Low genetic diversity after a bottleneck in a population of a Critically Endangered migratory marine turtle species

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Abstract

Hawksbill turtles (*Eretmochelys imbricata*), which are distributed throughout the world's oceans, have undergone drastic declines across their range, largely due to anthropogenic factors. Assessing sizes, genetic variability and structure of their populations at global and regional levels is critical to the development of conservation management strategies. Here, nuclear and mitochondrial markers were used to analyse patterns of parentage and population structure in hawksbill turtles in United Arab Emirates (UAE) waters, utilising samples from two life stages (hatchlings and juveniles), and to compare the UAE population with neighboring populations. Weak genetic differentiation was detected between juveniles and hatchlings and between the nesting sites of Dubai and Sir Bu Nair. Parentage analysis suggested that only 53 females and 74-80 males contributed to the hatchlings from 67 nests across three nesting sites in UAE (Dubai, Sir Bu Nair, Abu Dhabi). No females were identified as nesting in more than one location. In Dubai and Abu Dhabi, single paternity was the norm (75%), whereas on Sir Bu Nair, multiple paternity was detected in the majority of nests (67%). Polygyny was also frequently detected on Sir Bu Nair (15% of the overall number of males), but not in the other nesting sites. Comparison of the UAE population with published data from other populations suggests that population structure exists both within the Gulf and between the Gulf and Indian Ocean populations, and that the UAE population has lower genetic variability than the Seychelles population. Finally, our data suggest that the UAE population, and the Gulf population overall, experienced a bottleneck/founder event. The observed overall low genetic variability, evidence of population structure in the Gulf, and strong differentiation between the Gulf and the Indian Ocean populations, raises concerns about the sustainability of this species in this near-enclosed basin. Our results highlight the need for regional collaboration in the development of management measures for the long-term conservation of this Critically Endangered species.

Keywords: conservation management, hawksbill turtle, United Arab Emirates, population genetics, Arabian/Persian Gulf.

Introduction

The hawksbill turtle (*Eretmochelys imbricata*) occurs throughout the world's tropical oceans (Witzell, 1983), and is considered Critically Endangered across its range by the International Union for the Conservation of Nature Red List (IUCN, 2015). Worldwide, hawksbill populations have been drastically reduced by the harvesting of eggs for food and the hunting of adult turtles for the use of their carapace as curios (McClenahan et al., 2006). As hawksbill populations continue to decline in many parts of the world, there is a need to better understand this species' biology, life history, nesting ecology, population trends, movements/migrations, as well as population structure and connectivity, in order to develop appropriate management measures and assist the recovery of populations.

Inferences from molecular genetics have transformed the study of sea turtles (Bowen and Karl, 2007; Lee, 2008), and advanced our knowledge on topics such as natal philopatry (Meylan et al., 1990), migration patterns (Bowen et al., 2005), sex-biased gene flow (FitzSimmons et al., 1997a), mating systems (Phillips et al. 2013, Tedeschi et al., 2015), effective population size (Phillips et al., 2014), and even hybridization (Lara-Ruiz et al., 2006). Despite this body of research, the sea turtle molecular ecology literature remains biased towards green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles (Bowen and Karl, 2007; Jensen et al., 2016, Lee, 2008; Matsuzawa et al., 2016; Shamblin et al., 2015; Tedeschi et al., 2015). By contrast, hawksbill turtles have been less well studied, with a bias towards populations of the western Atlantic (Bowen et al., 2007; Vela-Zuazo et al., 2008; Vilaça et al., 2013). Until recently, hawksbill molecular research in the Indian Ocean consisted of a set of location-specific studies with a non-standardised set of markers (e.g. mtDNA, various suites of microsatellites; Mortimer & Broderick, 1999; Phillips et al., 2013, 2014; Tabib et al., 2011; Zolgharnein et al., 2011). A recent Indo-Pacific-wide mtDNA study set out a much broader picture of regional population structure, but also highlighted the lack of country-specific information on hawksbill populations at both nesting and foraging grounds (Vargas et al., 2015).

The hawksbills of the Arabian/Persian Gulf (henceforth 'the Gulf') were one of the eight Indo-Pacific genetic stocks identified by Vargas et al. (2015). Despite potentially harsh conditions (e.g. a 22°C range in annual water surface temperatures (Carpenter et al., 1997; Sheppard et al., 2010)), the area supports considerable numbers of hawksbills, with 100-1000 individuals nesting each year in each of Saudi Arabia, Iran, the United Arab Emirates (UAE), and Qatar (e.g. Al-Ghais, 2009; Al-Merghani et al., 2000; EAD, 2007, 2015; Miller, 1989; Mobaraki, 2004; Pilcher, 1999, 2000, 2015; SCENR,

2006), with smaller numbers (<10 annual nesters) on the offshore islands of Kuwait (Meakins and Al Mohanna, 2004). In recent years, anthropogenic threats, including the harvesting of eggs on remote islands (EAD, 2007; Pilcher et al., 2014), the stranding of juvenile turtles due to cold stunning (hypothermic reaction in cold water temperature; Caliendo et al., 2010), drowning in fishing gear (EAD, 2007), and accelerating coastal development (Sheppard et al., 2010) have negatively affected populations and their habitats, threatening the future of the species in the area. However, many aspects of the ecology of the hawksbills in the Gulf, including movements, migrations, and population connectivity, are poorly known. Addressing some of these outstanding questions will help the design and implementation of effective management plans for the area's hawksbills.

In the UAE, monitoring has shown that hawksbills nest on the mainland in Dubai, on Abu Dhabi's inshore and offshore islands, and on the offshore island of Sir Bu Nair (EAD, 2007, 2015; Pilcher et al., 2014). Still, the numbers of females and males that may be contributing to these nesting beaches is not known, and nor is the degree to which these nesting beaches are interconnected. Work on Iranian hawksbills has indicated genetic differentiation between nesting beaches only 350km apart (Zolgharnein et al., 2011), but studies in other regions have detected no significant differentiation at 500km (Phillips et al., 2014). It is also unknown how juvenile turtles feeding in Gulf waters relate to the region's nesting beaches (e.g. see Bowen et al., 2007) and how many breeding sites the area sustains. Establishing such boundaries and connections is important in defining management units and in assessing the benefits/risks associated with particular environmental interventions/impacts (e.g. Bowen et al., 2007; Godfrey et al., 2007; Mortimer, 2007a, 2007b). This is particularly true in the UAE, where large-scale coastal developments, increasing effluents from desalination and electricity generation, and other human stressors are substantially changing the environment (Sheppard et al., 2010).

Here, molecular markers were used to investigate the hawksbill population of the UAE. The parentage patterns, population connectivity among nesting beaches, and the relationship of juveniles to those nesting beaches were assessed. Then, the UAE population was compared with other hawksbill populations from elsewhere in the Gulf and Indian Ocean using published molecular datasets. The aim of this study is to provide information on the genetic and demographic health of the UAE hawksbill population, and contribute to a better understanding of this species within and beyond the Gulf.

Materials and Methods

Study sites and samples

Tissue samples were collected from hatchling and stranded juvenile hawksbill turtles in the UAE. Samples were preserved in DMSO 20% NaCl2 5M.

Hatchling sampling

Samples were collected during nest monitoring by the Emirates Marine Environmental Group (EMEG), which undertook daily beach patrols from 6 pm to 6 am at these sites and a number of others in the UAE during the nesting season from early March to April (Fig. 1). Nests were excavated and checked for dead hatchlings one week after the first observed hatchling emergence. One to five freshly dead hatchlings per nest for each of 68 nests across three nesting areas were sampled: (1) Dubai = 23 nests, 2008-2010; (2) Abu Dhabi (Sir Bani Yas, Bu Tinah, Saadiyat Island)= 5 nests, 2009-2010; (3) Sir Bu Nair Island = 40 nests, 2010 (total hatchling samples = 295).

Juvenile sampling

Samples were collected from 123 stranded juvenile hawksbills reported from Abu Dhabi (n=16), Dubai (n=100), Sharjah (n=5), Ras Al Khaimah (n=1) and Sir Bu Nair (n=1) in the winter seasons between 2007 and 2010. After rehabilitation and prior to release, tissue was taken from the trailing edge of the forelimb using a sterile 6 mm biopsy punch. Based on carapace dimensions and body weight, all sampled juveniles were considered to be less than one year old at the time of stranding (Caliendo et al., 2010) therefore to have been born during the previous nesting season.

Molecular methods

DNA was extracted using an ammonium acetate method (Nicholls et al., 2000) and diluted to a working concentration of 10 ng/µl. Samples were genotyped at 33 variable microsatellite loci in three multiplex PCRs, following the methodology of Phillips et al. (2013). Amplification was conducted using Qiagen Multiplex PCR kit in 2 µl PCRs (Kenta et al., 2008; Phillips et al., 2013). PCR products were separated and sized on an ABI 3730 automated sequencer with ROX 500 size standard, and the resulting genotype traces scored in GeneMapper 3.7 (all Applied Biosystems). Individuals were removed entirely from subsequent analysis if data were missing for more than ten loci in total. Loci

were checked for the presence of null alleles in CERVUS 3.0 (Marshall et al., 1998) using a subsample of 32 juveniles.

All juvenile samples and one hatchling sample per nest were amplified for the mitochondrial control region (D-loop) using the primer pair LCM 15382/H950 (Abreu-Grobois et al., 2006). Amplification was conducted following the methodology described in Abreu-Grobois et al. (2006). PCR products were purified with QIAgen PCR purification columns and sequenced using the ABI dye-terminator method as implemented by MACROGEN. Mitochondrial DNA sequences were aligned using ClustalX (Thompson et al. 1997) and edited with BioEdit Alignment Editor v.7.0.9 (Hall, 1999).

Data analysis

Parentage analysis

Parentage analysis was conducted in COLONY 2.0 (Wang & Santure, 2009), which uses a maximum-likelihood method to assign parentage and sibship groups. Hatchling microsatellite genotypes were entered into COLONY, along with: A) maternal sibships known from field data, B) excluded maternal sibships known from mtDNA data, and C) per-locus estimates of genotyping error (0.011-0.023) derived from repeat PCR of 96 samples. The program was allowed to infer both polyandry and polygyny, and to estimate and update allele frequencies during analysis. Five runs of COLONY were performed, with all runs having 'medium' length and 'medium' likelihood precision, and each run having a different random number seed. A second batch of five runs was performed that included stranded juvenile genotypes.

Estimates of the number of females contributing to our sample of hatchlings were obtained directly from the COLONY outputs, after verifying that nest lay dates were compatible with inferred patterns of mother-sharing. Considering that a maximum of five offspring per nest were analysed in this study, our data would give a minimum estimate of the occurrence and percentage of multiple paternity. Therefore, to estimate the number of contributing males, three approaches were used. Firstly, all inferred cases of polyandry (multiple paternity) and polygyny were simply accepted. Secondly, all polyandry was accepted, but polygyny was only accepted if it occurred across two nesting seasons (see Phillips et al., 2014). Finally, polyandry was only accepted when based on ≥ 2 offspring per father, and polygyny only accepted when based on ≥ 2 offspring per nest.

Population genetics

For hatchling microsatellites, population genetics analyses on three subsets of the main dataset (three as representing more than 50% of the samples based on five samples/nest) were performed, with each subset including one randomly selected hatchling per maternal family. For population genetics of the juveniles, all stranded individuals that were not inferred by COLONY as having a full sibling elsewhere in the dataset were used. If full-sib relationships were indicated, one individual per sibship was chosen at random. Microsatellite observed (Ho) and expected (He) heterozygosities (Schneider & Excoffier, 1999) were calculated in Arlequin 3.5 (Excoffier et al., 2005). Deviation from Hardy-Weinberg equilibrium was tested for using Fisher's exact test and the Markov chain method (dememorization number, number of batches, iterations per batch all = 1,000; sequential Bonferroni correction applied). Allelic richness for each locus was calculated in FSTAT 2.9.3.2 (Goudet, 2001).

Population differentiation based on microsatellite data was estimated as F_{ST} using Arlequin (Michalakis & Excoffier, 1996). STRUCTURE 2.3 (Pritchard et al., 2000) was used to estimate the most probable number of putative populations (K) that can explain the patterns of genetic variability in our sample. The admixture and correlated allele frequency models was used, with burn-in and simulation lengths of 1,000,000 steps each, to test a K-range of 1-4, with ten repeat runs per K-value. The Evanno method (Evanno et al., 2005) in Structure Harvester (Earl et al., 2012) was then applied to estimate the most probable number of populations. Whether any particular individual was an immigrant or had an immigrant ancestor was tested by using the model with prior population information, subdividing the individuals into K populations, according to the results of the previous analysis. v (migration rate) = 0.05 and 0.1 was assumed, and G (number of generations) = 0, 1 and 2 was tested.

The programme BOTTLENECK 1.2 (Piry et al., 1999) was used with the microsatellite genotypes to test for recent changes in the genetic effective population size (N_e; Wright, 1931). BOTTLENECK compares the observed heterozygosity of a population sample to that expected under mutation-drift equilibrium, with a significant heterozygosity excess indicating a recent population contraction and significant heterozygosity deficit indicating a population expansion (Piry et al., 1999). The two-phase model (TPM) using the settings recommended by Piry et al. (1999; non-stepwise = 5%, variance = 12) was considered. As an additional bottleneck test, we calculated the Garza-Williamson M-ratio (a measure of 'gappiness' in microsatellite allele size distributions; Garza and Williamson,

2001) in Arlequin 3.5.

For the mtDNA data, the genetic differentiation (F_{ST}) nesting sites and age groups as for microsatellite data was estimated. Gene diversity (H), nucleotide diversity (π), Tajima's D Tajima (1989a, 1989b, 1993) and Fu's F_S (Fu, 1997) in each population was also estimated to test for bottleneck/founder event using Arlequin 3.5. A mismatch distribution analysis (Rogers and Harpending, 1992) was conducted using Arlequin 3.5 to test for population expansion, and the parameters tau (τ) and theta (θ) were also estimated. Time since expansion after the bottleneck was estimated as $t=\tau/2\mu$ following Schenekar & Weiss (2011), utilizing the following parameters: τ as estimated from our data, estimated mutation rate (μ) set as 1.2-2.4% substitution/site/per million years (Encalada et al., 1996), and generation time of 35 years (Mortimer and Donnelly, 2008). We used median-joining networks generated in NETWORK 4.6 (Bandelt et al., 1999; http://www.fluxus-engineering.com) to infer phylogenetic relationships among mtDNA haplotypes.

Comparisons with published hawksbill datasets

UAE microsatellite data were compared with a published dataset of 389 individuals from the Republic of Seychelles that were genotyped at the same set of loci (Phillips et al., 2014). All amplifications of UAE hawksbill DNA included 'control' Seychelles samples to verify consistency of allele scoring. For mtDNA, we used 202 published hawksbill mtDNA control region (D-loop) sequences from Iran (n=82), Saudi Arabia (n=13), and Seychelles (n=107) (Vargas et al., 2015; Supplementary Table 1).

Results

Microsatellite genotypes (with > 23 loci) were obtained from 353 of the 418 initial samples, of these 241 were from hatchlings (67 nests) and 112 were from juveniles. Three loci (Eim31, CcP7C08, CcP2H12) were then omitted from downstream analyses due to their having an estimated null allele frequency > 0.1. mtDNA D-loop haplotypes (852 bp) were obtained from 182 samples (68 hatchlings and 114 juveniles), from which 15 different haplotypes were identified.

Parentage analysis

Across all sites (Dubai, Abu Dhabi, Sir Bu Nair) and years (2008-2010), COLONY suggested that a total of 53 females and 74-80 males contributed to the parentage of the hatchlings of our sample set. In

Dubai, 16 females were inferred as the mothers at 22 nests sampled across three seasons (Table1). At this site, the genotyped offspring of 12/16 inferred females were sired by only a single male each, while for two females, offspring were inferred as sired by two males. This resulted in an estimate of 16-17 males contributing to paternity. Two nests with <3 samples were excluded from this analysis, following the criteria described in the methods. Polyandry was strongly indicated (at least two offspring per father) for one female that nested twice in the same season, and polygyny was strongly indicated (at least two offspring per father) for two females nesting in the same season (Supplementary Table 2). In Abu Dhabi, parentage at the five sampled nests was explained by four inferred females and four inferred males (one female had two nests), with no multiple paternity detected. The 40 nests sampled from Sir Bu Nair were inferred to belong to 33 females, with 58-60 males responsible for paternity. At this site, multiple paternity was high: 11/33 females showing single paternity (five nests with < 3 samples were not considered), five females were fertilized by at least two males/nest, and 11 females had paternity of their hatchlings shared between more than two males per nest. All five cases of two-father families were supported by at least two offspring per inferred male, but no cases of ≥ 3 males passed this criterion for all inferred males. Similarly, 11 cases of polygyny, where the same male fertilized eggs from the nests of >1 female, were inferred, but all occurred in the same season and none were supported by more than one hatchling per nest (Supplementary Table 2). No cases of a female nesting in more than one location were detected. Two to four males were indicated as having sired offspring in at least two nesting sites, but this inference was not consistent across runs of COLONY (3-4 runs per male), and no case was supported by two or more offspring per nest per male.

Among the 112 juveniles included in the COLONY analysis, three showed a consistent association with a nest in the hatchling dataset, suggesting a potential shared parent (i.e. half-siblings). A juvenile from Dubai (stranded in winter 2007/2008) clustered with a nest from Dubai laid in 2010; a juvenile from Dubai and one from Sharjah (both from winter 2010/2011) clustered with two nests from Sir Bu Nair laid the season before (2010). Twelve juveniles showed consistent association with other juveniles, forming five clusters, and only in one cluster were the juveniles sampled/stranded in the same winter season.

Population analysis

Summary statistics for the microsatellite data are reported in Table 2. Loci deviating from HW equilibrium with $P < 0.05$ were detected in five cases (one in the hatchlings dataset, four in the juveniles data set; Table 2). However, no locus deviated significantly in both the hatchlings and juvenile datasets, or after sequential Bonferroni correction. Private alleles were detected in both datasets, but were more frequent among the juveniles.

The analysis of the 15 mtDNA haplotypes identified 33 polymorphic sites and overall low gene and nucleotide diversity (H = 0.722, π = 0.0017). One haplotype was present in 50% of the samples, with the other haplotypes shared between hatchlings and juveniles and across location, except for five unique haplotypes identified only among the juveniles and one only in Sir Bu Nair hatchlings.

Microsatellite data suggested marginally significant but quantitatively weak differentiation between nesting sites (Dubai and Sir Bu Nair), with F_{ST} across the three random subsets ranging between 0.006-0.009, (P range = 0.073-0.024). No significant genetic differentiation was detected for the mtDNA data (F_{ST} = -0.006, P = 0.45); the Abu Dhabi nesting site was excluded from this analysis due to its small sample size (n = 4). There was no significant microsatellite-based differentiation among juveniles stranded in different years, except between those stranded in winter 2009/2010 and those stranded in winter 2010/2011 (F_{ST} = 0.08, $P < 0.016$). However, this comparison was not significant when juveniles were excluded from Abu Dhabi on the grounds that this site had substantially greater representation in the winter 2010/2011 samples (n = 14 in 2010/2011; n = 1 in 2009/2010; n = 0 in all previous years). No pairwise comparisons among juveniles showed significant structure at mtDNA. Comparison between hatchlings and juveniles suggested marginal, near-significant differentiation at the nuclear DNA (F_{ST} = 0.007, P = 0.058), but did not show significant genetic differentiation at mtDNA (F_{ST} = -0.003, P = 0.6, respectively).

UAE hawksbill population identity

Comparison of the UAE population data with published mtDNA control region (D-loop) data from three other nesting locations in the Gulf (Iran NW, Iran SE and Saudi Arabia; Vargas et al., 2015) and one location from the Indian Ocean (Seychelles; Phillips et al., 2013; Vargas et al., 2015) suggested clear population structure, both within the Gulf, and between the Gulf and the Indian Ocean population. In a median joining network (Fig. 3), all the mtDNA sequences from the Gulf's populations clustered together, except for one haplotype observed in both the UAE and the Iran SE populations, that was

highly divergent from the main cluster (15 mutation steps). The UAE and the Iran NW populations showed five and three unique haplotypes respectively. Pairwise population comparison showed significant genetic differentiation between several population pairs within the Gulf (Table 3).

Based on mtDNA, the hawksbill populations of the Gulf were strongly differentiated from those of the Seychelles (Table 3). Differentiation was also high between the UAE and Seychelles populations based on the microsatellite genotypes (30 loci; F_{ST} = 0.193, $P < 0.001$). Average gene diversity for the UAE and Seychelles' populations was 0.60 and 0.68 respectively. Analysis of the microsatellite data from the UAE and Seychelles populations in STRUCTURE (no location prior, and model parameters as given above) returned K = 2 (Ln'(K) = 7378.45, Delta K = 59.8, after Evanno method; Supplementary Table 3) as the most likely number of cluster, identifying two well-separated populations represented by the UAE and the Seychelles samples (Supplementary Fig. 1). Minimal mixing was suggested between the two populations, with only one individual (one juvenile from Dubai's winter 2010/2011) identified as being a third generation immigrant from Seychelles to the UAE (probability 95%).

Demographic history

Bottleneck analysis of microsatellite data from the UAE population showed no clear support for either a recent population contraction or expansion. The two-phases mutation model (T.M.P.) was not consistent with a scenario of a bottleneck and the allele frequencies showed no mode shift. However, the Garza-Williamson ratio was 0.38, substantially lower than the threshold of 0.68 considered indicative of a bottleneck (Garza & Williamson, 2001).

MtDNA-based analysis supported the bottleneck/founder event scenario for the UAE and Gulf populations considered as a whole: the median joining network showed a star-like shape formation, typical of possible recent bottleneck followed by population expansion (Fig. 2 & 3). The mismatch distribution analysis for both the UAE population and the 'whole Gulf' population fitted the model distribution suggesting recent sudden demographic expansion, whereas for the Seychelles population it identified a bimodal distribution typical of a population in equilibrium (Fig. 3, a and b). Tajima's D and Fu's Fs values for the overall UAE population, were both negative and significant (-2.162, P = 0.001; -5.739, P = 0.026), and among the lowest when compared with those of the other populations in the Gulf and Indian Ocean. This supports the occurrence of a demographic change event. The estimated

mean values of τ were 1.227 for the UAE population and 1.080 for the overall Gulf population, giving an estimated time since expansion of 29,373 – 58,700 years BP.

Discussion

While sample sizes in this study were relatively small, variable rates of parentage patterns (monogamy vs polygamy) were observed, with polyandry and polygyny unexpectedly frequent at one nesting site,. Population structure was weak between different nesting sites and between different life stages (hatchlings vs juveniles) in the UAE, but clear among different populations inhabiting the Gulf, and strong between the UAE and a neighboring Indian Ocean population (Seychelles). Overall the observed genetic diversity was low among the UAE samples, adding further concerns to the relatively small number of nests, females and males observed in this study, compared to those of other populations studied around the world (Allen et al., 2010; Beggs et al., 2007). Highly mobile species are generally expected to show homogeneous populations across wide geographic ranges. An increasing number of genetic studies demonstrate however, that, despite being capable of long distance movements and the absence of obvious ecological barriers, many marine species show marked population structure (Ansmann et al., 2012; Chapman et al., 2015; Knutsen et al., 2003). This stresses the importance of assessing population structure on a fine geographic scale in order to develop effective conservation management plans. In this paper, the results of the parentage and populations structure analysis of hawksbill turtles in the UAE, and then the population structure analysis in the Gulf are discussed, while considering the nearest well studied Indian Ocean population.

In the nesting site along the Dubai coastline, where monitoring efforts were comparable across years, the total number of nests observed over three years was 28, but parentage analysis suggested only 16 females and 15-19 males were responsible for the 22 nests (of those 28) analysed in this study. The fact that no returning females have been detected across years in our sample set is likely a consequence of reproductive trends previously documented for this species, where females have an average inter-nesting season varying between two to three years (Mortimer & Bresson, 1999), and potentially up to eight years in Saudi Arabian Gulf waters (Al-Merghani et al., 2000; Miller, 1989). The Dubai nesting location was heavily affected by land reclamation and dredging activities during the study period, which could have resulted in female turtles either failing to nest or choosing alternative nesting sites (Miller, 1989) that were not monitored during this study. Furthermore, our study did not

detect any females nesting across multiple sites, consistent with the high site fidelity, often associated with natal homing, reported for marine turtles in other studies (Bowen & Karl, 2007).

The difference in inferred multiple paternity rates between the Dubai (low) and Sir Bu Nair (high) nesting sites may suggest different female mating strategies or breeding grounds between mainland (Dubai) and offshore (Sir Bu Nair) nesters. Such differences may also indicate local variation in the number, density and distribution of males (see Jensen et al., 2006; Phillips et al., 2013). Our rate of multiple paternity observed in Sir Bu Nair (67%) is also much higher than what reported in literature for this species (Joseph & Shawn, 2011: 20%; Phillips et al., 2013: 9.3%), but lower of what observed in other turtles species like green turtles (71%, Joseph, 2006) and olive ridley (92%, Jensen et al., 2006). That some polygyny was also inferred suggests that mating may be taking place closer to the nesting beaches than in the case of the population studied by Phillips et al. (2013). This may be because of the relatively small area utilized by this species in the Gulf compared to the areas utilized by populations in the Indian Ocean. Polygyny is rarely documented in turtles (Crim et al., 2002) and in the hawksbill turtle population in Seychelles, undergone an extensive paternity study of 1600 samples hatchlings across 85 nests, it was not detected (Philips et al., 2013). The results presented here also suggest that males rarely gained paternity at multiple nesting sites (only 2-4 males detected mating with females from our two different nesting sites separated by 75km). This may indicate some degree of 'roaming' around nesting sites by males (Wright et al., 2012), or some degree of mating on migration routes (FitzSimmons et al., 1997b). Females roaming in search of mates cannot be ruled out as a source of inter-site polygyny, but seems unlikely – the high rate of multiple paternity suggests males are not hard to come by. Further sampling of nesting females and their hatchlings are needed to obtain a more accurate estimate of male population size.

Several of the stranded juvenile individuals were inferred as potential half-siblings of sampled hatchlings, suggesting these juveniles may have originated from UAE waters. However, the majority of the juveniles likely originate from non-sampled nesting sites, either in the UAE or other locations in the Gulf. The overall values of heterozygosity and allelic richness for the juvenile dataset were higher than those observed among the hatchlings, suggesting a different, larger pool of females are producing these juveniles. Furthermore, marginally significant genetic differentiation was observed at the nuclear DNA but not at the mtDNA between juveniles and hatchlings, although several unique haplotypes were identified among the juveniles (Fig. 2). Recent radio tracking data of females across different areas of

the Gulf (Qatar, Iran and UAE) has shown that they converge and spend a considerable amount of time in UAE waters (Pilcher et al., 2014a,b). The authors also suggest that female dispersal patterns may reflect hatchling dispersal, which supports our hypothesis of juveniles coming from different nesting grounds within the Gulf. Our data also revealed small but significant differentiation among different years of juveniles, but only for the year that included juveniles from Abu Dhabi (2010/2011), suggesting that those individuals may originate from a different stock of fathers, and that females nesting in the Abu Dhabi area may utilize different breeding grounds from those that produce the Dubai juveniles. This is however a tentative inference constrained by the size of our sample.

The findings of this work support the previously suggested idea of the presence of multiple separate populations of hawksbill turtles in the Gulf (Tabib et al., 2014; Vargas et al., 2015; Zolgharnein et al., 2011). Based on microsatellites, genetic differentiation between the two main nesting sites sampled in the UAE (Dubai and Sir Bu Nair) and between nesting sites and juveniles was low and only marginally significant. Furthermore, based on mtDNA, the overall UAE population showed significant differentiation from the Iran NW population, though not from Iran SE or Saudi Arabia. These results suggest a possible population boundary for hawksbill turtles between the northern and southern regions of the Gulf basin (Fig. 1). Such an effect may reflect prevailing current patterns in the region, characterized by a front between Qatar and Iran (John et al., 1999; Pilcher et al., 2015) that may function as barrier for the dispersal of turtles between the northern and the central-south area of the basin. The fact that Saudi Arabian hawksbills did not show any significant differentiation from the UAE (this study) or other Gulf populations (Vargas et al., 2015) may indicate mixing of populations along the eastern coast of the Gulf, following the anticlockwise pattern of the coastal current (Sheppard et al., 1992). This result, however, could also be due to a limited power of the analysis due to significantly smaller sample sizes (only 13 sequences available from this population, Vargas et al., 2015) and considering the overall low mtDNA variation reported among all Gulf populations. Population boundaries of highly mobile marine species have been shown to coincide with oceanographic features such as current patterns and nutrients concentration (Amaral et al., 2012; Bourjea et al., 2007; Moura et al., 2012; Natoli et al., 2005). Additional data from different life stages and different locations, paired with oceanographic analysis, would help further clarify hawksbill population structure across the Gulf and determine what factors may drive this. The UAE population was strongly differentiated from that of the Seychelles, the geographically closest well-studied

population in the Indian Ocean, at both nuclear and mtDNA markers, suggesting mixing between these populations is extremely limited. Only a single recent migrant (one Dubai juvenile with potential third-generation Seychelles ancestor) was inferred in the sample, suggesting some limited reproductive mixing between Gulf and Indian Ocean populations.

Tests for bottlenecks based on microsatellites were contradictory, and did not unequivocally support a recent bottleneck event for the UAE population; however, tests based on microsatellites are not always reliable in detecting population reductions (Peery et al., 2012), especially if natural populations are interconnected through some degree of dispersal (Bush et al., 2007). The Garza Williamson ratio is recognized to be more sensitive especially in the case of older bottleneck events (Brook et al., 2011). In contrast, our mtDNA data suggest that the UAE, and the overall Gulf hawksbill population, originated from a single founder event, and then underwent a population expansion (see also Vargas et al., 2015). The UAE's Tajima's D and Fu's Fs values are among the lowest when compared with those of other populations in the Gulf and those of the Seychelles population (see Vargas et al., 2015). Furthermore, both values are negative and significant, suggesting that the population is still not at equilibrium. The mismatch distribution suggested a recent population expansion. The estimates for time since expansion (29,373 – 58,700 years BP) did not coincide with the recent formation of the Gulf (approx. 6,000 years BP (Kassler et al., 1973; Lambeck, 1996; Lambeck et al., 2002) and may suggest that a bottleneck may have affected an original turtle population inhabiting the adjacent Indian Ocean and only after colonizing the Gulf waters once they became available. It should be noted that in animals, long-term substitution rates are recognized to be lower (up to two orders of magnitude) than mutation rates observed across shorter periods (Ho et al., 2005, 2007). It is therefore likely that our estimates are overestimating the time since expansion. Utilizing a mutation rate of one order of magnitude higher, we obtain a time since expansion for the Gulf population between 5,993-2,998 years BP. Based on the original estimated dates (c.a. 29- 59 kya), the bottleneck happened before the colonization of Gulf waters. Another highly mobile species, the humpback whale (*Megaptera novaeangliae*), shows strong population differentiation, lack of migration and evidence of bottleneck in the northern Indian Ocean (Pomilla et al., 2014), coinciding with a geological period (60-18 kya) characterized by drastic climate changes in this basin (Banakar et al., 2010; Singh et al., 2011). Such an event may also be responsible for the pattern observed in the Gulf hawksbills. The analysis of hawksbill samples from populations from the adjacent northern Indian

Ocean, together with a more accurate estimate of the mutation rate for this species based on ancient DNA analysis, will help in clarifying the timing of colonization of the Gulf and the bottleneck.

Conclusions

Information about the population structure and identity of hawksbill turtles at a small geographic scale is still scarce in many regions, hampering the formulation of effective conservation measures. Our study furthers our understanding of the population structure of hawksbill turtles in the UAE and in Gulf waters, confirming the presence of population genetic structure in the Gulf, strong differentiation from the Indian Ocean populations, and suggesting fine population structure in UAE waters. This emphasizes the importance of regional cooperation in the research, management and conservation of this species in this semi-enclosed basin. Long-term monitoring of multiple nesting sites across the region, integration of different techniques (Dunbar et al., 2015, Williams et al., 2015), and methodical collection of samples would help address outstanding questions, such as whether mainland and offshore nesting females source different males or breeding grounds. Considering the low numbers of females confirmed in this study and the low genetic variability observed in the population, we suggest that immediate conservation measures be put in place to protect the remaining nesting sites and thereby help secure the future of this species in the UAE.

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Table 1: Summary information on nesting locations of hawksbill turtles in the United Arab Emirates with number of nests observed at each site during each nesting season, number of nests successfully analysed (samples with less than 10 missing loci), and number of mothers inferred by parentage analysis for each location for each nesting season. The juvenile sample groups with "*" include one (2009-2010) and two (2010-2011) samples from Sharjah respectively. 'NA' (Not Applicable) indicates locations where all nests were not monitored.

Nests					**Juveniles**	
Location	**Nesting season**	**Nests observed**	**Nests analysed**	**Mothers counted after analysis**	**Winter season**	**Samples analysed**
Dubai					2007-2008	18
	2008	2	2	2		
					2008-2009	27
	2009	7	7	5		
					2009-2010	10*
	2010	19	13	9		
					2010-2011	39*
	Subtotal	**28**	**22**	**16**		**94**
Sir Bu Nair					2009-2010	1
	2010	40	40	33		
	Subtotal	**40**	**40**	**33**		**1**
Abu Dhabi	2009	NA	2	2		
					2009-2010	1
	2010	NA	3	2		
					2010-2011	15
	Subtotal	NA	**5**	**4**		**16**
Ras Al Khaimah	NA	NA	NA	NA	2010-2011	1
	Subtotal					**1**
Total		**NA**	**67**	**53**		**112**

Table 2: Microsatellite genotypes from hawksbill turtles in the United Arab Emirates indicating allelic richness, number of alleles, number of private alleles (in parenthesis), heterozygosity expected (He) and observed (Ho) for hatchlings and juveniles.

Nests, N=53						**Juveniles N=104**				
Loci	**All. Rich**	**N alleles**	**Ho**	**He**	**P-value**	**All. Rich**	**N alleles**	**Ho**	**He**	**P-value**
Cc1	7.482	8	0.765	0.731	0.757	8.518	13 (5)	0.691	0.700	0.663
Cc13	6.632	7	0.708	0.732	0.393	6.488	9 (2)	0.716	0.727	0.352
Cc2	3.755	4 (1)	0.654	0.599	0.983	3	3	0.505	0.589	0.108
Cc28	3.942	4	0.538	0.616	0.525	3.778	4	0.569	0.574	0.505
CcP1G03	6.558	7 (1)	0.460	0.586	0.143	5.924	6	0.604	0.603	0.353
CcP7D04	6.833	7	0.723	0.834	0.070	6.435	7	0.826	0.832	0.545
CcP7E11	7.516	8	0.776	0.689	0.399	10.068	11 (3)	0.734	0.769	0.579
CcP8E07	10.55	11 (1)	0.769	0.792	0.739	8.581	12 (2)	0.657	0.707	*0.010*
Cm58	5.755	6	0.731	0.749	0.682	6.024	7 (1)	0.637	0.743	0.051
D1	12	12 (1)	0.513	0.582	0.137	13.202	16 (5)	0.685	0.702	0.678
D110	3.999	4	0.365	0.393	0.480	4.37	5 (1)	0.388	0.361	0.757
Ei8	7.538	8	0.804	0.781	0.835	7.557	8	0.760	0.781	0.910
Eim11kpb	8.989	9	0.940	0.846	0.940	9.992	11 (2)	0.794	0.822	0.277
Eim17	5.718	6	0.647	0.707	0.534	6.316	8 (2)	0.737	0.700	0.804
Eim19	3.937	4 (1)	0.569	0.541	*0.037*	2.971	3	0.598	0.533	0.421
Eim38high	4.985	5	0.408	0.368	0.763	4.948	5	0.451	0.442	0.741
Eim41	5.723	6 (2)	0.547	0.556	0.176	3.863	4	0.524	0.559	0.095
HKB17	3	3	0.500	0.605	0.082	3	3	0.705	0.590	0.070
HKB24	4.755	5 (1)	0.712	0.702	0.895	4.388	5 (1)	0.612	0.674	0.355
HKB25	4.8	5	0.673	0.695	0.986	4.392	5	0.667	0.641	0.353
HKB26	4.755	5 (1)	0.538	0.570	0.241	4.781	5 (1)	0.525	0.528	0.484
HKB29	4	4	0.538	0.643	0.105	4	4	0.718	0.689	0.263
HKB30	5	5	0.551	0.663	0.098	4.979	5	0.588	0.616	0.823
HKB31kpb	3.769	4	0.627	0.612	0.649	4.682	6 (2)	0.570	0.592	*0.005*
Or14	2	2	0.333	0.353	0.697	3.203	4 (2)	0.398	0.430	*0.017*
Or18	4	4	0.736	0.716	0.486	4.776	6 (2)	0.631	0.692	*0.001*
Or2	8	8	0.846	0.859	0.910	9.415	11 (3)	0.882	0.871	0.740
Or4	5.738	6	0.731	0.698	0.799	6.009	7 (1)	0.718	0.724	0.888
Or7	8.469	9 (2)	0.731	0.741	0.162	8.164	10 (3)	0.706	0.749	0.666
Cc117	7.741	8 (1)	0.808	0.818	0.368	8.236	9 (2)	0.786	0.825	0.405

Table 3: F_{ST} analysis based on mtDNA (control region D-loop sequence) data available on hawksbill turtles from this study (United Arab Emirates: UAE) and published data from Iran, Saudi Arabia and the Seychelles (Vargas et al., 2015). Number of samples analysed for each locations are reported in parenthesis. P values significance as follows: P ≤ 0.01 = **, P ≤ 0.001 = ***.

Population	UAE	Iran NW	Iran SE	Saudi Arabia
UAE (162)	-			
Iran NW (41)	0.066**	-		
Iran SE (41)	-0.004	0.084**	-	
Saudi Arabia (13)	0.026	0.028	0.05	-
Seychelles (107)	0.247***	0.380***	0.246***	0.318***

Supplementary Table 1: Summary table of all the hawksbill turtle mtDNA control region (D-loop) sequences utilized in this study. From left to right, name of the haplotype, Genebank accession number, origin of the haplotype and respective frequency in each population. Abbreviations of the locations as follows: UAE (United Arab Emirates; this study); Iran Northwest (Iran NW); Iran Southeast (Iran SE), Saudi Arabia and Seychelles (from Vargas et al., 2015).

Haplotype	GenBank #	UAE	Iran NW	Iran SE	Saudi Arabia	Seychelles	Total
EiIP-10	KT934057		1			0	1
EiIP-11	KT934058		1			0	1
EiIP-12	KT934059	2		1		0	3
EiIP-13	KT934060	7		2		0	9
EiIP-14	KT934061	7		3		0	10
EiIP-15	KT934062	3		1		0	4
EiIP-16	KT934063					12	12
EiIP-17	KT934064					44	44
EiIP-18	KT934065					29	29
EiIP-19	KT934066					5	5
EiIP-20	KT934067					1	1
EiIP-21	KT934068					2	2
EiIP-22	KT934069					2	2
EiIP-23	KT934070	2				0	2
EiIP-25	KT934072					2	2
EiIP-27	KT934074	9			3	0	12
EiIP-30	KT934077					3	3
EiIP-33	KT934080	80	32	20	9	5	146
EiIP-36	KT934082	7	5	4	1	0	17
EiIP-40	KT934085	15		7		0	22
EiIP-41	KT934086	18	1	3		0	22
EiIP-42	KT934087		1			0	1
EiIP-75	KT934098					1	1
EiIP-76	KT934099					1	1
EIUAE03		3				0	3
EIUAE08		2				0	2
EIUAE11		4				0	4
EIUAE13		1				0	1
EIUAE15		2				0	2
Total		162	41	41	13	107	364

Supplementary Table 2: Number of male hawksbill turtles detected at each nesting location in the United Arab Emirates according to three different approaches: 1) all inferred cases of polyandry (multiple paternity) and polygyny were accepted, 2) all polyandry was accepted, but polygyny was only accepted if it occurred across two nesting seasons, 3) polyandry was only accepted when based on ≥ 2 offsprings per father, and polygyny only accepted when based on ≥ 2 offsprings per nest. Nesting location and number of nests analysed for each location is reported for each column.

Approach	Dubai (22)	Abu Dhabi (5)	Sir Bu Nair (40)
1	16	4	59
2	19	4	79
3	15	4	33

Supplementary Table 3: Table output of the Evanno method implemented with STRUCTURE HARVESTER (Earl & vonHoldt, 2012) applied on the STRUCTURE analysis performed on the United Arab Emirates and Seychelles hawksbill turtle populations.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	Ln''(K)	Delta K
1	10	-54629.58	0.16	NA	NA	NA
2	10	-47251.13	121.74	7378.45	7278.28	59.78
3	10	-47150.96	173.93	100.17	193.66	1.11
4	10	-47244.45	180.48	-93.49	NA	NA

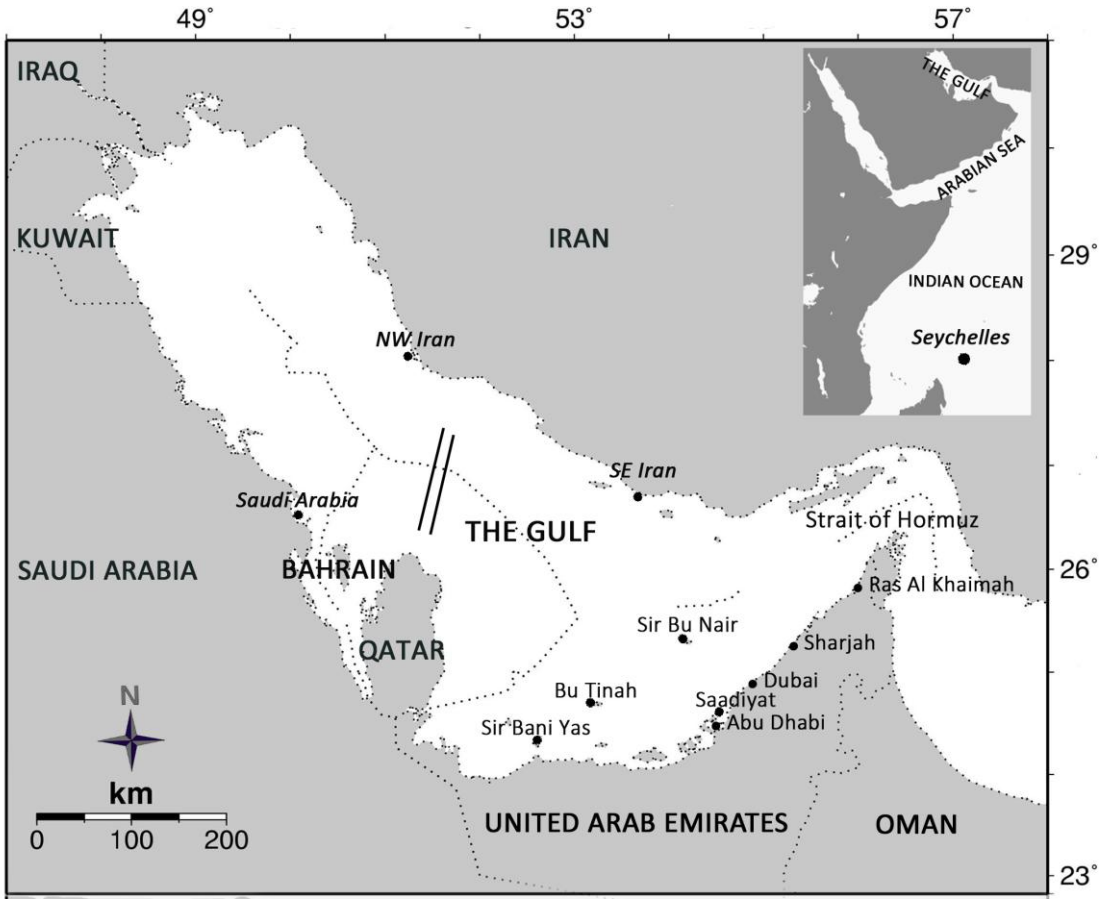
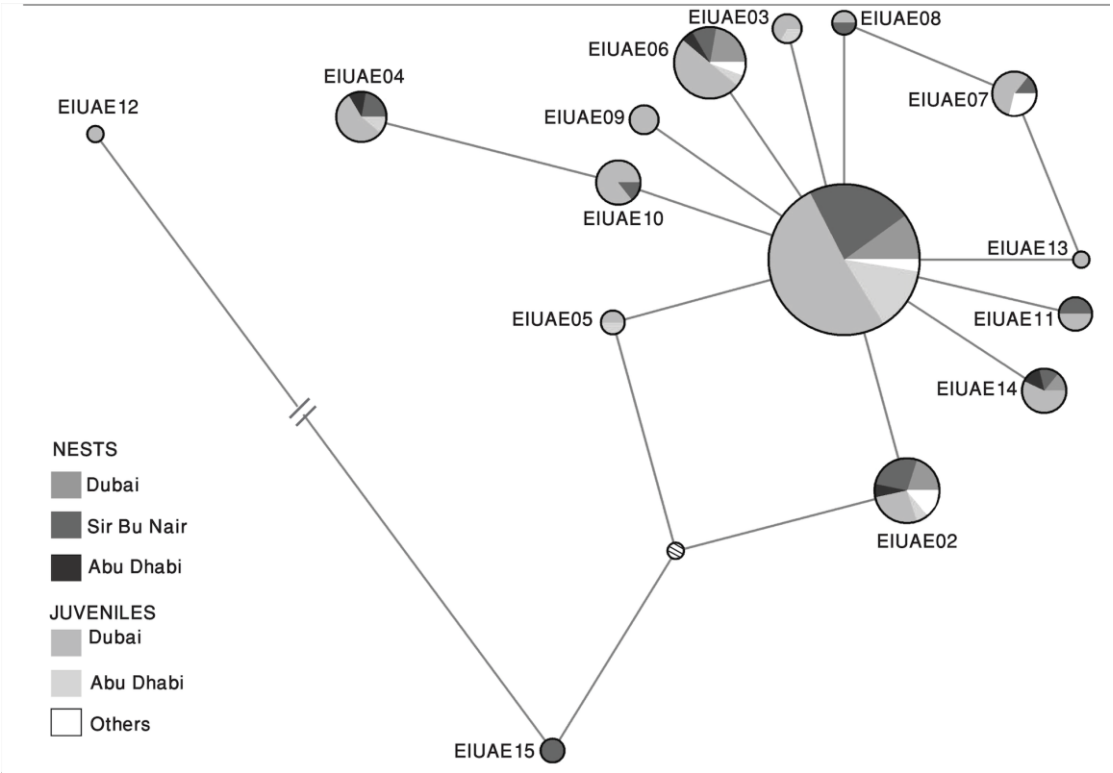


Figure 1: Hawksbill turtle nesting and stranding sampling locations across the United Arab Emirates considered in this study. The location of the published populations utilized as comparison in this study are italicized (Philips et al., 2014; Vargas et al., 2015). Parallel lines in the middle of the Gulf indicate the location of the central front that characterises the current pattern of the basin.



774
775 **Figure 2:** Media joining network based on 852 bps of the mtDNA control region (D-loop) of 162
776 hawksbill turtle individuals from the United Arab Emirates. Each circle represents a different haplotype
777 detected in the population. Size of circle is proportional to haplotype frequency. Color codes for each
778 site are shown in the illustration. Striped circles indicate ancestral extinct or un-sampled haplotypes.
779 The broken line indicates 15 bps mutations steps distance.
780

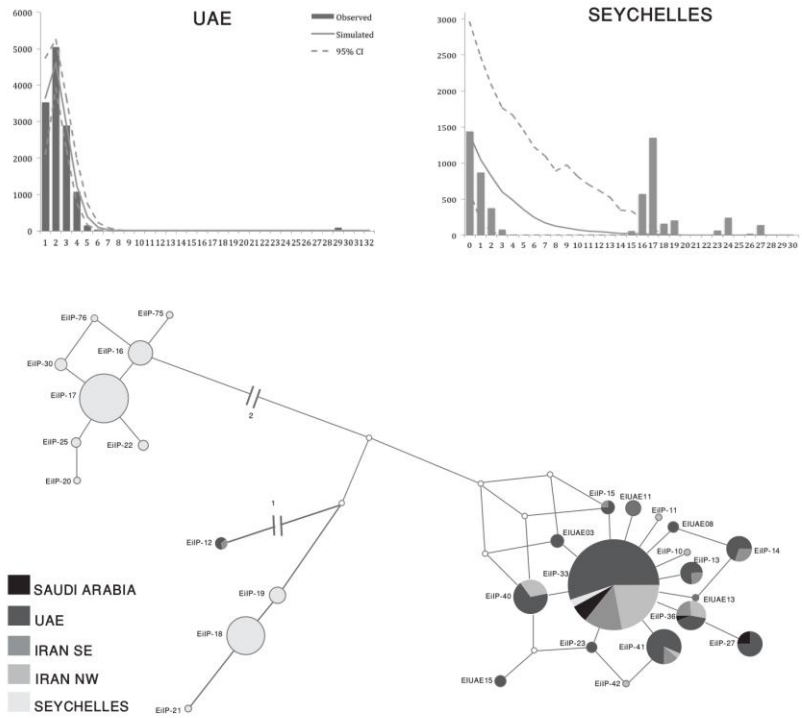


Figure 3: Median Joining Network based on 766 bps of the mtDNA control region (D-loop) of hawksbill turtle samples from the United Arab Emirates (UAE), Iran NW, Iran SE, Saudi Arabia and Seychelles populations. Number of sequences utilized for each population are reported in Supplementary Table 1. Each circle represents a different detected haplotype. The size of the circles is proportional to the frequency of each haplotype. Broken lines indicate 15 bps mutations steps distance (1) and 25 mutation steps distance (2). White circles indicate ancestral extinct or unsampled haplotypes. Graphs a) and b) show the mismatch distribution profile for the UAE population and the Seychelles population respectively.

Supplementary Figure 1: Structure analysis of the United Arab Emirates and the Seychelles hawksbill turtle populations based on 30 microsatellite loci. Estimated proportions of the coefficient of admixture of each individual's genome (y axes) that originated from population K, for K=2. Each individual is represented by a column (x axes). Geographic origin of the samples is reported below the x axis. The asterisk " * " indicates a third generation migrant individual.

Figure

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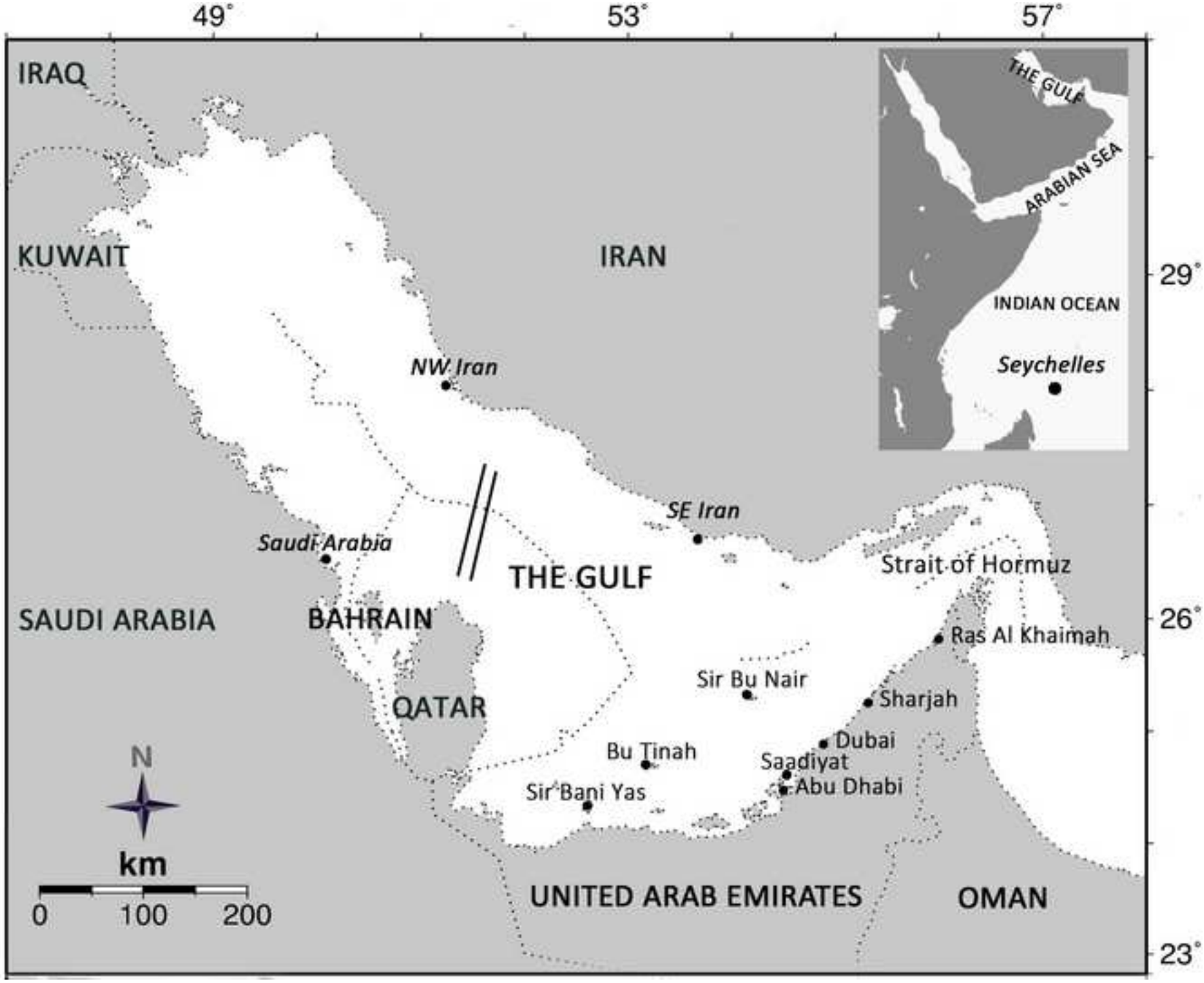

Figure

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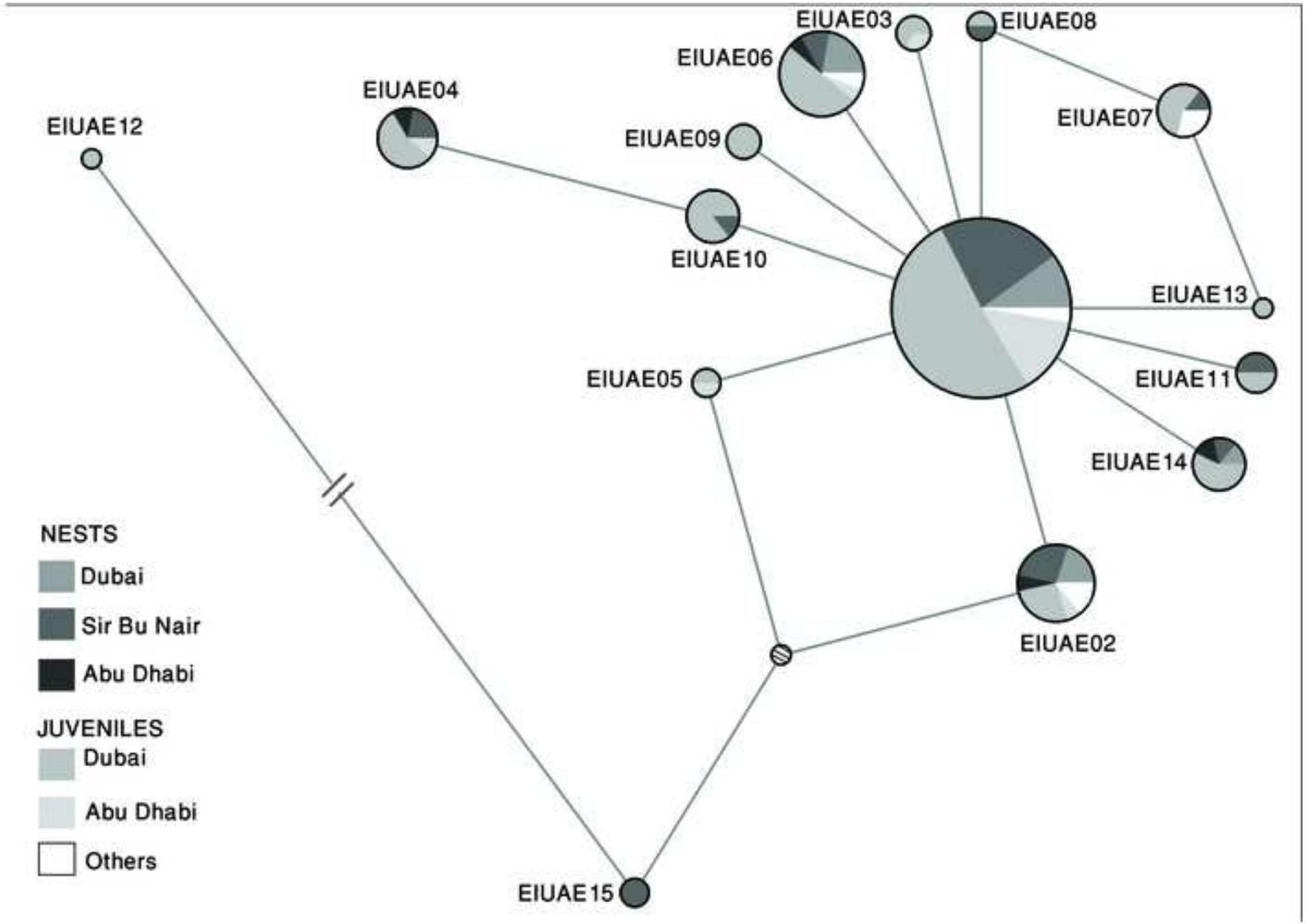

Figure

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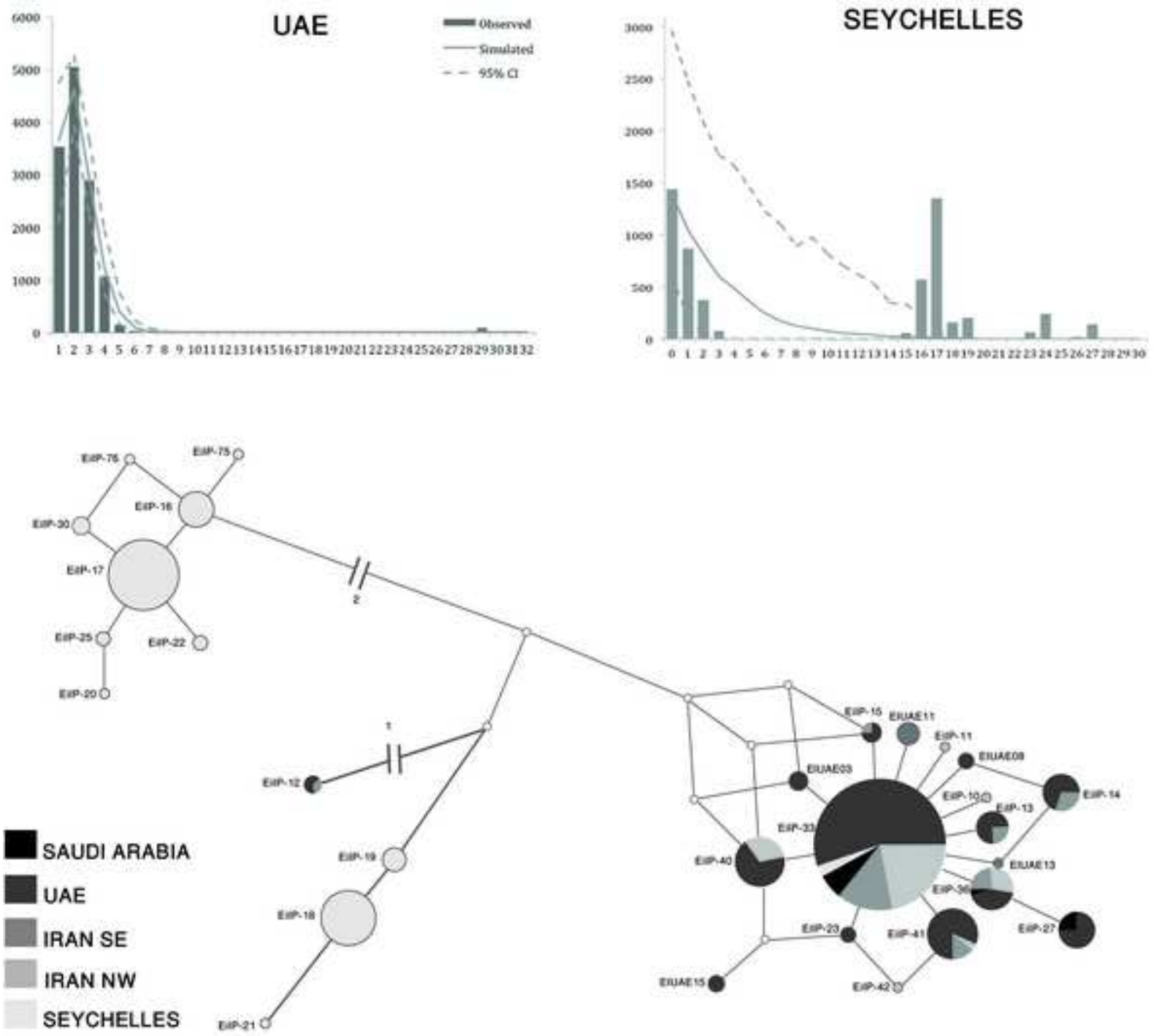

Table

Table 1: Summary information on nesting locations of hawksbill turtles in the United Arab Emirates with number of nests observed at each site during each nesting season, number of nests successfully analysed (samples with less than 10 missing loci), and number of mothers inferred by parentage analysis for each location for each nesting season. The juvenile sample groups with "*" include one (2009-2010) and two (2010-2011) samples from Sharjah respectively. 'NA' (Not Applicable) indicates locations where all nests were not monitored.

Nests					**Juveniles**	
Location	**Nesting season**	**Nests observed**	**Nests analysed**	**Mothers counted after analysis**	**Winter season**	**Samples analysed**
Dubai					2007-2008	18
	2008	2	2	2		
					2008-2009	27
	2009	7	7	5		
					2009-2010	10*
	2010	19	13	9		
					2010-2011	39*
	Subtotal	**28**	**22**	**16**		**94**
Sir Bu Nair					2009-2010	1
	2010	40	40	33		
	Subtotal	**40**	**40**	**33**		**1**
Abu Dhabi	2009	NA	2	2		
					2009-2010	1
	2010	NA	3	2		
					2010-2011	15
	Subtotal	NA	**5**	**4**		**16**
Ras Al Khaimah	NA	NA	NA	NA	2010-2011	1
	Subtotal					**1**
Total		**NA**	**67**	**53**		**112**

Table

Table 2: Microsatellite genotypes from hawksbill turtles in the United Arab Emirates indicating allelic richness, number of alleles, number of private alleles (in parenthesis), heterozygosity expected (He) and observed (Ho) for hatchlings and juveniles.

Nests, N=53						**Juveniles N=104**				
Loci	**All. Rich**	**N alleles**	**Ho**	**He**	**P-value**	**All. Rich**	**N alleles**	**Ho**	**He**	**P-value**
Cc1	7.482	8	0.765	0.731	0.757	8.518	13 (5)	0.691	0.700	0.663
Cc13	6.632	7	0.708	0.732	0.393	6.488	9 (2)	0.716	0.727	0.352
Cc2	3.755	4 (1)	0.654	0.599	0.983	3	3	0.505	0.589	0.108
Cc28	3.942	4	0.538	0.616	0.525	3.778	4	0.569	0.574	0.505
CcP1G03	6.558	7 (1)	0.460	0.586	0.143	5.924	6	0.604	0.603	0.353
CcP7D04	6.833	7	0.723	0.834	0.070	6.435	7	0.826	0.832	0.545
CcP7E11	7.516	8	0.776	0.689	0.399	10.068	11 (3)	0.734	0.769	0.579
CcP8E07	10.55	11 (1)	0.769	0.792	0.739	8.581	12 (2)	0.657	0.707	*0.010*
Cm58	5.755	6	0.731	0.749	0.682	6.024	7 (1)	0.637	0.743	0.051
D1	12	12 (1)	0.513	0.582	0.137	13.202	16 (5)	0.685	0.702	0.678
D110	3.999	4	0.365	0.393	0.480	4.37	5 (1)	0.388	0.361	0.757
Ei8	7.538	8	0.804	0.781	0.835	7.557	8	0.760	0.781	0.910
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HKB30	5	5	0.551	0.663	0.098	4.979	5	0.588	0.616	0.823
HKB31kpb	3.769	4	0.627	0.612	0.649	4.682	6 (2)	0.570	0.592	*0.005*
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Or7	8.469	9 (2)	0.731	0.741	0.162	8.164	10 (3)	0.706	0.749	0.666
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