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**Authors for correspondence:**

Lydia Hildebrand Furness

e-mail: [lydiafu@uio.no](mailto:lydiafu@uio.no)

Bastiaan Star

e-mail: [bastiaan.star@ibv.uio.no](mailto:bastiaan.star@ibv.uio.no)

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# Population structure of *Dugong dugon* across the Indo-Pacific revealed by historical mitogenomes

Lydia Hildebrand Furness<sup>1</sup>, Oliver Kersten<sup>1</sup>, Aurélie Boilard<sup>1</sup>, Lucy Keith-Diagne<sup>2</sup>, Cristina Brito<sup>3</sup>, James H. Barrett<sup>4</sup>, Andrew Kitchener<sup>5,6</sup>, Richard Sabin<sup>7</sup>, Shane Lavery<sup>8</sup>, Stephanie Plön<sup>9,10</sup> and Bastiaan Star<sup>1</sup>

<sup>1</sup>Center for Ecological and Evolutionary Synthesis, Department of Biosciences, University of Oslo, Oslo 0313, Norway

<sup>2</sup>African Aquatic Conservation Fund, BP 80 Joal 23015, Senegal, West Africa

<sup>3</sup>NOVA University Lisbon, Lisbon 1099-085, Portugal

<sup>4</sup>Norwegian University of Science and Technology, Trondheim 7491, Norway

<sup>5</sup>Department of Natural Sciences, National Museums Scotland, Edinburgh EH1 1JF, UK

<sup>6</sup>School of Geosciences, University of Edinburgh, Edinburgh EH10 5HF, UK

<sup>7</sup>Vertebrates Division, Natural History Museum, London SW7 5BD, UK

<sup>8</sup>School of Biological Sciences, University of Auckland, Auckland 1010, New Zealand

<sup>9</sup>Department of Pathology, Stellenbosch University, Stellenbosch, South Africa

<sup>10</sup>Bayworld Centre for Research and Education, Port Elizabeth, 7602, South Africa

LHF, 0009-0003-0964-0215; JHB, 0000-0002-6683-9891; AK, 0000-0003-2594-0827; SP, 0000-0002-9649-226X; BS, 0000-0003-0235-9810

*Sirenia*, an iconic marine taxon with a tropical and subtropical worldwide distribution, face an uncertain future. All species are designated 'Vulnerable' to extinction by the IUCN. Nonetheless, a comprehensive understanding of geographic structuring across the global range is lacking, impeding our ability to highlight particularly vulnerable populations for conservation priority. Here, we use ancient DNA to investigate dugong (*Dugong dugon*) population structure, analysing 56 mitogenomes from specimens comprising the known historical range. Our results reveal geographically structured and distinct monophyletic clades characterized by contrasting evolutionary histories. We observe deep-rooted and divergent lineages in the East (Indo-Pacific) and obtain new evidence for the relatively recent dispersal of dugongs into the western Indian Ocean. All populations are significantly differentiated from each other with western populations having approximately 10-fold lower levels of genetic variation than eastern Indo-Pacific populations. Additionally, we find a

significant temporal loss of genetic diversity in western Indian Ocean dugongs since the mid-twentieth century, as well as a decline in population size beginning approximately 1000 years ago. Our results add to the growing body of evidence that dugong populations are becoming ever more susceptible to ongoing human action and global climate change.

## 1. Background

The field of ocean research is undergoing a paradigm shift with increased awareness of and compassion for species threatened by extinction due to human activity [1]. However, the significance of marine resource exploitation by past human societies remains poorly understood on a global scale [2]. This lack of knowledge especially applies to tropical and subtropical regions, which also comprise biodiversity hotspots at a disproportionately high risk from global climate change [3]. Moreover, these regions have been significant focal points of human hunting in the past with evidence of marine exploitation going back thousands of years [4]. In order to obtain a better understanding of global anthropogenic impacts [5], we require further investigation and focus on these tropical and subtropical marine ecosystems, and their key components.

The dugong (*Dugong dugon*) is an iconic, large, herbivorous marine mammal that inhabits tropical and subtropical shallow coastal regions and seagrass forests across the Indo-Pacific from East Africa to Vanuatu [6]. The dugong is the only surviving species of the once diverse and widespread Family *Dugongidae* [7], and is one of four extant sea cow (*Sirenia*) species. Their herbivory has important ecological consequences, exerting a significant top-down influence within seagrass meadows that is integral to ecosystem dynamics, productivity [8,9] and carbon sequestration [10,11]. At present, the dugong occurs in specific areas of the Indian Ocean, Red Sea, Persian Gulf and western Pacific Ocean [12]. The species has a long history of cultural and economic importance. Humans have settled near and used riverine and nearshore environments for millennia [13], and so it is unsurprising that the animals humans shared these environments with were both hunted and revered by Indigenous cultures [14]. For example, a long history of exploitation is evident from extensive archaeological bone mounds in northeastern Australia [15–17] and Neolithic sites on the Arabian Peninsula [18]. For many Indigenous peoples, the dugong was an important part of spiritual/magico-religious practice; their unusual shape and elusive behaviour gave them a cryptic and supernatural quality, with communities from the Torres Straits and Flores to Madagascar having complex hunting rites associated with their capture [17,19]. Nowadays, dugongs have become important for tourism, particularly at attractive dive sites in the Red Sea where divers can observe individuals in clear waters in their natural habitat [20].

The conservation status of the dugong is classified as ‘Vulnerable’ by the IUCN [6] with the primary population of East Africa classified as ‘Critically Endangered’ and one in New Caledonia as ‘Endangered’ [21,22]. The dugong is threatened by coastal development, illegal hunting, pollution, environmental degradation, entanglements and vessel collisions [23–26]. Their near exclusive dependence on tropical marine seagrasses [27,28] in coastal habitats makes them particularly vulnerable to both direct and indirect impacts of human activity. The dugongs’ range has been reduced by increasing population fragmentation due to ongoing human action. While post-colonial hunting rates from commercial industries are known to have been wholly unsustainable [29], the duration of such intensive exploitation remains unknown. Based on archaeological and historical evidence [30–32], it is probable that unsustainable practices had been established long before the modern era, and that the dugong may have been suffering prolonged human exploitation [14,16]. To date, the majority of dugong studies comprise localized population studies, focusing on specific seascapes and regionally distinct threats [33–39]. Localized extinctions in the South China Sea and Okinawa have been reported in recent years [40,41]. The main, substantial populations now persist in regions including Australia [42], the Persian Gulf [36] and New Caledonia [43]. The general population trend is one of decline, and the risk of extinction is particularly high in island groups [43]. Given these conservation concerns, we urgently need to broaden our understanding of dugong population structure and dispersal potential.

Despite the lack of obvious physical barriers, and the observed ability of some individuals to travel long distances (i.e. greater than 100 km) [44], mtDNA control region and microsatellite data show that distinct population clusters exist within the dugong range [12,45], and that populations have

potentially greater genetic diversity in the Indo-Australian region [12,46]. In contrast, little geographic structuring has been detected among other populations, specifically in the western Indian Ocean [12]. A recent assessment of New Caledonian dugong population health, using the mtDNA control region, has revealed a genetically depauperate population, indicating that even some of the largest global dugong populations are now at significant risk of genomic degradation [43]. Nuclear whole genome data are limited to Australian waters, which still support the largest global population [47], and these data indicate a general decline in abundance since the last interglacial [48]. Our understanding of dugong population structure is therefore limited by the resolution of methods employed (e.g. [12]) or spatial scale, (e.g. [45] or [48]). As such, we are lacking a comprehensive population genetic assessment of the entire dugong range.

Recent advances in ancient DNA (aDNA) techniques have significantly altered our ability to obtain and analyse genomic data from historical and archaeological specimens [49] to be used in studying the biogeography of marine taxa [50–57] and conservation genomics [58]. Such advances have allowed the genomic analyses of historical museum specimens, which are of great relevance for species that are difficult or expensive to sample across their range.

In this study, we use established aDNA techniques to analyse the mitogenomes of 56 historical dugong individuals, from a sample set of 76 museum specimens, comprising the range of the species. Our main objective is for the improved resolution obtained by using entire mitogenomes to give a better insight into the population genetic structure and variation of the species. With these novel data, we expected to discover further geographic structuring that would elucidate prior findings, and possibly detect a loss of genetic diversity concurrent with archaeologically evidenced ancient hunting practices. Given the conservation concerns we have outlined, a comprehensive study such as this is urgently need to broaden our understanding of dugong population structure and dispersal potential in the context of ongoing environmental change.

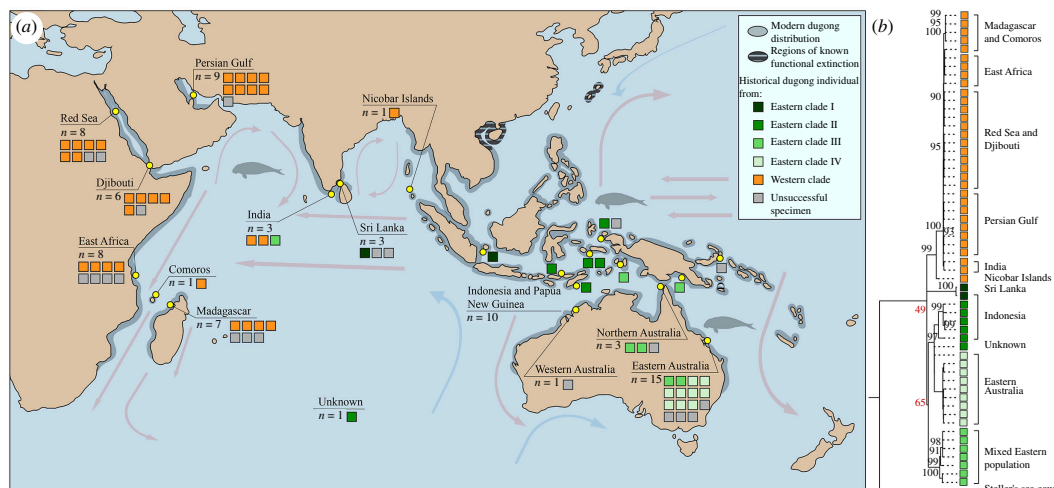
## 2. Materials

### 2.1. Taxon sampling

We sampled 76 individuals from known and major populations that span nearly all of the current and historical range of *D. dugon* (figure 1a). These individuals comprise 12 historical *D. dugon* bone specimens that were sampled *de novo* and 64 *D. dugon* DNA extracts that were used in a prior phylogeographic study targeting the mtDNA control region (D-loop) only (electronic supplementary material, table S1) [12]. We also sampled one archaeological Steller's sea cow (*Hydrodamalis gigas*) bone which was used as an outgroup in downstream analyses. These samples came from a diverse set of historical museum collections (electronic supplementary material, tables S1 and S2). These specimens have been identified to species genetically or morphologically. Collection and/or accession dates were recorded for 67 total sampled individuals. Where collection date was unavailable, we used the accession date (see electronic supplementary material text).

### 2.2. Museum sampling

Bone samples were taken using a Dremel and diamond cutting blade. We took approximately 2 g bone pieces (see electronic supplementary material, text). Sampling for powder for the DNA extracts was conducted similarly by [12] and samples were shipped from museums to the University of Auckland, New Zealand, in accordance with CITES regulations. These samples were extracted—see [12] for details—for amplification of the mtDNA control region, and the extracts were frozen and stored. All bone samples and DNA extracts were transferred to the University of Oslo, Norway, in accordance with CITES regulations. Specifically, we used CITES permit exemption codes (exemption for scientific transfer) to ship from the University of Auckland, New Zealand (NZ010), and Naturalis Biodiversity Centre, The Netherlands (NL001), to Naturhistorisk Museum, University of Oslo, Norway (NO001).



**Figure 1.** Distinct Indo-Pacific population structure of *D. dugong* revealed by historical mitogenomes. (a) Sample map of 56 dugong specimens (coloured boxes) showing the extant range of *D. dugong* (grey), including ranges of functional extinction (horizontal black lines). The specimens are coloured by the genetic clade (shades of green or orange) to which the individual was assigned. Arrows indicate major ocean currents, (b) Phylogenetic (ML) tree analyses of *D. dugong*, including a Steller's sea cow specimen. Branches with bootstrap values >90 are labelled, and major clades with bootstrap values <90 are highlighted in red. Metadata for the broad locality of each specimen's collection is given adjacent to the tree.

## 3. Methods

### 3.1. Milling and extraction

All pre-PCR (polymerase chain reaction) protocols were performed in a clean lab at the University of Oslo, Norway, following strict aDNA precautions [59,60]. Bone samples were exposed to UV light before milling using a stainless steel mortar [61], where samples were crushed into a chunky powder. DNA extraction comprised a pre-digestion protocol [62] with modifications from [63], without the prior bleach wash (see electronic supplementary material, text for more details). Bone powder of weight 120–160 mg was used for each pre-digestion, which was then followed by an overnight digestion of 48 hours. Eluates were concentrated (Amicon–30 kDa centrifugal filter units) and the DNA collected using Minelute (Qiagen) columns according to the manufacturer's instructions. DNA was eluted in 100  $\mu$ l elution buffer (EB), preheated to 60°C [64]. A TE-Tween mix (1% TE buffer and Tween-20 mix) was added to the [12] DNA extracts at a volume of 1  $\mu$ l per 20  $\mu$ l of DNA extract to facilitate the release of DNA strands bound to the wall of the tube from long-term storage. A Qubit measurement was generated for all DNA extracts to optimize the dilution of ET SSB and P5 and P7 splinted adapters in the library build for the amount of input DNA, as recommended for the Santa Cruz reaction protocol [65].

### 3.2. Library, PCR and clean-up

Negative controls were included in extraction and library-build experiments. We generated libraries from a total of 76 historical dugong specimens, and one Steller's sea cow. Extracted ancient and historical DNA was prepared for sequencing on the Illumina sequencing platform using the Santa Cruz reaction protocol, an approach that converts single-stranded and denatured double-stranded DNA into sequencing libraries in a single reaction [65]. Libraries were amplified using sample-specific P5 and P7 indexes. Amplification was initially carried out in triplicate 25  $\mu$ l reactions and later adapted to single 75  $\mu$ l reactions, as this made no difference to overall library complexity and sequencing results. Amplified libraries were cleaned and purified using AMPure® XP beads by Beckman-Coulter. All libraries were sequenced on an Illumina HiSeq 4000.

### 3.3. Mitogenomic analyses

Sequencing reads were processed with PALEOMIX [66] and mapping performed using BWA (algorithm: mem, MinQuality: 25). Reads were aligned to the *D. dugong* nuclear genome

assembly and associated mitogenome ([67]—dnazoo.org) and the Florida manatee (*Trichechus manatus latirostris*) mitochondrial genome assembly (accession: PRJNA68243) [68], to identify any individuals that may have been misidentified by museums (see electronic supplementary material, text). Genome assemblies were downloaded from DNA Zoo ([67]—dnazoo.org [69]). BAM files for all sequenced historical specimens in this study have been released under the ENA accession number PRJEB74084. aDNA damage was investigated with mapDamage v. 2.2.1 [70] (electronic supplementary material, figure S1). Variant calling was performed using BCftools v. 1.15.1 [71] with ploidy set to 1 (for haploid mitogenome data). Light VCF filtering was performed using BCftools v. 1.15.1 [71] and VCFtools v. 0.1.16 [72] with the following parameters: *minQ* > 30.0, *min-meanDP* = 3, *remove indels* = yes [51], *idepth* > 0.5. This produced a dataset of 56 mitogenomes with minimum of 0.5-fold coverage.

Principal component analysis (electronic supplementary material, figure S2) was conducted using PLINK v. 2.00a [73] and visualized in R Studio [74] using tidyverse v. 2.0.0 [75] and ggplot2 v. 3.4.4 [76]. The Steller's sea cow sample (ID: SHG017) was used as an outgroup for phylogenetic and haplotype network analyses. Filtered VCFs were indexed and consensus sequences were built and compiled using BCftools v. 1.15.1 *consensus (-H 1 a N -M N)* [71]. To visualize evolutionary relationships, IQ-TREE v. 2.2.2.3 [77] was used to generate a maximum likelihood (ML) tree using 1000 bootstrap replicates (*-m MFP -alrt 1000 -B 1000 AICc -bnni*), which was visualized and edited in FigTree v. 1.4.4 [78]. Tree confidence was assessed using bootstrap support values, and clades with branch values greater than 90 were considered strongly supported. An unrooted haplotype network was built using Fitchi [79] (with haploid used to specify mitochondrial (MT) data) and the required conversion from fasta to nexus file performed using ElConcatenero3 [80]. For further analyses, we used individuals with greater than 4.0-fold coverage (*idepth* > 4.0). Additionally, we assessed individual missingness (electronic supplementary material, figure S3) with VCFtools v. 0.1.16 [72], omitting any remaining individuals with considerable (*imiss* > 0.01) missing data. This subset of 41 individual mitogenomes (electronic supplementary material, table S4) was used for all subsequent analyses (see electronic supplementary material, text for details).

The multiple sequence alignment of our final subset (electronic supplementary material, table S4) was analysed in DnaSP v. 6 [81], where broad regional populations were specified according to museum location metadata and the data format edited for conversion to Arlequin file formats (*Genome = Haploid, Chromosome Location = Mitochondrial, Sites with alignment gaps = excluded*). Genetic distance between these populations was assessed using measures of absolute ( $D_{xy}$ ) and relative ( $\Phi_{ST}$ ) divergence, which were calculated in DnaSP v. 6 [81] and Arlequin [82], respectively.  $\Phi_{ST}$  is a measure of population differentiation due to genetic structure, while  $D_{xy}$  assesses the genetic distance between populations based on sequence divergence. When analysed together  $\Phi_{ST}$  and  $D_{xy}$  give a more nuanced understanding of population genetic structure and differentiation. In Arlequin, pairwise  $\Phi_{ST}$  was calculated based on a pairwise distance matrix.  $p$ -values (electronic supplementary material, table S6) were generated in Arlequin to test the significance of pairwise  $\Phi_{ST}$  using 1000 permutations. Genetic diversity was investigated on both spatial and temporal scales in DnaSP v. 6 [81], where we generated standard population genetic measurements under genetic differentiation and divergence analyses. Tajima's  $D$  [83] and Fu and Li's  $F$  [84]—statistics used in evolutionary biology to infer past population dynamics and detect selection—were generated for individual test groups within DnaSP v. 6 [81].

Female effective population size ( $N_e$ ) was modelled back in time for the species and for the eastern clades and western clade (as defined by the phylogeny) using a coalescent Bayesian skyline plot approach [85] implemented in BEAST v. 2.7.6 [86]. The multiple sequence alignment file was aligned using the MUSCLE alignment algorithm [87] in MEGA11 [88] and exported in nexus format. Jmodeltest2 v. 2.1.10 [89] was used to determine the best nucleotide model; best fitting models were determined using  $\Delta BIC$  (HKY+G). In BEAUti [86], the file was annotated: *site model = HKY; gamma category count = 4; strict clock; normal distribution; upper/lower bound rates:  $7.0 \times 10^{-9}$ – $7.5 \times 10^{-8}$  substitutions/site/year* (based on *Odobenus rosmarus*, see [51]). The output was then run in BEAST v. 2.7.6 [86] (10 000 000 iterations; 10% burn-in; logged every 10 000). Log and tree files were read into Tracer v. 1.7.2 [90] confirming convergence (ESS > 200) after which a coalescent Bayesian skyline plot was generated.

## 4. Results

### 4.1. DNA yield and library success

We obtained approximately 3.25 billion paired total sequencing reads for 66 dugong specimens from across the Indo-Pacific region (figure 1a). Specimens yielded between 0.06 and 65% endogenous DNA, with 3–54% mitochondrial clonality and an average read length of 88.4 bp (electronic supplementary material, table S3). Sequencing reads from all historical specimens show the typical fragmentation and deamination patterns expected with post-mortem DNA degradation, with recent specimens (i.e. from 1995) showing little post-mortem sequence modification (electronic supplementary material, figure S1). Of the 66 dugong libraries that were successfully amplified and sequenced, 56 passed our initial filtering with 0.5 $\times$ -fold coverage of the mitogenome.

### 4.2. Population structure and phylogenetic analyses

Principal component analysis of the 56 mitogenomes yielded three distinct clusters (electronic supplementary material, figure S2). An ML phylogenetic analysis shows that these three clusters can be broadly resolved into five major monophyletic clades. These clades are largely geographically restricted; they occur on either side of the Indo-Pacific range apart from a limited central zone off the Indian subcontinent where they co-occur (figure 1).

The phylogeny (figure 1b, see also electronic supplementary material, figure S4 for greater detail) and haplotype network (figure 2) of the 56 historical dugong samples reveal significant population structure across the Indo-Pacific range. The western clade (orange, figures 1 and 2) includes

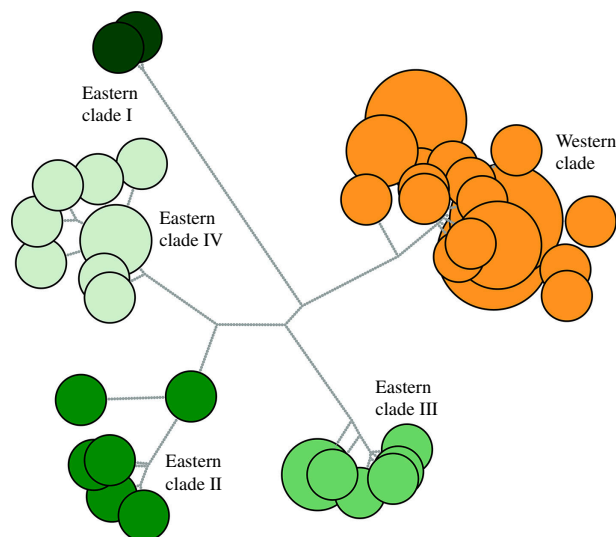
mitogenomes from eight broad regions across the western, and parts of the northern, Indian Ocean. In the eastern Indo-Pacific, there are at least three well-supported clades (II, III, IV; shades of green, figures 1 and 2). Each clade is supported with bootstrap support values  $>90$ . The exact relationships between these clades are less well defined, with a bootstrap support value of 65. We also observe a genetically distinct clade (I) in this region composed of two individuals (dark green, figures 1 and 2). The relationship of this clade with eastern clades II, III, IV, within the major east/west bifurcation, is less certain, with a bootstrap support value of 49 (see electronic supplementary material, text). Within the clades, subclades in the phylogeny are largely geographically structured. Many regionally defined clusters are supported with bootstrap support values  $>90$ , including those in the western clade such as Madagascar/Comoros (figure 1b).

The unrooted haplotype network (figure 2) has a total of 409 variable sites (including missing data given that the programme cannot discriminate) across the 56 mitogenomes. We observe lower levels of unique haplotypes among individuals from the western clade (with localities including the Persian Gulf, Red Sea and Djibouti) than those from the eastern clades (with localities including Indonesia and Australia; for figure 2 coloured according to museum location metadata, see electronic supplementary material, figure S5). Individuals in the eastern clades are separated by greater numbers of single nucleotide polymorphisms (SNPs) compared with their western clade conspecifics.

### 4.3. Genomic variation and population diversification

Nucleotide diversity ( $\pi$ ) estimates within *D. dugon* are based on a subset of 41 mitogenomes with sufficiently high coverage to reduce the impact of missing data (electronic supplementary material, table S4). This dataset includes 257 segregating sites (*S*) leading to a  $\pi$  of 0.0030 (table 1). We detect 31 unique haplotypes, six of which are shared by multiple individuals. Haplotype sharing occurs almost exclusively in the western clade (five out of six instances).

Between regionally defined populations (table 1),  $\pi$  ranges from 0.0001 to 0.0046, a 46-fold difference between the highest (ISN) and lowest (PER) populations measured. Haplotype diversity (*h*) and total *S* are significantly greater in populations in the eastern than the western Indo-Pacific region and significant variation in the spatial distribution of genetic variation ( $\chi^2$  *p*-value = 0.0019\*\*) is apparent across dugong populations. Neutrality tests show no significant values for Tajima's *D* (TD) and *F* statistics in any populations.



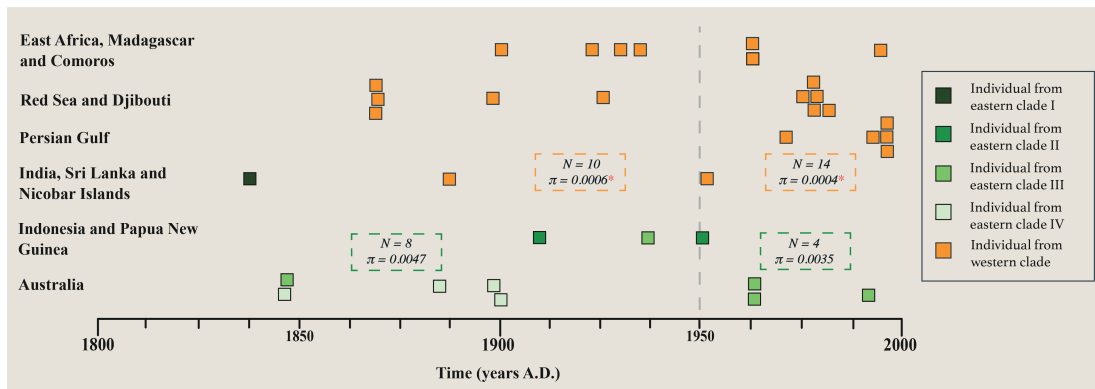
**Figure 2.** An unrooted haplotype network of 56 dugong individuals reflecting vastly different levels of divergence between eastern and western clades. The network nodes are coloured by the clade to which the individual mitogenomes were assigned genetically. Node size corresponds to number of individuals sharing that haplotype. Grey circles are indicative of single nucleotide polymorphisms (SNPs).

**Table 1.** Genetic diversity of *D. dugon* within six broad and regionally defined populations, and between eastern and western genetic clades. Standard measures given are the number of individuals ( $N$ ); haplotype diversity ( $h$ ); the number of haplotypes ( $Nh$ ); segregating sites ( $S$ ); nucleotide diversity ( $\pi$ ); Tajima's  $D$  ( $TD$ ); Fu and Li's  $F$  ( $F$ ). Significant  $p$ -values ( $<0.05$ ) in  $TD$  and  $F$  analyses are indicated with an asterisk. ID indicates insufficient individuals to compute the statistic.

population	code	$N$	$h$	$Nh$	$S$	$\pi$	$TD$	$F$
Australia	AUS	9	0.97	8	96	0.0027	1.343	2.082
Indonesia and Papua New Guinea	IPG	6	1.00	6	150	0.0036	-0.762	1.707
India, Sri Lanka and Nicobar Islands	ISN	3	1.00	3	109	0.0046	ID	ID
Persian Gulf	PER	5	0.80	3	2	0.0001	1.459	-0.186
Red Sea and Djibouti	RSD	10	0.80	5	9	0.0002	-0.139	1.072
East Africa, Madagascar and Comoros	EMC	8	0.89	6	15	0.0003	-0.261	-1.580
total		41	0.98	31	257	0.0030	-0.745	-0.335
eastern clades		16	0.99	15	202	0.0038	0.0843	0.353
western clade		25	0.95	16	49	0.0004	-1.8433*	-3.217*

Of the 257 variable sites, 193 sites are polymorphic in the eastern clades and monomorphic in the western clade, while 40 sites are polymorphic in the western clade and monomorphic in the eastern clades (figure 1b; see electronic supplementary material, tables S4 and S5). Overall, only nine sites were polymorphic in both the east and west. Nucleotide diversity ( $\pi$ ) in the western clade ( $n = 25$ ) is 0.0004, and 0.0038 in the eastern clades ( $n = 16$ ). The difference between average number of nucleotide differences ( $k$ ) is approximately 10-fold between east ( $k = 60.392$ ) and west ( $k = 6.887$ ). Although  $\pi$  is approximately 10-fold higher in the eastern clades (table 1), overall genetic differentiation between clades did not reach significance ( $\chi^2$   $p$ -value = 0.0869 ns). Neutrality tests show significantly negative values ( $p$ -value  $\leq 0.05$ ) for  $TD$  and  $F$  statistics within the western clade (table 1).

The potential for loss of diversity over time was tested for individuals with  $>4$ -fold coverage and for which we had a collection or accession date ( $n = 36$ ) (see electronic supplementary material, table S6 and text). We observe no significant difference in genetic variation in the eastern clades before ( $n = 8$ ) and since ( $n = 4$ ) 1950 ( $\chi^2$   $p$ -value = 0.2851 ns), although a decrease in nucleotide diversity ( $\pi$ ) from 0.0047 to 0.0035 is apparent (figure 3). Nevertheless, we do



**Figure 3.** Temporal genomics of *D. dugon* in the Indo-Pacific region demonstrate that there has been a significant loss of genetic diversity in the western clade over recent times. A timeline from 1800 to 2000 AD shows the temporal distribution of individuals used to measure the loss of genetic diversity over time. Data used are museum collection and accession dates (see electronic supplementary material, table S6). The dashed line at 1950 visually divides the two temporal periods tested for each clade following Plön *et al.* [12]. Individuals are coloured by clade. A number of individuals ( $n$ ) and nucleotide diversity ( $\pi$ ) for each tested clade subset are given within the dashed boxes, and a significant loss of diversity ( $\chi^2$   $p$ -value  $\leq 0.05$ ) is indicated with a red asterisk.

	AUS	IPG	ISN	PER	RSD	EMC
AUS	0.0027	0.0039	0.0051	0.0045	0.0046	0.0047
IPG	0.20*	0.0036	0.0048	0.0046	0.0047	0.0048
ISN	0.34*	0.17	0.0046	0.0026	0.0027	0.0028
PER	0.63**	0.59**	0.29*	0.0001	0.0003	0.0005
RSD	0.71**	0.70**	0.48**	0.49**	0.0002	0.0004
EMC	0.68**	0.66**	0.42**	0.47**	0.32**	0.0003

$\phi_{ST}$

$D_{xy}$

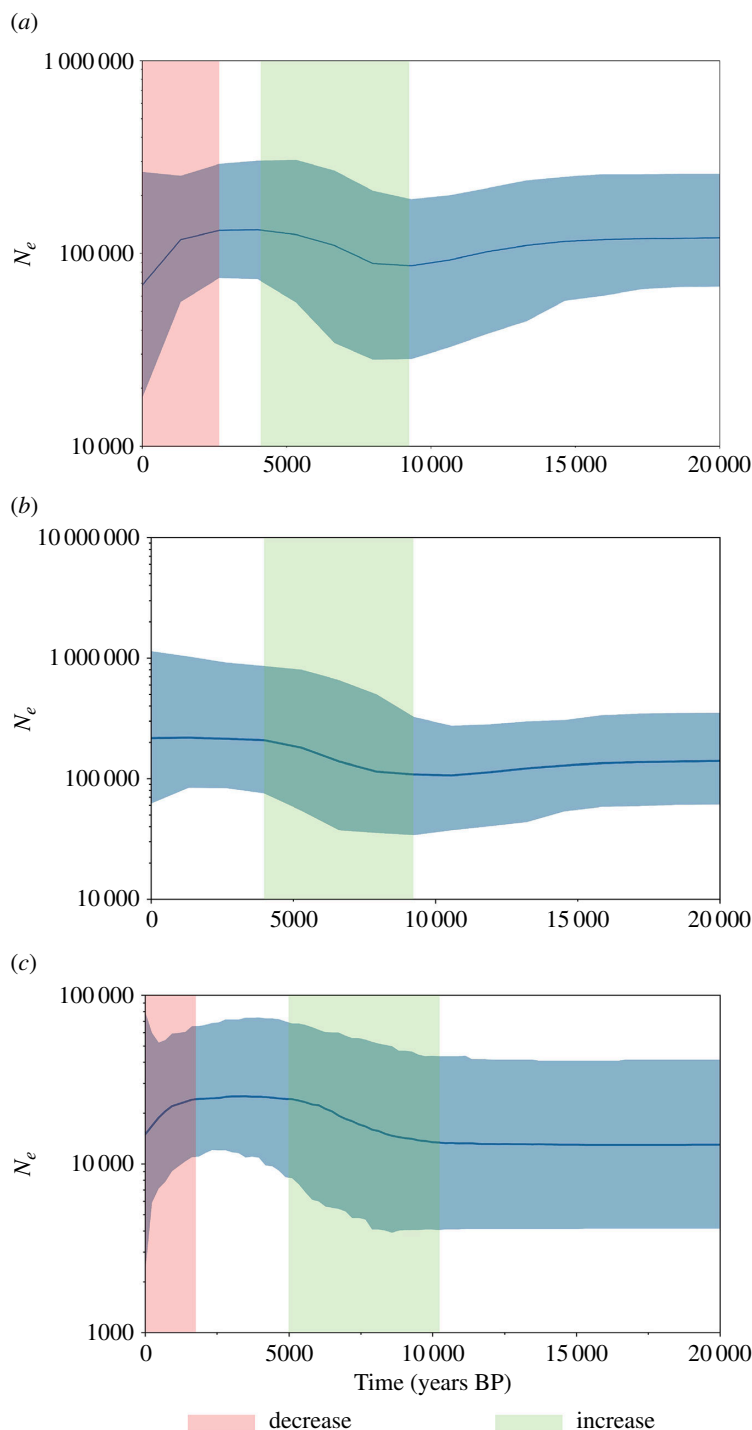
Nucleotide diversity ( $\pi$ )

**Figure 4.** Significant population structure between regionally defined dugong populations in the Indo-Pacific based on MT genome data. A heatmap depicts pairwise measures of absolute ( $D_{xy}$ ; pink) and relative ( $\Phi_{ST}$ ; grey) divergence between populations. Nucleotide diversity ( $\pi$ ; blue) within populations is given on the diagonal. Populations are coded as shown in table 1. Darker colours denote higher divergence or greater diversity. Significant  $p$ -values are indicated with a red asterisk (\* $\leq 0.05$ ; \*\* $\leq 0.01$ ).

identify a significant loss of genetic diversity in the western clade before ( $n = 10$ ) and since ( $n = 14$ ) 1950 ( $\chi^2$   $p$ -value = 0.0458\*). Neutrality tests show no significant values for TD and  $F$  statistics within these subsets (electronic supplementary material, table S7).

Substantial population diversification is observable between broad-scale regions based on pairwise measures of absolute and relative divergence, as well as intrapopulation genetic diversity and nucleotide diversity ( $\pi$ ) (figure 4). Levels of  $\pi$  were the greatest within eastern Indian Ocean populations, specifically ISN and IPG. Pairwise  $\Phi_{ST}$  values show significant ( $p$ -value  $\leq 0.01$ ) divergence between almost all population pairs (electronic supplementary material, table S8). Measures of pairwise  $\Phi_{ST}$  are concordant with the geographical proximity of populations, suggesting that the majority of structuring may be explained on a spatial scale.

$D_{xy}$  (absolute divergence) measurements are high within east–east and east–west pairwise tests (0.0051–0.0026), along with high pairwise  $\Phi_{ST}$  (0.20–0.71). One exception to this is IPG/ISN, which does not show significant differentiation ( $p$ -value  $\geq 0.05$ ); here we observe high  $D_{xy}$  (0.0048) and relatively low  $\Phi_{ST}$  (0.17). By contrast, west–west pairwise tests have high  $\Phi_{ST}$  (0.32–0.49) and low  $D_{xy}$  (0.0003–0.0005).



**Figure 5.** Long-term demography of *D. dugon* investigated using coalescent Bayesian skyline plots shows recent rapid reduction of female effective population size in western Indo-Pacific populations. These plots show female effective population size ( $N_e$ ) of (a) all *D. dugon* individuals, (b) the eastern clades' individuals and (c) the western clade individuals, based on an assumed rate of  $7.0 \times 10^{-9}$ – $7.5 \times 10^{-8}$  substitutions/site/year. Time is given in thousands of years ago (kya). Colour bands indicate increasing (green) and decreasing (red)  $N_e$ .

#### 4.4. Long-term population dynamics

The demographic history of *D. dugon* was investigated using coalescent Bayesian skyline plots and time estimates obtained based on a standard substitution rate of  $7.0 \times 10^{-9}$ – $7.5 \times 10^{-8}$  substitutions/site/year (figure 5). Overall, female  $N_e$  for *D. dugon* experienced two periods of reduction in recent times (figure 5a). This appears to be due to a slight reduction in  $N_e$  in the

east approximately 12 500 years ago (figure 5b), and a more dramatic reduction in the west over the last 1000 years (figure 5c). Notably,  $N_e$  in the east (figure 5b) has consistently been approximately 10-fold greater than that in the west (figure 5c).

## 5. Discussion

In this study, we used aDNA from historical specimens to improve our understanding of the population structure and temporal genomics of *D. dugon*. We observe significant population differentiation across the Indo-Pacific, associated with distinct evolutionary lineages. We find that levels of dugong genetic diversity are approximately 10-fold higher in the eastern compared with the western Indo-Pacific region. A significant decline in female  $N_e$  over the last millennium was observed in the West. We discuss the implications of our findings in the following text.

First, we find that the Indo-Pacific population divergence of *D. dugon* is largely associated with evolutionarily distinct clades that are largely geographically restricted to either the western or eastern parts of the range. Significant population structure is also found within these regions. For instance, in a previous study based only on mtDNA D-loop data, Madagascar/Comoros was the only western Indian Ocean population to be distinguished genetically [12]. Based on the higher resolution of our mitogenomic data, we find here that all of our sampled populations from this region (PER, RSD, EMC) are in fact significantly different ( $\Phi_{ST}$ ) from one another. Moreover, the observed patterns of high  $\Phi_{ST}$  and low  $D_{xy}$  among these populations suggest a more recent divergence than among the eastern clades. The data show a low sequence divergence, despite populations being significantly differentiated; absolute divergence tends to become high later relative to relative divergence because it reflects the accumulated changes in sequences over time. In conjunction with significantly negative TD and  $F$  values for the western clade, which are indicative of recent population expansion or a bottleneck [83,84,91], our findings point towards a relatively recent dispersal from a common ancestor of all modern western Indian Ocean dugongs across the Indo-Pacific.

Eastern clades, by contrast, comprised specimens with considerably deeper evolutionary divergence, and pairwise population divergence was driven by strong phylogeographic structure, with both high  $\Phi_{ST}$  and  $D_{xy}$ . Additionally, we found evidence of a genetically distinct clade (I) concordant with recent findings of potentially long-isolated and genetically distinct dugong aggregations in nearby Andaman seascapes [46,92]. Overall, the divergence patterns observed are consistent with the hypothesis that diversification and dispersal of *D. dugon* initially took place in the east, with some dispersal as far as South Asia, resulting in deeply rooted and diverse clades. The current western Indian Ocean clade resulted from a relatively recent colonization event. The biographical causes behind the current patterns of genetic variation are difficult to assess from these data alone; a greater sample size and use of whole genome sequencing may allow us to detect more detailed population structuring—specifically the placement of the deeper clades of which the branching could not be confidently resolved—as well as to better assess our hypothesis of a relatively recent dispersal of the modern western Indian Ocean dugong. Further, it should be considered that this dispersal could be linked to past environmental change, such as the disappearance of Sundaland after the Last Glacial Maximum [93] and/or the spread of seagrass meadows, which could have allowed dugongs to spread out across the Indo-Pacific.

Second, we determined that female  $N_e$  has been relatively stable over the last 20 000 years and that reductions in  $N_e$  have only occurred during the last millennium. This observation is in contrast to archaeological evidence, specifically the extensive dugong bone mounds excavated in the Arabian Peninsula, which spawned the hypothesis that dugong hunting has been intensive, and possibly unsustainable, since the Neolithic [14,16]. We did not observe the expected mitogenomic consequences, i.e. a loss of genetic diversity (e.g. [94,95]) that would support this. It is possible that the way in which people were hunting in the distant past could explain this finding. For example, if ancient hunting was on a local scale with heavy exploitation limited to populations that could be easily reached, the extirpated populations may not be represented in this historical dataset or the cumulative effect of such hunting practices on the entire global population may not be enough to leave a mitogenomic signal. It is possible that additional analyses (i.e. targeting the nuclear genome), could detect such an effect. Additionally, if hunting was sex-biased, i.e. males are targeted, a mitogenome study such as this would again not detect the expected genomic consequences of heavy exploitation. Finally, the recent reduction in  $N_e$  could be due to population structure [96]. Nonetheless, we find this reduction in the western populations which have considerably lower population structure compared with the eastern

populations. We, therefore, consider it unlikely that such a structure drives the reduction of  $N_e$  in western populations.

Third, mitogenomic variation differs greatly across dugong populations, with considerably lower levels of genetic diversity in the west than that in the east. Of particular concern is that one of the largest global dugong stocks, the Persian Gulf population [36], shows the lowest genetic diversity ( $\pi = 0.001$ ). This finding is similar to recent findings in New Caledonia of another of the largest global stocks comprising only three distinct haplotypes and having extremely low nucleotide diversity [43]. While we emphasize the urgent need for conservation management of the dugong, our findings demonstrate that additional conservation priority should be given to western populations who could be at disproportionate risk due to their considerably lower levels of genetic diversity, a limiting factor for adaptive potential, and relatively low population size. Nevertheless, with further anthropogenically induced extinctions [40,41], the higher level of unique genetic diversity in the eastern clades, and therefore, the uniqueness of the species remains under considerable threat. Overall, the distinct population structuring found throughout the Indo-Pacific adds to the existing evidence [97] for assigning conservation management units.

## 6. Conclusion

Our dataset represents the largest range-wide historical mitogenome study of *D. dugon* to date. The findings provide considerably greater resolution of species-wide genetic diversity and population structure across the Indo-Pacific. While prior studies had uncovered potentially divergent lineages of the dugong, our findings are biologically significant as a distinct split across the Indo-Pacific had not yet been reported and investigated at this level of detail. We have demonstrated that dugong female effective population size has been declining and that there has been a measurable loss of genetic diversity in the western portion of their range in recent history. Overall, our data add to the growing body of evidence that global dugong populations are significantly fragmented and becoming increasingly less genetically diverse, making them ever more susceptible to ongoing anthropogenic threats and global climate change.

**Ethics.** This work did not require ethical approval from a human subject or animal welfare committee.

**Data accessibility.** All raw sequence data has been released in the appropriate repositories.

The BAM files for all historical specimens are released under the ENA accession no. PRJEB74084.

Supplementary material is available online [98].

**Declaration of AI use.** We have not used AI-assisted technologies in creating this article.

**Authors' contributions.** L.H.F.: conceptualization, data curation, formal analysis, investigation, methodology, resources, visualization, writing—original draft, writing—review and editing; O.K.: formal analysis, methodology, writing—review and editing; A.B.: methodology, writing—review and editing; L.K.-D.: writing—review and editing; C.B.: funding acquisition, writing—review and editing; J.H.B.: funding acquisition, resources, supervision, writing—review and editing; A.K.: resources, writing—review and editing; R.S.: resources, writing—review and editing; S.L.: resources, writing—review and editing; S.P.: conceptualization, data curation, resources, writing—review and editing; B.S.: conceptualization, funding acquisition, project administration, supervision, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

**Conflict of interest declaration.** We declare we have no competing interests.

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