GOVERNMENT OF WESTERN AUSTRALIA

Department of **Biodiversity, Conservation and Attractions**

Are you from my mob (mummagul)?

Genetic connectivity of Australian snubfin dolphins (Orcaella heinsohni) between Prince Regent River and other Western Australian sites

Version: 1.1 Approved by:

Last Updated: 10 July 2021

Custodian: Review date:

Version number	Date approved DD/MM/YYYY	Approved by	Brief Description
1.1			

Biodiversity and Conservation Science

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Genetic connectivity of Australian snubfin dolphins (*Orcaella heinsohni*) between Prince Regent River and other Western Australian sites

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December 2021

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December 2021

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The recommended reference for this publication is:

Department Biodiversity, Conservation and Attractions, 2021, Genetic connectivity of Australian snubfin dolphins (Orcaella heinsohni) between Prince Regent River and other Western Australian sites, Department of Biodiversity, Conservation and Attractions, Perth.

This document is available in alternative formats on request.

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Acknowledgments

Data collection was assisted by Nyamba Buru Yawuru Country Managers (Roebuck Bay), Bardi-Jawi Rangers (Cygnet Bay), Dambeemangadee Rangers (Cone Bay, Prince Regent River), Balanggarra Rangers (Cambridge Gulf), Western and Eastern Kimberley Parks and Wildlife Rangers, the crew of PV Worndoom, and numerous volunteer field assistants. Special thanks to Danny Barrow, Ellen D'Cruz, Adrian Lane, Amon Jungine, Anthony Richardson (Richo), Brett Francis and Michael Hourn. Janet Oobagooma and Inga Pederson were generous in sharing their knowledge and sea country. Alexis Levengood, Bethan Littleford-Colquhoun and Celine Frère (University of the Sunshine Coast) contributed to the analysis of genetic samples collected under the WAMSI project Brown et al. (2017). Kym Ottewell and Shelley McArthur kindly assisted with laboratory analyses and interpretation.

Non-technical Summary

DNA stands for deoxyribonucleic acid and is the hereditary material or genetic code found in the cells of humans and most living organisms.

When animals (including humans) breed the parent's DNA is passed on to their offspring (one copy from each parent), for dolphins this is to the calves.

Mitochondrial DNA and microsatellite markers are two of the most common tools used to understand genetic population structure, dispersal of genes, and relatedness. Mitochondrial DNA (mtDNA) is maternally inherited, i.e., only from the mother to offspring/calf and evolves slowly over time (i.e., many generations can pass before changes occur). A haplotype is a specific region of this mitochondrial DNA that clusters with other mitochondrial sequences to show the origins of maternal lineages and show up during laboratory analyses.

Microsatellite markers are parts of DNA found in the genome (the genome is the complete set of genes and genetic instructions in an animal) and the microsatellite part of the DNA is bi-parentally inherited, i.e., from both parents to the offspring/calf and evolution or changes can occur more rapidly between generations.

Given the difference in the way it is inherited and speed of evolution, mitochondrial or microsatellite analyses give different levels of information and can be used for slightly different questions. Mitochondrial is the most basic as it is directly inherited from the mother with little change over time. It is useful for tracking maternal lineages. Microsatellites give more information as they are inherited from both parents and can have a lot of variation. They are useful for looking at relatedness, parentage and more recent fine-scale population structure, including contemporary gene flow between populations.

In this study we are trying to understand if snubfin dolphins seen in different places across the Kimberley are related to each other. We want to know if snubfins move between populations now, or if they have done so in the past. This can be done using their DNA as a clue because when they breed their genetics are passed on to their offspring.

This will help us make management decisions about any threats or pressures to snubfins in different places, in particular how important these pressures are to a local population or to all snubfins across the Kimberley. For example, if snubfins are isolated in an area and not breeding with others outside of their area this makes them very vulnerable to local extinction.

We collected 8 tissue samples from snubfins, using a dart gun in Prince Regent River in 2019 and 2020. Here we compare these samples with samples collected from snubfins by others in the past in Roebuck Bay, Cygnet Bay, Cone Bay and Yampi Sound.

The Prince Regent River snubfin dolphin DNA samples showed two haplotypes, one that was unique to Prince Regent River 'haplotype 1' and another that was also seen

in animals from Yawuru sea country (Roebuck Bay) and other sites in between ‘haplotype 2’, suggesting some ancestral relationship between Yawuru and Dambeemangadee snubfin dolphins. The Figure below shows the different haplotypes found at the four locations. Some haplotypes found at Roebuck, Cone and Cygnet Bays (6 and 7) were not among Prince Regent River samples suggesting that the Prince Regent River dolphins are more distantly related to the populations further west or that the DNA of snubfin dolphins has evolved, potentially losing the haplotypes 6 and 7 over time (see Figure 2, the network diagram to visualise this). These haplotypes could still be present among individuals not yet sampled in Prince Regent River. However, up to 20 snubfins were observed in each of the annual surveys (2018-2020) and of these 8 individual snubfins were sampled which may be a representative sample if a small number of snubfins use the Prince Regent River.

The microsatellite analyses provide more detailed information on genetic connectivity and suggests that Prince Regent River is genetically different from the three other places (Roebuck Bay, Cygnet Bay and Cone Bay) even though this is over a relatively short distance within Dambeemangadee sea country. See Figure 3 for a visual representation of the genetic structure into separate genetic clusters by place with Prince Regent River samples assigned to a separate genetic cluster from samples of other places.

Microsatellite genetic diversity appears slightly lower in Prince Regent River than other places that were sampled in the Kimberley, except Cone Bay which shows a similar result. But fewer samples were collected from Prince Regent River so this result must be interpreted cautiously in case it’s a result of small sample size rather than a true reflection of low genetic diversity. MtDNA genetic diversity for Prince Regent River was like most other sites sampled.

It is possible to provide details about the occurrence of contemporary gene flow and its direction between sites through genetic analyses and for this study it appears that Prince Regent River may be a sink meaning that dolphins come from surrounding areas and breed with dolphins in Prince Regent River, but dolphins are not apparently leaving the Prince Regent River to breed or move to other places. Indeed, the genetic differentiation found between Prince Regent River samples and other places suggested some level of site faithfulness where dolphins stay in the area where they were born.

Using the DNA from the snubfin dolphins, we can work out their gender. Such information can be valuable to further investigate the sex-dispersal bias (whether males or females are more likely to leave the area where they were born to breed) that we suspect based on what we’ve found so far.

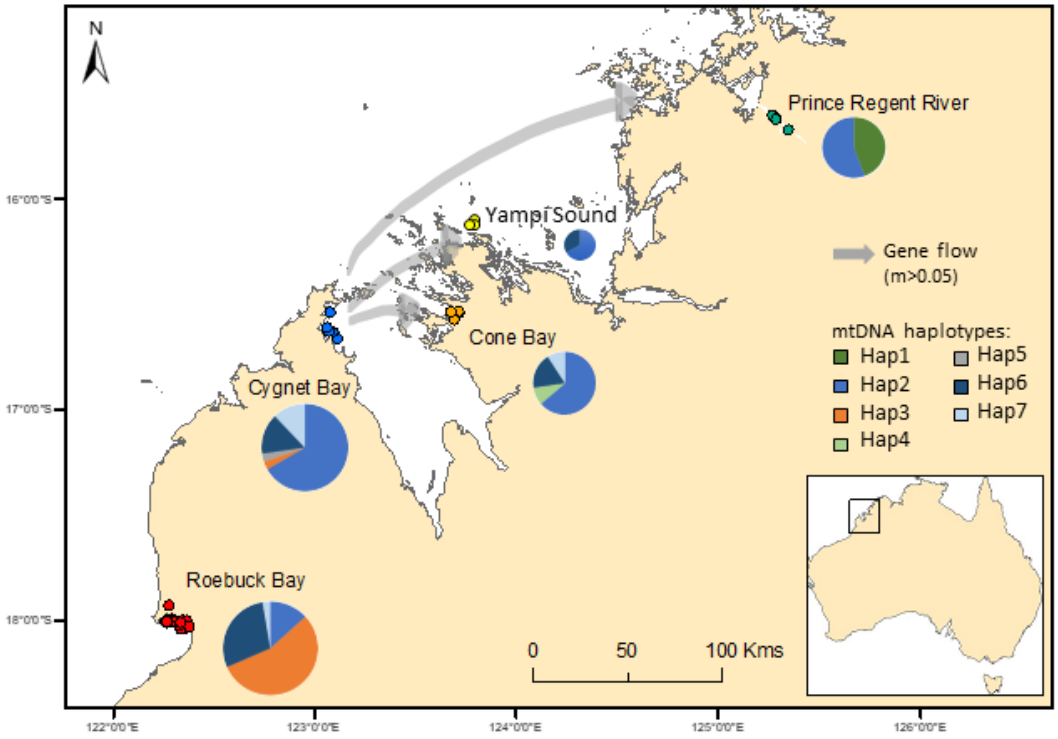
We have started to look at how related dolphins were to others from the same place or to other places in the Kimberley and some close relations were discovered (i.e., parents-offspring or full brothers/sisters (siblings)). Related individuals may bias the genetic analysis on population structure as it may reflect this family structure instead of the population if there are too many related individuals that have been sampled

from one place. We may need to do more analysis to work this out, such as by only using one individual from closely related pairs in our analyses.

Take home message:

Snubfin dolphins from Prince Regent River are not genetically isolated from other populations in the Kimberley. However, based on our limited samples it is likely that snubfin dolphins from Prince Regent River may stay in the place they are born, with gene flow limited to potential migration of snubfin dolphins from Cygnet Bay for breeding and/or staying with dolphins from Prince Regent River.

Therefore, snubfin dolphins from Prince Regent River should be regarded as a distinct management unit given the population is small, has limited connection with other snubfin populations in the Western Australian Kimberley region and is potentially vulnerable to pressures such as increased vessel traffic in the river



Interpretive map showing the places where snubfin dolphins were sampled, their mtDNA haplotypes (size of circle is proportional to the number of samples available) and gene flow and its direction (only showing gene flow that is moderate, m > 0.05).

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Introduction

The Australian snubfin dolphin (*Orcaella heinsohni*, 'snubfin dolphin' hereafter) is endemic to northern Australia and southern New Guinea (Beasley et al. 2005), with a distribution in Australian waters that is restricted to shallow coastal and estuarine waters of Western Australia, Northern Territory, and Queensland (Parra et al. 2002). Snubfin dolphins are classified as 'Vulnerable' in the *IUCN Red List of Threatened Species* (Parra et al. 2017) and considered Priority 4 (Rare, Near Threatened and other species in need of monitoring) by Western Australian State Government and formally protected in Australia under the *Environment Protection and Biodiversity Conservation Act 1999* and in Western Australia by the *Biodiversity Conservation Act 2016* (WA).

Understanding the population structure of a species, in particular genetic connectivity between populations is useful to inform management decisions at local and regional levels as well as assessing species' conservation status.

In this study, we provide information on the genetic connectivity of snubfin dolphins from the Prince Regent River, Dambeemangadee sea country, and to other sites in Western Australia's Kimberley region and for which preliminary analyses were previously conducted (Brown et al. 2014; Brown et al. 2017).

Materials and Methods

Sampling

Skin and blubber samples from non-calf snubfin dolphins were collected along the Kimberley coast of north-western Australia through several dolphin research projects conducted from 2014 to 2019, and covering five different sites: Roebuck Bay, Cygnet Bay, Cone Bay, Yampi Sound and Prince Regent River (Figure 1). Tissue samples were collected using either the DANinject or the PAXARMS remote biopsy system, the latter specifically designed for small cetaceans (Krützen et al. 2002). Once collected samples were stored in either 100% ethanol or saturated NaCl/20%

dimethyl sulfoxide (Amos and Hoelzel 1991) and, where possible, kept frozen until the time of analysis. Although samples obtained prior to 2017 have already been analysed (see Brown et al. 2017), eight new samples were collected in Prince Regent River between 2019 and 2020, providing further genetic connectivity information not previously available for this site. Given that only three samples were available for Yampi Sound (and only two of these samples could be used for microsatellite loci), we did not use this site for further analyses, except to identify the mtDNA haplotypes.

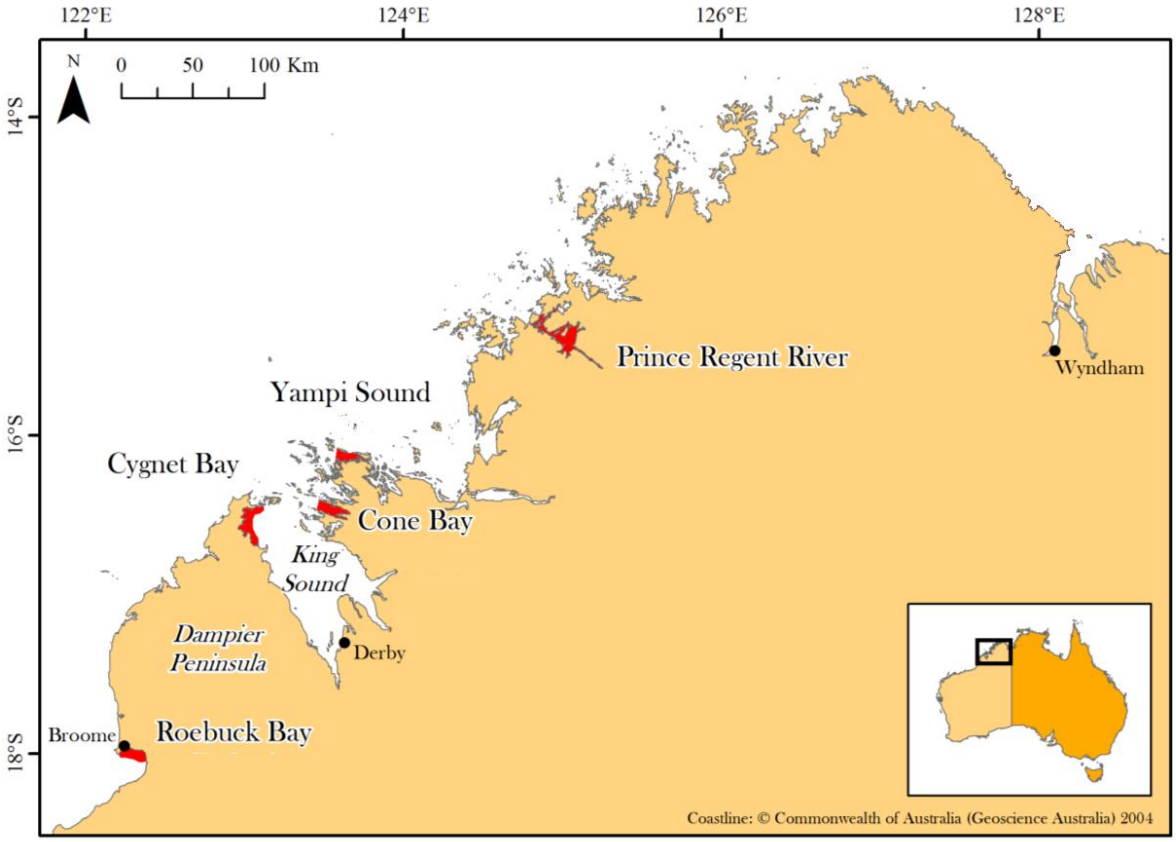


Figure 1. Location of the sampled individuals of snubfin dolphins along the Kimberley coastline of north-western Australia (Roebuck Bay, Cygnet Bay, Cone Bay, Yampi Sound and Prince Regent River). Map was modified from Brown et al. (2017).

DNA extraction for new samples

Genomic DNA was extracted from all skin/blubber samples using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions.

Genotyping and validation of microsatellites

Following sample preparation protocol used by Brown et al. (2017), we successfully genotyped newly collected samples from Prince Regent River (n =8) for eleven microsatellite loci: DIrFCB4, DIrFCB5 (Buchanan et al. 1996), LobsDi_19, LobsDi_21, LobsDi_24 (Cassens et al. 2005), SCA9, SCA22, SCA27, SCA39 (Chen and Yang 2009), TexVet5 and TexVet7 (Rooney et al. 1999). Using Geneious 9.1.8 (Kearse et al. 2012) with the microsatellite plugin 1.4 (Applied Biosystems), genotypes from previous and new samples (total of 112 samples) were scored all together (i.e., as opposed to scoring new samples independently to previous ones) to minimise scoring errors. Each microsatellite locus was checked for scoring errors using the software MicroChecker 2.2 with a confidence level of 95% (Van Oosterhout et al. 2004). Departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were tested using the Markov chain randomization in the R package "genepop" (Rousset 2002, 2008) with 10^5 dememorizations, 10^3 batches, and 10^4 iterations and the Bonferroni correction (Rice 1989). We also calculated the relatedness between all possible dyads using the R package "related" (Pew et al. 2014) using the TrioML estimator and tested differences within and between communities using a Mantel test and 10^4 permutations.

Mitochondrial DNA

We amplified a 475-bp mitochondrial fragment using the primers dlp1.5 (5'-TCA CCC AAA GCT GRA RTT CTA-3') and dlp5 (5'-CCA TCG WGA TGT CTT ATT TAA GRG GAA-3') (Baker et al. 1993) and following the PCR conditions described in Bacher et al. (2010) for new samples from Prince Regent River (n = 8). Their incorporation with samples previously analysed in Brown et al. (2017) provided successfully aligned mtDNA sequences of 408-bp for 94 samples (Roebuck Bay = 38; Cygnet Bay = 33; Cone Bay = 11; Yampi Sound = 3; Prince Regent River = 9) after manually editing in Geneious 9.1.8.

Genetic diversity

We assessed the level of microsatellite genetic diversity for each location site by computing the number of alleles (Na), effective number of alleles (Ne), private alleles

(NPA), and observed (Ho), expected, and unbiased expected (uHe) heterozygosities in GenAlEx (Peakall and Smouse, 2012), and allelic richness (AR) using FSTAT 2.9.3 (Goudet, 2001).

For mtDNA, we identified the number of haplotypes (NH), and estimated haplotype (h) and nucleotide (π) diversities using DnaSP 5.10 (Librado and Rozas 2009).

Genetic differentiation

We estimated pairwise genetic differentiation of microsatellite alleles (F_{ST}) (Weir and Cockerham 1984) and mtDNA Φ_{ST} (Tamura and Nei 1993) among communities using Arlequin 3.5 (Excoffier and Lischer 2010). For mtDNA, the choice of the model used was made after computing several nucleotide substitution models in jModelTest 2.1 (Posada 2008). Although the Akaike Information Criterion (AIC) suggested Hasegawa-Kishino-Yano (Hasegawa et al. 1985) as the best model, this one was unavailable in Arlequin, and therefore we used the Kimura 2P model (TPM2, Kimura 1981) as the next best model (ΔAICc < 2). All pairwise comparisons were testing for significance with 10^4 permutations and Bonferroni correction (Rice 1989).

Genetic population structure

We used two approaches to assess the number of distinct genetic clusters (K) among the snubfin dolphin sampling sites: (1) a Bayesian clustering algorithm implemented in STRUCTURE 2.3 (Pritchard et al. 2000), and (2) a discriminant analysis of principal components (DAPC) that maximises the differences between sites while minimising variation within sites (Jombart et al. 2010) using the R package 'adegenet' (Jombart and Ahmed 2011). DAPC does not assume a population genetics model such as STRUCTURE; instead, it transforms the data using PCA and then performs discriminant analysis on the number of principal components retained. In STRUCTURE, we conducted the analysis with LOCPRIOR models that assigned samples to their respective geographic site. Using a LOCPRIOR model improves clustering when the signal is weak without spuriously inferring structure (Hubisz et al. 2009) (see supplementary document for same analysis run without LOCPRIOR). We performed the analysis using the admixture

model with correlated allele frequencies (Falush et al. 2003), using a burn-in of 10^6 Markov Chain Monte Carlo (MCMC) steps followed by 10^7 MCMC steps. We repeated each run 10 times for *K* varying from one to seven (*K* = 6 and 7 being used to enable calculation of ΔK). The most likely value of *K* was determined by averaging the log probability LnP(D) among runs for each *K* value and selecting the highest mean LnP(D) (Pritchard et al. 2000). Individual genetic cluster assignment estimates (i.e., individual ancestry proportions) were generated for each set for *K* varying from two to five, using the web service software CLUMPAK (Kopelman et al. 2015). We then performed the DAPC analysis following Jombart and Collins (2015), also using location as priors. Cross-validation using the *xvalDapc* function from adegenet was used to choose the optimal number of principal components to retain. In addition to using locations as priors, we re-ran the analysis using the function *find.clusters* to assess the optimal number of groups with the Bayesian information criterion (BIC) method that was then used with the *snapclust* function (Beugin et al. 2018). The latter function uses maximum-likelihood estimations based on the expectation–maximization algorithm to investigate genetic clustering and admixture, assuming HWE and independence of loci (linkage equilibrium).

Gene flow

Contemporary migration rates among the sampling sites (including Yampi Sound) were estimated with the microsatellite loci and the Bayesian multilocus genotyping approach implemented in the program BayesAss 3.0 (Wilson and Rannala 2003). The acceptance rates of total iterations (i.e., between 20 and 60%) was reached by adjusting the parameters migration rates (m), allele frequencies (a) and inbreeding coefficient (f) to 0.3, 0.7 and 0.8, respectively. Five independent runs were performed using 10^7 Markov chain Monte Carlo (MCMC) iterations, 10^6 burn-in and sampled every 10^3 iterations, all using different random seed. Convergence was examined using the software Tracer 1.6 (Rambaut et al. 2013).

Results

Genetic diversity

A total of 112 individuals were genotyped at 11 polymorphic microsatellite loci (range 3-18 alleles) with 1.9% of missing data. Three loci (FCB4, SCA9, Lob24) showed

evidence of homozygosity excess possibly due to null alleles or stuttering, although results were inconsistent between sites (only found in one site each), and therefore were kept for further analyses. All 11 retained loci were in Hardy Weinberg Equilibrium (HWE) after Bonferroni correction, with the exception of one locus (FCB4). As this deviation from HWE was found in only one sampled site, the locus was included in the analyses. Current relatedness analysis suggested that about 5% of the pairs with individuals of any site were suspected parents-offspring or full-siblings (best relatedness parameter: dyadml $r \geq 0.5$, including RB 12.6%; CY 13.8%; CB 11%; YS 0%; PRR 16.7%). Given that only 11 loci were used in this study, false-positive for highly related individuals may occur and therefore it was decided to keep all individuals for further analyses.

Allelic diversity and heterozygosity values were generally higher for Roebuck Bay and Cygnet Bay samples compared with Cone Bay and Prince Regent River samples, although the difference in sample size between the two group sites may explain this variation (Table 1).

Table 1. Genetic diversity measures (SE) for snubfin dolphins using microsatellite loci (n = 11).

	N	**Na**	**Ne**	**NPA**	**AR**	**Ho**	**He**	**uHe**
Overall	112	8.182 (1.464)	3.648 (0.647)		8.135 (1.458)	0.608 (0.040)	0.659 (0.043)	0.662 (0.043)
Roebuck Bay	53	6.727 (1.161)	3.051 (0.449)	12	4.172 (0.561)	0.602 (0.043)	0.618 (0.040)	0.624 (0.033)
Cygnet Bay	40	6.455 (1.098)	3.610 (0.578)	10	4.098 (0.528)	0.616 (0.046)	0.654 (0.045)	0.663 (0.046)
Cone Bay	10	3.909 (0.625)	2.750 (0.362)	0	3.463 (0.494)	0.591 (0.072)	0.563 (0.062)	0.592 (0.065)
Prince Regent River	9	4.273 (0.843)	3.075 (0.575)	3	3.544 (0.471)	0.556 (0.068)	0.586 (0.052)	0.622 (0.055)

Note: **N** = number of screened samples; **Na** = number of found alleles; **Ho** = observed heterozygosity; **He** = expected heterozygosity; **uHe** = unbiased expected heterozygosity.

From the mtDNA analysis, a total of seven polymorphic sites were detected and seven unique haplotypes defined. The overall haplotype diversity was 0.716 (SD 0.0290) and ranged from 0.532 in Cygnet Bay to 0.667 in Yampi Sound (Table 2). There was one haplotype (Hap2) common to all sampled sites, and three unique haplotypes that each only occurred at one site, including one found in Cygnet Bay (Hap5, one sample), one in Cone Bay (Hap4, one sample) and one in Prince Regent River (Hap1, four samples).

Table 2. Genetic diversity measures (SE) for snubfin dolphins using mtDNA.

	N	**NH**	**h**	**π**
Overall	94	7	0.716 (0.0290)	0.006 (0.0003)
Roebuck Bay	38	4	0.609 (0.0580)	0.005 (0.0005)
Cygnet Bay	33	5	0.532 (0.0920)	0.003 (0.0006)
Cone Bay	11	4	0.600 (0.1540)	0.004 (0.0010)
Yampi Sound*	3	2	0.667 (0.3140)	0.005 (0.0023)
Prince Regent River	9	2	0.556 (0.0900)	0.007 (0.0011)

Note: **N** = number of screened samples; **NH** = number of haplotypes; **h** = haplotype diversity; **π** = nucleotide diversity. * To use for haplotype identification only.

The median-joining network showed no geographic grouping with most haplotypes separated by one or two mutations. The two haplotypes furthest apart were separated by five mutational steps and were found among Prince Regent River samples (Hap1 and Hap2). (Figure 2).

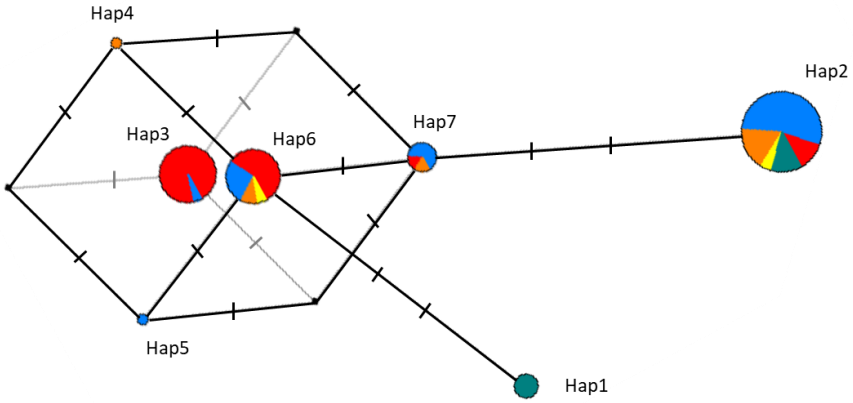


Figure 2. Median-joining network of mtDNA control region haplotypes in snubfin dolphins. The size of the circles is proportional to the total number of individuals carrying that haplotype. Different colours denote the five different sampled locations: Roebuck Bay = red; Cygnet Bay = blue; Cone Bay = orange; Yampi Sound = yellow; Prince Regent River = green. Number of mutational events between each haplotype is indicated by hash marks.

Genetic differentiation

Sampled snubfin dolphins at Roebuck Bay were significantly differentiated from all other sites using both microsatellites and mtDNA (Table 2). There was no significant differentiation between snubfin dolphins at Cygnet Bay and Cone Bay for mtDNA or microsatellites. Samples from Prince Regent River were significantly differentiated from all other sites using microsatellites but only with Roebuck Bay for mtDNA after Bonferroni correction.

Table 3. Microsatellite (above diagonal) and mtDNA (below diagonal) F_{ST} values and their significance levels.

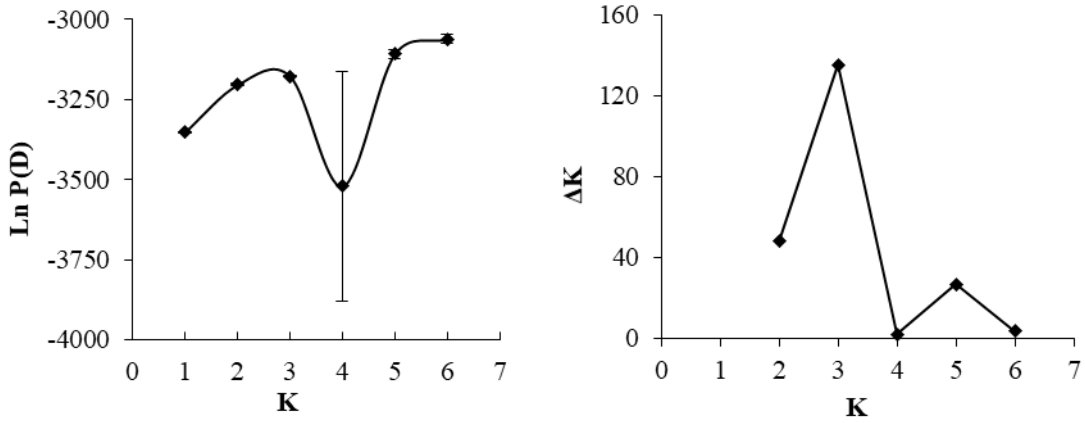
	Roebuck Bay	Cygnet Bay	Cone Bay	Prince Regent River
Roebuck Bay	-	**0.08301 *****	**0.06898 *****	**0.09945 *****
Cygnet Bay	**0.38651 *****	-	0.01591	**0.05990 ****
Cone Bay	**0.33251 *****	0.00000	-	**0.08107 ****

Prince Regent River	**0.37711 *****	*0.18293*	0.09640	-

Value in Italic was significant before Bonferroni correction.

Genetic structure

STRUCTURE using LOCPRIOR models (i.e., information about the site of sampling) showed a pattern for the most likely number of clusters (*K*) being 3 based on the high mean posterior probability (LnP(D)) reaching at *K* = 3 before dropping at *K* = 4 and Δ*K* index obtained by the method of Evanno et al. (2005) revealing a modal value of Δ*K* = 135 at *K* = 3 (Figure 3). Similar results were found when running the STRUCTURE analysis without prior information, although assignment of individuals was not as clear. Therefore, only assignment from the STRUCTURE analysis using LOCPRIOR models is shown here. Most individuals sampled at the same location were assigned to the same genetic cluster, with the greatest level of admixture found among samples from Cone Bay (admixed between a majority of Cygnet Bay cluster (*q* >50%) and a minority of Roebuck Bay cluster (*q* <50%).



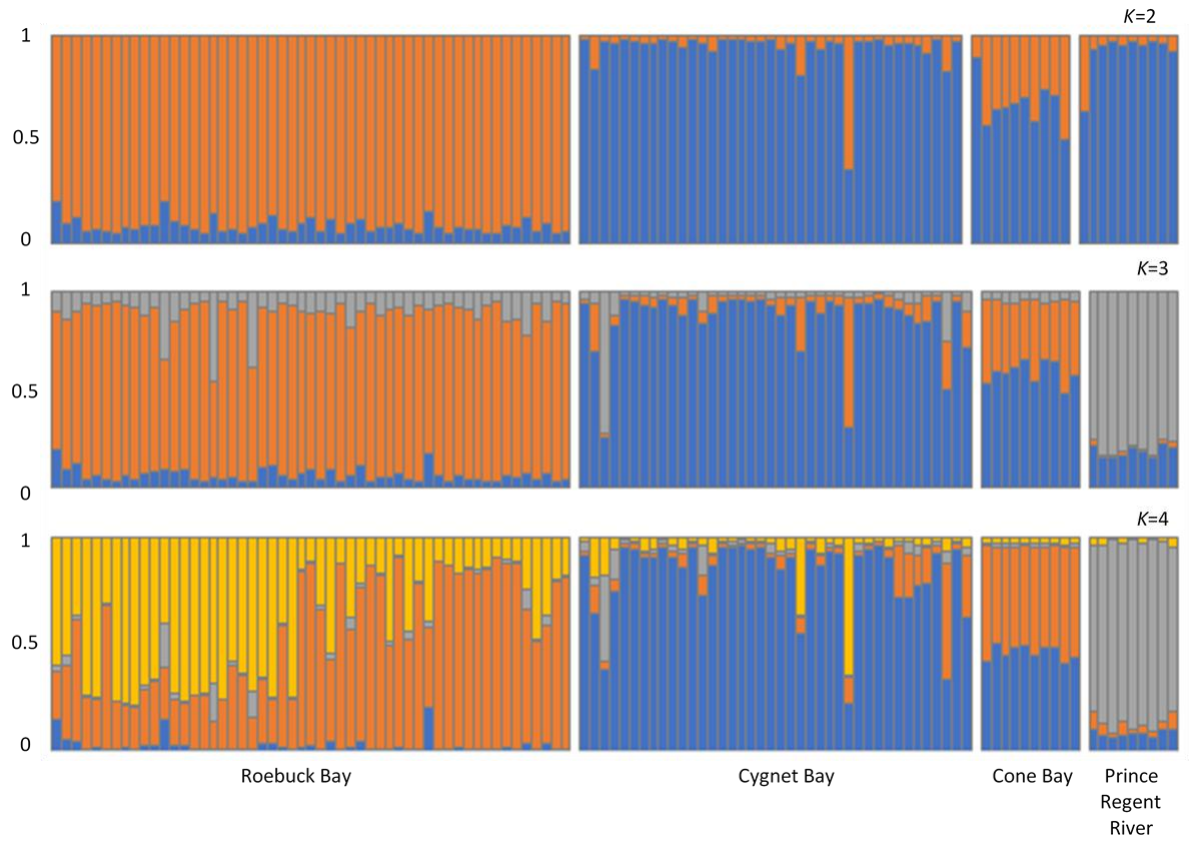


Figure 3. Mean of the estimated posterior probabilities (LnP(D)) and ΔK statistic (Evanno et al. 2005) over ten replicate runs for values of K = 1-6 using the Bayesian method in STRUCTURE with prior information (i.e., site of sampling). Genetic assignment probabilities from STRUCTURE (n = 112) with each vertical column corresponding to an individual dolphin and the colours indicating the membership proportions to each of the clusters (K =2,3 and 4 for comparison, although K = 3 was the most likely number of clusters).

The multivariate DAPC identified *K* = 2 or 3 or 4 as the optimal number of clusters according to the Bayesian information criteria (Figure 4-Top right). DAPC analysis using prior information indicated some genetic distance between Prince Regent River and the three other sites where some overlaps among samples occurred (Figure 4- Top left). When using *find.cluster* function, Roebuck Bay samples were similarly identified as a separate genetic cluster to the other locations, except for a few migrants (assigned [*q* > 0.9] to a different cluster than the one defined for the site of the samples) (Figure 4 – Bottom). While *K* = 4 did not bring further information, *K* = 3 identified the majority of samples from Cone Bay to be associated with a third genetic cluster, that is shared with a few samples from Cygnet Bay (i.e., migrant individuals, *q* > 0.90), while Prince Regent River samples are either assigned from

the genetic cluster identifying the majority of Cygnet Bay or Cone Bay samples (Figure 4 – Bottom).

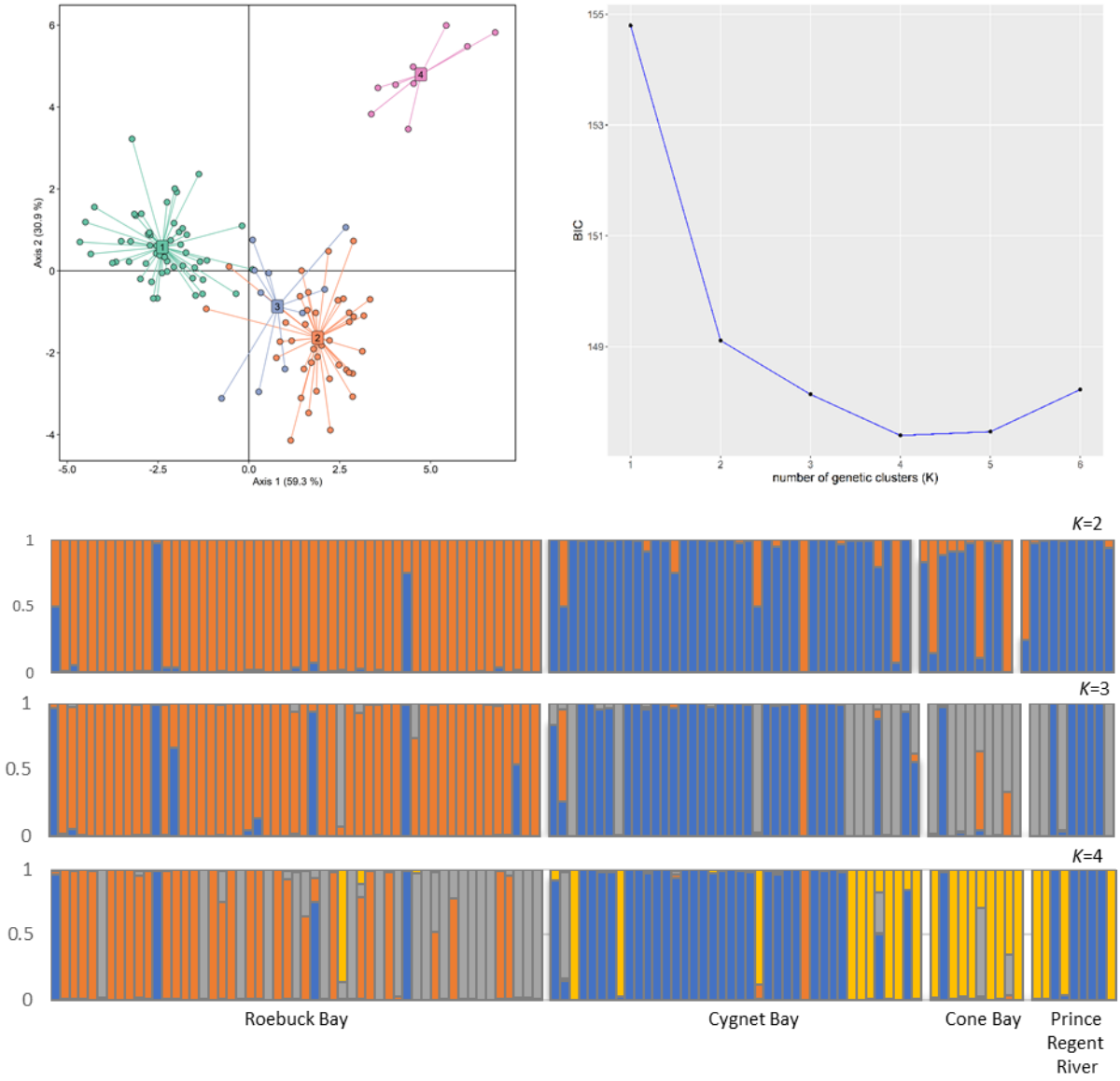


Figure 4. Discriminant analysis of principal components (DAPC): (Top left) PCA representation with prior information with 1-Roebuck Bay; 2-Cygnet Bay; 3- Cone Bay and 4-Prince Regent River; (Top right) BIC statistic obtained from find.clusters (no prior information); (Bottom) Genetic assignment probabilities with each vertical column corresponding to an individual dolphin. The colours indicate the membership proportions to each of the clusters (K =2, 3 and 4) based in snapclust outcomes.

Gene flow

Estimated contemporary migration rates inferred in BayesAss suggested low gene flow between the majority of the sites (m = 1-5%, Table). However, estimated migration rates from Cygnet Bay to Cone Bay and to Prince Regent River were

moderate (m = 24% and 22% of Cone Bay and Prince Regent River dolphins, respectively) while only negligible migration was estimated in the opposite direction (m < 1.35%). The proportion of non-immigrants from their respective origin site was high for Roebuck Bay and Cygnet Bay (0.95 and 0.94, respectively), while others show lower proportions (from 0.69 to 0.72).

Table 4. Mean (standard deviation) of the posterior distribution of the contemporary migration rates (m) in BayesAss (Wilson and Rannala 2003) among five sampled sites for snubfin dolphins. The site of which each dolphin belongs are listed in the rows (i.e., proportion of dolphins from that site), while the site from which they migrated are listed in the columns. Values along the diagonal (in bold) are the proportions of non-immigrants from the origin site for each generation. Moderate estimated migration rates (m > 0.10) are displayed in italic.

Migration	Origin:			
Into:	RBB	CYB	CNB	PRR
RBB	**0.9532 (0.0205)**	0.0283 (0.0179)	0.0104 (0.0089)	0.0080 (0.0079)
CYB	0.0257 (0.0164)	**0.9508 (0.0204**)	0.0135 (0.0103)	0.0100 (0.0093)
CNB	0.0478 (0.0354)	*0.2383 (0.0431)*	**0.6901 (0.0220**)	0.0238 (0.0222)
PRR	0.0307 (0.0278)	*0.2153 (0.0582)*	0.0255 (0.0240)	**0.7284 (0.0504)**

Discussion

Overall, the evidence for genetic differentiation and structure between Roebuck Bay and all other sampling sites in the Kimberley (Cygnet Bay, Cone Bay and Prince Regent River) confirmed previous investigations of population genetics in snubfin dolphins (Brown et al. 2014; Brown et al. 2017). The additional eight samples for Prince Regent River provided us with information not previously available, although interpretations should be made with caution given the low sample size (*i.e.*, number

of samples still low for Cone Bay and Prince Regent River and the general low number of loci).

Microsatellite loci analyses of samples of snubfin dolphins from Prince Regent River indicated genetic differentiation with all other sites, and only with Roebuck Bay for mtDNA. The dissimilarity in pairwise site differentiation between estimates of microsatellite F_{ST} (biparental inheritance) and mtDNA Φ_{ST} (maternal inheritance) may be caused by sex-biased dispersal, although not analysed here, in which males exhibit philopatry while females may disperse from the place they were born. The lack of differentiation in the mtDNA Φ_{ST} was also supported by the identification of a common mtDNA haplotype (H4) between Prince Regent River and all sampled sites, despite a second haplotype defined in Prince Regent River samples being unique to this site (Figure 4.3).

The contemporary migration rates inferred in BayesAss supported pattern of genetic differentiation with no gene flow occurring between Roebuck Bay and any of the other sites, including Prince Regent River. The analysis also indicated asymmetric gene flow (0.22-0.24) estimated from Cygnet Bay to Cone Bay and Prince Regent River, again suggesting a level of genetic dependence between those three sites. While asymmetric gene flow would suggest a source-sink dynamic (i.e., Cygnet Bay acting as a source and Cone Bay and Prince Regent River as a sink), the lower number of samples for Cone Bay and Prince Regent River may jeopardise the analysis because of the limitation to provide good representation of the genetic diversity for both sites. More samples from Cone Bay and potentially from Prince Regent River would provide better representation of the diversity at each site. However, only 20 individuals have been recorded in the Prince Regent River area (D'Cruz et al. 2020) meaning that the range of genetic diversity may already be captured in the 9 samples. New samples from sites further east in the Kimberley would expand our understanding of this species throughout Western Australia and comparison made with samples from the Northern Territory (Palmer et al. 2011) would provide a broader context and better understanding of the historic gene flow and connectivity across more of this species' range as well as the historical processes occurring among those populations (e.g., evolutionary processes).

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