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Kinship genomics approach to study mating systems in a depleted sea turtle rookery

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ABSTRACT

Knowledge of demographic processes and life history strategies is necessary for the conservation and management of endangered sea turtle populations, but it is difficult to ascertain because of the limited accessibility to marine environments that sea turtles use during the different stages of their life cycle. In such cases, molecular genetics and genomic approaches are useful to assess mating systems and operational sex ratios (OSR), which ultimately influence demography. This pilot study used genome-wide single nucleotide polymorphism (SNP) genetic markers for exploring kinship and mating systems in sea turtles where major obstacles prevent a comprehensive assessment in the wild. We sampled 217 young hawksbill turtles (*Eretmochelys imbricata*) of unknown parentage that had originally been collected locally from seven nests and were being temporarily kept in captivity by Treasure Island Ltd as part of its captive rearing conservation project at Bounty and Treasure Islands, Fiji, in the South Pacific. The raw dataset comprised 13,573 SNPs, of which we retained 639 SNPs for parentage and relatedness analyses. Our findings from seven different pairs of parents suggest a 1:1 male:female OSR and demonstrate that genome-wide SNP genotyping approaches can be used to infer OSR. Knowledge of OSR can help evaluate the magnitude of the impact of warming temperatures and consequent feminisation in sea turtles. Our approach can complement or substitute field observation of breeding males and nesting females when logistical or budgetary constraints prevent observation of OSR in wild sea turtle populations. This approach allows inference of OSR. Protection of beaches with a higher number of pairs of parents should be prioritised to increase genetic resilience. Conservation actions in rookeries with a female-skewed OSR should be prioritised to address population declines in the long run.

1. Introduction

Small declining populations are subject to demographic stochasticity that can exacerbate the threat of extinction, whereby skewed sex ratios can limit fitness and population growth (Lande, 1998; Stephens and Sutherland, 1999; Stephens et al., 1999; Bessa-Gomes et al., 2004). Small population dynamics are influenced by variations in the sex ratio, and reduction in population growth rates associated with the Allee effect can further influence the population extinction risk (Bessa-Gomes et al., 2004). In particular, in small populations, the extinction risk seems to be largely affected by the operational sex ratio (OSR), which is the ratio of sexually receptive males to receptive females (Emlen and Oring, 1977). The OSR reflects differences in mating systems and the direction of sexual selection (Weir et al., 2011; Janicke and Morrow, 2018). Skewed OSR that limits availability of one sex can increase the competition for mates and reduce mating opportunities (Emlen and Oring, 1977; Kvarnemo and Ahnesjo, 1996). A high female to male OSR can reduce successful mating by females in monogamous populations to a greater extent than in polygamous ones (Kvarnemo, 2018), as found in both terrestrial species (for example, in voles and rattlesnakes, see Klemme et al., 2007; Clark et al., 2014), and in marine species (such as pipefish, see Vincent et al., 1994).

Knowledge of demographic processes and life-history strategies are crucial to the conservation and management of endangered species, however, may be difficult to ascertain when there is limited human access to the environments used by the species

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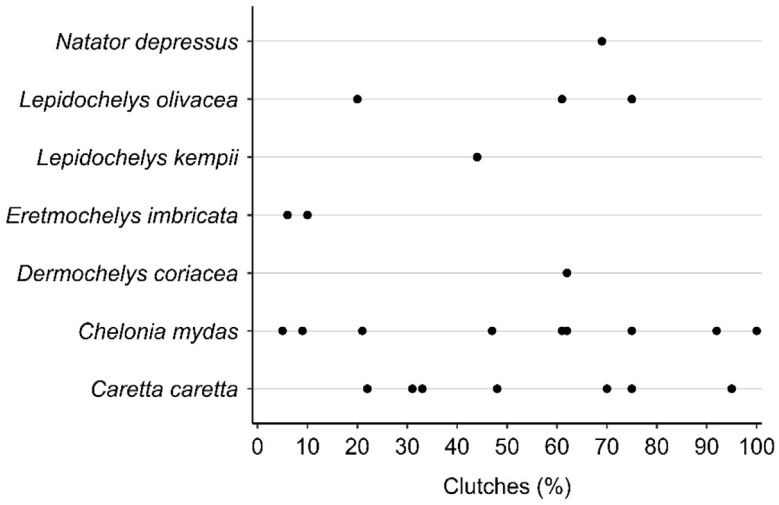

Fig. 1. Multiple paternity on egg clutches of wild sea turtles across species and rookeries (see Appendix S1 for details).

throughout its life cycle. Sea turtle species, with their complex life cycle spanning multiple life stages and marine habitats, are an example of situations where molecular genetics and genomic approaches are useful to assess factors influencing demography, e.g., mating systems and OSR (Jensen et al., 2013; Komoroske et al., 2017).

The mating system most often reported in sea turtles is polygynandry, whereby both male and female mate with more than one partner (Miller, 1997; Pearse and Avise, 2001; Holder and Holder, 2007; Jensen et al., 2013). Multiple mating can be advantageous to female sea turtles to ensure fertilisation and sperm competition, which increases the hatching success and/or offspring quality (Miller, 1997; Pearse and Avise, 2001). Female sea turtles usually lay multiple clutches of eggs during the same nesting season and use sperm storage instead of repeated mating during the internesting period (Joseph and Shaw, 2011; Stewart and Dutton, 2011; Phillips et al., 2013; Lasala et al., 2020).

Clutches of eggs with multiple fathers have been found in all of the seven sea turtle species, to a degree that varies among rookeries (see Fig. 1). Multiple paternity in wild sea turtle populations has been investigated with microsatellite markers in all except one study (Appendix S1), where electrophoretic allozymes were used (Harry and Briscoe, 1988). The majority of microsatellite studies focused on green turtles (*Chelonia mydas*) and the loggerhead sea turtle (*Caretta caretta*). In the green turtle, multiple paternity ranged from less than 10% of the clutches at Pangumbahan Coastal Park in Indonesia (Purnama et al., 2013) and the southern Great Barrier Reef in Australia (Fitzsimmons, 1998), to over 90% of the clutches at Tortuguero in Costa Rica (Alfaro-Núñez et al., 2015) and Ascension Island in the UK Overseas Territory (Ireland et al., 2003). For loggerheads, multiple paternity ranged from 22% of the clutches at Sanibel Island in Florida (Lasala et al., 2020) to 95% at Zakynthos, Greece (Zbinden et al., 2007). A complete list of the 31 studies is shown in Appendix S1.

Multiple paternity in sea turtles may be a consequence of male aggressiveness rather than female choice (Bowen and Karl, 2007), and low multiple paternity seems to be associated with a low encounter probability with males, either due to female-biased OSR or to the males' dispersed mating behaviour (Lee and Hays, 2004; Jensen et al., 2006; Phillips et al., 2013; Lee et al., 2018). The lowest frequencies of multiple paternity are reported for the hawksbill turtle (*Eretmochelys imbricata*), which ranged from 6% of clutches at the Yucatán Peninsula in Mexico (González-Garza et al., 2015) to 10% at Bahía de Jiquilisco in El Salvador (Gaos et al., 2018). However, this information should be interpreted with caution, as multiple paternity per clutch has been reported only in two hawksbill turtle studies (Appendix S1) and may not adequately reflect the species as a whole.

Although the hawksbill turtle is circumglobally distributed and its conservation status is classified as "critically endangered" in the International Union for Conservation of Nature Red List (Mortimer and Donnelly, 2008), little is known about its populations in the western and central South Pacific (Wallace et al., 2010). Indeed, four out of the five regional management units are "putative" due to a knowledge gap in hawksbill turtle's biogeography (Wallace et al., 2010). A small nesting population is present in Fiji, where nesting of hawksbill turtles occurs sporadically across several sites (Prakash et al., 2020).

To date, multiple paternity has been investigated by genotyping profiles of the mother and her clutch(es); the former by direct sampling of the nesting female, and the latter by assessing maternally-derived alleles in offspring. With this genotype exclusion approach, paternal alleles are those remaining in the offspring genotypes after accounting for the mother's alleles. However, the logistical constraints of accessing remote nesting sites combined with the depleted or rare status of nesting in some sea turtle populations, significantly reduce the chance of encountering, and thus sampling, nesting females.

Next generation sequencing (NGS) and single nucleotide polymorphism (SNP) markers can potentially shed new light on sea turtle mating systems (Roden et al., 2013). SNPs have been used to assess parentage and kinship in a variety of vertebrates (Heaton et al., 2002; Werner et al., 2004; Hauser et al., 2011; Weinman et al., 2015; Anderson et al., 2019). For sea turtles, SNPs were used to assess connectivity and population structure of green turtle populations in the Pacific (Roden et al., 2013; Hamabata et al., 2020; Álvarez-Varas et al., 2021), as well as to study leatherback (Komoroske et al., 2019) and hawksbill (Banerjee et al., 2020) turtle populations across the species global range. While these studies offered broad-scale insights into population genetic structure, a more targeted SNP discovery approach is required to identify markers for specific studies, such as investigation of fine-scale intra-population demographics or parentage and relatedness in small, isolated populations. Consequently, we used an NGS-based genotyping system called DArTseq which employs several innovations (see Kilian et al., 2012) for delivering high-quality genome-wide markers for non-model taxa.

Genome-wide SNP genotyping approaches can also inform wildlife management and traceability initiatives, by elucidating kinship in parentages. In the context of sea turtles, assessing

Table 1
Characteristics of the three sets of hawksbill turtles sampled. Mean size and standard deviation of hawksbill turtles refer to the curved carapace length notch to tip (CCLn-t). Mixed origins = individuals originated from multiple nests and kept together in the same tank; unique origin = all individuals hatched from a single nest.

Set	Location	Nesting season	Hawksbill turtles		Status (No. of nests)
			No. of hawksbill turtle sampled	Mean size ± S.D. (cm)	
A	Bounty Island	2014/2015	93	18.9 ± 2.1	Mixed origins (3)
B	Treasure Island	2015/2016	30	5.1 ± 0.3	Unique origin (1)
C	Treasure Island	2015/2016	94	5.2 ± 0.2	Mixed origins (3)

parentage and relatedness of hatchlings from single or multiple nests can offer insights into the maternity and/or paternity of cohorts, and therefore an understanding of how many mature males and females contribute towards a rookery.

This pilot study aimed to develop and test genomic approaches useful for exploring kinship and mating systems in sea turtles where major obstacles prevent a comprehensive assessment in the wild. We sampled young hawksbill turtles hatched in Fiji from unknown parents and used genome-wide SNP genetic markers to (1) reconstruct family relationships among the individuals sampled, (2) assign parentage and assess the occurrence of multiple paternity, and (3) infer operational sex ratio (OSR), i.e. the ratio of breeding adults.

2. Materials and methods

2.1. Study sites

Sampling was performed at two islands of the Mamanuca Group in Fiji, South Pacific; namely, Bounty Island (native name: *Kadavulailai*, 17.6732°S, 177.3055°E; ca. 0.22 km^2) and Treasure Island (native name: *Eluvuka*, 17.6552°S, 177.2669°E; ca. 0.07 km^2) (Fig. 2). The islands are separated by ca. 4.5 km of relatively shallow (max 30 m deep) seawater. A resort is present on each of these two otherwise uninhabited islands. Both islands have a low level of nesting activity for hawksbill turtles, whereby less than three nests per nesting season were recorded, on average, from 2014/2015 to 2018/2019 (Prakash et al., 2020). As part of conservation activities, a head-starting project sanctioned by the Fijian government and led by Treasure Island Ltd was underway at each island, whereby sea turtles locally hatched in the wild were collected upon emergence from nests, reared in captivity until reaching a minimum size of 20 cm curved carapace length, and then released at sea. We opportunistically sampled hatchlings being held at these head-start facilities to obtain a representation of nests from this severely depleted local nesting population.

2.2. Tissue sampling

Tissue sampling was performed within 11 months from hawksbills hatching, whereby a total of 217 individual samples were obtained from three sets of turtles (hereafter referred to as "Set A", "Set B" or "Set C"), representing seven nests (Table 1). One set (Set B) was made up of individuals hatched from the same nest whereas in the other two cases, each set comprised a mixture of individuals hatched from three nests. Each tissue sample was individually preserved in 90% molecular-grade ethanol. Tissues were sent to Diversity Arrays Technology Ltd (DArT PL) in Canberra, Australia for genomic deoxyribonucleic acid (gDNA) extraction and genotyping.

2.3. DArTseq 1.0 library preparation, sequencing, and genotype quality control

Genotyping by sequencing was performed using the DArTseq system (Sansaloni et al., 2011; Kilian et al., 2012), with many modifications targeted at the hawksbill turtle genome. Genome complexity reduction and reduced-representation library preparation were accomplished as per (Ren et al., 2015; Lal et al., 2021). Following library preparation, 77 bp single-end sequencing was performed on the Illumina HiSeq 2500 platform. Raw sequence reads were processed using several software packages including Illumina CASAVA v.1.8.2 for quality assessment (Cock et al., 2010) and DArTtoolbox for filtering, demultiplexing, alignment, and variant calling (Cruz et al., 2013; Kilian et al., 2012; Robasky et al., 2014). Final filtering of loci involved assessing homozygote and heterozygote call rates, frequency, polymorphic information content (PIC), average SNP count, read depth and repeatability, before DArt PL supplied final genotype scores.

After receiving genotypic data from DArT PL, the dataset was further filtered to retain only a single, highly informative SNP at each locus. This was achieved by filtering out duplicate SNPs (possessing identical Clone IDs), according to call rate and minor allele frequency (MAF; 2% per nest sample group). Subsequently, loci were screened for call rate (98% threshold), average polymorphic information content (PIC; 1%), MAF (2%), read depth ($\geq$7), and average repeatability (95%), to retain SNPs suitable for further analyses. All loci were then tested for departure from Hardy–Weinberg equilibrium (HWE) using Arlequin v.3.5.1.3 (Excoffier et al., 2005), using an exact test with 10,000 steps in the Markov Chain and 100,000 dememorisations. The final dataset was then created which contained only putatively neutral loci.

Screening for F_{st} outlier loci was carried out to identify markers potentially affected by selection, genetic drift, as well as hitch-hiking loci. The BayeScan v.2.1 (Foll and Gaggiotti, 2008; Foll, 2012) software package was employed to identify candidate loci under selection, at false discovery rate (FDRs) = 0.001, 0.005, 0.01, 0.05, 0.1 and 0.2. A further reduced dataset was generated to investigate parentage and relatedness. The first dataset containing 2555 SNPs was further filtered for MAF (>40%, after Huisman, 2017, 2021) followed by screening for linkage disequilibrium (LD). As the individuals sampled were presumed to be likely full-siblings within each nest site, higher levels of linkage were expected among pairs of loci. We used PLINK v. 1.07 (Purcell et al., 2007) to evaluate the squared allele count correlation (r^2) between all pairs of SNPs tested for LD in the dataset (Andrews et al., 2018), and filtered out one locus from each linked pair at an r^2 threshold of 0.5.

2.4. Resolution of genetic structure among nests

To resolve putative relationships between nest sample groups, genetic structure was examined using both a discriminant analysis of principal components (DAPC) to obtain a general overview, as well as the Netview R package (Neuditschko et al., 2012; Steinig et al., 2016) for a finer-scale assessment at the individual level. The DAPC was implemented in the R package *adegenet* v. 1.4.2 (Jombart, 2008; Jombart et al., 2010; Jombart and Ahmed, 2011) and carried out for all selectively-neutral loci, with an α-score optimisation used to determine the number of principal components to retain.

Netview R networks were visualised in the Cytoscape v. 2.8.3 network construction package (Smoot et al., 2011). First, the 1-IBS

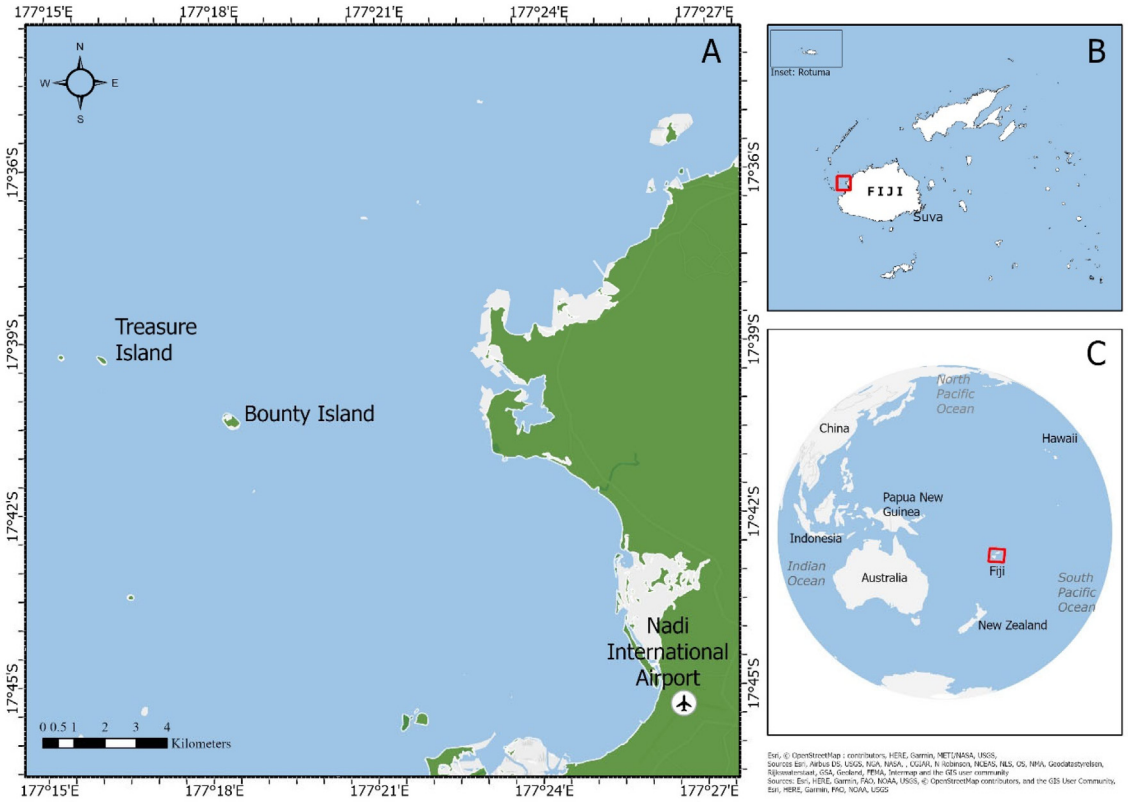

Fig. 2. A: The sampling sites; Bounty Island and Treasure Island. B: The study area in Fiji (red square). C: Location of Fiji in the region (red square).

(average proportion of shared alleles identical by state) matrices and corresponding networks were constructed at various thresholds of the maximum number of nearest neighbour (mk-NN) with values between 1 and 50, after which the optimal network for representation was selected based on cluster stability (Steinig et al., 2016).

2.5. Examination of relatedness and kinship

Family relationships among all individuals were assessed with COLONY2 v.2.0.5.8 (Jones and Wang, 2010) to identify any parent–offspring, and full-sibling or half-sibling pairs. COLONY2 utilises a full-pedigree maximum likelihood method to assign parentage and calculate sibship, taking into account null alleles, genotyping errors, and mutations (Jones et al., 2010). The original, filtered dataset (2555 SNPs) was further pruned to a dataset containing 639 SNPs as described earlier (see Section 2.3), and both datasets were used for all COLONY2 analyses. COLONY2 computations were run without updating allele frequencies with run progression, specifying the presence of inbreeding, specifying polygamy for both males and females, not inferring clones, and scaling full sibship relationships. Prior settings were as follows: (1) weak sibship priors for all relationship determinations were requested, (2) population allele frequency was specified as unknown (i.e. calculated during the run), (3) the full-likelihood (FL) method was used for all runs, and (4) the option for medium length runs at high precision selected. A total number of three runs were carried out using both datasets, each using a different random number seed, all assuming a genotyping error rate of 0.05. All other options remained at their default settings.

Full-sibling family assignments generated from each COLONY2 run were inspected in the ".*BestFSFamily" output file and sorted by family inclusion and exclusion probabilities. The numbers of full-sibling and half-sibling dyads detected during each run were inspected and ordered by probability. Then, each sample group was tallied using an inclusion threshold of $p \leq 0.05$. COLONY2 can infer single locus parental genotypes from multilocus genotype data (Jones and Wang, 2010; Harrison et al., 2013), using a likelihood framework based upon observed genotypes present in offspring input data. These are written to the ".*MumGenotype" and ".*DadGenotype" output files, and used to make parentage assignments for each nest sample group reported in the ".*BestConfig" output files. Finally, these inferred assignments were tabulated with putative mother and father identities (designations are arbitrary and represent only opposite sexes) assigned to individual offspring.

3. Results

3.1. DArTseq™ 1.0 genotyping and marker filtering

The raw dataset comprised 13,573 SNPs genotyped across all individuals at call rates ranging from 38.6% to 98.8%. The first filtering step removed 896 duplicate (clone) SNPs (6.6% loss), after which the dataset was filtered for call rate (95%), average PIC (1%), MAF (2%) and average repeatability (95%). All loci called at a read depth <7 were also excluded from the filtered dataset. No loci were found to deviate from HWE ($p < 0.001$), nor were any monomorphic between all sample sets. These steps collectively resulted in the retention of 2568 SNPs. Testing this filtered dataset for F_{st} outlier loci detected 13 SNPs putatively under the influence of balancing or directional selection. Their removal generated a final selectively-neutral dataset of 2555 SNPs. This dataset was used for performing all downstream genomic analyses. Further filtering to create a separate dataset for parentage and relatedness analyses using a MAF (>40%) filter and LD pruning ($r^2 > 0.5$) retained 639 SNPs.

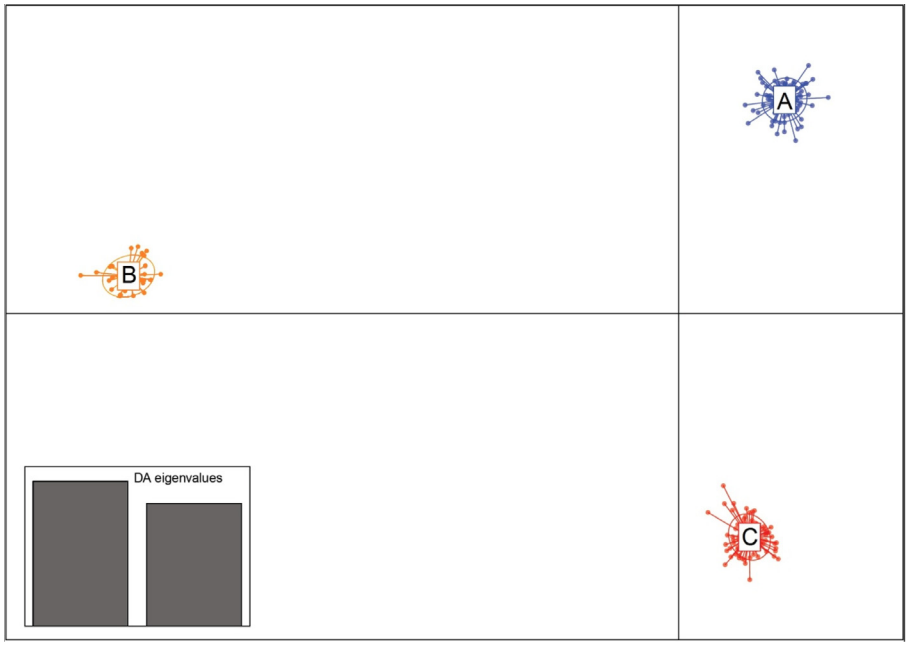

Fig. 3. Findings from discriminant analyses of principal components were carried out using the *R* package *adegenet* on the 217 individual hawksbill turtles sampled. Dots on scatterplots represent individuals, with colours denoting sample origin and inclusion of 95% inertia ellipses. The discriminant analysis (DA) eigenvalues inset barplot displays the F statistic levels for the first two (and most informative) discriminant functions retained in the analysis as per Jombart et al. (2010). Sample groups are Set A (Bounty Island, in blue), Set B (Treasure Island, in orange), and Set C (Treasure Island, in red).

3.2. Resolution of genetic structure among nests

Visualisation of broad-scale population structure with a DAPC (following α-score optimisation retained 13 principal components and two discriminant functions) revealed clear differentiation between all sample sets (Fig. 3). A fine-scale analysis with Netview R network generated at mk-NN = 15 revealed each set segregating into one or more discrete clusters, indicating putative family groups (Fig. 4). In particular, Set A and Set C were separated into three and two clusters, respectively. Set C also resolved three orphan nodes, which remained separate, potentially indicating the presence of a third family group. Hatchlings belonging to Set B consistently resolved to a single discrete cluster. These clusters persisted through an mk-NN range of 9–15, supporting the strength of these relationships in the data. While Netview R is sensitive to genetic differences at the individual level and has been used to resolve family-level groupings (Steinig et al., 2016), the ultimate resolution of family relationships required dedicated parentage and relatedness analyses.

3.3. Examination of relatedness and kinship

Family assignments generated with COLONY2 revealed the presence of a total of seven full-sibling families across the three sets sampled (see Appendix S2), with membership ranging 2–76 individuals. The results were identical when computed using the original (2555 SNPs) and reduced (639 SNPs) datasets. In addition, no half-sibling or unrelated individuals were detected, and all family inclusion probabilities were high ($p \geq 0.001$), except for two individuals from Set C (C_052 and C_084, $p = 0.067$). These results were concordant with the Netview R network reconstructions of genetic structure, supporting the presence of a single-family group among the individuals of Set B. Similarly, a total of three separate family groups were identified in each of the other two sets (i.e., Set A and Set C). These results indicate that, for these two sets, individuals may have originated from a total of six nests.

Reconstruction of the hypothetical parental genotypes of each individual hawksbill turtles permitted parentage assignment, which subsequently allowed for assessment of multiple paternity in these Fijian hawksbill turtles (see Appendix S3). Within all seven full-sibling family assignments made by COLONY2, all individuals were sired by a single parent pair, providing evidence of single paternity in Fijian hawksbill turtles. All individuals sampled from Set B were sired by a single father, whereas individuals in Set A were from three different parent pairs. One of these parent pairs contributed to 61 individuals, whereas the remaining two parent pairs accounted for 16 individuals. Given that these individuals were all reared in a single tank at the resort on Treasure Island, they likely belonged to the three different nests reported from the island at that time (Prakash et al., 2020). Similarly, the Set C nest sample group likely comprised individuals from three different nests from Treasure Island, containing 76, 2 and 16 individual hawksbill turtles, respectively. However, the assignment of two individuals from Set C (namely, C_052 and C_084) to a separate family may be questionable, as their family inclusion probability was very low ($p = 0.067$) compared to the inclusion probabilities of the other two families (both $p \geq 0.001$ across all COLONY2 runs). Nonetheless, the genotypes for both these individuals support the case for single paternity. Furthermore, these data suggest that the breeding sex ratio for Fijian hawksbill turtles is 1:1 male to female within the sets sampled in this study.

4. Discussion

This pilot study on Fijian hawksbill turtles demonstrates that genome-wide SNP genotyping approaches can be used to infer OSR in sea turtles. Knowledge of OSR is paramount for effective population management (Wedekind, 2012), but difficult to obtain due to sea turtles cyclic but non-annual breeding activity and their breeding grounds being at sea (Hamann et al., 2003). In wild populations, satellite telemetry of adult males and the use of drones for an aerial recording of breeding behaviour has helped shed light on the availability of males at breeding grounds (Hays

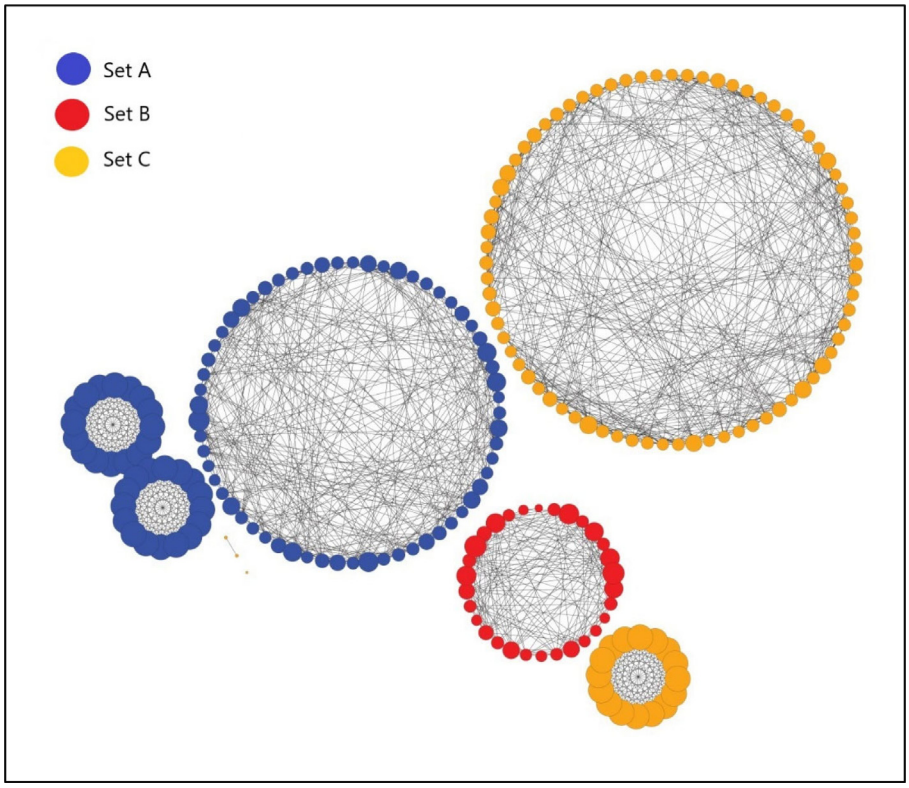

Fig. 4. Fine-scale genetic structure network of 217 individual hawksbill turtles analysed using Netview R. The network was produced from all sample groups analysed collectively and generated using a circular topology framework at mk-NN = 15, with node sizes mapped to the relatedness of individuals. Sample groups are Set A (Bounty Island, in blue), Set B (Treasure Island, in orange), and Set C (Treasure Island, in red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and Hawkes, 2018) and, upon comparison with the number of nesting females, to estimate the OSR (Rees et al., 2018; Schofield et al., 2019). For example, drones were used at the Laganas Bay loggerhead sea turtle breeding ground in the Mediterranean Sea, where a 1:1 ratio of receptive females to breeding males was found (Schofield et al., 2017). However, when logistic or budgetary constraints prevent obtaining field observation of breeding males and nesting females, our approach, which included the arbitrary designation of mother and father to represent opposite sexes, can be used to infer OSR.

It is worth noting though, that our approach requires sampling a high number of live hatchlings from each nest which, in our case, was possible because of the presence of a head-start facility. For populations with low nesting density distributed over vast areas, it may be possible to target a few key locations where nests distribution and/or hatching season are known. This would help maximising costs and efforts of sampling live hatchlings. Salvaging dead embryos or hatchlings from nests that have hatched is unreliable, because DNA quality may be poor and sample sizes insufficiently small from salvaged post-hatched nests. Where head-start facilities are not available, ethical considerations may further limit the ability to collect and sample live hatchlings without impacting their chances of survival (see Dutton and Stewart, 2013).

Sea turtles have temperature-dependent sex determination, where a greater number of males develop when the embryos are exposed to lower temperatures, and conversely a greater number of females develop when the embryos are exposed to higher temperatures (Mrosovsky and Yntema, 1980; Standora and Spotila, 1985). This process of environmental sex determination makes sea turtles particularly susceptible to climate change, with local warming temperatures leading to female-skewed hatchling sex ratios and predictions of extremely high (>90%) feminisation of hatchlings in some nesting beaches (Fuentes et al., 2010, 2011; Tapilatu and Ballamu, 2015; Patrício et al., 2019; Blechschmidt et al., 2020). For example, a high and increasing rate of feminisation was found in foraging green turtles sampled in winter 2014 and 2015 originating at the northern Great Barrier Reef in Australia, where 87% of the mature green turtles and almost all (>99%) immature individuals were females (Jensen et al., 2018).

Knowledge of the OSR can help evaluate the magnitude of the impact of warming temperatures on sea turtles, as a relatively balanced OSR may persist even at highly female-skewed primary sex ratios (Hays et al., 2014, 2017). Genetic analyses have been used to infer OSR; for example, parentage analysis of 14 polymorphic microsatellite loci suggested a ratio of 1.4 reproductive males to every breeding female at the green turtle rookery of Alagadi beach in Cyprus, in the Mediterranean Sea, despite a highly female-skewed offspring sex ratio (Wright et al., 2012). Furthermore, parentage analysis of six polymorphic microsatellite loci suggested a ratio of 1.0 breeding males to every breeding female at the leatherback rookery of Saint Croix, in the U.S. Virgin Islands, adding evidence that OSR may compensate for female-skewed sex ratio at birth (Stewart and Dutton, 2014).

The number of sea turtle nests in Fiji has declined over recent decades (Piovano et al., 2019; Piovano and Batibasaga, 2020; Prakash et al., 2020), resulting in an apparent but unquantified reduction in the number of breeders. The current low abundance of nests is spread over more than 300 islands and 500 islets, which greatly affects the possibility of encountering nesting females while patrolling only a few beaches during a nesting season that largely overlaps with the cyclone season. In this pilot study, the

identification of putative mothers and fathers via SNP analyses permitted an inference of OSR in hawksbill turtles nesting in the Mamanuca Group, suggesting a 1:1 ratio of breeding males to receptive females within the sets sampled in this study. The current OSR may reflect the sex ratios of hatchlings produced approximately two decades earlier, since hawksbill turtles mature when 17–22 years old (Snover et al., 2013). Long-term studies involving a larger number of nests can facilitate the identification of potentially adverse effects of increasing environmental temperatures on sea turtle OSR.

Conservation programs using sea turtle hatcheries, head-starting, or a combination of both to improve the chance of survival of local populations can also benefit from the genome-wide SNP genotyping approaches we have validated. In such cases, the parents are often unknown, and hatchlings from multiple nests and different pairs of parents are pooled together. In this study, genomic analyses identified seven family groups based on seven nests. Six groups were assigned to six distinct families, and another group comprising only two individuals was doubtful, as inclusion probability was not statistically significant. The number of individuals from each nest was unknown. The resulting smaller family in our sample consisted of 16 individuals, suggesting that a smaller number of individuals would lead to uncertainty.

Our findings show that hatchlings relatedness can be used to estimate the occurrence of multiple paternity, which allows for the estimation of the number of pairs of parents contributing to conservation programs. In this study, single paternity (monandry) was found in all of the three Fijian hawksbill turtle sets, where a total of seven full-sibling family groups were resolved across 217 hatchlings. Within each set, no offspring shared a putative mother with different putative fathers. Although this aligns with the current but limited knowledge of the hawksbill mating system, it most likely reflects the limited number of nests and hatchlings sampled. Indeed, the higher the percentage of hatchlings analysed and the larger the sample size of nests, the more likely multiple paternity will be found (Jensen et al., 2013). Nevertheless, the greater statistical power associated with use of many hundreds or thousands of SNPs can offset a low sample size by reducing the probability of incorrect assignment of parentage. The inclusion of maternal genotypes would further improve the capacity to precisely assess both maternal and paternal contributions.

Overall, this approach can also inform the conservation management of sea turtle rookeries: by prioritising the protection of those beaches with a higher number of pairs of parents to increase genetic resilience, and by directing conservation actions towards those rookeries where a female-skewed OSR inferred from high levels of multiple paternities can lead to population declines in the long run.

CRediT authorship contribution statement

Shritika S. Prakash: Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Monal M. Lal:** Data curation, Formal analysis, Visualization, Writing – review & editing. **Peter H. Dutton:** Writing – review & editing. **Ciro Rico:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Susanna Piovano:** Conceptualization, Funding acquisition, Supervision, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics statement

All procedures involving handling and sampling of young hawksbill turtles described herein were rigorously reviewed and subsequently approved by the University Research Ethics Committee of the University of the South Pacific, following the policies and guidelines stipulated in the Animal Research Ethics Handbook, before sample collection. The processes and procedures of live specimen handling and sampling complied with Fiji's regulation of animal protection as outlined in the Protection of Animals Act 1954 and authorised under the Fisheries Act Chapter 158, Regulation 27 (Ministry of Fisheries and Forests Certificate of Exemption by Public Officers to S.P., ref. 34/4/7 and FI/G/12-5). Individual turtles were placed on a sanitised polyethylene board, and their rear flipper was disinfected with isopropyl alcohol. A single 2 mm-long piece of skin tissue was removed from the trailing edge of the rear flipper using a single edge blade. Betadine was applied to avoid infection after sampling. Hatchlings were maintained for observation in a shallow water plastic basin for two hours and, upon resuming natural swimming behaviour, were released into their rearing tank. The permit to export samples from Fiji was issued by the Ministry of Environment (CITES Permit No. 16FJ/NV2000180) and the Ministry of Fisheries (Permit No. FJEXP-04302/15). The Department of Agriculture authorised the import permit from Australia (Permit No. IP15012938) under the Quarantine Act 1908 Section 13 (2AA).

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.rsma.2022.102174.

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