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Population genetic structure of bottlenose and whitespotted wedgefishes from the Southwest Indian Ocean using a dual marker approach

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ABSTRACT: Wedgefishes (Rhinidae) are threatened by unsustainable fishing globally, and especially in the Southwest Indian Ocean (SWIO), due to their high-value fins in the shark trade. The whitespotted wedgefish *Rhynchobatus djiddensis* and the bottlenose wedgefish *R. australiae* are both classified as Critically Endangered on the IUCN Red List of Threatened Species, yet a lack of species-specific knowledge and taxonomic uncertainty still exists within this genus. Genetic approaches aid in taxonomic classification and identifying distinct populations for targeted conservation. Morphological specimen identification of samples (n = 189) collected across the SWIO was confirmed based on the cytochrome oxidase c subunit I (*COI*) and/or nicotinamide adenine dehydrogenase subunit 2 (*ND2*) gene regions. The genetic diversity and population structure within and between species and sampling locations were investigated using a dual marker approach: (1) 2 concatenated mitochondrial gene regions, namely *COI* and the control region (n = 117), and (2) 9 nuclear microsatellite markers (n = 146). The overall genetic diversity was moderate, with an indication that different evolutionary forces are at play on a mitochondrial versus nuclear level. The 2 species were delineated based on both marker types, and for *R. djiddensis*, the sampling locations of South Africa and Mozambique were genetically homogeneous. For *R. australiae*, significant differentiation was found between sampling locations, with Madagascar and Tanzania being genetically the most similar. This information provides critical insights into the distribution range and population structure of the whitespotted wedgefish species complex that can support the sustainable management of wedgefishes.

KEY WORDS: *Rhynchobatus australiae* · *R. djiddensis* · Microsatellites · *COI* · Control region · *ND2* · Species identification · Rhino rays

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1. INTRODUCTION

Delineating populations and understanding the genetic connectivity of endangered and exploited species are important for their conservation management (Kardos 2021). An estimated one-third of all chondrichthyan species (sharks, rays, skates and chimaeras) have an increased extinction risk, with wedgefishes (Rhinidae) and giant guitarfishes (Glaucostegidae) considered the most at-risk marine fish families (Kyne et al. 2020, Dulvy et al. 2021). More specifically, 9 out of 10 species in the family Rhinidae were assessed as Critically Endangered by the IUCN Red List of Threatened Species (IUCN 2022). Fishing-induced mortality was identified as the primary threat, as wedgefishes are both targeted and retained as bycatch. The incentive for fishermen to target and retain bycaught individuals is driven by the exportation of wedgefish fins to international markets where they are considered the most valuable and highest quality within the global shark trade, fetching up to US$964 kg^{-1} in Hong Kong Special Administrative Region (Hau et al. 2018, Jabado 2019). Other wedgefish products, such as meat, are primarily consumed locally while their skin is increasingly used as leather (Dent & Clarke 2015, Moore 2017). Wedgefish products can easily be misidentified and traded as shark products, and the absence of trade data on rays hinders their detection in the fin trade (Boon 2017). Most countries only began tracking trade in ray products after 2012, which poses further enforcement and monitoring challenges (Dent & Clarke 2015). Despite a significant increase in fishing pressure, declining catch rates in trawl surveys and reductions in landings have been reported at fishing ports across the Indo-West Pacific (IWP) and Indian Ocean (Jabado 2018). Furthermore, the susceptibility of wedgefishes to overfishing is exacerbated by their K-selected life history traits (e.g. slow-growing, late to mature, long gestation) and coastal habitat destruction (Dulvy et al. 2014, Kyne et al. 2020).

There is significant taxonomic uncertainty associated with the genus *Rhynchobatus*, as most species are morphologically similar, and their patterns of spots and blotches change with growth, making in-field identification difficult (Kyne et al. 2020). Consequently, biological data from wedgefish species across the IWP have been largely synonymised under a single species complex referred to as the whitespotted wedgefish species complex, particularly prior to the species' reclarification by Last et al. (2016). This has led to a general lack of species-specific biological and ecological information, which has hindered species-level management and made enforcement of regulations challenging. Given that wedgefishes are landed primarily to supply global markets involved in the trade of elasmobranchs, international trade regulation is key to disincentivise harvest of local populations. In 2019, the family Rhinidae was listed on Appendix II of the Convention on International Trade in Endangered Species of Flora and Fauna (CITES), which means Parties to the Convention should implement measures to ensure international trade in these species is sustainable (Nakamura & Kuemlangan 2020, Pavitt et al. 2021). Furthermore, any continued trade must be regulated and require species-level landings as well as export data. Without species-level data, implementation and enforcement of the management measures that are already in place remain challenging. This is particularly evident in countries of the Great South (developing nations primarily located in Africa, Latin America and parts of Asia), where illegal, unreported and unregulated fisheries are extensive.

The Indo-Pacific is the centre of diversity for wedgefishes, with only 2 species occurring in the eastern Atlantic (Jabado 2019). In the Southwest Indian Ocean (SWIO) region that covers 15 000 km of the coastline from Kenya to South Africa, these species are predominantly caught as bycatch (with the fins retained) in artisanal, recreational, small-scale and commercial fisheries (Moore 2017, Kyne et al. 2020). Here, areas of high species richness often spatially overlap with areas experiencing substantial fishing pressure (Queiroz et al. 2019). Considering the conservation status of wedgefishes, such hotspots of overlap between the distribution of these species and fishing pressure should be considered priorities for management (Kyne et al. 2020). Overexploitation in this region has led to declines in the whitespotted wedgefish *R. djiddensis* in southern Mozambique and in KwaZulu-Natal on the east coast of South Africa (Daly et al. 2021), and in other wedgefish species in the SWIO region (including Madagascar and Tanzania), such as the bottlenose wedgefish *R. australiae* (Kyne et al. 2020, Cliff & Daly 2022). *R. djiddensis* and *R. australiae* have both been confirmed as present within the SWIO region and are heavily impacted by fisheries (Kyne et al. 2019a,b, 2020). SWIO *Rhynchobatus* spp. fisheries and regulations are summarised in Table S1 in the Supplement at www.int-res.com/articles/suppl/n053p409_supp.pdf.

Increased knowledge on the distribution range and population structure of the whitespotted wedgefish species complex will improve species-specific

data collection and fisheries monitoring (Giles et al. 2016, Kyne et al. 2020, Choo et al. 2021, Dulvy et al. 2021). Molecular methods provide some resolution to misidentification issues and further assessment of intraspecific genetic structure (Ward et al. 2008, Naylor et al. 2012). Elasmobranch population studies aim to elucidate the processes influencing genetic differentiation to identify marine stocks, defined as reproductively isolated biological units with significant genetic divergence from other stocks (Ovenden 1990). This leads to 2 concepts: evolutionary significant units (ESUs) and management units. ESUs represent historically isolated populations with unique ancestry and a certain capacity to adapt to specific environments (Moritz 1994, Funk et al. 2012), serving as long-term conservation units. Management units are identified as populations showing statistically significant differences in allele frequencies at mitochondrial or nuclear loci (Moritz 1994), aiming for short-term conservation goals. In this study, the detection of genetic divergence between sampling locations would warrant their consideration as distinct management units (Carvalho & Hauser 1994).

The interplay between different marine barriers and dispersal ecology, such as maximum depth of occurrence, maximum body size and habitat, influence elasmobranch dispersal potential (Hirschfeld et al. 2021), which can lead to lower population connectivity than expected. Failing to detect population structure can result in overexploitation or localised extirpation (Hueter et al. 2004, Karl et al. 2011, Ovenden 2013). When species are unable to cross open-ocean waters due to, for example, their inability to overcome oceanic currents (Ovenden 2013) or withstand temperature changes (Simpfendorfer & Heupel 2004), it can result in fragmented populations with lower overall diversity. Thus, understanding how these processes influence the underlying patterns of wedgefish population structure across a clearly defined distribution range is critical for the identification of putative management units.

The use of mitochondrial DNA (mtDNA), including gene regions such as nicotinamide adenine dehydrogenase subunit 2 (*ND2*), cytochrome oxidase *c* subunit I (*COI*) and the control region (*CR*), has been popular in the study of molecular diversity in animals and can be used for molecular specimen identification (Ward et al. 2005, Chabot & Allen 2009, Naylor et al. 2012, Pirog et al. 2019, Catalano et al. 2022, Sukumaran et al. 2023). The maternal inheritance, lack of recombination and faster mutation rate of mtDNA, as compared to nuclear DNA, make it well-suited for studying historical population dynamics. There is some published information on the molecular identification of fresh and processed specimens based on mtDNA in the context of wedgefish products in trade (Giles et al. 2016, Aisyah et al. 2021, Choo et al. 2021). These studies showed that *R. australiae* can be accurately differentiated from other Indo-Pacific species using *COI*-barcoding (Aisyah et al. 2021, Choo et al. 2021), as well as *ND2* and *CR* (Giles et al. 2016). Choo et al. (2021) found that wedgefishes, including *R. australiae*, were commercially available in forms of whole fish, fillet, fin, dried and cooked meats in Singapore. Aisyah et al. (2021) identified *R. australiae* and *R. springeri* from unidentified fins confiscated in South Bangka, Indonesia. A few studies have furthermore demonstrated population genetic structure at broader and more local scales. Simwanza & Rumisha (2023) found that populations of *R. australiae* from the Western Indian Ocean (WIO) are genetically distinct from those in the Eastern Indian Ocean (EIO) and Western Pacific (WP) based on *COI* sequences, whereas Giles et al. (2016) described moderate mtDNA differentiation in *R. australiae* across Southeast Asia and Australia based on the *CR* and/or *ND2* gene regions. Tapilatu et al. (2023) also observed moderate genetic divergence across east Indonesian populations of *R. australiae* using data from *COI* sequences. To our knowledge, there is presently no research on the mitochondrial or nuclear population structure of *R. djiddensis*, nor have any studies employing microsatellite markers been conducted for any wedgefish species. Nuclear microsatellite markers have been instrumental in improving our understanding of contemporary processes driving elasmobranch distribution patterns based on species-specific allele sizes and distinctive allele frequencies at multiple loci (Feldheim et al. 2001, Keeney et al. 2005, Karl et al. 2011, Vignaud et al. 2013, Maduna et al. 2017). Nuclear and mtDNA differ primarily in mode of inheritance and effective population sizes, thus their complementary use can enable more accurate quantification of genetic diversity parameters and investigation of population structure (Wright 1931, Ward 2000).

As such, this study aimed (1) to confirm the species-level taxonomic identification of *R. djiddensis* and *R. australiae* by performing molecular species identification based on the *ND2* and/or *COI* gene regions; and (2) to assess the distribution of genetic variation across SWIO populations of these 2 species, with a focus on *R. australiae*, using a dual marker approach comprising concatenated mitochondrial markers (*COI-CR*) and 9 nuclear microsatellites.

2. MATERIALS AND METHODS

2.1. Sampling and laboratory procedures

A total of 189 *Rhynchobatus* spp. samples, in the form of fin-clips or muscle tissue, were obtained from different locations across the SWIO region: Mozambique (n = 8), South Africa — Durban (n = 43), South Africa — Sodwana Bay (n = 10), Madagascar (n = 17), Réunion Island (n = 16), Seychelles (n = 58) and Tanzania (n = 25). Additionally, Australia (n = 12) was included through opportunistic sampling, despite it being outside the SWIO region. The specimens were initially morphologically identified based on criteria from species identification guides (Jabado 2019 and references therein) and then confirmed by molecular species identification. *R. djiddensis* is generally characterised by prominent black markings between the eyes with a large number of white spots and a black pectoral marking surrounded by 4 or more white spots. *R. australiae* has a distinctive bottle-shaped snout that is slightly constricted near the tip, 3 white spots aligned over the pectoral marking (usually 2 spots below), a short line of well-demarcated white spots on the mid dorsal surface and no spots on the tail.

Genomic DNA was extracted using a standard cetyltrimethylammonium bromide extraction protocol (Sambrook & Russell 2001). The purity and quantity were assessed using a NanoDrop™ ND 2000 spectrophotometer (Thermo Fisher Scientific). The *ND2* gene region was PCR-amplified using the primers ILEM and ASNM (Naylor et al. 2012), *COI* using VF2_tl and FishR2_tl (Ward et al. 2005) and *CR* using GWF (Pardini et al. 2001) and CL2 (Tillett et al. 2012). PCRs were carried out in a SimpliAmp™ Thermal Cycler in a 15 µl reaction volume. For *ND2,* the reaction mixture consisted of 50 ng template DNA, 1× PCR buffer, 200 µM of each dNTP, 0.33 µM of forward and reverse primers (1 pmol μl^{-1}), 2 mM of $MgCl_2$ and 0.5 U μl^{-1} of GoTaq® DNA polymerase (Promega). The thermocycling conditions were: 94°C for 2 min; followed by 40 cycles of 94°C for 30 s, 53°C for 30 s, 72°C for 90 s; and a final extension step at 72°C for 15 min. For *COI* and *CR*, the reaction mixture included 50 ng of template DNA, 1× PCR buffer, 200 µM of each dNTP, 0.3 µM of forward and reverse primers (1 pmol μl^{-1}), 2.5 mM of $MgCl_2$ and 0.4 U μl^{-1} of GoTaq®. The thermocycling conditions consisted of an initial denaturing step at 94°C for 5 min; followed by 35 cycles of 94°C for 30 s, either 54°C (*COI*) or 56°C (*CR*) for 30 s, 72°C for 90 s; and a final extension step at 72°C for either 10 min (*COI*) or 5 min (*CR*). The amplicons were sequenced with the appropriate forward primer using standard Sanger sequencing chemistry (BigDye® Terminator v.3.1 Cycle Sequencing Kit, Life Technologies), whereafter capillary electrophoresis was performed at the Central Analytical Facility (CAF) of Stellenbosch University, South Africa. For the amplification of microsatellite markers, 9 primer pairs previously developed in-house for *R. australiae* by J. Rumbelow (unpubl. data, Table S2) were used in 2 multiplex panels (M1 and M2, Table 1) with fluorescently labelled forward primers following the PCR conditions outlined in Table S2. Amplified products were run on an ABI 3730 DNA Analyzer with the LIZ-500 internal size standard (Applied Biosystems) by CAF, and Geneious® v.8.1.2 (Kearse et al. 2012) was used for binning and scoring of microsatellite alleles.

2.2. Mitochondrial sequence data analyses

2.2.1. Molecular specimen identification

Newly generated *ND2* (469 bp) and *COI* (559 bp) sequences were manually curated in Geneious. Specimen identification of the samples was performed by comparing sequences to the available DNA records on the Barcode of Life Data System (BOLD) (Ratnasingham & Hebert 2007) which accepts *COI* barcodes and returns a species-level identification when possible. *ND2* and *COI* sequences were also analysed against records available on the National Center for Biotechnology Information (NCBI) GenBank database (Benson et al. 2013) using the Basic Local Alignment Search Tool (BLASTn) which is an algorithm and program for comparing primary biological sequence information. To establish a threshold for species-level boundaries, the barcoding gap for the *COI* data set was calculated using the 'BarcodingR' package (Zhang et al. 2017) for R v2022.07.1 (R Development Core Team 2015), measured in K2P genetic distance.

2.2.2. Population genetic analyses

COI and *CR* sequences were manually curated and aligned using MAFFT (Katoh & Standley 2013) with the L-INS-i algorithm in Geneious. The trimmed alignments were concatenated to compile the final data sets which included a 559 bp fragment of *COI* and a 472 bp fragment of *CR* (*COI-CR*).

Diversity indices, including number of haplotypes (*H*), number of nucleotide changes (*k*), haplotype

Table 1. Details of 9 microsatellite loci primer pairs used in this study, developed for the bottlenose wedgefish *Rhynchobatus australiae* by J. Rumbelow (unpubl. data). T_A: annealing temperature; Panel: multiplex panel

Primer name	Primer sequence (5'- 3')	Motif	T_A (°C)	Expected product size (bp)	Panel	Dye
Rhyn2	F: GGTTGTGGATTCAGCCAGC R: GATCGCACACATCTTCACAATC	$(CCA)_5$	59	373	M2	FAM
Rhyn9	F: CCGGCCTACTGGTAAAAGTTC R: CCAGAGGTGGTGAACTGAATC	$(GAG)_6$	55	286	M1	VIC
Rhyn10	F: GGCTCTGCAATTCATCTCCC R: GCCGAGAATGTTTGATGGGAAC	$(GTT)_3$	60	300	M2	VIC
Rhyn11	F: GATATCTCCCCCTCTGTCTTTC R: CGTTTCTTCCTCTCTGCTACTG	$(AG)_{13}$	55	344	M2	PET
Rhyn13	F: CCACTTGCTGCATTCACTCC R: CGCGAGTCACTCTTTCATTGG	$(AGC)_5$	55	319	M1	NED
Rhyn17	F: GCGGCCAACAGATTTTGC R: CGCATGGGAGAATTCGTCTG	$(CT)_6$	58	225	M1	FAM
Rhyn19	F: GAGGGCCTTTTCAGAGTGC R: GGAAATGCAGGGATATGAACCG	$(GGA)_5$	55	242	M2	FAM
Rhyn20	F: GGATGACATGGTGTGCGTTG R: CAATGACGGCAATGATCACG	$(AAAT)_5$	58	291	M1	FAM
Rhyn27	F: CTGCATCAGTTAATCCCCTTTG R: GCTTTGCATCTTAGGCTTTGAG	$(GT)_6$	60	403	M2	NED

(h) and nucleotide (π) diversities, were calculated using DnaSP v.6 (Rozas et al. 2017). Subsequently, unique haplotypes were identified for each species in DnaSP, and the evolutionary relationships among haplotypes were inferred and visualised by constructing a median-joining inference network as implemented in PopART (Leigh & Bryant 2015). Genetic divergence among sampling populations was determined in Arlequin v3.5.1.2 (Excoffier & Luscher 2010), by means of pairwise Φ_{ST} tests (10 000 permutations, $p < 0.05$). A hierarchical analysis of molecular variance (AMOVA) was performed in Arlequin, with 10 000 permutations to determine statistical significance ($p < 0.05$). The null hypothesis of genetic homogeneity among groups was investigated using the *R. australiae* (*R. australiae* sampling locations grouped into Australia versus SWIO) and interspecific (*R. australiae* and *R. djiddensis* grouped separately) data sets. A Benjamini-Hochberg (B-H) test was performed to control for the false discovery rate using the 'p.adjust' function in R, and measures of genetic differentiation were considered significant if p-values were <0.05 following the B-H correction. Separate haplotype networks were also constructed, and diversity indices were calculated based on the individual *COI* and *CR* gene regions to enable comparison with published studies.

2.3. Microsatellite data analyses

Microsatellite genotypes were evaluated for stuttering, allelic dropout and the presence of null alleles using Micro-Checker v2.2.3 (van Oosterhout et al. 2004). GENEPOP ON THE WEB v4.2 (Rousset 2008) was used to test for linkage disequilibrium (LD; 10 000 iterations, 10 000 dememorizations, 500 batches), and deviations from Hardy-Weinberg Equilibrium (HWE) expectations were tested using the 'pegas' v1.1 package (Paradis 2010) for R. The inbreeding coefficient of each marker (F_{IS}) was estimated using the R package 'diveRsity' v1.9.90 (Keenan et al. 2013).

The R package 'poppr' v2.9.3 (Kamvar et al. 2014) was used to convert files for calculation of allelic richness (A_R), observed and expected heterozygosity (H_o and H_e) with 'hierfstat' v0.5-11 (Goudet 2005) and polymorphic information content (*PIC*) with 'polysat' v1.7-7 (Clark & Jasieniuk 2011). To test for genetic homogeneity across sampling sites for *R. djiddensis* and *R. australiae*, pairwise F_{ST} (Weir & Cockerham 1984) and significance (9999 permutations, $p < 0.05$) were calculated in Arlequin. The B-H method was applied to adjust p-values of tests using the 'p.adjust' function in R. An AMOVA was performed, as described above, for both the *R. australiae* and interspecific data sets. The genetic clustering patterns

were evaluated using 2 clustering methods: a multivariate discriminant analysis of principal components (DAPC) and a Bayesian clustering model-based method.

For the *R. australiae* data set, the DAPC was performed utilising the R package 'adegenet' v2.1.8 (Jombart 2008). The *K*-means method was run for $K = 1–10$ using the function 'find.clusters' to determine the number of groups that best describe the data. Based on the Bayesian information criterion (BIC) plotted as a function of *K* being inconclusive (Fig. S1A), *K* was chosen based on prior grouping, i.e. number of sampling locations for *R. australiae* ($K = 5$). Before running the DAPC, the alpha score was optimised (Fig. S1B), and cross-validation was performed (Fig. S1C) to determine the optimal number of principal components (PCs) to retain. Due to low levels of differentiation, it may be possible that both methods overestimate the number of PCs, hence the number of leading PC axes was restricted to 4 ($K - 1$ biologically informative PC axes that are expected for *K* effective populations; Thia 2023) (Fig. S1D).

The Bayesian clustering analyses were implemented in the program STRUCTURE v2.3.4 (Pritchard et al. 2000) assuming an admixture ancestry model with correlated allelic frequencies for the *R. djiddensis* and *R. australiae* data sets and independent allelic frequencies for the combined data set. For the latter, data from *R. djiddensis* was included to visualise the differences between the 2 species. Using prior location information, the model was applied for 10 iterations across $K = 5$, $K = 3$ and $K = 6$ for the *R. australiae*, *R. djiddensis* and combined data sets, respectively, with each iteration consisting of 500 000 Markov chain Monte Carlo (MCMC) generations and an initial burn-in phase of 50 000 generations. The best *K*-value was chosen based on 6 statistical tests employed by the program STRUCTURESELECTOR (Li & Liu 2018), namely Delta *K*, the corrected Evanno statistic Ln Pr(X|*K*) (Evanno et al. 2005) and the 4 tests of Puechmaille, i.e. MedMed *K*, MedMean *K*, MaxMed *K* and MaxMean *K* (Puechmaille 2016). Assignment plots were generated and visualised using POPHELPER (Francis 2017).

Due to the relatively low number of alleles, simulations were carried out using POWSIM v4.0 (Ryman & Palm 2006) to determine the power of the markers to detect low levels of population differentiation. Simulations were carried out with 1000 replicates for an effective population size of $N_E = 1000$, and the power of the analysis was indicated by the proportion of tests that were significant at $p < 0.05$.

3. RESULTS

3.1. Molecular specimen identification

From the barcoding gap analysis, the difference between intra- and interspecific distances was found to be 4.3%. Samples from southern Mozambique and South Africa were identified as *Rhynchobatus djiddensis* and those from Australia, Madagascar, Réunion Island, Seychelles and Tanzania as *R. australiae* (Fig. 1; Table S3). One sample from Mozambique was confirmed to be *R. australiae* but was excluded from most of the analyses, as a sample size of 1 is an insufficient representation of a putative population. All generated sequences matched to sequence entries of voucher specimens on GenBank.

3.2. Mitochondrial diversity and population differentiation

A concatenated *COI-CR* data set of 1031 bp in length was successfully generated for a total of 117 individuals (*R. djiddensis* n = 31, *R. australiae* n = 86) (Fig. 1), revealing 74 polymorphic sites.

The haplotype network showed 3 main groups of haplotypes (haplogroups) for the 8 sampling locations, with a single high-frequency haplotype per group (H1 = 4.27%, H6 = 51.23% and H15 = 24.79%) (Fig. 2). The first haplogroup (A) comprised all *R. djiddensis* sampling locations (South Africa — Durban, South Africa — Sodwana Bay and Mozambique), whereas the second (B) consisted of all *R. australiae* sampling locations (Réunion Island, Madagascar, Seychelles and Tanzania, i.e. SWIO) except for Australia, which formed the third haplogroup (C), separated by several mutations (A to B = 42, A to C = 34 and B to C = 24). It should be noted that the individual from Mozambique identified as *R. australiae* is not included here. No individuals of different species were shared between haplogroups, with *R. djiddensis* characterised by 3 private haplotypes and *R. australiae* by 14 private haplotypes, of which 4 were specific to the Australian individuals. *R. australiae* displayed higher overall diversity ($h = 0.506$, $\pi = 0.0066$) than *R. djiddensis* ($h = 0.127$, $\pi = 0.0001$) (Table 2). The degree of genetic diversity varied between sampling locations, with the highest haplotype and nucleotide diversity for *R. djiddensis* found in Mozambique ($h = 0.286$, $\pi = 0.0003$). For *R. australiae*, the lowest was in Seychelles ($h = 0.143$, $\pi = 0.0005$) and the highest in Australia ($h = 0.643$. $\pi = 0.0017$), with SWIO *R. australiae* ($h = 0.402$, $\pi = 0.0024$) also displaying lower

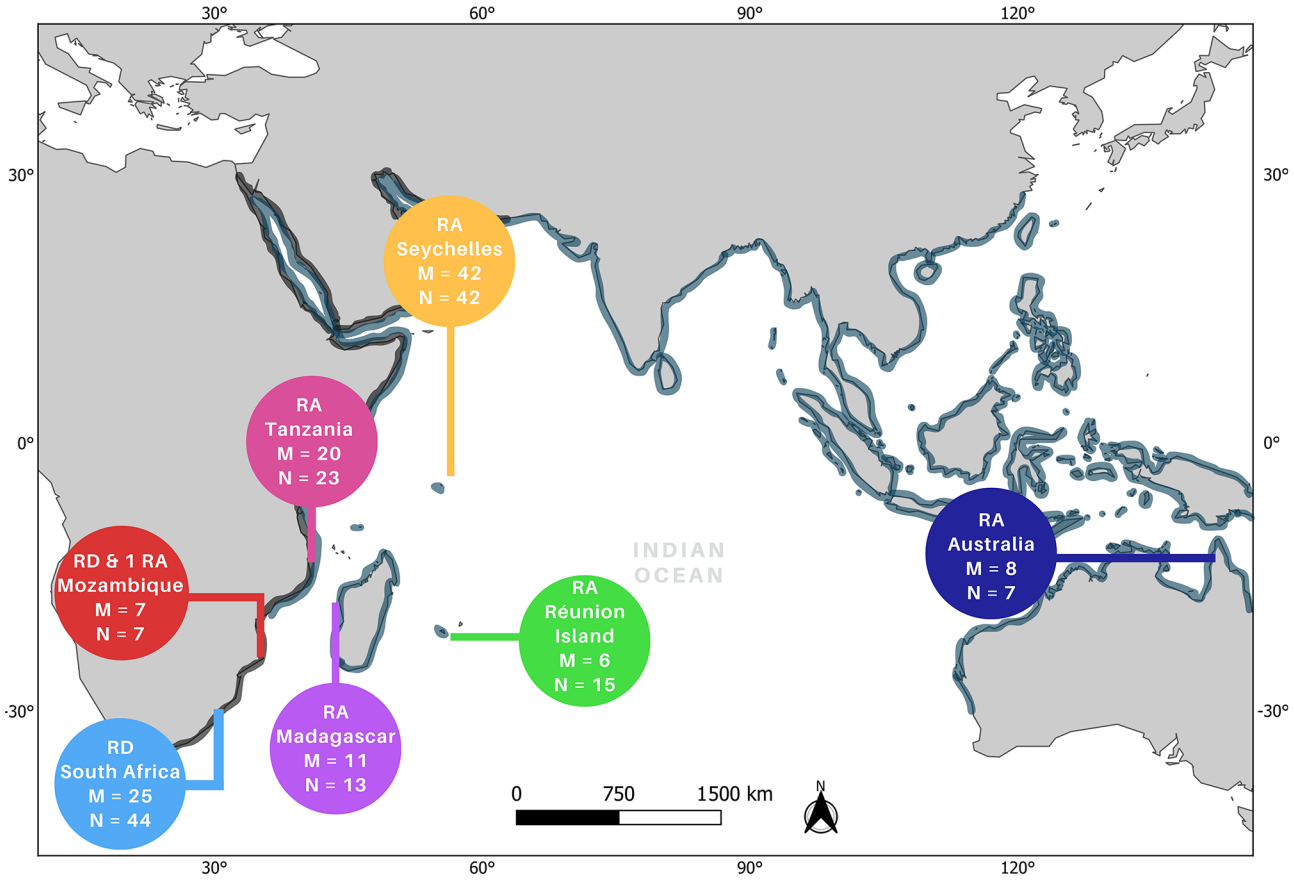

Fig. 1. Sampling locations across the Indian Ocean region. Distribution ranges are indicated in black for *Rhynchobatus djiddensis* (RD) and dark blue for *R. australiae* (RA). M is the number of samples sequenced for mitochondrial gene regions and used in analysis, and N is the number of samples genotyped for nuclear microsatellites. South Africa comprised Durban (M = 18, N = 31) and Sodwana Bay (M = 6, N = 9). One sample from Mozambique was molecularly confirmed to be *R. australiae* but was excluded from further analysis

diversity than Australia. The individual haplotype networks and diversity estimates based on *COI* and *CR*, separately, are shown in Table S4 and Fig. S2.

Significant interspecific differentiation between *R. djiddensis* and *R. australiae* was evident ($\Phi_{ST} > 0.9283$, $p < 0.001$), and no statistically significant intraspecific differentiation was observed for *R. djiddensis* ($\Phi_{ST} < 0.0483$, $p > 0.324$) (Table 3a). Regarding *R. australiae*, high levels of intraspecific differentiation were observed between sampling locations, particularly between Australia and all other locations ($\Phi_{ST} > 0.8820$, $p < 0.001$), whereas Réunion Island and Seychelles seem to be genetically the most similar ($\Phi_{ST} = 0$, $p = 0.999$). The AMOVA showed significant divergence among locations within groups ($\Phi_{SC} = 0.323$, $p < 0.001$) and within locations ($\Phi_{ST} = 0.932$, $p < 0.001$), but not among groups, i.e. Australia and SWIO ($\Phi_{CT} = 0.9$, $p = 0.198$) (Table 4). It also supports genetic discontinuity and restricted geneflow between *R. djiddensis* and *R. australiae* ($\Phi_{ST} = 0.8830$, $p < 0.001$) (Table S5).

3.3. Nuclear diversity and population differentiation

In total, 146 individuals were successfully genotyped for 9 species-specific microsatellite markers (*R. djiddensis* n = 47 and *R. australiae* n = 99) (Fig. 1).

Using Micro-Checker, it was revealed that no genotypes displayed stuttering, null alleles or large allelic drop-out. Across all sampling populations, all loci except Rhyn13 deviated from HWE ($p < 0.05$), and all loci had negative F_{IS} values. No significant LD was observed except for locus pairs Rhyn9 and Rhyn10, Rhyn2 and Rhyn20, and Rhyn13 and Rhyn27. This is primarily attributed to the fixation of alleles at these loci as well as a major sampling effect where observed values deviate considerably from expected values. Thus, all 9 loci were retained for downstream analyses. Genetic diversity estimates were moderate and consistent across all sampling populations, with *R. djiddensis* ($A_R = 2.346$, PIC = 0.344, $H_o = 0.82$) dis-

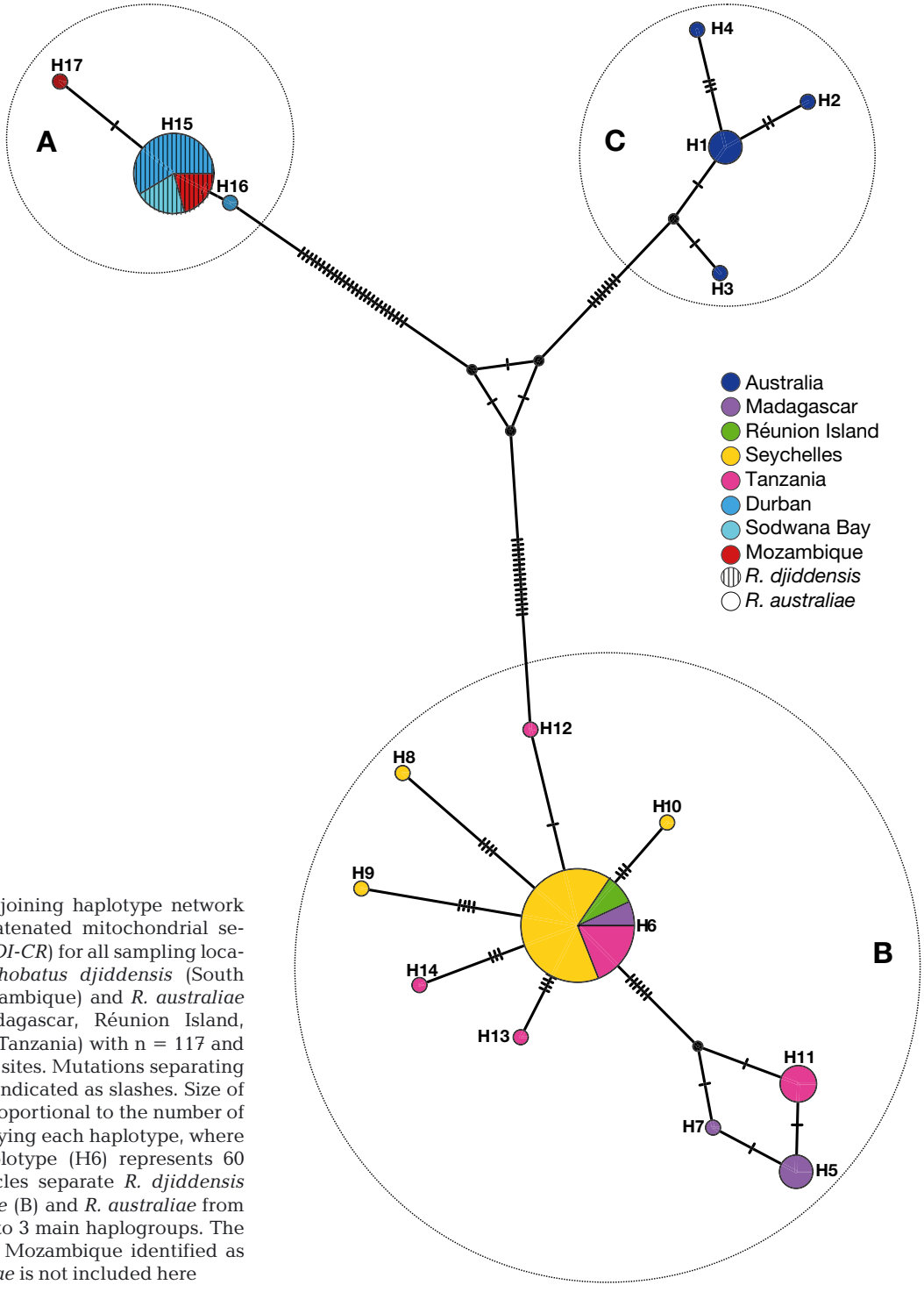

Fig. 2. Median-joining haplotype network based on concatenated mitochondrial sequence data (*COI-CR*) for all sampling locations of *Rhynchobatus djiddensis* (South Africa and Mozambique) and *R. australiae* (Australia, Madagascar, Réunion Island, Seychelles and Tanzania) with n = 117 and 79 polymorphic sites. Mutations separating haplotypes are indicated as slashes. Size of each circle is proportional to the number of individuals carrying each haplotype, where the largest haplotype (H6) represents 60 sequences. Circles separate *R. djiddensis* (A), *R. australiae* (B) and *R. australiae* from Australia (C) into 3 main haplogroups. The individual from Mozambique identified as *R. australiae* is not included here

playing slightly greater overall genetic diversity than *R. australiae* (A_R = 2.196, PIC = 0.336, H_o = 0.84) (Table 2).

Pairwise F_{ST} estimates indicated genetic differentiation between most sampling locations. Significant interspecific differentiation was observed between *R. australiae* and *R. djiddensis* ($F_{ST} > 0.0953$, $p < 0.001$) as well as within *R. australiae* ($F_{ST} > 0.0047$, $p < 0.024$) (Table 3b), although not between Australia, Tanzania and Madagascar ($F_{ST} = 0$, $p < 0.001$). The AMOVA corroborated this, with significant interspecific differentiation between *R. australiae* and *R. djiddensis*

Table 2. Genetic diversity indices for *Rhynchobatus djiddensis* and *R. australiae* based on (a) a 1031 bp concatenated alignment of *COI-CR* and (b) 9 nuclear microsatellite markers; n: sample size; *H*: number of haplotypes; *h*: haplotype diversity; π: nucleotide diversity; *k*: average number of nucleotide changes; PH: private haplotypes; A_R: allelic richness; PIC: polymorphic information content; A_N: number of alleles; H_o: observed heterozygosity; H_e: expected heterozygosity; F_{IS}: inbreeding coefficient; SA: South Africa; SWIO: Southwest Indian Ocean

(a) Mitochondrial DNA

Sampling location	n	*H*	*h*	π	*k*	PH
R. djiddensis	31	3	0.127	0.0001	0.1290	3
SA — Durban	18	2	0.111	0.0001	0.1111	1
SA — Sodwana Bay	6	1	—	—	—	0
Mozambique	7	2	0.286	0.0003	0.2857	1
R. australiae	86	14	0.506	0.0066	6.7975	14
Australia	8	4	0.643	0.0017	1.7500	4
Madagascar	11	3	0.636	0.0042	4.3636	2
Réunion Island	6	1	—	—	—	0
Seychelles	41	4	0.143	0.0005	0.5366	3
Tanzania	20	5	0.632	0.0038	3.8947	4
SWIO *R. australiae*	78	10	0.402	0.0024	2.4589	10
Global	117	17	0.674	0.0192	19.7172	—

(b) Nuclear DNA

Sampling location	n	A_R	PIC	A_N	H_o	H_e	F_{IS} (95% CI)
R. djiddensis	47	2.346	0.344	2.56	0.82	0.48	−0.71 (−0.88 to −0.53)
SA — Durban	31	2.384	0.377	2.56	0.81	0.47	−0.71 (−0.84 to −0.50)
SA — Sodwana Bay	9	2.320	0.289	2.22	0.75	0.44	−0.71 (−0.86 to −0.43)
Mozambique	7	2.333	0.367	2.44	0.92	0.53	−0.78 (−0.85 to −0.60)
R. australiae	99	2.196	0.336	2.78	0.84	0.47	−0.79 (−0.93 to −0.58)
Australia	7	2.389	0.346	2.45	0.73	0.44	−0.62 (−0.81 to −0.40)
Madagascar	13	2.094	0.309	2.22	0.88	0.46	−0.93 (−0.98 to −0.85)
Réunion Island	15	2.365	0.354	2.22	0.88	0.52	−0.75 (−0.87 to −0.38)
Seychelles	41	2.106	0.337	2.33	0.82	0.44	−0.88 (−0.99 to −0.59)
Tanzania	23	2.027	0.344	2.00	0.86	0.45	−0.83 (−1.00 to −0.77)
SWIO *R. australiae*	92	2.148	0.334	2.56	0.85	0.47	−0.81 (−0.95 to −0.63)
Global	146	2.252	0.431	3.11	0.83	0.47	−0.78 (−0.85 to −0.41)

Table 3. Genetic differentiation between 8 sampling locations of *Rhynchobatus djiddensis* (light grey) and *R. australiae* (dark grey) based on (a) the concatenated alignment of *COI-CR* and (b) 9 nuclear microsatellites. Φ_{ST} and F_{ST} estimates below the diagonal. SA: South Africa. *indicates statistical significance at an α level of 0.05; **bold** values indicate statistical significance after Benjamini-Hochberg correction

	Australia	Madagascar	Réunion Island	Seychelles	Tanzania	SA — Durban	SA — Sodwana Bay	Mozambique
Australia	0							
Madagascar	**0.8820***	0						
Réunion Island	**0.9632***	**0.3969***	0					
Seychelles	**0.9725***	**0.6233***	0	0				
Tanzania	**0.8840***	0.0932	0.0930	**0.2651***	0			
SA — Durban	**0.9846***	**0.9595***	**0.9980***	**0.9895***	**0.9501***	0		
SA — Sodwana Bay	**0.9736***	**0.9318***	**0.9999***	**0.9877***	**0.9283***	0.0000	0	
Mozambique	**0.9723***	**0.9333***	**0.9963***	**0.9871***	**0.9293***	0.0483	0.0000	0

(a)

Australia	0							
Madagascar	0.0000	0						
Réunion Island	**0.0473***	**0.0309***	0					
Seychelles	**0.0066***	**0.0047***	**0.0414***	0				
Tanzania	0.0000	0.0000	**0.0414***	**0.0259***	0			
SA — Durban	**0.1371***	**0.1571***	**0.1893***	**0.1861***	**0.1718***	0		
SA — Sodwana Bay	**0.1513***	**0.1742***	**0.2069***	**0.1918***	**0.1949***	0.0000	0	
Mozambique	**0.0953***	**0.1131***	**0.1209***	**0.1518***	**0.1306***	0.0000	0.0000	0

(b)

Table 4. Analysis of molecular variance (AMOVA) of *Rhynchobatus djiddensis* and *R. australiae* combined and *R. australiae* sampling locations grouped into Southwest Indian Ocean (SWIO) and Australia based on the concatenated mitochondrial alignment of *COI-CR* and 9 nuclear microsatellites. *indicates statistical significance at an α level of 0.05; **bold** values indicate statistical significance after Benjamini-Hochberg correction

Hypothesis tested	Source of variation	Variation (%)	Fixation index
Mitochondrial DNA			
R. australiae	Among groups	90	Φ_{CT} = 0.900
(SWIO and Australia)	Among locations within groups	3.23	Φ_{SC} = **0.323***
	Within locations	6.77	Φ_{ST} = **0.932***
Nuclear DNA			
R. australiae	Among groups	−1.29	F_{CT} = −0.013
(SWIO and Australia)	Among locations within groups	2.34	F_{SC} = **0.023***
	Within locations	98.95	F_{ST} = **0.01***

(F_{ST} = 0.1695, p < 0.001) (Table S5). For *R. australiae*, there was significant differentiation among locations within groups (F_{SC} = 0.023, p < 0.001) and within locations (F_{ST} = 0.01, p < 0.001), but not among groups (F_{CT} = 0, p = 0.780, Table 4). For both hypotheses tested, most of the variation was contained within and not between the populations. The multivariate DAPC plot of the *R. australiae* sampling locations (K = 5) showed very little clustering, with ellipses mostly overlapping (Fig. 3). Réunion Island showed the least amount of overlap with the rest, which corresponds to the small but significant pairwise F_{ST} values. The Bay-

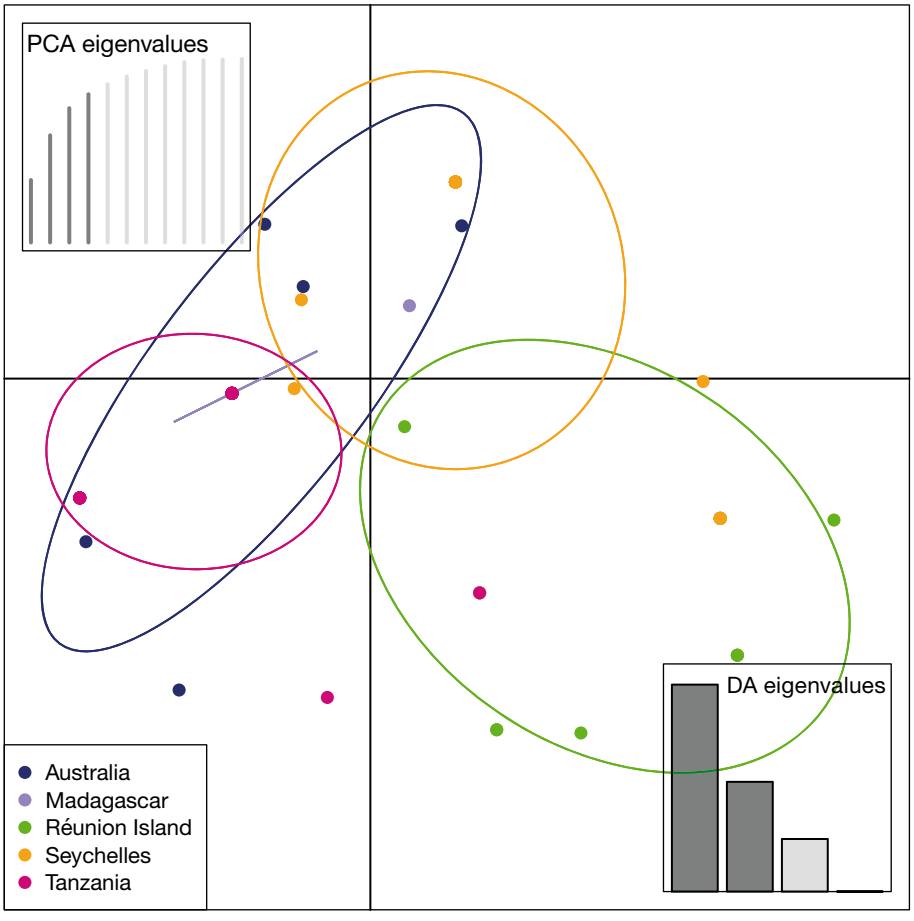

Fig. 3. Clustering patterns obtained from a discriminant analysis of principal components (DAPC) of *Rhynchobatus australiae* sampling locations, with n = 99, K = 5 and 4 principal components

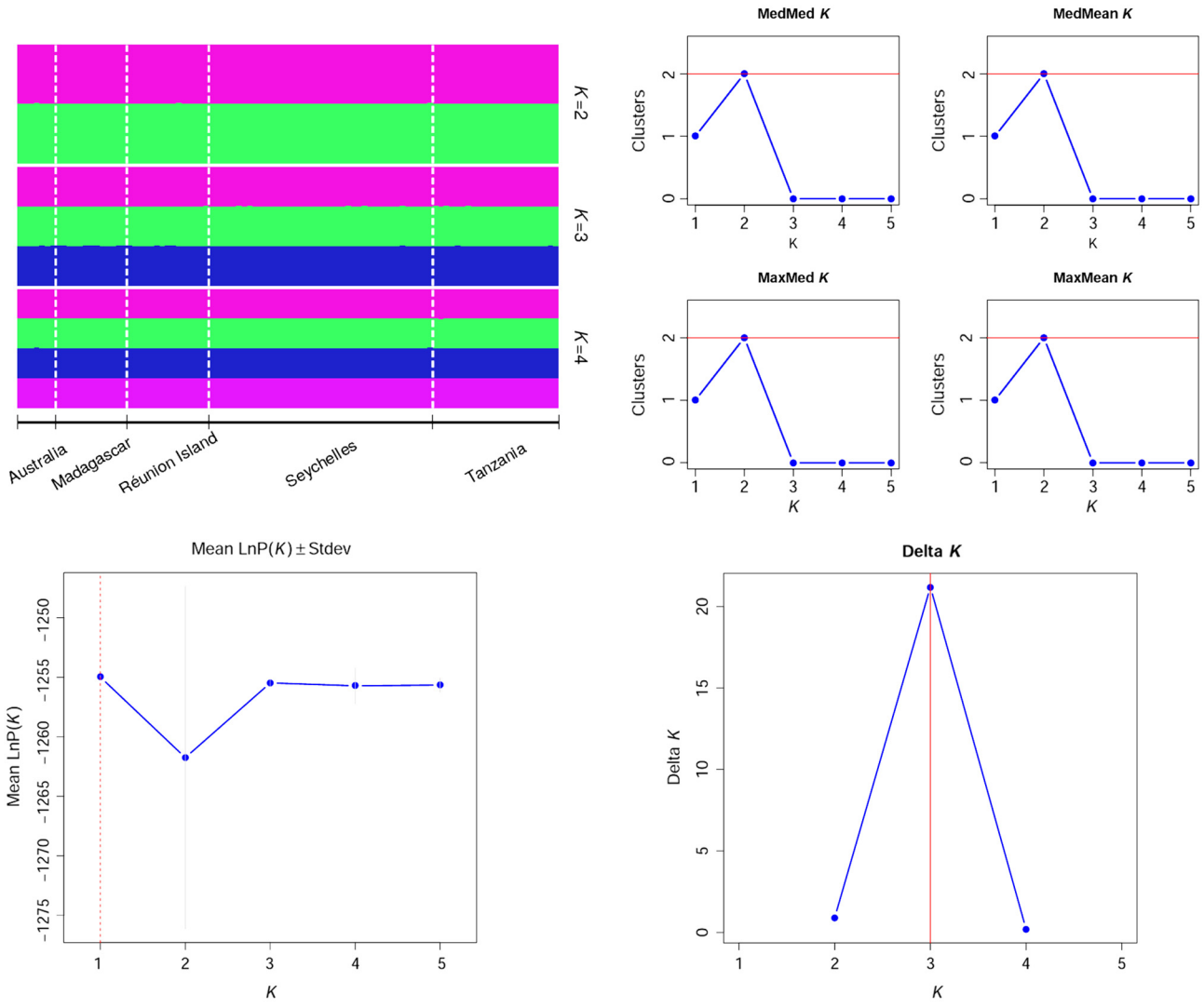

Fig. 4. Bayesian clustering assignments for *Rhynchobatus australiae* (K = 2–4) inferred by STRUCTURE based on the correlated allele frequency model, using 6 analytical methods to determine the most likely K: MedMed K, MedMean K, MaxMed K, MaxMean K (Puechmaille 2016), LnP(K) and Delta K (Evanno et al. 2005)

esian clustering analysis suggested a varying number of clusters in the *R. australiae* data set (K = 2–3) and the inferred ancestry coefficients showed no differentiation, which indicates high levels of gene flow between *R. australiae* sampling locations (Fig. 4). The STRUCTURE assignment plot for *R. djiddensis* is shown in the Supplementary material, demonstrating genetic homogeneity in the metapopulation (Fig. S3). A clear separation of species was evident; however, 1 *R. australiae* individual from Seychelles appears to have shared ancestry with *R. djiddensis* (Fig. S4).

Lastly, POWSIM analysis based on the proportion of significant chi-squared tests indicated a relatively low probability (36.4%) of detecting an F_{ST} value of as low as 0.005 using the microsatellite data set.

4. DISCUSSION

4.1. Molecular taxonomy

The application of molecular markers proved to be successful for the confirmation of morphological specimen identity within the whitespotted wedgefish species complex in the SWIO region. The correct identification of specimens at the species level is fundamental to effective management, as biological parameters, and therefore vulnerabilities, can vary widely, even between closely related taxa that are similar in appearance and overlapping in distribution (Moore 2017). If biological data from multiple species are mistakenly treated as being representative of a

single species, relevant estimates such as population size may be falsely inflated, and the species' threat level underestimated. This is particularly important amongst *Rhynchobatus* species which exhibit wide inter- and intraspecific variation in morphology and colouration as well as a general lack of baseline life history information (Moore 2017, Cliff & Daly 2022). Whole mitochondrial genomes of *R. djiddensis* and *R. australiae* from the SWIO were recently made available (Groeneveld et al. 2023), serving as another molecular resource for comparing individual mitochondrial gene regions for species identification. These authors also found that *ND2* was the most variable in terms of number of single nucleotide polymorphisms (SNPs), and Giles et al. (2016) showed that *R. australiae* can be reliably differentiated from other Indo-Pacific species using *ND2* and a section of *CR* short enough to amplify DNA from processed fins. In our study, we employed *ND2* in conjunction with *COI*, which effectively distinguished between *R. djiddensis* and *R. australiae* for initial molecular identification. Since the barcoding gap for our data set was estimated to be 4.3%, the standard ~98% sequence similarity threshold was considered reliable species-level identification (Barbuto et al. 2010).

Ideally, all sequences accessible in BOLD or GenBank should originate from vouchered specimens initially identified by taxonomic experts. However, due to the lack of this expertise and the inherent nature of public databases, erroneous entries of sequence data are unavoidable (Meiklejohn et al. 2019). Mitochondrial identification of *Rhynchobatus* samples encounters challenges due to the lack of curated reference sequences, potentially compromising accurate discrimination between different *Rhynchobatus* species (Giles et al. 2016). Caution should be exercised in using currently described mtDNA markers for species-level identification of *Rhynchobatus* spp., and additional whole mitogenome sequencing may be required to resolve taxonomic uncertainties.

All subsequent analyses using *COI-CR* and 9 microsatellites, namely the mitochondrial haplotype network, *F*-statistics, multivariate and Bayesian clustering analyses, consistently supported the delineation of specimens into 2 distinct species, which was congruent with their morphological identifications. One *R. australiae* specimen from Seychelles displayed ancestral genetic similarity to *R. djiddensis* in the combined Bayesian analysis (Fig. S4). The presence of individuals that display genetic characteristics from a different species or a common ancestry could indicate DNA introgression (e.g. Walter et al. 2017). However, this individual resides in the *R. australiae* clade under H6 in the haplotype network, while barcoding verified it as *R. australiae*, making introgression or misidentification unlikely. The most probable explanation is that this observation is an artefact of low marker polymorphism, where one unique allele can influence the results significantly. Additionally, when analysing the STRUCTURE results separately (Fig. 4), this individual does not show divergent ancestry from the other *R. australiae* samples.

4.2. Population structure

Our analyses suggest that the *R. djiddensis* populations sampled from South Africa (Durban and Sodwana Bay) and southern Mozambique are genetically homogeneous. The lack of genetic differentiation observed demonstrates that these 2 locations have a high degree of population connectivity and gene flow. Regarding *R. australiae*, the relatively high levels of genetic differentiation based on the mtDNA data set and the weak population structure based on the microsatellite data set indicate varying levels of genetic differentiation within *R. australiae*. Bayesian analyses overall suggested a lack of population structure, while the *F*-statistics and multivariate analyses demonstrated weak but significant differentiation. This may indicate reduced connectivity between some locations, as only those populations that are found adjacent to one another reflect shared genetic ancestry. The varying results obtained with the different population analysis approaches were not unexpected, as these methods differ in sensitivity to small sample sizes and subtle variation. Generally genetic differentiation among marine populations is both expected and observed to be low, thus it becomes more difficult to discriminate a signal of heterogeneity from limited sampling or lack of variation in the markers used (Ward 2000). The microsatellite markers used in the current study are inherently of low informativeness (global PIC = 0.431) and do not have the power to detect subtle differences in allele frequencies (power analysis = 36.4%), which must therefore be considered when interpreting the findings. There is a difference between evidence of panmixia versus the absence of evidence of population structure (i.e. lack of power) (Palm et al. 2009, Bailleul et al. 2018). Failing to detect genetic structure can be detrimental to a population. Thus, when taking all results into account, i.e. the mtDNA estimates demonstrating high differentiation and the nuclear DNA showing some evidence of differentiation despite lack of marker variability, we reject the hypothesis of

genetic homogeneity among the *R. australiae* sampling localities.

When different populations arise with limited connectivity between them, often driven by habitat features (Saitoh 2021), they can suffer a reduction in the level of genetic diversity which can be associated with range contraction and fragmentation of former distributions (Kenchington 2003). Coastal elasmobranchs, such as wedgefishes, are generally characterised by lower levels of diversity compared to highly mobile pelagic species that are capable of large-scale migration, enabling populations to remain connected across ocean basins (Ovenden 2013). Both *R. djiddensis* and *R. australiae* have tropical neritic habitats, thus the separation of these territories by deep oceanic waters and associated ocean currents, habitat discontinuities or temperature gradients likely constitute physical barriers to gene flow. This could explain the lower level of differentiation observed between geographically proximate populations, such as *R. djiddensis* from Mozambique and South Africa. Jordaan et al. (2021) further suggested that larger, reproductively active individuals move northwards to warmer waters in neighbouring Mozambique for reproductive purposes during winter in KwaZulu-Natal. This again highlights the connectivity between these 2 localities and the sensitivity of wedgefishes to temperature.

In contrast, *R. australiae* locations are more heterogeneous. Previous studies also reported genetic differentiation among *R. australiae* populations from the WIO, WP, Australia (Simwanza & Rumisha 2023) and East Indonesia (Tapilatu et al. 2023), as well as between Southeast Asia and Australia (Giles et al. 2016). We found that SWIO populations differ greatly from Australia, but within the SWIO, there might be more fine-scale population structure among Madagascar, Réunion Island, Seychelles and Tanzania but not between Madagascar and Tanzania ($\Phi_{ST} = 0.0932$, $F_{ST} = 0$, $p > 0.128$). The detection of population structure can imply that *R. australiae* may be susceptible to regional adaptation and location-specific selection pressures. Populations that occur at island locations (Madagascar, Réunion Island and Seychelles), or those that are geographically far apart (Australia and SWIO), are exposed to varying environmental selection pressures. Over time, these populations may develop genetic variations that increase their fitness and survival within their local habitat (Gregory 2009). The potential inability of the species to migrate across vast ocean expanses may also contribute to the genetic differentiation. While there is a general scarcity of data regarding its migratory behaviours, recent findings suggest episodic migration of *R. australiae* between Indonesia and Australia (Giles et al. 2016). Comparable migrations are likely happening across the species' range, particularly among neighbouring countries. Despite its high mobility, this species is rarely found below depths of 60 m and is predominantly associated with coastal habitats (Last et al. 2016); thus, deep-water barriers can impose constraints on its dispersal. From an evolutionary perspective, these factors influence the future trajectory of these populations, as they will likely not interbreed with other, more geographically distant *R. australiae* populations. This can lead to the fixation of alleles, ultimately driving divergence with restricted gene flow between smaller subpopulations. While there is a significant expanse of deep water between Madagascar and Tanzania, similar to the distance between Madagascar and Réunion Island, the genetic similarity observed may be attributed to historical connectivity, relatively recent colonisation events or similar selective pressures and environmental conditions.

Multiple types of molecular markers and additional samples from the SWIO are necessary to assist in establishing the effects of drift, mutation, isolation by distance and selection, and will enable better understanding of stock structure than any single genetic approach (Ward 2000).

4.3. Mitochondrial and nuclear diversity

Regarding mitochondrial diversity, a high level of haplotype diversity and low level of nucleotide diversity were observed, with this pattern comparable to other batoids (Li et al. 2015, Cruz et al. 2021). The *R. djiddensis* populations from both South Africa and Mozambique displayed notably low diversity, as did the *R. australiae* population from Seychelles, despite the latter having the largest sample size. These regions might host higher-risk populations as intraspecific genetic diversity is essential for long-term population persistence (Hoffman et al. 2014). It can also be indicative of restricted gene flow, which results in fragmented populations and reduced ability to adapt to changing environments (Chichorro et al. 2019). Isolated populations without substantial reproductive connectivity cannot reverse declining abundance through immigration and are therefore more vulnerable than connected populations (Patterson et al. 2022).

Despite consisting of multiple locations, our *COI* diversity estimates for SWIO *R. australiae* ($H = 2$, $h = 0.296$, $\pi = 0.0016$) (Table S4) was slightly lower than Australia ($H = 2$, $h = 0.389$, $\pi = 0.00139$). These esti-

mates are higher than WIO *R. australiae* from Simwanza & Rumisha (2023) (H = 2, h = 0.0778, π = 0.0004) but follow a similar trend where *R. australiae* from the EIO and WP had much higher levels of genetic diversity than the WIO populations. Our *CR* diversity estimates for SWIO *R. australiae* (H = 11, h = 0.384, π = 0.0029) are also much lower than *R. australiae* from Southeast Asia and Australia, Southeast of Timor Passage (H = 22, h = 0.707, π = 0.005) (Giles et al. 2016). *R. australiae* populations from the WIO region thus seem to be less diverse than other biogeographic regions. Additionally, all 3 haplotype networks (Fig. 2; Fig. S2) exhibited concordant relationships among the haplotypes of *R. australiae* from the above-mentioned studies, with slightly more mutational steps. This demonstrates that WIO populations differ from more Eastern populations (e.g. Australia). We hypothesise that this difference is mainly driven by substantial marine barriers preventing gene flow (Hirschfeld et al. 2021), but could be caused by multiple factors such as historical demography (Gubili et al. 2014), colonisation history (Hellberg 2009), environmental adaptation (Delaval et al. 2022) and ecological interaction (Pyron & Burbrink 2010).

Furthermore, the low level of nuclear diversity was not reflected in the mtDNA. The mito-nuclear discordance might indicate that different evolutionary forces are shaping the genetic variation at mitochondrial and nuclear loci. Nuclear DNA is bi-parentally inherited and mtDNA is matrilineally inherited, which makes mtDNA more susceptible to selection and other demographic processes (Dudgeon et al. 2012). Sex-biased dispersal is one such possibility, which can result in different gene frequencies between sexes within and among populations or subpopulations (Prout 1981). Although evidence for sex-biased dispersal in batoids has previously and increasingly been reported (Roycroft et al. 2019), including in species complexes, few studies have provided reliable evidence of this phenomenon (Flowers et al. 2016, Phillips et al. 2021). A passive acoustic telemetry study of *Rhynchobatus* spp. in a northern Australian embayment (White et al. 2014) reported no significant difference in philopatric behaviour (e.g. seasonal residency and site fidelity) between sexes, which may suggest that they are not philopatric to specific nearshore areas but rather move between them. However, the lack of correlation between *Rhynchobatus* spp. size and residency may be a result of the occurrence of 3 possible species, and the low number of males (n = 2) compared to females (n = 18) monitored may render the results of that study inconclusive. Thus, currently there is no research available supporting sex-biased dispersal in the genus *Rhynchobatus*.

In the current study, it is possible that the lack of microsatellite marker polymorphism could have influenced our results; thus, the mitochondrial estimates were considered to be more informative (Dudgeon et al. 2012). Further research on possible mito-nuclear discordance is needed, as elasmobranch reproductive strategies such as philopatry (Chapman et al. 2004) strongly determine spatial scale of management. Combining data from full mitochondrial genomes and a large number of nuclear markers (SNPs or microsatellites), employing a close-kin mark–recapture framework and sex-chromosome markers, and the use of collaborative sample-sharing platforms could address the challenges posed by low polymorphism and enhance our understanding of elasmobranch structuring patterns (Phillips et al. 2021).

4.4. Management implications

The distribution ranges of *R. djiddensis* and *R. australiae* have not been well defined due to confusion with other members of the species complex. Both species appear to have a disjunct distribution in the WIO. *R. djiddensis* was described in the Red Sea off Saudi Arabia and Yemen (Ebert et al. 2021), while *R. australiae* was described off the Manning River mouth, New South Wales, Australia. The current recognised distribution of *R. djiddensis* spans from the Red Sea to the Arabian/Persian Gulf, and off southern Mozambique and South Africa (Kyne et al. 2019a, Ebert et al. 2021), whereas *R. australiae* is found from Kenya to Mozambique, off Madagascar, and other WIO islands (Kyne et al. 2019b). The results from the present study confirm the distribution ranges for these species based on molecular data. Such information is important for fishery management and conservation because the geographic distribution of marine species is thought to affect extinction risk (Moore 2017). *R. djiddensis* was previously considered to be widespread throughout the IWP (Kyne et al. 2020), but this was likely based on the combined distribution patterns of several closely related *Rhynchobatus* species. Our samples from South Africa (Durban and Sodwana Bay) and southern Mozambique were confirmed to be *R. djiddensis*, with no samples from elsewhere in the SWIO identified as *R. djiddensis*. *R. djiddensis* is also officially listed in the Fish List of Réunion Island (Fricke et al. 2009), but these results, taken together with those of Jaquemet et al. (2023), indicate that *R. australiae* may be the only wedgefish

present off Réunion. Environmental DNA (Mariani et al. 2021) also supports the presence of only 1 species, and historically, no 2 species of *Rhynchobatus* have been recorded there. *R. djiddensis* thus seems to be restricted to discrete pockets within the WIO, with one pocket from South Africa to Mozambique and another from Oman into the Arabian Gulf (Last et al. 2016, Kyne et al. 2020).

Given that no genetic differentiation was observed in our *R. djiddensis* data set, South Africa and Mozambique can be considered as a single management unit. The frequency and extent of transboundary movements between South Africa and neighbouring Mozambique remains largely unknown, but *R. djiddensis* appears to move northwards as water temperatures cool with the onset of winter (Dunlop & Mann 2013, Jordaan et al. 2021). Their movement patterns in this area are therefore currently being investigated in greater detail using passive acoustic telemetry (R. Daly pers. comm.). *R. djiddensis* likely relies on nearshore habitats for pupping and mating, like the Inhambane Estuary in Mozambique (R. H. Bennett pers. obs.), but the multiple-sector fishery in Mozambique (and potentially other areas in the WIO) remains a threat to the conservation of this species (Daly et al. 2021). Because these sampling localities are genetically mixing, the inshore marine protected areas on the east coast of South Africa could provide some protection for the species as a whole (Cliff & Daly 2022). However, the potential fishery-induced reduction in abundance of the species in the SWIO (Kyne et al. 2020, Daly et al. 2021) should be closely monitored; if it is confirmed that *R. djiddensis* uses these specific nearshore habitats for reproduction or as nursery areas, localised effects in Mozambique could also have unfavourable consequences for the South African population (Daly et al. 2021).

Species with smaller distribution ranges, like *R. djiddensis*, are usually less resilient than others (Chichorro et al. 2019), and this is reflected in the low genetic diversity estimates. Thus, species with larger habitat ranges have greater resilience against multiple pressures due to greater dispersal ability (Chichorro et al. 2019). By comparison, *R. australiae* is widespread in the IWP from Mozambique through the WIO, the Arabian Sea, Southeast Asia, extending north to Taiwan, south to Australia and east to the Solomon Islands (Last et al. 2016, Kyne et al. 2019b). Our results support the widespread distribution of *R. australiae* in the SWIO, which may provide an additional buffer against extinction risk (Roberts & Hawkins 1999, Chichorro et al. 2019). In contrast, a recent alternative view is that the need for coherent international management often leads to failure of risk mitigation (Dulvy et al. 2014). If the species range is predominantly in one country, that country may make a concerted effort towards improved conservation of that species. If the range extends across multiple countries, no specific country takes ownership of the need to better manage the species. As such, the identification of appropriate management units for *R. australiae* is essential. Fishing pressure is considerably lower in northern Australia compared to other regions in the IWP, attributed to effective management regimes (Kyne et al. 2020). Nonetheless, this proportion of *R. australiae*'s range is not considered to be large enough to warrant a revision of their global status of Critically Endangered (Kyne et al. 2020). The lack of genetic connectivity further prevents other locations from benefiting from Australia's successful approach. Populations across the IWP should be managed as 3 different genetic stocks: WIO, WP and Australia (Simwanza & Rumisha 2023), and thus one distinct population such as Australia cannot replenish another such as WIO. Our study corroborates that Australia and WIO populations are genetically different, but within the SWIO, we found that there might be more fine-scale population structure. For this reason, it will be more cautionary to consider all sampling locations as separate managements units, except for Madagascar and Tanzania. Management efforts should be tailored to the needs of these isolated populations, and collaboration across regions is vital to conserve the meta-population of *R. australiae*.

Moreover, 1 specimen from Mozambique was molecularly identified as *R. australiae*. As *R. djiddensis* and *R. australiae* are confirmed present in Mozambique, this appears to be an important area of overlap of the distribution ranges of the 2 species. However, due to a lack of accurate location information on the single *R. australiae* and 2 of the *R. djiddensis* samples, limited conclusions can be drawn regarding the distribution of these 2 species in this region and potential genetic admixture. *R. australiae* has been observed at Ponta Zavora (B. Q. Mann pers. obs.), suggesting that this may be close to their southern distribution limit, but further sampling, especially in northern and central Mozambique, would provide considerably better resolution on the sympatry of these species.

5. CONCLUDING REMARKS

Here we demonstrated that *Rhynchobatus djiddensis* and *R. australiae* are genetically distinguishable based on a limited number of molecular markers.

Their distribution patterns were refined by confirming that our samples from southern Mozambique and South Africa are *R. djiddensis*, whereas those from Australia, Réunion Island, Madagascar, Seychelles and Tanzania are *R. australiae*. Preliminary population genetic analyses revealed potential mito-nuclear discordance and indicated some population differentiation for *R. australiae*, while *R. djiddensis* was homogeneous.

A combination of intensive fisheries, inadequate fisheries management measures and poor law enforcement commonly makes species conservation even more challenging in Great South countries (Moore 2017), as is the case for *R. djiddensis* and *R. australiae* in the SWIO region. Given their status as Critically Endangered and escalating fishing pressure globally, careful management of all populations across their entire distribution is warranted to preserve the genetic diversity of both species. However, conservation priority should be given to the SWIO region, especially the populations of *R. djiddensis*, and to *R. australiae* from Seychelles. Nevertheless, a cross-disciplinary approach using a combination of more direct methods (such as movement studies using telemetry or mark–recapture) and different genetic techniques are necessary to thoroughly understand the species distribution and processes that shape genetic composition and vulnerability, such as philopatry. Both traditional morphological taxonomy and molecular-based identification methods are needed to fully resolve the whitespotted wedgefish complex.

Data availability. The sequence data that support the findings of this study are openly available in NCBI GenBank (https://www.ncbi.nlm.nih.gov/) under accession numbers OQ221167–OQ221503.

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LITERATURE CITED

Aisyah S, Santia N, Supratman O, Syarif AF, Anggraeni A (2021) DNA barcoding and CITES-listed wedgefish (Rhynchobatidae, Rhinidae) from South Bangka, Indonesia. J Kelautan Trop 24:232–236

Bailleul D, Mackenzie A, Sacchi O, Poisson F, Bierne N, Arnaud-Haond S (2018) Large-scale genetic panmixia in the blue shark (*Prionace glauca*): a single worldwide population, or a genetic lag-time effect of the 'grey zone' of differentiation? Evol Appl 11:614–630

Barbuto M, Galimberti A, Ferri E, Labra M, Malandra R, Galli P, Casiraghi M (2010) DNA barcoding reveals fraudulent substitutions in shark seafood products: the Italian case of 'palombo' (*Mustelus* spp.). Food Res Int 43:376–381

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW (2013) GenBank. Nucleic Acids Res 41:D36–D42

Boon PY (2017) The shark and ray trade in Singapore. TRAFFIC Southeast Asia Regional Office, Petaling Jaya, Selangor

Carvalho GR, Hauser L (1994) Molecular genetics and the stock concept in fisheries. Rev Fish Biol Fish 4:326–350

Catalano G, Crobe V, Ferrari A, Baino R and others (2022) Strongly structured populations and reproductive habitat fragmentation increase the vulnerability of the Mediterranean starry ray *Raja asterias* (Elasmobranchii, Rajidae). Aquat Conserv 32:66–84

Chabot CL, Allen LG (2009) Global population structure of the tope (*Galeorhinus galeus*) inferred by mitochondrial control region sequence data. Mol Ecol 18:545–552

Chapman DD, Prodöhl PA, Gelsleichter J, Manire CA, Shivji MS (2004) Predominance of genetic monogamy by females in a hammerhead shark, *Sphyrna tiburo*: implications for shark conservation. Mol Ecol 13:1965–1974

Chichorro F, Juslén A, Cardoso P (2019) A review of the relation between species traits and extinction risk. Biol Conserv 237:220–229

Choo MY, Choy CPP, Ip YCA, Rao M, Huang D (2021) Diversity and origins of giant guitarfish and wedgefish products in Singapore. Aquat Conserv 31:1636–1649

Clark LV, Jasieniuk M (2011) Polysat: an R package for poly-

ploid microsatellite analysis. Mol Ecol Resour 11: 562–566

Cliff G, Daly R (2022) *Rhynchobatus djiddensis*. In: Cliff G, Olbers J (eds) Species profiles of South African sharks, rays and chimaeras. Spec Publ 2. WILDTRUST, Durban, p 361–367

Cruz VP, Adachi AMCL, Oliveira PH, Ribeiro GS and others (2021) Genetic diversity in two threatened species of guitarfish (Elasmobranchii: Rhinobatidae) from the Brazilian and Argentinian coasts: an alert for conservation. Neotrop Ichthyol 19:e210012

Daly R, Parker D, Cliff G, Jordaan GL, Nomfundo N, Bennett RH, Mann BQ (2021) Long-term catch trends and risk assessment of the Critically Endangered white-spotted wedgefish (*Rhynchobatus djiddensis*) from South Africa. Aquat Conserv 31:777–788

Delaval A, Frost M, Bendall V, Hetherington SJ and others (2022) Population and seascape genomics of a critically endangered benthic elasmobranch, the blue skate *Dipturus batis*. Evol Appl 15:78–94

Dent F, Clarke S (2015) State of the global market for shark products. FAO Fish Aquacult Tech Pap 590. FAO, Rome

Dudgeon CL, Blower DC, Broderick D, Giles JL and others (2012) A review of the application of molecular genetics for fisheries management and conservation of sharks and rays. J Fish Biol 80:1789–1843

Dulvy NK, Fowler SL, Musick JA, Cavanagh RD and others (2014) Extinction risk and conservation of the world's sharks and rays. eLife 3:e00590

Dulvy NK, Pacoureau N, Rigby CL, Pollom RA and others (2021) Overfishing drives over one-third of all sharks and rays toward a global extinction crisis. Curr Biol 31: 4773–4787.e8

Dunlop SW, Mann BQ (2013) Giant guitarfish (*Rhynchobatus djiddensis*). In: Mann BQ (ed) Southern African marine linefish species profiles. Oceanographic Research Institute Spec Publ 9. South African Association for Marine Biological Research, Durban, p 149–151

Ebert DA, Wintner SP, Kyne PM (2021) An annotated checklist of the chondrichthyans of South Africa. Zootaxa 4947: 1–127

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Excoffier L, Luscher H (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10: 564–567

Feldheim KA, Gruber SH, Ashley MV (2001) Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. Mol Ecol 10:295–303

Flowers KI, Ajemian MJ, Bassos-Hull K, Feldheim KA, Hueter RE, Papastamatiou YP, Chapman DD (2016) A review of batoid philopatry, with implications for future research and population management. Mar Ecol Prog Ser 562:251–261

Francis RM (2017) Pophelper: an R package and web app to analyse and visualize population structure. Mol Ecol Resour 17:27–32

Fricke R, Mulochau T, Durville P, Chabanet P, Tessier E, Letourneur Y (2009) Annotated checklist of the fish species (Pisces) of La Réunion, including a Red List of threatened and declining species. Stuttg Beitr Natkd Ser A (Biol) 2:1–168

Funk WC, McKay JK, Hohenlohe PA, Allendorf FW (2012) Harnessing genomics for delineating conservation units. Trends Ecol Evol 27:489–496

Giles JL, Riginos C, Naylor GJP, Dharmadi, Ovenden JR (2016) Genetic and phenotypic diversity in the wedgefish *Rhynchobatus australiae*, a threatened ray of high value in the shark fin trade. Mar Ecol Prog Ser 548:165–180

Goudet J (2005) Hierfstat, a package for R to compute and test hierarchical *F*-statistics. Mol Ecol Notes 5:184–186

Gregory TR (2009) Understanding natural selection: essential concepts and common misconceptions. Evolution 2: 156–175

Groeneveld MJ, Klein JD, Bennett RH, Bester-van der Merwe AE (2023) Characterization of the complete mitochondrial genomes of two Critically Endangered wedgefishes: *Rhynchobatus djiddensis* and *Rhynchobatus australiae*. Mitochondrial DNA B Resour 8:352–358

Gubili C, Sims DW, Veríssimo A, Domenici P and others (2014) A tale of two seas: contrasting patterns of population structure in the small-spotted catshark across Europe. R Soc Open Sci 1:140175

Hau CY, Abercrombie DL, Ho KYK, Shea KHS (2018) 'King of shark fins' not quite sharks...so what is in my shark fin soup? A rapid survey on the availability of shark-like batoid fins in Hong Kong SAR and Guangzhou, China retail markets. BLOOM Hong Kong Shark Foundation, https://www.bloomassociation.org/en/wp-content/uploads/2018/12/King-of-shark-fins-not-quite-sharks.pdf

Hellberg ME (2009) Gene flow and isolation among populations of marine animals. Annu Rev Ecol Evol Syst 40: 291–310

Hirschfeld M, Dudgeon C, Sheaves M, Barnett A, MacNeil A (2021) Barriers in a sea of elasmobranchs: from fishing for populations to testing hypotheses in population genetics. Glob Ecol Biogeogr 30:2147–2163

Hoffman JI, Simpson F, David P, Rijks JM and others (2014) High-throughput sequencing reveals inbreeding depression in a natural population. Proc Natl Acad Sci USA 111: 3775–3780

Hueter RE, Heupel MR, Heist EJ, Keeney DB (2004) Evidence of philopatry in sharks and implications for the management of shark fisheries. J Northwest Atl Fish Sci 35:239–247

IUCN (2022) The IUCN Red List of Threatened Species. Version 2022-2. https://www.iucnredlist.org (accessed 30 November 2022)

Jabado RW (2018) The fate of the most threatened order of elasmobranchs: shark-like batoids (Rhinopristiformes) in the Arabian Sea and adjacent waters. Fish Res 204: 448–457

Jabado RW (2019) Wedgefishes and giant guitarfishes: a guide to species identification. Wildlife Conservation Society, New York, NY

Jaquemet S, Oury N, Poirout T, Gadenne J, Magalon H, Gauthier A (2023) Elasmobranch diversity at Reunion Island (Western Indian Ocean) and catches by recreational fishers and a shark control program. Diversity 15: 768

Jombart T (2008) Adegenet: an R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405

Jordaan GL, Mann BQ, Daly R, Dunlop SW, Cowley PD (2021) Movement patterns and growth rate of the white-spotted wedgefish *Rhynchobatus djiddensis* in southern Africa based on tag-recapture data. Afr J Mar Sci 43: 201–213

Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2: e281

Kardos M (2021) Conservation genetics. Curr Biol 31: R1185–R1190

Karl SA, Castro ALF, Lopez JA, Charvet P, Burgess GH (2011) Phylogeography and conservation of the bull shark (*Carcharhinus leucas*) inferred from mitochondrial and microsatellite DNA. Conserv Genet 12:371–382

Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780

Kearse M, Moir R, Wilson A, Stones-Havas S and others (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649

Keenan K, McGinnity P, Cross TF, Crozier WW, Prodöhl PA (2013) DiveRsity: an R package for the estimation and exploration of population genetics parameters and their associated errors. Methods Ecol Evol 4:782–788

Keeney DB, Heupel MR, Hueter RE, Heist EJ (2005) Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. Mol Ecol 14:1911–1923

Kenchington EL (2003) The effects of fishing on species and genetic diversity. In: Sinclair M, Valdimarson G (eds) Responsible fisheries in the marine ecosystem. CABI Publishing, Wallingford, p 235–253

Kyne PM, Gledhill K, Jabado RW (2019a) Whitespotted wedgefish *Rhynchobatus djiddensis*. The IUCN Red List of Threatened Species 2019: e.T39394A121035795. https://dx.doi.org/10.2305/IUCN.UK.2019-2.RLTS.T39394A121035795.en

Kyne PM, Rigby CL, Dharmadi, Jabado RW (2019b) Bottlenose wedgefish *Rhynchobatus australiae*. The IUCN Red List of Threatened Species 2019: e.T41853A68643043. https://dx.doi.org/10.2305/IUCN.UK.2019-2.RLTS.T41853A68643043.en

Kyne PM, Jabado RW, Rigby CL, Dharmadi and others (2020) The thin edge of the wedge: extremely high extinction risk in wedgefishes and giant guitarfishes. Aquat Conserv 30:1337–1361

Last P, White W, de Carvalho M, Séret B, Stehmann B, Naylor G (2016) Rays of the world. CSIRO Publishing, Clayton

Leigh JW, Bryant D (2015) Popart: full-feature software for haplotype network construction. Methods Ecol Evol 6: 1110–1116

Li N, Chen X, Sun D, Song N, Lin Q, Gao T (2015) Phylogeography and population structure of the red stingray, *Dasyatis akajei* inferred by mitochondrial control region. Mitochondrial DNA 26:505–513

Li Y, Liu J (2018) STRUCTURESELECTOR: a web-based software to select and visualize the optimal number of clusters using multiple methods. Mol Ecol Resour 18: 176–177

Maduna SN, Rossouw C, da Silva C, Soekoe M, Bester-van der Merwe AE (2017) Species identification and comparative population genetics of four coastal houndsharks based on novel NGS-mined microsatellites. Ecol Evol 7: 1462–1486

Mariani S, Fernandez C, Baillie C, Magalon H, Jaquemet S (2021) Shark and ray diversity, abundance and temporal variation around an Indian Ocean Island, inferred by eDNA metabarcoding. Conserv Sci Pract 3:e407

Meiklejohn KA, Damaso N, Robertson JM (2019) Assessment of BOLD and GenBank — their accuracy and reliability for the identification of biological materials. PLOS ONE 14:e0217084

Moore ABM (2017) Are guitarfishes the next sawfishes? Extinction risk and an urgent call for conservation action. Endang Species Res 34:75–88

Moritz C (1994) Defining 'Evolutionarily Significant Units' for conservation. Trends Ecol Evol 9:373–375

Nakamura JN, Kuemlangan B (2020) Implementing the Convention on International Trade in Endangered Species of Wild Fauna and Flora through national fisheries legal frameworks: a study and a guide. Legal Guide No. 4. FAO, Rome

Naylor GJP, Caira JN, Jensen K, Rosana KAM, White WT, Last PR (2012) A DNA sequence-based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. Bull Am Mus Nat Hist 367:1–262

Ovenden J (1990) Mitochondrial DNA and marine stock assessment: a review. Mar Freshw Res 41:835–853

Ovenden JR (2013) Crinkles in connectivity: combining genetics and other types of biological data to estimate movement and interbreeding between populations. Mar Freshw Res 64:201

Palm S, Dannewitz J, Prestegaard T, Wickström H (2009) Panmixia in European eel revisited: no genetic difference between maturing adults from southern and northern Europe. Heredity 103:82–89

Paradis E (2010) Pegas: an R package for population genetics with an integrated-modular approach. Bioinformatics 26: 419–420

Pardini AT, Jones CS, Noble LR, Kreiser B and others (2001) Sex-biased dispersal of great white sharks. Nature 412: 139–140

Patterson TA, Hillary RM, Kyne PM, Pillans RD and others (2022) Rapid assessment of adult abundance and demographic connectivity from juvenile kin pairs in a critically endangered species. Sci Adv 8:eadd1679

Pavitt A, Malsch K, King E, Chevalier A, Kachelriess D, Vannuccini S, Friedman K (2021) CITES and the sea: trade in commercially exploited CITES-listed marine species. FAO Fish Aquacult Tech Pap 666. FAO, Rome

Phillips NM, Devloo-Delva F, McCall C, Daly-Engel TS (2021) Reviewing the genetic evidence for sex-biased dispersal in elasmobranchs. Rev Fish Biol Fish 31: 821–841

Pirog A, Ravigné V, Fontaine MC, Rieux A and others (2019) Population structure, connectivity, and demographic history of an apex marine predator, the bull shark *Carcharhinus leucas.* Ecol Evol 9:12980–13000

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Prout T (1981) A note on the island model with sex dependent migration. Theor Appl Genet 59:327–332

Puechmaille SJ (2016) The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Mol Ecol Resour 16:608–627

Pyron RA, Burbrink FT (2010) Hard and soft allopatry: physically and ecologically mediated modes of geographic speciation. J Biogeogr 37:2005–2015

Queiroz N, Humphries NE, Couto A, Vedor M and others (2019) Global spatial risk assessment of sharks under the footprint of fisheries. Nature 572:461–466

R Core Development Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Ratnasingham S, Hebert PDN (2007) BOLD: the Barcode of Life data system (www.barcodinglife.org). Mol Ecol Notes 7:355–364

Roberts CM, Hawkins JP (1999) Extinction risk in the sea. Trends Ecol Evol 14:241–246

Rousset F (2008) Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour 8:103–106

Roycroft EJ, Le Port A, Lavery SD (2019) Population structure and male-biased dispersal in the short-tail stingray *Bathytoshia brevicaudata* (Myliobatoidei: Dasyatidae). Conserv Genet 20:717–728

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol 34:3299–3302

Ryman N, Palm S (2006) POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. Mol Ecol Notes 6:600–602

Saitoh T (2021) High variation of mitochondrial DNA diversity as compared to nuclear microsatellites in mammalian populations. Ecol Res 36:206–220

Sambrook J, Russell D (2001) Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Simpfendorfer C, Heupel M (2004) Assessing habitat use and movement. In: Carrier JC, Musick JA, Heithaus MR (eds) Biology of sharks and their relatives. CRC Press, London, p 553–572

Simwanza RJ, Rumisha C (2023) Evidence of distinct genetic stocks of the bottlenose wedgefish (*Rhynchobatus australiae*) in the Indo-West Pacific. Tanzan J Sci 49:86–95

Sukumaran S, Sebastian W, Zacharia PU, Kizhakudan SJ and others (2023) Population genetic research on the spadenose shark, *Scoliodon laticaudus* (Chondrichthyes, Carcharhinidae), reveals the presence of two significantly differentiated clades along the Indian coast. Mar Biodivers 53:16

Tapilatu ME, Wijatanti DP, Subagiyo, Sembering A and others (2023) Genetic diversity of wedgefishes and guitarfishes at landing sites in east Indonesia using Cytochrome Subunit I (COI). Biodiversitas 24:3120–3127

Thia JA (2023) Guidelines for standardizing the application of discriminant analysis of principal components to genotype data. Mol Ecol Resour 23:523–538

Tillett BJ, Meekan MG, Field IC, Thorburn DC, Ovenden JR (2012) Evidence for reproductive philopatry in the bull shark *Carcharhinus leucas.* J Fish Biol 80:2140–2158

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Vignaud T, Clua E, Mourier J, Maynard J, Planes S (2013) Microsatellite analyses of blacktip reef sharks (*Carcharhinus melanopterus*) in a fragmented environment show structured clusters. PLOS ONE 8:e61067

Walter RP, Roy D, Hussey NE, Stelbrink B and others (2017) Origins of the Greenland shark (*Somniosus microcephalus*): impacts of ice-olation and introgression. Ecol Evol 7:8113–8125

Ward RD (2000) Genetics in fisheries management. Hydrobiologia 420:191–201

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. Philos Trans R Soc B 360:1847–1857

Ward RD, Holmes BH, White WT, Last PR (2008) DNA barcoding Australasian chondrichthyans: results and potential uses in conservation. Mar Freshw Res 59:57–71

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38: 1358–1370

White J, Simpfendorfer CA, Tobin AJ, Heupel MR (2014) Spatial ecology of shark-like batoids in a large coastal embayment. Environ Biol Fishes 97:773–786

Wright S (1931) Evolution in mendelian populations. Genetics 16:97–159

Zhang A, Hao M, Yang C, Shi Z (2017) BarcodingR: an integrated R package for species identification using DNA barcodes. Methods Ecol Evol 8:627–634

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