2	0.467	0.00334	21	5	0.614	0.00435
SWIO	90	2	0.296	0.00160	87	11	0.384	0.00290
<i>R. australiae</i>								
Global	143	7	0.635	0.01987	127	16	0.652	0.02696

Table S5: Analysis of molecular variance (AMOVA) of *Rhynchobatus djiddensis* and *R. australiae* based on the concatenated mitochondrial alignment of *COI-CR* and nine nuclear microsatellites.

Hypothesis tested	Source of variation	Variation (%)	Fixation index
Mitochondrial DNA			
Panmixia (<i>R. australiae</i> and <i>R. djiddensis</i>)	Among locations	88.30	$\Phi_{ST} = \mathbf{0.8830^*}$
	Within locations	11.70	
Nuclear DNA			
Panmixia (<i>R. australiae</i> and <i>R. djiddensis</i>)	Among locations	16.95	$F_{ST} = \mathbf{0.1695^*}$
	Within locations	83.05	

*indicates statistical significance at a 0.05 level; bold values indicate statistical significance after Benjamini-Hochberg correction.

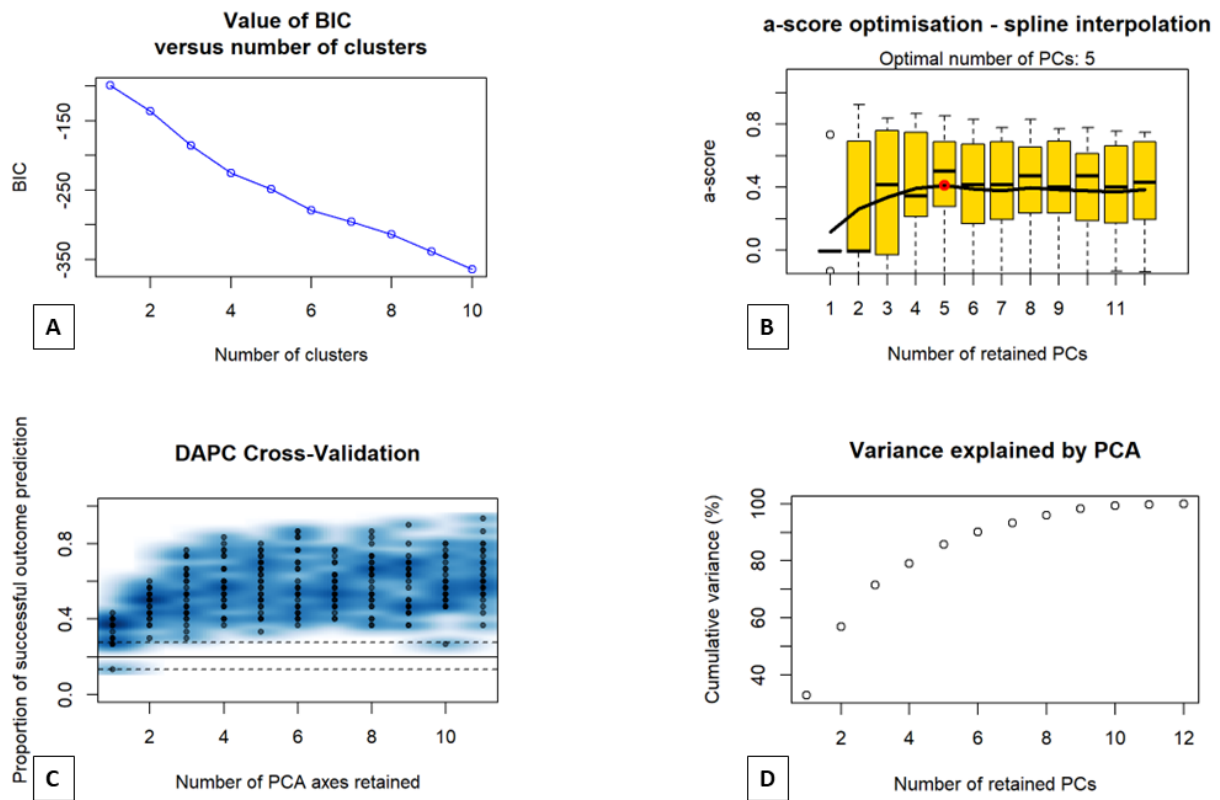


Figure S1: Optimisation of parameters for the *R. australiae* DAPC. (A) graph of BIC values for increasing values of K - inconclusive for identification of best K i.e., no clear inflection point; (B) a -score optimisation showing optimal number of PCs = 5 where the highest proportion of successful reassignment is obtained; (C) cross-validation where PCs = 11 had the lowest MSE and highest mean success; (D) graph of cumulated variance explained by the eigenvalues of the PCA - the first four PCs explain approximately 80% of the variance.

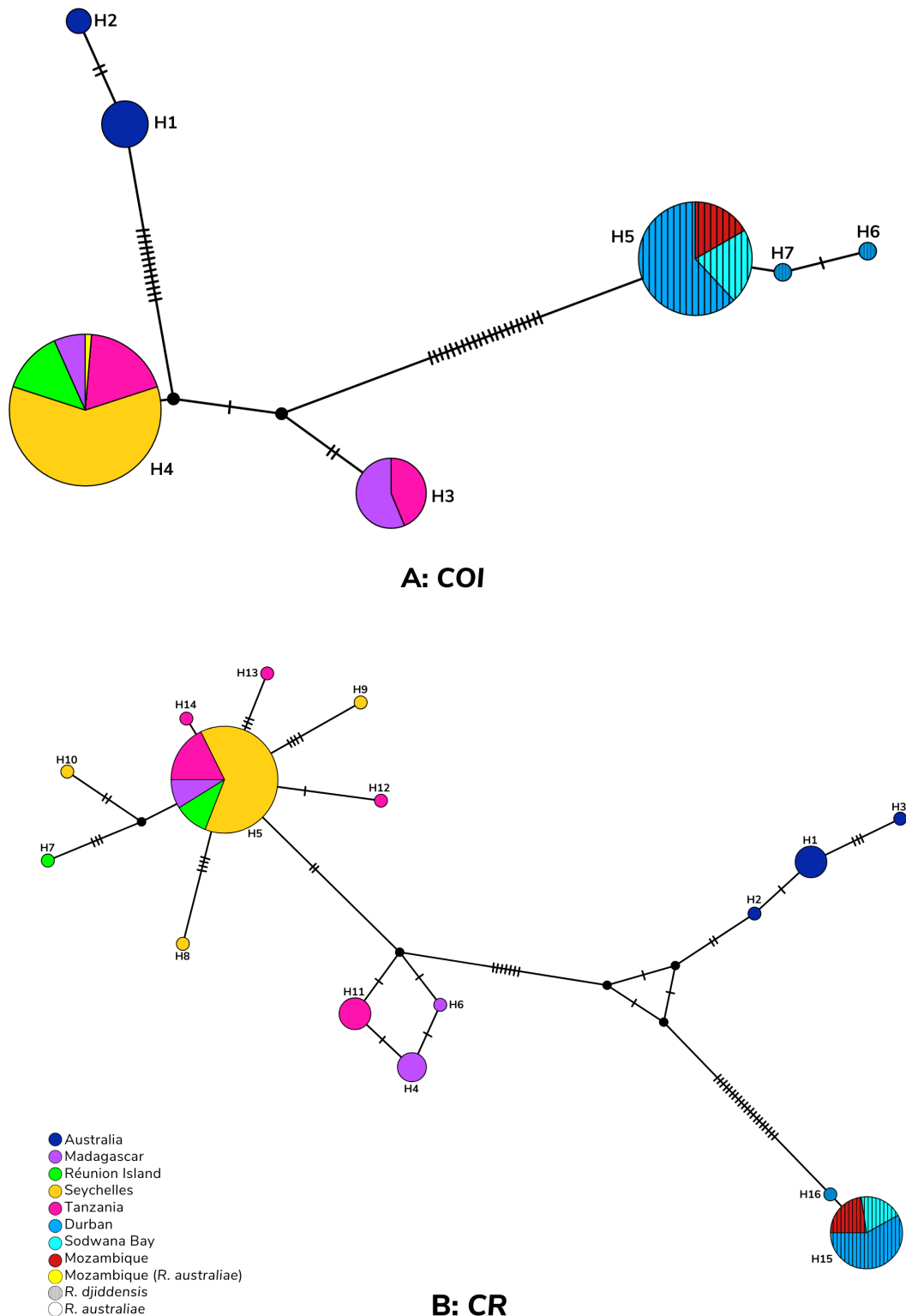


Figure S2: Median Joining haplotype networks based on alignments of (A) *COI* and (B) *CR* for all sampling populations of *Rhynchobatus djiddensis* (South Africa and Mozambique) and *Rhynchobatus australiae* (Australia, Madagascar, Réunion Island, Seychelles, Tanzania and one from Mozambique (yellow section in A, H4)). Mutations separating haplotypes indicated as slashes. Size of each circle is proportional to the number of individuals carrying each haplotype.

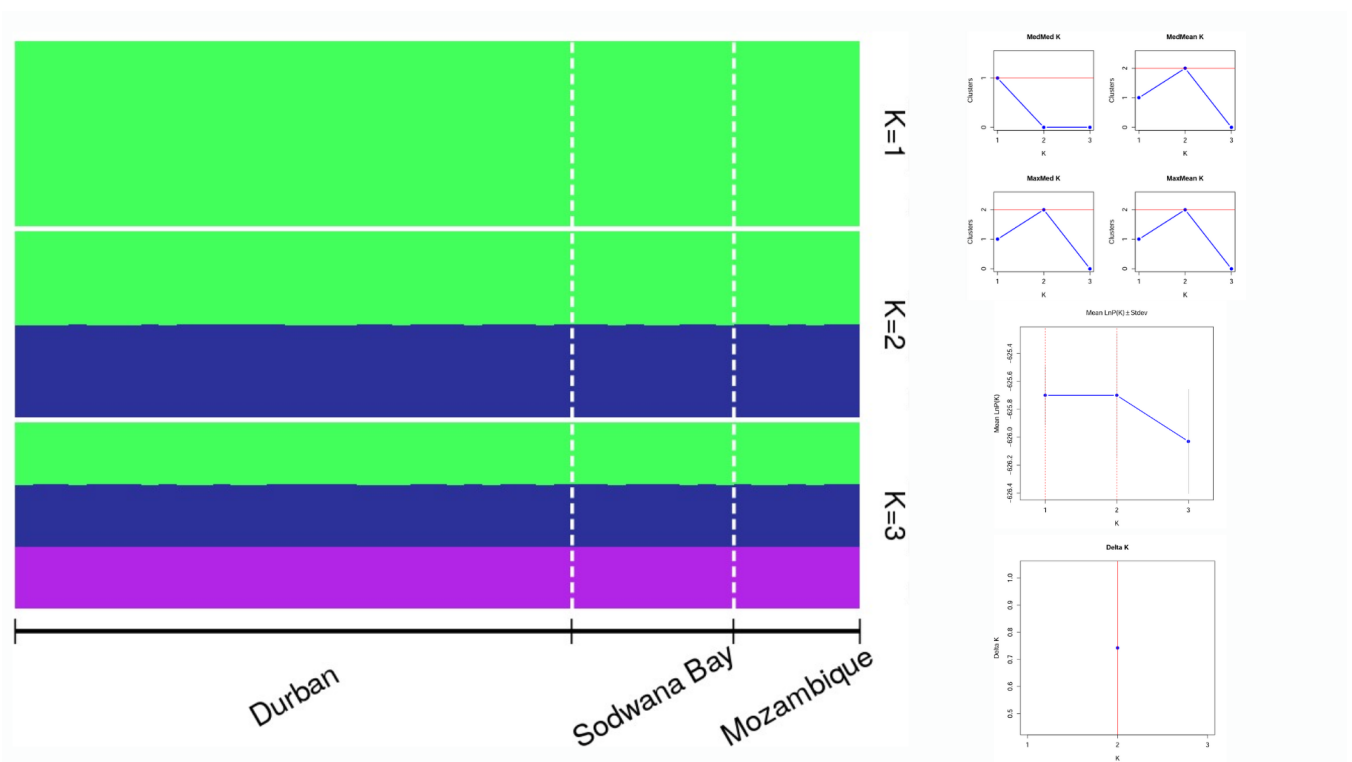


Figure S3: Barplot demonstrating Bayesian clustering assignments for *Rhynchobatus djiddensis* ($K = 1 - 3$) inferred by STRUCTURE based on the correlated allele frequency model, using six analytical methods to determine the most likely K : Delta K , Ln Pr($X|K$) (Evanno et al. 2005), MedMedK, MedMeaK, MaxMedK and MaxMeaK (Puechmaille 2016).

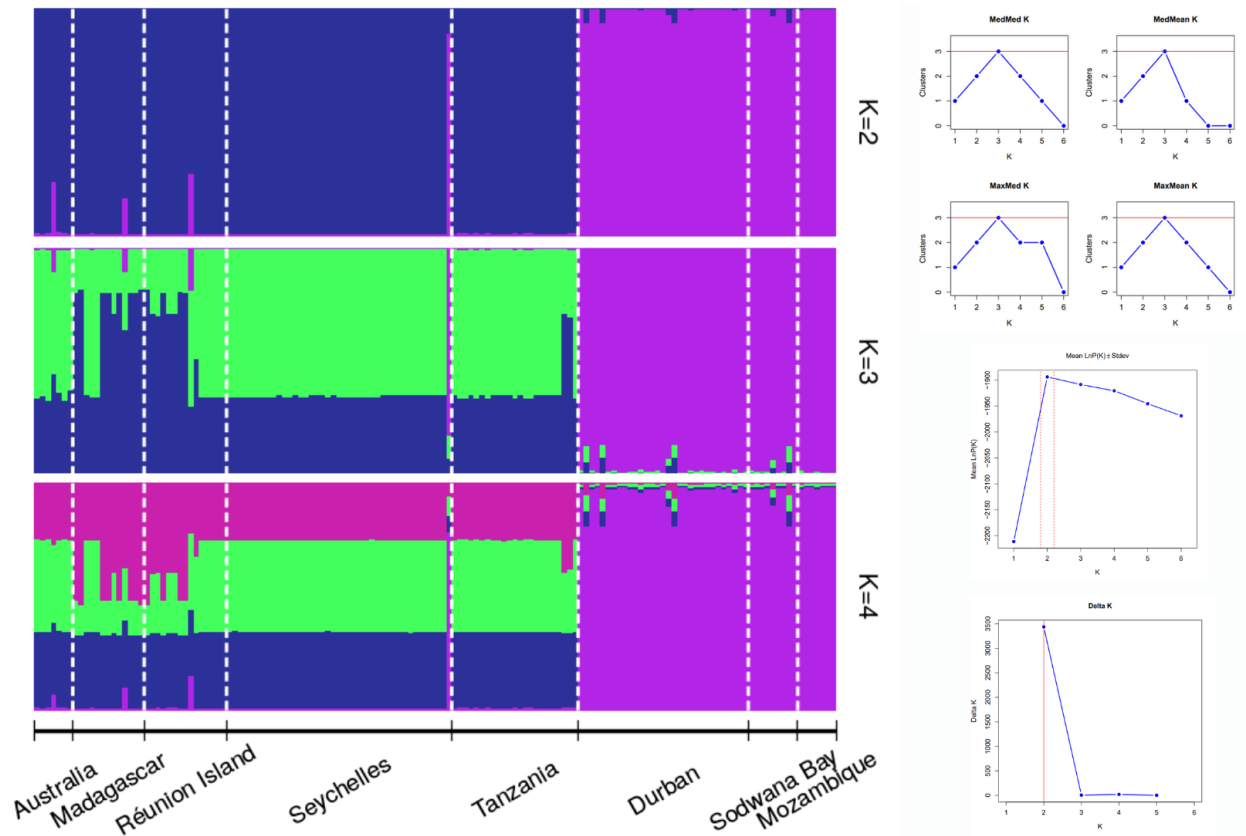


Figure S4: Barplot demonstrating Bayesian clustering assignments for *Rhynchobatus australiae* (Australia, Madagascar, Réunion Island, Seychelles and Tanzania) and *R. djiddensis* (Durban, Sodwana Bay and Mozambique) combined ($K = 2 - 4$) inferred by STRUCTURE based on the independent allele frequency model, using six analytical methods to determine the most likely K : Delta K , $\ln \Pr(X|K)$ (Evanno et al. 2005), MedMed K , MedMean K , MaxMed K and MaxMean K (Puechmaille 2016).

Literature Cited

- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology* 19:455–477.
- Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW (2012) GenBank. *Nucleic Acids Res* 41:36–42.
- CBD (no date) Convention on Biological Diversity: List of Parties
- CITES (no date) Convention on International Trade in Endangered Species of Wild Fauna and Flora: List of Parties
- Commonwealth of Australia (1999) Environment Protection and Biodiversity Conservation Act, 1999
- Commonwealth of Australia (1994) The Fisheries Management Act, 1994
- Government of Madagascar (1993) Loi n° 93-025 portant régime des pêches et de l'aquaculture à Madagascar [Fisheries and Aquaculture Act, 1993]
- Government of Mozambique (2019) Mozambican Fisheries Law: Law no. 7 of 2019.
- Government of Seychelles (2014) Marine Protected Areas Regulations, Regulation No. 5 of 2014. Victoria, Seychelles.
- Government of Seychelles (1998) The Fisheries Conservation and Management Act No. 18 of 1998. Victoria, Seychelles.
- Government of South Africa (1998) Marine Living Resources Act, 1998: Regulations relating to small-scale fishing.
- Government of South Africa (2004) National Environmental Management: Biodiversity Act, 2004: Lists of marine species that are threatened or protected, activities that are prohibited and exemption from restriction.
- Government of South Australia (2007) The Marine Parks Act, 2007
- Government of Tanzania (2003) Fisheries Act No. 22, 2003. Dar es Salaam, Tanzania.
- Government of Tanzania (1994) Marine Parks and Reserves Act No. 29, 1994. Dar es Salaam, Tanzania.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G (2013) QUILT: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649.
- Prefecture of Reunion Island (2009) Prefectural Order for the Protection of Marine Fauna and Flora of Reunion Island, 2009 (Order No. 1124). Saint-Denis, France.